# USE OF TITANIUM DIOXIDE AS EXCIPIENT IN HUMAN AND VETERINARY MEDICINES AND IDENTIFICATION OF ALTERNATIVES

## INDUSTRY FEEDBACK TO QWP EXPERTS/EMA QUESTIONS FINAL REPORT FEB 2024

efpia
European Federation of Pharmaceutical Industries and Associations

$TiO_2$ Alternatives Consortium

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# Introduction

This document is submitted on behalf of the European associations representing the human medicines manufacturers, veterinary medicines manufacturers and excipient producers. It is the interim feedback to the European Commission, EMA Quality Working Party (QWP) and Non-Clinical Working Party (NcWP) experts in relation to the requirement of the Regulation amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food additive Titanium Dioxide (E 171) (2022/63/EU). It aims at providing written answers to both the questions posed by QWP on 11 September 2023 and by QWP and NcWP experts at the QWP drafting group and industry associations meeting on Titanium Dioxide meeting of 16th October 2023.

The questions posed by the QWP to industry with the deadline of 2nd November 2023 were as follows:

**A. $TiO_2$ possible alternatives**

1. Please list the alternatives to replace / remove $TiO_2$ without negatively impacting the quality, safety and efficacy of medicine that you have investigated to date with the advantages and disadvantages and if applicable, any additional potential alternatives that are planned to be investigated in future.

2. Please supply a summary of the evidence /results from the ongoing studies comparing alternative formulations (for different dosage forms as available) with those containing $TiO_2$.

3. In 2021, you provided QWP with information on the methodology and timeline estimates on investigating potential alternatives to replace/remove $TiO_2$ without negatively impacting the quality, safety and efficacy in medicinal products. Please provide the updates to this information versus the last analysis.

**B. Industry impact assessment of the situation on the pharmaceutical sector and timelines**

4. In case an alternative to replace/remove $TiO_2$ is identified, please indicate approximate timelines to prepare and file for such a change (for subset of products/which ones/are there different issues for different products or dosage forms/types of products?).

5. Please, supply an updated summary of the calculated impact on availability, shortages, and costs of any requirement to replace/remove $TiO_2$ from medicines in Europe, considering the global nature of product development and supply.

# Overview

## Titanium Dioxide as a ubiquitous excipient in medicines globally

Titanium Dioxide ($TiO_2$, E171, anatase) is primarily used in medicinal products as a white colourant and opacifier in coatings and capsules. It has unique properties, such as providing light protection to many active ingredients and formulations and to ensure uniform appearance when used in in minimal quantities.

$TiO_2$ is ubiquitous in medicines globally. Although an exact number i difficult to establish, it is estimated that at least 100 000 human medicinal products and 1600 veterinary medicinal products in the EU contain $TiO_2$. The true number globally is likely to be significantly higher (EMA/504010/2021). Reformulation of even a proportion of these products would provide an enormous and unprecedented challenge which will be discussed in detail within this report.

$TiO_2$ has played a key role in the safety, efficacy and compliance for the majority of medicines in Europe for over 50 years; and as a pure mineral, $TiO_2$ meets the most stringent of requirements governing the safety of medicines, including those set by the European Pharmacopoeia, Japanese Pharmacopoeia and US Pharmacopoeia.

## Timeline of Developments

### 1. EFSA 2021

On the 6th May 2021, the European Food Safety Authority (EFSA) published their opinion on the safety assessment of E171 Titanium Dioxide, which states that it can no longer be considered safe when used as a food additive. EFSA found that, on the basis of a reassessment of the available safety data, a concern for genotoxicity "*could not be ruled out*" and, consequently, a "*safe level for daily intake of the food additive could not be established*". EFSA has previously reviewed the use of $TiO_2$ as a food additive in 2016, 2018 and 2019, however, all three previous EFSA investigations found no evidence indicating $TiO_2$ could present a risk to human health.

### 2. Industry Assessment and EMA report (EMA/504010 2021)[1]

On the 30 June 2021, three European associations representing the human medicines manufacturers (AESGP, EFPIA, Medicines for Europe) prepared a report[2] to feedback to the European Commission and EMA experts in relation to the opinion of EFSA on $TiO_2$ and its impact on human and veterinary medicinal products. The report provided written answers to the group of QWP experts on the use of titanium dioxide as an excipient and address three area: quantitative and qualitative presence of $TiO_2$ in medicinal products in EU/EEA, possible alternatives, and an impact assessment of a theoretical requirement to replace $TiO_2$.

Likewise, the two associations representing the Veterinary medicines sector (AnimalhealthEurope and Access VetMed (formerly EGGVP) also submitted a report[3] to feedback on the impact on veterinary medicines sector to the EMA updated on the 8th July 2021, the report included quantitative and

[1] https://www.ema.europa.eu/en/documents/report/final-feedback-european-medicine-agency-ema-eu-commission-request-evaluate-impact-removal-titanium_en.pdf

[2] https://www.ema.europa.eu/en/documents/other/annex-i-use-titanium-dioxide-excipient-human-medicines-industry-feedback-qwp-experts/ema-questions_en.pdf

[3] https://www.ema.europa.eu/en/documents/other/annex-ii-use-titanium-dioxide-excipient-veterinary-medicines-industry-feedback-qwp-experts/ema-questions_en.pdf

qualitative presence of $TiO_2$ in medicinal products in EU/EEA, possible alternatives and impact assessment of a theoretical requirement to replace $TiO_2$.

The EMA subsequently published their final feedback to the EU Commission request to evaluate the impact of the removal of $TiO_2$ from the list of authorised food additives on medicinal products in October 2021. It included the following conclusions:

- $TiO_2$ is extensively used as an opacifier and colourant in medicines due to its multiple functionalities.
- $TiO_2$ is used very frequently in oral solid dosage forms and in oral semi-solid dosage forms. $TiO_2$ is also present in dosage forms administered via routes other than oral.
- It is present in many essential medicines.
- To date [2021], no single material had been identified that provides the same combination of properties that are unique to $TiO_2$. Separating out the different functionalities of $TiO_2$ for those medicinal products in which it serves more than one function is difficult or might not be possible at all.
- Possible alternatives identified so far [2021] have a number of disadvantages versus $TiO_2$.
- The feasibility of replacing $TiO_2$ could not be confirmed at this stage. Each affected medicinal product will need an individual review and assessment.
- Europe would potentially be the only region globally to ban $TiO_2$ as excipient in medicines, which would require industry to develop new formulations.
- An acceptable transition period for phasing-out $TiO_2$ was difficult to envisage or estimate considering the scale of the use of this excipient, the time and costs involved in the reformulation and the volume of products impacted.
- Replacing $TiO_2$ in medicines will almost certainly cause significant medicines shortages and discontinuations/withdrawals of medicines from the EU/EEA market with major implications for patients and animals. Particular concerns arise in relation to certain vulnerable classes/types of products such as paediatric medicines, orphan medicines or low sales volume products.

## 3. Legislative requirements

On 14 January 2022, the Commission adopted a ban on the use of Titanium Dioxide as a food additive (E171), amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food additive Titanium Dioxide (E 171) (2022/63/EU. Since 2022, $TiO_2$ is not authorised in the food categories (with a transition period of 6 months (implemented 7 August 2022).

Regulation 2022/63 provisionally maintains the inclusion of E171 in the list of approved colours allowed for use in medicines. The recitals note that this is to avoid shortages of medicinal products containing $TiO_2$ as this could impact public health and animal health and welfare. It is also noted that the replacement of $TiO_2$ requires investigation and testing of suitable alternatives to ensure that quality, safety and efficacy of medicines are not negatively affected.

The Commission will review the necessity to maintain $TiO_2$ or to delete it from medicines by February 2025 based on a re-evaluation by EMA in April 2024.

## Summary of outreach and engagement between industry, EU institutions and the EU regulatory network

The industry has engaged extensively throughout the process with the EU institutions and EU Regulatory Network to build a good dialogue and align on the expectations from industry on the scientific investigation of $TiO_2$ and potential alternatives. Table 1 below outlines the dialogue since 2021.

**Table 1: Outreach and engagement touch points**

| Date | Engagement | From | To/With | Focus of interaction |
|---|---|---|---|---|
| 5 August 2021 | Letter | AESGP, EFPIA, Medicines for Europe | European Commission | • Request for scientific dialogue with the goal to arrive at an overarching risk assessment for the use of E171 in pharmaceuticals |
| 31 August 2021 | Letter | European Commission | AESGP, EFPIA, Medicines for Europe | • Response to letter of 5 August 2021<br>• Informed on no room for a separate scientific assessment on the use of $TiO_2$ in medicines<br>• Informed industry that on the 17 May 2021, the EC requested EMA to provide an analysis with the aim to define the technical purpose of $TiO_2$ in medicinal products. |
| 17 February 2022 | Letter | EMA, European Commission, HMA | AESGP, EFPIA, Medicines for Europe, AnimalhealthEurope, Access VetMed | • Informing industry of Reg 2022/63<br>• Informing industry of requirement to accelerate R&D of alternatives to $TiO_2$ |
| 25 February 2022 | Letter | AESGP, EFPIA, Medicines for Europe | EMA, European Commission, HMA | • Acknowledging receipt of letter 17/2<br>• Acknowledging the continued use of $TiO_2$ in medicines<br>• welcome the continued dialogue opportunities and the EU Regulatory Network |
| 2 May 2022 | Meeting | AESGP, EFPIA, Medicines for Europe | Commission, EMA, HMA | • Presentation and discussion with the Commission on the human pharmaceutical association's activities on $TiO_2$ and alternatives |

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|---|---|---|---|---|
| 3 May 2022 | Meeting | AESGP, EFPIA, Medicines for Europe, EUCOPE, AnimalhealthEurope, Access VetMed | QWP of EMA | • Presentation and discussion on the planned approach of industry on the scientific investigation of $TiO_2$ and potential alternatives |
| 24 June 2022 | Letter | AESGP, EFPIA, Medicines for Europe | EMA, HMA, cc European Commission | • Follow up from QWP meeting in May<br>• Requesting close collaboration on $TiO_2$ and alternatives<br>• Requesting support for the industry proposed integrated and technical plan to assess the safety of alternatives and establish the feasibility of replacing $TiO_2$ in medicinal products.<br>• Clarification of the EU Regulatory Network's expectations under Commission Regulation 2022/63 and EMA Q&A 384135/2021 |
| 23 September 2022 | Letter | EMA | AESGP, EFPIA, Medicines for Europe | • Acknowledged receipt of the letter of 24/6/22<br>• Welcomed the pharmaceutical industry's commitment to seeking safe potential alternatives to $TiO_2$ |
| 4 October 2022 | Meeting | AESGP, EFPIA, Medicines for Europe, Eucope | NcWP of EMA | • Presented on the Scientific Investigation of $TiO_2$ & Potential Alternatives |
| 27 February 2023 | Letter | AESGP, EFPIA, Medicines for Europe | EMA, HMA | • Industry thanked EMA for opportunities in 2022 for engagement and discussions with the EMA within the context of the QWP (May) and NcWP (October) on $TiO_2$ and alternatives<br>• Reiterated the need for close collaboration and request for a meeting |
| 18 April 2023 | Letter | EMA, HMA | AESGP, EFPIA, Medicines for Europe | • Responded to letter dated 27/2/23<br>• Recommended companies continue to explore possible alternatives to $TiO_2$ and the feasibility of such alternatives.<br>• Agreed to include the topic at the next QWP IP meeting however reiterated the need for a safety discussion |

| | | | | |
|---|---|---|---|---|
| | | | | • Requested information on the EMA re-evaluation processes |
| 26 May 2023 | Letter | AESGP, EFPIA, Medicines for Europe + $TiO_2$ Alternatives Consortium | EMA, HMA | Requested further clarifications from the EMA:<br>• Welcomed opportunity to discuss at the QWP<br>• Noted related article (27) of the adopted commission proposal for a directive of the EU general pharmaceutical legislation |
| 27 June 2023 | Meeting | AESGP, EFPIA, Medicines for Europe, EUCOPE, AnimalhealthEurope, $TiO_2$ Alternatives Consortium, IPEC | QWP of EMA | • Presentation and discussion updating on the approach of industry on the scientific investigation of $TiO_2$ and potential alternatives |
| 16 October 2023 | Meeting | AESGP, EFPIA, Medicines for Europe, EUCOPE, AnimalhealthEurope, $TiO_2$ Alternatives Consortium, IPEC | QWP, NcWP, Commission | • Presentation and discussion with industry associations to discuss the 5 proposed questions of the EMA |
| 10 November 2023 | Report | AESGP, EFPIA, Medicines for Europe, EUCOPE, AnimalhealthEurope, $TiO_2$ Alternatives Consortium, IPEC | EMA | • Industry Feedback to the QWP experts/EMA questions Interim report Nov 2024 |

# Investigation of Alternatives to Titanium Dioxide in Capsules and Coatings

Industry is continuing to address the requirements of Commission Regulation 2022/63 to assess alternatives to Titanium Dioxide. At the outset of investigations, it was established that alternatives to $TiO_2$ must:

1. Deliver products of equivalent or superior safety to those using $TiO_2$.
2. Deliver products of equivalent or superior efficacy and quality to those using $TiO_2$.
3. Be available and sustainable.

It was identified that although some materials had become commercially available (e.g., coatings and capsule shells) which did not contain $TiO_2$, there was lack of evidence to show whether these provided viable alternatives (e.g., assessing impact on medicine appearance, stability, light protection and/or the need for increased film coating quantities which can impact efficacy).

Most importantly, the safety of such alternatives (in general terms and relative to $TiO_2$) may not have been appropriately established. At the same time industry also noted that currently approved colours may also undergo EFSA re-assessment, particularly regarding assessment of the safety in relation to nanoparticles (see Annex 1).

## Excipients industry efforts to identify alternatives to Titanium Dioxide

The excipients industry has created a number of options for $TiO_2$ free coatings and capsules which are currently being evaluated by medicinal product manufacturers. The best options available are a culmination of each individual excipient company evaluating numerous excipients in different combinations over the last 2-3 years. It is estimated that over 2000 different combinations of excipients have been evaluated by suppliers. In the opinion of IPEC Europe, there is no 'like for like' replacement for $TiO_2$, and this document will illustrate some of the issues the pharmaceutical industry will face should $TiO_2$ be no longer be available as an excipient in Europe. IPEC Europe also notes the likelihood that in such an eventuality, the demand for replacement materials (eg titanium dioxide-free coatings and capsules) will surge and the time and costs required for any capacity expansion to meet this need must be taken into account.

$TiO_2$ is an inert material that gives film coatings and capsules an effective opacity and protection from UV light, it allows the rapid development of consistent colour regardless of the core colour and condition, and regardless of the process parameters used or the scale of production. One of its hidden values is that it makes the coating process and resulting product much more consistent and predictable. In order to find a suitable replacement, the material must meet as many of these characteristics as possible, otherwise the quality of the resulting drug product is likely to be negatively impacted.

## Process to assess alternatives to Titanium Dioxide

Film coating and capsule companies start by screening potential materials to assess their performance as an opacifier. Once a suitable material is identified different grades of the same material from different suppliers are screened to determine the most effective opacifier or the whitest source. The next step is to see how any material performs in film coating or capsule shell formulations compared to $TiO_2$. Depending on time pressures and demand some of these simple replacement coating or capsule shell formulations were made available commercially, but these remain non-optimised and

there are significant compromises that need to be evaluated. Once a viable material is identified the next step is to optimise that formulation and this may involve removing or adding additional excipients to counteract the lack of performance versus $TiO_2$ in one aspect or another. In all cases there are still compromises that need to be balanced against performance and quality of the coating or capsule, these will then be evaluated more closely and made into commercially available products if they are acceptable from a regulatory compliance standpoint (see General Compliance assessment below). It is only at this stage that these optimised coatings and capsules can be fully evaluated (opacity, stability, process parameters, scale, availability, safety and quality) in finished drug products, and which needs to be repeated for each dosage type and API. The optimised coating or capsule shell formulations being evaluated are the result of over 2000 different combinations of excipients being evaluated by excipient companies.

Generally speaking, IPEC Europe believes that there is no excipient that is the equivalent of $TiO_2$. $TiO_2$ free coatings and capsules are commercially available, but they are more sensitive to scale effects, process parameters, UV protection is lower, and colour is not as predictable. These formulations also tend to have more excipients added to them making any licence variation more complex. These points are further discussed later within this report.

## Titanium Dioxide Alternatives Consortium

To coordinate activities and deliver an industry-aligned assessment, a grouping of (>20) pharmaceutical companies was formed in 2022 to collectively address this via a new pre-competitive industry Consortium. **The aim of the Alternatives Consortium was to generate evidence that can be used by the EMA to support the re-evaluation of the feasibility of removing $TiO_2$ from the list of excipients for use in medicines.**

**What:**

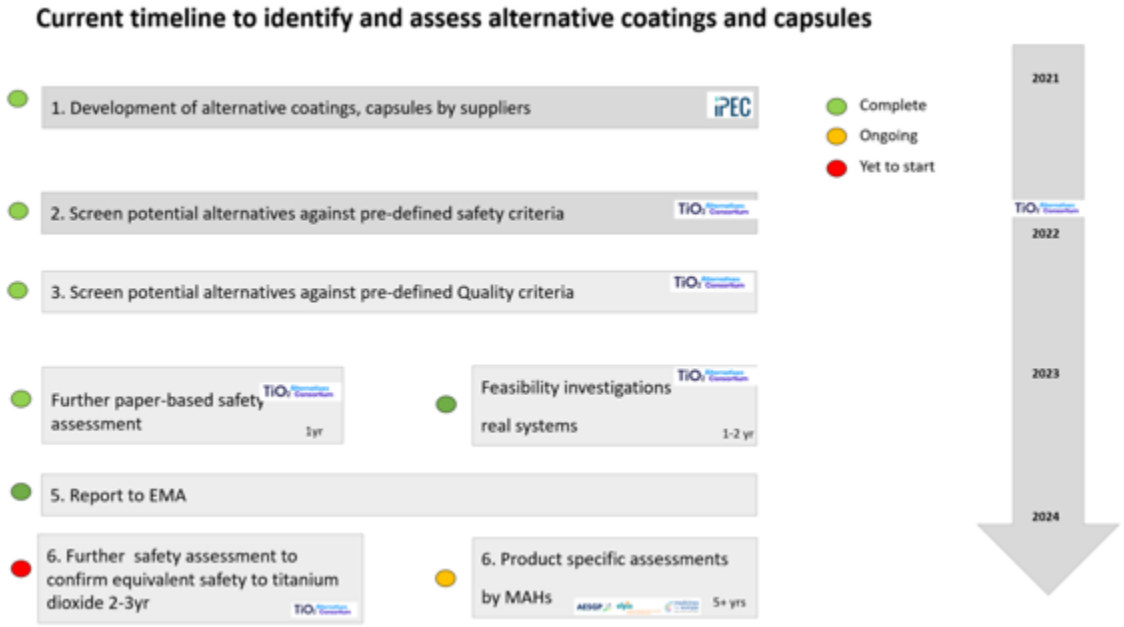
- These activities have been carried out by one, or several, Contract Research Organisations (CROs).
- They will be responsible for managing the work activities, and the associated financials, of this new consortium.

**How:**

- *Phase 1:* Comprised the technical evaluation of alternatives and manufacturing feasibility study running until approximately end 2023. Collect data and prepare final reporting to EMA IN February 2024.
- *Phase 2:* If required, in collaboration with the excipient industry and with input from EMA safety experts, would comprise *in-vivo* safety studies for the three most promising alternative candidates to complete their safety data set and would run beyond 2024.

## Summary and timeline of industry activities to identify and assess alternative coatings and capsules

The summary below presents an illustrated summary of the industry activities to identify alternative, $TiO_2$-free coatings and capsules, and to evaluate the safety and use of these in medicines.



# EMA Questions to Industry - 2023

## A. $TiO_2$ possible alternatives

**Question 1**

**Please list the alternatives to replace / remove $TiO_2$ without negatively impacting the quality, safety and efficacy of medicine that you have investigated to date with the advantages and disadvantages and if applicable, any additional potential alternatives that are planned to be investigated in future.**

### Assessment of $TiO_2$ alternative materials in film coat systems and hard capsule shells

The Consortium has undertaken a comprehensive assessment of alternative excipients to replace $TiO_2$ in film coats and hard capsule shells. The objective of the consortium has been to assess the potential impact of these alternative materials on the performance of immediate release film coated tablets and hard shell capsules. Immediate release products were selected for the evaluation as the impact on dissolution and disintegration would be easier to assess compared to the evaluation of controlled release dosage forms where any potential changes may have to be assessed through *in-vivo* studies.

The consortium has not evaluated the impact of alternative materials to $TiO_2$ in specialised dosage forms such as oral suspensions and soft capsules (softgels), where specialised manufacturing equipment and formulations which are designed for specific fill material result in a non-universal capsule shell formulation.

### Selection of Alternative $TiO_2$ Film Coat and Hard Capsule Shell Systems

To perform the assessment of these $TiO_2$-free alternatives, the consortium obtained ready-made coatings and hard capsules directly from the manufacturers. The manufacturers have the know-how and intellectual property related to the component selection, compositions, and manufacturing processes to match customer requirements.

For coatings and hard capsules there is several standard formulations depending on the film-forming polymer, structural additives (plasticizers, gelling agents), colorants and opacifiers and sometimes process aids. For coatings the main groups are Hypromellose (HPMC) versus polyvinylalcohol (PVA) polymers combined with different plasticizers. For capsules the main groups are Hypromellose (HPMC) versus gelatin with or without gelling agent.

During pharmaceutical development, multiple coatings or capsules are typically tested in parallel to determine the compositions yielding the most stable and robust drug product.

The following selection criteria for the $TiO_2$-alternatives were applied:

- **Suppliers:** all global suppliers known to the consortium were consulted, and included in the program if they offered alternatives.
- **Alternatives were selected based on**:
    - **Commercial readiness**: the alternatives had to be ready in terms of raw materials and manufacturing process. It was not a requirement that the alternative is effectively used in a commercial product.
    - **Compliance:** the alternatives and their components had to have a minimum compliance level with food or pharma quality monographs.

- For colored alternatives, the suppliers were consciously asked to avoid the use of organic dyes to avoid interferences in analytical and stability studies.

## General Compliance Assessment

Forty systems were studied (27 coats, 13 capsules). Key compliance considerations for alternative opacifying systems:

- Calcium carbonate ($CaCO_3$) and rice starch are included in 32 out of 40 systems, but for 15 systems the grades used have not been proven to meet multicompendial requirements limiting the potential for developing global formulations.
- Novel systems containing chemicals such as zinc oxide (ZnO) or sodium pyrophosphate are not globally approved for food use in oral medicines.
- Only calcium carbonate (white) and iron oxide (coloured)($Fe_2O_3$) are approved food colourants
- ECHA has submitted a dossier proposing 'suspected carcinogen' labelling for Talc, which is a component for all 8 PVA-coats out of various 27 coating systems studied.
- EFSA is re-evaluating the safety of iron oxides and hydroxides potentially affecting its status as approved food additive and colourant, which might impact all coloured coatings and capsule shells under evaluation.
- For 20 out of 40 alternatives, the system consists of an opacifier (e.g. calcium carbonate) and a component which boosts performance (e.g. isomalt). Most of the alternatives are used as an opacifier, not as colourant. The applicability of the food colourant requirement for these alternatives (opacifier, booster) is therefore unclear.

### 1. Tablet Film Coats – Compliance Assessment

Based on offerings from 8 global suppliers, the predicted best and most diverse $TiO_2$-free alternatives were selected.

These consisted of either Hypromellose, HPMC, (19 systems) or Polyvinyl alcohol, PVA, (8 systems) as these are the two most commonly used polymers in film coating. The coatings were initially assessed for compliance risks including food legislation (E-number, food colorant approval), investigation for nano-risk by EU-member states, global pharma approval for oral use, compliance to European Pharmacopoeia and to USP/NF & JP ('Other Pharm'), presence of talc and iron oxide ($Fe_2O_3$).

None of the 27 selected coat systems are considered risk-free with 24 out of 27 coats (~90%) considered to have 2 or more risks as shown in Figures 1 and 2.

**Figure 1: Compliance risk for hypromellose coatings**

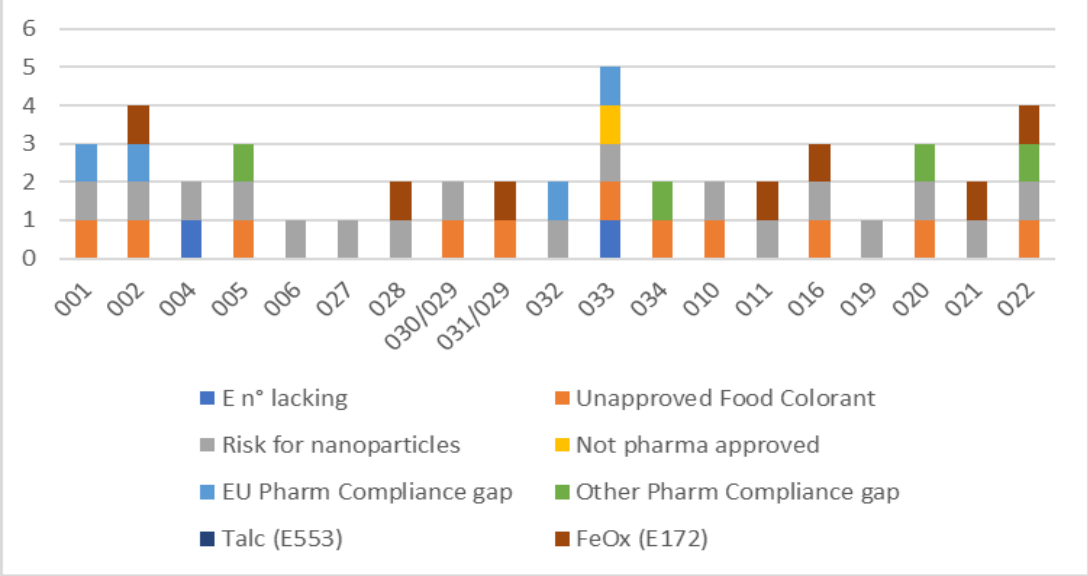
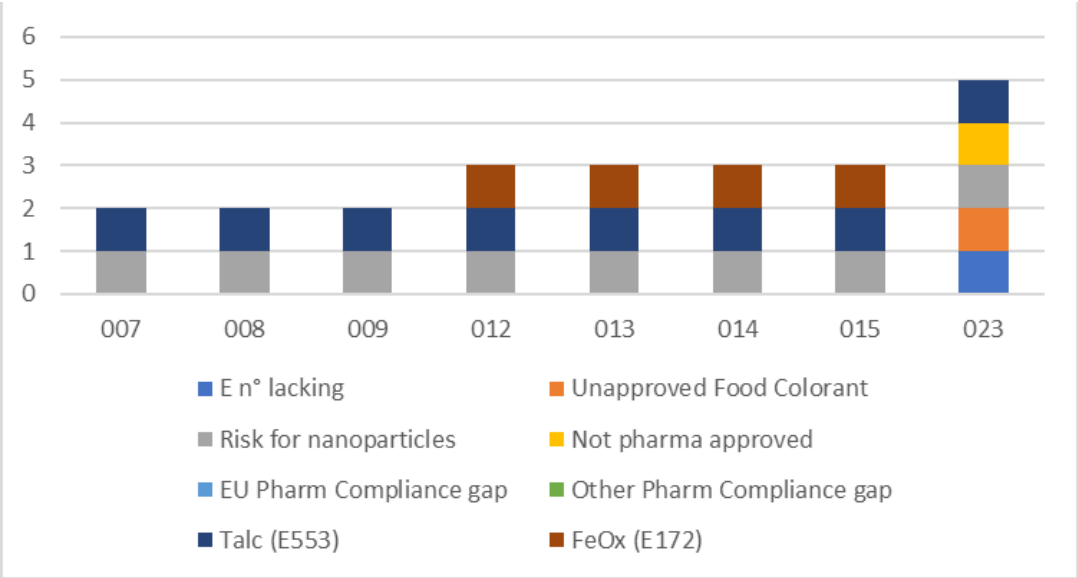



**Figure 2: Compliance risks for PVA coatings**




Note: supplier coat systems have been anonymised with random numbers

## 2. Capsule – Compliance Assessment

Based on offerings from 4 global suppliers, the predicted best and most diverse $TiO_2$-free alternatives were selected. The capsule shells with these alternatives include 8 HPMC & 5 gelatin capsules. The capsules were initially assessed for compliance risks including food legislation (E-number, food colorant approval), investigation for nano-risk by EU-member states, global pharma approval for oral use, compliance to European Pharmacopoeia and to USP/NF & JP ('Other Pharm'), presence of iron oxide.

None of the 13 selected systems are considered risk-free with 12 out of 13 capsules considered to have 2 or more risk as shown in Figures 3 and 4. For Iron Oxide ($Fe_2O_3$) exposure must be limited:

WHO-ADI E172 0.5 mg/Kg BW, JPN $Fe(OH)_3$ 5.67 mg/day, FDA 5 mg Fe/day. This typically limits the daily dose to 3 standard size #0[4] capsules per day.

**Figure 3: Compliance risks for hypromellose capsules**

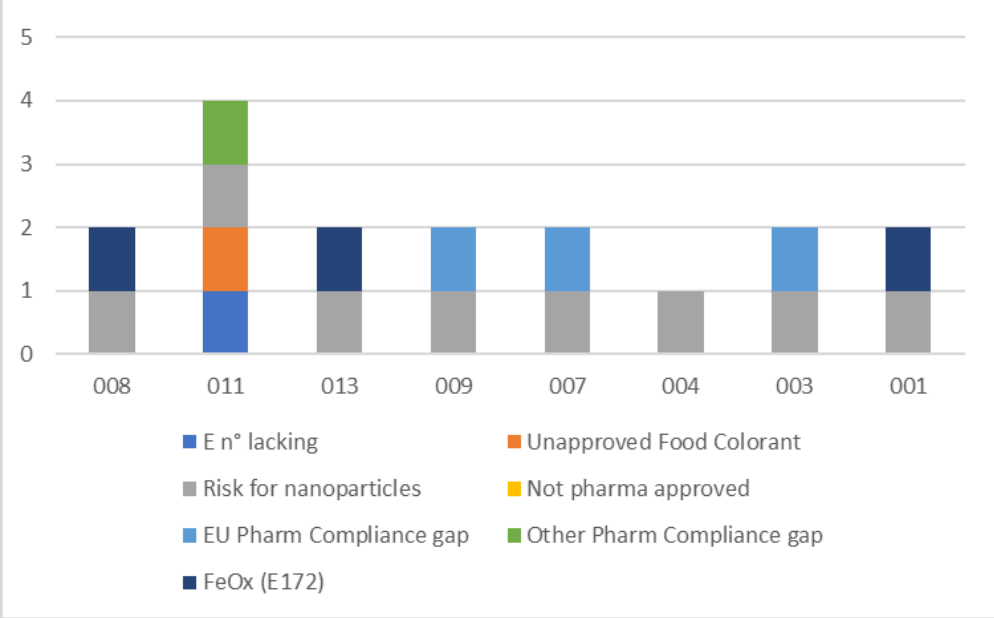
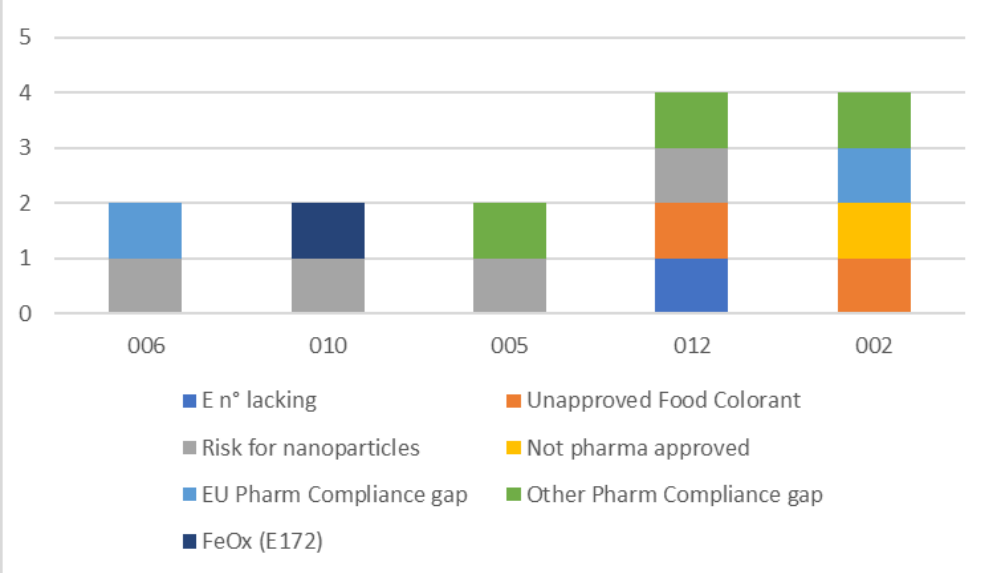



**Figure 4: Compliance risks for gelatin capsules**




Note: supplier capsules have been anonymised with random numbers

[4] Size 0 capsule corresponds to a capsule with a closed length of approximately 21.5 mm

## 3. Manufacturing and Quality Summary of $TiO_2$ Alternatives Consortium Assessment of Alternatives

The consortium has completed its activities, evaluating a significant number of film coat and capsule systems comparing their performance to reference $TiO_2$ containing systems. The detailed results and the conclusions of this analysis is provided in **(1) ANNEX 2: Alternatives to Titanium Dioxide in Tablet Coatings** and **(2) ANNEX 3: Alternatives to Titanium Dioxide in Hard Shell Capsules**.

From these activities the following conclusions can be determined:

**Film Coating**

**Table 2 List of coating materials selected for evaluation**

| Consortium Coat Reference | $TiO_2$-Free (Yes/No) | Color | Film Former A | Film Former B | Opacifier(s)[e] | Target[b] %Solids |
|---|---|---|---|---|---|---|
| COAT-001 | Yes | White | Hypromellose (HPMC)[d] | HPC[d] | Magnesium carbonate ($MgCO_3$) + A + B | 16 (15-17) |
| COAT-002 | Yes | Pink | HPMC | NA | Rice starch + A+B+D + ($Fe_2O_3$) | 16 (15-17) |
| COAT-003 | Yes | Clear | Polyvinyl Alcohol (PVA) | NA | Talc | 20 |
| COAT-004 | Yes | White | HPMC | NA | Calcium carbonate ($CaCO_3$) + C | 11 |
| COAT-005 | Yes | White | HPMC | NA | Magnesium oxide (MgO) | 11 |
| COAT-006 | Yes | White | HPMC | NA | $CaCO_3$ + D | 20 |
| COAT-007 | Yes | White | PEG- PVA graft copolymer[d] | PVA | $CaCO_3$ + Talc | 30 |
| COAT-008 | Yes | White | PVA | NA | $CaCO_3$ + Talc | 20 |
| COAT-009 | Yes | White | PVA | HPMC | $CaCO_3$ + Talc | 20 |
| COAT-010 | Yes | White | HPMC | NA | Rice starch + D | 20 |
| COAT-011 | Yes | Pink | HPMC | NA | $CaCO_3$ + D + $Fe_2O_3$ | 20 |
| COAT-012 | Yes | Pink | PEG- PVA graft copolymer | PVA | $CaCO_3$ + Talc + $Fe_2O_3$ | 30 |
| COAT-013 | Yes | Pink | PVA | HPMC | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-014 | Yes | Pink | PVA | NA | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-015 | Yes | Pink | PVA | NA | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-016 | Yes | Pink | HPMC | NA | Rice starch +D + $Fe_2O_3$ | 20 |
| COAT-017[a] | No | White | HPMC | NA | $TiO_2$ | 15 |
| COAT-018[a] | No | White | PVA | NA | $TiO_2$ + Talc | 25 |
| COAT-019 | Yes | White | HPMC | NA | $CaCO_3$ + D + E | 17 |
| COAT-020 | Yes | White | HPMC | HPC | Rice starch + D | 15 |
| COAT-021 | Yes | Pink | HPMC | HPC | $CaCO_3$ + D + $Fe_2O_3$ | 15 |
| COAT-022 | Yes | Pink | HPMC | HPC | Rice starch + D + $Fe_2O_3$ | 15 |
| COAT-023 | Yes | White | PVA | NA | F+ Talc | 18.5 (17-20) |
| COAT-024[a] | No | White | HPMC | NA | $TiO_2$ | 15 |
| COAT-025[a] | No | Pink | PVA | NA | $TiO_2$ + Talc + $Fe_2O_3$ | 18.5 (17-20) |

| | | | | | | |
|---|---|---|---|---|---|---|
| COAT-026[a] | No | Pink | HPMC | NA | $TiO_2$ + $Fe_2O_3$ | 15 |
| COAT-027 | Yes | White | HPMC | NA | $CaCO_3$ + D | 16.5 (15-18) |
| COAT-028 | Yes | Pink | HPMC | NA | $CaCO_3$ + D + $Fe_2O_3$ + FD&C Red #40 | 16.5 (15-18) |
| COAT-029 | Yes | White | HPMC | NA | B + G | 12 |
| COAT-030 | Yes | Clear | HPMC | NA | B + E | 12 |
| COAT-031[c] | Yes | Red | HPMC | NA | B + $Fe_2O_3$ | 12 |
| COAT-032 | Yes | White | HPMC | NA | $CaCO_3$ + H | 17.5 |
| COAT-033 | Yes | White | HPMC | NA | $CaCO_3$ + D + F | 18 |
| COAT-034 | Yes | White | HPMC | NA | Rice starch | 18 |

[a]$TiO_2$ reference coating materials bTarget or range %solids based on the manufacturers' recommendations.

[c]COAT-031 is a ready-to-use solid coloring agent preparation for addition to other film-coating admixes e.g., COAT-030.

[d]Hypromellose is described as hydroxypropylmethylcellulose (HPMC) hereafter in this report and macrogol-PVA graft copolymer as polyethylene glycol (PEG)-PVA graft copolymer. HPC = hydroxypropylcellulose

[e]$Fe_2O_3$ is not an opacifier per se but contributes to opacification through its colorant properties.

All of the 20 $TiO_2$-free coatings studied in detail were inferior to the $TiO_2$ reference coats based on the entire set of Key Performance Indicators (KPI). Some performed well when assessed against certain criteria but not others. Many did not achieve surface coverage and opacification at a 6% weight gain and those, which did, required a significantly higher coating level than the $TiO_2$ reference coats. In general, the performance of the coloured $TiO_2$-free coatings was poorer than the white $TiO_2$-free coatings.

In conclusion, none of the $TiO_2$-free coatings could match the properties of $TiO_2$. Their use will result in longer, more expensive and potentially less robust coating processes and may also impact on the stability and shelf-life of products. Colour matching between marketed products and $TiO_2$-free coatings will be extremely difficult and the colour palette available for product identification and anti-counterfeiting measures will be reduced due to the poor performance of the coloured coatings. There is also a risk to patient adherence due to the colour changes seen in some $TiO_2$-free coatings and to patient safety as a result of the limited colour palette available to distinguish between different products/strengths.

**Hard Shell Capsules**

The Consortium studied 13 $TiO_2$-free hard capsule shells and compare them with 4 $TiO_2$ reference capsule shells.

The results show that for white capsule shells, all of the $TiO_2$-free capsule shells have inferior properties to $TiO_2$ containing reference shells in terms of opacity and ability to camouflage the capsule shell contents. In some cases, they had reduced mechanical integrity than the $TiO_2$-containing counterparts. The gelatin-based $TiO_2$-free capsule shell, CAP-002's opacity varied significantly in response to changes in relative humidity. Therefore, none of the white $TiO_2$-free capsule shells evaluated were considered suitable replacements for $TiO_2$ containing capsule shells.

The red/orange $TiO_2$-free capsules containing the colorant, $Fe_2O_3$, performed well in the battery of tests. The capsule shells are opaque and therefore capable of camouflaging any colour differences in the capsule contents. $Fe_2O_3$ is not an opacifier per se but imparts opacification through its intense red colour. The intensity of colour makes it difficult for the human eye to detect colour changes in the capsule shell e.g., following accelerated stability storage, even though colorimetry data showed that

changes had occurred. However, exact colour matching for the purposes of reformulating an existing product as $TiO_2$-free may be difficult as CAP-014, the $TiO_2$ reference and the $TiO_2$-free CAP-001 from the same supplier, product line and tradename had colour difference values of above 2.

This pink semi-translucent capsule shell was the only non-red/orange coloured capsule shell evaluated. It does not contain $Fe_2O_3$. Its pink colour bleached to white in the photostability studies and it was found to be very brittle. In addition, its semi-transparency would not hide the colour and appearance of its contents. For the above reasons it is not considered a replacement for $TiO_2$ containing pink capsule shells. $TiO_2$-free capsule shells of other colours were not evaluated as part of the Consortium's work due to lack of availability at the start of the project.

Based on the results, only $TiO_2$-free red/orange capsule containing $Fe_2O_3$ could be suitable replacements for $TiO_2$ containing capsules. If $TiO_2$ was banned in medicines, this would severely restrict the colour palette available for new medicines or reformulating commercially available ones to be $TiO_2$-free, with a down-stream impact on the ability to identify medicines and prevent counterfeiting. In addition to a reduced colour palette caused by the darker colours imparted by iron oxides to the capsule shell, finding an imprinting ink with sufficient contrast to the capsule shell colour will be difficult because the lighter ink colours, e.g. white ink, contains $TiO_2$. The daily intake of iron oxide (E172) is restricted by authorities such as the World Health Organization, the FDA and the Japanese authorities for safety reasons. These limits translate approximately to the equivalent of 3 x Size 0 capsules per day. Based on these limitations, $Fe_2O_3$ would not be a suitable replacement for $TiO_2$ as it would not have global regulatory acceptability and could not be used in medicines developed for global markets, especially those involving multiple dosing or chronic use

## 4. Safety Assessment of Alternatives

The safety team of the consortium evaluated the potential colourants/opacifiers included in the $TiO_2$ alternative film coating and capsule systems assessed. A detailed safety report is attached as **ANNEX 4: Safety assessment of alternatives and comparison with Titanium Dioxide as an opacifier and colorant for oral administration**

All selected alternative colorants, which also serve as opacifiers, are already in use in medicinal product formulations and food supplements. The safety team considered all alternatives as safe, with comprehensive safety data sets in some cases and health authority assessments available. As with $TiO_2$, these opacifiers and colourants have been safely used in products for decades. However, some of the colourants/opacifiers have data gaps with regard to toxicity data (including genotoxicity, chronic toxicity, carcinogenicity, reproductive and developmental toxicity) compared to $TiO_2$, but given their history of safe human use, these non-clinical data gaps are not considered as being relevant.

- For a few opacifiers the presence of nanoparticles is unclear. Guidance from EMA/EFSA is needed to understand how to take into account the nanoparticle portions of opacifiers and if further safety testing is required to characterize those fractions. A critical review on the nanoparticle discussion in particular on the classification and the presence is attached in Annex 1 and is considered by the consortium as a basis for potentially seeking scientific advice from the EMA NcWP. However, current investigations demonstrated that the alternatives Zinc Oxide (ZnO), Calcium sulphate ($CaSO_4$), Calcium carbonate ($CaCO_3$), Magnesium carbonate ($MgCO_3$) and Magnesium oxide (MgO) may contain nanoparticles, but all are soluble at pH 1.2, therefore not falling under the EFSA definition of nanomaterials. In addition, Isomalt, Maltodextrin are freely soluble and do not pose a nanoparticle concern as well as Microcrystalline Cellulose and Rice Starch.
- There is an extensive data set for $TiO_2$ available, assessed by different authorities and expert groups ensuring its safety. Most notably, the carcinogenicity study (NCI TR-097, 1979) on $TiO_2$ using comparable material to the material used in medicines provided a robust conservative No Observed Adverse Effect Level (NOAEL) of 2250 mg/kg/day. Additionally, the JECFA concluded that there is no identifiable hazard for INS171 (similar to E171) and consequently no requirement for an ADI. However, the TiO2 Alternatives consortium have proposed establishing an oral permitted daily exposure (PDE) of 2250 mg/day which will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels. Also, the oral PDE can be applied to compare the safety of $TiO_2$ with the safety of alternative colourants/opacifiers.

Safety evaluations by Agencies are ongoing for some of the opacifiers and excipients, e.g.:

- *Talc:* ECHA is evaluating talc as a potential Category 2 carcinogen. The safety experts of the consortium concluded that talc (pharmacopoeia grade) can be considered as safe by the oral route. Furthermore, an EFSA opinion was published in June 2018 on talc as a food additive.
- *$Fe_2O_3$*: Currently, an EFSA re-evaluation is ongoing.

Of note, the risk assessments performed to date by the safety team of the consortium (see table below) have not taken into account that daily exposure of the selected opacifiers in the formulations will, in most cases, be higher compared to $TiO_2$ levels to reach the same effect (e.g. iron oxide ($Fe_2O_3$) would generally be 2-3 times higher than $TiO_2$).

It has to be mentioned and reiterated, that e.g., $Fe_2O_3$ exposure is limited: WHO-ADI E172 0.5 mg/Kg BW, JPN Fe(OH)3 5.67 mg/day, FDA 5 mg Fe/day. This typically limits the daily dose to 3 standard size #0 capsules per day from a safety perspective.

Overall, the consortium considers there is no relevant difference between the safety profile of $TiO_2$ and the investigated alternatives based on available data.

**Table 3: Current status of the safety assessments of $TiO_2$ alternatives**

| Chem Name CAS | Used in Food | Used in Drug Formulations | Other Assessments | Unintended Nanoparticles Present | Summary and potential safety Data gaps |
|---|---|---|---|---|---|
| Calcium Carbonate<br>$CaCO_3$<br>471-34-1 | E170 | FDA IID | JECFA 1965), SCF (1990) EFSA (2011, 2023) | Yes, but fast dissolution in the acidic environment of the stomach demonstrated (EFSA, 2011, 2023). Considered as no concern. | Comprehensive toxicology data package available, except chronic toxicity and carcinogenicity. However, for use in food, the EFSA Panel concluded that there is no need for a numerical acceptable daily intake (ADI) for calcium carbonate and that, in principle, there are no safety concerns with respect to the exposure to calcium carbonate per se at the currently reported uses and use levels in all age groups of the population, including infants below 16 weeks of age. No ADI specified |
| Calcium Sulfate<br>$CaSO_4$<br>anhydrous: 7778-18-9<br>hemihydrate: 10034-76-1<br>dihydrate: 10101-41-4 | E516 | FDA IID, US and EU Pharmacopoeia | GRAS, SIDS (2003), JECFA, (1973) | Yes, but soluble at pH1.2 | Basic toxicological data are available for calcium sulphate but long-term and carcinogenicity data in animals are lacking. In the available studies, the test item has often not been well characterised and i.e., information on particle size (i.e., nanoforms) is missing.<br>Calcium sulphate has a long history of safe use, an ADI was not specified, the tolerable upper intake limit is 2500 mg/d based on calcium intake. High doses of sulphate result in transient gastrointestinal effects. |
| Isomalt<br>64519-82-0 | E953 | FDA IID, US and EU Pharmacopoeia | GRAS, BfR (2014), SCF (1984, 1989), JECFA (1985) | No (freely soluble in water) | Extensive toxicological data, including repeat-dose (up to chronic) toxicity studies, multigeneration and teratogenicity studies, genotoxicity and carcinogenicity studies are available for isomalt. Even though many of the published studies are from 1970's to 1980's and may not fully comply to current standards, and no formal fertility and peri- and postnatal development studies are available (the multigeneration study covered many of the relevant endpoints). Overall, no relevant data gaps regarding toxicity data are seen. In humans, isomalt is well tolerated at doses <20 g/day. Gastrointestinal effects, in particular flatulence and diarrhoea, were observed at ≥20 g/day. |

| | | | | | |
|---|---|---|---|---|---|
| Magnesium Carbonate<br>$MgCO_3$<br>546-*93*-0 | E504 | FDA IID | Magnesium: JECFA (1986), EFSA (2015)<br>SCF (2006), BfR (2017) | Yes, but soluble at at pH 1.2 and 4.5 | Taking into account all available data, both the existing toxicological studies with magnesium carbonate and other Mg salts and that Mg is an essential trace element, it can be concluded that the use of magnesium carbonate as an excipient in pharmaceutical products is safe. The in vitro genotoxicity battery is missing, although there is no indication of a genotoxic potential for $MgCO_3$. |
| Magnesium Oxide<br>MgO<br>1309-48-4 | E530 | FDA IID, EU Pharmacopoeia | Magnesium: JECFA (1986), EFSA (2015), SCF (2006), BfR (2017)<br>MgO (GRAS) | MgO readily dissociates after a reaction with gastric HCl under formation of magnesium chloride ($MgCl_2$). | Considering the high NOAEL and relatively mild toxic effects associated with Mg intake, the available upper limit of 250 mg/day derived by regulatory authorities seems sufficient and it can be concluded that MgO is of low toxicity and concern. Whilst several routes of synthesis for MgO NP have been described, data on the particle size distribution of MgO for the use as a pharmaceutical excipient is lacking. Safety data of those MgO NP is rare and current studies do not fulfil the requirements by EFSA Guidance on risk assessment of nanomaterials to be applied in the food and feed chain [EFSA, 2021]. However, based on the dissociation of MgO in gastric fluid MgO is not considered a NP |
| Maltodextrin<br>471-34-1 | E1400 | FDA IID | GRAS<br>EFSA (2013) | No (freely soluble in water) | Maltodextrin is widely used across the food, cosmetic and pharmaceutical industry. Based on its metabolic profile, it has been considered non-hazardous by health authorities and is either an approved food additive or is considered safe but not classified as a food additive. No carcinogenicity studies or reproductive and developmental toxicity studies could be found for maltodextrin. |
| Microcrystalline Cellulose<br>9004-34-6 | E460-E469 indirect food additive (US FDA 2018) | FDA IID | JECFA (1998, 2000),<br>EFSA 2018 | No | The available data set and toxicity information with cellulose and derivative forms is extensive. Physical properties or particle size (including the nanoparticulate fraction) and distribution are not always available and represent a data gap. In alignment with US authorities, EFSA determined no numerical ADI for microcrystalline cellulose and based on the available toxicological dataset, considered no safety concern at the reported use levels (estimated exposure 660-900 mg/kg bw day) with unmodified and modified celluloses (EFSA, 2018). |
| Rice Starch | Nutrient | FDA IID | GRAS | No | Starch is GRAS listed and considered to be safe. It is already in use as an excipient for pharmaceuticals in different regions and REACH and EFSA reports are coming to the same conclusion. No genotoxicity and chronic toxicity data are available. |

| | | | | | |
|---|---|---|---|---|---|
| Tetrasodium pyrophosphate 7722-88-5 | E450 | FDA IID | GRAS<br>EFSA (2019), SCF (1997), JEFCA (2006) | TBD, No data on solubility in gastric fluid | The available toxicological information for each phosphate salt is limited and the overall phosphate assessment as a pharmaceutical excipient is based on read-across approaches and a group-specific toxicity assessment for several phosphate salts. While not assuming that there would be significant differences in toxicity, different salts could express different oral bioavailability or solubility in water.<br>The EFSA derived a group ADI for phosphates and its salt of 40 mg/kg bw per day (expressed as P). Both phosphates, E339 and E450, are considered to be of low toxicity concern for human exposure as pharmaceutical excipient. |
| Trisodium phosphate 7601-54-9 | E339 | FDA IID | GRAS<br>EFSA (2019), SCF (1997), JEFCA (2006) | TBD, No data on solubility in gastric fluid | |
| Zinc Oxide<br>ZnO | FDA Substances added to food list | FDA IID<br>UK, EU and US Pharmacopoeia | GRAS<br>SCF 2003<br>EFSA 2016 | Yes, fast but dissolution expected in the acidic environment of the stomach (EFSA, 2016), and soluble at pH 1.2 and pH 4.5 | For zinc oxide, no specific safety information was found in the open domain. However, as a food additive, zinc oxide is generally recognised as a safe substance. For zinc, detailed toxicological information can be found in the public space. In general, no adequate experimental studies are available to evaluate the carcinogenic potential of zinc or zinc compounds. In addition, the safety of zinc (oxide) nanoparticles is less well understood. |

## Question 2

**Please supply a summary of the evidence /results from the ongoing studies comparing alternative formulations (for different dosage forms as available) with those containing $TiO_2$.**

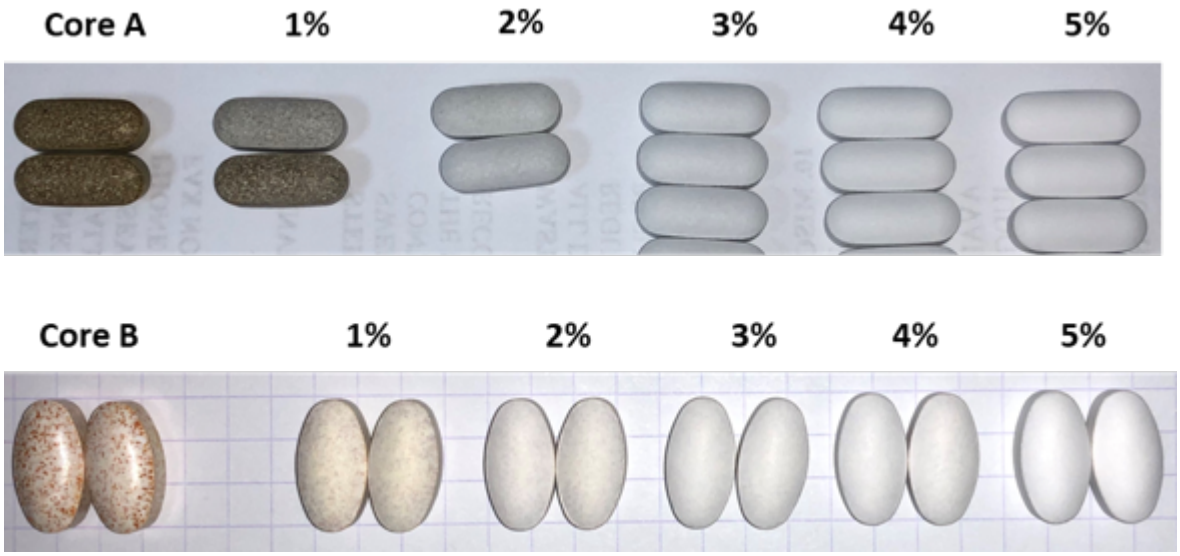
In the following sections examples of the performance of alternative materials to $TiO_2$ used in film coat systems and hard capsule shells is provided. As the $TiO_2$ Alternatives Consortium activities are still ongoing, some of the examples have been provided by individual pharmaceutical companies or material suppliers. Full detail is provided in **(1) ANNEX 2: Alternatives to Titanium Dioxide in Tablet Coatings** and **(2) ANNEX 3: Alternatives to Titanium Dioxide in Hard Shell Capsules**.

### Film Coating Systems

#### 1. Appearance: Opacity (Industry experience)

Two different coloured cores (Core A and Core B) were coated using a $TiO_2$ free film coat system to assess the ability for the system to mask the core appearance. The cores were coated to a weight gain of up to 5% w/w. Samples were taken throughout the coating process and were visually assessed for the coats ability to provide acceptable coverage. The results are presented in Figure 5.

**Figure 5: Visual appearance of different coloured cores coated with a $TiO_2$-free film coating system**

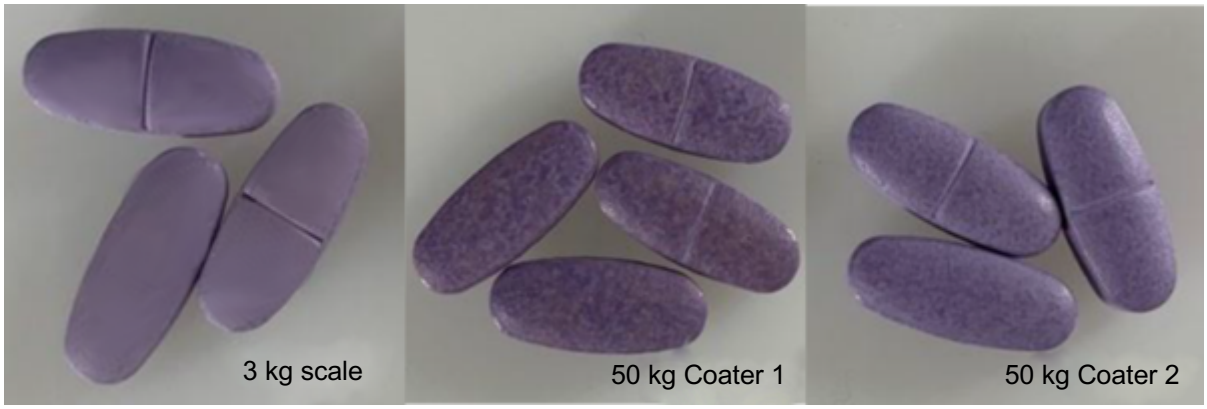



From this study it was observed that due to decreased opacity of the $TiO_2$-free system more coating needs to be applied to achieve an acceptable appearance. Also, any discolouration in the core and core defects were more challenging to cover.

**Manufacturability: Scale-Up (Industry Experience)**

A multivitamin tablet core was coated using a coloured (purple) $TiO_2$ free film coat system at small scale (3 kg) and at representative commercial scale (50 kg) using different types of coating equipment. The purpose of this study was to evaluate the impact of scale and the use of different coating equipment on the visual appearance of the coated tablets. The results are provided in Figure 6.

**Figure 6: Evaluation of the visual appearance of a multivitamin tablet core coated with a purple $TiO_2$ free coating system at different scales and equipment type**




In this study it was observed that at the 3 kg scale the visual appearance of the tablets was acceptable with no significant defects noted. However, at the 50 kg scale the visual appearance was poor with poor colour uniformity. Also, it was observed that there was a difference in the visual appearance between tablets coated in the two different types of equipment. Based on this study it was concluded that the coating scale can have an impact on the final coating appearance. Differences in the coater design (coating pan, spray gun positioning, air flow limitations, etc.) can impact the final film coating appearance.

**Colour Matching Capability (Industry Experience)**

A visual assessment of two $TiO_2$ free coating systems to match the colour of a $TiO_2$ based film coat system was performed. The results are presented in Table 4 below. Both the coating systems ($TiO_2$-free and the $TiO_2$ Based) were supplied from the same supplier.

**Table 4: Visual assessment of $TiO_2$ free film coating systems to colour match to a $TiO_2$ containing film coat system**

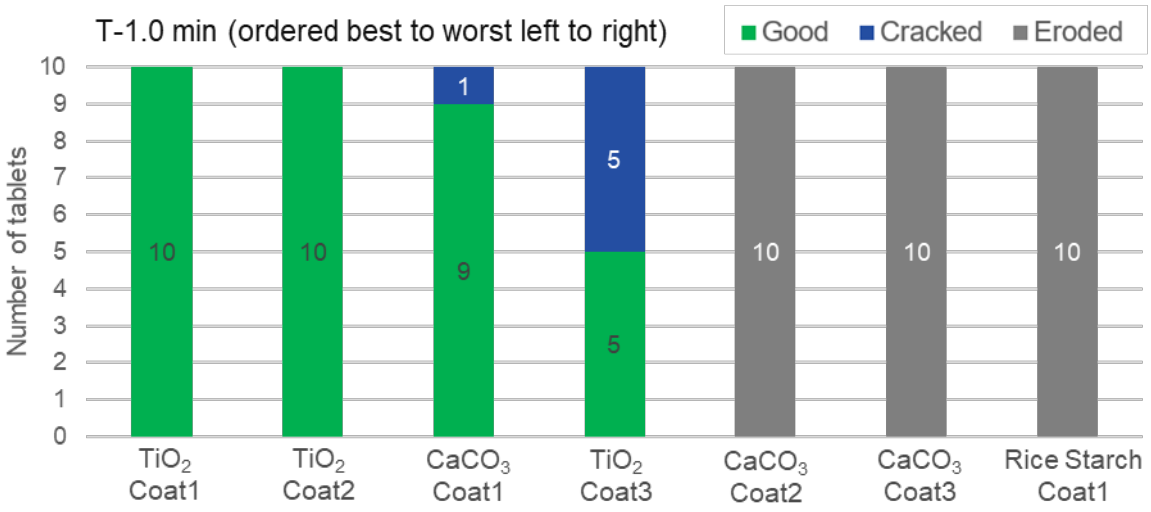
| Type (HPMC) | Colourants | Colour Match (3-4 % w/w gain) | Photo |
|---|---|---|---|
| TiO2 (Control) | Iron oxide | Control: Purple |  |
| CaCO3 | | No |  |
| Rice Starch | | No |  |

At equivalent film coat weight gains, it was not possible to match the visual appearance of the $TiO_2$ based film coat using the $TiO_2$ free alternatives. The film coating supplier confirmed that this was due to the removal of the $TiO_2$.

## 2. Mechanical Strength (industry experience of coat adhesion)

To-date, commercial scale experience of performance remains limited. As an example, film coats containing $TiO_2$, calcium carbonate ($CaCO_3$) and rich starch were assessed for their coat adhesion. Tablet cores were coated to a weight gain of approximately 3.5% w/w and then assessed for their friability using a Friabimat SA-400 (Born friabiliator). The results are provided in Figure 7.

**Figure 7: Bar chart representation of coated tablet defects after 1.0 min Friabimat® testing**



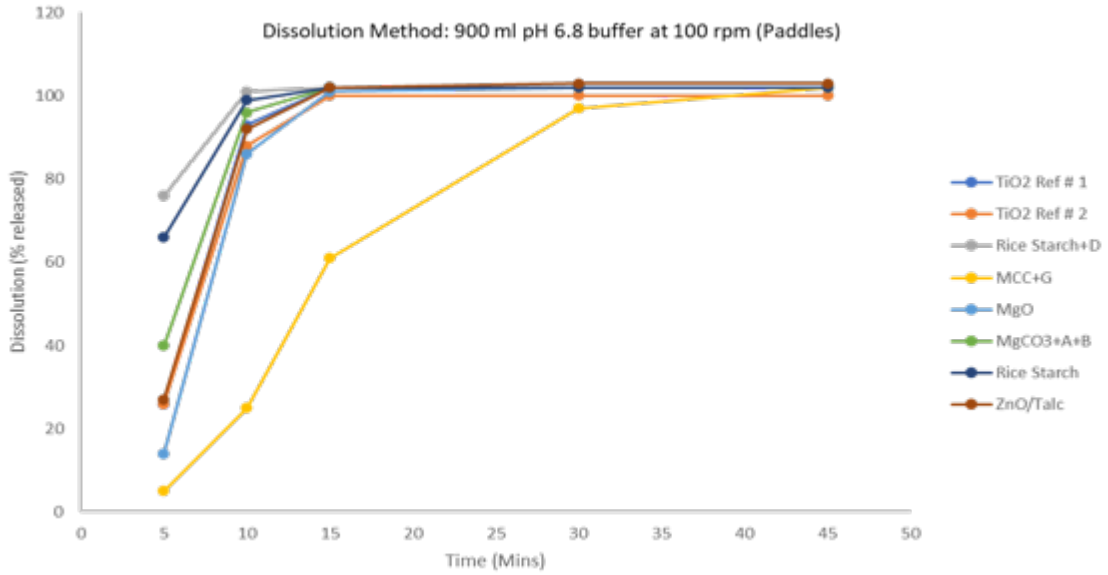

After 1 minute of using the friabiliator only the $TiO_2$ (coat 1 and coat 2) based film coats showed no erosion and cracking of the coat. One of the CaCO3 (Coat 1) film coating system showed minor erosion. The $CaCO_3$ coating systems (Coat 2 and Coat 3) and the rich starch showed significant erosion and cracking, with all the tablet samples failing. An example of the degree of failure is provided in Figure 8. However, it should be noted that one of the $TiO_2$ based film coat systems demonstrated a 50% failure rate for erosion and chipping (coat 3).

**Figure 8: Example of erosion and film cracking of a $CaO_3$ film coated tablets**

## 3. In-vitro Performance: (Consortium experience of impact on dissolution)

To assess the potential impact of the $TiO_2$ alternative film coating systems on dissolution performance, Rosuvastatin 10 mg cores were coated with a range of alternative systems and their dissolution performance was evaluated and compared to $TiO_2$ based film coat reference systems. The results are presented in Figure 9.

**Figure 9: Dissolution performance of Rosuvastatin tablet cores 10 mg coated with different film coating systems**




Compared to the $TiO_2$ references, most of the alternative systems demonstrated similar performance at 15 mins. The MCC based system demonstrated slower release compared to the other systems but was comparable by 30 minutes.

## 4. Chemical Stability: (Consortium experience)

Samples of Rosuvastatin tablet cores 10 mg were coated with different $TiO_2$ free and $TiO_2$ based film coating systems. The coated tablets were then placed on accelerated stability conditions (50°C / 30 % RH and 70°C / 75 % RH) in HDPE bottles. Samples were taken after 7, 14 and 21 days and tested for assay content. The results are presented in Figures 10 and 11.

**Figure 10: Assay of Rosuvastatin 10 mg tablets stored at 50°C/30% RH after 7, 14, & 21 days**

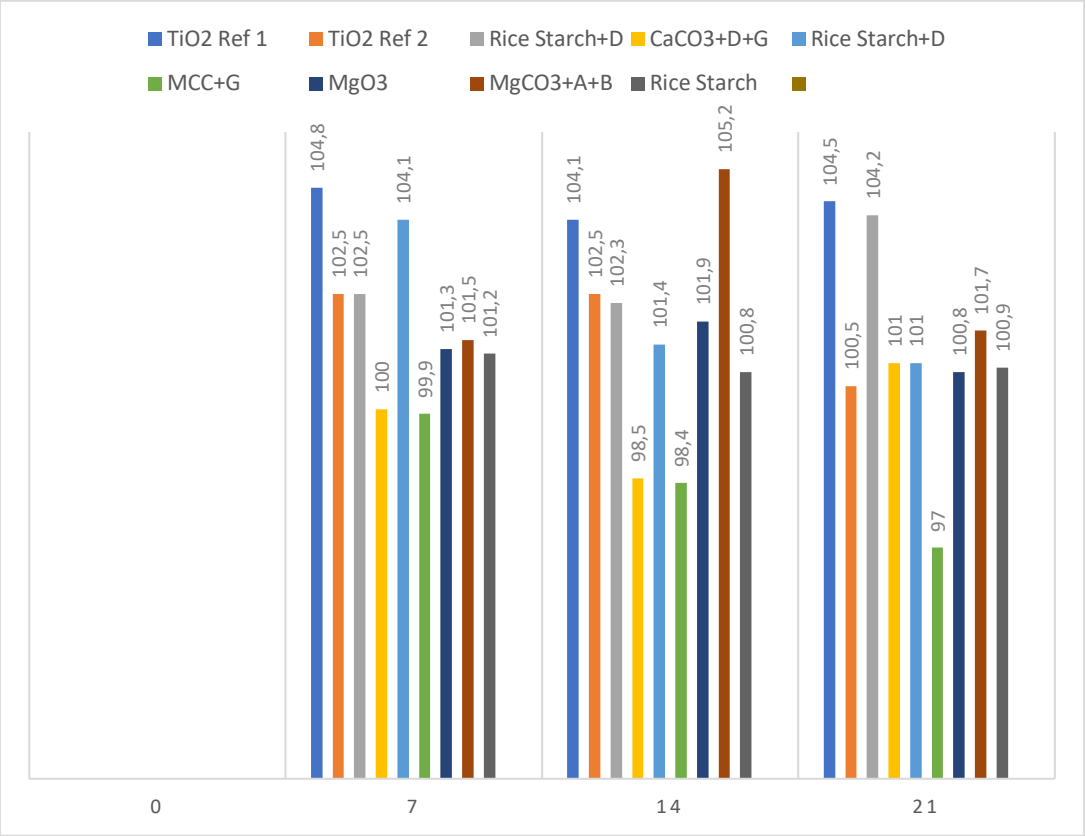
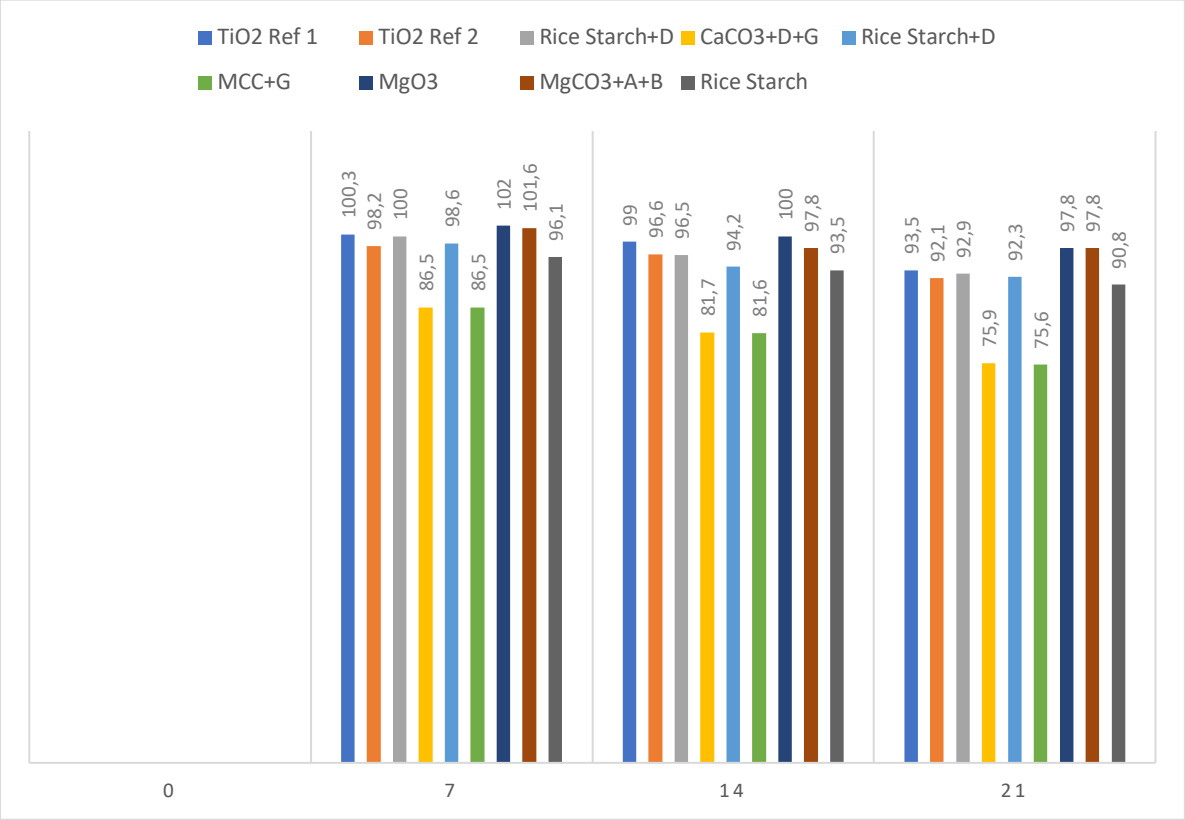



**Figure 11: Assay of Rosuvastatin 10 mg tablets stored at 70°C/75% RH after 7, 14 & 21 days**



Except for coating systems that contained material “G”, no trends in assay values were observed under all conditions and testing periods. Systems that contained material “G” demonstrate a comparable decrease at 70°C /75 % RH over the testing period compared to the $TiO_2$ references.

## 5. Photostability (Chemical) (Industry Experience)

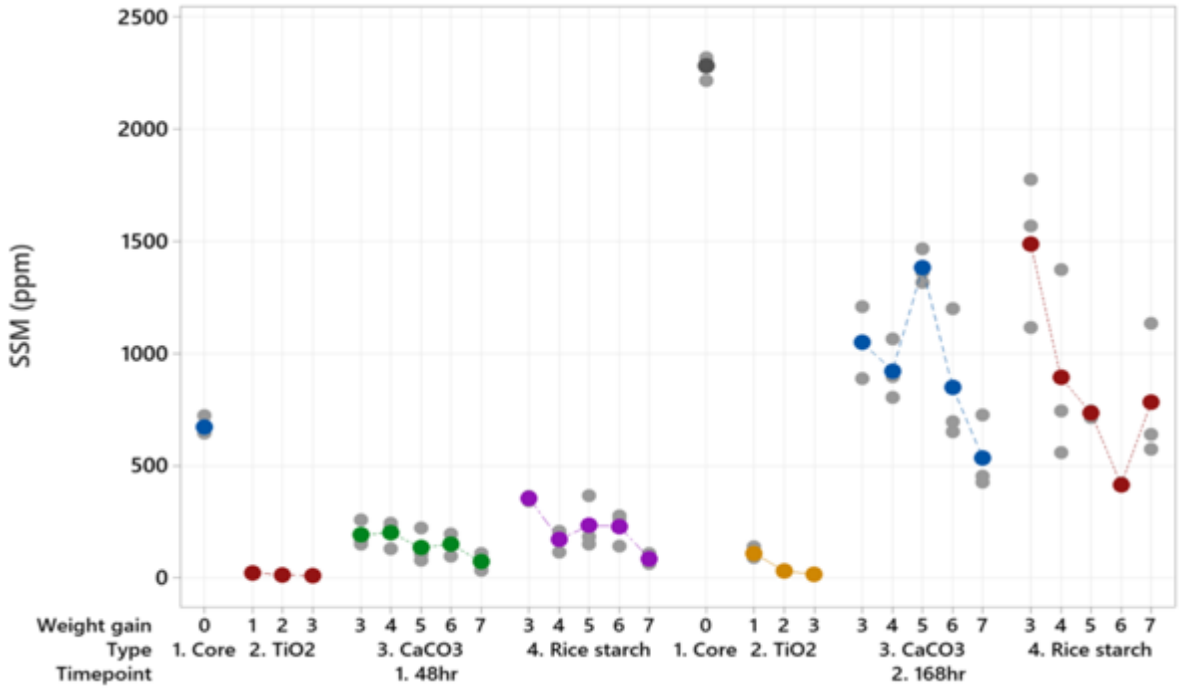
Tablets containing sodium stearyl fumarate were coated with $TiO_2$, $CaCO_3$ and rice starch-based coating systems. The weight gains applied are summarised in Table 5.

**Table 5: Amount of $TIO_2$ based $CaCO_3$ and rice starch film coat systems applied to tablets containing sodium stearyl fumarate**

| Film Coat | Coverage per tab (ug/mm$^2$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1% w/w | 2% w/w | 3% w/w | 4% w/w | 5% w/w | 6% w/w | 7% w/w |
| $TiO_2$ | 5.61 | 11.21 | 16.82 | n/a | n/a | n/a | n/a |
| $CaCO_3$ | n/a | n/a | 18.84 | 25.12 | 31.40 | 37.68 | 43.96 |
| Rice Starch | n/a | | 18.84 | 25.12 | 31.40 | 37.68 | 43.96 |

Coated tablet Samples of the different weight gains from the $TiO_2$, $CaCO_3$ and rice starch-based film coat systems were placed on photostability (using ICH option 2) for 48 and 168 hours. Samples were tested for photodegradant sodium stearyl malate (SSM). The results are presented in Figure 12.

**Figure 12: Formation of SSM after exposure to ICH photostability (Option 2) conditions of tablets containing Sodium Stearyl Fumarate coated with different film coat systems.**



Compared to the core, the amount of SSM formed with the $TiO_2$ based system was significantly less after 48 and 168 hours of exposure compared to core. After 48 hours both the $CaCO_3$ and rice starch systems demonstrated similar SSM formation which was less than the core and slightly higher than the $TiO_2$ system. After 168 hours both $CaCO_3$ and rice starch system demonstrated significant SSM formation compared to the $TiO_2$ system but less than the uncoated core. A relationship between coat weight gain and SSM formation can be established for all systems evaluated.

## Hard Capsule Shells

### 1. Mechanical Strength of Capsules (Consortium Experience)

Empty capsules (gelatin & HPMC) were assessed for their brittleness under a wide range of environmental conditions. Brittleness can be used as a surrogate how the shells may behave during encapsulation, long term stability and patient use. The results of the study are presented in Figure 13 and Figure 14.

**Figure 13: Brittleness assessment of empty gelatin capsule shells stored at different relative humidities for 72 hours**

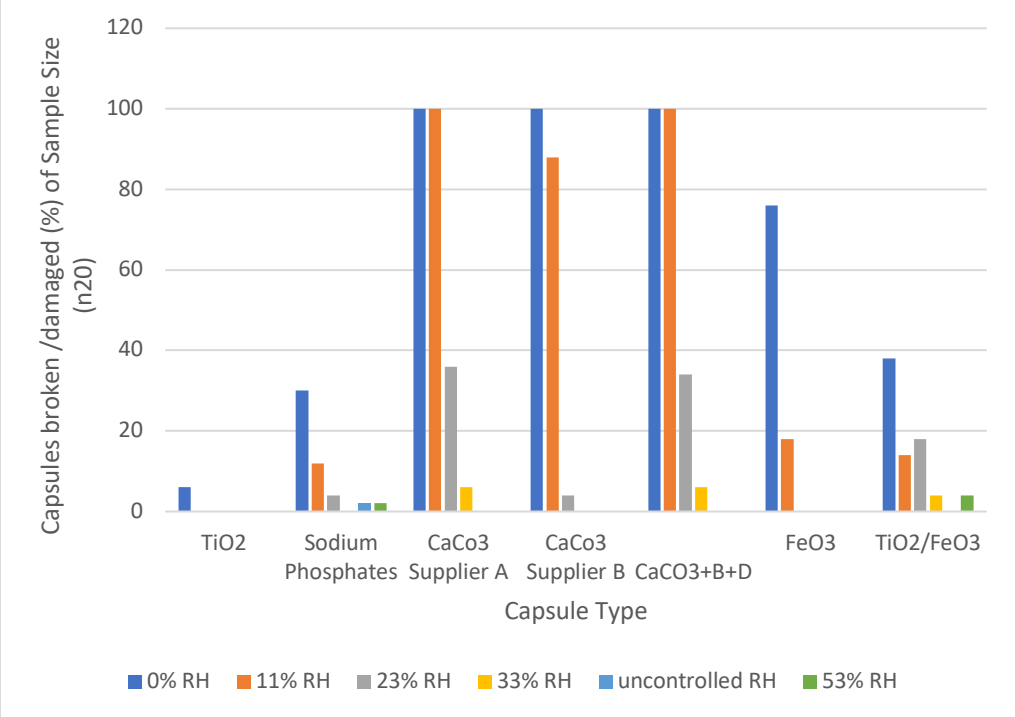
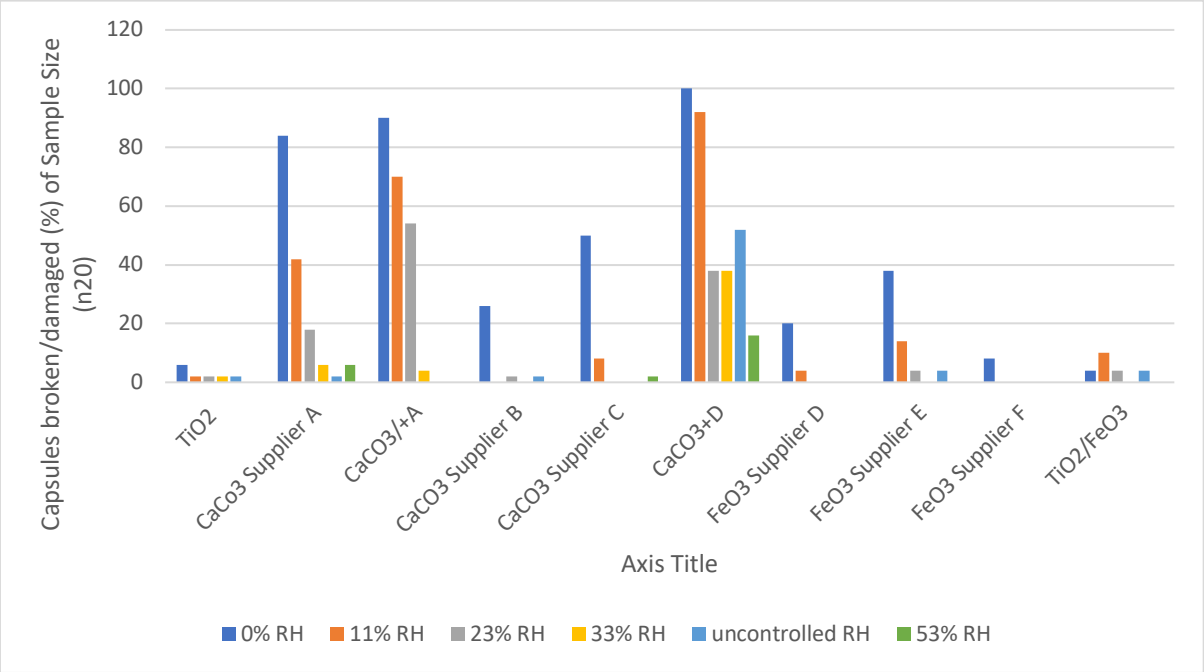


**Figure 14: Brittleness assessment of empty HPMC capsule shells stored at different relative humidities for 72 hours**

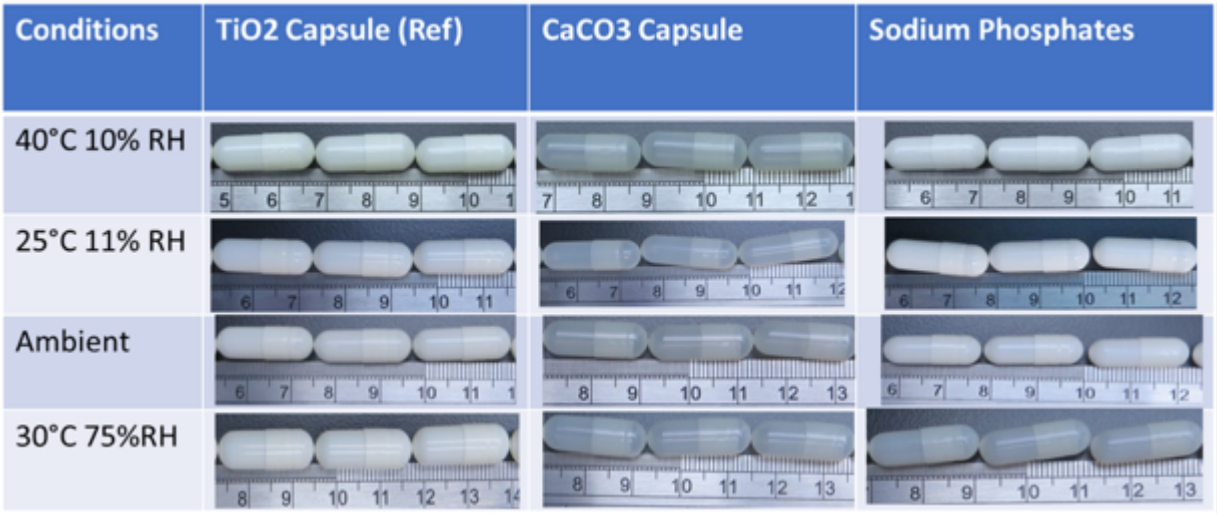



At lower humidities, $CaCO_3$ containing capsules were more brittle regardless of capsule shell evaluated (gelatin or HPMC). At uncontrolled and higher levels of humidity, >33% all the capsules demonstrated comparable brittleness except the $CaCO_3$+D HPMC. HPMC capsules showed less propensity for brittleness at the low humidities as expected when compared to gelatin comparator.

## 2. Appearance: Capsule (Industry Experience)

Empty $CaCO_3$ and Sodium Phosphates capsule shells placed under different storage conditions (open dish) for 7 days and compared for visual appearance with a $TiO_2$ reference capsule. The results are provided in Figure 15.

**Figure 15: Visual Appearance of $TiO_2$, $CaCO_3$ and Sodium Phosphate Based Capsule Shells Under Different Storage Conditions**

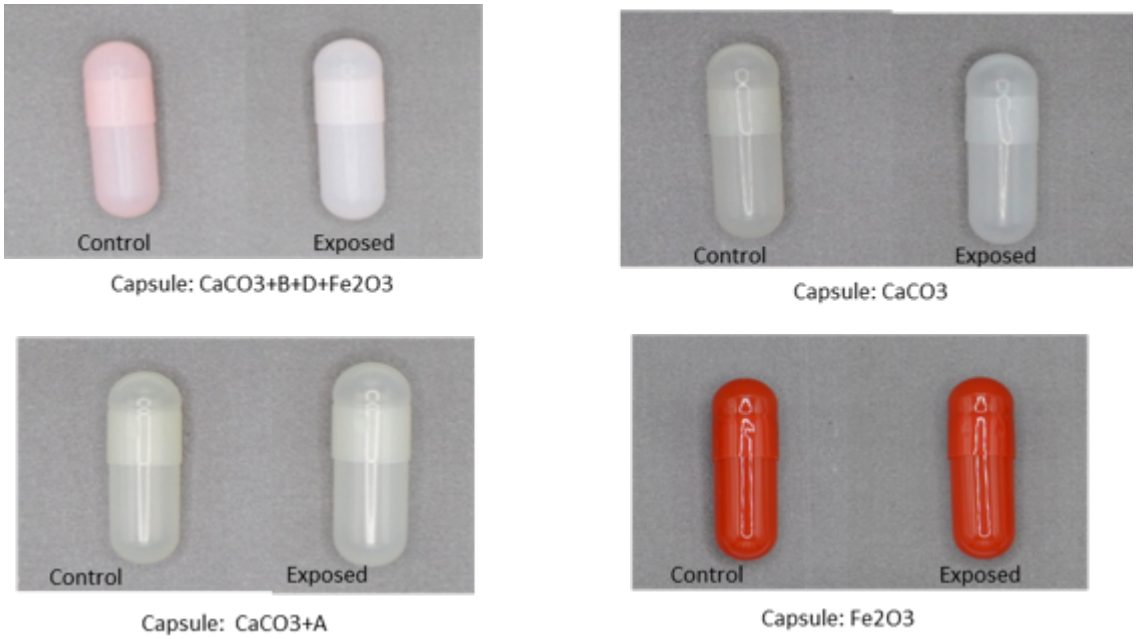


Under all conditions the $CaCO_3$ capsule remained more translucent than the $TiO_2$ reference. At Low %RH the Sodium Phosphates capsule demonstrated comparable appearance to the $TiO_2$ reference. However, at high humidity (30°C / 75% RH) the capsule became translucent. The change in opacity may have an impact on patient acceptability.

### 3. Photostability: Capsule Shell Appearance (Consortium Experience)

Empty $TiO_2$ free capsule shells using different opacifiers/components were assessed for their visual appearance stability under ICH photostability conditions (2.4 million Lux) and compared to a dark control sample. The results are presented in Figure 16.

**Figure 16: Visual appearance of empty capsule shells using alternative $TiO_2$ opacifier/components after exposure to 2.4 million lux with dark control for comparison**

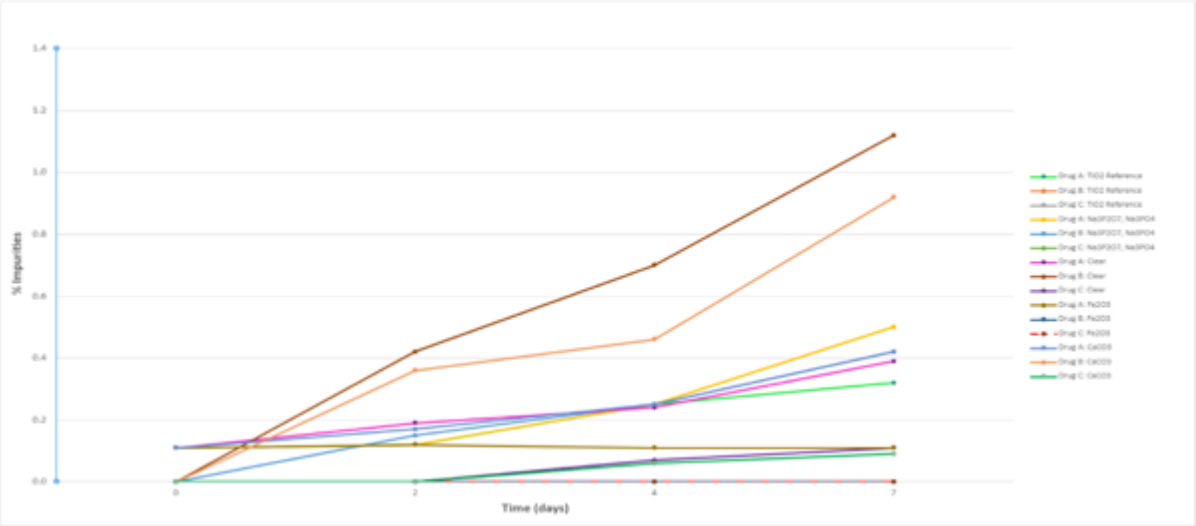



The $Fe_2O_3$ based capsule demonstrated no significant change under the stress conditions compared to the dark sample. Predominately $CaCO_3$ based capsules appear to become whiter/lighter under the stress conditions compared to the dark sample. The multicomponent opacifier system demonstrated significant loss of colour under the stress conditions compared to the dark sample.

### 4. Photostability (Chemical) of Capsules (Industry Experience)

Three model drugs (A, B, C) with different photo sensitivities were filled into gelatin capsules using $Fe_2O_3$, $CaCO_3$ and Sodium Phosphates as the primary opacifier. The capsules were then exposed to ICH Photostablity conditions (Option 2) for 7 days. Samples of the different capsule shell types were then assessed for the formation of each compounds impurities and compared to $TiO_2$ and clear gelatin capsule shells filled with the same model drugs and stored under the same conditions. The results are summarised in Figure 17.

**Figure 17: Formation of impurities for model compounds A, B and C filled into $TiO_2$, $Fe_2O_3$, $CaCO_3$, Sodium Phosphates based gelatin capsule shells and clear gelatin capsules shells after exposure to ICH photostability (Option 2) conditions for 7 days**




Under the conditions used, different amounts of photodegradation were observed for the different model compounds in the different capsule shell types. The $Fe_2O_3$ based capsule shell provided equivalent or improved photo protection for the 3 model drugs compared to the $TiO_2$ reference capsule shell. For model drug A; the Sodium Phosphates and $CaCO_3$ capsule shells demonstrated similar impurity profiles after 4 and 7 days but higher compared to the $TiO_2$ reference capsule shell. For model drug B; $CaCO_3$ capsule shell demonstrated significant degradation observed compared to $TiO_2$ reference capsule shell but less than the clear capsule shell reference. For model drug C: Sodium Phosphate and $CaCO_3$ capsule shells demonstrated comparable degradation but were higher than $TiO_2$ and $Fe_2O_3$ capsules shells. Based on this study it was possible to rank order the different capsule shell performance to inhibit the formation of the model compounds' impurities:

**$TiO_2$ = $Fe_2O_3$ capsule shells> Sodium Phosphates capsule shell > $CaCO_3$ = Clear capsule shells**

## Question 3

**In 2021, you provided QWP with information on the methodology and timeline estimates on investigating potential alternatives to replace/remove $TiO_2$ without negatively impacting the quality, safety and efficacy in medicinal products. Please provide the updates to this information versus the last analysis.**

## Timeline estimates

The $TiO_2$ Alternatives Consortium timelines for the planned assessments of film coating systems and hard capsule shells using alternative materials as potential replacement for $TiO_2$ are provided in Figures 18 and Figure 19. This has been completed to plan and the outcomes are summarised in **(1) ANNEX 2: Alternatives to Titanium Dioxide in Tablet Coatings** and **(2) ANNEX 3: Alternatives to Titanium Dioxide in Hard Shell Capsules**.

**Figure 18: Timeline of $TiO_2$ Alternatives Consortium activities to assess film coating systems with different components as opacifiers**

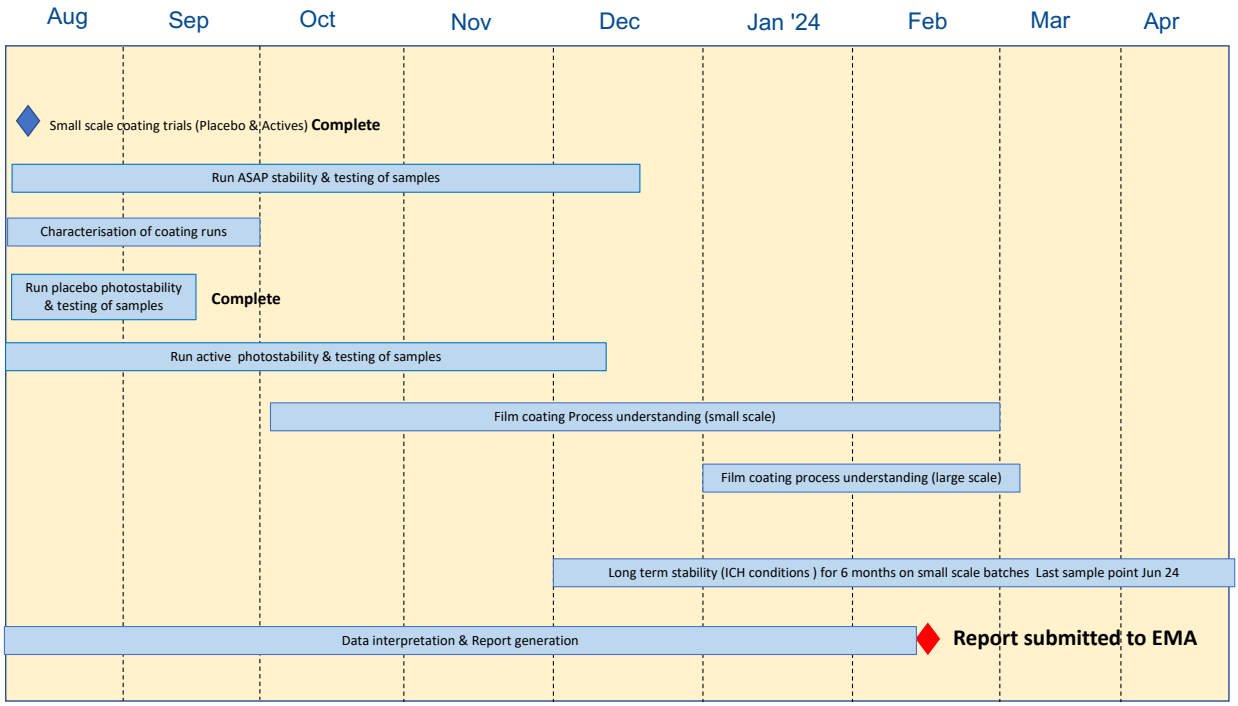
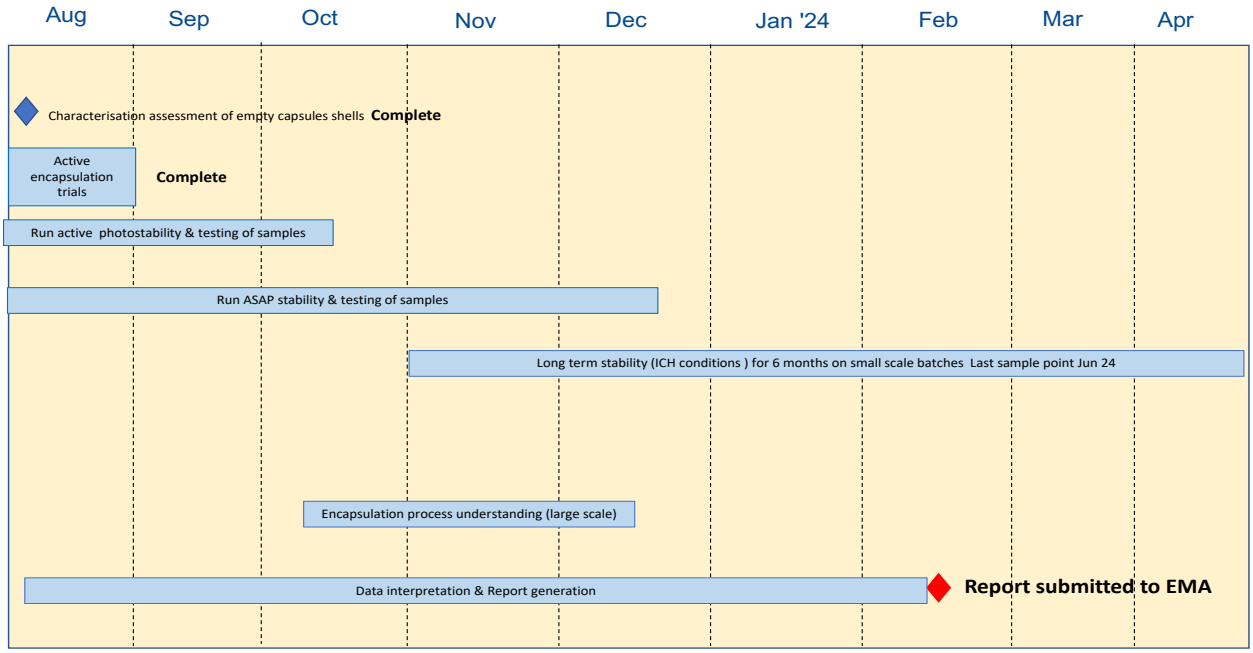


**Figure 19: Timeline of $TiO_2$ Alternatives Consortium activities to assess hard capsule shells with different components as opacifiers**




Some of the real-time and modelled stability data will be available but, due to the nature of long-term real time ICH stability studies, the full 6 m data will only be available from April 2024.

## B. Industry impact assessment of the situation on the pharmaceutical sector and timelines

**Question 4**

**In case an alternative to replace/remove $TiO_2$ is identified, please indicate approximate timelines to prepare and file for such a change (for subset of products/which ones/are there different issues for different products or dosage forms/types of products?).**

### 1. Introduction

Since late 2021, industry has been evaluating the potential impact of a $TiO_2$ ban and the challenges of switching to coatings and capsules containing potential alternative excipients. Specifically, industry have been looking at the technical feasibility for different types of medicines, the potential impact on patients and healthcare providers, the global regulatory impact, and supply chain challenges (such as capacity, timelines, and cost). The following section describe the results of an in-depth evaluation with a focus on the processes and timelines needed to remove or replace $TiO_2$ in European medicines. This description builds upon the preliminary estimates provided to the EMA in 2021.[2]

The summary herein of the industry evaluations describes the risk factors medicines suppliers must consider in reformulating different products and dosage forms and the various possible reformulation options. The assessment of timelines is then presented in two parts:

- The timelines for registering a single product considering the added complexity of $TiO_2$-alternative formulations (for both new products and marketed products).
- The estimated timelines for the reformulation of the thousands of products currently on the market in Europe considering business, regulatory and supply chain factors.

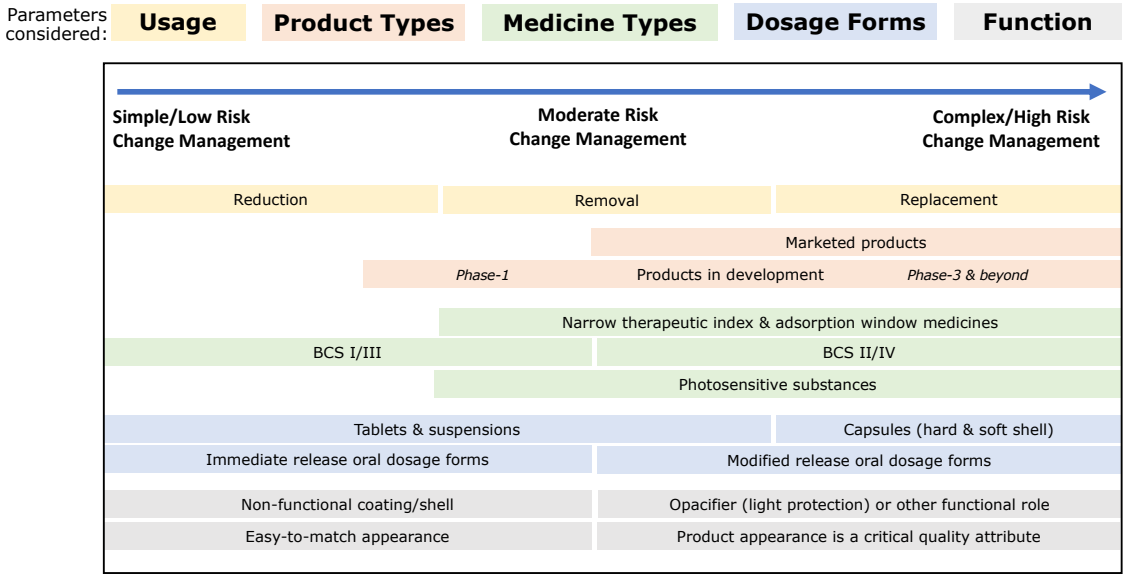
### 2. Risk Factors for Reduction / Removal / Replacement

Initially, it is important to note which factors were considered in the evaluation of the timelines for the reformulation of European medicines to eliminate the use of $TiO_2$. These are summarized in Figure 20 Depending on the product type, dosage form, usage, and function, the complexity and risks associated with the reformulation effort can vary considerably.

On the left side of Figure 20 those factors that create a lower risk, relatively simple, reformulation scenario are shown. Moving to the right of Figure 20, the factors that add significant complexity and risk to reformulation are illustrated.

It is estimated that more than half of the medicines currently marketed in Europe would map to the higher risk, right-hand side of Figure 20. For these higher complexity products there are currently no generally proven alternatives to the use of $TiO_2$ as an excipient and successful reformulation of these products is not guaranteed. If reformulation were not technically feasible or economically viable, potentially such medicines would have to be withdrawn from the European market, even though they may continue to be marketed elsewhere in the world.

**Figure 20: Summary of the factors considered when estimating the timelines for the removal or replacement of $TiO_2$ in European medicines.**




- It is estimated that complex products comprise a significant percentage (>50%) of the medicines currently marketed in Europe.
- There are currently no proven $TiO_2$ alternatives for these complex products. Successful reformulation is not guaranteed.

More details on each of the risk factors illustrated in Figure 20 are provided in Table 6. It is important to note that for many of these risk factors there are no proven technical solutions (for example, for most capsule products) or the reformulation approach has to be customized for each individual product and then tested to ensure no impact on critical products attributes (such as drug release rate, product shelf-life, patient acceptability, *etc*).

**Table 6: Detailed description of the risk factors considered by industry in their timeline analysis**

| Risk factor | Description of issue |
|---|---|
| Narrow therapeutic index & limited adsorption window drugs; BCS II/IV compounds | • Bio-performance of alternative coatings and capsules is still quite poorly understood currently for these types of medicines.<br>• Minimal clinical experience with $TiO_2$ -free coatings and capsules. |
| Photosensitive products | • Currently available $TiO_2$ -free coatings and capsule shells do not provide a sufficiently high level of protection from light.<br>• Protective primary packaging not always a suitable alternative (e.g., for in-use stability). |
| Capsules (hard & soft shell) | • Globally acceptable alternative capsule shell options are not available (e.g., FeO2 levels).<br>• Available alternatives demonstrate lack of robustness (e.g., brittleness). |
| Modified release products | • Impact of alternatives on medicine release performance is not predictable and thus each product needs to be studied on a case-by-case basis. |
| Coloured tablet cores or capsule fills | • Masking or colour matching is very challenging, and subsequent change of product appearance can lead to non-compliance. |

| | |
|---|---|
| Globally registered products | • Formulation and process changes are slow to be approved in some regions or are contingent on prior EMA approval.<br>• Criteria for demonstration of equivalent performance may vary between regulatory agencies. |
| Long-established products | • For some long-established products, developed via traditional approaches, a lack of product or process knowledge may make changes to formulation or manufacturing process highly challenging. |
| Patient Access | • Costs of changing formulation composition or manufacturing process may exceed revenues for many generic products. |

## 3. Reformulation Options Considered

The scenarios considered for reformulation were **replacement**, **removal** or **reduction** of the $TiO_2$ content in medicines. The EMA and European Commission have emphasized removal and replacement as their preferred approaches, but for completeness (and in the light of potential future scientific advances to establish a safe "permitted daily exposure" (PDE) for $TiO_2$) the possibility of reducing the amount of $TiO_2$ in medicines has also been considered as a potential approach.

### *- Replacement*

After analyzing the current offering of medicines in Europe it is clear that there are very few cases where a simple 1:1 substitution of $TiO_2$ with another material would be possible. The work of the $TiO_2$ Alternatives Consortium has clearly shown that in almost every case a more extensive change in the formulation composition and concomitant manufacturing process changes would be required, even for the simplest formulations. For example, changes will often be needed to the film forming polymer, plasticizers, extenders, and the final film thickness in addition to replacing the opacifier or pigment. Similarly processing conditions (such as coating solution spray rate) will also need to be modified in many cases.

For each product the impact of these composition and process changes on the performance and stability of the medicines needs to be studied in detail. In addition, any downstream impact on analytical methods (such as specificity) and packaging configurations (such as tablet size and thickness) would need to be evaluated.

It is important to note that the replacement of $TiO_2$ with alternative materials will in most cases increase the thickness of the tablet coating or capsule shell. This is expected to lead to longer processing times and increased manufacturing capacity demands beyond today's norms.

Finally, in cases where clinical bioequivalence study is required to demonstrate comparable *in-vivo* performance, reformulation timelines would be extended significantly.

### *- Removal*

Non-adherence to medications is a common problem and the WHO estimate that fifty percent of patients with chronic conditions deviate from their initial treatments. $TiO_2$ is crucial for the optimum appearance of tablets and capsules, and plays a significant role in patient compliance by enabling the differentiation of different dosage forms and different product strengths.

The Consortium experimental studies have shown that removal of $TiO_2$ from most film coated tablets and encapsulated products results in a significant impact on product appearance. The product color, smoothness and elegance can all change markedly, and thus patient acceptability and adherence can be negatively affected.

Thus, this reformulation approach (that is, removal of $TiO_2$) is only likely to be feasible for a very small percentage of existing products (estimated to be <<5%).

*- Reduction*

Based on the initial guidance of the EMA and the European Commission, *reduction* in $TiO_2$ levels in European medicines is not generally being considered for any product. However, this is a potentially valuable approach that could minimize patient exposure to $TiO_2$ whilst maintaining product performance and minimizing product shortages. A similar approach to that used for preservatives might be feasible, with manufacturers being required to demonstrate the need for a certain level of $TiO_2$ to provide the necessary functionality (light protection, *etc*). To enable this approach, a permitted daily exposure (PDE) would need to be established based on toxicological data.

## 4. Timeline for elimination of $TiO_2$ from European medicines

In 2021 the industry provided a preliminary estimate of the costs and timelines for eliminating $TiO_2$ from European medicines. This was communicated in the table shown below (Table 7) and the estimated time varied from 31 to 63 months per product based on the complexity of the reformulation project.

**Table 7: Preliminary estimate of costs and timelines for eliminating $TiO_2$ from European medicines**

| Type of Product | Type of Regulatory Variation | Timing for R&D per formulation (months) | Timing to produce validation/stability batches per formulation (months) | Stability Requirements per formulation (months) | Batch analysis and regulatory documentation preparation per formulation (months) | Bioequivalence study (months) | High level project costs per formulation (excluding API) (€) | Fees/MA (€) per marketing authorisation | Regulatory Assessment Timetable (realistic) per marketing authorisation | worst-case total per marketing authorisation (months) |
|---|---|---|---|---|---|---|---|---|---|---|
| SIMPLE Hard Capsule / Coated Tablet – coating non-functional i.e. differentiation strategy | IAIN | 9 to 12 | 3 to 6 | 3 to 6 | 6 | 0 | 500.0000 | pan EU RMS with 1 strength product: 16,000 + 9,000 per additional strength | 30 days acceptance/rejection | 31 |
| COMPLEX Coated Tablet – coating functional i.e. gastro resistance | II | 12 to 18 | 6 to 9 | 6 to 12 | 9 | 9 | 1.500.000 | pan EU RMS with 1 strength product: 118,000 + 30,000 per additional strength | 3 - 6 months | 63 |

Where companies decide that the only viable supply option is to replace titanium dioxide globally costs and timelines will be significantly increased (eg 3-4 years)

These preliminary estimates have been refined by industry following a more in-depth analysis and the updated estimates will be presented in the next few paragraphs. These updated timeline estimates have been confirmed by recent experiences with reformulation for the purposes of nitrosamine reduction in products developed for the European market.

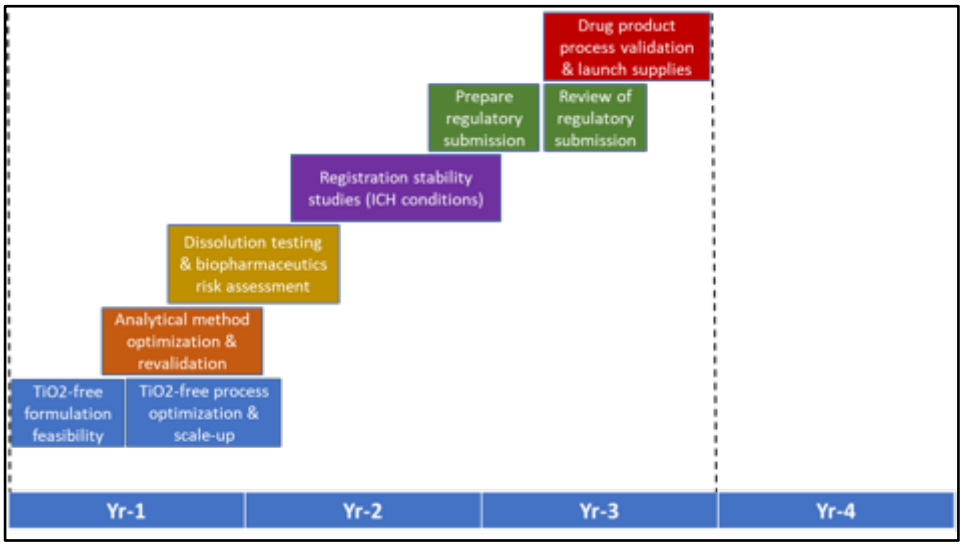
For ease of understanding, the timelines for reformulating individual products will be presented first, and after that the timelines for reformulation an entire product portfolio (one company's products) will be presented.

*Low-risk / Simple case*

For a low-risk (or relatively simple) reformulation project the estimated EU submission time is about three years per product (Figure 21). This scenario would be for a typical immediate release tablet

where reformulation is possible with standard excipients and the formulation and manufacturing changes are minor. These changes would need to have a minimal impact on product appearance, stability and performance, and no bioequivalence study would be required to demonstrated similar in-vivo functioning (hence, probably a BCS Class 1 or 3 product).

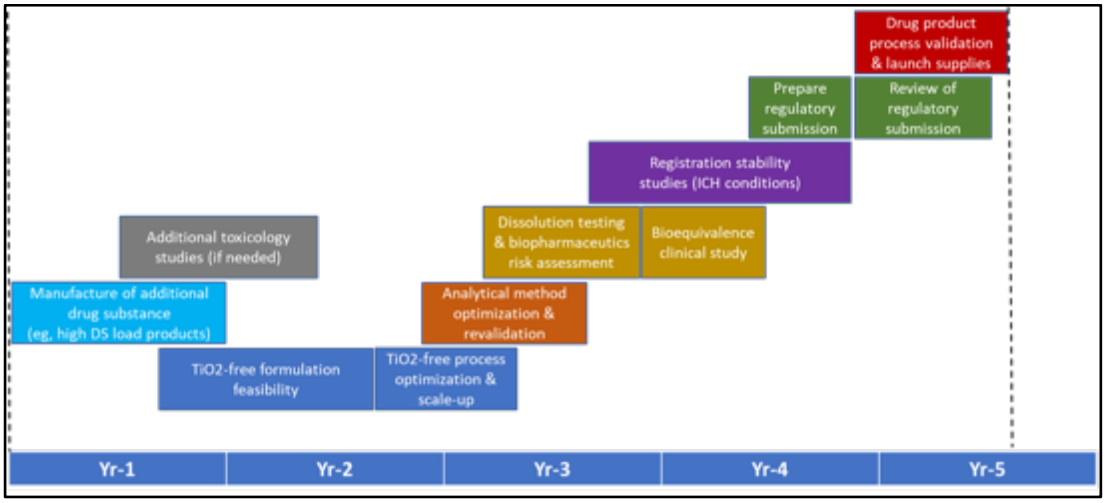
**Figure 21: Estimated timelines for the reformulation of a single low-risk/simple product**




*High-risk / Complex case*

In the case of a high-risk or more complex formulation scenario, the updated timeline for an individual product to be reformulated is about five years (Figure 22). This would be the case when supplies of the active drug substance are limited, or additional toxicology data needs to be collected on the alterative material(s) in the formulation. The added complexity could also be driven by the need to provide extensive light protection, or for a modified release dosage form where the film coating controls the drug release rate. If significant formulation or process changes were required, or if they had a marked impact on the product appearance, stability, or performance, then these could all increase the time needed to develop a $TiO_2$ -free medicine. The need for bioequivalence studies (perhaps in patients) could also extend the timelines for reformulating a complex product.

**Figure 22: Estimated timelines for the reformulation of a single high-risk/complex product**



## 5. Timelines for reformulating multiple products

Over 100,000 authorized medicinal products in Europe currently contain titanium dioxide. Reformulating all of these products would be the biggest reformulation effort ever undertaken by the pharmaceutical industry and there is a high probability that the supply of some medicines would be disrupted.

If business and supply chain factors are taken into account, it is possible to roughly estimate the time required for a typical medicines manufacturer to reformulate their entire product portfolio. However, there are many unknown variables or external influences that could have an impact on the timelines for remediation, therefore a detailed schedule for eliminating $TiO_2$ from European medicines cannot be provided at this time. These unknowable factors include the following:

- Cost and availability of commercial quantities of $TiO_2$ -free film coatings and capsule shells
- Long term stability and process robustness for $TiO_2$ -free medicines
- Patient responses to changes in the appearance of their medicines
- Speed of regulatory approvals in Europe and other markets
- Global economic factors (such as pandemics, recessions, regional conflicts, etc)
- Competing regulatory priorities (such as nitrosamine remediation and EG/DEG testing)
- Availability of contract manufacturing capacity for reformulation activities
- Ability to recoup reformulation costs by raising prices
- What competitor companies are doing
- Patient / consumer sentiment regarding continued use of $TiO_2$ (in Europe and other regions)

For most medicines manufacturers, remediation of multiple products concurrently would need to be staged over multiple years due to R&D and manufacturing capacity limitations. The consortium studies have demonstrated that thicker film coatings will be needed and this will equate to longer processing times and reduced manufacturing throughput for each company. There is also a finite and limited capacity for stability sample storage, analytical testing, and bioequivalence testing within the industry as a whole. The reformulation efforts for existing products would have to compete for these facilities with new products that are being developed to meet unmet medical needs. Even if new facilities for manufacturing and testing are commissioned immediately it would take several years for these GLP/GMP facilities to come online.

Other factors that need to be considered include the need to continue to supply existing products to patients (in Europe and the rest of the world) whilst the reformulation efforts are underway. There may also be a finite capacity at EMA/national competent authorities for the review of updated regulatory dossiers. It will be very important to minimize the impact on patients (due to product appearance changes, taste changes, package changes, *etc*) by education and outreach via pharmacists and doctors. In some regions, pandemic supply chain issues continue.

Finally, there may be unintended or unexpected impacts on global product registrations that cannot be easily 40foreseen. Many companies develop globally standardized products to simplify their supply chains and regulatory obligations, and any requirement to provide different products for the European market will add technical, commercial and regulatory complexity which could have unintended negative impacts on the supply of medicines for Europe.

In conclusion, it is conservatively estimated that for it would take between 7 and 12 years for a typical company to reformulate their entire portfolio of new and existing medicines. For some large multi-national companies, it would take even longer and lack of a long enough transition period would likely increase product withdrawals and/or lead to shortages of some medicines.

## 6. Summary of potential timelines

Based on studies completed with $TiO_2$-alternatives to date the feasibility of replacing $TiO_2$ in every European medicine still cannot be confirmed at this stage. Consortium studies confirm that certain subsets of products (such as capsules, photosensitive actives, narrow therapeutic index medicines) will be very challenging to reformulate. Based on previous reformulation experiences (e.g., to reduce nitrosamine levels) the industry confirms its initial estimate that reformulation of individual products will likely take from 3 to 5 years (and this could be longer for certain products).

Taking individual product timeframes, capacity constraints, unknowable risk factors, and the large number of products involved into account, the industry also estimates that removal of $TiO_2$ from all European medicines should be expected to take more than a decade. Based on analysis of the technical, commercial, and regulatory complexity of reformulating global products, the industry also confirms that the banning of titanium dioxide from European medicines could result in the withdrawal of hundreds (or possibly thousands) of products from the market and supply shortages for a significant number of medicines.

Also of note is that, at present, the majority of medicines suppliers have not yet developed any detailed plans for reformulation en-masse of medicines' portfolios, and that only approximations such as those described in this report, achievable at this stage. This is due to critical factors outlined in this report, including:

- That generally usable and suitable alternative coating and capsules have not been identified that give medicines of proven equivalent quality, safety and efficacy.
- That the safety of titanium dioxide has been evaluated by many groups and regulatory authorities as presenting no concern.
- That many alternative materials on coating and capsules do not yet have the same cumulative evidence of safety as titanium dioxide.
- That complexity on scale for such a multi-product activity (which requires technical, safety, manufacturing capacity and commercial assessment, including considerations of global considerations) is such that clarity is first required on timelines, available capacity and scope

## Question 5

**Please, supply an updated summary of the calculated impact on availability, shortages, and costs of any requirement to replace/remove titanium dioxide from medicines in Europe, considering the global nature of product development and supply.**

### 1. Recent Global Safety Evaluations of $TiO_2$

A key factor effecting the calculated impact on availability, shortages and costs is the status of titanium dioxide globally. This is due to the fact that many medicines are developed with global supply chains in mind, and without specific manufacture or formulations for the EU market. As such, in updating industry's summary, it is essential to first outline the updated assessment made by other countries of the safety of titanium dioxide. This summary is provided below:

**UK FSA 2022**

COT (2022) Interim position paper on titanium dioxide[5]. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, UK:
The UK's Food Standards Agency (FSA) published after reviewing the evidence of the data, that no safety concerns have been identified, and that the weight of evidence does not support the EFSA conclusion. Consequently, there will not be a change to regulation in England and Wales. Food Standards Scotland (FSS) reached the same conclusion. In essence, they do not agree with the EFSA assessment and do not see a need to replace $TiO_2$ in pharmaceuticals. It is anticipated that UK COT will publish the outcome of the evaluation in Q4 2023/Q1 2024 based on a further analysis of the UK COM (Committee on Mutagenicity).

**Health Canada (HC) June 2022:**

The Food Directorate's comprehensive review[6] of the available science of $TiO_2$ as a food additive summarized:

- HC re-evaluated the cancer study with new data on compound characteristics that were not available for the EFSA evaluation. Unitane 0-220 particle size and purity is highly comparable to recent food grade $TiO_2$, E171 and HC concluded there was no evidence of cancer in mice and rats exposed to high concentrations of food-grade $TiO_2$.
- HC concludes that there were no changes to DNA in various animal studies after treatment with $TiO_2$. In addition. No adverse effects on reproduction, development, immune, gastrointestinal or nervous systems, or general health when rats were exposed from pre-conception to adulthood.
- Whilst HC acknowledged the uncertainties in the database that would benefit from further research, the weight of evidence (WoE) suggests that these gaps are not significant enough to warrant a precautionary approach.
- In summary, the Food Directorate's position is that there is no conclusive scientific evidence that the food additive $TiO_2$ is a concern for human health.

[5] https://cot.food.gov.uk/2021-statementsandpositionpapers (Accessed on October 29, 2023)

[6] https://www.canada.ca/en/health-canada/services/food-nutrition/reports-publications/titanium-dioxide-food-additive-science-report.html (Accessed on October 29, 2023)

**USA FDA**

The FDA reviewed the findings of EFSA's 2021 Opinion on titanium dioxide. The FDA notes that EFSA's 2021 Opinion continued to confirm no general and organ toxicity, as well as no effects on reproductive and developmental toxicity. Based on this evaluation, FDA published in the Code of Federal Regulations. Title 21, Volume 1 (21CFR73.575] updated in June 07 2023, the acceptable use of $TiO_2$ in food up to 1% (w/w)[7].

**Australian / New Zealand September 2022**

The Authorities (FSANZ) highlighted in their risk assessment[8] that absorption of food-grade titanium dioxide following ingestion in food is very low. Recent studies with food-grade titanium dioxide in rats suggest that less than 0.01% of the amount eaten is absorbed. FSANZ discussed that pre-neoplastic lesions in the colon were observed in a drinking water study with sonicated food-grade $TiO_2$ at 10 mg/kg bw/day, but these findings were not replicated in two studies in which food-grade $TiO_2$ was administered via the diet up to considerably higher doses (up to 267 or 1000 mg/kg bw/day).

They considered the results of feeding studies a being more relevant than studies after sonification.

In addition, they mentioned that the observations of pre-neoplastic lesions are also inconsistent with the findings of a 2-year bioassay of $TiO_2$ in rats and mice conducted by the US NCI. No evidence of toxicity or carcinogenicity was observed at dietary concentrations up to 50,000 ppm in this study.

A recent OECD TG-compliant EOGRT study in rats with food-grade $TiO_2$ administered via the diet at doses up to 1000 mg/kg bw/day found no evidence of systemic toxicity, developmental or reproductive toxicity or developmental neurotoxicity. and no evidence of developmental immunotoxicity was observed with $TiO_2$ in this study.

In conclusion, based on the data currently available, FSANZ concludes there is no evidence to suggest that dietary exposures to food-grade $TiO_2$ are of concern for human health.

**Ministry of Health, Labour and Welfare of Japan 2023**

National Institute of Health Sciences (NIHS) experts stated it is difficult to support the EFSA opinion. Additionally, based on the results from Agaki et al. 2023[9], it is thought that the absorption of $TiO_2$ from the gastrointestinal tract is extremely low. Therefore, it is difficult to rationally explain the EFSA interpretation, which assumes that orally administered $TiO_2$ reached target tissues such as the bone marrow at a concentration that would explain its induction of genotoxicity.

***Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2023***

The JECFA discussed all available data in its Ninety-seventh meeting (Safety evaluation of certain food additives) from 31 October–9 November 2023[10]. In this meeting, the Committee considered additional toxicological studies relevant to the safety assessment of INS171 that investigated the toxicokinetics, acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity, and

[7] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=73.575 (Accessed October 29, 2023)
[8] https://www.foodstandards.gov.au/consumer/foodtech/Documents/FSANZ_TiO2_Assessment_report.pdf (Accessed October 29, 2023)
[9] Akagi, J. et. al. (2023) Oral toxicological study of titanium dioxide nanoparticles with a crystallite diameter of 6 nm in rats. Akagi et al. Particle and Fibre Toxicology. https://doi.org/10.1186/s12989-023-00533-x
[10] https://cdn.who.int/media/docs/default-source/food-safety/jecfa/summary-and-conclusions/jecfa97-summary-and-conclusions.pdf?sfvrsn=1b8ecced_5&download=true (Accessed February 2024)

reproductive and developmental toxicity, as well as special studies addressing the short-term initiation/promotion potential for colon cancer.

JECFA also evaluated estimates of dietary exposure to $TiO_2$, estimating the maximum 95th percentile to be 10 mg/kg bw/day, which was used for the risk evaluation of INS 171 in the diet.

INS 171 consists of uncoated $TiO_2$ anatase particles including a minor fraction of nano size particles. Food-grade $TiO_2$ is identified and labelled as E171 by the EU. INS 171 and E171 are equivalent except that INS 171 does not include the $TiO_2$ coating of pearlescent pigments (INS 176). Therefore, in line with the HC review, the JECFA also considered the historical carcinogenicity data from the NCI to be relevant for the risk assessment of INS 171 and by extension, E171.

The JECFA took into account that INS 171 was not carcinogenic in an adequately conducted 2-year study in mice and rats at gender-averaged doses of up to 7500 mg/kg bw/day for mice and 2500 mg/kg bw/day for rats, the highest doses tested.

The JECFA confirmed the assessments of other agencies that there was no evidence of reproductive or developmental toxicity in studies in rats at INS 171 doses of up to 1000 mg/kg bw/day, the highest doses tested.

JECFA stated that they reviewed all available research on genotoxicity risk and determined that the evidence is insufficient, owing mostly to the lack of suitable testing methodologies for nanoparticles." This indirectly implies, that value of the indicator assays like the comet assay in vitro is not relevant to describe the genotoxic potential, at least in the current format.

Therefore, JECFA recommended more research to address the current uncertainty about the distribution of $TiO_2$ particle sizes in food and to develop genotoxicity tests that are more appropriate for nanoparticles.

Finally, the JECFA concluded that considering the very low oral absorption of INS 171, and in the absence of any identifiable hazard associated with INS 171 in the diet, i**t was appropriate to reaffirm the ADI "not specified"** established at the Thirteenth meeting in 1969.

## 2. Further EU assessments on $TiO_2$ safety

***Scientific Committee on Consumer Safety (SCCS) 2023***

The expert panel concluded:

- There exists insufficient evidence to exclude the genotoxic potential of almost all $TiO_2$ particles, with the exception of the two nano-grades RM09 and RM11, where a negative hypoxanthine-guanine-phosphoribosyl-transferase test (HPRT) and micronucleus test (MNT) *in vitro* confirmed the absence of a genotoxic potential,
- In line with this interpretation, SCCS felt unable to recommend any safe levels for $TiO_2$ (including pigmentary grade) in cosmetics,
- Overall, the SCCS evaluation is in line with the EFSA statement but acknowledges that the situation for cosmetics is different from food ingredients in that oral uptake of cosmetics is usually incidental and thus quantitatively much lower, and primarily via oral buccal exposure versus through the GIT,
    - In contrast to others, their assessment is based on *in vitro* data from the Comet Assay, whereas elsewhere this assay is given much less weight as an indicator test as it is not equivalent to stable mutations or chromosome damage,

- A valid *in vitro* micronucleus or chromosomal aberration test (assuring all nanotoxicology state-of-the-art principles are applied) with adequately selected E171-equivalent material(s) would be needed to overrule the current conclusion,
- A lot of weight is given to the Kirkland et al. (2022)[11] review and the SCCS conclusions are in agreement with the Kirkland et al. conclusions ("the profile of genotoxicity results from the most robust studies with titanium dioxide does not fit the response pattern which would be expected for a genotoxic carcinogen"),
- SCCS is of the opinion that the Applicants should draw up a proposal for specifications of the different $TiO_2$ grades used in cosmetics.

Thus, SCCS is the only committee that follows EFSA's opinion that a genotoxic potential of $TiO_2$ cannot be excluded. However, in both cases this interpretation is based on data from assays that are considered by most other groups as not providing data reliable enough for such a conclusion. Of note, the SCCS suggest that well conducted OECD-compliant in vitro tests (micronucleus or chromosome aberration test) would adequately mitigate the genotoxicity concern (data that is currently lacking). In addition, the suggested to draw up a proposal for specifications for the different grades of $TiO_2$ used in those cosmetic products that could lead to oral and inhalation exposure. The SCCS will be able to assist the Commission in reviewing the proposal.

## 3. Recently Published $TiO_2$ Quality Evidence

**Titanium Dioxide (E171 Grade) and the Search For Replacement Opacifiers and Colorants: Supplier Readiness Survey, Case Studies and Regulatory Perspective[11]**

In a comprehensive review published in 2023, the IQ Consortium summarised a number of surveys and practical assessments conducted with alternative materials by IQ member companies. In this, review, they note that a range of technical challenges and regulatory hurdles were identified which mean that, in the short term, it will be difficult to replace titanium dioxide with the currently available alternative materials while readily achieving the same drug product quality attributes. The assessment summarised that this was linked to higher variability, colour fading and identified scale up risk, of E171 free film coatings[12], and the consequent negative impact on development costs and timelines and product quality. The review also highlighted the regulatory and supply chain hurdles that would have to be overcome if a titanium dioxide replacement was required for the EU market but was not mandated by others.

## 4. Recently Published $TiO_2$ Safety Evidence

The conclusions from non-EU regulators' assessment are further supported by assessments and published since the EFSA Assessment. These are summarised below:

**Chronic Toxicity Study in rats with genotoxicity endpoint conducted at the National Institute of Health Sciences, Japan, 2023[13]**

[11] Bruno Hancock, et al 2023 Titanium Dioxide (E171 Grade) and the Search For Replacement Opacifiers and Colorants: Supplier Readiness Survey, Case Studies and Regulatory Perspective, Journal of Pharmaceutical Sciences, ISSN 0022-3549, https://doi.org/10.1016/j.xphs.2023.12.006. https://www.sciencedirect.com/science/article/abs/pii/S0022354923005154

[12] https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/film-coating

[13] Akagi, Ji., Mizuta, Y., Akane, H. et al. Oral toxicological study of titanium dioxide nanoparticles with a crystallite diameter of 6 nm in rats. Part Fibre Toxicol 20, 23 (2023). https://doi.org/10.1186/s12989-023-00533-x

- *In June* 2023 an Oral toxicological study of titanium dioxide nanoparticles with a crystallite diameter of 6 nm in rats was published
- Overall, the result of the study demonstrated that there were no toxic changes, including general toxicity, induction of colonic abnormalities, DNA-damaging potential, and accumulation of $TiO_2$ in the liver, kidney, or spleen following the oral administration of anatase $TiO_2$ NPs with a crystallite size of 6 nm for 28 or 90 days.
- The NOAEL in both 28- and 90-day studies observed was 1000 mg/kg bw/day. The results provide further evidence for evaluating the safety of oral exposure to $TiO_2$ that may contain very small crystallites because Immunohistochemical analysis of colonic crypts showed no extension of the proliferative cell zone or preneoplastic cytoplasmic/nuclear translocation of β-catenin either in the male or female 1000 mg/kg bw/day group.
- In addition, no significant increase in micronucleated or γ-H2AX positive hepatocytes was observed, demonstrating an absence of double strand breaks.
- This is in particular important as the induction of γ-H2AX was not observed at the deposition sites of yellowish-brown materials.
- Overall, the authors concluded there are NO safety concerns even with these extremely small nano-sized particles of 6 nm.

**Expert Review of the genotoxicity of titanium dioxide ($TiO_2$), 2022**

A panel of experts (not employed by companies that manufacture and sell $TiO_2$, was convened to perform the review of the genotoxicity of $TiO_2$ (expertise in genetic toxicology, general toxicology, bioavailability, carcinogenicity, and nanoparticle characterisation)[14].

- Only Studies with Genetic Toxicology endpoints covered by validated OECD protocols were reviewed.
- From 337 datasets with available genotoxicity data on $TiO_2$, by using a structured WoE approach, taking into account the relevant endpoints, study protocols and material characterizations, only 34 (10.1%). Studies eventually provided relevant data.
- Of these 34, 10 were positive, all of which were from studies of DNA strand breakage or chromosome damage. All the positive findings were associated with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis, or combinations of these. Considering that DNA and chromosome breakage can be secondary to physiological stress, it is highly likely that the observed genotoxic effects of titanium dioxide, including those with nanoparticles, are secondary to physiological stress.
- Expert Panel re-evaluated the data in each dataset included in the final assessment (and sometimes did not confirm the authors findings), whereas EFSA accepted the authors' conclusions without further review for datasets included in the final assessment.
- "Existing evidence does not therefore support a direct DNA damaging mechanism for titanium dioxide (nano and other forms)"
- However, carefully designed studies of apical endpoints (gene mutation, MN or CA), following OECD recommended methods, performed with well characterised preparations of $TiO_2$, would allow firmer conclusions to be reached.

[14] Kirkland, D., et al A weight of evidence review of the genotoxicity of titanium dioxide ($TiO_2$), Regulatory Toxicology and Pharmacology (2022), doi: https://doi.org/10.1016/j.yrtph.2022.105263

In addition, two $TiO_2$ cosmetic grades were tested negative in the in vitro gene mutation assay (HPRT) and MNT vitro assay (data presented at the Genetic Toxicology Association (GTA) Meeting 2023), in accordance with the published EFSA protocol for testing of Nanomaterials.

## 5. Ongoing safety testing

In addition, several safety evaluations considering the different grades of $TiO_2$ are planned or ongoing outside the pharmaceutical industry, involving high quality OECD compliant studies with $TiO_2$/E171, adequately designed to assess the nanomaterial fraction via:

- in vitro gene mutation assays (SCCS reported two negative in vitro assays for cosmetic grade $TiO_2$)
- In vivo Comet assay
- Transgenic animal mutagenicity studies

For example, the HESI GTTC MGRA working group is working on and Adverse Outcome Pathway for $TiO_2$ to support Risk assessment based on Mode of Action[15].

It should be noted that the different $TiO_2$ grades showed different physicochemical properties that may lead to different biological consequences.

## 6. Safety Summary and Industry Assessment of the EFSA Opinion

TiO2 has an extensive toxicological data set, demonstrating no evidence for potential hazard to human health. Since the EFSA Evaluation, new data were generated and should be considered in an updated risk:benefit assessment. These data provide supportive evidence to consider $TiO_2$ as non-mutagenic/carcinogenic.

So far, authorities outside EU assessing the available data considered $TiO_2$ as no risk for medicinal products. Some non-EU authorities followed the EFSA recommendation without their own assessments. Recently SCHEER followed also the EFSA conclusion, but only for nano-grade materials (<100 ng/day), i.e., toys containing pigmentary grade $TiO_2$ can be used with no or negligible risk.

Industry concludes in their assessment that there is no evidence that $TiO_2$ (E171) has mutagenic potential in vitro or in vivo. Genotoxic effects observed are primary DNA damage (stand breaks) and chromosomal damage (clastogenicity) mainly in indicator assay like the comet assay in vitro which have limitations in their relevance for hazard identification. However, these genotoxic effects seem not to result in gene mutation. The effects were observed at cytotoxic doses and/or considered to be secondary to oxidative/physiological stress. Several modes of actions inducing primary DNA lesion may exist, including formation of reactive (oxygen) species (induced directly, via inflammation or mitochondrial dysfunction) and direct DNA interaction only in vitro, but there is no proof for covalent binding of $TiO_2$ to DNA, no proof that $TiO_2$ enters the nucleus and no proof this results in gene mutations. Occurrence of primary DNA damage and clastogenicity in the absence of mutation induction is not novel and has been identified for situations where primary DNA damage is efficiently repaired and does not result in tumour induction.

Emergent data of the material characterisation (including the nanoparticulate fraction) that was representative of Unitane-0-220 used in the negative oral carcinogenicity studies conducted by the NTP are key (consequently, carcinogenicity data were accepted by HC, FSANZ, FDA). These carcinogenicity data are essential for informing the biological significance of in vitro and in vivo genotoxicity study results for the benefit:risk assessment of medicinal products, providing a NOAEL of 2250 mg/kg/day. With this NOAEL it should be possible to calculate a PDE supported by the new data

[15] https://hesiglobal.org/genetic-toxicology-gttc/

from Agaki et al[16], highlighting that Immunohistochemical analysis of colonic crypts showed no extension of the proliferative cell zone or preneoplastic cytoplasmic/nuclear translocation of β-catenin either in the male or female 1000 mg/kg bw/day group. Regarding genotoxicity, no significant increase in micronucleated or γ-H2AX positive hepatocytes was observed. Additionally, the induction of γ-H2AX was not observed at the deposition sites of yellowish-brown materials.

Overall, Industry does not agree with the EFSA assessment and considers $TiO_2$ as being safe when used as an opacifier or colorant in medicinal products. Industry requests the opportunity to work with EMA on any potential new safety studies with $TiO_2$ and/or potential alternatives.

The $TiO_2$ Alternatives Consortium Safety team examined the data on the potential health hazards of $TiO_2$. A review of the many decades of data on $TiO_2$ found that:

- Any genotoxicity observed with $TiO_2$ is likely secondary to physiological stress and not due to direct DNA damage.
- One study that suggested $TiO_2$-related effects, i.e., Bettini et al., 2017, is flawed and not reproducible.
- Nearly all regulatory agencies have reached a different conclusion compared to the EU and state that the food additive E171 does not pose a human health concern.
- The National Cancer Institute (NCI, 1979) carcinogenicity study is considered valid and is the most appropriate study for assessing the long-term effects of $TiO_2$ and setting an oral PDE. Although a PDE is not normally necessary for low hazard substances, a PDE for $TiO_2$ was determined and Scientific Advice requested. Scientific information and establishment of the PDE will serve for risk-benefit evaluation on the use of low amounts of $TiO_2$ contained in tablets and capsules in oral medicinal products and will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels.

## 7. Availability & shortages following a requirement to replace/remove titanium dioxide from medicines

The requirement to produce $TiO_2$ -free medicinal products in Europe, considering the global landscape wherein it remains fully available in other countries, creates the need for separate EU-only supply chains, and a greater likelihood of unforeseen issues leading to EU medicines shortages.

Many pharmaceutical companies supply or subcontract production to supply chains producing medicines for global markets. There is still uncertainty on whether these MAHs or their subcontractors would be supportive of reformulation to remove $TiO_2$ only in EU medicines, considering the effort required on regarding human resources and material resources.

Availability of $TiO_2$-free excipients is already problematic following the EU-wide ban in food, with suppliers having limited capacities to provide these excipients. Alternative options, regardless of suitability, currently available on the market cannot at present satisfy the volumes required by all the EU Pharmaceutical industry. Considering there is no 'one size fits all' alternative available today each reformulation has its own special consideration. Any requirement to replace $TiO_2$ would certainly lead to supply chain shortages.

Furthermore, a negative shelf-life impact is foreseen for many products following the removal/replacement of $TiO_2$. This will lead to further strains on the supply chains impacting availability.

[16] https://particleandfibretoxicology.biomedcentral.com/articles/10.1186/s12989-023-00533-x (https://doi.org/10.1186/s12989-023-00533-x)

For some products it will likely not be possible technically to remove $TiO_2$ from their formulation. These products may need to be withdrawn from the market.

The pharmaceutical industry does not have the capacity to reformulate all impacted medicines in parallel. Considering the findings of the Alternatives Consortium, companies will likely have to prioritize certain products above others for any reformulation work, leading to some low margin products either disappearing from the market for a certain period of time or even being completely removed, depending on the commercial perspective.

All reformulations will have to undergo regulatory variation procedures. Considering the hypothetical number of reformulations required delays from Competent Authorities are expected in this scenario, which can only lead to additional shortages of medicinal products. (to keep in mind the recent QRD template update and issues created for veterinary medicines) – bottleneck for both companies and authorities.

## 8. Costs of reformulation for the pharmaceutical industry

Considering the previously presented technical disadvantages created by the removal of $TiO_2$ from medicinal products, each point has a cost associated with it which varies from product to product. As an industry we cannot produce exact numbers associated with each of these points as every company has its own specificities when it comes to manufacturing distribution and overall efficiency of these steps, but it is unanimously agreed that each of the cost-producing arguments are not negligible. It is possible to split the costs into two different categories: **one-time costs** and **additional running costs**.

### *One-time costs*

One-time costs include all the R&D (reformulation, production, stability testing etc) costs and the authorization costs. It is worth noting that depending on the function $TiO_2$ serves in each individual product, the R&D one-time costs vary by a ten-fold factor or even more in some cases. Gastroresistant coatings are much more expensive to reformulate compared to products where $TiO_2$ has an opacifier function.

In some cases, the additional drug substance costs needed for reformulation development, repetition of stability studies, and repetition of drug product validation costs could add up to millions of euros. We have a similar situation for toxicology studies.

Inventory write-off is also something to be taken into account, for stocks of products not yet placed on the market that cannot be sold anymore.

For nationally approved products all authorization costs will be multiplied by the number of Member States where these products are available, in some cases also by strength and species in case of veterinary medicines.

Looking at these one-time costs from a broad perspective, considering the number of products impacted it is easily estimated that the financial impact is well into billions of Euros.

### *Additional running costs*

Additional running costs will be generated by:

- Raw material/excipients prices and fluctuations of pricing based on additional demand;
- Production costs associated with the de-coupling of EU production from the rest of the world;
- Longer film-coating processing times;

- Shelf life and overall medicines' stability and costs implied by trying to reverse these negative impacts (changes in packaging for example, or additional requirements in the distribution chain).

All such costs will have to be absorbed by manufacturers considering that medicines pricing is most often regulated from a reimbursement perspective in Europe. Even in free pricing pharmaceutical market settings, there may be measures that limit the possibility to increase prices (i.e., maximum price caps/minimum rebate levels in procurement or other civil contractual arrangements having a similar effect).

In the case of over-the-counter (OTC) products, the reformulation costs may result in price increase and may discourage people to practice self-care and push them to seek healthcare and reimbursed medication, thus adding additional costs and strain on the health systems

# Summary

## Summary of Evidence on Alternatives

Industry is continuing to investigate potential alternatives to titanium dioxide ($TiO_2$) with a clear plan to ensure there is no impact on patients from any replacements. Since 2021 there has been significant investigation and investment by suppliers of coatings and capsules and MAHs. A substantial amount of evidence has been generated and there have been many peer-reviewed publications. Industry's summary of the evidence is that:

- **For Coatings:** For many products alternative coating can replace $TiO_2$, although significant increased amounts will be required and appearance matching will not generally be possible. It may not be possible to replace for complex, modified release dosage forms and products sensitive to light may be at risk of increased impurities and lower quality and safety.
- **For Capsules**: the overall evidence generated to-date, suggests that it will be challenging to identify alternatives that can deliver products of the appropriate quality.
- Overall, the evidence confirms that for some medicines, use of $TiO_2$ as an excipient can be critical to safety and efficacy (e.g. as an opacifier to protect from light and prevent degradation, or to ensure that the minimal amount to coating is used to enable tablet dissolution).

Many international regulatory authorities have reviewed the safety of $TiO_2$ and concluded there is no safety concern in food or medicines. Furthermore, many alternative coatings and capsules contain colourants and opacifiers that do not have the same evidence of safety as $TiO_2$.

Industry refers to the 2022 article in J Pharm Sci as a review of all currently available literature on alternative coatings and the unique properties of $TiO_2$[17]. This document provides significant scientific assessment and concludes that:

> *"At the time of writing, in the view of the authors,* ***no system or material which could address both current and future toxicological concerns of Regulators and the functional needs of the pharmaceutical industry and patients has been identified****. This takes into account the assessment of materials such as calcium carbonate, talc, isomalt, starch and calcium phosphates. In this paper an IQ Consortium team outlines the properties of titanium dioxide and criteria to which new replacement materials should be held"*

A further detailed review, including a summary of surveys of capsule and coating supplier readiness and case studies on the use and issued encountered in real systems was published by the IQ Consortium in Dec 2023. This further supports the conclusions summarised in this report.[18]

Based on the existing comprehensive safety package for titanium dioxide, in particular, as additional scientifically sound data has been made available, industry is of the opinion that a permitted daily exposure (PDE) for titanium dioxide can be calculated. This PDE will provide a safe exposure limit and will finally support the comparison of Safety Data of the alternatives and will ensures the use of titanium dioxide as an excipient in pharmaceuticals. Industry asked EMA NcWP for scientific Advice

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[17] Blundell et al *J. Pharm. Sci*, **2022** The Role of Titanium Dioxide (E171) and the Requirements for Replacement Materials in Oral Solid Dosage Forms: An IQ Consortium Working Group Review DOI: 10.1016/j.xphs.2022.08.011

[18] *Titanium Dioxide (E171 Grade) and the Search For Replacement Opacifiers and Colorants: Supplier Readiness Survey, Case Studies and Regulatory Perspective,* Hancock, Melnick et al*, J Pharm Sci,* Dec **2023**, https://doi.org/10.1016/j.xphs.2023.12.006

on this topic. Taking all currently available data (low bioavailability, negative in vivo mutagenicity and carcinogenicity) and calculations together, Industry is proposing an oral PDE of 2250 mg/day to support the risk-benefit assessment of E171 as an excipient in oral pharmaceutical products, despite the fact that no hazardous properties have been identified for this material. Establishing the PDE will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels. Request on Scientific Advice from EMA was submitted on January 31, 2024 by Sanofi.

## Summary of Potential Impact on EU Medicines Supply of Restrictions on the Use of Titanium Dioxide

Depending on the unique requirements of each medicine, any individual reformulation (if possible) may take 3-5 years from lab to patient. Furthermore, it is conservatively estimated that for it would take between 7 and 12 years for a typical company to reformulate their entire portfolio of new and existing medicines.

Wholesale changes to medicines' formulations in Europe will be a significant and unnecessary resource drain for companies supplying medicines to Europe and to the European medicines regulatory authorities and will require significantly more than a decade to implement.

At the same time, Titanium dioxide continues to be assessed outside of Europe as having **no safety concern** (e.g. following assessment by Health Authorities in Japan, UK, Canada, Aus/NZ and the preliminary review of US). Titanium dioxide also continues to meet the most stringent of requirements governing the safety of medicines, including those set by the European pharmacopoeia, Japanese pharmacopoeia and US pharmacopoeia.

For products with global supply chains, consideration as to the viability of any new EU-only formulation would need to be assessed on a case-by-case basis, and many products could be discontinued for the EU. In addition, many clinical programs for innovative drugs are ongoing via multi-region clinical trials with titanium dioxide-containing formulations (eg in EU, US, China, & Japan simultaneously)

Overall, an isolated, EU-only restriction on titanium dioxide use for medicines in the EU will likely have a significant impact on medicines supply and innovative clinical programs.

## Colours permitted for use in human and veterinary medicinal products other than those included in the Union list of authorised food additives

Industry has reviewed the recently adopted proposal for a directive of the EU general pharmaceutical legislation[19] and notes with interest Directive Article 27 and the process wherein the Commission may establish a new list of colours permitted for use in medicinal products, other than those included in the Union list of authorised food additives.

Industry welcomes this important new element in the legislation, given the different benefit/risk considerations for medicines versus food and the impact on patient access of changing colours in medicines. Industry's interpretation is a similar process should apply to titanium dioxide, and that, following the assessment of the EMA, the Commission could potentially add titanium dioxide to the new list of colours permitted for use in medicinal products.

Industry also notes that the provisions of regulation 2022/63 (14) apply **only to the use of titanium dioxide as a colourant** (eg not as an excipient/opacifier) and that considerations per the *2007 CHMP opinion on CMR aspects of excipients*[20] should apply to titanium dioxide when used as an opacifier or

[19] https://health.ec.europa.eu/medicinal-products/pharmaceutical-strategy-europe/reform-eu-pharmaceutical-legislation_en

[20] https://www.ema.europa.eu/en/documents/other/chmp-scientific-article-53-opinion-potential-risks-carcinogens-mutagens-substances-toxic_en.pdf

any other excipient uses than colourant. Industry also noted the EMA position that "*$TiO_2$ is monographed in the European Pharmacopoeia and is considered to be suitable for use in the medicinal products as an excipient*." (EMA/504010/2021).

# Conclusions and Recommendations from Industry

## Evidence to-date supports the ongoing use of $TiO_2$ in medicines:

There is a long experience of safe use of $TiO_2$ in medicines and scientific evaluation of the currently available safety data does not raise any safety concerns.

To date, for some alternatives to $TiO_2$, there is no such level of evidence and safety risks cannot be assessed with the same level of confidence as for $TiO_2$. Moreover, EMA conclusions from September 2021 (EMA/504010/2021) are still valid:

- *"[…] The feasibility of replacing $TiO_2$ cannot be confirmed at this stage*
- *Any requirement to replace $TiO_2$ in medicines will almost certainly cause significant medicines shortages and discontinuations/withdrawals of medicines from the EU/EEA market with major implications for patients and animals […]"*

As is clear from the assessment and data generated by the Alternatives Consortium, no alternative system or material to $TiO_2$ has been identified for use as an opacifier in coatings and capsules with the functional requirements to ensure that the same high-quality medicines can be supplied to patients.

As such, and based on the current understanding, industry recommends that, in order to ensure ongoing supply of medicines to EU patients, titanium dioxide remains on the list of colours available for use in medicines (per the provisions of Regulation 2022/63, Article 16) and that $TiO_2$ is included in the new list of colours permitted for use in medicinal products, other than those included in the Union list of authorised food additives, per Article 27 in the 2023 draft of the general pharmaceutical legislation (Com (2023)192 final). In addition, based on the existing comprehensive safety package for titanium dioxide, in particular, as additional scientifically sound data has been made available, industry is of the opinion that a permitted daily exposure (PDE) for titanium dioxide can be calculated. This PDE will provide a safe exposure limit and will finally support the comparison of Safety Data of the alternatives and will ensures the use of titanium dioxide as an excipient in pharmaceuticals. Industry asked the EMA NcWP for scientific Advice on this topic.

Although it is unusual from a toxicological perspective to derive a PDE for a non-hazardous compound, a PDE calculation using scientifically robust data will increase confidence of patients in the safety of medicinal products containing $TiO_2$ and will allow the pharmaceutical industry to continue to provide patient access to life-saving medicines and to develop innovative high-quality medicines in the future.

Taking all currently available data (low bioavailability, negative in vivo mutagenicity and carcinogenicity) and calculations together, Industry is proposing an oral PDE of 2250 mg/day to support the risk-benefit assessment of E171 as an excipient in oral pharmaceutical products, despite the fact that no hazardous properties have been identified for this material. Establishing the PDE will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels. Request on Scientific Advice from EMA was submitted on January 31, 2024 by Sanofi.

# Annex 1

## General Considerations for Safety Assessment of Nanoparticles in Excipients

Nanoparticles were mentioned in the E171 EFSA opinion and also in the exchange of information between the EMA and Industry on the 16th October 2023, this is a topic that is not well understood and this summary will help to clarify the situation.

Classification on the status of a material as nano or non-nano have been and are still an area of ongoing discussion for academia, industry and policy makers leading to a variety of definitions, guidance, and legislation. Moreover, the ongoing development on the best applicable analytical techniques to provide evidence on the nano content adds uncertainty to the nano discussion.

The major intention of policy makers in defining nanomaterials is to focus on material which might merit additional safety evaluation for the purpose of protecting human health. The underlying rationale for this approach is that material in nano form might have a different physiological distribution and consequently a different risk/safety profile compared to the non-nano form. The risk assessment approach varies around the world with the EU (EFSA) taking a precautionary principle approach compared to other regions where a balanced risk assessment approach is favoured.

While currently there are no specific regulations for nanomaterials which may be contained in medicinal products and their components, crossover between industrial sectors is leading to related questions being asked of marketing authorisation holders and in turn to excipient manufacturers.

In 2022 the EC published a new "Recommendation on the definition of a nanomaterial" (2022/C229/EC) this is an update of the previous version published in 2011. This definition is intended to be incorporated into any new or revised EU or National Regulations by policy makers and regulators as they get written, but as of today this has not yet happened. The definition on the size of a nanoparticle is the same (<100nm), but the concentration of nanoparticles present to define a nanomaterial has been confirmed and this is >50%.

Almost all solid food ingredients, additives and excipients contain nanoparticles, particles of <100nm in size. Nanoparticles in food generally dissolve in the body's GIT. Many nanoparticles are created naturaly. For example, cow's milk naturally contains casein micelles, which are nanocapsules created by nature to deliver nutrients to newborn calves. Others are created by standard technologies commonly used during production for food additives or excipients such as drying, milling grinding etc These can be described as unintentional or incidental nanoparticles that are not essential for the function of the excipient, but they are simply generated by the manufacturing process. The vast majority of excipients will contain incidental nanoparticles and they will have always been present since they were first used in drug products many decades ago. Excipients would have been assessed for safety at the time of first use although it is unlikely that the presence of nanoparticles would have been known at the time as the ability to accurately measure particles of this size was not widespread and this is still the case today.

Engineered nanomaterials are intentionally created to perform a specific function which is dependent on their nanoscale properties. For example, iron hydroxide adipate tartrate is an engineered nanomaterial developed for use in food supplements as a source of bioavailable iron[21]. Its nano properties enable it to be more bioavailable and therefore easier for the body to absorb and use.

There are currently no pharmacopeial monographs or food additive specifications where there is a specification for the nano content. The only region where there is some guidance on the presence of

[21] Understanding Nanoparticles and Engineered Nanomaterials: Use and Labelling. EU Speciality Food Ingredients Factsheet. Dec 2022

nanoparticles in excipients is the United States where FDA guidance acknowledges nanoparticles can be present in excipients and that they are likely to have always been present. In their view if these excipients with a history of use in humans are used in the same way as they have been used historically with the same dose level and in drug products with the same route of administration then they are considered low risk. However, if an excipient is created, or modified, to give it the benefit of nanoscale properties then this needs to be fully characterized based on their functionality and intended use. Proper controls, including test methods and acceptance criteria, a description of material source, and grade should be defined in a premarket application, with justification for how those acceptance criteria enable the product to meet its desired quality target product profile[22].

In the European Union there is no such guidance for excipients used in medicines, which means that the guidance from other industries plays a role in the excipient selection process. Considering currently available information, the parameters to define nanomaterials are not applied consistently. This inconsistency is a drawback for manufacturers of engineered nanomaterials or excipients containing incidental nanoparticles that are used as pharmaceutical raw material (pharmaceutical excipient and active pharmaceutical ingredient), and for drug manufacturers in complying with multiple regulatory requirements.

EFSA (European Food Safety Authority) takes the role as risk assessor and widens the risk evaluation from nanomaterials only as defined by the relevant food regulation to material not covered by regulatory definition but keeping some parameters of nano as described above. The definition of nanomaterial in use by EFSA is not aligned with the new 2022 EC definition. To set the scene on required risk evaluation EFSA published guidance documents on the technical requirements to establish the presence of small particles (<500nm) including nanoparticles (<100nm)[23]. A second document on risk assessment of nanomaterials to be applied in the food and feed chain as also published[24].

To determine if the EFSA assessment should take into account nano-specific considerations it splits the criteria into three sections:

1. Addresses solubility and dissolution rate as key physicochemical properties to assess whether consumers will be exposed to particles.
2. Establishes the information requirements for assessing whether the conventional material contains a fraction or consists of small particles, and its characterisation.
3. Describes the information to be presented for existing safety studies to demonstrate that the fraction of small particles, including particles at the nanoscale, has been properly evaluated.

If the material in question is a nanomaterial then it will need to undergo full assessment by EFSA, it is interesting to note that in the case of titanium dioxide it does not fulfil the particle size criteria for a nanomaterial but was selected based on its perceived nanoscale properties (e.g. Specific Surface Area) which have been artificially generated using sonication in many studies and this is not an industrial process in either the food or pharmaceutical industries. Since the EFSA opinion was published in 2021 further evidence has come to light that demonstrates that the titanium dioxide samples used in the 1979 National Cancer Institute (NCI) NIH Carcinogenicity study are representative of the E171 grades used in Europe today. It could be argued that if titanium dioxide was submitted to EFSA today it would not necessarily be subject to the same nano assessment that was conducted in 2020. Reference to this NCI study, and its outcome that titanium dioxide is not carcinogenic by the oral route, is made in

[22] Drug Products, Including Biological Products, that Contain Nanomaterials. Guidance for Industry. FDA, April 2022 Pharmaceutical Quality/CMC

[23] EFSA Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles, EFSA Journal 2021;19(8):6769

[24] EFSA Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health. EFSA Journal 2021;19(8):6768

both the Health Canada and FSANZ reports and allows them to conclude that consumption of titanium dioxide (E171) as a food additive is not a concern for human health.

Guidance from EMA on the assessment of incidental nanoparticles in excipients would be welcome.

**ANNEX 2**

# Alternatives to Titanium Dioxide in Tablet Coatings

Evaluation of Titanium Dioxide-free Tablet Coatings

Report for the European Medicines Agency

08 Feb 2024

| **Written By** | **Affiliation** | **Signature** | **Date** |
|---|---|---|---|
| Louise Rosenmayr-Templeton | Consultant,<br>Tower Pharma Consulting | | 08 Feb 2024 |
| **Reviewed/Approved By** | | | |
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## List of Abbreviations

| | |
|---|---|
| °C | Degrees Celsius |
| Agg | Aggregation |
| API | Active Pharmaceutical Ingredient |
| $CaCO_3$ | Calcium Carbonate |
| Consort | Consortium |
| Diss | Dissolution |
| E of D | Ease of Dispersion |
| EC | European Commission |
| EMA | European Medicines Agency |
| EP | European Pharmacopoeia |
| FCT | Film Coated Tablet |
| g | Gram |
| HDPE | High Density Polyethylene |
| HPC | Hydroxypropylcellulose |
| HPMC | Hydroxypropylmethylcellulose (Hypromellose) |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IPC | In-Process Control |
| JP | Japanese Pharmacopoeia |
| kg | Kilogram |
| KPI | Key Performance Indicator |
| $MgCO_3$ | Magnesium Carbonate |
| MgO | Magnesium Oxide |
| m3/hr | Metres-Cubed Per Hour |
| mbar | Millibar |
| MCC | Microcrystalline Cellulose |
| mg | Milligram |
| min | Minute |
| mm | Millimetre |
| MPa | Megapascal |
| N | Newton |
| NF | National Formulary |
| PB | Pre-Blend |
| PE | Polyethylene |
| Ph.Eur | European Pharmacopoeia |
| PVA | Polyvinyl Alcohol |
| RPM | Revolutions Per Minute |
| Sec | Second |
| Sett | Settling (Sedimentation) |
| $TiO_2$ | Titanium Dioxide |
| USP | United States Pharmacopeia |
| UV | Ultra-violet |
| w/w | Weight for Weight |
| XRPD | X-Ray Powder Diffraction |

# Executive Summary

Titanium dioxide ($TiO_2$) (E171) is a ubiquitous opacifier and colorant in pharmaceuticals and is estimated to be present in at least 100,000 human medicinal products and 1600 veterinary medicinal products in the European Union. On 14 January 2022, the European Commission (EC) banned $TiO_2$ as a food additive based on safety concerns and, as a result, E171 was removed from the permitted food additives list. At present $TiO_2$ is still allowed for use in medicines per EC Regulation 2022/63. However, given the impact of a potential $TiO_2$ ban on medicine availability, and in response to a request from the EC to the pharmaceutical industry, the $TiO_2$ Alternatives Consortium was formed to conduct a comprehensive evaluation of potential alternatives to $TiO_2$ in tablet coatings and hard gelatin capsules. This report concerns the evaluation studies carried out on the $TiO_2$-free film-coatings for tablets in comparison with $TiO_2$ containing coating systems.

The Consortium worked with numerous coating material manufacturers to identify $TiO_2$-free coating materials that were either commercially available or close to commercialization. From a review of over 100 different materials, 29 were selected for initial evaluation. The selection included coatings containing a variety of available alternative opacifiers, coating polymers and suppliers. 20 of these were taken forward to comprehensive small scale (3 kg) coating studies with associated analytical testing, photostability and accelerated stability studies. These included the coating of yellow placebo tablets and active tablet cores containing APIs (nifedipine, olmesartan, rosuvastatin and prasugrel) with known sensitives to factors of relevance to the replacement of $TiO_2$ in tablet coatings with $TiO_2$ alternatives. The studies were designed to evaluate key performance indicators (KPIs) for the $TiO_2$-free coated batches against those coated with $TiO_2$ containing coating systems. The key performance indicators were as follows:

- Acceptable coat appearance and coverage at ≤ 6% weight gain
- Potential for wide color palette which enables a match with existing tablet colors
- Manufacturability
- Mechanical strength of the coat and its adherence to the tablet surfaces
- In vitro performance
- Chemical and physical stability of $TiO_2$-free coatings to light and accelerated stability conditions
- Ability of $TiO_2$-free coatings to protect susceptible actives from chemical and physical instability during storage

## Results and Conclusion

Table 1 provides an overview of the key findings from the studies. All of the 20 $TiO_2$-free coatings studied in detail were inferior to the $TiO_2$ reference coats based on the entire set of KPIs. Some performed well when assessed against certain criteria but not others. Many did not achieve surface coverage and opacification at a 6% weight gain and those, which did, required a significantly higher coating level than the $TiO_2$ reference coats. In general, the performance of the colored $TiO_2$-free coatings and the clear COAT-030 was poorer than the white $TiO_2$-free coatings.

In conclusion, none of the $TiO_2$-free coatings could match the properties of $TiO_2$. Their use will result in longer, more expensive and potentially less robust coating processes and may also impact on the stability and shelf-life of products. Color matching between marketed products and $TiO_2$-free coatings will be extremely difficult and the color palette available for product identification and anti-counterfeiting measures will be reduced due to the poor performance of the colored coatings. There is also a risk to patient adherence due to the color changes seen in some $TiO_2$-free coatings and to patient safety as a result of the limited color palette available to distinguish between different products/strengths.

Table 1: Overview of key findings

| Opacifier Group[a] | Consortium Coat Reference | Color | Key Findings of Evaluation Against the KPIs |
|---|---|---|---|
| $TiO_2$-free hypromellose (hydroxypropylmethylcellulose (HPMC)) film coatings containing $CaCO_3$ | COAT-004 | White | All of the white HPMC-based $TiO_2$-free coating containing $CaCO_3$ were inferior to the corresponding $TiO_2$ reference batch when compared against the KPIs. Overall, COAT-006 and COAT-027 performed best and COAT-004, the worst. However, the former two coating were not subjected to the same stability challenges as COAT-019 and COAT-004 as they were only used to coat placebo tablets and therefore their ability to protect actives from extreme light exposure (2 x ICH Q1B) and accelerated stability conditions was not assessed. However, both COAT-006 and COAT-027 did not change in appearance during the photostability study on the placebo tablets. |
| | COAT-006 | White | |
| | COAT-019 | White | |
| | COAT-027 | White | |
| | COAT-032 | White | |
| $TiO_2$-free polyvinyl alcohol (PVA) film coatings containing $CaCO_3$<br><br>(COAT-013 also contains HPMC) | COAT-013 | Pink | COAT-013, COAT-014 and COAT-015 performed poorly against the KPIs for manufacturability, tablet appearance and color matching to the $TiO_2$ reference. They were also less effective at protecting olmesartan from the effects of moisture. |
| | COAT-014 | Pink | |
| | COAT-015 | Pink | |
| $TiO_2$-free macrogol-PVA graft copolymer film coatings containing $CaCO_3$ | COAT-007 | White | COAT-007 performed poorly against the KPIs for manufacturability, coated tablet visual appearance at 6%weight gain and color matching with the $TiO_2$ reference batch. Coating solids sedimented during the coating process and the coat thickness was thin. |
| $TiO_2$-free film coatings containing other divalent metal opacifiers<br><br>(COAT-002 also contains rice starch)<br>(COAT-033 also contains $CaCO_3$) | COAT-001 | White | None of the $TiO_2$-free coatings were equivalent to $TiO_2$ coatings for all of the KPIs, although some like COAT-023 and COAT-019 were equivalent or almost equivalent to $TiO_2$ reference coatings for a very limited number of KPIs e.g. COAT-023 protected rosuvastatin from photodegradation. COAT-005 was difficult to prepare as a coating suspension and agglomerates resulted in a failed batch due to gun blockages. COAT-033 had poor flow properties, although a homogeneous suspension could be prepared. Based on assay and related impurity levels, COAT-005 and COAT-001 seemed better at protecting the acid-sensitive rosuvastatin at 70°C/75%RH than the $TiO_2$ reference coatings. However, both coats turned brown after 21 days under these conditions. The effectiveness of the coating with $TiO_2$-free coats may depend on the extent of the coating and opacification challenge e.g. COAT-001 was successful in coating and color matching |
| | COAT-002 | Pink | |
| | COAT-005 | White | |
| | COAT-023 | White | |
| | COAT-033 | White | |

| Opacifier Group[a] | Consortium Coat Reference | Color | Key Findings of Evaluation Against the KPIs |
|---|---|---|---|
| | | | with the corresponding $TiO_2$ reference rosuvastatin batch but did not produce enough coverage/opacification to hide the yellow color of the nifedipine cores. |
| $TiO_2$-free film coatings rice starch | COAT-010 | White | The $TiO_2$-free coatings containing rice starch were inferior to the $TiO_2$ reference coatings in terms of their ability to protect sensitive APIs and in coating the colored praugrel core tablets. However, COAT-034 was the best $TiO_2$-free coating of those tested with respect to mechanical strength as evaluated by extended friability testing. |
| | COAT-016 | Pink | |
| | COAT-020 | White | |
| | COAT-034 | White | |
| $TiO_2$-free film coatings containing other opacifiers<br><br>(COAT-031 is an colored admix designed for addition to other coatings. It was tested in combination with COAT-030) | COAT-030 | Clear | COAT-030, either alone or in combination with COAT-031, performed very poorly in the coating studies, not only in comparison to the $TiO_2$ reference coatings but also the other $TiO_2$-free coatings. It was difficult to disperse and had to be sieved prior to coating to prevent gun blockages. It was by far the worst-performing $TiO_2$-free coating in the extended friability testing, used to assess and compare the mechanical strength of the coats, and the COAT-030 coated rosuvastatin batch on accelerated stability had one of the lowest assay and highest related impurities results of all of the batches. COAT-031 is not designed for use on its own. It dispersed easily during coating suspension manufacture. |
| | COAT-031 | Red | |

[a]Other excipients in the coatings will also contribute to opacification.

# Introduction

Titanium dioxide ($TiO_2$) is a commonly used opacifier and colorant in in tablet coatings and capsules shells and is estimated to be present in at least 100,000 human medicinal products and 1600 veterinary medicinal products in the European Union [1]. Until recently it was listed under the European food additive list as E171. However, there have been recent concerns about its safety when administered orally [2].

On 14 January 2022, the European Commission (EC) banned $TiO_2$ as a food additive, with the result that Annexes II and III to Regulation (EC) No. 1333/2008 of the European Parliament and European Council were amended and E171 removed from the permitted food additives list [4].

At present $TiO_2$ is still allowed for use in medicines. However, given its presence in numerous medicines on the European market and the impact of a potential ban on medicine availability, the EC has carried out the following:

- Requested that the European Medicines Agency (EMA) conduct a re-evaluation of the impact in preparation for a Commission review by 01 April 2024,
- Stated that it is critical for the pharmaceutical industry to work towards identifying alternatives to $TiO_2$ addressing quality, safety and efficacy.

In order to carry out a thorough evaluation of $TiO_2$-free coating systems and $TiO_2$-free hard capsule shells, a consortium was formed between a number of pharmaceutical companies (listed in Appendix A). This consortium worked collaboratively with color mixture and capsule shell suppliers to identify potential $TiO_2$-free alternatives which would not impact the quality, safety and efficacy of medicinal products as outlined in the EC Regulation 2022/63.

This report concerns the experimental work and results from small scale tablet coating experiments which compared $TiO_2$-free coating systems with $TiO_2$ containing coating systems.

# $TiO_2$ – Uses and Excipient Properties

Titanium dioxide, in the high purity form used in foods and pharmaceuticals (E171), has a dual role as opacifier and colorant in tablet coatings and hard capsule shells. It is also used for similar reasons in soft gel capsules and sprinkles and also suspensions [1][4]. However, its use in other pharmaceutical products is outside of the scope of the Consortium's work.

$TiO_2$ has many useful physicochemical properties that make it an excellent opacifier and colorant for pharmaceutical products. It exists in a number of crystalline forms but only the rutile and anatase polymorphs are of commercial relevance [4][6]. $TiO_2$ has very high heat stability, both in terms of chemical stability and conversion to other crystalline forms (anatase to rutile conversion) occurs at 915°C). The anatase polymorph changes color from white to grey under high energy conditions and this has been exploited for laser printing of tablets and capsules [6][8].

$TiO_2$ has a high refractive index (2.55 for anatase and 2.72 for rutile). The anatase polymorph is used mainly in pharmaceuticals as it is less hard and abrasive and results in a more lustrous finish [4]. The ability of $TiO_2$ to scatter visible light means that it confers a vivid, opaque, white color to tablet coatings and capsule shells, and in combination with other colorants, opacifies colored capsule shells and tablet coatings. It therefore significantly broadens the range of colors which can be obtained. This makes for elegant solid dosage forms, facilitates medicine identification, while conversely hindering counterfeiting, and prevents batch-to-batch color variations which may raise patient concerns and negatively impact on patient adherence to therapy. Its ability to opacify capsule shells enables the use of over-encapsulation as a commonly used and effective method of blinding investigational medicines for clinical trials. $TiO_2$ is also both tasteless and odourless, an important property given the role of coatings and capsules to mask taste, and $TiO_2$'s presence in the outer layers of the dosage form [4].

$TiO_2$ absorbs ultra-violet light [9] and this, together with its scattering of visible light, plays an important role in protecting photo-sensitive drugs in solid dosage forms from degradation [4][6]. In addition, it is chemically inert at the temperatures and conditions used during manufacturing processes and dosage form storage. $TiO_2$ is very poorly water-soluble and non-hygroscopic. Its non-hygroscopicity means that its presence in tablet coatings and capsule shells does not impact adversely on moisture-sensitive compounds. In addition, its presence neither hydrates nor dehydrates coatings or capsule shells which can lead to cracking or softening. It also does not result in an extreme acidic or alkaline microenvironment within the coat or capsule shell which could impact on acid or alkaline instable drugs or result in physical form conversion e.g., salt to base.

Overall, from a formulation perspective, $TiO_2$'s has numerous useful functional properties when it is incorporated into tablet coatings and capsule shells [1][4][6], and has been only rarely associated with instability of active compounds [10].

From a processing perspective, the $TiO_2$ used in pharmaceuticals has a particle size of around 200 nm but forms larger aggregates which facilitate particle flow and easy processing [4]. In addition, at the $TiO_2$ concentration used in coating suspensions (10%w/w - 30% w/w), the suspensions formed with coating polymers, plasticizers and other coating ingredients are of suitable viscosity to flow, be pumped and sprayed. Similarly at the 5%w/w concentration typically found in capsules, there is no interference with capsule formation.

Any $TiO_2$-free coating or capsule shell needs to possess many of the functional excipient properties of $TiO_2$ in a comparable way. To date, very little has been published on comparing $TiO_2$-free systems with $TiO_2$ ones [6][11]. However, both $TiO_2$-free ready-to-use admixes for the preparation of coating suspensions for tablet coating and $TiO_2$-free hard capsule shells are available from a variety of vendors.

# Objectives of Evaluation and $TiO_2$ Alternatives Assessment

## Objectives

As previously stated, this report deals with the data generated by the $TiO_2$ Alternatives Consortium comparing tablet coating with $TiO_2$-free systems with $TiO_2$ containing ones. The study details and results from the comparative work on $TiO_2$-free hard capsule shells versus $TiO_2$-containing ones are recorded in a separate report [11].

The objectives of the study on tablet coatings are as follows:

- To conduct a comprehensive comparative study on $TiO_2$-free and $TiO_2$ -containing coating systems involving various types of $TiO_2$-free coating systems which were available at the start of the study,
- Evaluate and compare $TiO_2$-free versus $TiO_2$- containing coating suspensions with regard to processing. This evaluation included coating suspension viscosity and overall handling during coating,
- Evaluate and compare the quality of the coat achieved on oval and round placebo tablets using $TiO_2$-free versus $TiO_2$-containing coating systems at small scale,
- Carry out a photostability study of film-coating color on the coated placebos,
- Evaluate and compare the quality of the coat achieved on four different active cores using $TiO_2$-free versus $TiO_2$-containing coating systems at small scale. Each active core contained an active pharmaceutical ingredient (API) which is already marketed in the EU. Each of the APIs selected is known to be unstable under certain conditions of relevance to the replacement of $TiO_2$ in tablet coats e.g., light exposure,
- Conduct photostability and accelerated stability studies on the coated active tablets.

## Assessment of $TiO_2$ Alternatives - Key Performance Indicators

The experimental work described in this report was carried out at Almac Pharma Services, Craigavon, UK on behalf of the Consortium according to experimental designs developed by technical experts from

the Consortium member companies. Certain experiments were outsourced to Almac's Physical Sciences Group at Craigavon and to Reading Scientific Services Ltd., Reading, UK (RSSL).

In all of the experiments described the results obtained with the $TiO_2$-free systems were compared to one or more $TiO_2$-containing reference coatings. Table 2 shows the key performance indicators (KPI) for the evaluation of $TiO_2$-free coatings versus $TiO_2$-containing coating systems, together with the rationale for their selection:

*Table 2: Key performance indicators and rationale for their selection*

| Key Performance Indicator | Rationale |
|---|---|
| **Manufacturability** in terms of the following:<br>• How easy it is to prepare and obtain an agglomerate-free suspension. Coating suspensions must be stable with little or no sedimentation.<br>• $TiO_2$-free coating suspension viscosity must enable pumping and spray rates typical for coating operations with no increased risk of spray line or spray gun blockage. However, it should also be sufficient to keep the coat constituents suspended during the coating process.<br>• Coating operations are not compromised or made more difficult or time-consuming by using $TiO_2$-free coating materials. | Manufacturability was selected as a KPI because a more difficult, time-consuming manufacturing process would add to the cost of manufacturing medicines, making them more expensive. It could also potentially result in a less robust process, increasing the risk of batch-to-batch variability in drug product quality. In turn, this could impact on the reliability of stable supplies to the market and potentially lead to medicine shortages. |
| **Acceptable coat appearance and coverage at ≤ 6% weight gain** (as evaluated by visual appearance, colorimetry, digital optical microscopy).<br>• The appearance of the coat obtained should be as good or better than the $TiO_2$-containing reference coat(s) with respect to visual elegance and underlying tablet surface coverage and opacity at coating %weight gains of ≤ 6%.<br>• The change in coating system should not impact the quality of any debossed image. | Appearance was chosen as a key performance indicator as consistent batch-to-batch color performance is important to ensure consistent product quality and patient confidence in their medicines. The quality of the debossed image is important for patient compliance, medication identification and to tackle counterfeiting.<br><br>**Rationale for selection of ≤6%w/w coating levels**<br>A weight gain of 6 %w/w is two to three times more than that typically required for $TiO_2$ coatings (2-3% w/w). However, in order to give $TiO_2$-free coatings the best chance of success, the acceptance criteria was a comparable coating to the $TiO_2$ reference coatings at a weight gain of ≤6% w/w. This is despite the increase in processing times and costs that are incurred with higher coating weight gains. |
| **Potential for wide color palette which enables a match with existing tablet colors** (as evaluated by visual appearance and colorimetry) so that existing tablet colors can be maintained. | The ability of the $TiO_2$-free coatings to enable a wide color palette and allow color matching was considered key to their performance, as it is important to differentiate between medicines to facilitate patient compliance and medication identification. The ability to match existing tablet coat colors is important if there were to be a requirement to replace $TiO_2$ coats in pre-existing products or when blinding products for clinical trial purposes. |
| **Mechanical strength of the coat and its adherence to the tablet surface (**as assessed by extended friability studies). | This performance indicator was chosen as poor mechanical strength could lead to issues with coat adhesion to the tablet core creating coating and tablet core defects in downstream processes, such |

| | |
|---|---|
| The mechanical strength of the coat should not be compromised by a change from $TiO_2$-containing coating systems to $TiO_2$-free ones. | as packaging and transportation/shipment, which would impact product quality and could result in patient complaints. Dealing with the issues caused by poor mechanical coat strength would increase production costs as processes, such tablet sorting to remove defective tablets, are time-consuming. |
| **In vitro performance** (as assessed by disintegration, dissolution) | Dissolution is a critical quality attribute for solid dosage forms. The use of $TiO_2$-free coats should not compromise tablet disintegration and/or release of active compounds from the tablets. |
| **Photostability of the coat** (as assessed by visual appearance, colorimetry, coat thickness).<br><br>The appearance of the $TiO_2$-free coats should be as stable or more stable than the $TiO_2$-containing reference coats to conditions of extreme light exposure (2 x ICH Q1B requirements). | Photostability is a KPI because color fading/change on exposure to UV light could result in product not meeting its appearance specification which is typically a drug product critical quality attribute.<br><br>Light exposure could also potentially cause degradation or changes in the properties of the film coating, which can in turn affect the thickness of the coating. |
| **Ability of $TiO_2$-free coatings to protect light-sensitive actives against photodegradation** (as assessed by assay, related impurities, disintegration and dissolution on samples exposed to the equivalent of 2 x ICH Q1B conditions).<br><br>$TiO_2$-free coatings should provide equivalent or greater light exposure protection to photosensitive actives than $TiO_2$-containing systems. | $TiO_2$ has the ability to block ultra-violet (UV) light, thus, $TiO_2$ coatings can provide protection to light-sensitive actives and excipients. The loss of this protection through replacement of a $TiO_2$ containing coating with a $TiO_2$-free one could result in a loss of light-protection with consequences for product stability. Therefore, it is important that $TiO_2$ coatings provide equivalent or greater protection against photodegradation as $TiO_2$ containing ones. |
| **Chemical and physical stability of $TiO_2$-free coatings** (as assessed by tablet visual appearance, colorimetry and coat thickness on samples stored under accelerated stability conditions versus $T_0$ results).<br><br>The stability of $TiO_2$-free coats during accelerated studies should be equivalent or greater than $TiO_2$-containing ones. | The chemical and physical properties of the coat should not change on storage as this would result in the medicine failing its appearance specification, potentially reduced protection for light sensitive APIs, product recalls and, most importantly, a reduction in patient faith in their medicine. |
| **Ability of $TiO_2$-free coatings to protect susceptible APIs from chemical and physical instability during storage** (as measured by assay, related impurities, disintegration and dissolution on samples stored under accelerated stability conditions versus $T_0$ results).<br><br>The ability of $TiO_2$-free coats to protect susceptible APIs from degradation in accelerated stability studies should be equivalent or greater than $TiO_2$-containing ones. In addition, the properties of the $TiO_2$ alternative should not promote API or excipient instability. | $TiO_2$ is non-hygroscopic, chemically inert and its presence does not result in a strongly acidic or alkaline microenvironment. Therefore, its inclusion in coatings facilitates the protection of moisture-sensitive compounds and does not promote degradation of actives. Any $TiO_2$-free coating must also provide similar protection and not promote degradation. |

# Experimental Part 1: Coating Suspension Characterization

## Selection of Coating Materials for Evaluation

In total 34 coating materials were selected for initial evaluation, 29 of which were $TiO_2$-free and 5 contained $TiO_2$. The selection process involved outreach to all known suppliers of film coating materials. The coating colors chosen for assessment were white and pink. The Consortium then carried out a screen of > 100 $TiO_2$-free film coating systems and the ultimate selection of the 29 was based on a number of criteria including the following:

- Coating material constituents are either compendial or supported by an adequate safety package,
- Coating material constituents are suitable for pediatric formulations for children of 2 years and above,
- Samples of coating materials were available for Consortium evaluation at the start of the project,
- Coating materials are available for white or pink coatings or both,
- The composition of the coating materials was disclosed so that coatings could be chosen to enable the evaluation of coatings containing a variety of substitutes for $TiO_2$ in combination with different polymer and plasticizer systems. They included coating materials based on hypromellose (described hereafter as hydroxypropylmethylcellulose (HPMC)), polyvinyl alcohol (PVA) or macrogol-PVA graft copolymer (described hereafter as polyethylene glycol (PEG)- PVA graft co-polymer),
- Coating materials were chosen from a variety of vendors.

The $TiO_2$-free coating materials selected best represented the range of available opacifiers, coating polymers and suppliers. They, together with the $TiO_2$ reference coating materials used for comparison, are shown in Table 3 overleaf. The coating materials constituents for all of the coating materials either met pharmacopoeial standards (Ph.Eur. and/or USP/USP-NF) or are food grade.

## Anonymization of $TiO_2$ and $TiO_2$-free Coating Material Details

For confidentiality purposes the trade name and description, the vendor and full details of the composition of the coating materials are not disclosed. Each $TiO_2$-free and $TiO_2$ containing coating material studied was given a Consortium Coat Reference (COAT-001 to COAT-034). Some of the alternative opacifiers have been disclosed, while others have been given an identifier letter. Iron red oxide ($Fe_2O_3$), where present, has been listed as an opacifier. It is not an opacifier per se but contributes to opacification through its colorant properties.

Table 3: List of coating materials selected for evaluation

| Consortium Coat Reference | $TiO_2$-Free (Yes/No) | Color | Film Former A | Film Former B | Opacifier(s)[e] | Target[b] %Solids |
|---|---|---|---|---|---|---|
| COAT-001 | Yes | White | Hypromellose (HPMC)[d] | HPC[d] | Magnesium carbonate ($MgCO_3$) + A + B | 16 (15-17) |
| COAT-002 | Yes | Pink | HPMC | NA | Rice starch + A+B+D + ($Fe_2O_3$) | 16 (15-17) |
| COAT-003 | Yes | Clear | Polyvinyl Alcohol (PVA) | NA | Talc | 20 |
| COAT-004 | Yes | White | HPMC | NA | Calcium carbonate ($CaCO_3$) + C | 11 |
| COAT-005 | Yes | White | HPMC | NA | Magnesium oxide (MgO) | 11 |
| COAT-006 | Yes | White | HPMC | NA | $CaCO_3$ + D | 20 |
| COAT-007 | Yes | White | PEG- PVA graft copolymer[d] | PVA | $CaCO_3$ + Talc | 30 |
| COAT-008 | Yes | White | PVA | NA | $CaCO_3$ + Talc | 20 |
| COAT-009 | Yes | White | PVA | HPMC | $CaCO_3$ + Talc | 20 |
| COAT-010 | Yes | White | HPMC | NA | Rice starch + D | 20 |
| COAT-011 | Yes | Pink | HPMC | NA | $CaCO_3$ + D + $Fe_2O_3$ | 20 |
| COAT-012 | Yes | Pink | PEG- PVA graft copolymer | PVA | $CaCO_3$ + Talc + $Fe_2O_3$ | 30 |
| COAT-013 | Yes | Pink | PVA | HPMC | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-014 | Yes | Pink | PVA | NA | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-015 | Yes | Pink | PVA | NA | $CaCO_3$ + Talc + $Fe_2O_3$ | 20 |
| COAT-016 | Yes | Pink | HPMC | NA | Rice starch +D + $Fe_2O_3$ | 20 |
| COAT-017[a] | No | White | HPMC | NA | $TiO_2$ | 15 |
| COAT-018[a] | No | White | PVA | NA | $TiO_2$ + Talc | 25 |
| COAT-019 | Yes | White | HPMC | NA | $CaCO_3$ + D + E | 17 |
| COAT-020 | Yes | White | HPMC | HPC | Rice starch + D | 15 |
| COAT-021 | Yes | Pink | HPMC | HPC | $CaCO_3$ + D + $Fe_2O_3$ | 15 |
| COAT-022 | Yes | Pink | HPMC | HPC | Rice starch + D + $Fe_2O_3$ | 15 |
| COAT-023 | Yes | White | PVA | NA | F+ Talc | 18.5 (17-20) |
| COAT-024[a] | No | White | HPMC | NA | $TiO_2$ | 15 |
| COAT-025[a] | No | Pink | PVA | NA | $TiO_2$ + Talc + $Fe_2O_3$ | 18.5 (17-20) |
| COAT-026[a] | No | Pink | HPMC | NA | $TiO_2$ + $Fe_2O_3$ | 15 |
| COAT-027 | Yes | White | HPMC | NA | $CaCO_3$ + D | 16.5 (15-18) |
| COAT-028 | Yes | Pink | HPMC | NA | $CaCO_3$ + D + $Fe_2O_3$ + | 16.5 |

| Consortium Coat Reference | $TiO_2$-Free (Yes/No) | Color | Film Former A | Film Former B | Opacifier(s)[e] | Target[b] %Solids |
|---|---|---|---|---|---|---|
| | | | | | FD&C Red #40 | (15-18) |
| COAT-029 | Yes | White | HPMC | NA | B + G | 12 |
| COAT-030 | Yes | Clear | HPMC | NA | B + E | 12 |
| COAT-031[c] | Yes | Red | HPMC | NA | B + $Fe_2O_3$ | 12 |
| COAT-032 | Yes | White | HPMC | NA | $CaCO_3$ + H | 17.5 |
| COAT-033 | Yes | White | HPMC | NA | $CaCO_3$ + D + F | 18 |
| COAT-034 | Yes | White | HPMC | NA | Rice starch | 18 |

[a]$TiO_2$ reference coating materials [b]Target or range %solids based on the manufacturers' recommendations.

[c]COAT-031 is a ready-to-use solid coloring agent preparation for addition to other film-coating admixes e.g., COAT-030.

[d]Hypromellose is described as hydroxypropylmethylcellulose (HPMC) hereafter in this report and macrogol-PVA graft copolymer as polyethylene glycol (PEG)-PVA graft copolymer. HPC = hydroxypropylcellulose

[e]$Fe_2O_3$ is not an opacifier per se but contributes to opacification through its colorant properties.

## Grouping of Coating Materials for Analysis Purposes

With respect to analysis of the various studies described in this report, the coatings are often grouped in tables and graphs based on whether they are based on HPMC, PVA and/or PEG- PVA graft copolymer. In some studies, the results have been grouped based on the opacifier type such as those containing $CaCO_3$, other divalent metal opacifiers or rice starch or miscellaneous opacifiers. In Sections 0, 0 and 0 the coated tablets containing active pharmaceutical ingredients (API) are grouped for analysis on the basis of the core tablet used.

## Characterization of Coating Suspensions

### 2. Methodology

The coating suspensions were prepared from the materials listed in Table 3 at the target % solids concentration recommended by the manufacturer or the mid-point of the recommended concentration range. COAT-029 was not prepared. It had been originally planned to use COAT-029 in combination with the COAT-030 clear coating. However, the Consortium later decided to trial the COAT-030 only as both these systems are standalone coating systems and are not designed for combination as a single suspension.

The suspensions were prepared as per each manufacturer's instructions.

For each prepared suspension, the following were recorded:

- Ease of dispersion
- Agglomeration
- Presence of foam on dispersion, after mixing for the recommended preparation time and prior to spraying
- Evidence of sedimentation in the coating suspension after the coating process was complete
- Suspension appearance
- Suspension pH

Some of the suspensions were prepared in advance of coating the small scale (3 kg) tablet batches described in Section 0 and Section 0 (some twice as required for two separate batches). Others were prepared at the 500 g scale to evaluate the preparation and suspension properties alone. For the coating suspensions prepared to evaluate suspension properties only, the suspensions were screened through a 500 µm screen to test for the presence of agglomerates. The acceptance criteria are shown in Table 4.

Table 4: Acceptance criteria for coating suspension preparation

| Criteria | Evaluation | | |
|---|---|---|---|
| | **Easy to prepare & use** | **Acceptable** | **Not Satisfactory** |
| Ease of Dispersion (E of D) | Easy = Immediate/Readily (within approx. 1-2 min) | Fairly easy to disperse = (within approx. 5 min) | Difficult to disperse |
| Agglomeration (Agg) | None or minimal which dispersed within few minutes | Agglomerates still present after 10 min | Coating suspension had to be sieved &/or spray gun blocking &/or agglomerates observed after coating |
| Foaming (Foam) | None or Low = minimal or thin layer | High level of foam but did not interfere with coating process | High level of foam which interferes with coating process |
| Settling (Sett) (Sedimentation) | None | Minimal | Significant |
| Appearance at end of suspension preparation | Report color | Report color | Report color |
| | Homogeneous | Minor inhomogeneity but easily redispersed | Non-homogeneous |
| pH[a] | Report results | Report results | Report results |
| Overall evaluation | No or minor issues observed during preparation | Satisfactory but some difficulties experienced in preparation | Many difficulties experienced in suspension preparation and use |

[a]Impact of coating suspension pH on compound and coat stability was assessed during the stability studies.

## 3. Results and Discussion

The observations and measurements made during the preparation of the various coating suspensions are shown in Table 5, Table 6, Table 7 and Table 8. For this analysis, the suspensions were grouped into those which contain contained $TiO_2$, those which contain $CaCO_3$, those which contain divalent metal opacifiers other than $CaCO_3$ and those which contained rice starch or other types of opacifier.

Most of the coating materials resulted in coating suspensions that were easy to disperse and use with no agglomerates or excessive foam or settling (sedimentation) at the end of the mixing process. However, COAT-030 did not disperse well and coating both with it alone, or in combination with COAT-031, led to gun blockages. COAT-007 and COAT-005 also resulted in unsatisfactory results. There was solid material remaining in the suspension container after the end of mixing for COAT-007. This suggested non uniform dispersion or sedimentation. COAT-005 was difficult to disperse, and resulted in many agglomerates and coating spray gun blockages. Spray gun blockages can cause issues in product appearance quality as spraying can become uneven or stop completely and the reduction in atomisation of the coating suspension can result in over-wetting of the tablet bed leading to issues such as coat sticking and tablet twinning.

COAT-030 and COAT-005 are HPMC-based and of low solids content, 12% and 11 %, respectively. COAT-005 contains magnesium oxide as the opacifier, while COAT-030 is described by its manufacturer as a clear coating, although its constituents will impart some opacity to the coating.

COAT-007 has a high solids content (30%) and is PEG-PVA graft copolymer based. It contains calcium carbonate plus talc as the opacifiers. A ring of solid material was found in the coating suspension vessel after coating completion. Poor dispersion and/or sedimentation was not observed for COAT-012, which has an almost identical qualitative composition except for the addition of iron oxide colorants and was provided by the same supplier. It may be that the presence of the iron ions facilitates suspension dispersion through absorption to coating suspension particle surfaces resulting in electrostatic repulsion.

COAT-033, which contains $CaCO_3$ and Opacifiers D + F, resulted in a satisfactory homogeneous suspension. However, the coating material contained clumps and its poor flow properties are likely to make it difficult to use at a larger scale.

Suspension pH

The pH of the $TiO_2$- containing suspensions ranged from 6.9 to 7.7. The pH of the $TiO_2$- free suspensions containing $CaCO_3$ and/or other metal opacifiers ranged from 7.6 to 11.0, with the majority having a pH of 7.6 to 9. Only two, those produced from COAT-001 and COAT-005 had a pH above this. COAT-001 contains $MgCO_3$, while COAT-005 contains MgO and the pH of these suspensions reflect the higher pH resulting from the dissolution of the two magnesium compounds. The pH of suspensions containing rice starch ranged from 3.4 to 7.9, with only one suspension (that produced using COAT-034) being outside the range of pH 6-8. Its pH is due a weak acid constituent in COAT-034.

The acidic or alkaline nature of excipients can impact on drug solubility and/or stability and also on the stability and/or function of other excipients. In Sections 0 and 0 of this report the results of photostability and accelerated stability studies are reported.

## 4. Conclusion

The following $TiO_2$-free coating systems proved difficult to prepare due to issues such as poor dispersion, formation of agglomerates and/or sedimentation:

- COAT-005 (HPMC, MgO)
- COAT-007 (PEG-PVA graft copolymer, $CaCO_3$+ talc)
- COAT-030 (HPMC, a clear coat containing excipients, B+E which contribute to its opacification)

COAT-033, which contains $CaCO_3$ and Opacifiers D + F, resulted in a satisfactory homogeneous suspension. However, clumping and its poor flow properties are likely to reduce its manufacturability at a larger scale.

Suspensions from the other $TiO_2$ free coating systems and the $TiO_2$ containing reference materials could be prepared without major difficulties.

Table 5: Observations and pH measurements during preparation of $TiO_2$-containing coating suspensions

| Consortium Coat Ref | Film Former | %Solids | E of D | Agg | Foam | Appearance End of Prep | Sett. | pH[a] | Comments/Overall Assessment |
|---|---|---|---|---|---|---|---|---|---|
| COAT-017 | HPMC | 15% | Easy | None | Low | White Homogeneous | None | 7.3 | Used for Coating Run 1 & 21 (Placebo & Rosuvastatin). No issues observed during suspension preparation. Variable spray rates and a potential gun blockage on Run 1 led to a higher tablet bed temperature than planned during spraying. For Run 21 a 1.0 mm spray gun was used instead of the 0.8 mm gun for Run 1 and the preparation before spraying and stirring time for suspension manufacture was extended. |
| COAT-018 | PVA | 25% | Easy | None | Low | White Homogeneous | None | 7.1 | Used for Coating Run 6 and 20 (Placebo & Rosuvastatin) No issues observed during suspension preparation. |
| COAT-024 | HPMC | 15% | Easy | None | None | Very white Homogeneous | None | 6.9 | Used for Coating Run 11 (Nifedipine) No issues observed during suspension preparation. |
| COAT-025 | PVA | 18.5% | Easy | None | Low | Pink Homogeneous | None | 7.7 | Used for Coating Run 16 (Olmesartan) No issues observed during suspension preparation. |
| COAT-026 | HPMC | 15% | Easy | None | High | Pink Homogeneous | None | 7.3 | Used for Coating Run 30 (Prasugrel). High foam levels noted at end of process but did not interfere with coating. |

[a]If coating prepared for two runs, pH is the average of two runs.

Table 6: Observations and pH measurements during preparation of $TiO_2$-free coating suspensions containing $CaCO_3$

| Consortium Coat Ref. | Film Former | %Solids | E of D | Agg | Foam | Appearance | Sett. | pH[a] | Comments/Overall Assessment |
|---|---|---|---|---|---|---|---|---|---|
| COAT-004 | HPMC | 11% | Easy | Dispersed overnight | Low | Creamy white Homogeneous | None | 7.9 | Used for (Nifedipine) Coating Run 14<br>Agglomerates dispersed overnight<br>Creamy white on preparation, white on use. |
| COAT-006 | HPMC | 20% | Easy | None | Low | White Homogeneous | None | 7.9 | Used for Coating Run 4 and 9 (Placebo).<br>No issues observed during suspension preparation. |
| COAT-007 | PEG-PVA copolymer+ PVA | 30% | Easy | None | Low | White Homogeneous | Solids present | 8.6 | Used for Coating Run 5 and 10 (Placebo). Appeared homogeneous on preparation. Ring of solid around bottom of container after coating. |
| COAT-008 | PVA | 20% | Easy | None | Low | Bright, white homogeneous | None | 9.0 | Suspension prepared only – no coating |
| COAT-009 | PVA HPMC | 20% | Easy | None | None | Bright white Homogeneous | None | 8.1 | Suspension prepared only – no coating<br>No issues observed during suspension preparation. |
| COAT-011 | HPMC | 20% | Easy | None | None | Dusky pink Homogeneous | None | 8.4 | Suspension prepared only – no coating<br>No issues observed during suspension preparation. |
| COAT-012 | PEG-PVA copolymer PVA | 30% | Easy | None | None | Dusky pink Homogeneous | None | 8.6 | Suspension prepared only – no coating<br>No issues observed during suspension preparation |
| .COAT-013 | PVA+ HPMC | 20% | Easy | None | Low | Pink Homogeneous | None | 8.1 | Used for Coating Run 17 (Olmesartan)<br>No issues observed during suspension preparation. |
| COAT-014 | PVA | 20% | Easy | None | High | Pink Homogeneous | None | 8.1 | Used for Coating Run 19 (Olmesartan) Substantial foam at start of spraying but did not interfere with coating. |
| COAT-015 | PVA | 20% | Easy | Minimal | Low | Pink, Initial color variation on surface | None | 8.7 | Used for Coating Run 18 (Olmesartan)<br>Slight color variation on coating surface - cleared with mixing. |
| COAT-019 | HPMC | 17% | Easy | None | High | White Homogeneous | None | 7.6 | Used for Coating Run 23 (Rosuvastatin). Substantial layer foam after mixing and before spraying but did not interfere with coating process. |
| COAT-021 | HPMC HPC | 15% | Fairly easy | Present initially | None | Pink Homogeneous | None | 8.6 | Suspension prepared only – no coating<br>~5 min for dispersion - agglomerates only at start. |
| COAT-027 | HPMC | 16.5% | Easy | None | Low | White Homogeneous | None | 8.5 | Used for Coating Run 3 and 8 (Placebo). No issues during suspension preparation. Spray rates variable on Run 3 but more consistent on Run 8. |
| COAT-028 | HPMC | 16.5% | Easy | None | Low | Bright pink Homogeneous | None | 8.2 | Suspension prepared only – no coating<br>Powder built up due to rapid addition but dispersed |

| Consortium Coat Ref. | Film Former | %Solids | E of D | Agg | Foam | Appearance | Sett. | pH[a] | Comments/Overall Assessment |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | within 1 min with increased stirrer speed. |
| COAT-032 | HPMC | 17.5% | Some issues Run 2 | None | None | White Homogenous | None | 8.2 | Used for Coating Runs 2 & 7 (Placebo)<br>Some dispersion issues observed with Run 2. Required overnight stirring. No issues with Run 7. |

[a] If coating prepared for two runs, pH is the average of two runs.

Table 7: Observations and pH measurements during preparation of $TiO_2$-free coating suspensions containing divalent metal opacifiers other than $CaCO_3$

| Consortium Coat Ref. | Film Former | %Solids | E of D | Agg | Foam | Appearance | Sett. | pH[a] | Comments/Overall Assessment |
|---|---|---|---|---|---|---|---|---|---|
| COAT-001 | HPMC+ HPC | 16% | Easy | Present | None | White Homogeneeus | None | 10.1 | Used for Coating Run 12 (Nifedipine) & Run 27 (Rosuvastatin)<br>Run 27 – A few agglomerates present initially but cleared rapidly. |
| COAT-002[b] | HPMC | 16% | Easy | None | Low | Bright pink Homogeneous | None | 8.0 | Used for Coating Run 32 (Prasugrel) |
| COAT-005 | HPMC | 11% | Difficult | Difficult to disperse | Low | Dark cream/beige suspension Not Homogeneous | None | 11.0 | Used for Coating Run 26 & 26 repeat (Rosuvastatin)<br>Prepared twice due to gun blockages. Material contained lumps and did not disperse readily. Had to be screened after further gun blockages. Many agglomerates on 1000 & then 500 µm screen<br>Colour – not as white as expected. |
| COAT-023 | PVA | 18.5% | Easy | None | High for Run 15 | White Homogeneous | None | 7.6 | Used for Coating Run 15 (Nifedipine) & Run 29 (Rosuvastatin)<br>Run 15 - Substantial foam prior to spraying but did not interfere with coating.<br>Run 29 - Minimal foam throughout |
| COAT-033[c] | HPMC | 18% | Difficult | Initially present None at 60 min | Low | White Homogeneous | None | 8.6 | Used for Coating Run 13 (Nifedipine)<br>Material contained lumps and had poor flow.<br>Agglomerates dispersed after 1 hour. |

[a]If coating prepared for two runs, pH is the average of two runs.
[b]Contains rice starch but included in this table as also contains a divalent metal opacifier.
[c]Also contains $CaCO_3$ but included in this table as it contains a divalent metal opacifier.

Table 8: Observations and pH measurements during preparation of $TiO_2$-free coating suspensions containing rice starch and/or miscellaneous opacifiers

| Consortium Coat Ref | Film Former | %Solids | E of D | Agg | Foam | Appearance | Sett. | pH[a] | Comments/Overall Assessment |
|---|---|---|---|---|---|---|---|---|---|
| COAT-010 | HPMC | 20% | Easy | None | None | Creamy white | None | 7.1 | Used for Coating Run 24 (Rosuvastatin)<br>Added very gradually due to loss of vortex on addition. However, dispersed easily. |
| COAT-016 | HPMC | 20% | Easy | Present initially | Low | Dark pink/brown | None | 7.9 | Used for Coating Run 31 (Prasugrel) Some agglomerates at start but dispersed rapidly. |
| COAT-020 | HPMC+HPC | 15% | Difficult | Many | Low | White Homogeneous | None | 7.3 | Used for Coating Run 22 (Rosuvastatin)<br>Difficult to disperse and prolonged mixing required for approx. 120 min to disperse agglonerates. However, homogeneous suspension achieved. |
| COAT-022 | HPMC HPC | 15% | Easy | None | None | Orange/pink Homogeneous | None | 6.4 | Suspension prepared only – no coating.<br>No issues observed during suspension preparation. |
| COAT-034 | HPMC | 18% | Easy | Present initially | None | Creamy white Homogeneous | None | 3.4 | Used for Coating Run 28 (Rosuvastatin)<br>Few agglomerates on first addition. |
| COAT-030/031 | HPMC | 12% | Difficult | Sieving required | Low | Pink Sieving required for homogeneity | None | 4.9 | Used for Coating Run 31 (Prasugrel)<br>COAT-030 did not disperse easily. Multiple agglomerates present after slow addition. COAT-O31 dispersed easily. Suspension screened through 500 µm screen after overnight mixing. Solid granules retained on screen - no powdery agglomerates. |
| COAT-003[b] | PVA | 20% | Easy | None | Low | White Homogeneous | None | 8.0 | Suspension prepared only – no coating.<br>No issues observed during suspension preparation. |
| COAT-030[b] | HPMC | 12% | Difficult | Sieving required | Low | Creamy white Not homogeneous | Surface color difference | 4.7 | Used for Coating Run 25 & 25 repeat (Rosuvastatin)<br>Colour differences on suspension surface observed during decanting. Second preparation required due to gun blockages. After 2 hr mixing suspension screened - large agglomerates (wetted powder) retained on 500 µm screen. |

[a]If coating prepared for two runs, pH is the average of two runs.

*[b]Coating suspensions described as being clear coatings by the suppliers. However, certain excipients contribute to a degree of opacification.*

## Viscosity Measurements

### 5. Methodology

The coating material suspensions were prepared according to the manufacturers' instructions and were stirred for at least 60 min, prior to viscosity analysis. The measurements were carried out on suspensions at the target or mid-range concentration recommended by the manufacturer. Analysis was carried out using a TA DHR-1 rheometer equipped with a 40 mm/4° cone geometry. Each dispersion was analyzed in duplicate under the following conditions:

- Temperature – 25°C
- Shear rate 10.0 1/s to 1000.0 1/s
- 4 points per decade
- Equilibration time of 5.0s
- Averaging time – 10.0s

### 6. Viscosity Results and Discussion

The viscosity of coating suspensions is important to maintain the coating components in uniform suspension during coating process. However, it must also be low enough for the suspension to be pumped and atomized. Viscosity measurements on the coating suspensions are shown in Figure 1, Figure 2 and Figure 3.

Figure 1 shows the average viscosity of the $TiO_2$ containing coating suspensions versus shear rate.

Figure 1: $TiO_2$ containing coating suspensions - average viscosity versus shear rate

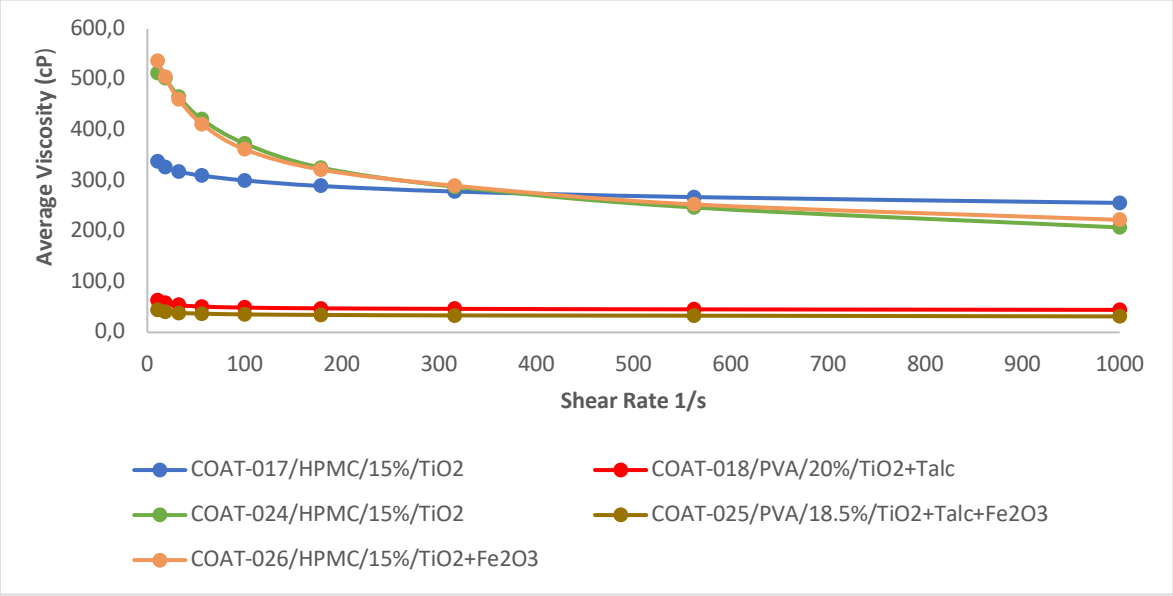
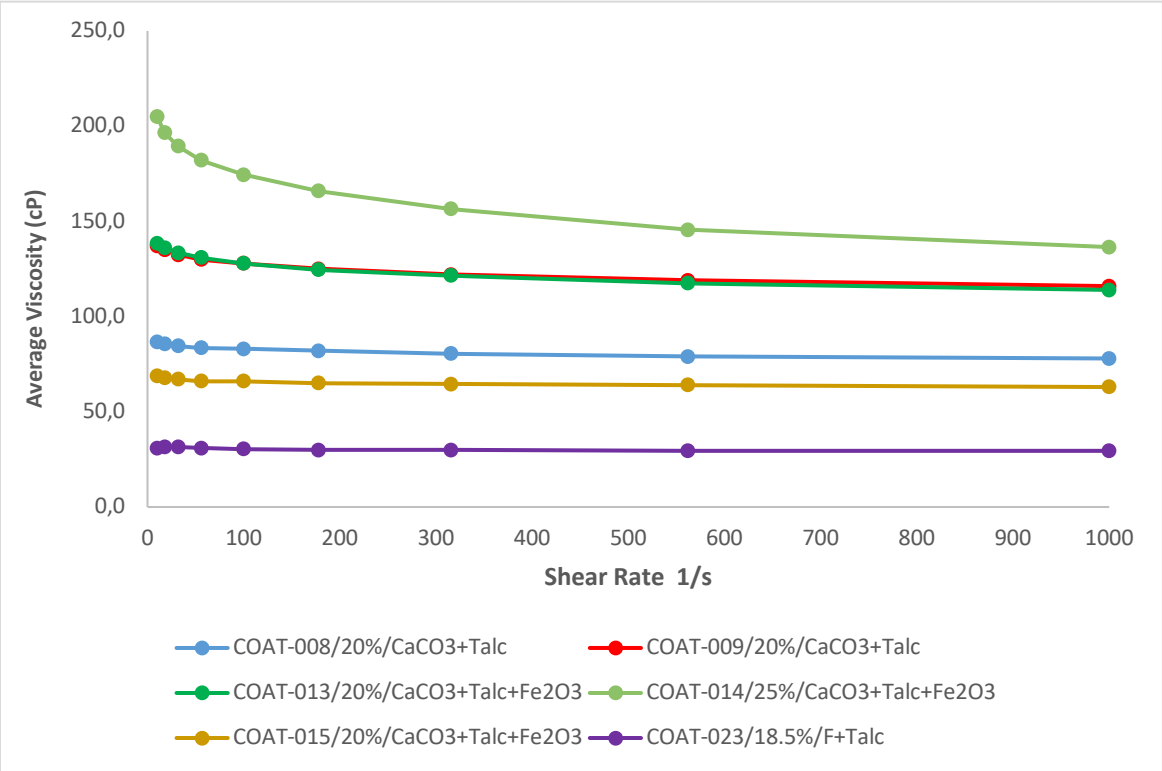


Figure 2 shows the average viscosity of the PVA-based $TiO_2$-free coating suspensions versus shear rate, while Figure 3 shows the data for a selection of the $TiO_2$-free suspensions based on HPMC.

Figure 2: PVA based $TiO_2$-free coating suspensions - average viscosity versus shear rate

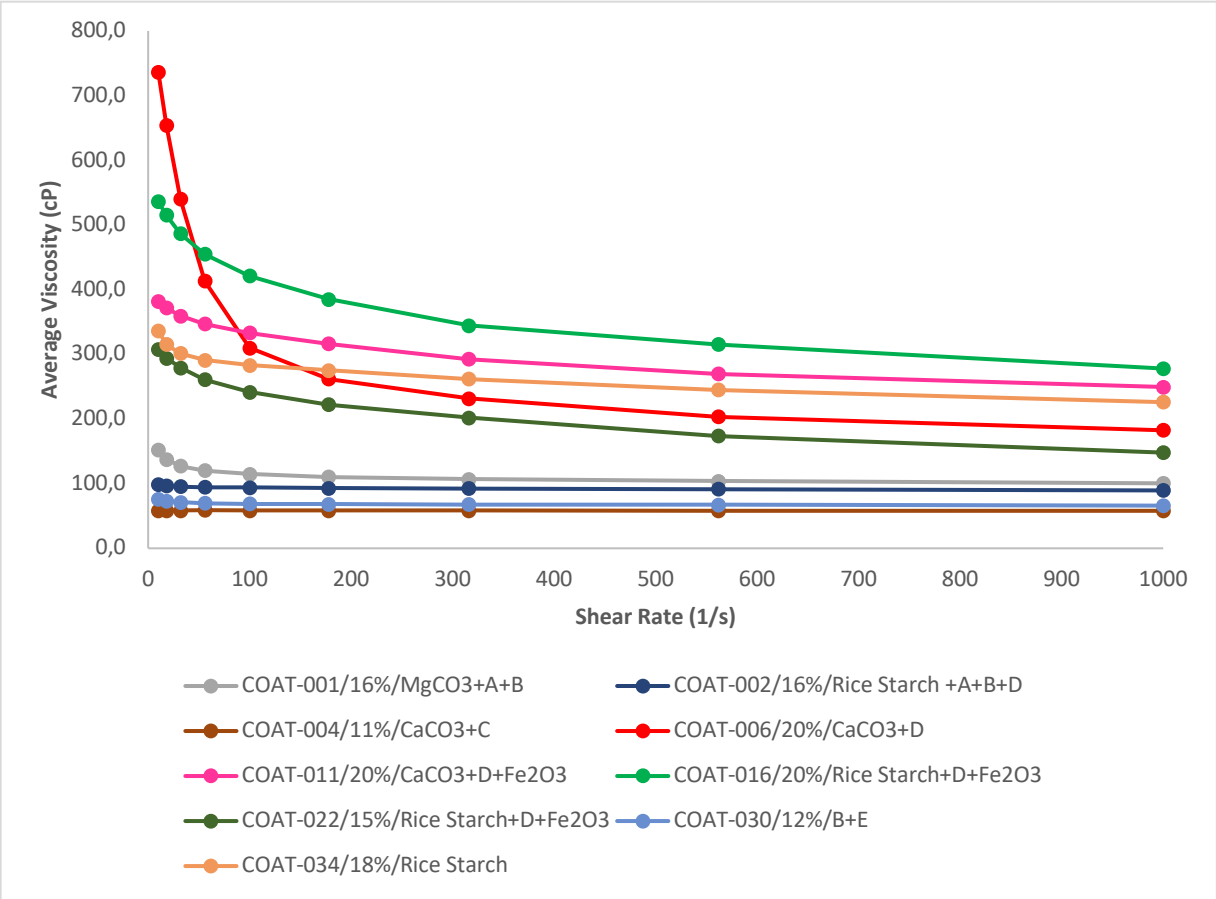



In general, the PVA-based $TiO_2$-free coating suspensions had relatively low viscosities and a low degree of shear thinning. However, with the exception of COAT-023, they all had higher viscosities than the PVA-based $TiO_2$ reference coatings shown in Figure 1. COAT-014 had the highest viscosity, followed by COAT-009 and COAT-013, which additionally contain HPMC. There was a slight trend with regard to increasing viscosity and % solids content as COAT-014, with the highest % solids, had the highest viscosity and COAT-023, with the lowest %solids, had the lowest viscosity. However, the composition of the coatings had a greater influence with COAT-013 and COAT-015 having different viscosities despite both containing 20% solids.

In general, HPMC-based suspensions had higher viscosity than PVA-based ones and showed a greater extent of shear thinning. Viscosity differences between coating suspensions manufactured from HPMC are likely to be due to differences in the %polymer and HPMC grade(s) employed in the preparation of the coating material admix, although % solids may also play a role. At very low shear rates, COAT-006 had higher viscosity than any of the HPMC-based $TiO_2$ reference coatings. However, at higher shear rates its viscosity was in a similar range to the corresponding $TiO_2$ reference coats.

The type of opacifier present did not make any significant difference to suspension viscosity, The PVA-based $TiO_2$-free coats contained mainly $CaCO_3$ and talc as opacifiers but had different viscosities. Similarly, $CaCO_3$ and rice starch are used as opacifiers in both higher and lower viscosity HPMC-based $TiO_2$-free coatings.

Figure 3: HPMC-based $TiO_2$-free coating suspensions - average viscosity vs shear rate




The two $TiO_2$-free coating suspensions based on PEG-PVA graft copolymer, COAT-007 and COAT-012, showed minimal shear-thinning. Their viscosity was low and was 51.5 cP and 54.5 cP at the lowest shear rate and 49 cP and 50 cP at the highest shear rate, respectively. This was despite their having a solids content of 30%, higher than any of the other coating suspensions.

## 7. Conclusion

Coating suspension viscosity was influenced mainly by the film-forming polymer used. HPMC-based coating suspensions typically had higher viscosity than ones containing PVA or PEG-PVA graft polymer.

# Experimental Part 2: Manufacture of Placebo Tablets

## Introduction

The coating suspensions were initially evaluated for their ability to coat colored placebo tablets. Both round (9 mm diameter) and oval tablets (15 x 7 mm) were manufactured from a placebo blend containing yellow iron oxide. Iron oxide was selected to provide the core tablets with an intense yellow color which would facilitate evaluation of the different coatings (see Section 0) and their ability to completely hide it. The manufacture of oval and round tablets allowed the assessment of coating the two most commonly used tablet shapes. Both sets of tablet tooling were embossed to assess the ability of the different coatings to coat debossed tablets. The tooling drawing are shown In Appendix B (Section 0).

## Materials and Formulation

The materials used in the manufacture of the placebo tablets for the coating evaluation are listed in Table 9, together with the composition of the blend and the round and oval tablets. The batch size was 300 kg.

Table 9: Materials used and composition of placebo blend and tablets

| Material | Function | Batch formula (%w/w) | Quantity per 300 kg batch (kg) | Round Tab (mg) | Oval Tab (mg) |
|---|---|---|---|---|---|
| Lactose Monohydrate Spray Dried NF, EP, JP (Spray Dry Fast Flo 316) | Soluble diluent | 61.7 | 185.1 | 185.1 | 308.5 |
| Microcrystalline Cellulose (MCC) EP/USP-NF (Avicel PH102) | Diluent/Binder | 34.5 | 103.5 | 103.5 | 172.5 |
| Croscarmellose Sodium NF,Ph.Eur., JP (AcD-DiSol SD-711) | Disintegrant | 2.8 | 8.4 | 8.4 | 14.0 |
| Magnesium Stearate Ph.Eur/USP-NF/JP (LIGAMED MF-2-V-MB) | Lubricant | 0.5 | 1.5 | 1.5 | 2.5 |
| Yellow Iron oxide (Sicovit Yellow 10 E172, Yellow Iron Oxide 17017) | Colourant | 0.5 | 1.5 | 1.5 | 2.5 |
| **Total:** | | **100.0** | **300.0** | **300.0** | **500.0** |

## Manufacture

### 8. Manufacture of Blend

In order to ensure adequate dispersion to the iron oxide within the blend, a geometric mixing strategy was used. This involved first mixing the colorant with aliquots of microcrystalline cellulose (MCC) in a series of steps to form a pre-blend (PB) so that at each stage the iron oxide was increasingly diluted.

### 9. Manufacture of Placebo Tablets

A portion of the placebo blend was compressed on Kilian S250 tablet press fitted with 9 mm round, concave Euro-D embossed tooling. The round tablets had a target weight of 300 mg and a target tensile strength of 2MPa. The remainder was compressed using the same equipment but fitted with the oval

shaped concave Euro-D embossed 15 x 7mm tooling. The oval tablets had a target weight of 500 mg and a target tensile strength of 2MPa. The tablets were passed through a deduster following compression.

Prior to the start of each compression run, a five-point compression profile was carried out to determine the limits for thickness and hardness. Following profiling, the tablet press was set up to achieve tablets of a target tensile strength of 2.0 MPa. In-process controls (IPCs) were performed at the start of compression, at 20 min intervals and at the end of compression. The tablets were stored in double PE bags within HPDE drums.

## Results

The IPC results from Batch ENQ3822/PIRT/001/01 (round tablets) and Batch ENQ3822/PIRT/002/01 (oval tablets) are shown in Table 10.

Table 10: Results of IPC tests on round and oval tablets

| Test | Specification | Batch No. ENQ3822/PIRT/001/01 | Batch No. ENQ3822/PIRT/002/01 |
|---|---|---|---|
| | | Round | Oval |
| Target Weight | | 300 mg (round) | 500 mg (oval) |
| Individual Tablet Weight | 285 - 315 mg (round)<br>475 - 525 mg (oval) | 291 mg - 307 mg | 496 mg - 502 mg |
| Average Tablet Weight | 295 - 305 mg (round)<br>491.7 - 508.3 mg (oval) | 299.4 mg (297.3-301.9) | 499.0 mg (498.4 - 499.4) |
| % RSD | NMT 1.7% | 0.47% - 1.17% | 0.18% - 0.32% |
| Average Thickness | Target 4.6 (4.5-4.7) mm (round)<br>Target 5.45 (5.18 - 5.68) mm (oval) | 4.64 mm<br>(4.61 - 4.67 mm individuals) | 5.39 mm<br>(5.36 - 5.40 mm individuals) |
| Hardness | NA | 107.8 - 147.1 N (individuals) | 170.5 - 183.4 N (individuals) |
| Average Hardness | Target 120 (96-145) N (round)<br>Target 180 (150 -240) N (oval) | 111.5 - 128.6 N (average hardness across IPCs) | 175.3 - 180.3 N (average hardness across IPCs) |
| Average Tensile Strength | Target 2 MPa | 2.02 - 2.34 MPa across IPCs<br><br>2.07 MPa at set up | 2.19 - 2.26 MPa across IPCs<br><br>2.27 MPa at set up |
| Appearance | Matches intended shape and free from chips, defects and other markings | Conforms<br>Dark yellow/amber round embossed tablets with dark spots of iron oxide | Conforms<br>Dark yellow/amber oval embossed tablets with dark spots of iron oxide |
| Friability | ≤ 0.5 %w/w | 0.0 % (at set up) | 0.0 % (at set up) |

Based on the results ENQ3822/PIRT/001/01 (round) and ENQ3822/PIRT/002/01 were considered suitable for the coating runs.

# Experimental Part 3: Coating of Placebo Tablets

## Materials, Processing and Testing

### 10. Materials

Placebo round and oval tablets, produced as described in Section 0, were coated in the following 10 small-scale trials at a batch size of 3 kg. Various $TiO_2$-free and $TiO_2$ containing coating suspensions were used to coat these placebo tablets and also the active core tablets described in Section 0. Since some of these coating materials had similar compositions, it was decided to select 20 of the $TiO_2$-free coating materials from Table 3, while maintaining a variety of film-former types, plasticizers, $TiO_2$ alternative opacifiers and suppliers within the final selection.

The coating materials used for the placebo tablets are shown in Table 11. The $TiO_2$-free coating materials were selected from a variety of vendors. However, all contained calcium carbonate as an opacifier. This was to assess $CaCO_3$, the most commonly used replacement for $TiO_2$, versus $TiO_2$ as an opacifier. Other opacifiers were also evaluated as part of the Consortium's studies in the course of the active tablet trials (see Section 0). The $TiO_2$-free coating materials were HPMC-based, except for COAT-007 which contains PEG-PVA graft copolymer. All $TiO_2$-free coating suspensions were used to coat both round and oval tablets, and compared to two $TiO_2$ containing coatings for reference, one HPMC-based and the other PVA-based.

The coating suspensions were prepared at the manufacturer's target or the middle of the target range for solids content and according to the instructions given by the manufacturer. The properties of these suspensions and their ease of manufacture are described in Section 3.

Table 11: Coating materials used for coating the placebo tablets

| **Round placebo tablet cores (Batch No. ENQ3822/PIRT/001/01)** | | | | | |
|---|---|---|---|---|---|
| **Coating Run** | **Coated Tab Batch No.** | **Consortium Coat Ref** | **Film Former** | **Opacifier(s)** | **%Solids (w/w)** |
| 1 | 003/01 | COAT-017[a] | HPMC | $TiO_2$ | 15 |
| 2 | 004/01 | COAT-032 | HPMC | $CaCO_3$+H | 17.5 |
| 3 | 005/01 | COAT-027 | HPMC | $CaCO_3$+D | 16.5 |
| 4 | 006/01 | COAT-006 | HPMC | $CaCO_3$+D | 20 |
| 5 | 007/01 | COAT-007 | PEG-PVA graft copolymer+PVA | $CaCO_3$+Talc | 30 |
| **Oval placebo tablet cores (Batch No. ENQ3822/PIRT/001/02)** | | | | | |
| **Coating Run** | **Coated Tabl Batch No.** | **Consortium Ref No.** | **Film Former** | **Opacifier**** | **%Solids (w/w)** |
| 6 | 008/01 | COAT-018[a] | PVA | $TiO_2$+Talc | 25 |
| 7 | 009/01 | COAT-032 | HPMC | $CaCO_3$+H | 17.5 |
| 8 | 010/01 | COAT-027 | HPMC | $CaCO_3$+D | 16.5 |
| 9 | 011/01 | COAT-006 | HPMC | $CaCO_3$+D | 20 |
| 10 | 012/01 | COAT-007 | PEG-PVA graft copolymer+PVA | $CaCO_3$+Talc | 30 |

[a]$TiO_2$ containing coating materials used for comparison.

## 11. Equipment and Manufacture

The small-scale (3 kg) placebo batches were coated using an O'Hara Labcoat coater fitted with a 15-inch pan. For each batch the equipment was pre-warmed until the exhaust temperature was between 40°C and 50°C before adding the tablets. The tablet bed was then pre-warmed until the exhaust temperature reached between 40°C and 50°C. The tablets were coated to a target 6% weight gain. 250 tablets were removed after a 2%, 3%, 4%, 5% and 6% weight gain for analysis.

Spray rates of 20 g/min were targeted across all runs. Coating gun nozzle sizes of 0.8 mm or 1.0 mm were used during the 10 coating trials. Coating process parameters were tailored based on the suppliers' literature. However, the majority of the coating runs were performed with identical set points for atomising and pattern air pressure (1.5 bar), inlet air flow (250 $m^3$/hr), pan differential pressure (-0.25 mbar) and pan speed (18 rpm). Inlet air temperatures were adjusted to maintain the manufacturers recommended exhaust or tablet bed temperatures as appropriate.

After coating the tablet batches were dried for between 6 min to 15 min until the exhaust temperature was approximately 50°C and cooled for 10 to 15 min. Each batch was stored in cable-tied double PEG bags in a HDPE drum.

## 12. Analytical Testing

Approximately 250 film-coated tablet samples at weight gains of 2%, 3%, 4% 5% and 6% were assessed for appearance to evaluate the %weight gain required to achieve complete coverage of the tablets' yellow color. The point of sampling was determined based on the theoretical amount of coating suspension sprayed to achieve a desired weight gain. However, some samples may have been taken earlier, if IPC testing showed that the weight gain had already been achieved. Coating was not stopped until a 6% weight gain had been reached as determined through IPC testing.

250 tablets were sampled from the bulk for other potential testing including a photostability (see Section 0). The initial testing of the tablets was as described in Table 12.

Table 12: Testing of placebo film coated tablets

| Test | Methodology | Samples to be tested by % weight gain |
|---|---|---|
| Appearance | Visual using photography | 2%, 3%, 4%, 5%, 6% |
| Appearance | Colorimetry | 2%, 3%, 4%, 5%, 6% |
| Coat thickness | Digital optical microscopy | 2%, 3%, 4%, 5%, 6% |
| Quality of debossed image | Digital optical microscopy | 2%, 3%, 4%, 5%, 6% |
| Disintegration | Ph.Eur. | 2%, 4%, 6% |

## 13. Analytical Methodology - Visual Appearance

The visual appearance of the samples at different %coat weight gains from each of the coated placebo batches were assessed visually from photographs according to the following procedure:

10 tablets were sampled from each batch of tablets (2 tablets per coating level). They were photographed using a Sony a6000 camera fitted with a Tamron 35mm F/2.8 Di OSD M1:2 lens. The visual assessment focused on the coat coverage of the tablets at different coating levels on both bellyband and tablet faces as well as inspection of debossing for any signs of infilling. The acceptance criteria for an acceptable coating are as described in Table 13.

Table 13: Acceptance criteria for coatings based on visual appearance

| Criteria | Evaluation | | |
|---|---|---|---|
| | **Acceptable** | **Acceptable with Caveats** | **Not Satisfactory** |
| Color and appearance at ≤6% coating level | White at 6% weight gain | Tablet core hidden but off-white coat, not white[a] | Tablet core color visible Spray pattern visible |
| Coverage | Even and underlying yellow core not visible | Uneven at low % weight gain | Uneven at high 6% weight gain and/or yellow core still visible |
| Debossing | Legible and no in-filling | Legible with minor amount of in-filling | Not legible |
| Surface | Smooth and glossy/matt | Slight surface roughness | Surface not smooth |
| Color and coverage at same %weight gain as $TiO_2$ reference | Yes | Acceptable coat achieved but ≤2% greater weight gain required | Coat inacceptable or acceptable but >2% greater weight gain required. |
| Overall acceptable coat achieved at ≤6% weight gain | Yes | Overall coat acceptable with caveats | Acceptable coat not achieved |

[a]Off-white coatings may not be acceptable in some international markets and will not enable color matching to white tablets whether these are coated with $TiO_2$ containing coats or not.

Visual observations of tablet appearance were also recorded during manufacture on the samples at the different %coating weight gains from each placebo batch. This separate analysis also gives an impression of the quality of the coating.

## 14. Analytical Methodology - Colorimetry

The DigiEye Version 7 equipment was used for the colorimetry experiments. This equipment consists of a D65 illuminant and additional LEDs to produce a calibrated D65 source, and a Nikon Z6II Mirrorless Digital Camera with Nikon Nikkor Z f/4-6.3 VR Lens for image capture.

Twenty tablets of each sample were placed into the custom tablet holder. The average result was then calculated from the twenty total samples.

The following parameters were measured and calculated.

L* = Lightness defined on a scale of 0 black/total absorption to 100 white/total reflection.

a* = Red/green value from negative 100 as green to positive 100 as red values.

b*= Yellow/blue value from negative 100 as blue to positive 100 as yellow values.

C = Specifies chroma which describes the vividness of the color.

h = Hue angle which specifies how the color is perceived ranging from 0° (red) through 90° (yellow), 180° (green), 270°(blue) and back to 0°.

$\Delta E^*_{00}$ = Total color difference value based on the average values of L*a*b* obtained for the $TiO_2$-containing reference coat versus the $TiO_2$-free coats. This was carried out to evaluate the ability of the $TiO_2$-free coatings to match the reference coat. It was calculated according to the Delta E 2000 equation.

$$\Delta E^*_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H}}$$

This International Commission on Illumination (CIE) equation provides the most accurate color difference values currently available [13].

The $\Delta E^*_{00}$ values were interpreted as follows [14]:

White Tablets

The $TiO_2$-free coatings were determined to match the color of the corresponding $TiO_2$ reference if $\Delta E^*_{00} \leq 1.0$. $\Delta E^*_{00} \leq 1.0$ is considered to mean a color difference which is not perceptible to the eye. A $\Delta E^*_{00} > 1.0$ was considered to be noticeable to a patient.

Colored Tablets

For colored tablets, the $\Delta E^*_{00}$ values were interpreted as follows:

| | |
|---|---|
| $\Delta E^*_{00} \leq 1.0$ | Color difference not perceptible to the human eye. |
| $\Delta E^*_{00}$ 1 - 2 | Color difference perceptible through close observation. |
| $\Delta E^*_{00} > 2$ | Color difference noticeable at a glance. |

The acceptance criterion for color matching of colored $TiO_2$-free coated tablets to the corresponding $TiO_2$ reference was $\Delta E^*_{00} < 2$.

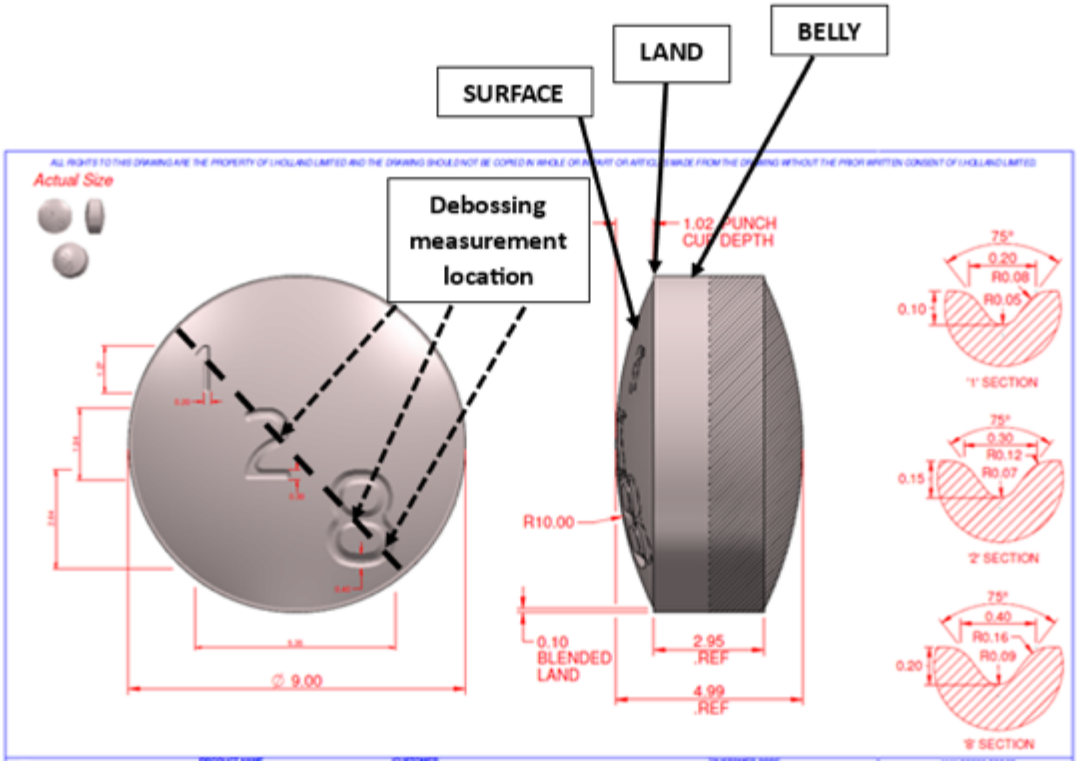
Rationale for Differences in Acceptance Criteria for White and Colored Tablets

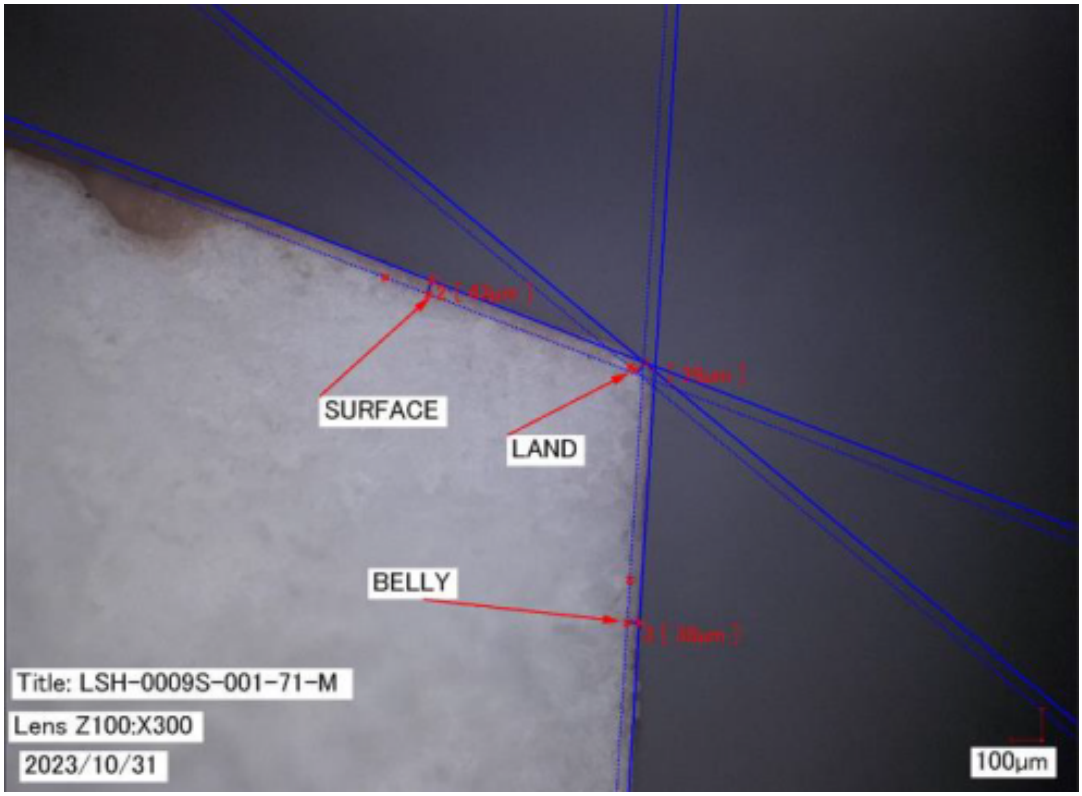
White is a color associated in many cultures with cleanliness, purity and the health professions. White surfaces reflect light back to the human eye, while colored surfaces reflect only a portion. Therefore, surface imperfections and color differences are more perceptible to the human eye when two white objects are being compared than when the comparison is made between two colored objects.

## 15. Analytical Methodology - Optical Microscopy

Digital microscopy images were acquired using a Keyence VHX-2000 at x100 and x300 magnification. Coating thickness was measured at four different areas: on the tablet land, belly, surface and at the debossed image.

*Figure 4: Diagrams showing the land, belly, surface and debossed image measurement locations*



## Results and Discussion - Testing of the Placebo Film-coated Tablets

### 16. Visual Appearance and Comparison at Various Weight Gains

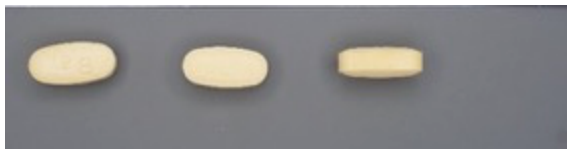
The photographs of the coated placebo tablet batches at various %coating weight gains are shown in Figure 5 together with photographs of the cores. Since there is a slight yellow tinge to the photographs due to the conditions used, a detailed visual description of the tablets' appearance is included.

Figure 5: Visual appearance of coated round and oval placebo tablets and uncoated cores

Tablet Cores

Batch ENQ3822/PIRT/001/01

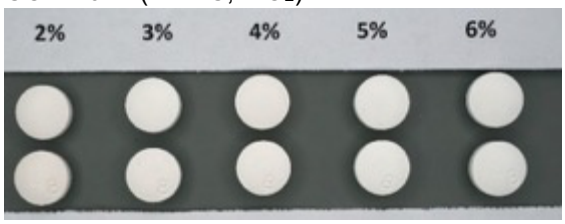
Batch ENQ3822/PIRT/001/02



Coated Tablets

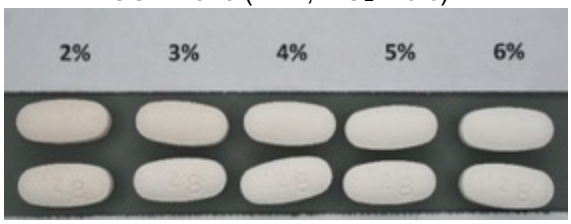
Batch ENQ3822/PIRT/003/01

COAT-017 (HPMC, $TiO_2$)




Batch ENQ3822/PIRT/008/01

COAT-018 (PVA, $TiO_2$+Talc)

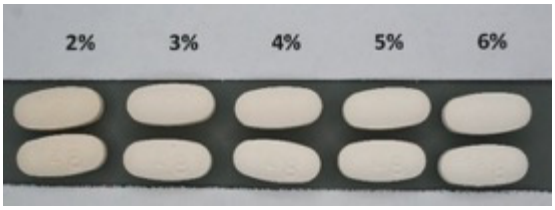



Batch ENQ3822/PIRT/004/01

COAT-032 (HPMC, $CaCO_3$+H)


Batch ENQ3822/PIRT/009/01

COAT-032 (HPMC, $CaCO_3$+H)

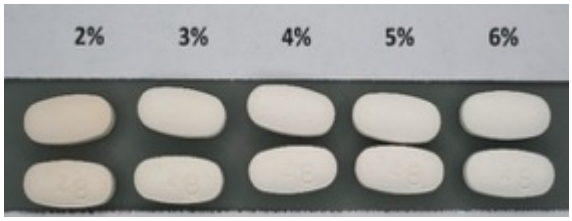



Batch ENQ3822/PIRT/005/01

COAT-027 (HPMC, $CaCO_3$+D)

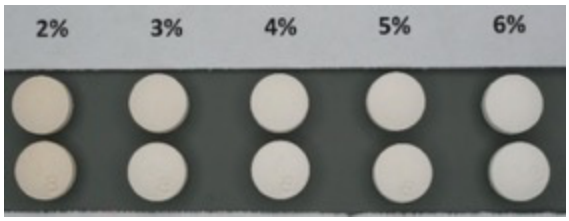

Batch ENQ3822/PIRT/010/01

COAT-027 (HPMC, $CaCO_3$+D)



Batch ENQ3822/PIRT/006/01

COAT-006 (HPMC, $CaCO_3$+D)

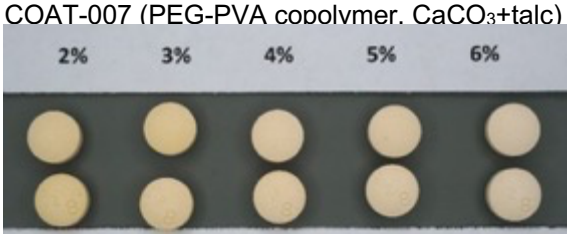



Batch ENQ3822/PIRT/011/01

COAT-006 (HPMC, $CaCO_3$+D)

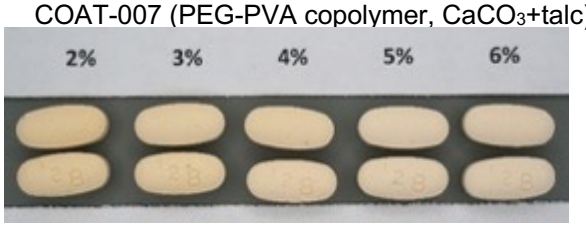

Batch ENQ3822/PIRT/007/01

COAT-007 (PEG-PVA copolymer, $CaCO_3$+talc)




Batch ENQ3822/PIRT/012/01

COAT-007 (PEG-PVA copolymer, $CaCO_3$+talc)




The coatings were considered acceptable when they met the criteria in Table 13. The evaluation against these criteria is shown in Table 14 for the round tablets and in Table 15 for the oval tablets. Visual observations on the appearance of the tablets sampled at the different %coating weight gains from each placebo batch during manufacture are given in Table 16.

The photographs and the descriptions in Table 14 and Table 15 show that for the HPMC-based coated round tablets (Batches ENQ3822/PIRT/003/01 to ENQ3822/PIRT/006/01) it was possible to achieve an adequate coating coverage with both $TiO_2$-containing and $TiO_2$-free coatings. However, for the $TiO_2$-free coating this was achieved at significantly higher weight gains than for the $TiO_2$-containing reference (COAT-017) (3% versus 5 to 6%). The appearance results from the photography are supported by the observations made during manufacture (see Table 16).

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Table 14: Visual appearance of round placebo batches at different %coating weight gains

| Coating Run | Batch No. ENQ3822/PIRT/ | Consort Coat Ref | Color at 6% Weight Gain | Coverage | Debossing | Surface | Color & Coverage Achieved at Same %Weight Gains as $TiO_2$ Reference | Overall Acceptable Coat |
|---|---|---|---|---|---|---|---|---|
| Round Cores | 001/01 | NA | Yellowish tablet core | NA | 1, 2 and 8 debossed on one side | Glossy bellyband and tablet face | NA | NA |
| 1 | 003/01 | COAT-017[a] | White<br>2% off-white compared to 6% | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth, slightly glossy | NA | Yes at 3% |
| 2 | 004/01 | COAT-032 | Off-white - 4 - 6%<br>2 % - 3 % levels have slight orange tint. | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Tablets had slight orange tinge at 3% weight gain | Off-white at 6% |
| 3 | 005/01 | COAT-027 | White<br>2% - orange tinge<br>3% - 4% off-white compared to 5% & 6%. | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | White tablets achieved at 5% vs 3% for $TiO_2$ reference | Yes at 5% |
| 4 | 006/01 | COAT-006 | White<br>2% - off-white with orange tinge<br>3% - 6% - off-white to white | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | White tablets achieved at 5% vs 3% for $TiO_2$ reference | Yes at 5% |
| 5 | 007/01 | COAT-007 | Pale Yellow at 6%<br>Darker yellow at 2% | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Acceptable coat not achieved | Not at 6% |

[a]$TiO_2$ reference coat

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

Table 15: Visual appearance of oval placebo batches at different % coating weight gains.

| Coating Run | Batch No. ENQ3822/PIRT/ | Consort Coat Ref | Color at 6% Weight Gain | Coverage | Debossing | Surface | Color & Coverage Achieved at Same %Weight Gains as $TiO_2$ Reference | Acceptable Coat at 6% Weight Gain |
|---|---|---|---|---|---|---|---|---|
| Oval Cores | 002/01 | NA | Yellowish tablet core | NA | 1, 2 and 8 debossed on one side | Glossy bellyband and tablet face. | NA | NA |
| 6 | 008/01 | COAT-018[a] | White<br>Off-white at 2 %<br>White at 5 % | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Surface slightly coarse at all coating levels[b] | NA | Yes at 5% |
| 7 | 009/01 | COAT-032 | Off-white - 3 - 6%<br>2 % - have slight orange tint | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Off-white at 5% weight gain | Off-white at 6 % |
| 8 | 010/01 | COAT-027 | White<br>2 % - off-white with pink tint<br>Off-white at 3 % to white at 6 %. | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Off-white at 5% weight gain | Yes at 6% |
| 9 | 011/01 | COAT-006 | Off-white<br>Off-white at 2 %<br>Becomes less off-white at higher %. | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Off-white at 5% weight gain | Off-white at 6% |
| 10 | 012/01 | COAT-007 | Pale Yellow at 6%<br>Darker yellow at 2%<br>Dark specs & spray pattern visible. | Even on bellyband & tablet faces – all % levels. | Legible<br>No in-filling | Smooth and matt | Acceptable coat not achieved | Not at 6% |

[a] $TiO_2$ reference coat

[b] Coating suspension prepared at 25% w/v, the maximum recommended %solids concentration. The manufacturer has now recommended 20%w/v solids for an improved finish.

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

Table 16: Descriptions of placebo tablet appearance during manufacture

| Coating Run | Batch No. ENQ3822/PIRT/ | Consort Coat Ref No. | Recom. % Wt Gain | Comment on Appearance of Samples and Bulk | Acceptable Coat at 6% Weight Gain Manufacturing Observations | Acceptable Coat at 6% Weight Gain Photography |
|---|---|---|---|---|---|---|
| Round Cores | 001/01 | NA | NA | Dark yellow/amber round embossed tablets with dark spots of iron oxide. | NA | NA |
| 1 | 003/01 | COAT-017[a] | 2 -3% | 2%w/w good surface coverage. Bright white film coated tablets (FCTs) at 3 %w/w. | Yes at 3% | Yes at 3% |
| 2 | 004/01 | COAT-032 | 5% | 2%w/w good surface coverage but dark spots (iron oxide) noticed up to 4 %w/w. Edge color noticeable at 5% w/w. Less obvious at 6%w/w, creamy white bulk FCTs. | Yes, but off-white at 6% | Off-white at 6% |
| 3 | 005/01 | COAT-027 | 2 -3% | 4 % good surface coverage but edges darker until 5 % w/w. White bulk FCTs. | Yes at 6% | Yes at 5% |
| 4 | 006/01 | COAT-006 | 3 – 5% | 4% good coverage. Whlte bulk FCTs | Yes at 4% | Yes at 5% |
| 5 | 007/01 | COAT-007 | 5% | Core color not covered at end of coating process. Dark spots still noticeable. Beige bulk FCTs. | Not at 6% | Not at 6% |
| Oval Cores | 002/01 | NA | NA | Dark yellow/amber oval embossed tablets with dark spots of iron oxide. | NA | NA |
| 6 | 008/01 | COAT-018[a] | 2 -3% | 4%w/w good coverage. | Yes at 4% | Yes at 5% |
| 7 | 009/01 | COAT-032 | 5% | 5%w/w decent surface coverage but edges still yellow for all tablets including bulk. | Not at 6% | Off-white at 6% |
| 8 | 010/01 | COAT-027 | 2 -3% | 4%w/w decent surface coverage but edges still yellow for all tablets including bulk. | Not at 6% | Yes at 6% |
| 9 | 011/01 | COAT-006 | 3 - 5% | 4% w/w decent surface coverage but edges still dark. Laboratory lighting made it hard to discern if the 6%w/w sample edges were yellow/shadowed. | Edges at 6% may be yellow | Off-white at 6% |
| 10 | 012/01 | COAT-007 | 5% | Bulk tablets still very obviously colored. | Not at 6% | Not at 6% |

[a]$TiO_2$ reference coats

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

No coating issues such orange peel roughness or picking or sticking were observed for any of the batches.

The requirement to coat to higher %coating weight gains to achieve a satisfactory coating with the $TiO_2$-free coating materials has several disadvantages. It results in prolonged processing times which, in turn, increases the time the tablets are exposed to conditions of higher temperature and humidity, with a potential impact on product stability. It also increases the amount of coating components that the patient ingests as the coat forms a higher percentage of the overall tablet weight. Finally, it increases processing costs due to the extended manufacturing times and the higher amounts of materials used, and reduces the equipment capacity since less product can be produced within a defined time period which can be a limitation for reliable supply.

COAT-032 resulted in an off-white coat on both round and oval tablets. It is not clear whether this is a feature of the suspension color or not, as coverage was described as good and the bulk as creamy white. Off-white tablets present issues when color matching with white tablets. They also may not have global regulatory acceptability. Therefore, based on the photography and manufacturing observations, this batch is considered to be coated satisfactorily with caveats, the caveats being that it could not be used for color matching with white tablets or have regulatory acceptability on a global basis.

For the HPMC-based coated oval placebo tablets (ENQ3822/PIRT/009/01 to ENQ3822/PIRT/011/01) the results are less clear cut. The photography results carried out on just 12 tablets per batch would suggest that all HPMC-based $TiO_2$-free coatings could achieve good or satisfactory results at 6% weight. However, Batch ENQ3822/PIRT/011/01 (COAT-006) was described as being off-white as opposed to white. The observations during manufacturing described the coated bulk tablets from Batch ENQ3822/PIRT/009/01 and ENQ3822/PIRT/010/01 (COAT-032 and COAT-027) at 6 % weight gain as having yellow edges, while it was difficult to discern under the light conditions used whether this was also the case for Batch ENQ3822/PIRT/011/01 (COAT-006).

None of the HPMC-based coatings resulted in in-filling of the debossed image. The tablet dimensions and debossed images presented a challenge representative of typical tablets. However, the coating of smaller debossed tablets with $TiO_2$-free coatings would result in a greater challenge with respect to potential in-filling and maintenance of image legibility, given the higher % coating weight gain required. The $TiO_2$-free coatings resulted in a smooth, matt finish, while the $TiO_2$-containing reference produced a smooth, slightly glossy one.

The coats produced by the PVA and PEG-PVA copolymer-based coatings (COAT-018 and COAT-007) were less successful. The coating with the $TiO_2$-containing reference (ENQ3822/PIRT/008/01, COAT-018) produced good coverage on oval tablets at 4 to 5% on the basis of both photographic and manufacturing observations. However, the surface texture appeared slightly coarse at all coating levels. The COAT-018 suspension was prepared at 25% w/v, the maximum recommended %solids concentration. The manufacturer has now recommended 20%w/v solids for an improved finish. COAT-007 (Batch ENQ3822/PIRT/007/01 and ENQ3822/PIRT/012/01) failed to produce a satisfactory coat on both round and oval tablets at a 6% weight gain as shown in both the photographs and visual observations. For both COAT-007 coating suspensions prepared for the round and oval tablets, a thick ring of settled material was found on decanting the suspension at the end of coating (see Table 6). It is postulated that the loss of solids from the suspension may be the cause of the poor coverage and opacification found with this $TiO_2$-free coat.

## 17. Colorimetry

Figure 6 shows the L* a* and b* values for the $TiO_2$-free coatings compared with the $TiO_2$-containing reference coatings at 2% to 6% coated tablet weight gain. The graph for the L* values for the round tablets clearly shows that the HPMC-based $TiO_2$-containing reference coating (COAT-017) results in a greater increase in tablet whiteness at all coating percentages than all of the $TiO_2$-free coatings. The L* value is over 80 even at a 2 % weight gain. This is in line with the visual appearance results (Figure 5, Table 14). This superior performance at low % coating levels is also reflected in the ability of COAT-O17 to reduce a* and b* values, the latter being a measure of blue and yellowness. As well as elegance, the increased whiteness may facilitate masking tablet core color which is useful in placebo-controlled trials and commercial products. It also provides a good background for ink printing on the tablet which may be used to help tackle counterfeiting and/or tablet identification.

The next best performing coating materials are COAT-027 and COAT-006 which achieve similar L*, a* and b* values to the $TiO_2$ HPMC-based comparator at a 5% coating level. Again, this agrees well with the visual appearance results. COAT-O32 results in slightly lower L* and higher a* and b* values than the other HPMC-based coatings and was visually assessed as being off-white at a 6% coating level from photographs. COAT-007, based on a PEG-PVA graft copolymer, was the worst performing coat in the colorimetry tests which aligns with the visual appearance of the tablets coated with it.

The $TiO_2$ reference coat for the oval tablets was a PVA-based coating (COAT-018). Its performance compared to COAT-O27 and COAT-006 was only slightly better in terms of L* values and a* values. However, its ability to reduce b* values was better than the other coatings at all coating levels, showing its ability to hide the yellow color of the cores efficiently. Again, COAT-032 was slightly inferior to the $TiO_2$ reference coat (COAT-018) and the other two HPMC-based $TiO_2$-free coatings on the oval tablets based on the colorimetry results, while COAT-007 was the worst performing in respect to L* values and a* and b* values. All of the above results are in line with the visual appearance results based on photography and manufacturing observations (Figure 5, Table 15, and Table 16).

In order to compare the results across both round and oval tablets, the L* values, b* values and hue angles were plotted for all the placebo tablets batches. Figure 7 shows the L* values at a 6% coating level, Figure 8, the b* values, and Figure 9, the hue angle values.

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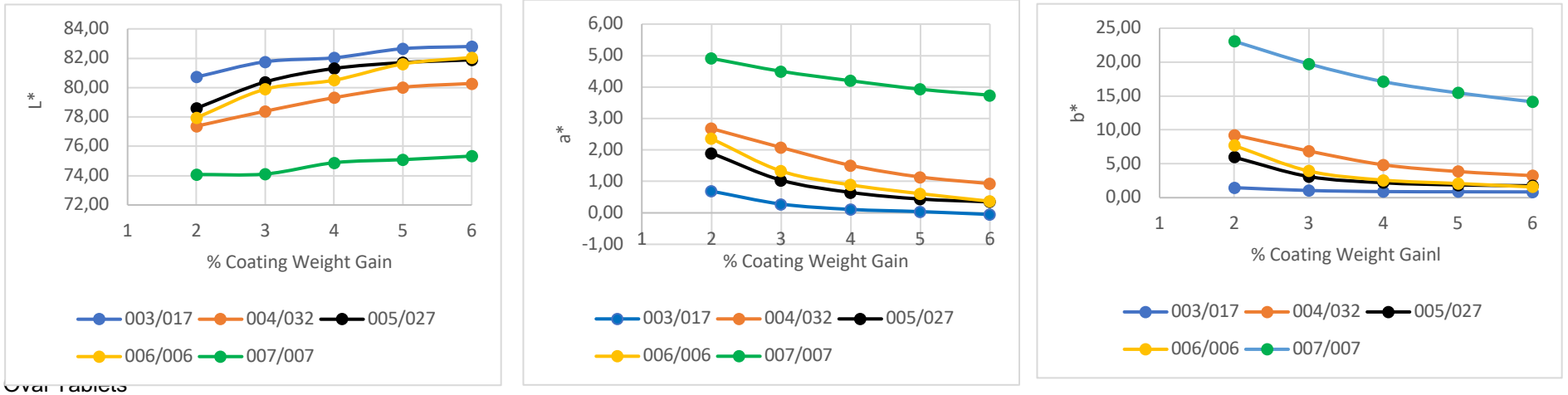
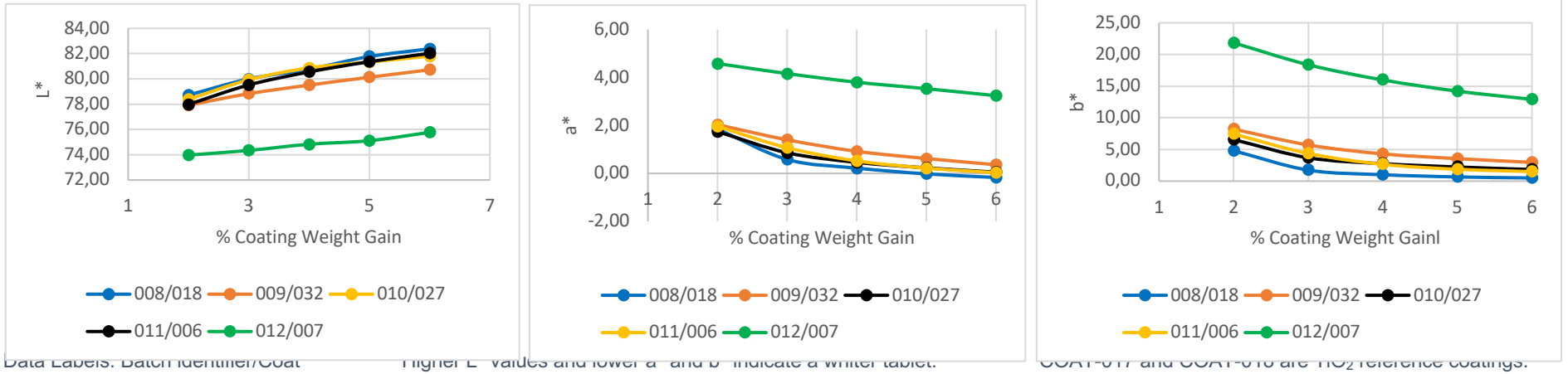
Figure 6: L*a*b* values for placebo round and oval tablets at different % coating levels

Round Tablets

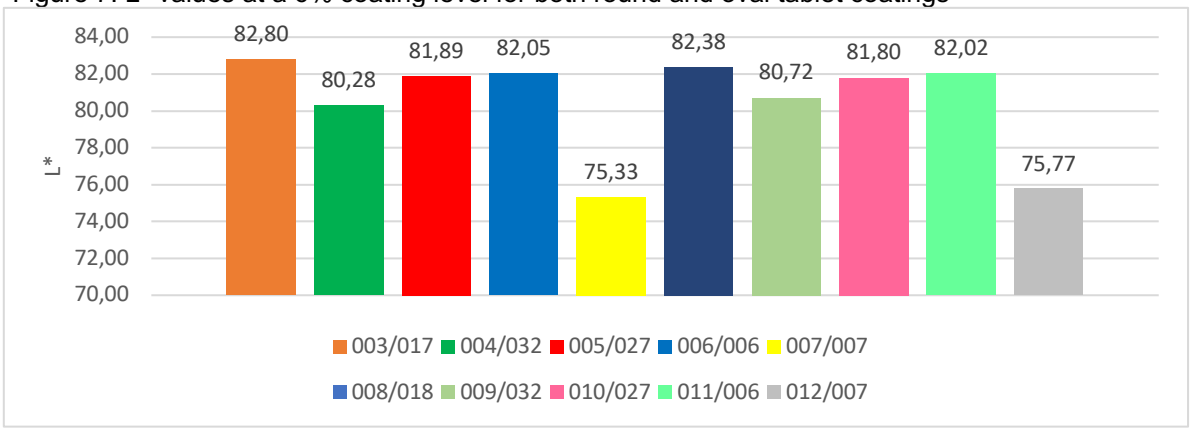



Oval Tablets




Data Labels: Batch Identifier/Coat Higher L* values and lower a* and b* indicate a whiter tablet. COAT-017 and COAT-018 are $TiO_2$ reference coatings.

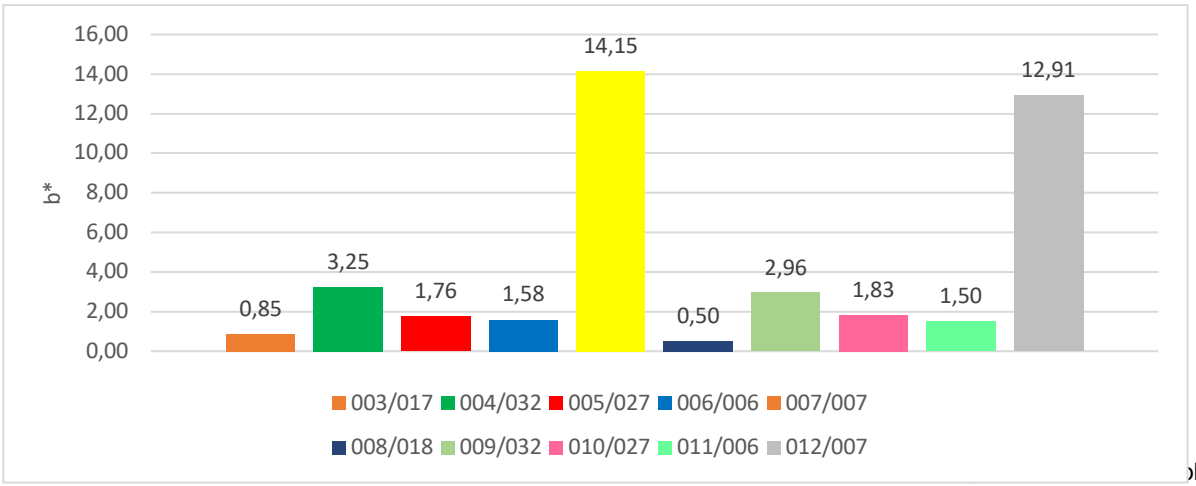
Figure 7: L* values at a 6% coating level for both round and oval tablet coatings




Data Labels: Batch identifier/Coat Batch identifier 003 – 007 = round tablets, 008 – 012 = oval tablets

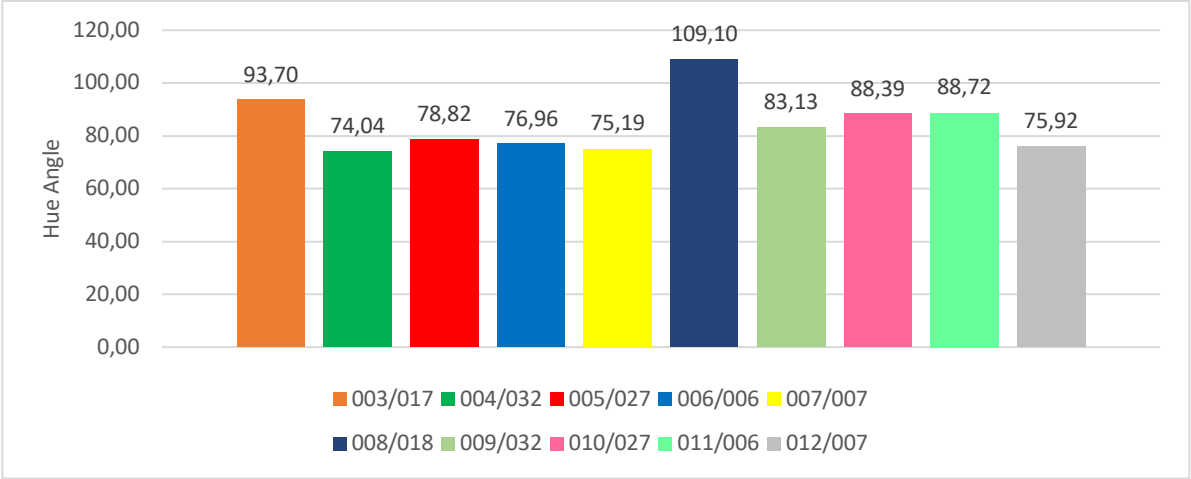
Higher L* values indicate a whiter tablet.

Figure 8: b* values at a 6% coating level for both round and oval tablet coatings




Lower b* values indicate a whiter tablet.

Figure 9: Hue angle values at a 6% coating level for both round and oval tablet coatings




Data Labels: Batch identifier/Coat Data labels - Batch identifier 003 – 007 = round tablets, 008 – 012 = oval tabletHue angles should be close to the $TiO_2$ reference as possible.

The results show that very similar colorimetry results are obtained for the coating of both round and oval tablets and the rank order of coat superiority/inferiority remains the same. The values of L* and b* for the $TiO_2$-free coatings, which resulted in white tablets as assessed by visual appearance at the 6% coating level, lie close to those of the $TiO_2$-reference coatings.

The hue angle results (see Figure 9) show very different values for the reference coatings compared to the $TiO_2$-free coatings, indicating that use of $CaCO_3$ as the opacifier in the coatings resulted in white of a different hue. The hue angle differences are important as this colorimetry parameter closely correlates with the human perception of color.

The hue angle differences may be due to the differences in surface texture (slightly glossy for tablets coated with COAT-017, slightly coarse for tablets coated with COAT-018 and smooth and matt for the $TiO_2$-free coatings) (see Table 14 and Table 15) or be due to the optical scattering properties of $TiO_2$ itself. The differences in surface texture of the different tablet batches may also have an impact on the ease of down-stream processing as glossy tablets are more likely to slip and slide over each other, making packaging easier.

Based on these findings, $\Delta E^*_{00}$ values based on L* chroma and hue angle values were calculated for each % coating weight gain for the various $TiO_2$-free HPMC-based coatings using Batch ENQ3822/PIRT/003/01 (COAT-017) as the $TiO_2$ reference coat. The values were calculated using the equation in Section 14.

A $\Delta E^*_{00}$ of ≤ 1 was considered to mean that the $TiO_2$-free tablets were of comparable color to the $TiO_2$ reference coating (COAT-017). The $\Delta E^*_{00}$ values for COAT-007, a $TiO_2$-free coating based on PEG-PVA graft copolymer, were not calculated as the tablet appearance was so visually different from either the HPMC-based or PVA-based reference coatings (COAT-017 and COAT-018 respectively).

Table 17: $\Delta E^*_{00}$ of $TiO_2$-free HPMC-based coatings versus $TiO_2$ HPMC-based reference coat

| Batch No. ENQ3822/PIRT | Consortium Coat Reference | $\Delta E^*_{00}$ | | | | |
|---|---|---|---|---|---|---|
| | | 2% Coat | 3% Coat | 4% Coat | 5 % Coat | 6 % Coat |
| 003/01 | COAT-017 ($TiO_2$) | NA | NA | NA | NA | NA |
| 004/01 | COAT-032 ($CaCO_3$+H) | 6.99 | 5.85 | 4.43 | 3.57 | 3.12 |
| 005/01 | COAT-027 ($CaCO_3$+D) | 4.35 | 2.34 | 1.53 | 1.30 | 1.24 |
| 006/01 | COAT-006 ($CaCO_3$+D) | 5.79 | 3.19 | 2.18 | 1.60 | 1.07 |
| 009/01 | COAT-032 ($CaCO_3$+H) | 6.03 | 4.72 | 3.67 | 3.09 | 2.50 |
| 010/01 | COAT-027 ($CaCO_3$+D) | 4.76 | 2.79 | 1.97 | 1.62 | 1.19 |
| 011/01 | COAT-006 ($CaCO_3$+D) | 5.50 | 3.48 | 2.02 | 1.35 | 0.89 |

The results show that only the oval tablet batch coated with the $TiO_2$-free coating, COAT-006, meets the color matching criterion of $\Delta E^*_{00} \leq 1$ with the white HPMC-based $TiO_2$ reference, although the round tablets coated with COAT-006 at a 6% coating weight gain have a $\Delta E^*_{00}$ value just outside of the

criterion. COAT-027 is the next best $TiO_2$-free coating with $\Delta E^*_{00}$ values between 1 and 2 at a 6% coating weight gain, suggesting that a difference would be only noticeable on close inspection.

Neither COAT-006 and COAT-027 have $\Delta E^*_{00}$ values <2 versus the $TiO_2$ reference at 3% weight gain, the coating level at which the reference batch was considered fully coated. This is line with the visual appearance results which showed that coverage and opacification of the yellow tablet core surfaces with the $TiO_2$-free coatings required higher coating weight gains than COAT-017 and COAT-018, the two $TiO_2$ reference coatings.

It should be noted that the COAT-006 produced an off-white coat on the oval tablets as judged by visual inspection (see Table 15). However, a color difference $\Delta E^*_{00}$ value of < 1 suggests there is no perceptible color difference between it and the $TiO_2$-reference, COAT-017, coated batch which was assessed visually as being white. The discrepancy in the results may be due to the subjective nature of color perception despite the use of standardized experimental conditions and/or intra-batch variation in coat quality coupled with the small sample size used (only 2 tablets per %weight gain per batch were used for the photography experiments and 20 for colorimetry). Colorimetry can also only measure the coating color on one tablet face and not the other or the tablet sides.

## 18. Coat Thickness and Quality of Debossed Image

Due to poor contrast in digital microscopy, for some samples it was difficult to define the exact boundary between the coating and the core and therefore some values reported for coating thickness are approximate.

Figure 10 shows typical digital microscopy images of the coated placebo tablets. The examples shown are from Placebo Run 1, at a 4% coating weight gain, Batch ENQ3822/PIRT/003/01, which was coated with COAT-017, a HPMC-based coat containing $TiO_2$.

Figure 10: Digital microscopy images from Placebo Run 1, at a 4% coating weight gain, Batch ENQ3822/PIRT/003/01

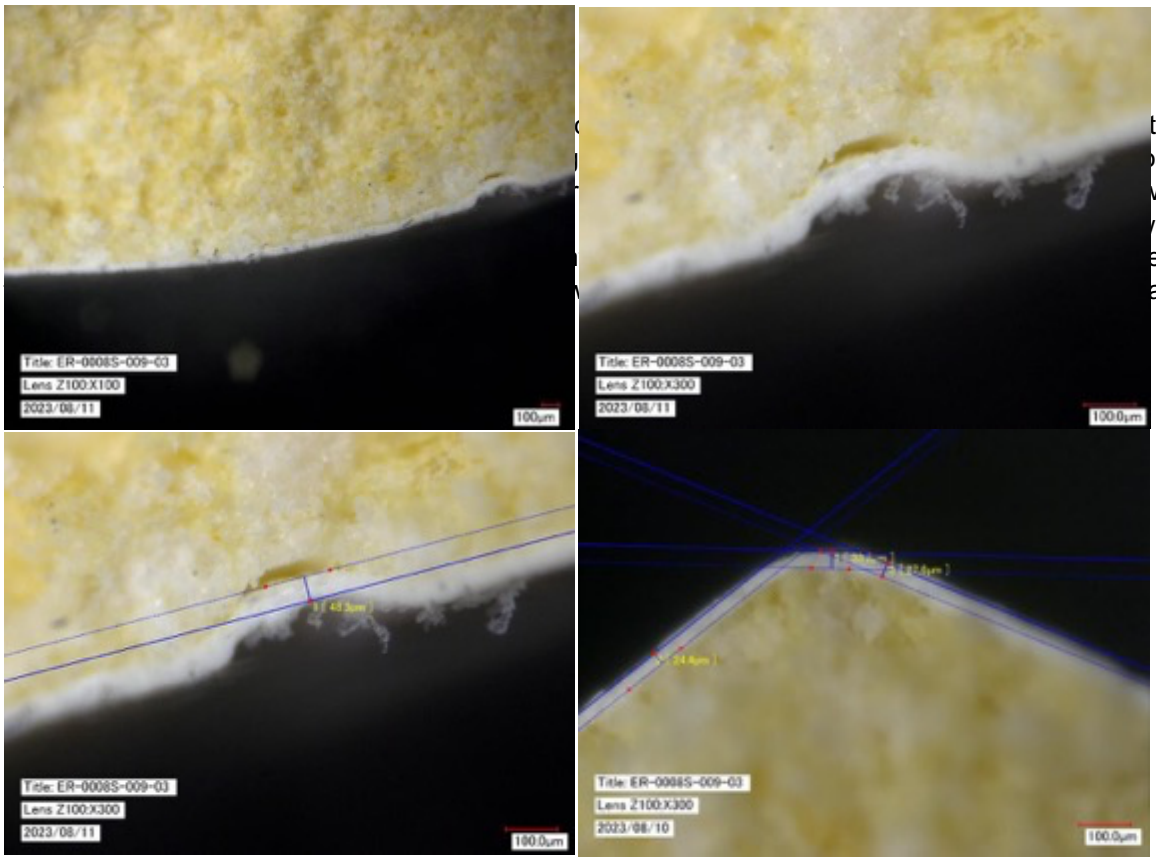
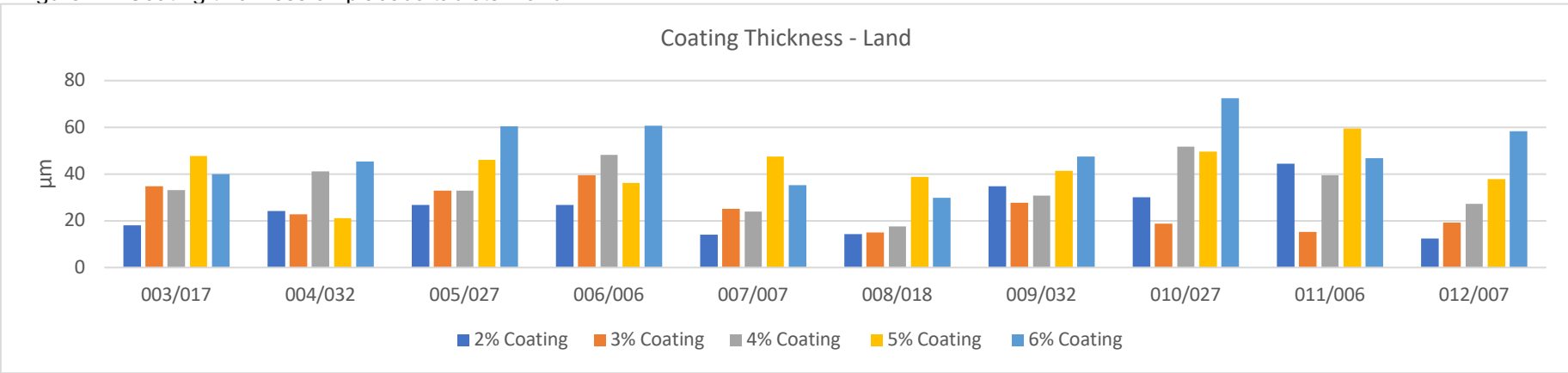


Figure 11: Coating thickness on placebo tablets - land




Data labels - Batch identifier/Coat  Data labels - 003 – 007 = round tablets, 008 – 012 = oval tablets  COAT-017 and COAT-018 are $TiO_2$ reference coats

Figure 12: Coating thickness on placebo tablets - belly

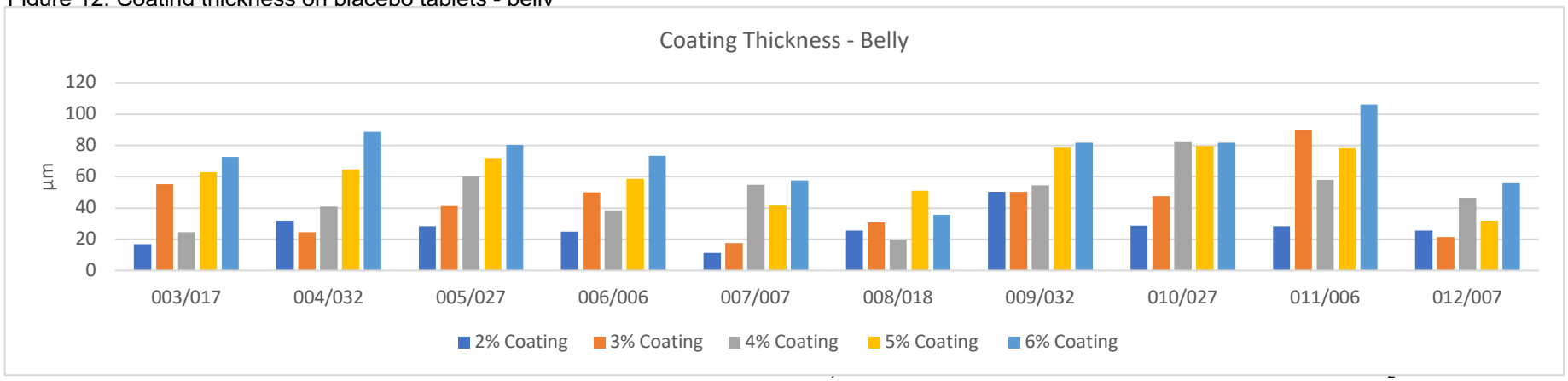


Figure 13: Coating thickness on placebo tablets - surface

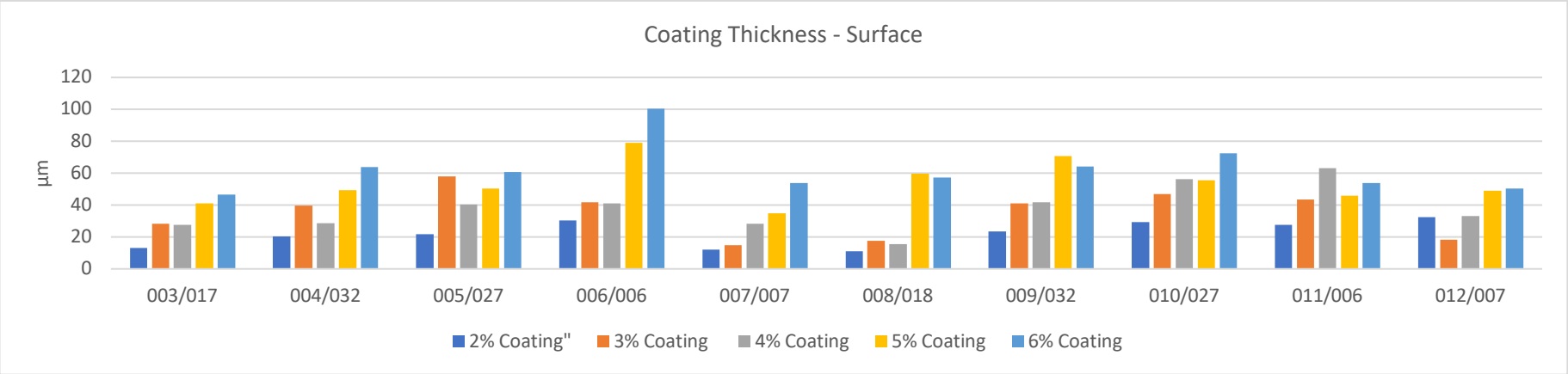
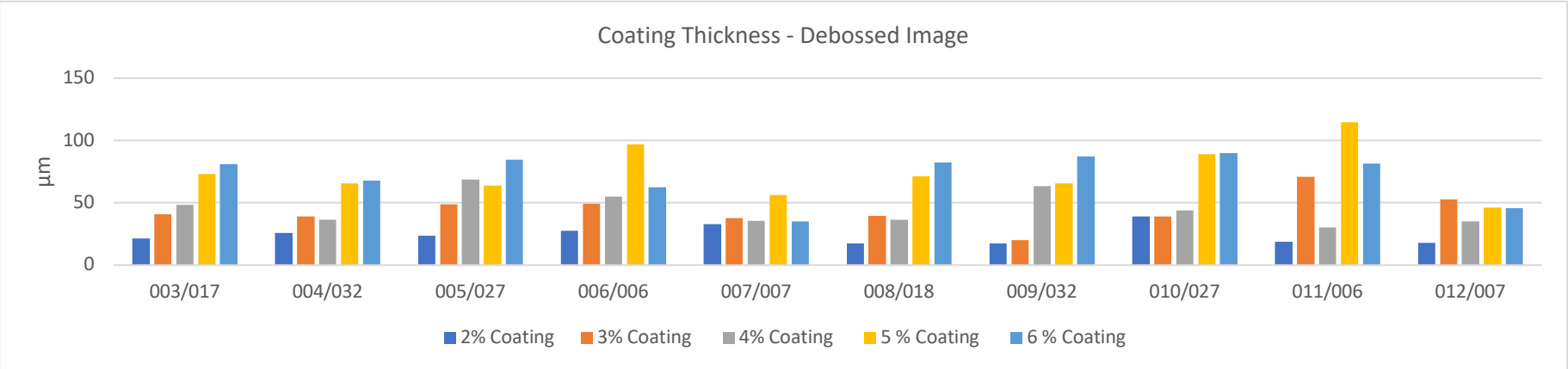



Figure 14: Coating thickness on placebo tablets - debossed image




Data labels - Batch identifier/Coat  Data labels Batch identifier 003 – 007 = round tablets, 008 – 012 = oval tablets  COAT-017 and COAT-018 are $TiO_2$ reference coats.

For the $TiO_2$ reference coated batches (ENQ3822/PIRT/003/01, COAT-017, round tablets) and ENQ3822/PIRT/008/01, COAT-018, oval tablets), coating thickness is relatively low even at 6% weight gain compared with some of the batches coated with the $TiO_2$-free coatings. This is despite the batch coated with COAT-017 and the batch coated with COAT-018 being assessed as visually white and completely coated at a 3% coating weight gain and 5% coating level respectively based on the photography results.

Table 18 compares the coat thickness levels at 3% and 6% weight gain for the $TiO_2$-free and $TiO_2$ reference coated round tablet batches ($TiO_2$ reference ENQ3822/PIRT/003/01) and at 5% and 6% for the oval tablets ($TiO_2$ reference ENQ3822/PIRT/008/01). This comparison also includes the average coat thickness based on the four individual values.

Table 18: Tablet coat thickness comparison

| **Round Tablets** | | | | **Coating Thickness (µm)** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Batch No. ENQ3822/PIRT/** | **Consortium Coat Ref.** | **Opacifier** | **% Wt Gain** | **Land** | **Belly** | **Surface** | **Deboss** | **Mean** |
| 003/01 | COAT-017 | $TiO_2$ | 3 | 35 | 55 | 28 | 41 | 39.8 |
| 004/01 | COAT-032 | $CaCO_3$+H | 3 | 23 | 24 | 40 | 39 | 31.5 |
| 005/01 | COAT-027 | $CaCO_3$+D | 3 | 33 | 41 | 58 | 49 | 45.2 |
| 006/01 | COAT-006 | $CaCO_3$+D | 3 | 39 | 50 | 42 | 49 | 45.1 |
| 007/01 | COAT-007 | $CaCO_3$+Talc | 3 | 25 | 18 | 15 | 38 | 23.9 |
| 003/01 | COAT-017 | $TiO_2$ | 6 | 40 | 73 | 47 | 81 | 60.1 |
| 004/01 | COAT-032 | $CaCO_3$+H | 6 | 45 | 89 | 64 | 68 | 66.4 |
| 005/01 | COAT-027 | $CaCO_3$+D | 6 | 60 | 80 | 61 | 84 | 71.4 |
| 006/01 | COAT-006 | $CaCO_3$+D | 6 | 61 | 73 | 100 | 63 | 74.2 |
| 007/01 | COAT-007 | $CaCO_3$+Talc | 6 | 35 | 58 | 54 | 35 | 45.5 |
| **Oval Tablets** | | | | **Coating Thickness (µm)** | | | | |
| **Batch No. ENQ3822/PIRT/** | **Consortium Coat Ref.** | **Opacifier** | **% Wt Gain** | **Land** | **Belly** | **Surface** | **Deboss** | **Mean** |
| 008/01 | COAT-018 | $TiO_2$+Talc | 5 | 39 | 51 | 60 | 71 | 55.2 |
| 009/01 | COAT-032 | $CaCO_3$+H | 5 | 41 | 79 | 71 | 66 | 64.1 |
| 010/01 | COAT-027 | $CaCO_3$+D | 5 | 50 | 80 | 56 | 89 | 68.4 |
| 011/01 | COAT-006 | $CaCO_3$+D | 5 | 59 | 78 | 46 | 115 | 74.6 |
| 012/01 | COAT-007 | $CaCO_3$+ Talc | 5 | 38 | 32 | 49 | 46 | 41.3 |
| 008/01 | COAT-018 | $TiO_2$+Talc | 6 | 30 | 36 | 57 | 83 | 51.4 |
| 009/01 | COAT-032 | $CaCO_3$+H | 6 | 48 | 82 | 64 | 87 | 70.2 |
| 010/01 | COAT-027 | $CaCO_3$+D | 6 | 72 | 82 | 72 | 90 | 79.1 |
| 011/01 | COAT-006 | $CaCO_3$+D | 6 | 47 | 106 | 54 | 81 | 72.0 |
| 012/01 | COAT-007 | $CaCO_3$+Talc | 6 | 58 | 56 | 50 | 46 | 52.6 |

At the 3% and 6% coating levels on round tablets, Batch ENQ3822/PIRT/007/01 (COAT-007, PEG-PVA copolymer based) had the lowest average coat thickness. This is despite this coating suspension being prepared at a 30% solids content. The other coats were sprayed at a %solids content which ranged from 15% to 25%.

With respect to the round tablets, at a 3% weight gain Batch ENQ3822/PIRT/004/01 (COAT-032) has a slightly lower average coat thickness than the $TiO_2$ reference batch, while for Batches ENQ3822/PIRT/005/01 (COAT-027) and ENQ3822/PIRT/006/01 (COAT-006) the average coat thickness is slightly higher. However, the visual appearance and colorimetry data indicate that at a 3% coating level, the yellow surface of the $TiO_2$ reference batch (ENQ3822/PIRT/003/01) is fully hidden,

while this is not the case for the $TiO_2$-free coatings. At a 6 % weight gain the round tablet $TiO_2$ reference batch has the lowest average coating thickness with the exception of Batch ENQ3822/PIRT/007/01.

A similar picture emerges from the oval tablet data. The reference $TiO_2$ coated tablet batch (Batch ENQ3822/PIRT/008/01) is PVA-based and was sprayed at a solids content of 25%. It had the lowest average coat thickness at a 6% weight gain (51.4 µm) of all the oval tablet lots and the second lowest at 5%, despite being considered fully coated at 5% on the basis of visual appearance and colorimetry data. The three batches that were considered having good surface coverage with $TiO_2$-free coatings at 6 % based on photography data (Batches ENQ3822/PIRT/010/01, ENQ3822/PIRT/011/01 and ENQ3822/PIRT/009/01) had a much higher average coating thickness at 6 % weight gain of over 70 µm.

The above data show that HPMC-based $TiO_2$-free coatings require a higher coating thickness compared with either HPMC-based or PVA-based $TiO_2$ containing counterparts to hide the yellow color of the tablet cores completely. Use of COAT-007, the only PEG-PVA-based $TiO_2$-free coating, (Batches ENQ3822/PIRT/007/01 and ENQ3822/PIRT/012/0) resulted in poorly coated tablets with a low average coat thickness on both round and oval tablets.

The increase in coat thickness required to achieve opacification with the HPMC-based $TiO_2$-free coatings could provide challenges in terms of debossing/logo definition which is important for tackling counterfeiting and facilitating medication identification and also for primary packaging operations e.g. in blister pockets. Both the round and oval tablets studied have typical tablet dimensions and a logo which presented a reasonable challenge during coating. However, in-filling and debossed image legibility may be more of an issue on smaller tablets e.g, 6 mm tablets.

### 19. Disintegration Times

The disintegration times of the placebo tablets are shown in Table 19.

Table 19: Disintegration times of the placebo tablets

| **Round Tablets** | | | | | |
|---|---|---|---|---|---|
| **Batch No. ENQ3822/PIRT/** | **Consortium Coat Ref.** | **Opacifier** | **Disintegration Time at %Wt Gain (min:sec)** | | |
| | | | **2%** | **4%** | **6%** |
| 003/01 | COAT-017 | $TiO_2$ | 01:37 | 02:11 | 02:30 |
| 004/01 | COAT-032 | $CaCO_3$+H | 02:07 | 02:14 | 02:22 |
| 005/01 | COAT-027 | $CaCO_3$+D | 01:35 | 01:40 | 02:01 |
| 006/01 | COAT-006 | $CaCO_3$+D | 01:38 | 01:53 | 02:01 |
| 007/01 | COAT-007 | $CaCO_3$+Talc | 02:16 | 02:25 | 02:45 |
| **Oval Tablets** | | | | | |
| **Batch No. ENQ3822/PIRT/** | **Consortium Coat Ref.** | **Opacifier** | **Disintegration Time at %Wt Gain (min:sec)** | | |
| | | | **2%** | **4%** | **6%** |
| 008/01 | COAT-018 | $TiO_2$+Talc | 01:18 | 01:33 | 01:53 |
| 009/01 | COAT-032 | $CaCO_3$+H | 01:31 | 01:48 | 02:14 |
| 010/01 | COAT-027 | $CaCO_3$+D | 01:24 | 01:39 | 01:47 |
| 011/01 | COAT-006 | $CaCO_3$+D | 01:26 | 01:47 | 01:55 |
| 012/01 | COAT-007 | $CaCO_3$+Talc | 02:06 | 02:20 | 02:25 |

Overall, the disintegration times ranged from 1.3 min and 2.75 min. For each batch the disintegration times increased very slightly with %coating weight gain. There was no significant differences between the results for the $TiO_2$-free coated tablet batches and the $TiO_2$ reference coated batches and similar results were obtained for both round and oval tablets.

## Section Summary and Conclusions

With respect to the acceptance criteria in Table 13, the visual and colorimetry data show that white to off-white round coated tablets can be achieved from yellow-colored cores using three HPMC-based $TiO_2$-free coating materials at ≤6% weight gain. COAT-006 was the only $TiO_2$-free coating to achieve surface coverage at 6% weight gain on the oval tablets based on the observations during manufacturing. The coat was judged off-white on visual assessment but had a color difference $\Delta E^*_{00}$ value of < 1 suggesting no perceptible color difference between it and the $TiO_2$-reference, COAT-017 coated batch. The discrepancy in the results may be due to the subjective nature of color perception despite the use of standardized experimental conditions, variation in coat quality and the small sample size (only 2 tablets per %weight gain per batch were used for the photography experiments and 20 for colorimetry). Colorimetry can also only measure the coating color on one tablet face and not the other or the tablet sides.

With respect to color matching of the HPMC-based coatings with the HPMC-based $TiO_2$ reference based on colorimetry, COAT-006 performed best, followed by COAT-027. However, only the COAT-006 on oval tablets met the acceptance criterion of $\Delta E^*_{00} \leq 1$ for no perceptible color difference, while COAT-006 on round tablets and COAT-027 on round and oval tablets had $\Delta E^*_{00}$ values of between 1 to 2, which suggests a difference in color could be discerned on close inspection.

The rank order of coating quality for the HPMC-based $TiO_2$-free coatings was as follows: COAT-006 = COAT-027 > COAT-032. However, a higher %weight gain and coating thickness were required compared with the $TiO_2$ containing coatings to ensure the yellow color of the tablet core surface was completely hidden. Therefore, the $TiO_2$-free coatings were less effective at opacification than the $TiO_2$ reference coatings for the placebo tablets.

The only $TiO_2$-free PEG-PVA graft copolymer-based coat (COAT-007) tested in this experimental section did not result in satisfactory tablet surface coverage and the coating thickness was low. This poor result may be due to loss of opacifying components in the coating suspension due to sedimentation.

# Experimental Part 4: Photostability Study on Placebo Coated Tablets

## Protocol

Samples from the round and oval coated tablets from the small-scale coating runs underwent photostability testing. The samples tested and testing methods are shown in Table 20. Tablets from each sample were placed in a petri dish and exposed to light in a stability cabinet corresponding to total illumination of not less than 2.4 million lux hours. This is equivalent of 2 x ICH Q1B conditions, where 1 x ICH Q1B is light exposure of not less than 1.2 million lux hours and an integrated near UV exposure of not less than 200 Watt hours/$m^2$. The results were compared with controls kept in the dark (petri dishes covered in aluminium foil) under the same conditions.

Table 20: Photostability testing of placebo coated tablets

| Test | Samples tested by % weight gain) |
|---|---|
| Appearance (Colorimetry) | 2%, 3%, 4%, 5%, 6% |
| Appearance (photographs taken of the exposed and corresponding control samples side-by-side to allow comparison) | 2%, 3%, 4%, 5%, 6% |
| Coat thickness (digital optical microscopy) | 2%, 4%, 6% |
| Quality of debossed image (digital optical microscopy) | 2%, 4%, 6% |
| Disintegration | 2%, 4%, 6% |

The visual appearance, colorimetry method, digital optical microscopy and disintegration methods are as described in Section 0.

## Results and Discussion

### 20. Colorimetry and Visual Appearance

The visual appearance data and $\Delta E^*_{00}$ color difference values for the light exposed and dark control coated placebo tablet samples are shown in Table 21. The visual appearance data show that for all 10 placebo batches, there was no visible difference in appearance between the exposed and control samples at any of the coating levels studied. This means that both the $TiO_2$ containing reference coatings and all of $CaCO_3$ based $TiO_2$-free coatings remained stable under conditions of severe light exposure. The visual appearance results were supported by color difference $\Delta E^*_{00}$ values calculated from the colorimetry data generated on the light exposed and control samples. All $\Delta E^*_{00}$ were < 1.5 and the majority < 1.

Among the round tablet batches, ENQ3822/PIRT/003/01 ($TiO_2$ HPMC-based reference, COAT-017), ENQ3822/PIRT/004/01 (COAT-032), ENQ3822/PIRT/005/01 (COAT-027) and ENQ3822/PIRT/007/01 (COAT-007) had $\Delta E^*_{00}$ values < 1 at all % coating levels. ENQ3822/PIRT/006/01 (COAT-006) had a $\Delta E^*_{00}$ value just over 1 at 1.17 at the 2% coating level only.

Among the oval tablet batches, ENQ3822/PIRT/011/01 (COAT-006) and ENQ3822/PIRT/009/01 (COAT-032) had $\Delta E^*_{00}$ values of <1 at all coating levels. Batches ENQ3822/PIRT/012/01 (COAT-007), and ENQ3822/PIRT/010/01 (COAT-027) had $\Delta E^*_{00}$ values > 1 at 2% and 4% coating levels respectively. Batch ENQ3822/PIRT/008/01 ($TiO_2$ PVA-based reference, COAT-018) had $\Delta E^*_{00}$ values > 1 at 2% and 3% coating weight gains. However, for all coated oval tablet batches at coating levels above 4%, the color difference between the exposed samples and the controls was <1.

Table 21: Colorimetry data and visual appearance for the light exposed and control coated placebo tablets

| Batch No. ENQ3822/PIRT | Consortium Coat Ref. | 2 % Coating | 3 % Coating | 4 % Coating | 5 % Coating | 6 % Coating | Appearance Exposed versus Control |
|---|---|---|---|---|---|---|---|
| | | $\Delta E^*_{00}$ Exp vs Control | $\Delta E^*_{00}$ Exp vs Control | $\Delta E^*_{00}$ Exp vs Control | $\Delta E^*_{00}$ Exp vs Control | $\Delta E^*_{00}$ Exp vs Control | |
| 003/01 | COAT-017 ($TiO_2$) | 0.54 | 0.40 | 0.46 | 0.29 | 0.30 | No visible difference at all % coatings |
| 004/01 | COAT-032 ($CaCO_3$+H) | 0.83 | 0.70 | 0.62 | 0.76 | 0.70 | No visible difference at all % coatings |
| 005/01 | COAT-027 ($CaCO_3$+D) | 0.78 | 0.50 | 0.44 | 0.46 | 0.38 | No visible difference at all % coatings |
| 006/01 | COAT-006 ($CaCO_3$+D) | 1.17 | 0.85 | 0.62 | 0.57 | 0.43 | No visible difference at all % coatings |
| 007/01 | COAT-007 ($CaCO_3$+Talc) | 0.69 | 0.83 | 0.85 | 0.73 | 0.93 | No visible difference at all % coatings |
| 008/01 | COAT-018 ($TiO_2$+Talc) | 1.46 | 1.33 | 0.80 | 0.69 | 0.95 | No visible difference at all % coatings |
| 009/01 | COAT-032 ($CaCO_3$+H) | 0.94 | 0.75 | 0.90 | 0.81 | 0.39 | No visible difference at all % coatings |
| 010/01 | COAT-027 ($CaCO_3$+D) | 0.77 | 0.90 | 1.04 | 0.48 | 0.46 | No visible difference at all % coatings |
| 011/01 | COAT-006 ($CaCO_3$+D) | 0.87 | 0.89 | 0.70 | 0.67 | 0.62 | No visible difference at all % coatings |
| 012/01 | COAT-007 ($CaCO_3$+Talc) | 1.09 | 0.77 | 0.71 | 0.84 | 0.66 | No visible difference at all % coatings |

Color code: Green = $\Delta E^*_{00} \leq 1$ (no perceptible color difference), Yellow= $\Delta E_{00}$* 1-2 (color difference perceptible on close inspection)

Overall, the data indicate that none of these tablet coatings were adversely affected by exposure to conditions equivalent of 2 x ICH Q1B photostability requirements and there was no difference in this regard between the $TiO_2$-free coats and the $TiO_2$ reference coats.

## 21. Coating Thickness

Table 22 shows the average coating thickness data (land, bellyband, surface and debossed image) for each of the light-exposed placebo tablet batches and their corresponding dark controls. There is some variation in the average coat thickness with some samples having thicker coats on the exposed sample and others on control. Since no visible difference could be discerned between the light-exposed and control samples from each of the 10 placebo batches and this result is supported by the colorimetry data, these coating thickness differences are simply variation and have no impact on the ability of each of the coatings to withstand extreme light exposure.

Table 22: Comparison average coat thickness for the light-exposed and control placebo samples

| Batch No. ENQ3822/PIRT/ | Consortium Coat Ref. | % Wt Gain | Coating Thickness (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean Exp | Min | Max | Mean Control | Min | Max |
| 003/01 | COAT-017[a] | 2 | 22.3 | 19 | 25 | 13.9 | 10 | 17 |
| 004/01 | COAT-032 | 2 | 25.7 | 15 | 34 | 26.1 | 22 | 29 |
| 005/01 | COAT-027 | 2 | 25.5 | 17 | 35 | 21.0 | 8 | 29 |
| 006/01 | COAT-006 | 2 | 27.1 | 23 | 32 | 29.3 | 21 | 42 |
| 007/01 | COAT-007 | 2 | 30.3 | 15 | 51 | 38.7 | 23 | 47 |
| 008/01 | COAT-018[a] | 2 | 23.6 | 17 | 34 | 15.9 | 11 | 28 |
| 009/01 | COAT-032 | 2 | 25.3 | 19 | 36 | 24.2 | 22 | 28 |
| 010/01 | COAT-027 | 2 | 22.3 | 18 | 28 | 24.3 | 10 | 43 |
| 011/01 | COAT-006 | 2 | 27.3 | 23 | 33 | 36.7 | 20 | 52 |
| 012/01 | COAT-007 | 2 | 28.0 | 17 | 42 | 23.0 | 18 | 35 |
| f | | | | | | | | |
| 003/01 | COAT-017[a] | 4 | 52.0 | 49 | 55 | 38.9 | 23 | 53 |
| 004/01 | COAT-032 | 4 | 40.0 | 18 | 60 | 38.3 | 21 | 54 |
| 005/01 | COAT-027 | 4 | 53.3 | 30 | 67 | 63.2 | 48 | 77 |
| 006/01 | COAT-006 | 4 | 56.4 | 26 | 74 | 37.6 | 23 | 58 |
| 007/01 | COAT-007 | 4 | 19.4 | 13 | 27 | 41.0 | 34 | 53 |
| 008/01 | COAT-018[a] | 4 | 32.6 | 17 | 55 | 50.0 | 42 | 60 |
| 009/01 | COAT-032 | 4 | 47.3 | 24 | 68 | 51.0 | 38 | 61 |
| 010/01 | COAT-027 | 4 | 60.3 | 32 | 89 | 50.7 | 33 | 68 |
| 011/01 | COAT-006 | 4 | 47.3 | 24 | 58 | 60.3 | 40 | 80 |
| 012/01 | COAT-007 | 4 | 30.6 | 11 | 44 | 39.8 | 22 | 59 |
| | | | | | | | | |
| 003/01 | COAT-017[a] | 6 | 67.4 | 45 | 97 | 57.1 | 51 | 68 |
| 004/01 | COAT-032 | 6 | 60.7 | 36 | 78 | 47.7 | 32 | 70 |
| 005/01 | COAT-027 | 6 | 69.4 | 42 | 90 | 84.3 | 54 | 131 |
| 006/01 | COAT-006 | 6 | 84.5 | 55 | 110 | 77.0 | 54 | 94 |
| 007/01 | COAT-007 | 6 | 60.8 | 37 | 73 | 51.0 | 31 | 74 |
| 008/01 | COAT-018[a] | 6 | 55.6 | 17 | 100 | 63.0 | 37 | 85 |
| 009/01 | COAT-032 | 6 | 70.1 | 34 | 94 | 69.5 | 40 | 85 |
| 010/01 | COAT-027 | 6 | 76.0 | 43 | 113 | 67.2 | 50 | 94 |
| 011/01 | COAT-006 | 6 | 57.8 | 44 | 74 | 64.4 | 44 | 91 |
| 012/01 | COAT-007 | 6 | 54.8 | 41 | 80 | 29.8 | 24 | 36 |

[a]$TiO_2$ reference coatings

## 22. Disintegration Times

Table 23 shows the disintegration times for each of the light-exposed placebo tablet batches and their corresponding dark controls.

Table 23: Comparison of the disintegration times for the light-exposed and control placebo tablets

| Round Tablets | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3822/PIRT/ | Consortium Coat Ref. | Opacifier(s) | Disintegration Time at %Wt Gain (min:sec) | | | | | |
| | | | 2% | | 4% | | 6% | |
| | | | Exp | Cont | Exp | Con | Exp | Con |
| 003/01 | COAT-017 | $TiO_2$ | 01:32 | 01:02 | 02:04 | 01:41 | 02:32 | 02:15 |
| 004/01 | COAT-032 | $CaCO_3$+H | 01:59 | 01:17 | 02:19 | 01:14 | 02:12 | 01:36 |
| 005/01 | COAT-027 | $CaCO_3$+D | 01:43 | 00:57 | 01:41 | 01:01 | 01:24 | 01:22 |
| 006/01 | COAT-006 | $CaCO_3$ | 01:43 | 00:44 | 01:32 | 01:11 | 01:28 | 01:18 |
| 007/01 | COAT-007 | $CaCO_3$+Talc | 00:59 | 00:47 | 01:13 | 01:04 | 01:32 | 01:33 |
| **Oval Tablets** | | | | | | | | |
| Batch No. ENQ3822/PIRT/ | Consortium Coat Ref. | Opacifier | Disintegration Time at %Wt Gain (min:sec) | | | | | |
| | | | 2% | | 4% | | 6% | |
| | | | Exp | Con | Exp | Con | Exp | Con |
| 008/01 | COAT-018 | $TiO_2$+Talc | 01:42 | 01:08 | 01:50 | 01:38 | 02:11 | 02:04 |
| 009/01 | COAT-032 | $CaCO_3$+H | 01:00 | 01:10 | 01:35 | 01:18 | 01:42 | 01:40 |
| 010/01 | COAT-027 | $CaCO_3$+D | 01:43 | 01:16 | 01:50 | 01:22 | 02:06 | 01:40 |
| 011/01 | COAT-006 | $CaCO_3$ | 01:55 | 01:17 | 02:03 | 01:13 | 02:05 | 01:44 |
| 012/01 | COAT-007 | $CaCO_3$+Talc | 02:03 | 01:37 | 02:19 | 01:51 | 03:03 | 02:09 |

The results show that light exposure did not alter the disintegration times significantly compared to the control samples for either the placebo tablets coated with the $TiO_2$-free coatings or the $TiO_2$ reference coatings. There is a trend for slightly increased disintegration times following light exposure. However, this occurs for both $TiO_2$ containing and $TiO_2$-free coated batches and may be related due to tablet variability or determination of the disintegration test end-point.

## Section Summary and Conclusion

Overall, the data indicate that none of these tablet coatings were adversely affected by exposure to conditions equivalent of 2 x ICH Q1B photostability requirements and there was no difference in this regard between the $TiO_2$-free coats and the $TiO_2$ reference coats.

# Experimental Part 5: Coating of Active Batches

## Rationale for Selection of Active Core Tablets

In order to assess the impact of $TiO_2$-free coatings on API stability, small-scale (3 kg) coating runs were carried out on active tablet cores of four different compounds. All of the compounds are included in film-coated tablet products authorised in the EU. The compounds were selected due to their known instability under certain conditions e.g., light, moisture etc. Therefore, their stability may be compromised as a result of a change in tablet coating composition. Details of the active tablet cores, their sourcing, batch numbers and the rationale for selection are shown in Table 114.

Table 24: Active core tablet details

| Tablet Core | Description | Rationale for Selection | Batch No. | Manufacturer/Supplier |
|---|---|---|---|---|
| Corinfar (nifedipine) 10 mg retard tablet cores | Round, yellow biconvex tablets No embossing | Potential for photodegradation | G170349 | PLIVA Croatia Ltd. Prilaz baruna, Filipovica 25, 10000 Zagreb, Croatia. |
| Olmesartan 20 mg tablet cores | White, round, biconvex tablets Embossed OL20 on one side only | Potential moisture sensitivity | G174627 | Actavis Ltd. BLB015-016 Bulebel Industrial Estate Zejtun ZTN3000, Malta. |
| Rosuvastatin 10 mg tablet cores | White, round, bioconvex tablets No embossing | Potential for photodegradation Potential for hygroscopicity and salt metathesis or disproportionation | G174604 G174605 | Hemofarm A.D. Beogradiski put b.b. 26300 Vršac Serbia. |
| Prasugrel HCl 10 mg tablet cores | White, oval, bioconvex tablets No embossing | Potentially sensitive to alkali and potential for salt disproportionation | G175131 | Hemofarm A.D. Beogradiski put b.b. 26300 Vršac Serbia. |

## Materials, Processing and Testing

### 23. Materials

The active core tablets, described in Section 0, were coated with either $TiO_2$-free or $TiO_2$-containing coating suspensions in 23 small-scale trials at a batch size of 3 kg. The coating materials used are shown in Table 25. Five coating runs were conducted with the nifedipine cores, four runs with olmesartan tablet cores, three with rosuvastatin (Batch No. G174604) and seven (plus two repeat batches) with rosuvastatin (Batch No. G174605) and four with prasugrel. One coating run for nifedipine, olmesartan and prasugrel core tablets was carried out with a $TiO_2$-containing coating suspension (COAT-024, COAT-025 and COAT-026) as a reference. Two reference coating runs were carried out for rosuvastatin (COAT-017 and COAT-018).

The coating suspensions were prepared at the manufacturer's target or the middle of the target range for %solids content and according to the instructions given by the manufacturer. The properties of these suspensions and their ease of manufacture are described in Section 3.

Table 25: Coating materials used for coating the active cores

| Corinfar (nifedipine) Retard 10 mg Tablet Cores | | | | | Batch No. G170349 |
|---|---|---|---|---|---|
| **Coating Run** | **Coated Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Reference** | **Film-Former** | **Opacifier** | **% Solids (w/w)** |
| 11 | 001/01 | COAT-024[a] | HPMC | $TiO_2$ | 15 |
| 12 | 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 16 |
| 13 | 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 18 |
| 14 | 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 11 |
| 15 | 005/01 | COAT-023 | PVA | F+Talc | 18.5 |
| **Olmesartan 20 mg Tablet Cores** | | | | | **Batch No. G174627** |
| **Coating Run** | **Coated Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Reference** | **Film-Former** | **Opacifier** | **% Solids (w/w)** |
| 16 | 006/01 | COAT-025[a] | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 18.5 |
| 17 | 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | 20 |
| 18 | 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 20 |
| 19 | 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 20 |
| **Rosuvastatin 10 mg Tablet Cores** | | | | | **Batch Nos. G174604 and G174605** |
| **Coating Run** | **Coated Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Reference** | **Film-Former** | **Opacifier** | **% Solids (w/w)** |
| 20 | 010/01[b] | COAT-018[a] | PVA | $TiO_2$+Talc | 25 |
| 21 | 011/01[b] | COAT-017[a] | HPMC | $TiO_2$ | 15 |
| 22 | 012/01[b] | COAT-020 | HPMC+HPC | Rice Starch+D | 15 |
| 23 | 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 17 |
| 24 | 014/01 | COAT-010 | HPMC | Rice Starch+D | 20 |
| 25[d] | 015/01 | COAT-030[e] | HPMC | B+E | 12 |
| 25 (repeat) | 015/02 | COAT-030[e] | HPMC | B+E | 12 |
| 26[d] | NA (Failed Batch) | COAT-005 | HPMC | MgO | 11 |
| 26 (repeat) | 016/01 | COAT-005 | HPMC | MgO | 11 |
| 27 | 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 16 |
| 28 | 018/01 | COAT-034 | HPMC | Rice starch | 18 |
| 29 | 019/01 | COAT-023 | PVA | F+Talc | 18.5 |
| **Prasugrel HCl 10 mg Tablet Cores** | | | | | **Batch No. G175131** |
| **Coating Run** | **Coated Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Reference** | **Film-Former** | **Opacifier** | **% Solids (w/w)** |
| 30 | 020/01 | COAT-026[a] | HPMC | $TiO_2$+$Fe_2O_3$ | 15 |
| 31 | 021/01 | COAT-016 | HPMC | Rice Starch+D+ $Fe_2O_3$ | 20 |
| 32 | 022/01 | COAT-002 | HPMC | Rice starch+ A+B+D+$Fe_2O_3$ | 16 |
| 33 | 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 12[c] |

[a]$TiO_2$ containing coating material used as a comparison.

[b]Tablet cores Batch No. G174604 used.

[c]% Solids - COAT-030 plus COAT-031 (95%:5%)

[d]Batches not used for further work.

[e]Coating material labelled as producing a clear coat. However, coating material constituents will result in some opacification.

## 24. Equipment and Coating of Active Cores

The small-scale (3kg) batches of active-containing cores were coated in an O'Hara Labcoat coater fitted with a 15-inch pan. Since nifedipine and rosuvastatin are light-sensitive, coating was carried out under yellow lighting. However, the appearance checks were carried out under standard laboratory lighting. The other tablet cores were coated under standard laboratory lighting.

Spray rates of 20 g/min were targeted across all runs. Coating gun nozzle sizes of 1.0 mm or 1.2 mm were used during the 23 coating trials. Coating process parameters were tailored based on the suppliers' literature. However, the majority of the coating runs were performed with identical set points for atomising and pattern air pressure (1.5 bar), inlet air flow (250 $m^3$/hr), pan differential pressure (-0.25 mbar) and pan speed (18 rpm). Inlet air temperatures were adjusted to maintain the manufacturers recommended exhaust or tablet bed temperatures as appropriate.

The tablets were coated to a target 6% weight gain. Approximately 640 tablets were removed after a 2%, 3%, 4% and 5% weight gain for analysis. The point of sampling was determined based on the theoretical amount of coating suspension sprayed to achieve a desired weight gain. However, some samples may have been taken earlier, if IPC testing showed that the weight gain had already been achieved. Coating was not stopped until a 6% weight gain had been reached as determined through IPC testing. After coating the tablet batches were dried and cooled. The batches were stored in cable-tied PE bags in a HDPE drum with or without a foil bag depending on the nature of the cores.

250 coated tablets were sampled from the bulk. These samples served as the $T_0$ samples for the accelerated stability studies described in Section 0.

The majority of batches were coated with no or only minor issues. However, Run 25 (coating of rosuvastatin with COAT-030), had to be repeated due to issues with gun blockages. The initial attempt at coating rosuvastatin with COAT-005 failed (Run 26) and the run was restarted again twice due to gun blockages. Issues were also found on preparation of the coating suspensions (see Section 3).

## 25. Analytical Testing

The film-coated tablets samples after a weight gain of 2%, 3%, 4% 5% and 6% were assessed for appearance to evaluate the %weight gain required to achieve complete coverage and opacification of the tablets' surface plus other testing including photostability (see Section 0). The testing of the tablets was as described in Table 26.

Table 26: Testing of active coated tablets

| Attribute | Methodology | Samples tested by %weight gain |
|---|---|---|
| **Samples from all 23 coating trials** | | |
| Appearance – Visual Assessment | Photography | 2%, 3%, 4%, 5%, 6% |
| Appearance - Colorimetry | DigiEye | 2%, 3%, 4%, 5%, 6% |
| Coat thickness | Digital optical microscopy | 2%, 4%, 6% |
| Quality of debossed image | Digital optical microscopy | 2%, 4%, 6% if present[a] |
| Solid state | X-ray powder diffraction (XRPD) | 2%, 4%, 6% |
| Disintegration | Ph.Eur. 2.9.1 | 2%, 4%, 6% |
| **Corinfar (Nifedipine) 10 mg Retard Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/UV spectroscopy | 2%, 4%, 6% |
| **Olmesartan 20 mg Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/UV spectroscopy | 2%, 4%, 6% |
| **Rosuvastatin 10 mg Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/HPLC | 2%, 4%, 6% |
| **Prasugrel HCL 10 mg Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/HPLC | 2%, 4%, 6% |

[a]Only olmesartan tablets are debossed

## 26. Analytical Testing Methodology - Visual Assessment

10 tablets were sampled from each batch of tablets (2 tablets per coating level). They were photographed using a Sony a6000 camera fitted with a Tamron 35mm F/2.8 Di OSD M1:2 lens. The visual assessment focused on the coat coverage of the tablets at different coating levels on both bellyband and tablet faces as well as inspection of debossing for any signs of infilling. The acceptance criteria for an acceptable coating are as described in Table 27.

Table 27: Acceptance criteria for coatings based on visual appearance

| Criteria | Evaluation | | |
|---|---|---|---|
| | **Acceptable** | **Acceptable with Caveats** | **Not Satisfactory** |
| Color and appearance at ≤6% coating level | Tablet same color as coat at 6% weight gain | For white coatings only -tablet core hidden but off-white coat, not white[a] | Tablet core color visible Spray pattern visible |
| Coverage | Even and underlying core not visible | Uneven at low % weight gain | Uneven at high 6% weight gain and/or yellow core still visible |
| Debossing | Legible and no in-filling | Legible with minor amount of in-filling | Not legible |
| Surface | Smooth and glossy/matt | Slight surface roughness | Surface not smooth |
| Color and coverage at same % weight gain as $TiO_2$ reference | Yes | Acceptable coat achieved but ≤2% greater weight gain required | Coat inacceptable or acceptable but >2% greater weight gain required. |
| Overall acceptable coat achieved at ≤6% weight gain | Yes | Overall coat acceptable with caveats | Acceptable coat not achieved |

[a]Off-white coatings may not be acceptable in some international markets and will not enable color matching to white tablets whether these are coated with $TiO_2$ containing coats or not.

## 27. Analytical Testing Methodology - Colorimetry

The DigiEye Version 7 equipment was used for the colorimetry experiments and as described in Section 14.

The $\Delta E^*_{00}$ color difference values were interpreted as follows [14]:

White Tablets

The $TiO_2$-free coatings were determined to match the color of the corresponding $TiO_2$ reference if $\Delta E^*_{00} \leq 1.0$. $\Delta E^*_{00} \leq 1.0$ is considered to mean a color difference which is not perceptible to the eye. A $\Delta E^*_{00} > 1.0$ was considered to be noticeable to a patient.

Colored Tablets

For colored tablets, the $\Delta E^*_{00}$ values were interpreted as follows:

$\Delta E^*_{00} \leq 1.0$ Color difference not perceptible to the human eye.

$\Delta E^*_{00}$ 1 - 2 Color difference perceptible through close observation.

$\Delta E^*_{00} > 2$ Color difference noticeable at a glance.

The acceptance criterion for color matching of colored $TiO_2$-free coated tablets to the corresponding $TiO_2$ reference was $\Delta E^*_{00} < 2$.

The rationale for the difference in acceptance criteria for white and colored tablets is as described in Section 14.

## 28. Analytical Testing Methodology - Optical Microscopy

Digital microscopy images were acquired using a Keyence VHX-2000 at x100 and x300 magnification as described in Section 15. Coating thickness was measured at four different areas: on the tablet land, belly, surface and at the debossed image (olmesartan tablets only).

### 29. Analytical Testing Methodology - X-ray Powder Diffraction

Pre-cut active coated and core tablets were ground and analysed by XRPD. XRPD analyses were carried out using a Panalytical Empyrean diffractometer equipped with a Cu X-ray tube and a PIXcel 1D-Medipix3 detector system. The diffractograms of the coated tablets were compared with those of the uncoated tablet cores.

### 30. Assay, Related Impurities and Dissolution

The methods for assay, related impurities and dissolution for nifedipine, olmesartan, rosuvastatin and prasugrel were based on the methods provided by the manufacturers of the active cores.

## Results - Testing of Active 2%, 4% and 6% Film-coated Tablets

### 31. Visual Appearance and Comparison at Various Weight Gains

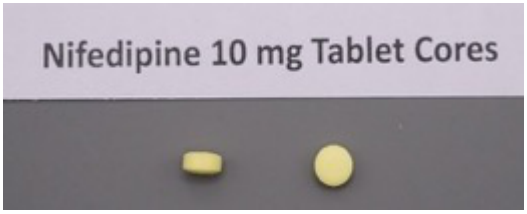
Nifedipine Retard Coated Tablets

Figure 15 shows photographs of the coated tablet batches of nifedipine retard at various %coating weight gains and the uncoated cores. Since there is a slight yellow tinge to the photographs due to the conditions used, Table 28 provides a detailed visual description of the tablets' appearance.

Figure 15: Visual appearance of coated nifedipine retard tablets and uncoated cores

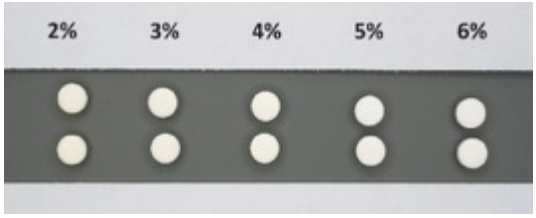
Nifedipine retard 10 mg tablet cores

Batch No. G170349



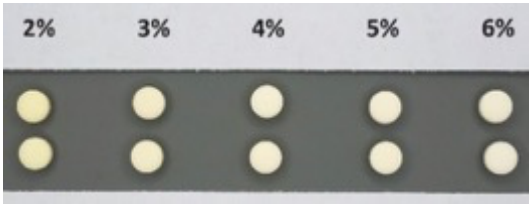

ENQ3822/AIRT/001/01

COAT-024 (HPMC, $TiO_2$)



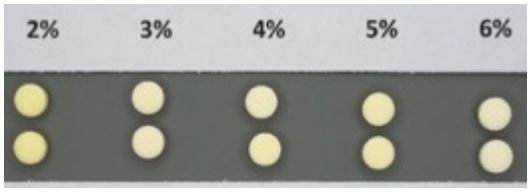

ENQ3822/AIRT/002/01

COAT-001 (HPMC+HPC, $MgCO_3$+A+B)



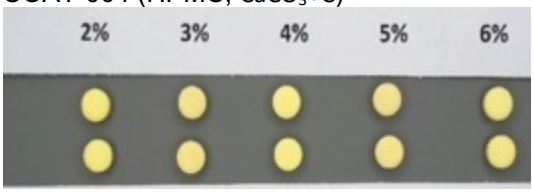

ENQ3822/AIRT/003/01

COAT-033 (HPMC, $CaCO_3$+D+F)



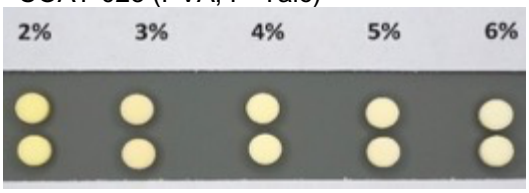

ENQ3822/AIRT/004/01

COAT-004 (HPMC, $CaCO_3$+C)




ENQ3822/AIRT/005/01

COAT-023 (PVA, F+Talc)




The coating experiments on the nifedipine retard cores evaluated the ability of three HPMC-based $TiO_2$-free coatings and one PVA-based $TiO_2$-free coating to cover and opacify the surface of round yellow core tablets with no debossed image. All of the coating materials used in this experiment contained either $CaCO_3$ or divalent metal opacifiers. The $TiO_2$-containing reference coat was COAT-024 which is HPMC-based. The photographs clearly show that only the $TiO_2$-reference was capable of opacifying the yellow surface.

Based on the visual description of the tablets, tablet surface coverage with the $TiO_2$ reference coating (COAT-024) was complete at a 5% weight gain. This agreed with visual appearance checks during manufacture which found that completely white tablets were obtained at a 5% coating level.

With COAT-001, COAT-033 and COAT-023, the yellow color became paler as the %coating level increased. Therefore, it may have been possible to achieve white tablets with much higher coating weight gains (> 6%) using these coatings. However, coating weight gains of 7% and above would prolong coating times approximately 1.5 to 2 times compared with that required for the $TiO_2$ reference coat. This increases the risk of tablet damage and API thermal instability. The visual appearance descriptions for the $TiO_2$-free coated tablets are in line with the appearance checks made during the manufacturing process.

Table 28: Visual appearance of the coated nifedipine retard tablets and uncoated cores

| Coating Run | Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Color at 6% Weight Gain | Coverage | Surface | Color & Coverage Achieved at Same %Weight Gain as $TiO_2$ Reference | Acceptable Coat at 6% Weight Gain |
|---|---|---|---|---|---|---|---|
| Nifedipine Cores | G170349[a] | NA | Round bright yellow tablet[a] | NA | Glossy bellyband and slightly less glossy tablet face[a] | NA | NA |
| 11 | 001/01 | COAT-024[b] | 2% slightly yellow<br>3% -slight tint<br>4-6% white | Even on bellyband and tablet faces at all coating levels. | Smooth and matt | NA | Yes at 5% |
| 12 | 002/01 | COAT-001 | 2% yellow became paler with increasing %coating. | Even on bellyband and tablet faces at all coating levels. | Smooth and matt | No | No - tablets still pale yellow |
| 13 | 003/01 | COAT-033 | Color changes from yellow to paler yellow from 2% to 6%. | Even on bellyband and tablet faces at all coating levels | Smooth | No | No - tablets still pale yellow |
| 14 | 004/01 | COAT-004 | Bright yellow and similar on all coating levels. Spraying pattern is visible on all tablets. | Even on bellyband and tablet faces at all coating levels. | Smooth and slightly glossy | No | No - tablets bright yellow |
| 15 | 005/01 | COAT-023 | Pale yellow and became paler with increasing coating level. | Even on bellyband and tablet faces at all coating levels. | Smooth and matt | No | No - tablets still pale yellow |

[a]Core tablet batch no, appearance and surface [b]$TiO_2$ reference

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

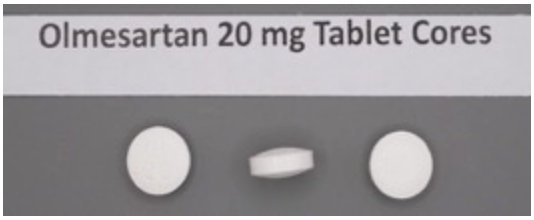
Olmesartan Coated Tablets

Figure 16 shows photographs of the coated tablet batches of olmesartan at various %coating weight gains and the uncoated cores. A detailed visual description of the tablets' appearance is included in Table 29. This description is evaluated against the acceptance criteria in Table 27.

Figure 16: Visual appearance of coated olmesartan tablets and uncoated cores

Olmesartan 20 mg tablet cores

Batch No. G174627

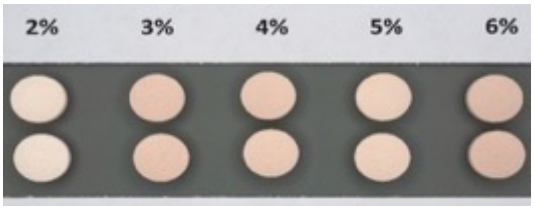



ENQ3822/AIRT/006/01

COAT 25 (PVA, $TiO_2$)


ENQ3822/AIRT/007/01

COAT-013 (PVA+HPMC, $CaCO_3$+Talc+$Fe_2O_3$)




ENQ3822/AIRT/008/01

COAT-015 (PVA, $CaCO_3$+Talc+$Fe_2O_3$)


ENQ3822/AIRT/009/01

COAT-014 (PVA, $CaCO_3$+Talc+$Fe_2O_3$)

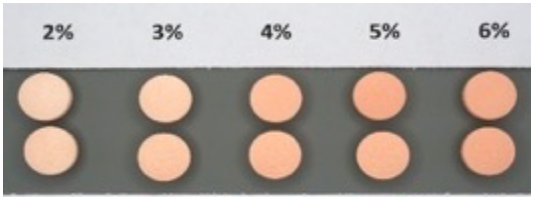


Table 29: Visual appearance of the coated olmesartan tablets and uncoated cores

| Coating Run | Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Color at 6% Weight Gain | Coverage | Debossing | Surface | Color & Coverage Achieved at Same %Weight Gain as $TiO_2$ Reference | Acceptable Coat at 6% Weight gain |
|---|---|---|---|---|---|---|---|---|
| Olmesartan Cores | G174627[a] | NA | White tablets[a] | NA | OL20 on one side | NA | Glossy on bellyband and tablet face[a] | NA |
| 16 | 006/01 | COAT-025[b] | Light pink at all coating levels | Even on bellyband and tablets faces on all coating levels. | Legible No infilling at any coating level. | Smooth and matt | NA | Yes at 3% |
| 17 | 007/01 | COAT-013 | Light pink at 2%. Darkens as %coating increases. Dark pink at 6 %. | Even on bellyband and tablets faces on all coating levels. | Legible, no infilling at any coating level. | Smooth and matt | Yes, pale pink at 2% | Pale pink at 2%, however, color darkens with increasing weight gain. |
| 18 | 008/01 | COAT-015 | Light pink at 2%. Darkens as %coating increases. Salmon/pink at 6 % level. | Even on bellyband and tablets faces on all coating levels. Spray pattern observed at all coating levels. | Legible, no infilling at any coating level. | Smooth and slightly glossy | No, spray pattern observed | No, spray pattern observed Color darkens as %coating increases. |
| 19 | 009/01 | COAT-014 | Light pink at 2%. Darkens as %coating increases. Salmon/pink at 6 % level. | Even on bellyband and tablets faces on all coating levels. Spray pattern observed at all coating levels. | Legible, no infilling at any coating level. | Smooth and slightly glossy | No, spray pattern observed | No, spray pattern observed Color darkens as %coating increases. |

[a]Core tablet batch no, appearance and surface [b]$TiO_2$ reference

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

The coating experiments with the olmesartan white, round tablet cores with a debossed image on one side evaluated the ability of three pink, $TiO_2$-free PVA-based coating suspensions to coat the white tablet surfaces. In each case $CaCO_3$ was considered to be the main opacifier. Although other excipients such as talc and the colorant $Fe_2O_3$ in the different coatings will contribute to opacification. The results were compared to tablets coated with a pink $TiO_2$-containing reference, COAT-025.

The visual descriptions of the coated tablet batches are in line with observations on the coated tablet batches' appearance recorded during manufacture. Based on the photographs, the tablets coated with the reference, COAT-025, were considered completely coated at a 2% weight gain, while based on the manufacturing observations, this was thought to occur at the 3 % weight gain. The latter fits with the manufacturer's recommendation of a 3% to 7% weight gain for this coating material.

The tablets coated with COAT-013, a $TiO_2$-free coating, were also considered to be completely coated at a 2% weight gain based on both the photographs and manufacturing observations. However, while the $TiO_2$ reference coated tablets remained a light pink color at all %coating weight gains studied, the tablets coated with COAT-013 darkened with increasing % weight gain. This would suggest that coverage was not complete at 2%, despite the apparent light pink color being similar to that of the reference coat. The color development on the tablets coated with COAT-014 and COAT-015 followed a similar pattern, with the tablets coated at a 2% coating level being pale pink in color and higher %weight gains resulting in a darker shade of pink. This phenomenon (ie, different tablet colors/appearance as a result of variations in %weight gain) is likely to result in less robust coating processes using these materials as it will be theoretically possible to see differences in product appearance as a result of variations in coating efficiency, or differences in process parameters, material attributes or other factors. This would suggest that COAT-013, COAT-014 and COAT-015 are inferior to the $TiO_2$ reference coat.

In addition, based on the photographs, the tablets coated with COAT-014 and COAT-015 displayed a spray pattern at all coating levels suggesting issues with the homogeneity of the coating suspension and/or uneven spraying conditions. The observations recorded during manufacturing support this with some tablets displaying red specks. However, overall COAT-015 produced a more homogenous coat than COAT-014 with complete coverage occurring at a 4% weight gain, while the coating on the tablets sprayed with COAT-014 was still non-homogeneous at the 6% weight gain.

Since the coating parameters were set as per the coating material manufacturer's recommendations, sub-optimal coating suspension formulation and/or preparation were suspected. Although it should be noted that for both these coating materials, no significant issues were reported during coating suspension preparation. Some color variations on the surface of the coating suspension were observed for COAT-015 during preparation but these cleared with mixing and were not observed during coating or at the end of the run.

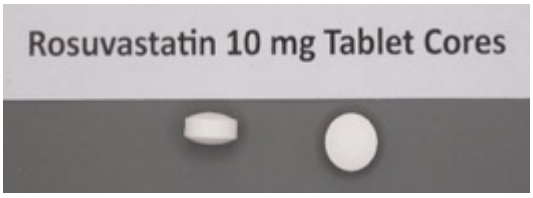
Rosuvastatin Coated Tablets

Figure 17 shows the photographs of the coated tablet batches of rosuvastatin at various %coating weight gains and the uncoated cores. A detailed visual description of the tablets' appearance is included in Table 30. This description is evaluated against the acceptance criteria in Table 27.

Figure 17: Visual appearance of coated rosuvastatin tablets and uncoated cores

Rosuvastatin 10 mg tablet cores

Batch No. G174604

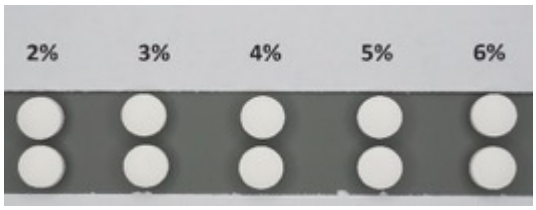



ENQ3822/AIRT/010/01

COAT-018 (PVA, $TiO_2$+Talc)

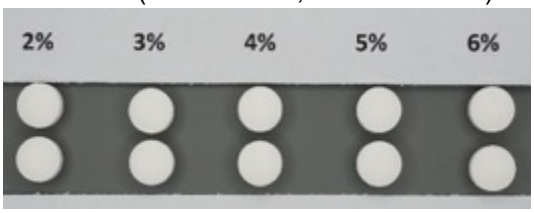

ENQ3822/AIRT/011/01

COAT-017 (HPMC, $TiO_2$)




ENQ3822/AIRT/012/01

COAT-020 (HPMC+HPC, Rice Starch+D)




ENQ3822/AIRT/013/01

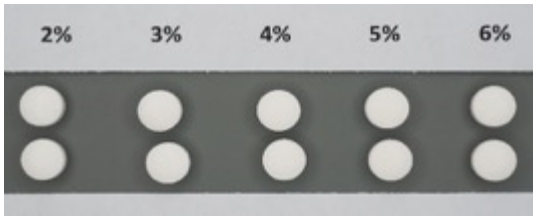
COAT-019 (HPMC, $CaCO_3$+D+E)


ENQ3822/AIRT/014/01

COAT-010 (HPMC, Rice Starch+D)

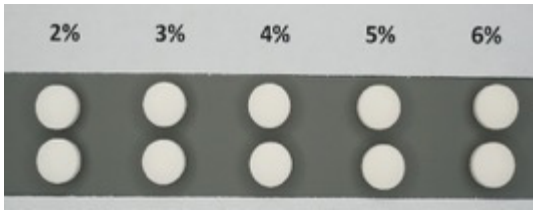

ENQ3822/AIRT/015/02

COAT-030 (HPMC, B+E)



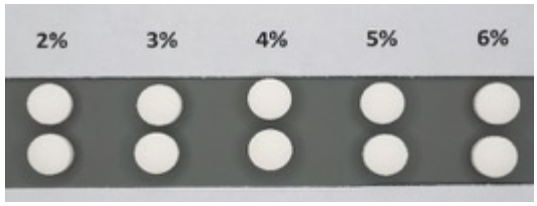
ENQ3822/AIRT/016/01

COAT-005 (HPMC, MgO)



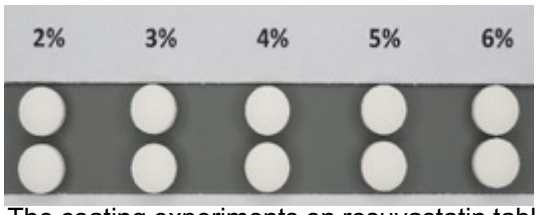

ENQ3822/AIRT/017/01

COAT-001 (HPMC+HPC, $MgCO_3$+A+B)



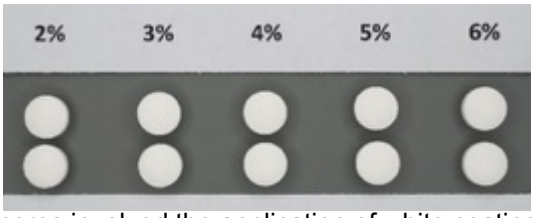

ENQ3822/AIRT/018/01

COAT-034 (HPMC, Rice Starch)




ENQ3822/AIRT/019/01

COAT-023 (PVA, F+Talc)




The coating experiments on rosuvastatin tablet cores involved the application of white coatings to white round tablets with no debossed image. Two of the batches were coated with $TiO_2$-containing coating suspensions, one based on HPMC (COAT-017) and the other on PVA (COAT-018) . The $TiO_2$-free coatings were HPMC-based with the exception of COAT-023 (coating used for Batch ENQ3822/AIRT/019/01). The white $TiO_2$-free coatings contained a variety of opacifiers such as $CaCO_3$, divalent metal opacifiers or rice starch.

As the tablet cores were white and coated with white coatings, for the majority of batches it was difficult to determine at which %weight gain coverage was complete, and how this compared with the $TiO_2$ reference coatings. The exception was COAT-005, used for Batch ENQ3822/AIRT/016/01, which produced off-white tablets at lower %weight gains and off-white to cream tablets at the 5% and 6 % coating levels. The surface texture of Batch ENQ3822/AIRT/011/01 coated with the $TiO_2$ reference, COAT-017, changed from slightly glossy and coarse at 2% and 3% weight gain to smoother at higher coating levels. This may indicate that surface coverage is complete at around 4% weight gain. However, given the similarity in color between the tablets coated at the different coating levels, this could not be confirmed.

In summary, for the rosuvastatin coated tablets, it was not possible to compare the $TiO_2$-free coatings versus the reference coatings based on visual assessment of the coated rosuvastatin tablets alone.

Table 30: Visual appearance of the coated rosuvastatin tablets and corresponding uncoated cores

| Coating Run | Coated Tablet Batch No. ENQ3822/AIRT/ | Consort Coat Ref | Color at 6% weight gain | Coverage | Surface | Color & Coverage Achieved at Same %Weight Gain as $TiO_2$ Reference | Acceptable Coat at 6% Weight Gain |
|---|---|---|---|---|---|---|---|
| Rosuvastatin Cores | G174604[a] | NA | White tablets[a] | NA | Smooth and glossy on bellyband and face[a] | NA | NA |
| 20 | 010/01 | COAT-018[bc] | White tablets at all coating levels. | Even on bellyband and tablets faces. | Slightly glossy and coarse[c] | Not possible to assess as both core and coat are white. | |
| 21 | 011/01 | COAT-017[b] | White tablets at all coating levels. | Even on bellyband and tablets faces. | Slightly glossy and coarse at the 2 % and 3 % levels Becomes smoother at higher coating levels | Not possible to assess as both core and coat are white. | |
| 22 | 012/01 | COAT-020 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Matt | Not possible to assess as both core and coat are white. | |
| 23 | 013/01 | COAT-019 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Slightly glossy | Not possible to assess as both core and coat are white. | |
| 24 | 014/01 | COAT-010 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Matt and slightly coarse | Not possible to assess as both core and coat are white. | |
| 25 (repeat) | 015/02 | COAT-030 | White tablets at all coating levels. | Even on bellyband and tablets faces.<br>Edges looks smoother on the 5% and 6% coated tablets. | Slightly glossy and smooth | Not possible to assess as both core and coat are white. | |
| 26 (repeat) | 016/01 | COAT-005 | 2 % tablets are off-white and become off-white/cream at 5 % and 6 %. | Even on bellyband and tablets faces.<br>Edges looks smoother on the 5% and 6% coated tablets. | Smooth and slightly glossy | Difficult to compare with the $TiO_2$ reference batches as not possible to determine when they are fully coated as both the core and coat are white. Based on color development alone, it would suggest that coverage is complete at 5% weight gain. | |
| 27 | 017/01 | COAT-001 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Matt and smooth | Not possible to assess as both core and coat are white. | |
| 28 | 018/01 | COAT-034 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Matt and smooth | Not possible to assess as both core and coat are white. | |
| 29 | 019/01 | COAT-023 | White tablets at all coating levels. | Even on bellyband and tablets faces. | Glossy and slightly coarse | Not possible to assess as both core and coat are white. | |

[a]Core tablet batch no, appearance and surface [b]$TiO_2$ reference

[c]Coating suspension prepared at 25% w/v, the maximum recommended %solids concentration. The manufacturer has now recommended 20%w/v solids for an improved finish.

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

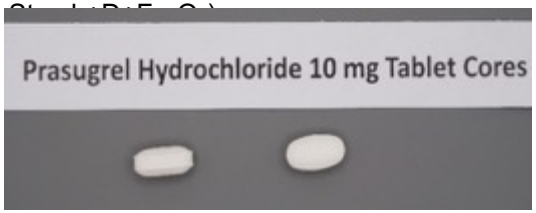
Prasugrel Coated Tablets

Figure 18 shows the photographs of the coated tablet batches of prasugrel at various %coating weight gains and the uncoated cores. A detailed visual description of the tablets' appearance is included in Table 31. This description is evaluated against the acceptance criteria in Table 27.

Figure 18: Visual appearance of coated prasugrel tablets and corresponding uncoated cores

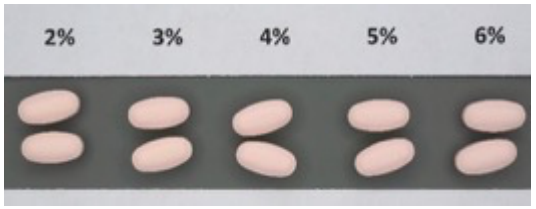
Prasugrel 10 mg tablet cores

Batch No. G175131



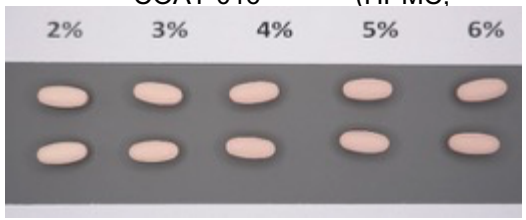

ENQ3822/AIRT/020/01

COAT-026 (HPMC, $TiO_2$+$Fe_2O_3$)



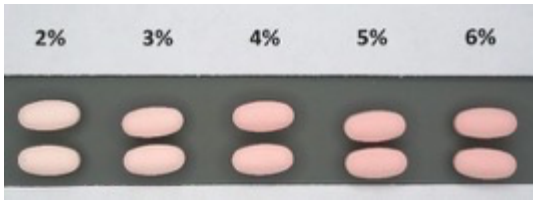

ENQ3822/AIRT/021/01

COAT-016 (HPMC, Rice




ENQ3822/AIRT/022/01

COAT-002(HPMC, Rice Starch+A+B+D+$Fe_2O_3$)




ENQ3822/AIRT/023/01

COAT-030 & COAT-031 (HPMC, B+E+$Fe_2O_3$)

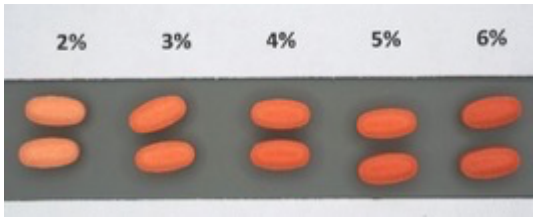


Table 31: Visual appearance of the coated prasugrel tablets and uncoated cores

| Coating Run | Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Color at 6% Weight gain | Coverage | Surface | Color & Coverage Achieved at Same %Weight Gain as $TiO_2$ Reference | Acceptable Coat at 6% Weight Gain |
|---|---|---|---|---|---|---|---|
| Prasugrel Cores | G175131[a] | NA | Off-white tablets[a] | NA | Glossy bellyband and matt faces[a] | NA | NA |
| 30 | 020/01 | COAT-026[b] | Similarly pink at all coating levels. | Even on bellyband and tablets faces. | Glossy and smooth | NA | 2% |
| 31 | 021/01 | COAT-016 | Light pink at 2% Slightly darker at higher levels. Spray pattern visible on all levels Less pronounced on the 4 %, 5 % and 6 % tablets. | Even on bellyband and tablets faces. | Matt and smooth | No, spray pattern visible at all coating levels. | No, spray pattern visible at all coating levels. |
| 32 | 022/01 | COAT-002 | Light pink at 2% and pink at 6 % level. | Even on bellyband and tablets faces. | Matt and smooth | No | At 6% |
| 33 | 023/01 | COAT-030/COAT-031 | Light pink/red at 2%/ light red at 3% and red at 4%, 5% and 6% levels. | Even on bellyband and tablets faces Spray pattern visible at all levels. | Glossy and smooth | No, spray pattern visible at all coating levels. | No, spray pattern visible at all coating levels. |

[a]Core tablet batch no, appearance and surface [b]$TiO_2$ reference

Color Code: Green = Acceptable, Yellow = Acceptable with caveats, Red = Not satisfactory

The prasugrel tablet cores were coated with HPMC-based colored coatings, three of which were $TiO_2$-free. Two contained rice starch or rich starch in combination with Opacifier A for opacification. The third was a combination of a clear coat and a colored admix which produced a red coating. The mixture of the clear coat (COAT-030) and the red admix (COAT-031) contained $Fe_2O_3$ and Opacifiers B and E. The $TiO_2$-free coated tablets were compared with those coated with the HPMC-based, pink, $TiO_2$ reference (COAT-026).

Use of COAT-030/COAT-031 and COAT-016 resulted in tablets with a visible spray pattern at all coating levels. Issues with dispersibility and agglomeration were identified with use of the COAT-030/031 combination (see Section 3). However, COAT-016 coating suspension was found to be easy to prepare (see Table 8). It is not clear why COAT-016 should have resulted in a spray pattern. COAT-002 produced a light pink coat at 2% weight gain which developed into an acceptable pink coat at the 6% coating level. Therefore, it required a much higher coating weight gain than the $TiO_2$ reference to achieve tablet surface coverage.

The observations made during manufacture of the $TiO_2$ reference coated tablets (Batch ENQ3822/AIRT/020/01) indicated that tablet surface coverage was complete at the 2% coating level. This is in agreement with the photography results. With respect to COAT-016 (Batch ENQ3822/AIRT/020/01) and COAT-002 (Batch ENQ3822/AIRT/021/01), good coverage was achieved at ≥4% weight gain and at 6% weight gain respectively. In contrast to the photography results, no spray pattern was observed on the tablets coated with COAT-016 during manufacturing. However, bearding was reported 20 minutes into the coating run and the nozzle was cleaned. This early issue may be the cause of the spray pattern on the tablets. In Batch ENQ3822/AIRT/021/01 two tablets with pink dots were observed in the tablet sample coated with COAT-002. The tablet sample coated with COAT-030/COAT-031 (Batch ENQ3822/AIRT/023/01) appeared homogenous at 5% and 6% weight gain with no spray pattern observed. The slight differences between the visual appearance results from the photographed samples and those noted during manufacturing may reflect the small size of the samples tested.

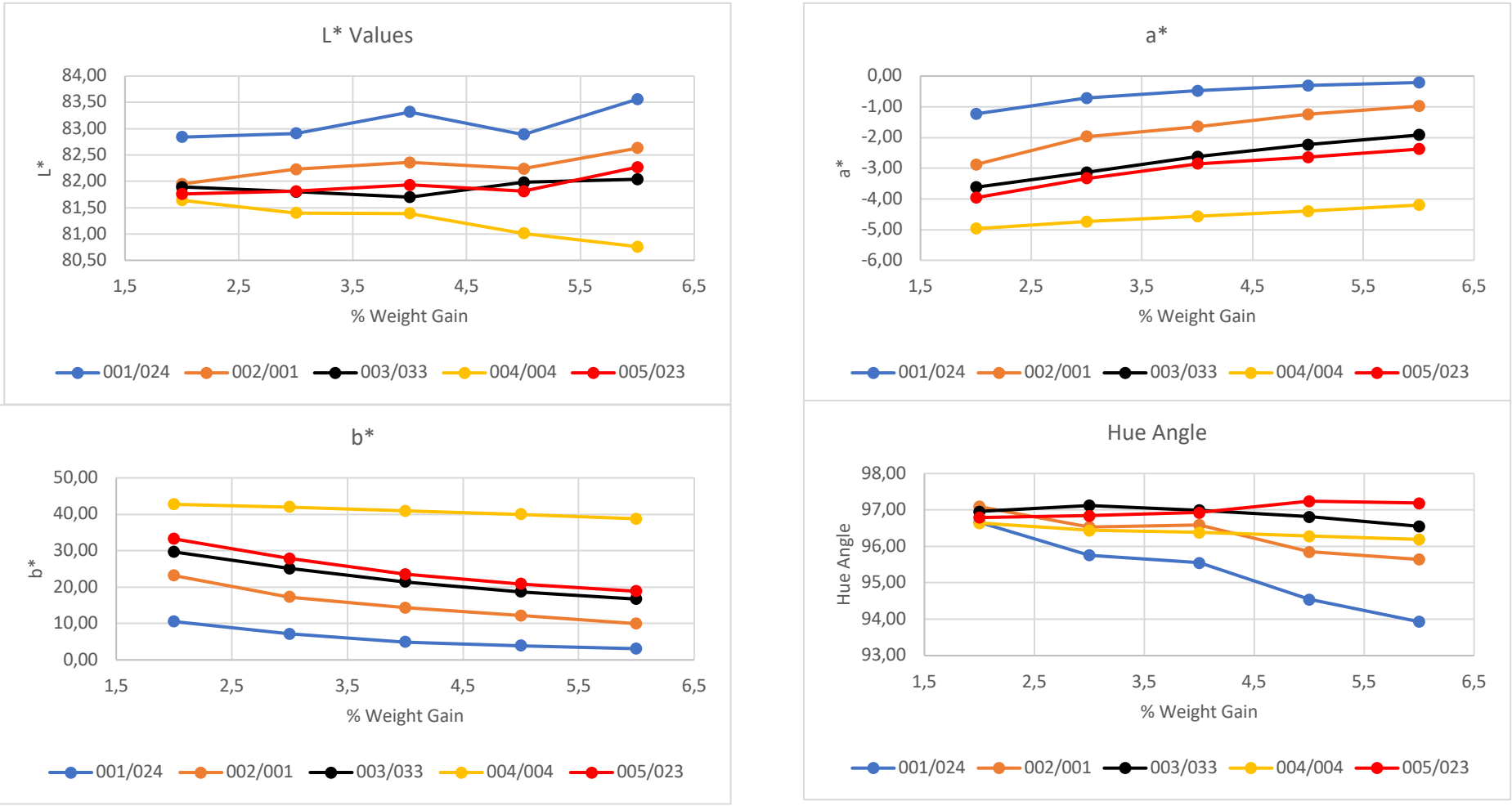
### 32. Visual Appearance - Colorimetry

Nifedipine Coated Tablets

Figure 19 shows the L*, b* and the hue angle values for the nifedipine retard coated tablets. The chroma values are not shown as they were very similar and follow the same trend to the b* values. The graphs in Figure 19 clearly show that the $TiO_2$ reference coat (COAT-024) results in very different colorimetry data to the $TiO_2$-free coats. The L* values, representing lightness, are higher, the a* and b* values, representing green-red and blue-yellow respectively, are lower than the $TiO_2$-free coatings even at 2% weight gain. The L* values reach their maximal values around a 6% weight gain. The hue angle of the $TiO_2$ reference coat also fell to a greater extent with %coating weight gain compared with the $TiO_2$-free coatings. It reached its lowest level at the 6% weight gain.

As expected, the L* values for all of the coatings slightly increased and the a* and b* values decreased with increasing %weight gain. The exception was L* values for the tablets coated with COAT-004 (Batch ENQ3822/AIRT/004/01) which decreased at higher %weight gains. Its a* and b* values also decreased only minimally with increasing %weight gain. These data are in line with the photographs and visual appearance data data which showed that this batch was still yellow at a 6% coating weight gain. The colorimetry data for the other $TiO_2$-free coated batches also agree with the visual descriptions and photographs. Coating with COAT-001, COAT-023 and COAT-033 resulted in pale yellow tablets which became lighter as the %coating level increased. As expected, given the appearance of the tablets, all of the $\Delta E_{00}$ values calculated against the $TiO_2$ reference at the same % weight gains were > 2.

Figure 19: Colorimetry data on the coated nifedipine tablets




Data Labels: Batch identifier/Coat Higher L* values and lower a* and b* indicate a whiter tablet.

In conclusion, none of the $TiO_2$-free coatings could cover and opacify the surface of the nifedipine tablet cores even at a 6% coating level, while the $TiO_2$ containing reference could achieve this at a 5% coating weight gain (based on photographic and visual appearance checks during manufacturing).

Olmesartan Coated Tablets

Figure 20 shows the L*, a*, b* and the hue angle values for the olmesartan coated tablets. The chroma values are not shown as they were very similar and follow the same trend to the b* values The L* values decrease with increasing %weight gain for both the $TiO_2$-free coatings (COAT-013, COAT-014 and COAT-015) and the $TiO_2$ reference (COAT-025). However, at all %coating levels the $TiO_2$ reference has higher L* values than the $TiO_2$-free coatings and the decrease in L* over the coating level range is slight. This reflects the visual color of the tablets which remains light pink even at higher % weight gains.

The L* values for the batches coated with $TiO_2$-free coatings are lower than that of the $TiO_2$ reference at all coating levels and the decrease in L* steeper across the %coating range, as would be expected given their darker color which deepens at higher %weight gain. The L* value plots for the $TiO_2$-free coated tablet batches follow the same trend but are clearly differentiated from each other, indicating that the individual tablet batches have a different degree of lightness.

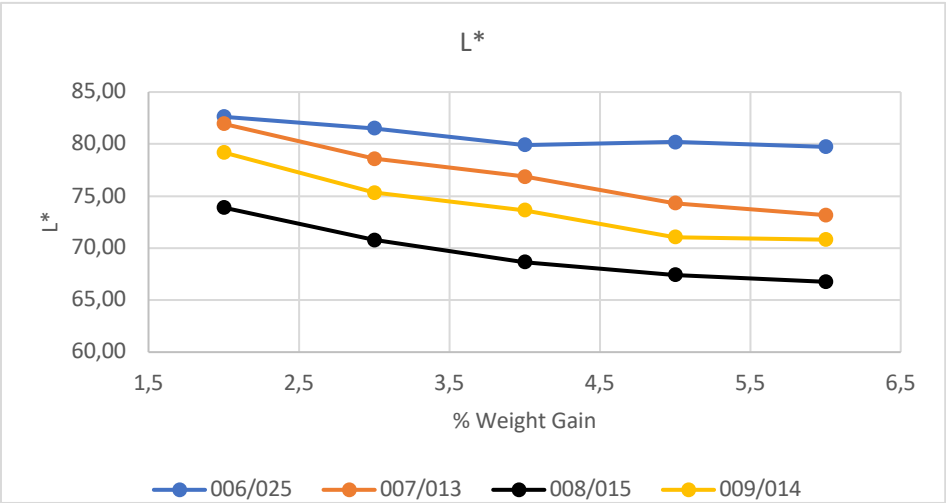
Overall, the a* values (green-red) increase with the %coating level as would be expected given they are pink coatings. However, the a* value plot of the tablets coated with COAT-013 (Batch ENQ3822/AIRT/007/01) is similar to that of the $TiO_2$-reference, while the a* values of Batch ENQ3822/AIRT/008/01 and ENQ3822/AIRT/009/01, coated with COAT-015 and COAT-014 respectively, are higher across the coating range. A similar pattern is observed for the b* value plots with the $TiO_2$ reference and COAT-013 coated tablets hardly changing across the coating range, while the values for COAT-014 and COAT-015 coated tablets increased.

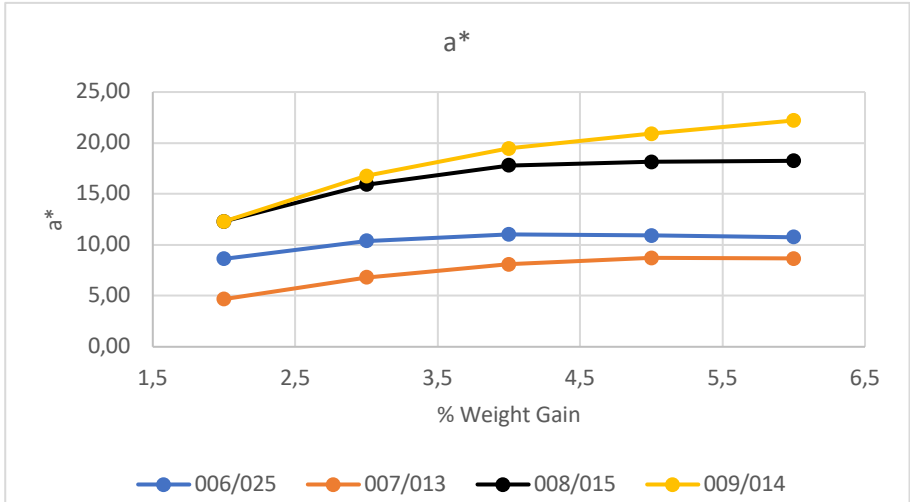
The hue angle plots for the $TiO_2$-free coated tablet batches are very similar, while that of the $TiO_2$ reference is clearly different. This aligns with visual appearance results which showed that the $TiO_2$ reference coat produced a light pink coating, while the $TiO_2$-free coated tablet batches were pink to salmon pink at the higher %coating levels. As expected, given the appearance of the tablets, all of the $\Delta E^*_{00}$ values calculated against the $TiO_2$ reference at the same %weight gains were > 2.

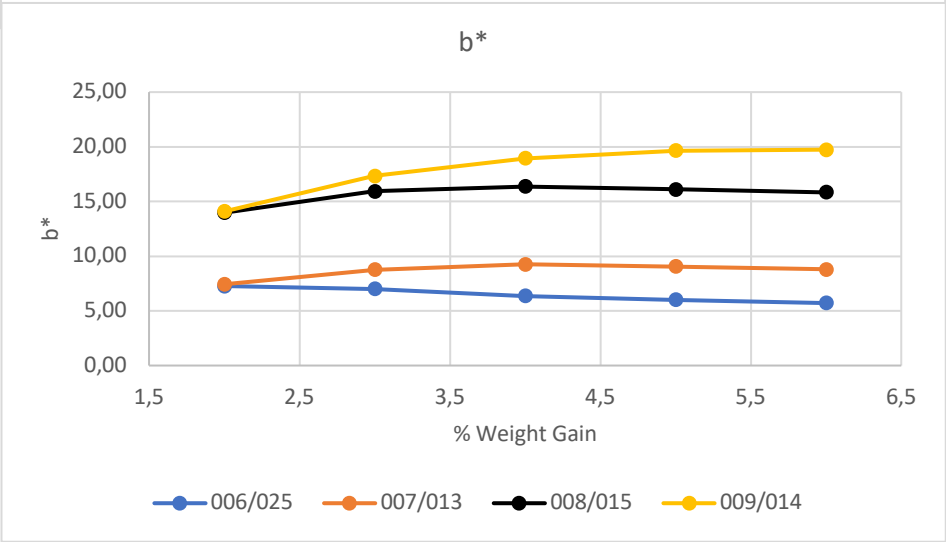
Rosuvastatin Coated Tablets

Figure 21 and Figure 22 show the colorimetry data for the coated rosuvastatin batches. The chroma values are not plotted due to their similarity to the b* data. However, they are shown in Table 32. This experiment involved applying white $TiO_2$ -free coatings to white core tablets and comparing them with two white $TiO_2$-containing reference coatings, one based on HPMC (COAT-017) and one based on PVA (COAT-018). As the coatings were white and the core tablets were white, in most cases it was difficult to discern visually if the tablet surface coverage was complete (see Section 31.). Figure 21 shows the L* and a* value data. The L* values for Batch ENQ3822/AIRT/010/01 coated with the PVA-based $TiO_2$ reference was higher than both the Batch ENQ3822/AIRT/011/01 coated with the HPMC-based $TiO_2$ reference and the batches coated with the $TiO_2$-free coatings at all weight gains. For the other batches the L* values were very similar and there was some variation in the values between the different % coating weight gains. Therefore, it was difficult to differentiate between the batches.

Figure 20: Colorimetry data on the coated olmesartan tablets



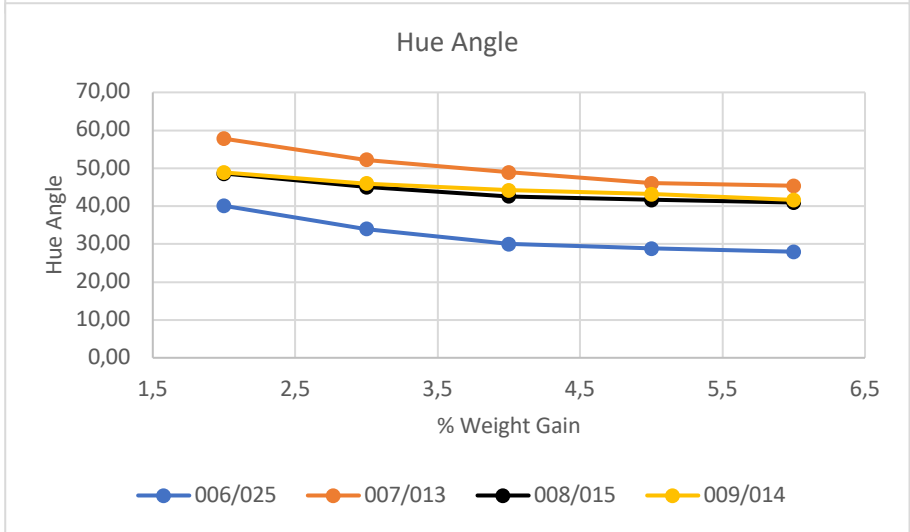
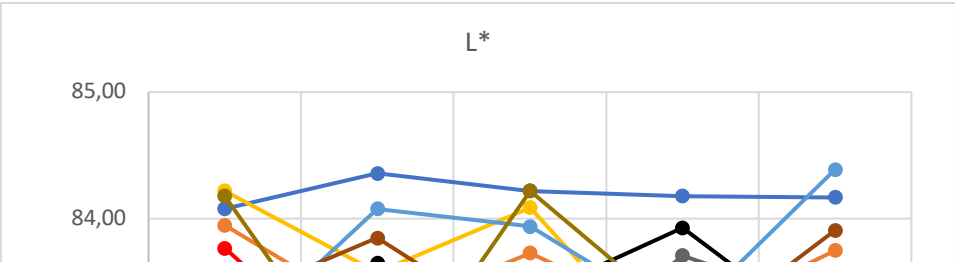
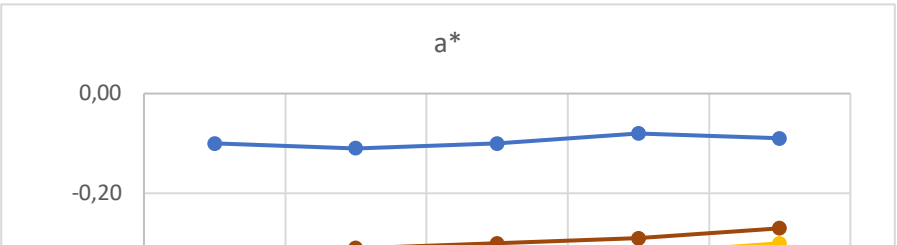




Figure 21: L* and a* values for the coated rosuvastatin tablets

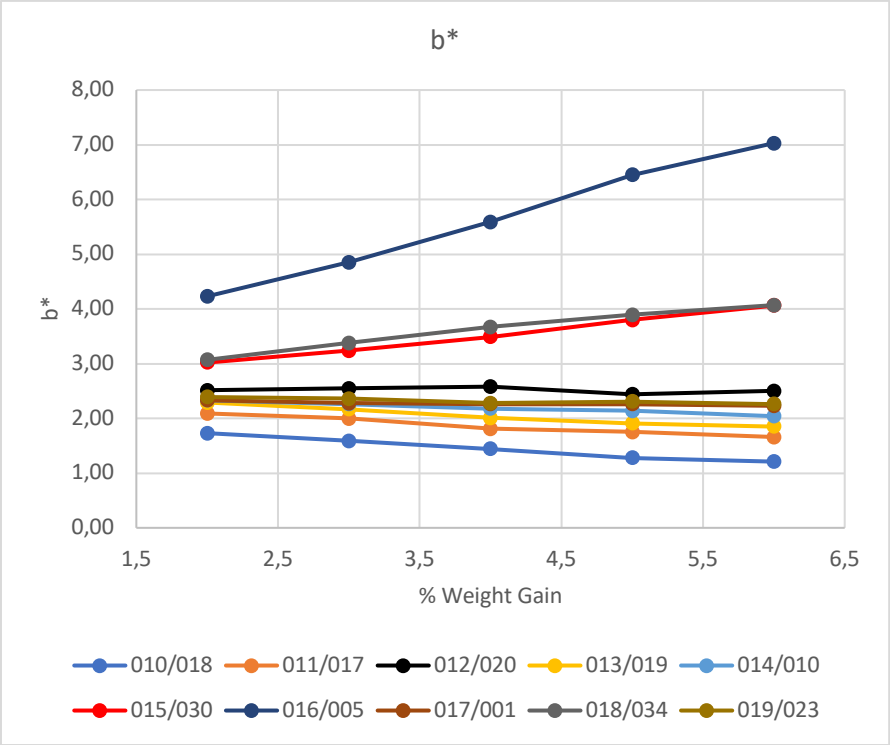


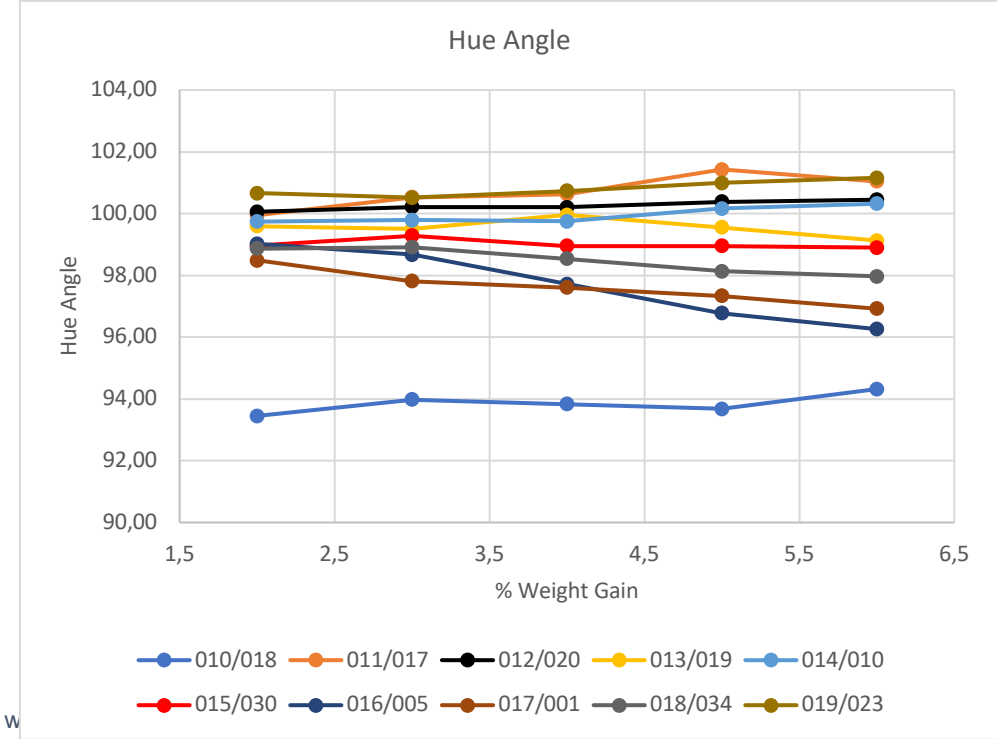



Data Labels: Batch/Coat

Higher L* values and lower a* and b* indicate a whiter tablet.

Figure 22: b* and hue angle values for coated rosuvastatin tablets



The a* values for the batches remained fairly constant with increasing coating weight gain and increased or decreased only very slightly. Most of the a* data for the coated rosuvastatin batches was very similar. However, two batches showed clear differences to the rest: Batch ENQ3822/AIRT/010/01, which was coated with the PVA-based $TiO_2$ reference, COAT-018, and Batch ENQ3822/AIRT/016/01, which was coated with the HPMC-based $TiO_2$-free coat, COAT-005 with MgO as the opacifier. The a* values of the former lay closer to zero than the others (less green), while the latter had slightly larger negative a* values than the other batches (more green).

With respect to the b* values (as shown in Figure 22) the values decreased slightly for most of the batches with increasing %weight gain. Again, the batch coated with COAT-018, the PVA-based $TiO_2$ reference, had the lowest values. Batch ENQ3822/AIRT/016/01, coated with the HPMC-based $TiO_2$-free coat, COAT-005, showed a clear upward trend in b* values with increasing coating weight gain (more yellow). This batch was described as off-white to creamy white at higher coating levels (see Table 30). Batch ENQ3822/AIRT/018/01, coated with the HPMC-based $TiO_2$-free coat, COAT-034, with rice starch as the opacifier and the Batch ENQ3822/AIRT/015/01, coated with the HPMC-based $TiO_2$-free coat clear coat, COAT-030, also showed an upward trend in b* values with increased % coating but to a lesser extent.

The hue angle values (see Figure 22) for the different coated batches were fairly constant with increasing %weight gain with some batches showing a minor decrease. The batch coated with the PVA-based $TiO_2$ reference had clearly different hue angle values to the other coated batches including Batch ENQ3822/AIRT/011/01 which was coated with COAT-017, the HPMC-based $TiO_2$ reference. The hue angles for this batch were consistently lower than the others.

As previously mentioned, it was difficult to discern visually when surface coverage and opacification was complete due to a white coating being applied to a white core. In order to evaluate the data further, the $\Delta E_{00}$ values were compared using the $TiO_2$ HPMC-based reference (COAT-017) for the HPMC-based $TiO_2$-free coatings and the $TiO_2$ PVA-based reference (COAT-018) for the one PVA-based $TiO_2$-free coating. The reference and $TiO_2$-free coated batches were compared at equivalent % coating levels. These individual results are presented in detail in Table 32. In the case of the nifedipine, olmesartan and praugrel tablets, the coating was a different color to the core tablet and the $\Delta E_{00}$ values against the corresponding $TiO_2$ reference batch are all > 2 with the exception of prasugrel batch ENQ3822/AIRT/032/01 at a 3% weight gain only. They just confirm the visual appearance assessment and therefore the individual values have not been included in this report. The results show that COAT-001 ($MgCO_3$+A+B), COAT-019 ($CaCO_3$+D+E), COAT-010 (rice starch+D) and COAT-020 ((rice starch+D) all had $\Delta E_{00}$ values less than 1 at all coating levels suggesting there was no perceptible color difference between these batches and the $TiO_2$ reference batch coated with COAT-017. The batches coated with COAT-023 and COAT-030 had $\Delta E_{00}$ values < 1, but only at specific % weight gains. This would indicate that the color of the tablets diverged from the relevant $TiO_2$ reference depending on the amount sprayed, and may reflect tablet surface coverage variation at the different tablet weight gains and also the color of the coatings (COAT-030 is described as a clear coating).

In summary, only COAT-019, COAT-001, COAT-020 and COAT-010 could achieve color matching to the HPMC-based $TiO_2$-reference at coating levels at which the reference coat, COAT-017, had previously achieved surface coverage (≥3% weight gain – see Section 16). It was not possible to ascertain visually when coating was complete for all of the batches as the white coatings were being sprayed onto white cores.

Table 32: Colorimetry data on the coated rosuvastatin tablets.

| Run No. | Batch No. ENQ3822/AIRT | Consortium Coat Reference | Film Former | Opacifier | % Weight Gain | L* | a* | b* | C | h | $\Delta E_{00}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 2 | 84.08 | -0.10 | 1.73 | 1.73 | 93.45 | NA |
| | | | | | 3 | 84.36 | -0.11 | 1.59 | 1.59 | 93.98 | NA |
| | | | | | 4 | 84.22 | -0.10 | 1.44 | 1.44 | 93.83 | NA |
| | | | | | 5 | 84.18 | -0.08 | 1.28 | 1.28 | 93.68 | NA |
| | | | | | 6 | 84.17 | -0.09 | 1.21 | 1.21 | 94.32 | NA |
| 21 | 011/01 | COAT-017 | HPMC | $TiO_2$ | 2 | 83.95 | -0.37 | 2.09 | 2.12 | 99.95 | NA |
| | | | | | 3 | 83.23 | -0.37 | 2.00 | 2.04 | 100.53 | NA |
| | | | | | 4 | 83.73 | -0.34 | 1.81 | 1.84 | 100.63 | NA |
| | | | | | 5 | 83.17 | -0.35 | 1.75 | 1.78 | 101.43 | NA |
| | | | | | 6 | 83.75 | -0.32 | 1.66 | 1.69 | 101.04 | NA |
| 22 | 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 2 | 82.86 | -0.45 | 2.51 | 2.55 | 100.06 | 0.84 |
| | | | | | 3 | 83.65 | -0.46 | 2.55 | 2.59 | 100.22 | 0.65 |
| | | | | | 4 | 83.38 | -0.46 | 2.58 | 2.62 | 100.20 | 0.79 |
| | | | | | 5 | 83.93 | -0.45 | 2.44 | 2.49 | 100.38 | 0.87 |
| | | | | | 6 | 83.03 | -0.46 | 2.50 | 2.55 | 100.45 | 0.95 |
| 23 | 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 2 | 84.22 | -0.39 | 2.29 | 2.32 | 99.59 | 0.43 |
| | | | | | 3 | 83.58 | -0.36 | 2.17 | 2.20 | 99.50 | 0.38 |
| | | | | | 4 | 84.09 | -0.35 | 2.01 | 2.04 | 99.96 | 0.40 |
| | | | | | 5 | 82.77 | -0.32 | 1.91 | 1.94 | 99.55 | 0.35 |
| | | | | | 6 | 83.19 | -0.30 | 1.85 | 1.87 | 99.13 | 0.44 |
| 24 | 014/01 | COAT-010 | HPMC | Rice Starch+D | 2 | 82.96 | -0.40 | 2.33 | 2.36 | 99.74 | 0.72 |
| | | | | | 3 | 84.08 | -0.39 | 2.26 | 2.29 | 99.79 | 0.66 |
| | | | | | 4 | 83.94 | -0.38 | 2.18 | 2.22 | 99.75 | 0.51 |
| | | | | | 5 | 83.21 | -0.38 | 2.14 | 2.17 | 100.16 | 0.46 |
| | | | | | 6 | 84.39 | -0.37 | 2.04 | 2.08 | 100.32 | 0.63 |

| Run No. | Batch No. ENQ3822/AIRT | Consortium Coat Reference | Film Former | Opacifier | % Weight Gain | L* | a* | b* | C | h | $\Delta E_{00}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25B | 015/02 | COAT-030 | HPMC | B+E | 2 | 83.77 | -0.48 | 3.02 | 3.06 | 98.96 | 0.90 |
| | | | | | 3 | 82.69 | -0.53 | 3.24 | 3.28 | 99.28 | 1.20 |
| | | | | | 4 | 82.93 | -0.55 | 3.49 | 3.53 | 98.95 | 1.64 |
| | | | | | 5 | 82.61 | -0.60 | 3.80 | 3.85 | 98.95 | 1.91 |
| | | | | | 6 | 82.44 | -0.64 | 4.06 | 4.11 | 98.90 | 2.35 |
| 26 | 016/01 | COAT-005 | HPMC | MgO | 2 | 82.86 | -0.67 | 4.23 | 4.28 | 99.02 | 2.06 |
| | | | | | 3 | 82.96 | -0.74 | 4.85 | 4.91 | 98.67 | 2.53 |
| | | | | | 4 | 82.76 | -0.76 | 5.59 | 5.64 | 97.72 | 3.35 |
| | | | | | 5 | 82.03 | -0.77 | 6.45 | 6.49 | 96.78 | 4.08 |
| | | | | | 6 | 82.50 | -0.77 | 7.03 | 7.08 | 96.26 | 4.61 |
| 27 | 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 2 | 83.34 | -0.35 | 2.33 | 2.36 | 98.49 | 0.48 |
| | | | | | 3 | 83.85 | -0.31 | 2.28 | 2.30 | 97.81 | 0.55 |
| | | | | | 4 | 83.08 | -0.30 | 2.26 | 2.28 | 97.60 | 0.63 |
| | | | | | 5 | 83.00 | -0.29 | 2.26 | 2.28 | 97.34 | 0.59 |
| | | | | | 6 | 83.91 | -0.27 | 2.23 | 2.25 | 96.92 | 0.61 |
| 28 | 018/01 | COAT-034 | HPMC | Rice Starch | 2 | 82.91 | -0.48 | 3.07 | 3.10 | 98.87 | 1.14 |
| | | | | | 3 | 83.60 | -0.53 | 3.38 | 3.42 | 98.91 | 1.29 |
| | | | | | 4 | 82.70 | -0.55 | 3.67 | 3.71 | 98.54 | 1.83 |
| | | | | | 5 | 83.71 | -0.56 | 3.89 | 3.93 | 98.13 | 1.98 |
| | | | | | 6 | 83.30 | -0.57 | 4.07 | 4.11 | 97.97 | 2.20 |
| 29 | 019/01 | COAT-023 | PVA | F+Talc | 2 | 84.18 | -0.45 | 2.39 | 2.43 | 100.66 | 0.85 |
| | | | | | 3 | 82.48 | -0.44 | 2.36 | 2.40 | 100.52 | 1.53 |
| | | | | | 4 | 84.22 | -0.43 | 2.28 | 2.32 | 100.73 | 0.99 |
| | | | | | 5 | 83.21 | -0.45 | 2.31 | 2.35 | 100.99 | 1.28 |
| | | | | | 6 | 82.53 | -0.45 | 2.26 | 2.31 | 101.16 | 1.56 |

Prasugrel Coated Tablets

Figure 23 shows the L*, a*, b* and hue angle plots for the coated prasugrel batches. The chroma data (not shown) followed the same trend as the b* values. The data for the three colored HPMC-based $TiO_2$-free coatings were compared to the $TiO_2$ reference (COAT-026). The colorimetric data for Batch ENQ3822/AIRT/023/01, coated with the COAT-030/COAT-O31 combination, was very different to the other batches, reflecting its red as opposed to pink color. The L*, a* and b* values for the other two coatings evaluated, COAT-002 and COAT-016, were closer to that of the $TiO_2$ reference. The hue angle values for the tablets coated with COAT-002 (Batch ENQ3822/AIRT/022/01) were closer to that of the $TiO_2$ reference coated tablets than those of the other two lots. COAT-002 contains rice starch+A+B+D+$Fe_2O_3$ as opacifiers. COAT-016 employs rice starch as the opacifier and iron oxide as the colorant. Neither of these two coatings could match the $TiO_2$ reference coat with respect to $\Delta E^*_{00}$ values which were all greater than 2, except in the case of the 3% weight gain sample from Batch ENQ3822/AIRT/022/01 (COAT-002) which was 0.92. The colorimetry data align with the visual appearance descriptions (see Figure 18 and Table 31).

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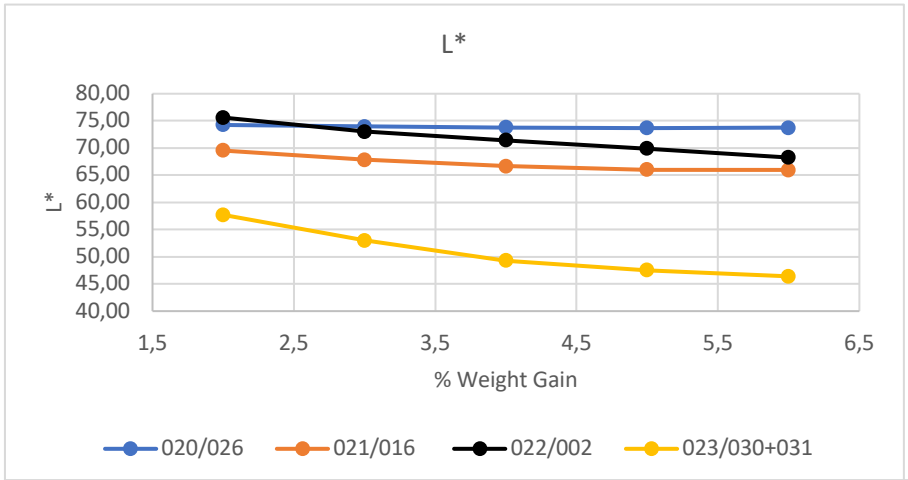
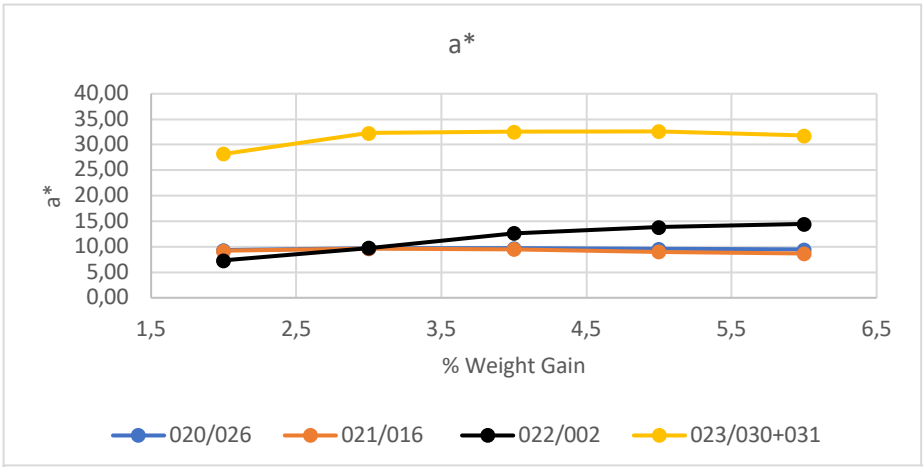
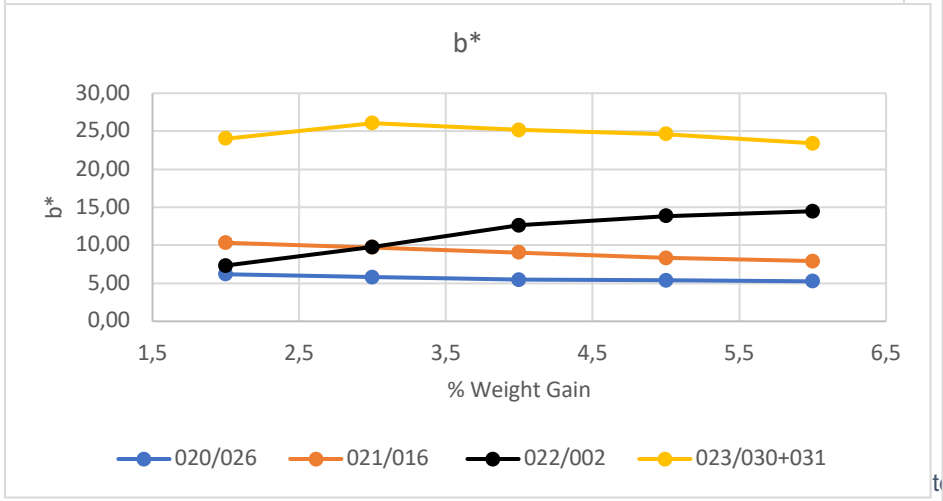
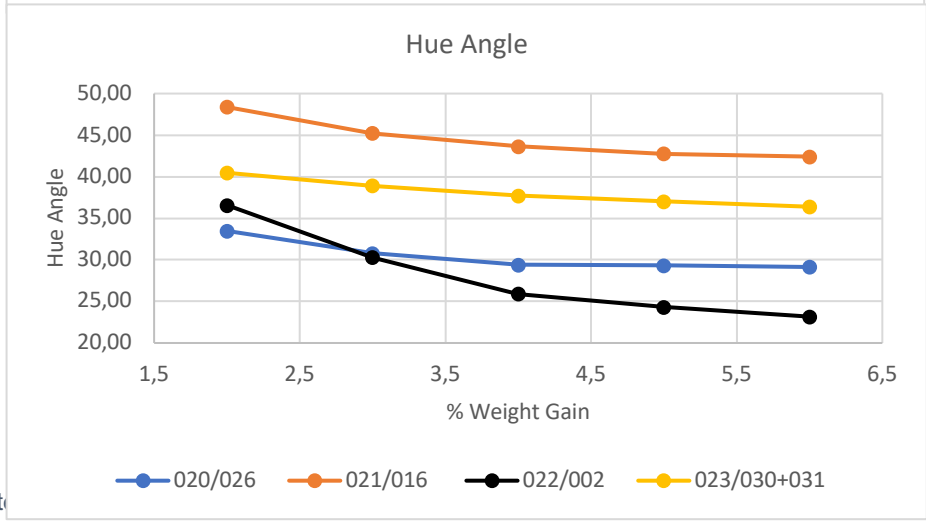
Figure 23: Colorimetry data for the coated prasugel tablets



## 33. Coating Thickness

The coating thickness measured for the nifedipine, olmesartan, rosuvastatin and prasugrel coated tablets are discussed in this section. Since the experimental objective was to compare the coat thickness achieved on the tablet batches coated with $TiO_2$-free coatings versus the corresponding $TiO_2$ reference batch, the results are shown for 2%, 4% and 6% coating weight gains and have not been calculated on a tablet surface area basis. For this reason, the coating thickness achieved on the nifedipine, olmesartan, rosuvastatin and prasugrel tablets, whether coated with $TiO_2$-free or $TiO_2$ reference coatings, cannot be directly compared between different tablet types as the tablets are of different sizes and shapes.

Nifedipine Coated Tablets

The coating thickness results for coated nifedipine retard tablets are shown in Table 33. In general, coating thickness increased with %coating weight gain, although there was some variation between values for land, belly and surface at the different coating levels.

Table 33: Coating thickness on the coated nifedipine retard tablets at 2%, 4% and 6% weight gain

| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Opacifier | % Weight Gain | Coating Thickness (µm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Land | Belly | Surface | Mean |
| 001/01 | COAT-024 | $TiO_2$ | 2% | 50 | 16 | 13 | 26.2 |
| | | | 4% | 10 | 18 | 21 | 16.4 |
| | | | 6% | 33 | 35 | 47 | 38.4 |
| 002/01 | COAT-001 | $MgCO_3$+ A+B | 2% | 18 | 12 | 17 | 15.7 |
| | | | 4% | 27 | 34 | 29 | 30.4 |
| | | | 6% | 41 | 47 | 39 | 42.1 |
| 003/01 | COAT-033 | $CaCO_3$+ D+F | 2% | 14 | 11 | 11 | 12.0 |
| | | | 4% | 18 | 27 | 14 | 19.7 |
| | | | 6% | 44 | 42 | 31 | 38.9 |
| 004/01 | COAT-004 | $CaCO_3$+C | 2% | 36 | 24 | 14 | 24.7 |
| | | | 4% | 56 | 32 | 38 | 42.1 |
| | | | 6% | 33 | 29 | 40 | 34.0 |
| 005/01 | COAT-023 | F+Talc | 2% | 12 | 13 | 13 | 12.6 |
| | | | 4% | 18 | 19 | 20 | 18.9 |
| | | | 6% | 34 | 32 | 24 | 29.7 |

In general, the mean coating thickness for the batches coated with COAT-001, COAT-033 and COAT 004 were similar to that of the $TiO_2$ reference although there was some variation at the different coating levels reflecting the variation in the individual values. This is despite only the reference coat achieving adequate coverage of the tablets' yellow surface. The mean coating thickness of the coat produced using the COAT-023 suspension (Batch ENQ3822/AIRT/005/01) was lower than that of the $TiO_2$ reference and that of the other $TiO_2$-free coatings. Again, coverage was inadequate to completely hide the yellow color of the core tablet surface.

Olmesartan Coated Tablets

The coating thickness results for coated olmesartan tablets are shown Table 34. In general, coating thickness increased with %coating weight gain, although there was some variation between values for land, belly, surface and debossing at the different coating levels. The results show that the coating thickness achieved at the different %weight gains for the $TiO_2$-free coated tablet batches was similar to the $TiO_2$ reference, although again there was some variation at the different coating levels. Therefore, differences in color and surface coverage could not be attributed to significant coating thickness variation.

Table 34: Coating thickness on the coated olmesartan tablets at 2%, 4% and 6% weight gain

| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Opacifier | % Weight Gain | Coating Thickness (µm) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Land | Belly | Surface | Deboss | Mean |
| 006/01 | COAT-025 | $TiO_2$+Talc +$Fe_2O_3$ | 2% | 15 | 31 | 21 | 28 | 23.7 |
| | | | 4% | 31 | 34 | 23 | 136 | 55.9 |
| | | | 6% | 58 | 42 | 33 | 66 | 49.7 |
| 007/01 | COAT-013 | $CaCO_3$+ Talc+ $Fe_2O_3$ | 2% | 39 | 33 | 31 | 43 | 36.5 |
| | | | 4% | 26 | 28 | 27 | 44 | 31.1 |
| | | | 6% | 41 | 51 | 41 | 83 | 53.9 |
| 008/01 | COAT-015 | $CaCO_3$+ Talc+ $Fe_2O_3$ | 2% | 22 | 27 | 28 | 30 | 26.7 |
| | | | 4% | 36 | 51 | 19 | 55 | 40.4 |
| | | | 6% | 97 | 53 | 37 | 54 | 60.3 |
| 009/01 | COAT-014 | $CaCO_3$+ Talc+ $Fe_2O_3$ | 2% | 23 | 22 | 28 | 25 | 24.6 |
| | | | 4% | 13 | 27 | 32 | 58 | 32.7 |
| | | | 6% | 70 | 79 | 47 | 68 | 65.9 |

Rosuvastatin Coated Tablets

The coating thickness results for coated rosuvastatin tablets are shown in Table 35. In general, coating thickness increased or remained similar with %coating weight gain for the rosuvastatin tablets and there was some variation between values for land, belly and surface at the different coating levels. For some batches a higher coating thickness was recorded for the 2% or 4% weight gain tablet than at higher weight gains, demonstrating the variation that can occur when measuring coating thickness based on one individual tablet.

The HPMC-based $TiO_2$ reference coat (COAT-017) produced a thicker coat at all %weight gains than the PVA-based $TiO_2$ reference (COAT-018). However, with the exception of Batch ENQ3822/AIRT/016/01 (COAT-005), all of the $TiO_2$-free coated batches had a mean coating thickness lower than Batch ENQ3822/AIRT/011/01 which was coated with the $TiO_2$ reference, COAT-017. COAT-001, COAT-034 and COAT-023 resulted in a mean coating thickness which was also often lower than the $TiO_2$ PVA-based reference, COAT-018. The other $TiO_2$-free coated batches, with the exception of COAT-005, produced coatings in the same thickness range as COAT-018. This was despite two of these coating materials being HPMC-based (COAT-001 and COAT-034) and only one, PVA-based (COAT-023).

Coating thickness is measured by digital optical microscopy on one individual tablet per batch. Therefore, the results for batches with coating thicknesses lower than the $TiO_2$ reference tablets may not be reflective of the batch as a whole. Coating thickness of these batches was measured again as part of photostability and accelerated stability studies.

The $TiO_2$-free coat, COAT-005 (Batch ENQ3822/AIRT/016/01), produced a much thicker coat on the rosuvastatin tablet cores than either of the $TiO_2$ reference coatings. The visual appearance and colorimetry data of the batch was also clearly different to the $TiO_2$-reference and other $TiO_2$-free coatings with the tablets being off-white to cream at higher coating levels (see Sections 31 and 32). COAT-005 contains MgO as the opacifier.

Table 35: Coating thickness on the coated rosuvastatin tablets at 2%, 4% and 6% weight gain

| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Opacifier | Coating Thickness (µm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | % Weight Gain | Land | Belly | Surface | Mean |
| 010/01 | COAT-018 | $TiO_2$+Talc | 2% | 13 | 14 | 13 | 13.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 4% | 28 | 48 | 27 | 34.4 |
| | | | 6% | 39 | 26 | 34 | 33.0 |
| 011/01 | COAT-017 | $TiO_2$ | 2% | 55 | 51 | 48 | 51.4 |
| | | | 4% | 50 | 49 | 30 | 42.8 |
| | | | 6% | 27 | 48 | 41 | 38.9 |
| 012/01 | COAT-020 | Rice Starch +D | 2% | 23 | 22 | 31 | 25.1 |
| | | | 4% | 36 | 33 | 30 | 33.0 |
| | | | 6% | 17 | 21 | 30 | 22.7 |
| 013/01 | COAT-019 | $CaCO_3$+D+E | 2% | 16 | 12 | 21 | 15.9 |
| | | | 4% | 19 | 21 | 22 | 20.7 |
| | | | 6% | 23 | 34 | 27 | 27.8 |
| 014/01 | COAT-010 | Rice Starch +D | 2% | 30 | 30 | 31 | 30.3 |
| | | | 4% | 26 | 22 | 27 | 25.0 |
| | | | 6% | 20 | 25 | 35 | 26.4 |
| 015/02 | COAT-030 | B+E | 2% | 32 | 29 | 26 | 29.0 |
| | | | 4% | 36 | 29 | 29 | 31.1 |
| | | | 6% | 24 | 35 | 36 | 31.6 |
| 016/01 | COAT-005 | MgO | 2% | 17 | 27 | 29 | 24.2 |
| | | | 4% | 48 | 61 | 47 | 51.8 |
| | | | 6% | 62 | 59 | 78 | 66.3 |
| 017/01 | COAT-001 | $MgCO_3$+A+B | 2% | 18 | 15 | 21 | 17.9 |
| | | | 4% | 26 | 23 | 27 | 25.1 |
| | | | 6% | 18 | 14 | 18 | 16.3 |
| 018/01 | COAT-034 | Rice Starch | 2% | 21 | 35 | 29 | 28.5 |
| | | | 4% | 24 | 28 | 16 | 22.5 |
| | | | 6% | 27 | 20 | 23 | 23.2 |
| 019/01 | COAT-023 | F+Talc | 2% | 29 | 20 | 18 | 22.1 |
| | | | 4% | 21 | 23 | 26 | 23.3 |
| | | | 6% | 35 | 26 | 12 | 24.3 |

Prasugrel Coated Tablets

The coating thickness results for coated prasugrel tablets are shown in Table 36.

Table 36: Coating thickness on the coated prasugrel tablets at 2%, 4% and 6% weight gain

| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Opacifier | Coating Thickness (µm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | % Weight Gain | Land | Belly | Surface | Mean |
| 020/01 | COAT-026 | $TiO_2$+$Fe_2O_3$ | 2% | 35 | 19 | 47 | 33.8 |
| | | | 4% | 31 | 51 | 24 | 35.3 |
| | | | 6% | 69 | 58 | 44 | 56.8 |
| 021/01 | COAT-016 | Rice Starch+ D+$Fe_2O_3$ | 2% | 23 | 18 | 16 | 19.0 |
| | | | 4% | 31 | 43 | 49 | 40.7 |
| | | | 6% | 44 | 9 | 47 | 33.3 |
| 022/01 | COAT-002 | Rice Starch+ A+B+D+ $Fe_2O_3$ | 2% | 19 | 17 | 28 | 21.3 |
| | | | 4% | 38 | 45 | 49 | 44.0 |
| | | | 6% | 24 | 39 | 31 | 31.4 |
| 023/01 | COAT-030 + COAT-031 | B+E+$Fe_2O_3$ | 2% | 18 | 21 | 20 | 19.7 |
| | | | 4% | 25 | 41 | 29 | 31.6 |
| | | | 6% | 62 | 85 | 65 | 70.7 |

Again for the prasugrel tablets, the coating thickness increased with %coating weight gain and there was some variation between values for land, belly and surface at the different coating levels. For Batches ENQ3822/AIRT/021/01 and ENQ3822/AIRT/022/01, a higher coating thickness was recorded for the 4% weight gain tablet, than at the 6% weight gain, demonstrating the variation that can occur when measuring coating thickness based on one individual tablet. The highest mean coating thickness was achieved on Batch ENQ3822/AIRT/023/01 which was coated with the COAT-030/COAT-031 combination. However, the visual appearance data for this batch (see Table 31) showed that coating was unsatisfactory with a spray pattern visible on the tablets at all coating levels.

Based on the visual appearance data, the $TiO_2$ reference coat, COAT-026, achieved tablet surface coverage at 2% weight gain and the $TiO_2$-free coat, COAT-002, at the 6% coating level. The mean coating thickness for both batches coated with these coating materials was around 30 µm, showing again, as for the placebo tablets (see 18), that surface coverage and opacification depends on the composition and properties of the coating, provided sufficient material has been deposited on tablet surface, and not the coating thickness alone.

### 34. X-ray Powder Diffraction

The results of the X-ray powder diffraction studies are shown in Table 37, Table 38, Table 39 and Table 40. All of the coated nifedipine and olmesartan batches were nifedipine pattern A and olmesartan pattern A, respectively, showing that coating with the $TiO_2$-free coatings did not impact on the solid state of the API at least initially. An elevated baseline was observed in the XRPD patterns for nifedipine which may be indicative of some disorder or amorphous content (see Figure 24).

All of the rosuvastatin batches displayed the characteristic rosuvastatin pattern A. However, others also showed peak shifting and additional peaks. The batches, which showed minor peak shifting, included ENQ3822/AIRT/010/01 and ENQ3822/AIRT/011/01, the batches coated with the PVA-based and HPMC-based $TiO_2$ references, COAT-018 and COAT-017. $TiO_2$-free coated batches displaying peak shifting included ENQ3822/AIRT/015/02, and ENQ3822/AIRT/016/01 at a 4% and 6% weight gain only. These samples also included an additional peak at 21.5 °2θ. Batch ENQ3822/AIRT/017/01 contained an additional peak at 25.4 °2θ but only in the 4% weight gain sample. Two other samples showed evidence of amorphous material: ENQ3822/AIRT/014/01 at 4% weight gain and ENQ3822/AIRT/023/01 at a 6% weight gain. Although no amorphous material was evident in the other samples from these batches. All of the samples of the prasugrel tablets displayed the prasugrel pattern A. Only the 6% weight gain sample of ENQ3822/AIRT/030/01, coated with the $TiO_2$ reference, COAT-026, had an additional peak at 37.7 °2θ.

In summary, there are no major differences in the XRPD results for the $TiO_2$-free coated batches and the corresponding $TiO_2$ reference batches. The cause of the additional peaks in the certain prasugrel and rosuvastatin tablet batches is unknown and would require further investigation. The elevated baseline observed in the XRPD patterns for nifedipine may be indicative of some disorder or amorphous content.

Table 37: XRPD results for nifedipine coated tablets at 2%, 4% and 6% weight gain

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Film Former | Opacifier | % Weight Gain | XRPD Pattern |
|---|---|---|---|---|---|
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 2 | Nifedipine Pattern A |
| | | | | 4 | Nifedipine Pattern A |
| | | | | 6 | Nifedipine Pattern A |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 2 | Nifedipine Pattern A |
| | | | | 4 | Nifedipine Pattern A |
| | | | | 6 | Nifedipine Pattern A |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 2 | Nifedipine Pattern A - low signal |
| | | | | 4 | Nifedipine Pattern A |
| | | | | 6 | Nifedipine Pattern A |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 2 | Nifedipine Pattern A |
| | | | | 4 | Nifedipine Pattern A |
| | | | | 6 | Nifedipine Pattern A |
| 005/01 | COAT-023 | PVA | F+Talc | 2 | Nifedipine Pattern A |
| | | | | 4 | Nifedipine Pattern A |
| | | | | 6 | Nifedipine Pattern A |

Figure 24: Example of the XRPD pattern for the nifedipine batches (Batch ENQ3822/AIRT/001/01)

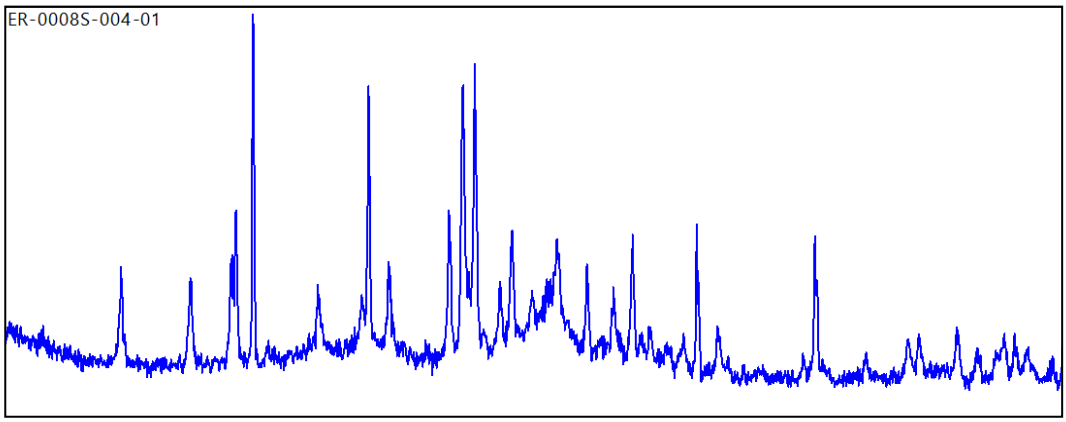


Table 38: XRPD results for olmesartan coated tablets at 2%, 4% and 6% weight gain

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Film-Former | Opacifier | % Weight Gain | XRPD Pattern |
|---|---|---|---|---|---|
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 2 | Olmesartan Pattern A |
| | | | | 4 | Olmesartan Pattern A |
| | | | | 6 | Olmesartan Pattern A |
| 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | 2 | Olmesartan Pattern A |
| | | | | 4 | Olmesartan Pattern A |
| | | | | 6 | Olmesartan Pattern A |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 2 | Olmesartan Pattern A |
| | | | | 4 | Olmesartan Pattern A |
| | | | | 6 | Olmesartan Pattern A |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 2 | Olmesartan Pattern A |
| | | | | 4 | Olmesartan Pattern A |
| | | | | 6 | Olmesartan Pattern A |

Table 39: XRPD results for rosuvastatin coated tablets at 2%, 4% and 6% weight gain

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Film Former | Opacifier | % Weight Gain | XRPD Pattern |
|---|---|---|---|---|---|
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 2 | Rosuvastatin Pattern A + minor peak shifting |
| | | | | 4 | Rosuvastatin Pattern A + minor peak shifting |
| | | | | 6 | Rosuvastatin Pattern A + minor peak shifting |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 2 | Rosuvastatin Pattern A + minor peak shifting |
| | | | | 4 | Rosuvastatin Pattern A + minor peak shifting |
| | | | | 6 | Rosuvastatin Pattern A + minor peak shifting |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch +D | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A |
| | | | | 6 | Rosuvastatin Pattern A |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A |
| | | | | 6 | Rosuvastatin Pattern A |
| 014/01 | COAT-010 | HPMC | Rice Starch +D | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A + amorphous content |
| | | | | 6 | Rosuvastatin Pattern A |
| 015/02 | COAT-030 | HPMC | B+E | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A + peak shifting + peak 21.5 °2θ |
| | | | | 6 | Rosuvastatin Pattern A + peak shifting + peak 21.5 °2θ |
| 016/01 | COAT-005 | HPMC | MgO | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A + peak shifting + peak 21.5 °2θ |
| | | | | 6 | Rosuvastatin Pattern A + peak shifting + peak 21.5 °2θ |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A + additional peak @ 25.4 °2θ |
| | | | | 6 | Rosuvastatin Pattern A |
| 018/01 | COAT-034 | HPMC | Rice starch | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A |
| | | | | 6 | Rosuvastatin Pattern A |
| 019/01 | COAT-023 | PVA | F+Talc | 2 | Rosuvastatin Pattern A |
| | | | | 4 | Rosuvastatin Pattern A |
| | | | | 6 | Rosuvastatin Pattern A + amorphous content |

Table 40: XRPD results for prasugrel coated tablets at 2%, 4% and 6% weight gain

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Film Former | Opacifier | % Weight Gain | XRPD Pattern |
|---|---|---|---|---|---|
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 2 | Prasugrel Pattern A |
| | | | | 4 | Prasugrel Pattern A |
| | | | | 6 | Prasugrel Pattern A + additional peak @ 37.7 °2θ |
| 021/01 | COAT-016 | HPMC | Rice Starch+ D+$Fe_2O_3$ | 2 | Prasugrel Pattern A |
| | | | | 4 | Prasugrel Pattern A |
| | | | | 6 | Prasugrel Pattern A |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+$Fe_2O_3$ | 2 | Prasugrel Pattern A |
| | | | | 4 | Prasugrel Pattern A |
| | | | | 6 | Prasugrel Pattern A |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 2 | Prasugrel Pattern A |
| | | | | 4 | Prasugrel Pattern A |
| | | | | 6 | Prasugrel Pattern A |

## 35. Disintegration Times - $TiO_2$-free Coated versus $TiO_2$ Reference Batches

Table 41 shows the disintegration times of the various $TiO_2$-free coated batches and their corresponding $TiO_2$ references at a 2%, 4% and 6% coating weight gain. For all of the nifedipine batches, the disintegration times increased with %coating weight gain with the exception of Batch ENQ3822/AIRT/004/01 whose disintegration time decreased slightly at the 4% coating level compared with the 2%. However, the extent to which the disintegration times increased varied significantly. For Batch ENQ3822/AIRT/001/01 coated with the $TiO_2$ reference coating, COAT-024, and Batch ENQ3822/AIRT/002/01 coated with the $TiO_2$-free coating, COAT-001, the increase is insignificant with both batches disintegrating after approximately 7 min at the 6% coating level, compared with around 5.5 min at 2%. In comparison the core tablets disintegrated in 4 min 18 sec showing that coating with these two coats had not increased disintegration time significantly.

Batch ENQ3822/AIRT/003/01 coated with the $TiO_2$-free coating, COAT-033, also disintegrated at between 5 and 6 min at the 2% coating level. However, the disintegration times increased significantly as the coating layer increased to reach over 18 min at a 6% weight gain, the longest disintegration time of all the nifedipine batches. The other two nifedipine coated batches had significantly increased disintegration times compared with the $TiO_2$ reference and COAT-001 coated batches, even at the 2% level, and it was over 10 min at the 6% coating level. COAT-033, COAT-004 and COAT-023 contain $CaCO_3$+D+F, $CaCO_3$ +C and F+talc as opacifiers respectively. The nifedipine tablets coated with these three $TiO_2$-free batches failed to achieve full coverage at the 6% coating level. Despite this, the disintegration times were prolonged.

All of the olmesartan and prasugrel coated tablet batches had disintegration times of less than 3 minutes and less than 4 minutes respectively. Therefore, the presence of the excipients used to replace $TiO_2$ in the $TiO_2$-free coatings did not affect disintegration of these tablets adversely. Disintegration times increased very slightly with the %weight gain. In comparison, the core olmesartan tablets disintegrated in 1 min 1 sec and the prasugrel core tablets in 1 min 35 sec.

Table 41: Disintegration times of $TiO_2$-free coated batches and the corresponding $TiO_2$ references

| Coated Tablet Batch No. | Consortium Coat Ref | Film Former | Opacifier | Disintegration Time at %Weight Gain (min:sec) |
|---|---|---|---|---|

| ENQ3822/AIRT/ | | | | | | |
|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 05:33 | 06:28 | 07:18 |
| 002/01 | COAT-001 | HPMC+ HPC | $MgCO_3$+A+B | 05:28 | 06:05 | 06:51 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 05:38 | 08:47 | 18:06 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 08:55 | 07:31 | 12:47 |
| 005/01 | COAT-023 | PVA | F+Talc | 06:04 | 09:06 | 10:46 |
| **Olmesartan 20 mg Coated Tablets** | | | | 2% | 4% | 6% |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+ $Fe_2O_3$ | 01:39 | 02:06 | 02:14 |
| 007/01 | COAT-013 | PVA+ HPMC | $CaCO_3$+Talc+ $Fe_2O_3$ | 01:45 | 01:57 | 02:22 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 01:41 | 01:58 | 02:27 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 01:47 | 02:09 | 02:35 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | 2% | 4% | 6% |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 04:28 | 05:29 | 05:23 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 05:20 | 05:31 | 05:56 |
| 012/01 | COAT-020 | HPMC+ HPC | Rice Starch+D | 04:57 | 05:10 | 04:47 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 04:22 | 05:35 | 06:00 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 04:58 | 05:13 | 04:23 |
| 015/02 | COAT-030 | HPMC | B+E | 06:10 | 07:02 | 09:03 |
| 016/01 | COAT-005 | HPMC | MgO | 05:34 | 06:13 | 06:17 |
| 017/01 | COAT-001 | HPMC+ HPC | $MgCO_3$+A+B | 04:29 | 04:33 | 05:44 |
| 018/01 | COAT-034 | HPMC | Rice Starch | 04:21 | 05:07 | 05:07 |
| 019/01 | COAT-023 | PVA | F+Talc | 04:49 | 05:18 | 05:37 |
| **Prasugrel 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 01:56 | 02:32 | 03:05 |
| 021/01 | COAT-016 | HPMC | Rice Starch+D+ $Fe_2O_3$ | 02:05 | 02:30 | 02:48 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+$Fe_2O_3$ | 01:23 | 02:24 | 03:02 |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 02:11 | 02:50 | 03:57 |

Most of the rosuvastatin batches disintegrated between 4 and 7 minutes irrespective of whether the coating contained $TiO_2$ or not. The exception was Batch ENQ3822/AIRT/015/02 which took just over 9 min to disintegrate at the 6% coating level. In comparison, the disintegration time for the rosuvastatin cores was 2 min 58 sec. This batch was coated with COAT-030, a clear coat. This coating was also used in combination with COAT-031 to coat prasugrel tablets (Batch ENQ3822/AIRT/023/01). This batch also disintegrated more slowly compared with the other prasugrel lots but did so within 4 minutes. It should be noted that COAT-030 is designed as a coating to protect moisture and pH-sensitive drugs and so the increase in disintegration times may reflect its water barrier properties.

For most batches the disintegration time increased with the %coating level to varying extents, the largest increase being seen with the tablets coated with COAT-030. For the batches coated with COAT-010, COAT-020 and COAT-034, all of which contain rice starch, the disintegration times only increased at

4% coating level and then decreased or remained constant at the 6% weight gain. This may be due to the ability of starch to absorb water.

## 36. Assay and Related Impurities - $TiO_2$-free Coated versus $TiO_2$ Reference Batches

Table 42 and Table 43 show respectively the assay and total related impurity results for the various $TiO_2$-free coated and $TiO_2$ coated reference batches at a 2%, 4% and 6% coating weight gain. The assay results for the coated nifedipine batches were within expectation and ranged from 98.0 %label claim (%LC) to 99.4 %LC.

The total related impurity results for the coated nifedipine batches were ranged from 0.06 %LC to 0.12 %LC. There was no clear trend in the assay or related impurities across the batches or between coating levels. However, for the batches coated with COAT-004 and COAT-023, the assay results were slightly lower and the related impurity values slightly higher than for the other lots.

For the coated olmesartan batches the assay values varied from 100.2 %LC to 101.4 %LC and the total related impurities were 0.3 %LC for all batches. There was no trend in the assay results across the batches or between coating levels.

The assay values for the rosuvastatin batches ranged from 98.4 %LC to 103.2 %LC and the total impurities from 0.47 %LC to 0.61 %LC. There was no trend in the assay or related impurity results across the batches or between coating levels.

The assay values for the prasugrel coated batches ranged from 98.6 %LC to 101.1 %LC and the total impurities from 0.6 %LC to 0.8 %LC. There was no trend in the assay or related impurity results across the batches or between coating levels.

Table 42: Assay results for the $TiO_2$-free batches and corresponding $TiO_2$ references

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consort Coat Ref | Film Former | Opacifier | Assay (%LC) at %Weight Gain | | |
|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | 2% | 4% | 6% |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 98.5 | 98.6 | 98.9 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 99.1 | 98.4 | 99.4 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 98.5 | 98.9 | 99.1 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 98.6 | 98.3 | 98.1 |
| 005/01 | COAT-023 | PVA | F+Talc | 98.5 | 98.0 | 98.2 |
| **Olmesartan 20 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+ $Fe_2O_3$ | 100.2 | 100.3 | 100.5 |
| 007/01 | COAT-013 | PVA+ HPMC | $CaCO_3$+Talc+ $Fe_2O_3$ | 100.3 | 100.8 | 100.7 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 100.6 | 101.2 | 101.4 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 101.3 | 100.6 | 100.6 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 101.4 | 99.8 | 101.4 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 101.2 | 100.7 | 103.2 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 103.0 | 100.6 | 101.9 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 100.7 | 98.8 | 100.9 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 102.4 | 99.1 | 101.6 |
| 015/02 | COAT-030 | HPMC | B+E | 101.3 | 99.3 | 99.4 |
| 016/01 | COAT-005 | HPMC | MgO | 100.1 | 98.4 | 98.4 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 101.1 | 98.9 | 98.9 |
| 018/01 | COAT-034 | HPMC | Rice Starch | 100.9 | 100.1 | 100.0 |
| 019/01 | COAT-023 | PVA | F+Talc | 101.0 | 98.4 | 99.5 |
| **Prasugrel 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 98.6 | 99.1 | 98.6 |
| 021/01 | COAT-016 | HPMC | Rice Starch+D+$Fe_2O_3$ | 99.0 | 99.4 | 98.6 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+$Fe_2O_3$ | 99.7 | 99.3 | 100.4 |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 99.0 | 100.1 | 100.1 |

Table 43: Related impurity results for the $TiO_2$-free batches and corresponding $TiO_2$ references

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | Film Former | Opacifier | Total Related Impurities at %Weight Gain (%LC) | | |
|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 0.09 | 0.09 | 0.06 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 0.07 | 0.08 | 0.08 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 0.07 | 0.07 | 0.07 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 0.12 | 0.11 | 0.12 |
| 005/01 | COAT-023 | PVA | F+Talc | 0.10 | 0.10 | 0.10 |
| **Olmesartan 20 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 0.3 | 0.3 | 0.3 |
| 007/01 | COAT-013 | PVA+ HPMC | $CaCO_3$+Talc+ $Fe_2O_3$ | 0.3 | 0.3 | 0.3 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 0.3 | 0.3 | 0.3 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 0.3 | 0.3 | 0.3 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 0.52 | 0.61 | 0.51 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 0.52 | 0.60 | 0.52 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 0.49 | 0.61 | 0.50 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 0.51 | 0.61 | 0.50 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 0.47 | 0.57 | 0.52 |
| 015/02 | COAT-030 | HPMC | B+E | 0.59 | 0.59 | 0.59 |
| 016/01 | COAT-005 | HPMC | MgO | 0.47 | 0.51 | 0.50 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 0.49 | 0.51 | 0.50 |
| 018/01 | COAT-034 | HPMC | Rice Starch | 0.53 | 0.52 | 0.56 |
| 019/01 | COAT-023 | PVA | F+Talc | 0.53 | 0.54 | 0.54 |
| **Prasugrel 10 mg Coated Tablets** | | | | **2%** | **4%** | **6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 0.8 | 0.7 | 0.8 |
| 021/01 | COAT-016 | HPMC | Rice Starch+D+$Fe_2O_3$ | 0.7 | 0.7 | 0.6 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+$Fe_2O_3$ | 0.8 | 0.7 | 0.7 |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 0.7 | 0.6 | 0.6 |

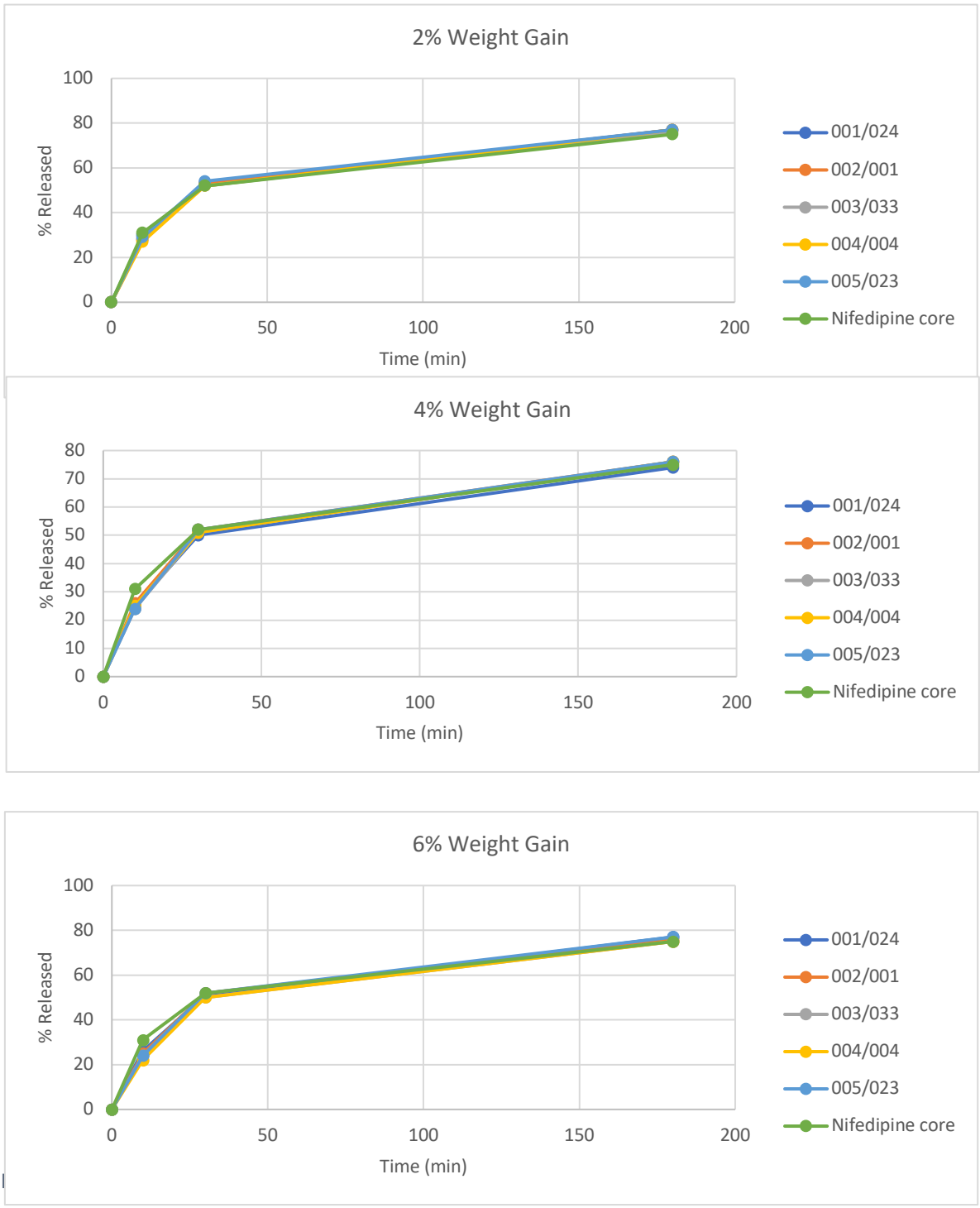
In summary, the results indicate that coating with the $TiO_2$-free coats and the $TiO_2$ reference coats up to 6% weight gain did not affect the assay or impurity results of the active tablets chosen for evaluation.

## 37. Dissolution

Coated Nifedipine Batches

The dissolution results for the $TiO_2$-free and corresponding $TiO_2$ reference coated batches are shown in Figure 25 for the nifedipine tablets.

Figure 25: Dissolution of the coated nifedipine tablets at 2%, 4% and 6% weight gain




All of the coated nifedipine tablet batches released 75% of the API in 180 min. The % released at each time-point was almost identical. This was despite Batch ENQ3822/AIRT/004/01 having a disintegration time of over 18 min at the 6% coating level (see Table 41) which was significantly greater than that of the other nifedipine batches.

Coated Olmesartan Batches

The dissolution results for the olmesartan coated tablets are shown in Table 44. All of the coated batches released over 90% in 15 minutes regardless of the coating used or the % coating weight gain. The %released varied from 92% to 99%. Therefore, coating had a negligible effect on dissolution as might be expected from the rapid disintegration of these tablets which occurred within 3 min.

Table 44: Dissolution results for the coated olmesartan tablets at 0%, 2%, 4% and 6% weight gain

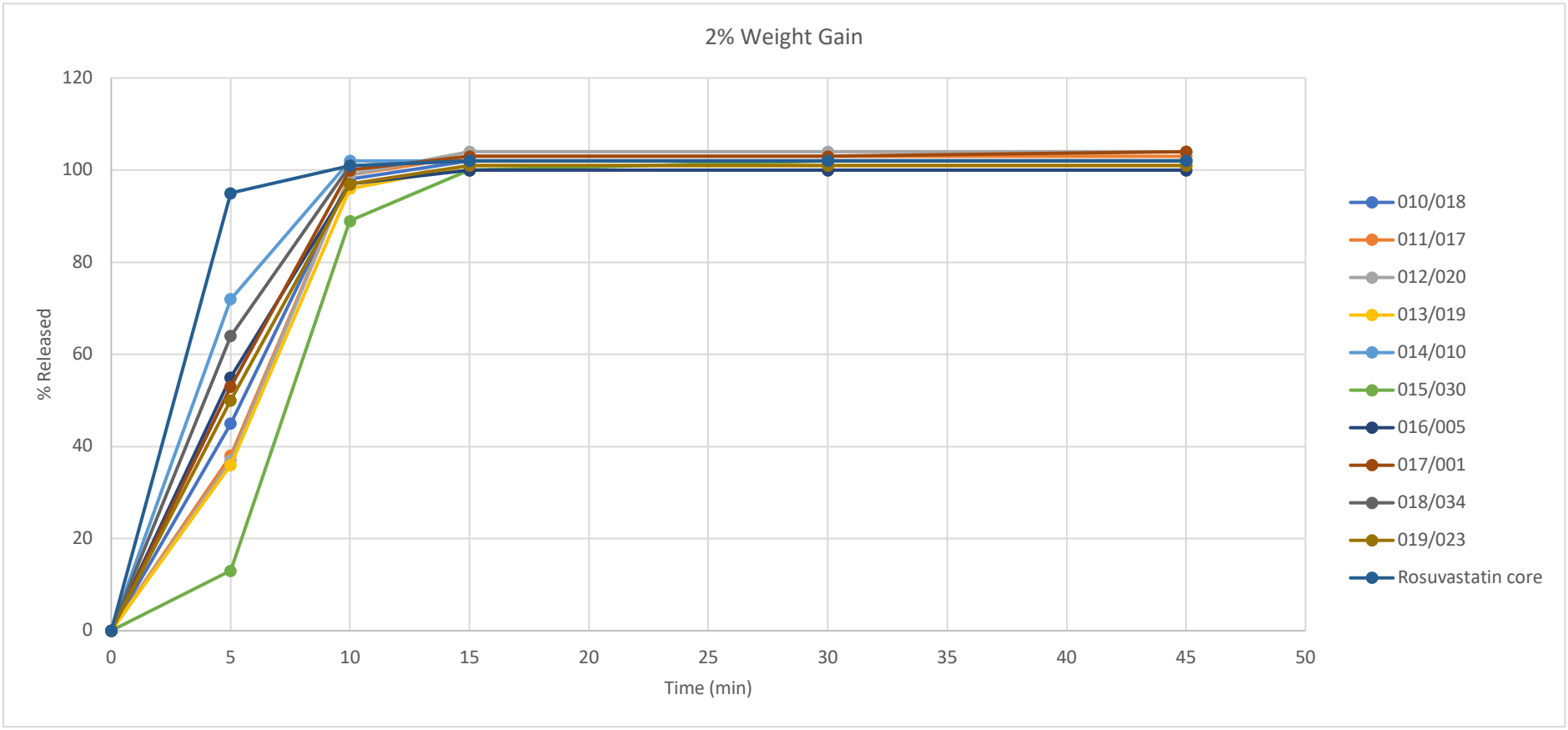
| **Batch No. ENQ3822/AIRT/** | **006/01P1** | | **007/01P1** | | **008/01P1** | | **009/01P1** | |
|---|---|---|---|---|---|---|---|---|
| **Consort. Cap Ref.** | **COAT-025** | | **COAT-013** | | **COAT-015** | | **COAT-014** | |
| **Film Former/Opacifier** | **PVA/$TiO_2$+Talc+$Fe_2O_3$** | | **PVA+HPMC/$CaCO_3$+Talc+$Fe_2O_3$** | | **PVA/$CaCO_3$+Talc+$Fe_2O_3$** | | **PVA/$CaCO_3$+Talc+$Fe_2O_3$** | |
| **Diss. Time (min)** | **Mean (%)** | **RSD (%)** | **Mean (%)** | **RSD (%)** | **Mean (%)** | **RSD (%)** | **Mean (%)** | **RSD (%)** |
| **%Weight Gain** | **0%** | | **0%** | | **0%** | | **0%** | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 97 | 2 | 97 | 2 | 97 | 2 | 97 | 2 |
| **%Weight Gain** | **2%** | | **2%** | | **2%** | | **2%** | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 95 | 1 | 97 | 1 | 99 | 2 | 98 | 1 |
| **%Weight Gain** | **4%** | | **4%** | | **4%** | | **4%** | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 97 | 1 | 97 | 2 | 97 | 1 | 96 | 1 |
| **%Weight Gain** | **6%** | | **6%** | | | | **6%** | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 93 | 2 | 92 | 5 | 97 | 2 | 94 | 1 |

The dissolution results for the rosuvastatin tablets are shown in Figure 26, Figure 27 and Figure 28. The tablet cores released an average of 95% in the first 5 minutes and almost all of the coated batches had completely released the rosuvastatin by 10 to 15 min regardless of the %coating level or the coat used. There was some variation in the release rates between 0 and 15 minutes. The exception was Batch ENQ3822/AIRT/015/02 which only released 5% of the API at 5 min, 56% after 10 min and 93% within 15 minutes at the 6% coating level. All of the rosuvastatin was released by the 30 minutes time-point. This batch had longer disintegration time of just over 9 min compared with between 4 min to 7 min for the other batches (see Table 41). The fastest dissolution occurred with the batches coated with $TiO_2$-free coatings containing rice starch (ENQ3822/AIRT/012/01, ENQ3822/AIRT/014/01 and ENQ3822/AIRT/018/01). This again is in line with their slightly faster disintegration.

Figure 29 shows the dissolution data for the coated prasugrel tablets. The recovery from the prasugrel cores was only 83% at 60 min, while that from the coated batches was only slightly higher. Overall, the data were more variable than with the other active batches. There was no significant difference in the dissolution profiles from the $TiO_2$-free coated batches and that of the $TiO_2$ reference batch.

In summary, coating the batches of nifedipine, olmesartan, rosuvastatin and prasugrel cores with the $TiO_2$-free coatings under evaluation did not affect their dissolution to any great extent and the results were comparable to those coated with the $TiO_2$ reference coats.

Figure 26: Dissolution data for the rosuvastatin coated tablets at a 2% weight gain



Data Labels: Batch/Coat

Figure 27: Dissolution data for the rosuvastatin coated tablets at a 4% weight gain

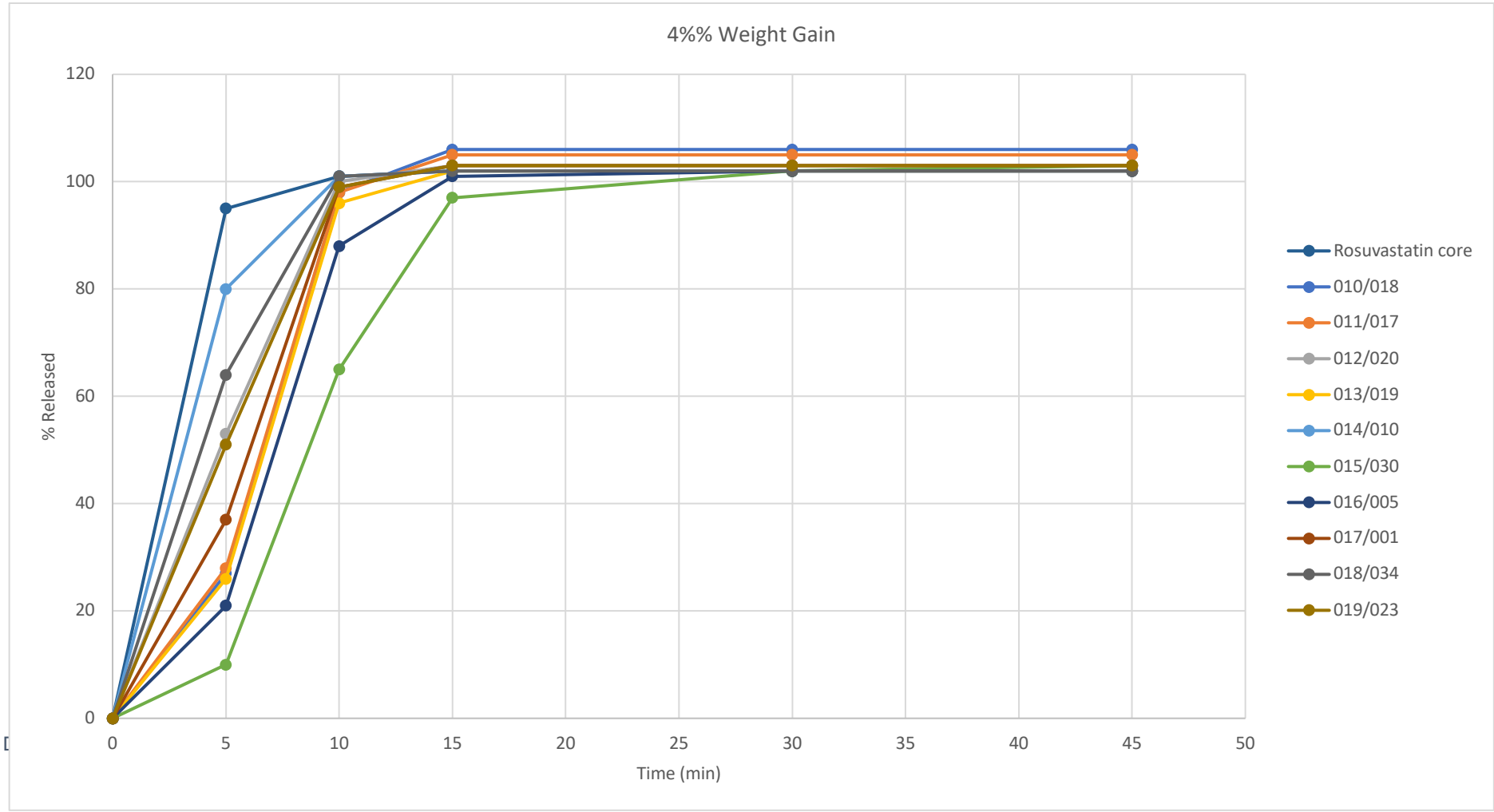
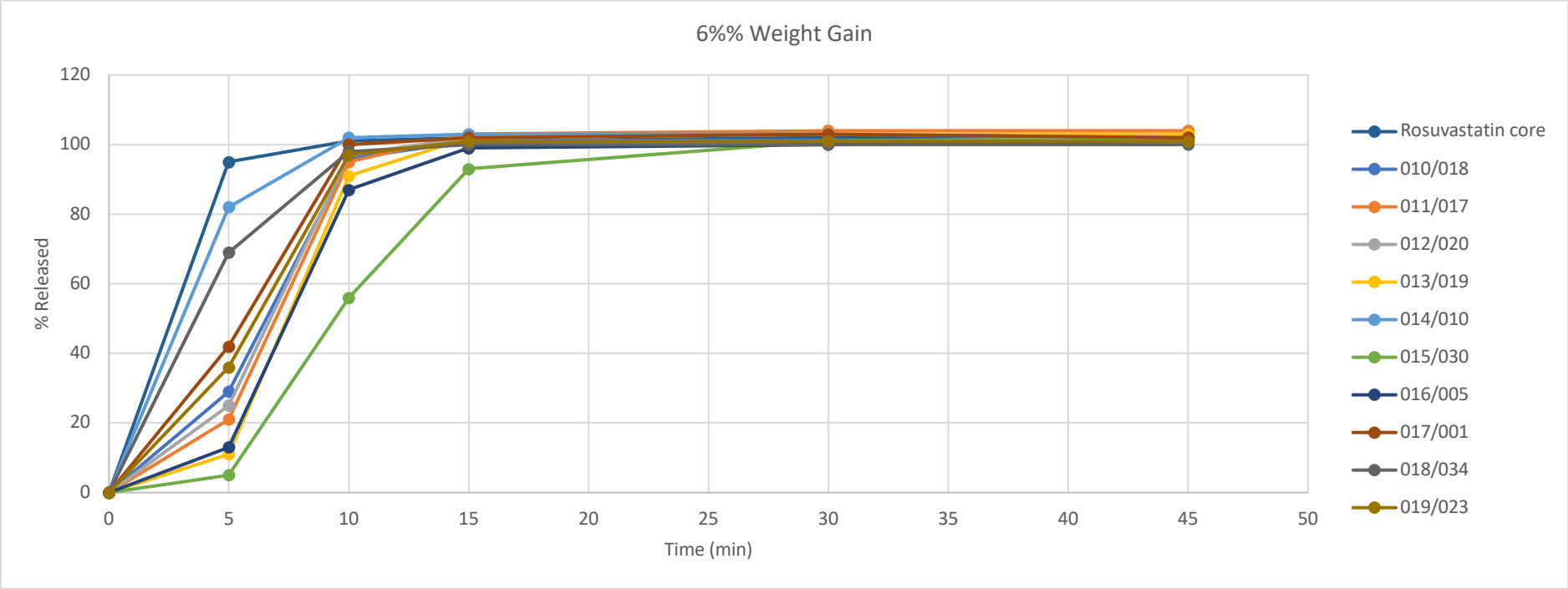
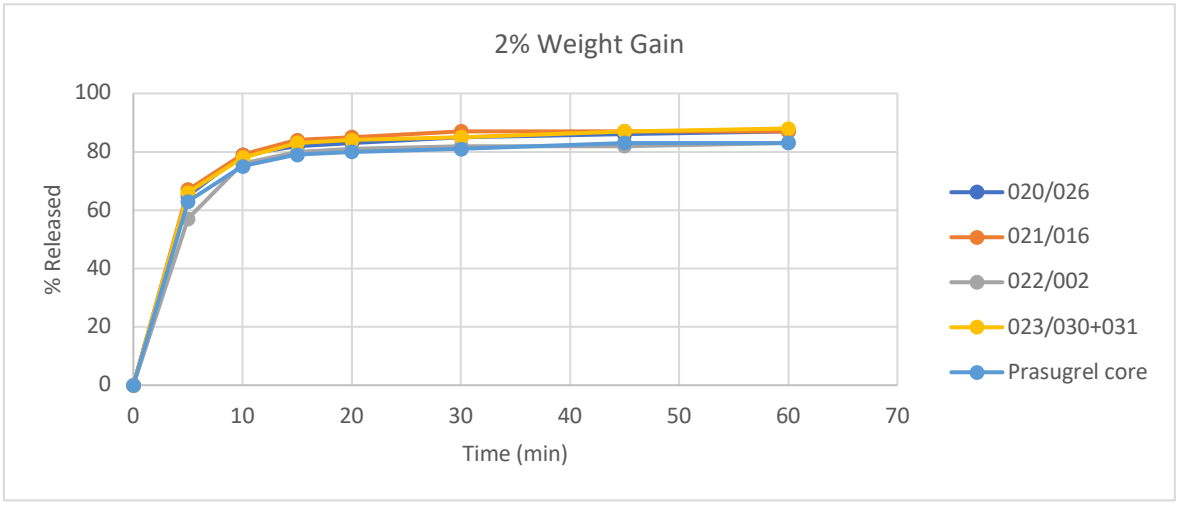


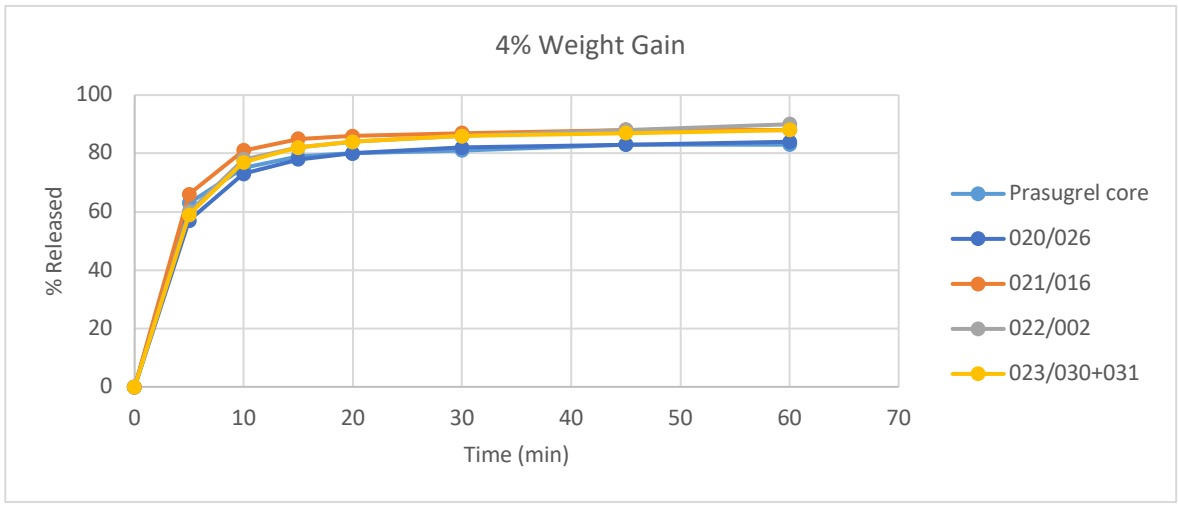
Figure 28: Dissolution data for the rosuvastatin coated tablets at a 6% weight gain

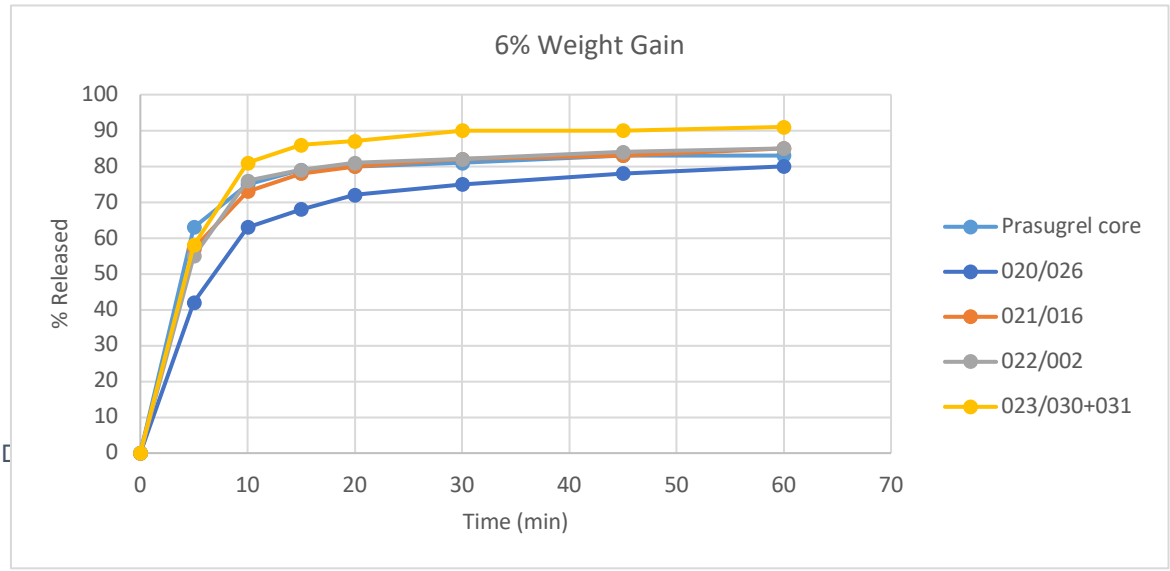



Data Labels: Batch/Coat

Figure 29: Dissolution data for the prasugrel coated tablets at a 2%, 4% and 6% weight gain



## Section Summary and Conclusions

Coated Nifedipine Batches

Four $TiO_2$-free coatings and one $TiO_2$-reference coat were used to coat the nifedipine tablet cores. The $TiO_2$-reference, COAT-024, covered and opacified the yellow surface of the nifedipine tablet cores at a ≥ 5% coating level based on visual appearance data. All of the $TiO_2$-free coatings failed to do so and the coated tablets were still yellow in color even at a 6% coating weight gain. The visual description data are in agreement with the results from colorimetry, with none of the $TiO_2$-free coated batches being a color match for the $TiO_2$ reference batch.

There was no difference in the XRPD pattern obtained from the tablets coated with the $TiO_2$-free coatings and the batch coated with the $TiO_2$-reference coat, and all displayed the nifedipine Pattern A. This showed that the $TiO_2$-free coatings and $TiO_2$ reference coat had no immediate impact on the solid-state characteristics of the API.

The $TiO_2$-free coated batches had similar assay and %total related impurities values and dissolution profiles to the $TiO_2$ coated reference batch. Disintegration times increased with % coating weight gain for all batches and varied between the different batches, increasing significantly for three of the $TiO_2$-free coated batches especially for the 6% weight gain samples. However, dissolution of nifedipine was not significantly affected by whether the coating was $TiO_2$-free or the reference and was almost identical to that of the core tablet.

Coated Olmesartan Batches

The three $TiO_2$-free coatings tested, COAT-013, COAT-014 and COAT-015, gave unsatisfactory results compared with the pink $TiO_2$ reference coat, COAT-025. In all cases the coating color intensity increased with the %coating level suggesting that the end-point of surface coverage would be difficult to determine and may be susceptible to changes in coating efficiency, process parameters, material attributes and process scale. In addition, the tablets coated with COAT-014 and COAT-015 displayed a spray pattern. In contrast, the $TiO_2$ reference coat resulted in tablet surface coverage at a 3% weight gain based on visual appearance observations, and the color remained constant as the %coating weight gain increased. Therefore, these three $TiO_2$-free coatings are inferior to the $TiO_2$ reference and, as expected from the visual data, none were a color match for the $TiO_2$ reference coating based on colorimetry data.

There were no or only minor differences between the XRPD, assay, related impurity and dissolution results for the $TiO_2$-free and the $TiO_2$ reference coated olmesartan batches showing that use of the $TiO_2$-free coatings did not impact on the chemical and physical stability of the API and the invitro performance of the coated olmesartan tablets.

Coated Rosuvastatin Batches

The white rosuvastatin tablet cores were coated with white coatings. Therefore, it was not possible to compare the $TiO_2$-free coatings against the reference coatings based on visual assessment alone, as completion of surface coverage was difficult to discern. Colorimetry data showed only COAT-019, COAT-001, COAT-020 and COAT-010 could achieve color matching to the HPMC-based $TiO_2$ reference at coating levels at which the reference coat, COAT-017, had previously achieved surface coverage (≥3% weight gain – see Section 16). It was not possible to ascertain when coating was complete for all of the other batches as the white coatings were sprayed onto white cores.

All of the rosuvastatin batches displayed the characteristic rosuvastatin pattern A. However, some also displayed peak shifting and additional peaks. The source of these additional peaks and the reason for peak shifting are unknown and would require further investigation.

The $TiO_2$-free coated batches had similar assay and %total related impurities values to the $TiO_2$ coated reference batch. Disintegration times increased slightly with %coating weight gain. Most of the rosuvastatin batches disintegrated between 4 and 7 minutes irrespective of whether the coating contained $TiO_2$ or not. The exception was the batch coated with COAT-030 which took slightly longer to disintegrate. However, dissolution of rosuvastatin was not significantly affected by whether the coating was $TiO_2$-free or the reference, with release being complete or almost complete by 15 min regardless of the % coating level or the coat used. The batch with slowest release was Batch

ENQ3822/AIRT/015/02, which was coated with COAT-030, whose disintegration time was also the longest. However, even its 6% weight gain sample had released 93% of rosuvastatin at the 15-min time-point.

Coated Prasugrel Batches

The prasugrel tablet cores were coated with HPMC-based colored coatings, three of which were $TiO_2$-free. Two contained rice starch or rich starch in combination with Opacifier A for opacification. The third was a combination of a clear coat and a colored admix which produced a red coated coating. The $TiO_2$-free coated tablets were compared with those coated with the HPMC-based, pink, $TiO_2$ reference (COAT-026).

Tablet surface coverage appeared complete for the $TiO_2$ reference coated batch at the 2% coating level based on photography and visual appearance observations during manufacture. The batches coated with the $TiO_2$-free coats, COAT-016 and COAT-002, had good coverage at a ≥4% weight gain and at 6% weight gain respectively, while coverage was obtained with the COAT-030/031 combination at 5% or 6% weight gain. However, coating with COAT-016 and the COAT-030/031 combination resulted in a spray pattern on the tablets which was observed in the photographed samples but not during manufacturing. Again, the results show that higher % weight gains are required for $TiO_2$-free coatings to achieve surface coverage than when the coating contains $TiO_2$.

All of the prasugrel batches displayed the characteristic prasugrel pattern A regardless of whether coated with $TiO_2$-free coating or the $TiO_2$ reference coat, thus, showing that coating had not impacted on the physical stability of the API. However, the $TiO_2$ reference batch XRPD trace included one additional peak. The source of this peak is unknown and would require further investigation.

The use of $TiO_2$-free coatings had no significant impact on prasugrel coated batch assay, % related impurities, disintegration or dissolution.

# Experimental Part 6: Photostability Study on Active Tablets

## Protocol

Coated tablet samples from the 23 coating trials on active-containing cores (see Section 9, Table 25) were subjected to photostability testing. Samples were tested at a 2 %, 3%, 4%, 5% and 6% coating weight gain. The conditions used were not less than 2.4 million lux hours which is equivalent to 2 x ICH Q1B cycles (where 1 x ICH Q1B cycle equals light not less than 1.2 million lux hours and UV not less than 200 Watt-hours/$m^2$). 210 tablets from each sample were placed in a clear borosilicate petri dish. A further 210 tablets from each sample were placed in a clear borosilicate petri dish which was then wrapped in aluminium foil to act as dark controls. Following exposure, the samples were stored at laboratory room temperature. The samples were analysed as shown in Table 45.

Table 45: Tests carried out on the stability study samples

| **Attribute** | **Methodology** | **Samples Tested by %Weight Gain** |
|---|---|---|
| **All photostability samples** | | |
| Appearance - Visual | Photography | 2%, 4%, 6% |
| Appearance - Colorimetry | DigiEye | 2%, 3%, 4%, 5%, 6% |
| Coat thickness | Digital optical microscopy | 2%, 4%, 6% |
| Solid state | XRPD | 2%, 4%, 6% |
| Disintegration | Ph.Eur. 2.9.1 | 2%, 4%, 6% |
| **Corinfar (nifedipine) 10 mg Retard Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/UV spectroscopy | 2%, 4%, 6% |
| **Olmesartan 20 mg Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/UV spectroscopy | 2%, 4%, 6% |
| **Rosuvastatin 10 mg Tablets only** | | |
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/HPLC | 2%, 4%, 6% |
| **Prasugrel 10 mg Tablets only** | | |

| | | |
|---|---|---|
| Assay | HPLC | 2%, 4%, 6% |
| Impurities | HPLC | 2%, 4%, 6% |
| Dissolution | USP Apparatus II (Paddles)/HPLC | 2%, 4%, 6% |

## Analytical Methods

### 38. Visual Appearance

The visual appearance checks were conducted immediately after removal from the stability chamber. Only one side of each tablet was exposed in the photostability chamber, therefore, some color variation could be present in the light-exposed samples. For this reason, two tablets were used from each sample to assess visual appearance of the front and back of the tablets.

### 39. Colorimetry

Colorimetry was carried out as described in Section 14. The sides of the tablets exposed to light were examined to determine which tablet face was more visually different from the corresponding control sample. This was carried out as only one face would have been exposed to light during the photostability study. Colorimetry was carried out on the more visually different tablet face except in cases where the two sides were indistinguishable to the human eye.

In order to assess the color differences between the light-exposed versus the dark control tablets, the $\Delta E^*_{00}$ values for each batch were calculated for individual tablets compared to the mean average (n=20) of the corresponding control. The values for the individual tablets were then averaged to give the mean $\Delta E^*_{00}$. The acceptance criteria for there being no color difference between the exposed and control samples were $\Delta E^*_{00} \leq 1$ for white tablets and $\Delta E^*_{00} < 2$ for colored tablets. The rationale for these criteria is given in Section 14.

### 40. Coating Thickness

Coating thickness was measured by digital optical microscopy as described in Section 28. In some samples, it was difficult to clearly define the boundary between coating and core due to poor contrast in digital microscopy. Therefore, some values reported for coating thickness were approximate. The measurements for each stability sample were averaged to give the mean result and the minimum and maximum thickness for each sample reported. The mean thickness (quoted to one decimal place) for each exposed sample was then compared with the mean thickness of the corresponding control sample

### 41. Other Methods

These were as described in Section 0.

## Results and Discussion

### 42. Visual Appearance and Colorimetry

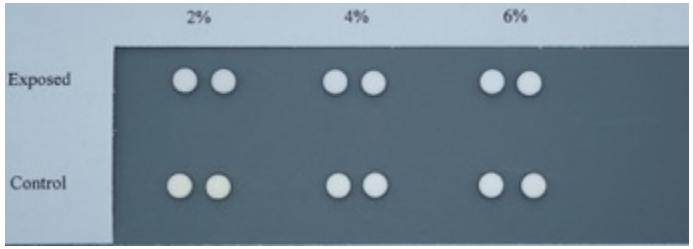
Nifedipine Coated Tablets

Figure 30 shows the photographs of the light-exposed and control samples from the coated nifedipine retard batches and Table 46 the visual description of the samples. Table 47 shows the $\Delta E^*_{00}$ values for the light exposed coated nifedipine tablets versus the corresponding controls.

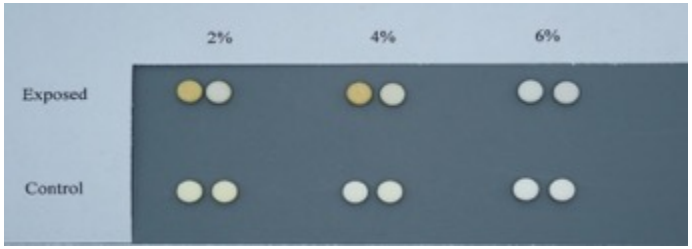
Figure 30: Photographs of light exposed and control coated nifedipine tablet samples at different film coat percentage weight gains

For all photographs, the exposed (upward facing) tablet surface is on the left and the downward facing tablet surface on the right.

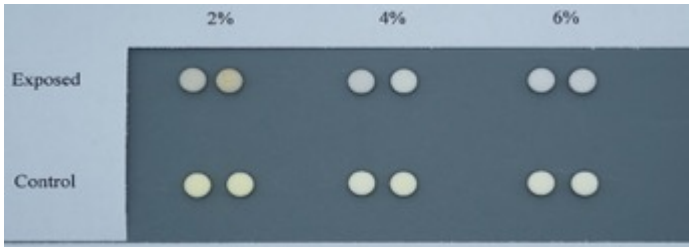
Batch ENQ3822/AIRT/001/01



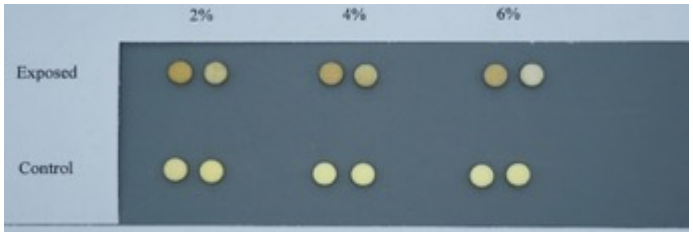

Batch ENQ3822/AIRT/002/01




Batch ENQ3822/AIRT/003/01




Batch ENQ3822/AIRT/004/01




Batch ENQ3822/AIRT/005/01

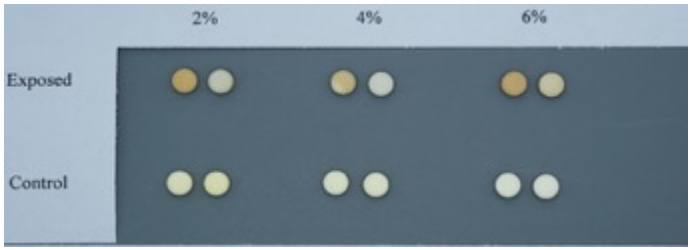


Table 46: Visual appearance of the light-exposed nifedipine tablet samples versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | Weight Gain | Appearance Light-Exposed | Appearance Dark Control | Difference Exp vs Con |
|---|---|---|---|---|
| 001/01 COAT-024 (HPMC/$TiO_2$) | 2% | Slightly off-white round tablets, no color variation, no visible defects. | Pale yellow round tablets, no color variation, no visible defects. | Yes |
| | 4% | White round tablets, no color variation, no visible defects. | Paler (compared to 2%) yellow round tablets, no color variation, no visible defects. | Yes |
| | 6% | White round tablets, no color variation, no visible defects. | Off-white round tablets, no color variation, no visible defects. | Yes |
| 002/01 COAT-001 (HPMC+HPC $MgCO_3$+A+B) | 2% | Yellow round tablets, color variation between front/back of tablets, no visible defects. | Pale yellow round tablets, no color variation, no visible defects. | Yes |
| | 4% | Yellow round tablets, color variation between front/back of tablets, some splotching on coat. | Paler (Compared to 2%) yellow round tablets, no color variation, no visible defects. | Yes |
| | 6% | Off-white round tablets, some color variation between front/back of tablets, no visible defects. | Pale yellow round tablets, no color variation, no visible defects. | Yes |
| 003/01 COAT-033 (HPMC/$CaCO_3$+D+F) | 2% | Light yellow/brown round tablets, color variation between front/back of tablets, no visible defects. | Bright yellow round tablets, no color variation, no visible defects. | Yes |
| | 4% | Pale yellow round tablets, no color variation, some splotching on coat. | Pale yellow round tablets, no color variation, no visible defects. | Yes |
| | 6% | Off-white round tablets, color variation between front/back of tablets, no visible defects. | Pale yellow round tablets, no color variation, no visible defects. | Yes |
| 004/01 COAT-004 (HPMC/$CaCO_3$+C) | 2% | Dark yellow round tablets, color variation between front/back of tablets, some splotching on coat. | Yellow round tablets, no color variation, no visible defects. | Yes |
| | 4% | Dark yellow round tablets, color variation between front/back of tablets, some splotching on coat. | Yellow round tablets, no color variation, no visible defects | Yes |
| | 6% | Dark yellow round tablets, color variation between front/back of tablets, some splotching on coat. | Yellow round tablets, no color variation, no visible defects. | Yes |
| 005/01 COAT-023 (PVA/F+Talc) | 2% | Dark yellow/orange round tablets, color variation between front/back of tablets, no physical defects. | Yellow round tablets, no color variation, no visible defects. | Yes |
| | 4% | Dark yellow/orange round tablets, color variation between front/back of tablets, no physical defects. | Yellow round tablets, no color variation, no visible defects. | Yes |
| | 6% | Dark yellow/orange round tablets, color variation between front/back of tablets, no physical defects. | Yellow round tablets, no color variation, no visible defects. | Yes |

Color Code: Red = Difference in appearance

Table 47: $\Delta E^*_{00}$ values for light-exposed coated nifedipine tablets versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | Consort Coat Reference | Film Former | Opacifier | % Coating | $\Delta E^*_{00}$ |
|---|---|---|---|---|---|
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 2 | 7.24 |
| | | | | 3 | 5.91 |
| | | | | 4 | 4.15 |
| | | | | 5 | 3.74 |
| | | | | 6 | 2.74 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 2 | 7.82 |
| | | | | 3 | 4.37 |
| | | | | 4 | 7.03 |
| | | | | 5 | 5.10 |
| | | | | 6 | 4.29 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 2 | 10.11 |
| | | | | 3 | 10.35 |
| | | | | 4 | 10.39 |
| | | | | 5 | 9.94 |
| | | | | 6 | 9.47 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 2 | 13.57 |
| | | | | 3 | 13.53 |
| | | | | 4 | 13.35 |
| | | | | 5 | 11.50 |
| | | | | 6 | 12.81 |
| 005/01 | COAT-023 | PVA | F+Talc | 2 | 10.96 |
| | | | | 3 | 8.27 |
| | | | | 4 | 9.34 |
| | | | | 5 | 10.42 |
| | | | | 6 | 12.51 |

Color Code: Red = Does not meets color difference acceptance criterion for white tablets

Both the visual and colorimetry data show that there was a color difference between the photoexposed samples and the controls. This is particularly obvious when the two faces of the exposed samples are compared in the photographs. In the colorimetry experiments the side of the tablets more obviously different to the control was selected for the color difference comparison with the dark controls. All of the $\Delta E^*_{00}$ values were > 2 (meaning that a color difference between the photoexposed samples and the controls is obvious at a glance).

Nifedipine is well-known for its sensitivity to photodegradation by UV light and visible light below 500 nm in wavelength [14]. The The data show that neither the $TiO_2$-free coatings nor the $TiO_2$ reference coating could fully protect nifedipine from photodegradation. However, there were differences in the extent to which there was a color change between the exposed sample and the control for the different coatings.

The $TiO_2$ reference coating, COAT-024, gave the best results with a color difference $\Delta E^*_{00}$ value of 2.74 at a 6%w/w weight gain. The $TiO_2$ reference coated tablets (Batch ENQ3822/AIRT/001) were deemed to be fully coated based on visual appearance at a weight gain of 5%w/w. However, the colorimetry data indicate that a 6% w/w coating weight gain provided further but still incomplete protection against photodegradation based on the reduction in $\Delta E^*_{00}$ from 3.74 at 5% weight gain to 2.74 at the 6% coating level.

None of the $TiO_2$-free coatings tested were able to hide the yellow color of the cores completely, as can be seen from the control samples, and therefore their ability to protect nifedipine against photodegradation will be reduced. Therefore, it is not surprising that their $\Delta E^*_{00}$ values for the exposed and corresponding control samples are higher than for the $TiO_2$ coated batch.

Olmesartan Coated Tablets

Table 48 shows the visual appearance and Table 49 the $\Delta E^*_{00}$ values for the light-exposed olmesartan samples versus the corresponding controls.

Table 48: Visual appearance of the light-exposed coated olmesartan tablet samples versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | % Coat Wt. Gain | Appearance Light-exposed | Appearance Dark Control | Difference Exp vs Con |
|---|---|---|---|---|
| 006/01 COAT-025 (PVA/$TiO_2$+Talc+$Fe_2O_3$) | 2% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| | 4% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| | 6% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| 007/01[a] COAT-013 (PVA+HPMC/ $CaCO_3$+Talc+$Fe_2O_3$) | 2% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| | 4% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| | 6% | Pink round tablets, no color variation, no visible defects. | Pink round tablets, no color variation, no visible defects. | No |
| 008/01[a] COAT-015 (PVA/$CaCO_3$+Talc+$Fe_2O_3$) | 2% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, no color variation, no visible defects. | Yes[b] |
| | 4% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, no color variation, no visible defects. | Yes[b] |
| | 6% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, no color variation, no visible defects. | Yes[sb] |
| 009/01[a] COAT-014 (PVA/$CaCO_3$+Talc+$Fe_2O_3$) | 2% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, slight color variation on tablet bellybands, no visible defects. | No |
| | 4% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, slight color variation on tablet bellybands, no visible defects. | No |
| | 6% | Pink round tablets, some color variation on tablet bellybands, no visible defects. | Pink round tablets, slight color variation on tablet bellybands, no visible defects. | No |

[a]Intensity of pink color increases with % coating

[b]Color variation on bellyband may be due to a coating issue as opposed to light exposure as Batch ENQ3822/AIRT/009/01 has the same color variation but on both exposed and control samples.

Color Code: Green = No visible difference between samples, Yellow = Slight difference but may be due to coating variation.

There was no significant color difference between the light-exposed samples and the corresponding controls for the batches coated with $TiO_2$-free coatings and the $TiO_2$ reference except for Batch ENQ3822/AIRT/008/01 where there was some color variation on the bellyband of the tablets exposed to light. This reflects that the olmesartan is not photosensitive and that the coatings themselves are not changing color significantly on light exposure. All of the batches met the colorimetry acceptance criterion of $\Delta E^*_{00}$ values < 2 and most $\Delta E^*_{00}$ values were < 1.

Table 49: $\Delta E^*_{00}$ values for light-exposed coated olmesartan tablets versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | Consort Coat Reference | Film Former | Opacifier | % Coating | $\Delta E^*_{00}$ |
|---|---|---|---|---|---|
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+ $Fe_2O_3$ | 2 | 0.59 |
| | | | | 3 | 0.47 |
| | | | | 4 | 0.36 |
| | | | | 5 | 0.51 |
| | | | | 6 | 0.42 |
| 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc +$Fe_2O_3$ | 2 | 1.00 |
| | | | | 3 | 0.76 |
| | | | | 4 | 0.81 |
| | | | | 5 | 0.86 |
| | | | | 6 | 0.74 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 2 | 0.98 |
| | | | | 3 | 1.01 |
| | | | | 4 | 0.59 |
| | | | | 5 | 0.47 |
| | | | | 6 | 0.44 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+ $Fe_2O_3$ | 2 | 1.27 |
| | | | | 3 | 1.42 |
| | | | | 4 | 1.13 |
| | | | | 5 | 0.86 |
| | | | | 6 | 0.59 |

Color Code: Green = Meets color difference acceptance criterion for colored tablets, $\Delta E^*_{00} < 2$

The color variation on the bellyband of Batch ENQ3822/AIRT/008/01 may be due to variation in the coating coverage. Such variation would not be picked up by colorimetry as data are collected from one face of each of the 20 tablets, not the bellyband. Color variation on the bellyband was also observed for Batch ENQ3822/AIRT/009/01 but on both the light-exposed and control samples.

Rosuvastatin Coated Tablets

Table 50 contains the visual descriptions of the light-exposed and control tablets and Table 51 contains the $\Delta E^*_{00}$ values. Rosuvastatin is sensitive to light and undergoes photodegradation [15]. The colorimetry data results show that only the batches coated with the two $TiO_2$ reference coats (Batches ENQ3822/AIRT/010/01 and ENQ3822/AIRT/011/01) and the $TiO_2$-free coating, COAT-023 (Batch ENQ3822/AIRT/019/01) showed no discernable color difference between the exposed and control samples at all coating levels and met the acceptance criterion of $\Delta E^*_{00} \leq 1$ for white tablets. For the batches coated with the $TiO_2$ reference coatings the colorimetry data are in line with the visual descriptions which showed no visible differences between the light-exposed and control samples. However, the light-exposed samples of Batch ENQ3822/AIRT/019/01 were described as slightly off-white and the controls as white. However, no color variation was observed between the

exposed and non-exposed faces of the exposed tablet samples. This would suggest that light exposure was not an influence in this perceived color difference and that the visual appearance results are in line with the colorimetry data. COAT-023 contains Opacifier F.

Table 50: Visual appearance of the light-exposed coated rosuvastatin tablets versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | % Coat Wt. Gain | Appearance Light-exposed | Appearance Dark Control | Difference Exp vs Con |
|---|---|---|---|---|
| 010/01 COAT-018 (PVA/$TiO_2$+Talc) | 2% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| | 4% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| | 6% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| 011/01 COAT-017 (HPMC/$TiO_2$) | 2% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| | 4% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| | 6% | Round white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | No |
| 012/01 COAT-020 (HPMC+HPC/ Rice Starch+D) | 2% | Round cream tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Round cream tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Round cream tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| 013/01 COAT-019 (HPMC/$CaCO_3$+D+E) | 2% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects | Yes |
| | 6% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| 014/01 COAT-010 (HPMC/Rice Starch+D) | 2% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Round off-white tablets, slight color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| 015/02 COAT-030 (HPMC/B+E) | 2% | Pale yellow round tablets, slight color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Pale yellow round tablets, slight color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Pale yellow round tablets, slight color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| 016/01 | 2% | Pale yellow round tablets, | Round off-white tablets, no | Yes |

| Batch No. ENQ3822/AIRT/ | % Coat Wt. Gain | Appearance Light-exposed | Appearance Dark Control | Difference Exp vs Con |
|---|---|---|---|---|
| COAT-005 (HPMC/MgO) | | color variation between front/back of tablets, no visible defects. | color variation, no visible defects. | |
| | 4% | Pale yellow round tablets, color variation between front/back of tablets, no visible defects. | Round off-white tablets, no color variation, no visible defects. | Yes |
| | 6% | Pale yellow round tablets, color variation between front/back of tablets, no visible defects. | Round off-white tablets, no color variation, no visible defects. | Yes |
| 017/01 COAT-001 (HPMC+HPC $MgCO_3$+A+B) | 2% | Off-white round tablets, color variation between front/back of tablets, damage visible on a small number of tablets but not representative of the whole exposed condition. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Off-white round tablets, color variation between front/back of tablets, damage visible on a small number of tablets but not representative of the whole exposed condition. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Off-white round tablets, color variation between front/back of tablets, damage visible on a small number of tablets but not representative of the whole exposed condition. | Round white tablets, no color variation, no visible defects. | Yes |
| 018/01 COAT-034 HPMC/Rice Starch | 2% | Pale yellow round tablets, color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Pale yellow round tablets, color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Pale yellow round tablets, color variation between front/back of tablets, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| 019/01 COAT-023 (PVA/F+Talc) | 2% | Round slightly off-white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 4% | Round slightly off-white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |
| | 6% | Round slightly off-white tablets, no color variation, no visible defects. | Round white tablets, no color variation, no visible defects. | Yes |

Color Code: Green = no visible difference between samples, Red = visible difference.

The light-exposed samples from all other batches coated with $TiO_2$-free coatings were visibly different from the control samples, and visible difference could be discerned between the exposed and non-exposed tablet faces within the light-exposed samples.

$\Delta E^*_{00}$ values for the other $TiO_2$-free coated batches were > 1 and most >2 suggesting that a difference between the light-exposed samples and the controls would be obvious, at the very least on close inspection. This was supported by the visual data. There was a downward trend in the $\Delta E^*_{00}$ values with increasing %weight gain for the majority of the $TiO_2$-free coated batches. Batch ENQ3822/AIRT/015/02, coated with the $TiO_2$-free clear coat, COAT-030, gave variable results for the $\Delta E^*_{00}$ values and there was no clear trend with %weight gain.

Table 51: $\Delta E^*_{00}$ values for light-exposed coated rosuvastatin tablets versus corresponding controls

| Batch No. ENQ3822/AIRT/ | Consort Coat Reference | Film Former | Opacifier | % Coating | $\Delta E^*_{00}$ |
|---|---|---|---|---|---|
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 2 | 0.62 |
| | | | | 3 | 0.60 |
| | | | | 4 | 0.61 |
| | | | | 5 | 0.73 |
| | | | | 6 | 0.59 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 2 | 0.72 |
| | | | | 3 | 0.74 |
| | | | | 4 | 0.65 |
| | | | | 5 | 0.51 |
| | | | | 6 | 0.56 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 2 | 8.23 |
| | | | | 3 | 6.71 |
| | | | | 4 | 4.63 |
| | | | | 5 | 3.78 |
| | | | | 6 | 3.22 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 2 | 6.08 |
| | | | | 3 | 4.57 |
| | | | | 4 | 2.23 |
| | | | | 5 | 1.88 |
| | | | | 6 | 1.48 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 2 | 6.68 |
| | | | | 3 | 4.83 |
| | | | | 4 | 4.45 |
| | | | | 5 | 4.15 |
| | | | | 6 | 1.89 |
| 015/02 | COAT-030 | HPMC | B+E | 2 | 9.29 |
| | | | | 3 | 8.56 |
| | | | | 4 | 6.21 |
| | | | | 5 | 7.44 |
| | | | | 6 | 7.27 |
| 016/01 | COAT-005 | HPMC | MgO | 2 | 8.31 |
| | | | | 3 | 7.28 |
| | | | | 4 | 6.14 |
| | | | | 5 | 5.91 |
| | | | | 6 | 3.86 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 2 | 4.42 |
| | | | | 3 | 3.26 |
| | | | | 4 | 2.50 |
| | | | | 5 | 1.81 |
| | | | | 6 | 1.75 |
| 018/01 | COAT-034 | HPMC | Rice Starch | 2 | 7.83 |
| | | | | 3 | 6.80 |
| | | | | 4 | 5.39 |
| | | | | 5 | 4.41 |
| | | | | 6 | 3.50 |
| 019/01 | COAT-023 | PVA | F+Talc | 2 | 0.52 |
| | | | | 3 | 0.40 |
| | | | | 4 | 0.43 |
| | | | | 5 | 0.32 |

| Batch No. ENQ3822/AIRT/ | Consort Coat Reference | Film Former | Opacifier | % Coating | ΔE*00 |
|---|---|---|---|---|---|
| | | | | 6 | 0.28 |

Color Code: Green = Meets criterion for color difference for white tablets, Red = Does not meets acceptance criterion

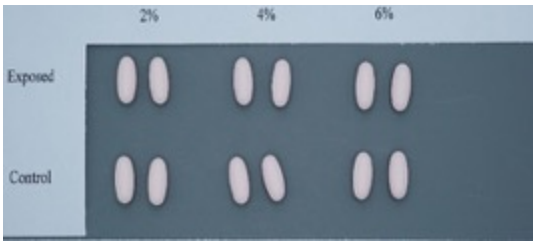
Prasugrel Coated Tablets

Figure 31 shows the photographs of the light-exposed and control samples of the coated prasugrel tablet batches, while Table 52 contains the visual descriptions of the tablets and Table 53 the ΔE*00 values.

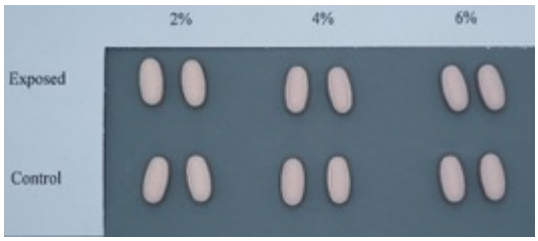
Figure 31: Photographs of light-exposed and control coated prasugrel tablet samples at different film coat percentage weight gains

For all photographs, the exposed (upward facing) tablet surface is on the left and the downward facing tablet surface on the right.

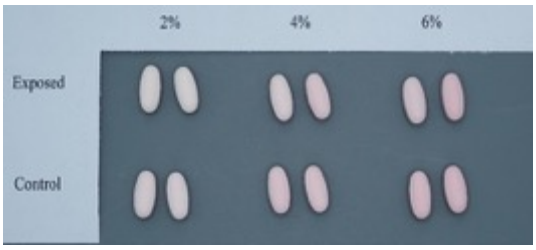
Batch ENQ3822/AIRT/020/01



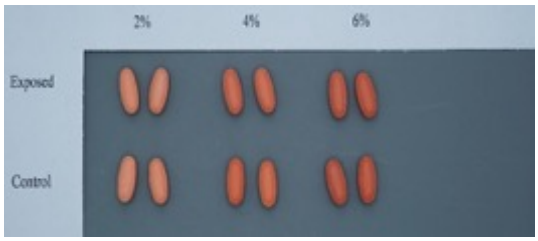

Batch ENQ3822/AIRT/021/01




Batch ENQ3822/AIRT/022/01




Batch ENQ3822/AIRT/023/01




Prasugrel undergoes photodegradation when in solution, although no significant photodegradation was found for this API in the solid-state following exposure to 1 x ICH Q1B conditions [16].

The photographs and visual descriptions show that only Batch ENQ3822/AIRT/020/01 coated with the $TiO_2$ reference coat, COAT-026, had no visually perceptible change in appearance following extreme light exposure, while a color difference can be discerned between the exposed and non-exposed faces of the light-exposed tablet samples in the batches coated with the $TiO_2$-free coatings.

Table 52: Visual appearance of the light-exposed coated prasugrel tablets versus corresponding controls

| Batch No. ENQ3822/AIRT/ | % Coat Wt. Gain | Appearance Light-exposed | Appearance Dark Control | Difference Exp vs Con |
|---|---|---|---|---|
| 020/01 COAT-026 (HPMC/$TiO_2$+$Fe_2O_3$) | 2 | Pink oval tablets, no color variations, no visible defects. | Pink oval tablets, no color variations, no visible defects. | No |
| | 4 | Pink oval tablets, no color variations, no visible defects. | Pink oval tablets, no color variations, no visible defects. | No |
| | 6 | Pink oval tablets, no color variations, no visible defects. | Pink oval tablets, no color variations, no visible defects. | No |
| 021/01 COAT-016 (HPMC/Rice Starch+ D+$Fe_2O_3$) | 2 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pale pink oval tablets, no color variation, no visible defects. | Yes |
| | 4 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pale pink oval tablets, no color variation, no visible defects. | Yes |
| | 6 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pale pink oval tablets, no color variation, no visible defects. | Yes |
| 022/01[a] COAT-002 (HPMC/ Rice Starch+ A+B+D+$Fe_2O_3$) | 2 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pink oval tablets, slight color variations on bellyband of tablets, no visible defects. | Yes |
| | 4 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pink oval tablets, slight color variations on bellyband of tablets, no visible defects. | Yes |
| | 6 | Pale pink oval tablets, some color variations between front/back of tablets, no visible defects. | Pink oval tablets, slight color variations on bellyband of tablets, no visible defects. | Yes |
| 023/01[a] COAT-030 & COAT-031 (HPMC/B+E+$Fe_2O_3$) | 2 | Red oval tablets, color variations between front/back of tablets, no visible defects. | Red oval tablets, no color variations, no visible defects. | Yes |
| | 4 | Red oval tablets, color variations between front/back of tablets, no visible defects. | Red oval tablets, no color variations, no visible defects. | Yes |
| | 6 | Red oval tablets, color variations between front/back of tablets, no visible defects. | Red oval tablets, no color variations, no visible defects. | Yes |

[a]Color intensity varies with %coating weight gain

Color Code: Green = no visible difference between samples, Red = visible difference.

Table 53: ΔE* values for light exposed coated prasugrel tablets versus the corresponding controls

| Batch No. ENQ3822/AIRT/ | Consort Coat Reference | Film Former | Opacifier | % Coating | ΔE*$_{00}$ |
|---|---|---|---|---|---|
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 2 | 3.94 |
| | | | | 3 | 4.26 |
| | | | | 4 | 4.49 |
| | | | | 5 | 3.69 |
| | | | | 6 | 3.80 |
| 021/01 | COAT-016 | HPMC | Rice Starch +D+$Fe_2O_3$ | 2 | 4.49 |
| | | | | 3 | 3.78 |
| | | | | 4 | 3.86 |
| | | | | 5 | 0.56 |
| | | | | 6 | 0.71 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+$Fe_2O_3$ | 2 | 3.20 |
| | | | | 3 | 3.61 |
| | | | | 4 | 3.39 |
| | | | | 5 | 3.26 |
| | | | | 6 | 3.64 |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 2 | 1.14 |
| | | | | 3 | 0.88 |
| | | | | 4 | 1.48 |
| | | | | 5 | 1.24 |
| | | | | 6 | 0.92 |

Color Code: Green = Meets criterion for color difference for colored tablets, Red = Does not meets acceptance criterion

However, the colorimetry results show that there was a significant color change in the batches coated with the $TiO_2$ reference coating, COAT-026 and the $TiO_2$-free coating, COAT-002, containing Rice Starch+A+B+D+$Fe_2O_3$, following extreme light exposure. In contrast, the batch coated with COAT-016 met the acceptance criterion for the color difference of < 2 for colored tablets at least at certain coating weight gains, while the batch coated with the COAT-030 and COAT-031 combination met it all %coating weight gains.

The colorimetry results for Batch ENQ3822/AIRT/020/01 are very different to the visual data which indicated no change in visual appearance as a result of light exposure. This discrepancy was investigated and the reason for it is currently unknown.

## 43. Coating Thickness

Nifedipine Coated Tablets and Olmesartan Coated Tablets

The average coating thickness (land, belly, surface) for the exposed and control nifedipine tablets are shown in Table 54. The average coating thickness (land, belly, surface and debossed image) for the exposed and control olmesartan tablets are shown in Table 55.

Table 54: Average coating thickness for the exposed and control nifedipine tablets

| Batch No. ENQ3822/ | Consort Coat Ref | Weight Gain | Coating Thickness (µm) | | | | | | Difference Mean Exp vs Cont (µm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Exposed | | | Control | | | |
| | | | Mean | Min | Max | Mean | Min | Max | |
| AIRT/001/01 | COAT-024 | 2% | 19.7 | 18 | 22 | 18.0 | 15 | 21 | 1.7 |
| | | 4% | 33.3 | 30 | 38 | 29.3 | 25 | 37 | 4.0 |
| | | 6% | 37.0 | 30 | 45 | 36.0 | 28 | 40 | 1.0 |
| AIRT/002/01 | COAT-001 | 2% | 18.3 | 12 | 25 | 27.7 | 22 | 33 | -9.3 |
| | | 4% | 40.3 | 30 | 46 | 50.7 | 43 | 65 | -10.3 |
| | | 6% | 56.7 | 46 | 69 | 55.0 | 52 | 57 | 1.7 |
| AIRT/003/01 | COAT-033 | 2% | 30.3 | 26 | 34 | 27.0 | 22 | 35 | 3.3 |
| | | 4% | 36.7 | 30 | 40 | 35.0 | 33 | 37 | 1.7 |
| | | 6% | 57.7 | 47 | 69 | 50.7 | 45 | 58 | 7.0 |
| AIRT/004/01 | COAT-004 | 2% | 24.0 | 21 | 26 | 22.3 | 21 | 23 | 1.7 |
| | | 4% | 28.0 | 21 | 39 | 41.7 | 36 | 49 | -13.7 |
| | | 6% | 51.0 | 42 | 58 | 32.3 | 30 | 34 | 18.7 |
| AIRT/005/01 | COAT-023 | 2% | 35.3 | 24 | 50 | 28.3 | 23 | 35 | 7.0 |
| | | 4% | 40.3 | 37 | 42 | 30.7 | 24 | 38 | 9.7 |
| | | 6% | 49.7 | 47 | 53 | 43.0 | 40 | 45 | 6.7 |

Table 55: Average coating thickness for the exposed and control olmesartan tablets

| Batch No. ENQ3822/ | Consort Coat Ref | Weigh Gain | Coating Thickness (µm) | | | | | | Difference Mean Exp vs Cont (µm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Exposed | | | Control | | | |
| | | | Mean | Min | Max | Mean | Min | Max | |
| AIRT/006/01 | COAT-025 | 2% | 26.3 | 19 | 34 | 28.8 | 25 | 32 | -2.5 |
| | | 4% | 40.0 | 25 | 53 | 43.3 | 25 | 86 | -3.3 |
| | | 6% | 60.8 | 53 | 73 | 57.8 | 45 | 75 | 3.0 |
| AIRT/007/01 | COAT-013 | 2% | 23.0 | 19 | 26 | 27.3 | 23 | 30 | -4.3 |
| | | 4% | 38.0 | 32 | 49 | 28.8 | 23 | 40 | 9.3 |
| | | 6% | 60.8 | 48 | 82 | 65.3 | 54 | 71 | -4.5 |
| AIRT/008/01 | COAT-015 | 2% | 44.3 | 42 | 49 | 27.0 | 19 | 41 | 17.3 |
| | | 4% | 48.8 | 32 | 76 | 47.3 | 44 | 51 | 1.5 |
| | | 6% | 58.8 | 44 | 91 | 55.3 | 42 | 72 | 3.5 |
| AIRT/009/01 | COAT-014 | 2% | 38.5 | 25 | 49 | 20.8 | 13 | 40 | 17.8 |
| | | 4% | 43.3 | 31 | 51 | 48.5 | 38 | 74 | -5.3 |
| | | 6% | 67.3 | 53 | 94 | 72.3 | 55 | 100 | -5.0 |

There is no trend in the thickness data for both the nifedipine and olmesartan coated tablets with sometimes the exposed sample having a thicker mean coating than the corresponding control and sometimes it is vice-versa. Differences in coating thickness are not the reason for the differences in the visual data and $\Delta E^*_{00}$ values found for the various coated nifedipine batches. The least color difference was found for Batch ENQ3822/001/01 coated with the $TiO_2$ reference, COAT-024, at a %weight gain of 6%. However, the exposed tablet sample at 6% weight gain had a mean coating thickness which was comparable to the $TiO_2$-free coated tablets.

The exposed sample of Batch ENQ3822/AIRT/008/01 of the coated olmesartan tablets displayed color variation on the tablet bellyband at all coating levels. For this reason, the individual values of the coating thickness on the tablet bellyband were also compared as well as the mean coat thickness values. It was 43 µm, 47 µm and 44 µm for the exposed sample and 28 µm, 50 µm and 72 µm at a coating weight gain of 2%, 4% and 6% for the control sample respectively. Therefore, coating thickness does not account for the color variation on the bellyband of the exposed tablets. However, the tablets from this batch were previously observed to display a spray pattern (see Table 31) and this may account for the color variation.

Rosuvastatin Coated Tablets

The average coating thickness (land, belly, surface) for the exposed and control rosuvastatin coated tablets are shown in Table 56.

Table 56: Average coating thickness for the exposed and control rosuvastatin tablets

| Batch No. ENQ3822/ | Consort Coat Ref | Weight Gain | Coating Thickness (µm) | | | | | | Diff Mean Exp vs Cont (µm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Exposed | | | Control | | | |
| | | | Mean | Min | Max | Mean | Min | Max | |
| AIRT/010/01 | COAT-018 | 2% | 27.3 | 26 | 30 | 26.7 | 26 | 27 | 0.7 |
| | | 4% | 40.3 | 38 | 42 | 42.3 | 38 | 45 | -2.0 |
| | | 6% | 51.0 | 38 | 65 | 55.7 | 50 | 59 | -4.7 |
| AIRT/011/01 | COAT-017 | 2% | 25.0 | 22 | 29 | 29.3 | 27 | 31 | -4.3 |
| | | 4% | 45.3 | 38 | 49 | 35.7 | 35 | 36 | 9.7 |
| | | 6% | 57.3 | 44 | 71 | 50.7 | 48 | 53 | 6.7 |
| AIRT/012/01 | COAT-020 | 2% | 36.3 | 35 | 38 | 26.0 | 24 | 28 | 10.3 |
| | | 4% | 58.7 | 56 | 61 | 43.0 | 41 | 46 | 15.7 |
| | | 6% | 64.3 | 56 | 75 | 50.3 | 46 | 54 | 14.0 |
| AIRT/013/01 | COAT-019 | 2% | 31.0 | 29 | 34 | 34.7 | 30 | 44 | -3.7 |
| | | 4% | 39.3 | 37 | 42 | 43.3 | 43 | 44 | -4.0 |
| | | 6% | 68.0 | 48 | 80 | 52.7 | 49 | 55 | 15.3 |
| AIRT/014/01 | COAT-010 | 2% | 29.7 | 24 | 33 | 29.3 | 25 | 33 | 0.3 |
| | | 4% | 54.7 | 52 | 59 | 53.3 | 52 | 55 | 1.3 |
| | | 6% | 77.3 | 60 | 88 | 74.7 | 68 | 80 | 2.7 |
| AIRT/015/02 | COAT-030 | 2% | 34.0 | 25 | 50 | 42.0 | 39 | 46 | -8.0 |
| | | 4% | 49.0 | 36 | 62 | 53.7 | 51 | 56 | -4.7 |
| | | 6% | 65.0 | 58 | 74 | 87.3 | 72 | 100 | -22.3 |
| AIRT/016/01 | COAT-005 | 2% | 32.3 | 27 | 36 | 48.3 | 44 | 54 | -16.0 |
| | | 4% | 49.0 | 47 | 51 | 50.3 | 46 | 57 | -1.3 |
| | | 6% | 54.7 | 52 | 59 | 74.0 | 68 | 85 | -19.3 |
| AIRT/017/01 | COAT-001 | 2% | 27.7 | 27 | 28 | 26.0 | 25 | 28 | 1.7 |
| | | 4% | 39.3 | 29 | 46 | 55.7 | 53 | 61 | -16.3 |
| | | 6% | 49.3 | 38 | 57 | 64.7 | 60 | 73 | -15.3 |
| AIRT/018/01 | COAT-034 | 2% | 25.7 | 24 | 28 | 11.7 | 9 | 13 | 14.0 |
| | | 4% | 52.7 | 45 | 59 | 40.3 | 35 | 45 | 12.3 |
| | | 6% | 55.3 | 52 | 59 | 65.7 | 62 | 70 | -10.3 |
| AIRT/019/01 | COAT-023 | 2% | 26.0 | 24 | 28 | 21.7 | 18 | 24 | 4.3 |
| | | 4% | 45.7 | 41 | 49 | 36.0 | 32 | 38 | 9.7 |
| | | 6% | 49.0 | 46 | 53 | 48.0 | 45 | 52 | 1.0 |

There is no trend in the thickness data for the rosuvastatin coated tablets with sometimes the exposed sample having a thicker mean coating than the corresponding control and sometimes it is vice-versa.

Differences in coating thickness are not the reason for the differences in the $\Delta E^*_{00}$ values found for the various coated rosuvastatin batches in the colorimetry experiments (see Table 51). The lowest $\Delta E^*_{00}$ values were found for the batches coated with the two $TiO_2$ reference coats COAT-017 and COAT-018 (Batches ENQ3822/AIRT/010/01 and ENQ3822/AIRT/011/01), and the $TiO_2$-free coating, COAT-023 (Batch ENQ3822/AIRT/019/01). This is despite these batches mainly having average coating thicknesses either lower or in the same range as the other coated rosuvastatin lots.

Prasugrel Coated Tablets

The average coating thickness (land, belly, surface) for the exposed and control prasugrel coated tablets are shown in Table 57.

Table 57: Average coating thickness for the exposed and control prasugrel tablets

| Batch No. ENQ3822 | Consort Coat Ref | % Weight Gain | Coating Thickness (µm) | | | | | | Diff Mean Exp vs Cont (µm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Exposed | | | Control | | | |
| | | | Mean | Min | Max | Mean | Min | Max | |
| AIRT/020/01 | COAT-026 | 2 | 31.0 | 29 | 33 | 23.7 | 21 | 25 | 7.3 |
| | | 4 | 47.7 | 43 | 53 | 36.0 | 32 | 41 | 11.7 |
| | | 6 | 45.0 | 38 | 51 | 60.7 | 55 | 66 | -15.7 |
| AIRT/021/01 | COAT-016 | 2 | 19.7 | 17 | 24 | 24.0 | 23 | 25 | -4.3 |
| | | 4 | 27.7 | 23 | 35 | 62.0 | 38 | 74 | -34.3 |
| | | 6 | 69.7 | 62 | 75 | 87.0 | 79 | 100 | -17.3 |
| AIRT/022/01 | COAT-002 | 2 | 29.0 | 26 | 32 | 20.7 | 19 | 22 | 8.3 |
| | | 4 | 56.0 | 54 | 59 | 39.3 | 38 | 40 | 16.7 |
| | | 6 | 52.3 | 50 | 54 | 58.0 | 49 | 63 | -5.7 |
| AIRT/023/01 | COAT-030 + COAT-031 | 2 | 24.7 | 19 | 30 | 39.0 | 28 | 52 | -14.3 |
| | | 4 | 38.7 | 32 | 49 | 47.7 | 41 | 53 | -9.0 |
| | | 6 | 74.7 | 68 | 80 | 64.0 | 60 | 72 | 10.7 |

Again, there is no trend in the thickness data for the prasugrel coated tablets with sometimes the exposed sample having a thicker mean coating than the corresponding control and sometimes it is vice-versa.

### 44. X-ray Powder Diffraction

The results of the X ray powder diffraction studies are shown in Table 58, Table 59, Table 60 and Table 61. The exposed and control samples from the nifedipine all showed a nifedipine pattern A. An elevated baseline was observed in the XRPD patterns for nifedipine which may be indicative of some disorder or amorphous content. This elevated baseline had been previously observed when the batches were first tested (see Figure 24). The exposed and control samples from the coated olmesartan, rosuvastatin and prasugrel tablets all displayed the characteristic olmesartan pattern A, rosuvastatin pattern A and prasugrel pattern A respectively.

In addition to the characteristic pattern A of the relevant API, almost all of the 23 exposed and all of the 23 control samples showed additional peaks. Additional peaks had been previously seen in certain of the rosuvastatin and prasugrel coated tablet samples. However, the additional peaks in the exposed and control samples were observed in greater numbers than when the batches were first manufactured, and were now present in all batches as opposed to only certain of the rosuvastatin and prasugrel coated tablet lots. The cause of the additional peaks in the exposed and control batches is unknown and would require further investigation.

In summary, there were no major differences in the XRPD results for the light-exposed and control samples for any of the 23 coated batches.

Table 58: XRPD results for the exposed and control nifedipine tablets

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | %Weight Gain | XRPD Pattern Exposed Sample | XRPD Pattern Control Sample |
|---|---|---|---|---|
| 001/01 | COAT-024 | 2 | Nifedipine Pattern A + additional peak at 9.8, 25.3, 28.2 and 31.4°2θ | Nifedipine Pattern A + additional peak at 33.2°2θ |
| | | 4 | Nifedipine Pattern A + additional peaks at 33.2 and 36.8°2θ | Nifedipine Pattern A + additional peak at 24.9 and 33.2°2θ |
| | | 6 | Nifedipine Pattern A + additional peak at 33.1°2θ | Nifedipine Pattern A + additional peaks at 8.5, 12.2, 23.2, 26.2, 28.5, 32.7 and 33.2°2θ |
| 002/01 | COAT-001 | 2 | Nifedipine Pattern A + additional peaks at 9.8, 18.3 and 25.4°2θ | Nifedipine Pattern A + additional peaks at 23.2 and 33.2°2θ |
| | | 4 | Nifedipine Pattern A | Nifedipine Pattern A + additional peaks at 18.3 and 38.7°2θ |
| | | 6 | Nifedipine Pattern A + additional peak at 12.2 and 39.8°2θ | Nifedipine Pattern A + additional peaks at 28,2 and 37.6°2θ |
| 003/01 | COAT-033 | 2 | Nifedipine Pattern A + additional peaks at 28.5, 29.0, 31.8, 33.2 and 34.4°2θ | Nifedipine Pattern A + additional peaks at 23.3 and 33.1°2θ |
| | | 4 | Nifedipine Pattern A + additional peaks at 18.3, 31.7, 36.2 and 38.6°2θ | Nifedipine Pattern A + additional peaks at 8.2, 16.0, 25.6, 31.7, 32.3 and 33.1°2θ |
| | | 6 | Nifedipine Pattern A + additional peaks at 9,7, 13.4, 28.2 and 33.1°2θ | Nifedipine Pattern A + additional peak at 33.1°2θ |
| 004/01 | COAT-004 | 2 | Nifedipine Pattern A + additional peaks at 18.3 and 29.4°2θ | Nifedipine Pattern A + additional peaks at 30.2 and 33.2°2θ |
| | | 4 | Nifedipine Pattern A + additional peaks at 9.8, 18.3, 20.3, 34.6, 34.9 and 37.7°2θ | Nifedipine Pattern A + additional peaks at 20.3, 23.1, 28.1 and 33.1°2θ |
| | | 6 | Nifedipine Pattern A + additional peaks at 9.8, 31.7 and 33.2°2θ | Nifedipine Pattern A + additional peaks at 29.4, 31.8 and 33.2°2θ |
| 005/01 | COAT-023 | 2 | Nifedipine Pattern A + additional peaks at 18.4, 18.7, 33.2 and 38.9°2θ | Nifedipine Pattern A + additional peaks at 18.4, 31.8 and 33.2°2θ |
| | | 4 | Nifedipine Pattern A + additional peaks at 13.7, 20.3, 26.2 and 31.7°2θ | Nifedipine Pattern A + additional peak at 18.4, 31.8 and 33.2°2θ |
| | | 6 | Nifedipine Pattern A + additional peaks at 9.8, 18.3, 31.7, 33.2, 34.4, 36.2, 39.8 and 39.9°2θ | Nifedipine Pattern A + additional peaks at 20.2, 23.1 and 31.7°2θ |

Table 59: XRPD results for the exposed and control olmesartan tablets

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | %Weight Gain | XRPD Pattern Exposed Sample | XRPD Pattern Control Sample |
|---|---|---|---|---|
| 006/01 | COAT-025 | 2 | Olmesartan Pattern A + additional peaks at 12.2, 16, 18.6, 29.2, 30.2 and 35.9°2θ | Olmesartan Pattern A + additional peaks at 12.0, 12.2, 15.8, 16 and 18.6°2θ |
| | | 4 | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6 and 35.3°2θ | Olmesartan Pattern A + additional peaks at 12.2, 15.8, 16.0, 18.6, 20.6, 24.7, 26.2 and 32.3°2θ |
| | | 6 | Olmesartan Pattern A + additional peaks at 12.2, 14.4, 15.7, 16.0 and 18.6°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 32.8 and 34.1°2θ |
| 007/01 | COAT-013 | 2 | Olmesartan Pattern A + additional peaks at 12.2, 14.4, 16.0, 18.7, 20.7, 29.4 and 38.9°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ |
| | | 4 | Olmesartan Pattern A + additional peaks at 12.2, 14.4, 15.7, 16.0, 18.6, 29.4 and 35.3°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16, 18.6 and 29.4°2θ |
| | | 6 | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6, 29.4 and 37.7°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ |
| 008/01 | COAT-015 | 2 | Olmesartan Pattern A + additional peaks at 12.2, 15.7, 16.0, 18.6, 29.4, 32.3, 35.4 and 37.6°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6, 29.4, 33.6 and 34.1°2θ |
| | | 4 | Olmesartan Pattern A + additional peaks at 12.2, 15.7, 16.0, 18.7, 29.4, 30.2, 32.3, 34.0 and 35.9°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 29.4, 31.7, 34.0 and 34.1°2θ |
| | | 6 | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6, 29.4 and 37.6°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ |
| 009/01 | COAT-014 | 2 | Olmesartan Pattern A + additional peaks at 12.2, 15.8, 16.0, 18.6, 29.4 and 32.3°2θ | Olmesartan Pattern A + additional peaks at 12.0, 12.2, 16.0, 18.6, 29.4 and 38.9°2θ |
| | | 4 | Olmesartan Pattern A + additional peaks at 12.2, 14.4, 15.8, 16.0, 18.7, 27.5 and 29.4°2θ | Olmesartan Pattern A + additional peaks at 12.2, 15.8, 16.0, 18.6 and 29.4°2θ |
| | | 6 | Olmesartan Pattern A + additional peaks at 12.2, 15.7, 16.0, 18.6, 29.4 and 34.0°2θ | Olmesartan Pattern A + additional peaks at 12.2, 16.0 18.7, 28.5 and 29.4°2θ |

Table 60: XRPD results for the exposed and control rosuvastatin tablets

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | %Weight Gain | XRPD Pattern Exposed Sample | XRPD Pattern Control Sample |
|---|---|---|---|---|
| 010/01 | COAT-018 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.7, 32.3, 35.9 and 36.6°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0, 18.6 and 32.3°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 23.2°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 18.7°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 23.2°2θ |
| 011/01 | COAT-017 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2 and 16.0°2θ | Rosuvastatin Pattern A + additional peaks at 16.0, 18.6 and 18.7°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peak at 12.2 and 16.0°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 18.7°2θ | Rosuvastatin Pattern A + additional peaks at 12.2 and 16.0°2θ |
| 012/01 | COAT-020 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2 and 16.0°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2,16.0 and 18.7°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peak at 12.2, 15.7, 16.0 and 18.7°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 18.7 and 32.3°2θ |
| 013/01 | COAT-019 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6, 29.4 and 32.8°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 29.4°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 29.4°2θ | Rosuvastatin Pattern A + additional peaks at 15.2, 16.0, 17.7, 18.6, 21.6 and 29.4°2θ |
| 014/01 | COAT-010 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16 and 18.6°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12, 12.2, 16.0, 18.3 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16,18.6, 33.7 and 34.1°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0, 18.6, 31.8 and 36.3°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16 and 18.6°2θ |
| 015/02 | COAT-030 | 2 | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.7°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 15.7, 16.0, | Rosuvastatin Pattern A + additional peaks at 12.2, 16 and |

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | %Weight Gain | XRPD Pattern Exposed Sample | XRPD Pattern Control Sample |
|---|---|---|---|---|
| | | | 18.6 and 33.2°2θ | 18.6°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.7°2θ | Rosuvastatin Pattern A + plus peaks at 12.2, 16.0, 18.7, 21.6°2θ |
| 016/01 | COAT-005 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6, 34.0 and 38.8°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| 017/01 | COAT-001 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 15.8, 16.0 and 18.6°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.7, 35.6 and 37.8°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 32.3°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 15.8, 16.0 and 18.6°2θ |
| 018/01 | COAT-034 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.0, 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| 019/01 | COAT-023 | 2 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6, 23.2 and 32.3°2θ |
| | | 4 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ |
| | | 6 | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0 and 18.6°2θ | Rosuvastatin Pattern A + additional peaks at 12.2, 16.0, 18.6 and 32.4°2θ |

Table 61: XRPD results for the exposed and control prasugrel tablets

| Coated Tablet Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | %Weight Gain | XRPD Pattern Exposed Sample | XRPD Pattern Control Sample |
|---|---|---|---|---|
| 020/01 | COAT-026 | 2 | Prasugrel Pattern A + additional peaks at 18.2 and 25.3°2θ | Prasugrel Pattern A + additional peaks at 18.2, 25.3 and 37.7°2θ |
| | | 4 | Prasugrel Pattern A + additional peak at 18.3, 19.7 and 20.0°2θ | Prasugrel Pattern A + additional peaks at 14.2 and 18.2°2θ |
| | | 6 | Prasugrel Pattern A + additional peak at 18.3°2θ | Prasugrel Pattern A + additional peaks at 14.2 and 22.7°2θ |
| 021/01 | COAT-016 | 2 | Prasugrel Pattern A | Prasugrel Pattern A + additional peaks at 12.5, 18.2°2θ and minus peak at 8.3°2θ |
| | | 4 | Prasugrel Pattern A + additional peak at 33.1°2θ | Prasugrel Pattern A + additional peak at 18.2°2θ |
| | | 6 | Prasugrel Pattern A | Prasugrel Pattern A + additional peak at 37.6°2θ |
| 022/01 | COAT-002 | 2 | Prasugrel Pattern A + additional peaks at 14.2 and 37.8°2θ | Prasugrel Pattern A + additional peaks at 18.2 and 19.9°2θ |
| | | 4 | Prasugrel Pattern A | Prasugrel Pattern A + additional peaks at 18.2°2θ |
| | | 6 | Prasugrel Pattern A + additional peaks at 14.2 and 18.2°2θ | Prasugrel Pattern A + additional peaks at 18.2, 19.9, 20.8 and 25.4°2θ |
| 023/01 | COAT-030 & COAT-031 | 2 | Prasugrel Pattern A + additional peak at 32.2°2θ | Prasugrel Pattern A + additional peak at 18.2°2θ |
| | | 4 | Prasugrel Pattern A + additional peak at 18.0 and 18.2°2θ | Prasugrel Pattern A + additional peaks at 18.2 and 37.6°2θ |
| | | 6 | Prasugrel Pattern A + additional peaks at 12.5, 18.0 and 18.3°2θ | Prasugrel Pattern A + additional peaks at 18.2 and 37.6°2θ |

## 45. Disintegration Times - Exposed vs Control Samples from the Active Coated Tablets

The disintegration times of the light-exposed and control samples from the 23 coated batches are shown in Table 62. The disintegration times of the exposed nifedipine samples ranged from 4 min 24 sec to 5 min 30 sec, while those for the corresponding control samples ranged from 3 min 51 sec to 5 min 38 sec. The light-exposed olmesartan samples disintegrated within 1.5 min to 3 min, as did the control samples.

There was also no significant change in the disintegration times of the rosuvastatin and prasugrel coated tablets following light exposure compared with control. Disintegration times of the rosuvastatin samples were more dependent on coating composition and for some batches on %coating weight gain than on light exposure. The disintegration times of the exposed rosuvastatin samples ranged from 1 min 39 sec to 7 min 30 sec, while those for the control samples ranged from 1 min 58 sec to 6 min 50 sec. The disintegration times for the light-exposed prasugrel samples ranged from 1 min 31 sec to 4 min 4 sec, while the range for the controls was 1 min 29 sec to 3 min 46 sec.

In summary, light exposure had no significant impact on the disintegration of the tablet samples.

Table 62: Disintegration times of the exposed and control samples from the 23 coated tablet batches

| Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | Film Former | Opacifier | Disintegration Time at %Weight Gain (min:sec) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 4:37 | 4:16 | 5:10 | 4:04 | 5:10 | 4:36 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 4:57 | 4:31 | 5:14 | 4:54 | 5:16 | 5:27 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 4:47 | 3:51 | 4:53 | 5:20 | 5:08 | 5:23 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 4:37 | 4:03 | 5:06 | 4:48 | 5:30 | 5:38 |
| 005/01 | COAT-023 | PVA | F+Talc | 5:22 | 4:57 | 4:24 | 4:55 | 5:22 | 5:09 |
| **Olmesartan 20 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 1:46 | 1:31 | 2:08 | 2:18 | 2:13 | 1:56 |
| 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | 1:39 | 1:40 | 1:58 | 2:21 | 1:43 | 2:02 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 1:39 | 1:30 | 2:18 | 1:59 | 2:26 | 2:37 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+$FeO_3$ | 1:55 | 1:47 | 2:12 | 1:49 | 2:56 | 2:40 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 3:41 | 3:23 | 5:04 | 4:25 | 4:33 | 4:37 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 2:39 | 3:30 | 3:19 | 3:31 | 3:05 | 3:40 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 1:39 | 1:58 | 4:14 | 3:34 | 4:33 | 3:55 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 3:46 | 3:25 | 4:57 | 5:13 | 5:26 | 4:05 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 3:11 | 3:24 | 3:53 | 3:22 | 3:23 | 2:45 |
| 015/02 | COAT-030 | HPMC | B+E | 4:35 | 5:09 | 5:45 | 5:42 | 7:30 | 6:50 |
| 016/01 | COAT-005 | HPMC | MgO | 4:01 | 4:40 | 4:54 | 4:54 | 5:25 | 5:42 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 3:50 | 4:11 | 4:11 | 4:08 | 2:47 | 4:48 |
| 018/01 | COAT-034 | HPMC | Rice starch | 1:58 | 3:29 | 2:35 | 4:04 | 2:31 | 3:39 |
| 019/01 | COAT-023 | PVA | F+Talc | 4:14 | 3:53 | 5:29 | 4:22 | 4:16 | 5:12 |
| **Prasugrel 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 1:51 | 1:53 | 2:01 | 2:05 | 2:45 | 2:09 |
| 021/01 | COAT-016 | HPMC | Rice Starch + D+$Fe_2O_3$ | 1:31 | 1:29 | 2:12 | 2:07 | 2:39 | 2:25 |
| 022/01 | COAT-002 | HPMC | Rice Starch+A+B +D+$Fe_2O_3$ | 1:53 | 1:41 | 3:08 | 2:27 | 3:03 | 3:11 |
| 023/01 | COAT-030 & | HPMC | B+E+$Fe_2O_3$ | 2:32 | 2:04 | 3:12 | 2:44 | 4:04 | 3:46 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | COAT-031 | | | | | | | | |

## 46. Assay and %Total Related Impurities in Light-Exposed and Control Samples

The average assay and %total impurities in the various light exposed and control samples in the photostability study are shown in Table 63 and Table 64 respectively.

Coated Nifedipine Batches

The average assay values for all light-exposed samples were approx. 25% to 35% less than the results obtained for the corresponding control samples. The %total related impurities also increased significantly. This shows that none of the $TiO_2$-free coats and also the $TiO_2$ reference coat could fully protect the nifedipine against photodegradation. Therefore, the data are in line with the visual appearance and colorimetry results.

Based on the highest assay and lowest % total related impurities values at a 6% coating weight gain, the best results were obtained for Batch ENQ3822/AIRT/001/01 which was coated with the $TiO_2$ reference coat, COAT-024. The rank order thereafter was Batch ENQ3822/AIRT/002/01 (COAT-001), which gave the closest results to the $TiO_2$ reference batch, Batch ENQ3822/AIRT/005/01 (COAT-023), ENQ3822/AIRT/003/01 (COAT-033) and ENQ3822/AIRT/004/01 (COAT-004). For all coatings an increase in %weight gain made only minor improvements in terms of increase in assay and/or decrease in %total related impurities when the 2% and 4% weight gain data were compared to those at a 6% weight gain.

Coated Olmesartan Batches

The assay value for the 4 olmesartan batches lay between 98% and 100% and there was no significant difference between the light-exposed and control samples. Similarly, the %total related impurities values did not significantly change following light exposure. The results are in line with the visual and colorimetry data.

Coated Rosuvastatin Batches

With respect to assay, the light-exposed samples from Batch ENQ3822/AIRT/010/01, Batch ENQ3822/AIRT/011/01 and Batch ENQ3822/AIRT/019/01 had values close to those of the corresponding control. The largest difference in exposed versus control assay for these three batches was Batch ENQ3822/AIRT/019/01 at a 6% weight gain (assay value difference of just under 5%). The assay difference for the 2% and 4% weight gain samples of this batch and for the other two lots is < 3% at all %coating weight gains studied. However, the total % related impurities for these batches showed considerably more degradation than would be anticipated given the assay results.

The %total related impurities in the exposed samples of Batch ENQ3822/AIRT/011/01 at a 4% and 6% coating level and Batch ENQ3822/AIRT/010/01 at a 6% weight gain were approximately double that found in the corresponding dark controls. These batches are coated with the HPMC-based $TiO_2$ reference, COAT-017 and the PVA-based $TiO_2$ reference, COAT-018, respectively. The light-exposed sample of Batch ENQ3822/AIRT/019/01 at a 6% weight gain contained around three times the %total related impurities compared with its control sample. This batch is coated with the PVA-based $TiO_2$-free coating, COAT-023. It also was the best performing $TiO_2$-free coating in the visual appearance and colorimetry tests (see Section 42). The other batches coated with $TiO_2$-free coatings had assay values which ranged from 86.3% and 92.1 %LC and %total related impurity values of 7.6% to 10.0%.

Table 63: Average assay - exposed vs control samples

| Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | Film Former | Opacifier | Average Assay at %Weight Gain (%LC) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 70.2 | 98.4 | 73.3 | 97.6 | 76.2 | 100.4 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 70.1 | 101.5 | 71.2 | 99.6 | 73.0 | 100.9 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 65.2 | 100.5 | 66.9 | 98.3 | 67.4 | 98.7 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 62.4 | 100.6 | 63.5 | 95.2 | 66.0 | 101.3 |
| 005/01 | COAT-023 | PVA | F+Talc | 68.0 | 101.5 | 66.1 | 96.7 | 68.5 | 99.1 |
| **Olmesartan 20 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 99.5 | 99.2 | 99.7 | 98.7 | 99.2 | 98.9 |
| 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | 99.4 | 99.1 | 99.4 | 98.8 | 99.4 | 98.6 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 99.5 | 99.9 | 99.6 | 99.3 | 99.4 | 99.0 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 98.9 | 99.0 | 99.6 | 98.6 | 99.2 | 98.4 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 99.0 | 100.0 | 101.5 | 104.2 | 101.9 | 102.0 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 100.8 | 102.1 | 102.9 | 102.6 | 103.2 | 101.3 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 89.1 | 102.2 | 89.1 | 101.3 | 89.6 | 98.1 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 88.3 | 102.8 | 92.1 | 103.6 | 91.2 | 101.7 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 88.3 | 101.1 | 90.1 | 101.5 | 90.9 | 102.1 |
| 015/02 | COAT-030 | HPMC | B+E | 89.7 | 102.0 | 89.1 | 104.3 | 88.4 | 104.0 |
| 016/01 | COAT-005 | HPMC | MgO | 87.5 | 102.6 | 86.4 | 102.8 | 87.0 | 102.4 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 86.3 | 103.2 | 91.0 | 100.8 | 91.2 | 101.9 |
| 018/01 | COAT-034 | HPMC | Rice starch | 87.5 | 101.0 | 87.8 | 101.2 | 89.6 | 101.7 |
| 019/01 | COAT-023 | PVA | F+Talc | 100.6 | 101.7 | 99.3 | 101.8 | 97.7 | 102.5 |
| **Prasugrel 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 97.7 | 98.8 | 97.9 | 99.3 | 98.2 | 99.0 |
| 021/01 | COAT-016 | HPMC | Rice Starch+ D+$Fe_2O_3$ | 96.7 | 99.8 | 98.0 | 99.6 | 98.0 | 99.9 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ A+B+D+Fe2O3 | 96.1 | 99.5 | 97.1 | 99.6 | 95.9 | 99.8 |
| 023/01 | COAT-030 & | HPMC | B+E+$Fe_2O_3$ | 95.4 | 99.8 | 96.9 | 100.1 | 98.9 | 100.8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | COAT-031 | | | | | | | | |

Color Code: Red = Significant change in assay (>5%)

Table 64: %Total related impurities - exposed vs control samples

| Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | Film Former | Opacifier | %Total Related Impurities at %Weight Gain (%LC) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nifedipine Retard 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 001/01 | COAT-024 | HPMC | $TiO_2$ | 23.71 | 0.09 | 20.75 | 0.12 | 18.54 | 0.10 |
| 002/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 22.30 | 0.20 | 21.01 | 0.16 | 19.95 | 0.10 |
| 003/01 | COAT-033 | HPMC | $CaCO_3$+D+F | 26.50 | 0.10 | 25.16 | 0.11 | 24.70 | 0.10 |
| 004/01 | COAT-004 | HPMC | $CaCO_3$+C | 27.96 | 0.41 | 27.06 | 0.13 | 25.74 | 0.14 |
| 005/01 | COAT-023 | PVA | F+Talc | 23.92 | 0.15 | 25.67 | 0.13 | 22.82 | 0.10 |
| **Olmesartan 20 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 006/01 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | 0.3 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 |
| 007/01 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 | 0.4 |
| 008/01 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 0.4 | 0.4 | 0.3 | 0.4 | 0.3 | 0.4 |
| 009/01 | COAT-014 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | 0.3 | 0.4 | 0.3 | 0.5 | 0.3 | 0.4 |
| **Rosuvastatin 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 010/01 | COAT-018 | PVA | $TiO_2$+Talc | 3.78 | 0.70 | 1.89 | 0.67 | 1.35 | 0.68 |
| 011/01 | COAT-017 | HPMC | $TiO_2$ | 2.82 | 0.70 | 1.35 | 0.65 | 1.14 | 0.71 |
| 012/01 | COAT-020 | HPMC+HPC | Rice Starch+D | 9.23 | 0.70 | 8.99 | 0.66 | 8.93 | 0.69 |
| 013/01 | COAT-019 | HPMC | $CaCO_3$+D+E | 8.95 | 0.72 | 8.28 | 0.70 | 7.77 | 0.73 |
| 014/01 | COAT-010 | HPMC | Rice Starch+D | 9.10 | 0.67 | 8.56 | 0.64 | 8.24 | 0.67 |
| 015/02 | COAT-030 | HPMC | B+E | 9.78 | 0.79 | 9.55 | 0.82 | 10.00 | 0.76 |
| 016/01 | COAT-005 | HPMC | MgO | 9.77 | 0.57 | 9.39 | 0.58 | 9.39 | 0.54 |
| 017/01 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | 9.54 | 0.61 | 8.65 | 0.64 | 7.64 | 0.59 |
| 018/01 | COAT-034 | HPMC | Rice starch | 9.72 | 0.64 | 9.56 | 0.99 | 8.84 | 0.63 |
| 019/01 | COAT-023 | PVA | F+Talc | 2.78 | 0.71 | 2.79 | 0.67 | 2.26 | 0.70 |
| **Prasugrel 10 mg Coated Tablets** | | | | **Exp 2%** | **Control 2%** | **Exp 4%** | **Control 4%** | **Exp 6%** | **Control 6%** |
| 020/01 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | 1.3 | 0.9 | 1.1 | 1.0 | 1.0 | 0.9 |
| 021/01 | COAT-016 | HPMC | Rice Starch+D+ $Fe_2O_3$ | 1.3 | 0.9 | 1.1 | 0.8 | 0.9 | 0.9 |
| 022/01 | COAT-002 | HPMC | Rice Starch+ | 1.7 | 1.0 | 1.6 | 0.9 | 1.7 | 1.0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | A+B+D+$Fe_2O_3$ | | | | | | |
| 023/01 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | 1.7 | 1.0 | 1.5 | 1.0 | 1.1 | 0.9 |

In summary, none of the coatings tested were able to protect rosuvastatin fully from photodegradation. However, the two $TiO_2$ reference coats, COAT-017 and COAT-018, and the $TiO_2$-free coat, COAT-023 performed significantly better than the other $TiO_2$ coats in protecting rosuvastatin against the extreme light exposure experienced in the photostability study.

Coated Prasugrel Tablets

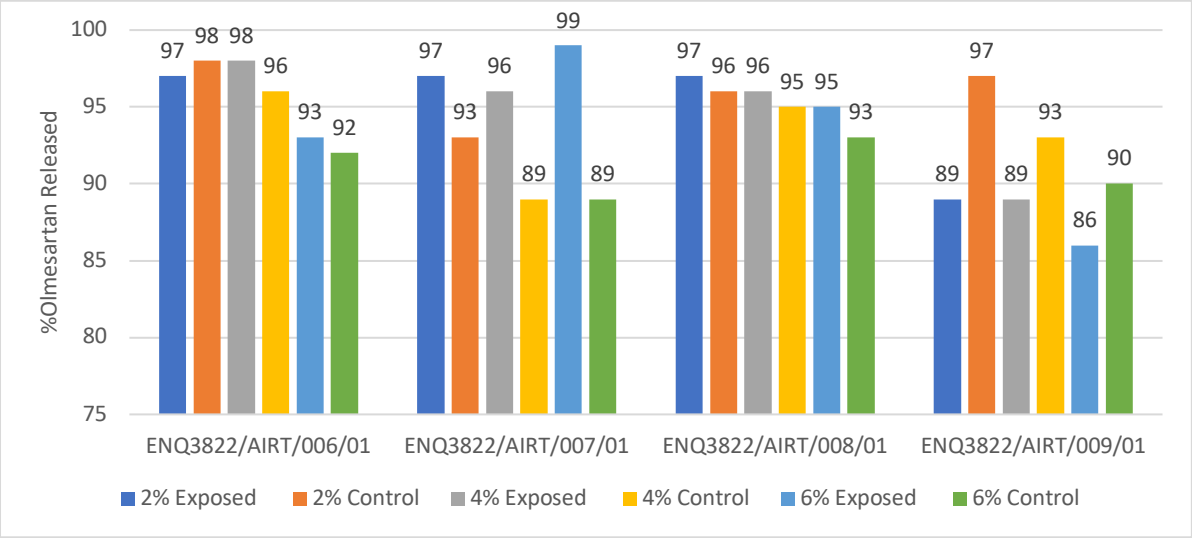
The assay values for all of the light-exposed prasugrel samples were slightly less than the corresponding controls. The least difference in assay was observed for Batch ENQ3822/AIRT/020/01 at all coating levels and Batch ENQ3822/AIRT/021/01 at coating weight gains ≥4% (assay difference < 2%). These batches were coated with the $TiO_2$ reference coat, COAT-026 and the $TiO_2$-free coat, COAT-016. The %total related impurity results show that for the ≥4% weight gain samples, the level of impurities was also very similar to that of the control samples. For the other two batches, the %total related impurities in the 4% coating level exposed samples were significantly greater compared with Batch ENQ3822/AIRT/020/01 and Batch ENQ3822/AIRT/021/01. For ENQ3822/AIRT/022/01 the level of related impurities was also significantly higher in the 6% weight gain sample. This shows that these coatings were less well able to protect prasugrel from photodegradation than COAT-026 and COAT-016.

## 47. Dissolution

Coated Olmesartan Batches

The dissolution of the coated olmesartan batches are shown in Figure 32.

Figure 32: %Release of olmesartan at 15 minutes

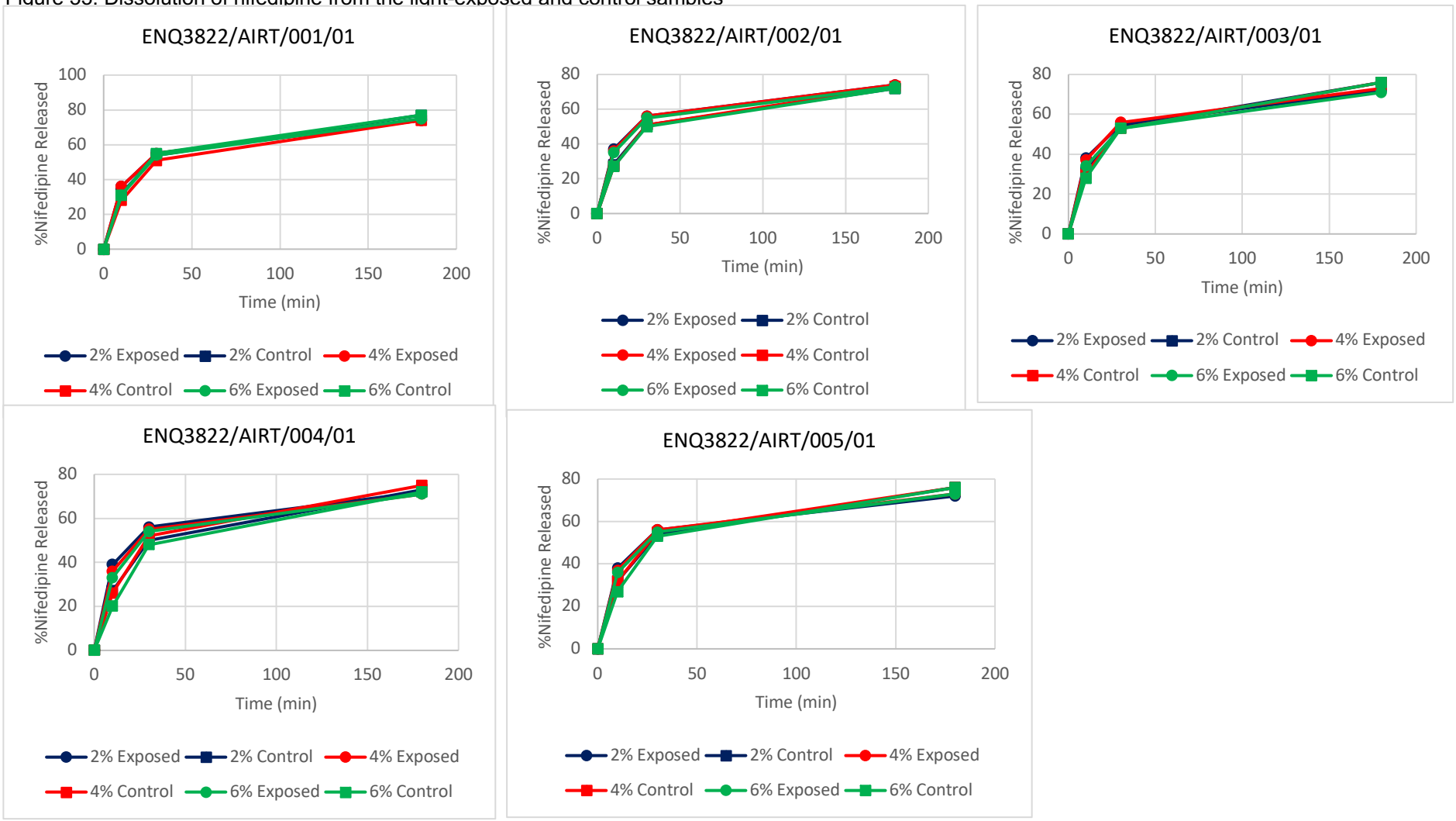



For Batches ENQ3822/AIRT/006/01 and ENQ3822/AIRT/008/01 there is very little difference in the %release of olmesartan from the light-exposed and control samples. For ENQ3822/AIRT/007/01 the light-exposed samples released more API than the control samples, while for ENQ3822/AIRT/009/01 the opposite was the case. However, for all batches the %released at the 15-min time-point was greater than 85%. This would suggest that light exposure had minimal impact on the dissolution of the olmesartan from the coated tablet batches.

Coated Nifedipine Batches

The dissolution results are shown in Figure 33. The dissolution profiles of the exposed nifedipine samples and the control samples were not significantly different when the profile shape and %LC released were considered. The greatest difference was at the 10 min time-point. The exposed samples of the $TiO_2$-free coated nifedipine batches were faster at this time-point than the corresponding controls, the greatest difference being observed with Batch ENQ3822/AIRT/004/01 (the exposed samples were 10% to 13% faster). At the 30 min time-point the %released was similar for all of the nifedipine samples regardless of whether the samples had been exposed to light or not.

Figure 33: Dissolution of nifedipine from the light-exposed and control samples

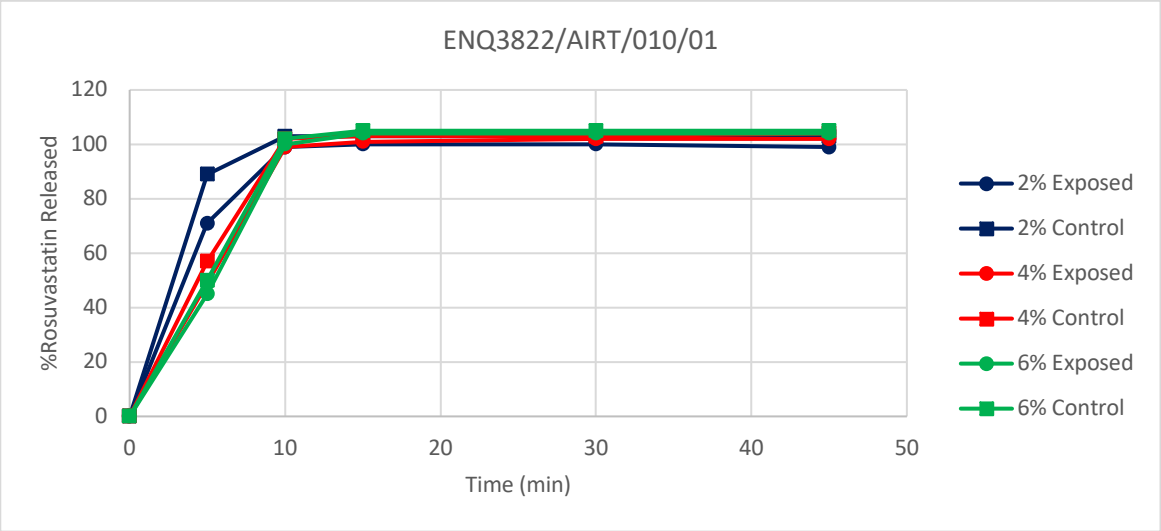


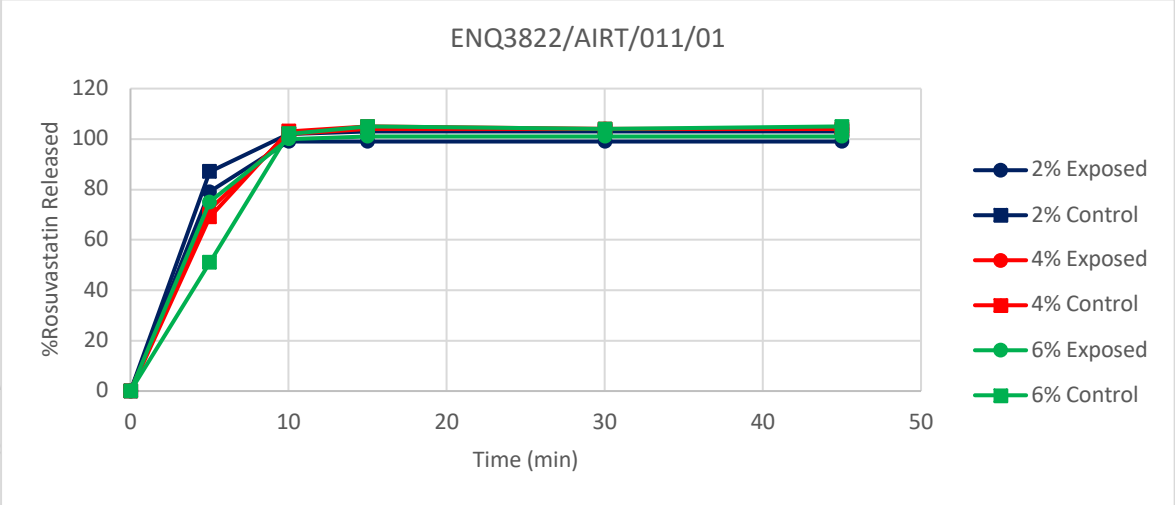
However, the exposed samples had significantly reduced assay values compared with the controls (see Table 63) and therefore the % release at 180 min will be similar to 100% of the available nifedipine if the assay values are considered.

Coated Rosuvastatin Batches

The dissolution results are shown in Figure 34 for the batches coated with the $TiO_2$ reference coatings.

Figure 34: Dissolution from the light-exposed and control samples from the $TiO_2$ reference batches

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significantly higher than the light-exposed one. However, release from all other samples from this batch were similar at this time-point. Release of rosuvastatin from Batch ENQ3822/AIRT/011/01 at the 5-min time-point was similar for all samples except for the 4% control sample which was significantly slower than the rest.

Overall, light exposure had no impact on rosuvastatin release from the batches coated with the two $TiO_2$ reference coats.

The dissolution results are shown in Figure 35 and Figure 36 for the batches coated with the $TiO_2$-free coatings. With the exception of Batch ENQ3822/AIRT/015/02, the graphs show that the dissolution profile shape remained the same for all samples of all batches, in that there was a steep rise in the %rosuvastatin released until the 10 min time-point and thereafter there was no further or minimal change. For Batch ENQ3822/AIRT/015/02 release of rosuvastatin was extended until the 30 min time-point after which there was minimal change in the amount of rosuvastatin measured. For this batch only the 2% control sample released approx. 100% rosuvastatin release at 15 min. This batch was coated with COAT-030 which contains an acidic component whose hydrophobicity may be contributing to the slow-down in rosuvastatin dissolution.

For the majority of the $TiO_2$-free coated batches there was variation in the %rosuvastatin released at the 5 min time-point. This variation depended on the batch, the %coating and whether the sample had been subjected to extreme light exposure or not, with the light-exposed samples typically showing slower release than the corresponding controls. The exception to this was Batch ENQ3822/AIRT/018/01, coated with COAT-034, whose samples showed little variation in rosuvastatin release at the 5-min time-point regardless of the %coating weight gain or whether they had been exposed to light or not. For Batch ENQ3822/AIRT/015/02 with its extended dissolution profiles, most variation in release occurred at the 10-min time-point with the exposed samples clearly releasing at a slower rate than the corresponding controls.

For Batch ENQ3822/AIRT/019/01 coated with COAT-023, approximately 100% of rosuvastatin label claim was released for both the exposed and control samples. %Release was similar at the 5-min time-point for all of the samples except for the 2% coated control sample whose % release was significantly greater than the other samples. The recovery results are in line with the assay results for this batch.

For the other $TiO_2$-free coated batches recovery from the exposed samples at the 45-min time-point was approximately 10 to 15% lower than that of control samples which is in line with the lower assay results for these batches.

Overall, light-exposure did not impact the dissolution of rosuvastatin significantly from the $TiO_2$-free coated samples and the level of recovery at the 45 min time-point reflected the assay values for the exposed and control samples. The exception was for Batch ENQ3822/AIRT/015/02 which clearly showed a significant slowdown in release for the light-exposed samples at the 10 min time-point, the extent to which also depended on the %coating weight gain. The lower recovery values for its light-exposed samples at the end of dissolution are in line with the lower assay values obtained.

Figure 35: Dissolution of rosuvastatin from the light-exposed and control samples coated with $TiO_2$-free coatings (Runs 22 to 25B)

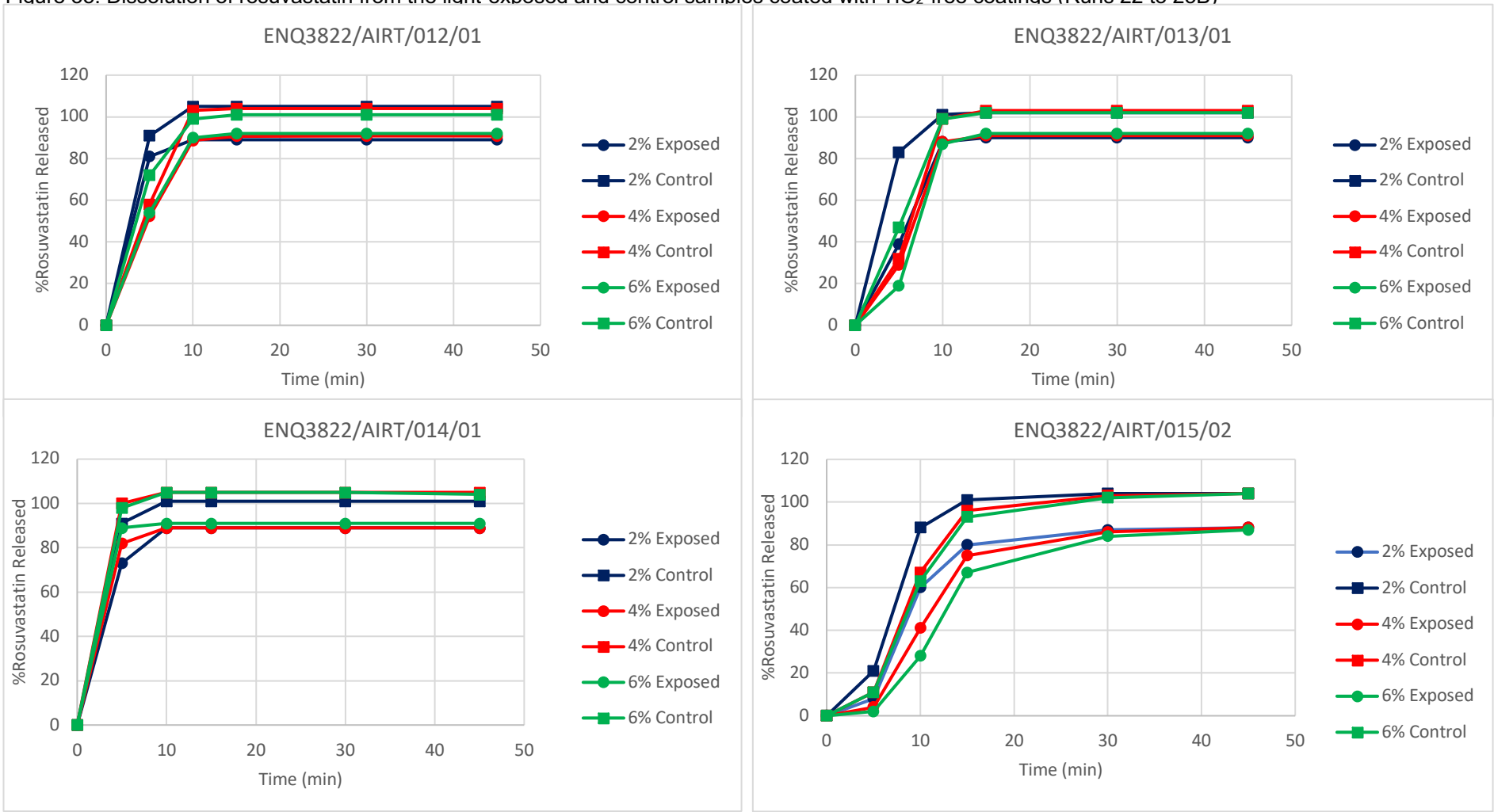
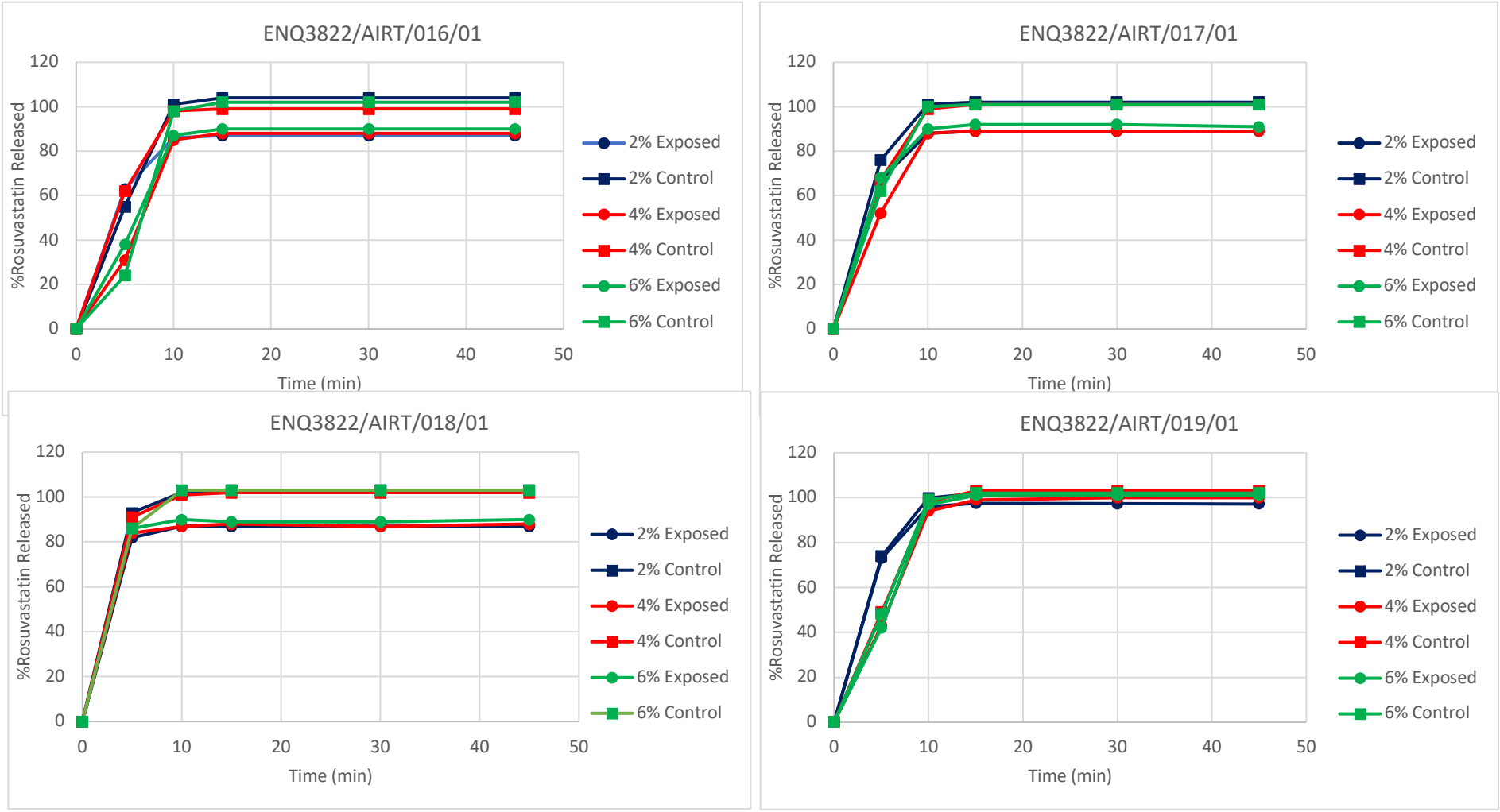


Figure 36: Dissolution of rosuvastatin from the light-exposed and control samples (Runs 26 to 29)

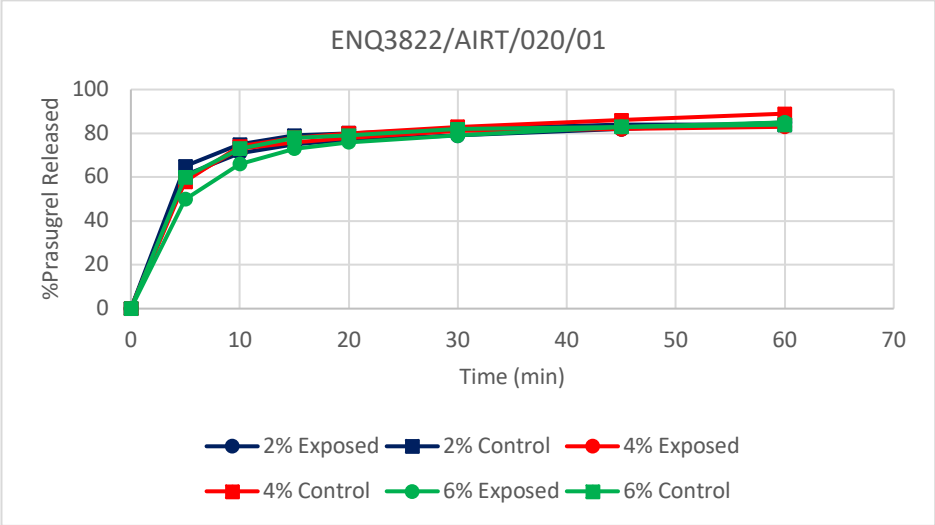


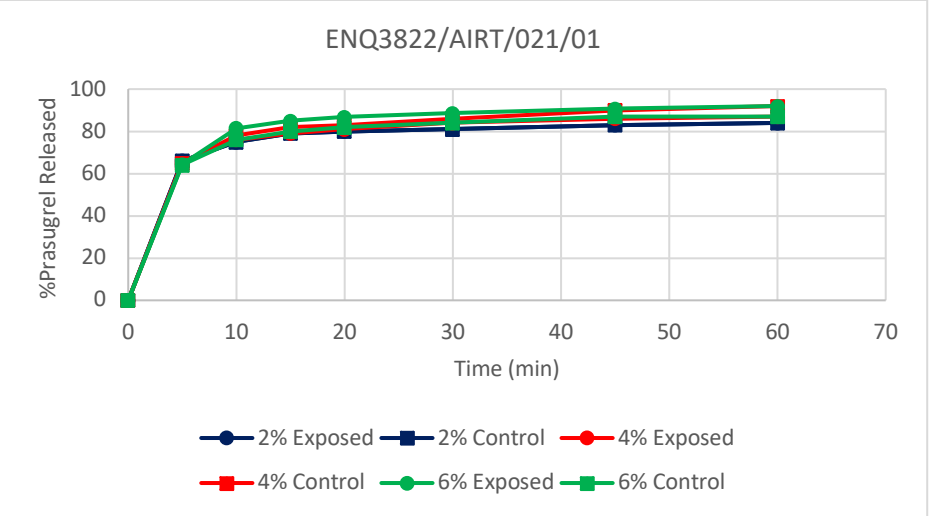
Coated Prasugrel Tablets

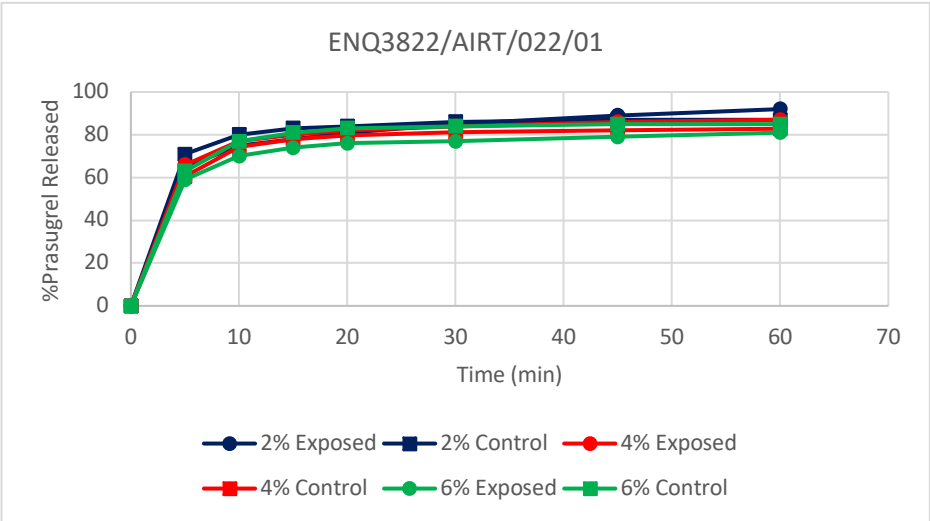
Figure 37 shows the dissolution data for the light-exposed and corresponding dark control prasugrel samples. Light exposure did not impact the release of prasugrel significantly for Batches ENQ3822/AIRT/020/01, ENQ3822/AIRT/021/01 and ENQ3822/AIRT/022/01 coated respectively with the $TiO_2$ reference coat, COAT-026, and the $TiO_2$-free coatings, COAT-016 and COAT-002. The largest %difference between the release from the exposed and corresponding control samples for these batches was 7%, in both cases for the 6% coating weight gain samples at the 10-min time-point.

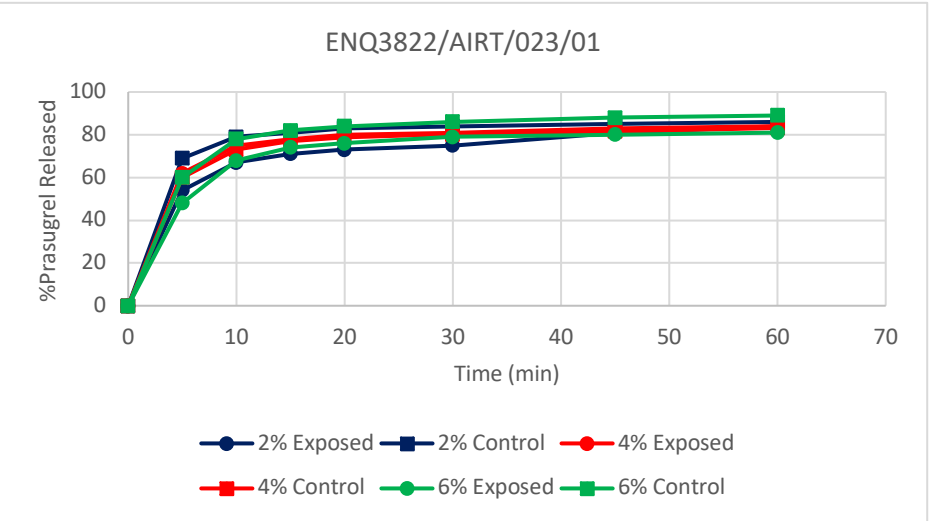
Release from the light-exposed samples from Batches ENQ3822/AIRT/023/01 was slower than the controls at the earlier time-points for the 2% and 6% weight gain samples. However, the difference was much less for the 4%weight gain samples. As with the other prasugrel batches, ENQ3822/AIRT/023/01 exposed and control samples released over 80% of LC at 60 min. In addition, the shape of the dissolution profile did not change as a result of light exposure. Therefore, it can be concluded that light exposure did not significantly influence dissolution from any of the prasugrel batches.

Figure 37: Dissolution of prasugrel from the light-exposed and control samples



## Section Summary and Conclusions

The results from the photostability study depended on the stability challenge presented by the API in the core and not just on the coating used. Nifedipine and rosuvastatin are prone to photodegradation, while olmesartan and prasugrel (at least in the solid state) are not. Therefore, it is not possible to compare directly the $TiO_2$-free coatings used to coat the batches containing these two latter compounds, with those containing the former, for their ability to protect light-sensitive compounds.

Coated Nifedipine Batches

None of the four $TiO_2$-free coatings or the $TiO_2$ reference could protect nifedipine from degradation under the severe light conditions. However, both the visual appearance, colorimetry, assay and the %total impurity data indicated that the $TiO_2$ reference performed better than the $TiO_2$-free coatings.

Coated Olmesartan Batches

Light exposure did not impact on the visual appearance, colorimetry data, assay, related impurity levels, disintegration and dissolution of any of the olmesartan coated batches regardless of whether the coating was $TiO_2$-free or not. This would be as expected given that this compound is not sensitive to light.

Coated Rosuvastatin Batches

COAT-023 was the only $TiO_2$-free coat that proved to be similar to the two $TiO_2$ reference coats, COAT-017 and COAT-018, in its ability to protect rosuvastatin against photodegradation as measured by visual appearance, colorimetry, assay and %related impurities. The other $TiO_2$-free coatings were inferior.

Coated Prasugrel Batches

Visual appearance data showed that the appearance of the $TiO_2$ reference batch was not affected by undergoing photostability, while the appearance of the three $TiO_2$-free coatings were. However, the colorimetry data did not support this finding and found that the $TiO_2$-free COAT-030/COAT-031 combination was the only coated prasugrel batch to have $\Delta E^*_{00}$ values < 2 at all %coating levels. The reason for this discrepancy between the visual and colorimetric data is not known.

For all batches prasugrel assay was similar between the exposed and the control samples, while %related impurities increased slightly in the exposed samples. The results would suggest that the $TiO_2$ reference and the $TiO_2$-free COAT-016 provided protection against this increase at ≥4% coating weight gain, while the $TiO_2$-free COAT-030/COAT-031 combination achieved this at a 6% coating level.

# Experimental Part 7: Accelerated Stability Studies on Active Tablets

## Protocol

Coated tablets with a 6% weight gain from the 23 coating trials (see Table 25) were packed into either 30 mL or 60 mL HDPE bottles with an induction seal and capped with HPDE caps. They were subjected to accelerated testing over 21 days. During stability storage the samples were stored open with the induction seal removed. The packed tablet batch numbers, bottle size and number of tablets per bottle are shown in Table 65 and Table 66 shows the time-points and storage conditions.

Table 65: Packed tablet batch numbers and packaging used

| **Corinfar (nifedipine) Retard 10 mg Tablets** | | | | **Tablet cores Batch No. G170349** | |
|---|---|---|---|---|---|
| **Stability Protocol No. STP/023/** | **Packed Tablets Batch No. ENQ3822/AIRT/** | **Bulk Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Ref No.** | **HDPE Bottle** | **Tablets per Bottle** |
| 058/02/P | 001/01P1 | 001/01 | COAT-024[a] | 30 mL | 210 |
| | 002/01P1 | 002/01 | COAT-001 | 30 mL | 210 |
| | 003/01P1 | 003/01 | COAT-033 | 30 mL | 210 |
| | 004/01P1 | 004/01 | COAT-004 | 30 mL | 210 |
| | 005/01P1 | 005/01 | COAT-023 | 30 mL | 210 |
| **Olmesartan 20 mg Tablets** | | | | **Tablet cores Batch No. G174627** | |
| **Stability Protocol No. STP/023/** | **Packed Tablets Batch No. ENQ3822/AIRT/** | **Bulk Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Ref No.** | **HDPE Bottle** | **Tablets per Bottle** |
| 101/02/P | 006/01P1 | 006/01 | COAT-025[a] | 60 mL | 105 |
| | 007/01P1 | 007/01 | COAT-013 | 60 mL | 105 |
| | 008/01P1 | 008/01 | COAT-015 | 60 mL | 105 |
| | 009/01P1 | 009/01 | COAT-014 | 60 mL | 105 |
| **Rosuvastatin 10 mg Tablets** | | | | **Tablet cores Batch Nos. G174604 and G174605** | |
| **Stability Protocol No. STP/023/** | **Packed Tablets Batch No. ENQ3822/AIRT/** | **Bulk Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Ref No.** | **HDPE Bottle** | **Tablets per Bottle** |
| 102/02/P | 010/01P1[b] | 010/01[b] | COAT-018[a] | 60 mL | 210 |
| | 011/01P1[b] | 011/01[b] | COAT-017[a] | 60 mL | 210 |
| | 012/01P1[b] | 012/01[b] | COAT-020 | 60 mL | 210 |
| | 013/01P1 | 013/01 | COAT-019 | 60 mL | 210 |
| | 014/01P1 | 014/01 | COAT-010 | 60 mL | 210 |
| | 015/02P1 | 015/02 | COAT-030 | 60 mL | 210 |
| | 016/01P1 | 016/01 | COAT-005 | 60 mL | 210 |
| | 017/01P1 | 017/01 | COAT-001 | 60 mL | 210 |
| | 018/01P1 | 018/01 | COAT-034 | 60 mL | 210 |
| | 019/01P1 | 019/01 | COAT-023 | 60 mL | 210 |
| **Prasugrel HCl 10 mg Tablets** | | | | **Tablet cores Batch No. G175131** | |
| **Stability Protocol No. STP/023/** | **Packed Tablets Batch No. ENQ3822/AIRT/** | **Bulk Tablet Batch No. ENQ3822/AIRT/** | **Consortium Coat Ref No.** | **HDPE Bottle** | **Tablets per Bottle** |
| 103/02/P | 020/01P1 | 020/01 | COAT-026[a] | 60 mL | 105 |
| | 021/01P1 | 021/01 | COAT-016 | 60 mL | 105 |
| | 022/01P1 | 022/01 | COAT-002 | 60 mL | 105 |
| | 023/0P1 | 023/01 | COAT-030 & COAT-031 | 60 mL | 105 |

[a]$TiO_2$ containing coating material used as a comparison [b]Tablet cores Batch No. G174604 used.

Table 66: Storage conditions and time-points

| Condition | T = 7 Days | T = 14 Days | T = 21 Days |
|---|---|---|---|
| 50°C/30%RH | x | x | x |
| 50°C/75%RH | o | o | o |
| 60°C/30%RH | o | o | o |
| 60°C/75%RH | o | o | o |
| 70°C/11%RH | o | o | o |
| 70°C/75%RH | x | x | x |

**X** = Scheduled testing

O = Optional testing if requested by the Consortium

After removal of the samples from the stability chamber they were stored at laboratory room temperature. The tests carried out on the samples are shown in Table 67.

Table 67:Tests carried out on the accelerated stability study samples

| Attribute | Methodology |
|---|---|
| **All stability samples from the selected storage conditions and time-points** | |
| Visual assessment[a] | Photography |
| Appearance - Colorimetry | DigiEye |
| Coat thickness | Digital optical microscopy |
| Solid state | XRPD |
| Disintegration | Ph.Eur. 2.9.1 |
| **Corinfar (nifedipine) 10 mg Retard Tablets only** | |
| Assay | HPLC |
| Impurities | HPLC |
| Dissolution | USP Apparatus II (Paddles)/UV spectroscopy |
| **Olmesartan 20 mg Tablets only** | |
| Assay | HPLC |
| Impurities | HPLC |
| Dissolution | USP Apparatus II (Paddles)/HPLC |
| **Rosuvastatin 10 mg Tablets only** | |
| Assay | HPLC |
| Impurities | HPLC |
| Dissolution | USP Apparatus II (Paddles)/HPLC |
| **Prasugrel HCL 10 mg Tablets only** | |
| Assay | HPLC |
| Impurities | HPLC |
| Dissolution | USP Apparatus II (Paddles)/HPLC |

[a]Visual assessment performed at each time-point immediately after removal of the samples from the chamber. Photographs were taken and the tablet color and any defects were noted.

$T_0$ testing was carried out on bulk tablets from each batch.

The analytical methodology was as described in Section 0 unless otherwise stated.

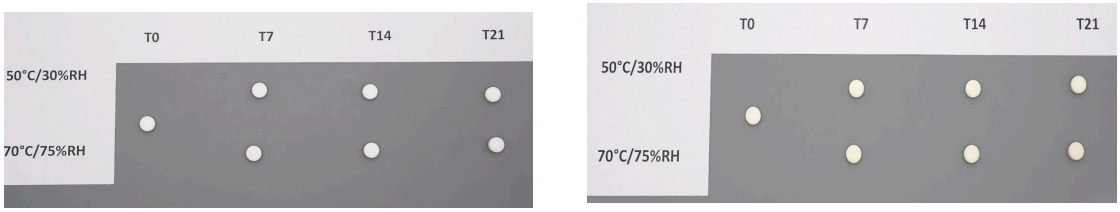
## Results and Discussion

### 48. Visual Appearance and Colorimetry

Coated Nifedipine Tablets

Figure 38 shows two examples of photographs of the 5 coated nifedipine batches on accelerated stability versus the $T_o$ control, one of the batch coated with the $TiO_2$ reference coat and the other with a $TiO_2$-free coating. The visual appearance of the coated nifedipine batches on accelerated stability are described in Table 68 and the color differences ($\Delta E^*_{00}$ values) between the coated nifedipine tablet samples versus $T_0$ presented in Table 69.

Figure 38: Photographs of the coated nifedipine tablets from the accelerated stability study




The photographs and the visual descriptions suggest that the samples stored at 50°C/30%RH did not visually change in appearance compared to the $T_0$ sample. However, the colorimetry data for these samples show significant color differences can be detected. The visual data for the samples stored at 70°C/75%RH indicated that a color change had taken place with the colorimetry data showing high $\Delta E^*_{00}$ values. The $\Delta E^*_{00}$ values are higher than those found for the samples stored at 50°C/30%RH and the visible color change and the $\Delta E^*_{00}$ values increased with time for the samples stored at 70°C/75%RH.

In order to investigate the difference in the visual results and the colorimetry data for the 50°C/30%RH samples, photographs were retaken and the individual values of L* a* b* chroma and hue angle compared in more detail. The visual data again showed no difference in appearance for the 50°C/30%RH samples and the $T_o$ sample, while the review of the colorimetry data found that differences in the L* values between the 50°C/30%RH samples and the $T_0$ control were the main driver for the high $\Delta E^*_{00}$ values. Therefore, whilst the nifedipine colorimetry results suggest a visual difference ($\Delta E^*_{00}$ >2), this difference is mainly due to the measured change in lightness value which is not always perceptible in standard photographs and visual observations and, thus, is thought to be of little practical relevance.

Table 68: Description of the visual appearance of the coated nifedipine tablet batches following storage under accelerated conditions

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Film Former | Opacifier | Appearance | |
|---|---|---|---|---|---|
| | | | | 50°C/30%RH | 70°C/75%RH |
| 001/01P1 | COAT-024 | HPMC | $TiO_2$ | No noticeable change in color observed throughout the duration of the study. | A slight change in color from white to off-white can be observed at 21 days. |
| 002/01P1 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | No noticeable change in color observed throughout the duration of the study. | A slight change in color from white to off-white can be observed after 7 days. The change is more pronounced after 14 days and a significant change can be observed after 21 days. |
| 003/01P1 | COAT-033 | HPMC | $CaCO_3$+D+F | No noticeable change in color observed throughout the duration of the study. | A slight change in color can be observed at 21 days. |
| 004/01P1 | COAT-004 | HPMC | $CaCO_3$+C | No noticeable change in color observed throughout the duration of the study. | A slight change in color from pale yellow to yellow/orange can be observed after 7 days. The change is more pronounced after 14 days and a significant change, with color shift to orange, can be observed after 21 days. |
| 005/01P1 | COAT-023 | PVA | F+Talc | No noticeable change in color observed throughout the duration of the study. | A slight change in color can be observed at 7 days. The change in color is more visible after 14 and 21 days. |

Color Code: Green = No change in appearance throughout stability study, Red = Change in appearance

Table 69: Color differences between the coated nifedipine tablet samples on accelerated stability versus $T_0$

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Color Difference - 50°C/30%RH | | | Color Difference 70°C/75%RH | | |
|---|---|---|---|---|---|---|---|
| | | T=7 Days | T=14 Days | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 001/01P1 | COAT-024 | 4.72 | 4.10 | 4.27 | 6.05 | 6.22 | 6.99 |
| 002/01P1 | COAT-001 | 5.06 | 5.09 | 4.94 | 10.10 | 14.06 | 17.94 |
| 003/01P1 | COAT-033 | 4.99 | 4.48 | 4.63 | 6.91 | 7.20 | 8.34 |
| 004/01P1 | COAT-004 | 4.85 | 4.28 | 4.56 | 8.90 | 10.89 | 11.62 |
| 005/01P1 | COAT-023 | 5.00 | 4.80 | 4.32 | 6.52 | 7.08 | 7.18 |

Color Code: Green = $\Delta E^*_{00}$ values ≤ 1 (acceptance criterion for white coated tablets). Red = $\Delta E^*_{00}$ values > 1

Coated OlmesartanTablets

The visual appearance of the coated olmesartan batches on accelerated stability are described in Table 70 and the color differences ($\Delta E^*_{00}$ values) between the coated olmesartan tablet samples versus $T_0$ presented in Table 71.

For Batch ENQ3822/AIRT/006/01P1 coated with the $TiO_2$ reference coat, COAT-025, there was no color change observed in the tablets compared with $T_0$ for any of the stability samples subjected to accelerated stability and the colorimetry results are in agreement with the appearance results with all $\Delta E^*_{00}$ values being less than the acceptance criterion for colored tablets of ≤2 and most of the values < 1.

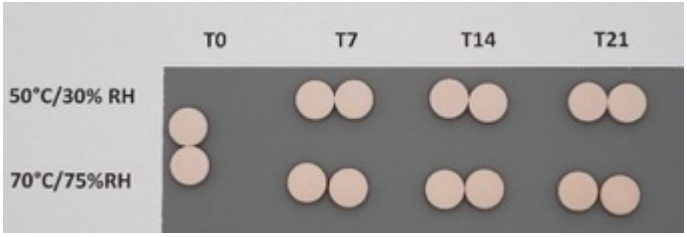
For Batch ENQ3822/AIRT/009/01P1 coated with the $TiO_2$ free coat, COAT-014, there was also agreement between the visual results and the colorimetry data. The 50°C/30%RH samples showed no change in physical appearance and had $\Delta E^*_{00}$ values of < 1, while those stored at 70°C/75%RH became darker with the time spent under these conditions and had $\Delta E^*_{00}$ values > 2.

Batch ENQ3822/AIRT/007/01P1, coated with COAT-013, changed very slightly in color after 21 days at 50°C/30%RH based on visual observations (see Figure 39). The $\Delta E^*_{00}$ values were all < 1 for all the samples stored at 50°C/30%RH, indicating no perceptible color difference at any of the time-points. This coating was observed to darken over the course of the stability study at 70°C/75%RH. The samples stored under 70°C/75%RH condition had higher $\Delta E^*_{00}$ values than those stored at 50°C/30%RH which increased at the later time-points suggesting a higher color difference between these samples and the $T_0$ sample. However, only the 21-day sample had $\Delta E^*_{00}$ values of around 2, while the 7-day and 14-day sample were close to 1 and < 2 respectively, suggesting that a color difference would only be noticeable on close observation.

Batch ENQ3822/AIRT/008/01P1, coated with COAT-015, was observed not to change in color at 50°C/30%RH at any of the time-points, with only a small change being observed at 70°C/75%RH at the 21-day time-point (see Figure 39). However, in the case of this batch, the $\Delta E^*_{00}$ values are all in excess of 2, suggesting that there is a clear color difference. The reason for the differing results between the visual and colorimetry data are under investigation.

Figure 39: Photographs of Batch ENQ3822/AIRT/007/01P1 and ENQ3822/AIRT/008/01P1

Batch ENQ3822/AIRT/007/01P1




Batch ENQ3822/AIRT/008/01P1

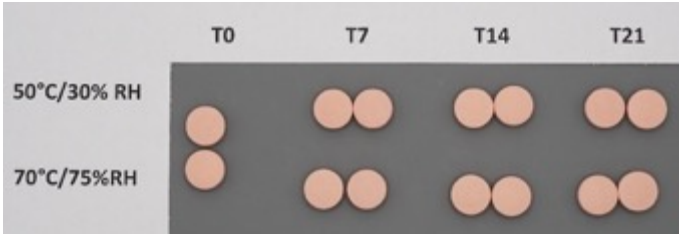


Table 70: Description of the visual appearance of the coated olmesartan tablet batches following storage under accelerated conditions

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Film Former | Opacifier | Appearance | |
|---|---|---|---|---|---|
| | | | | 50°C/30%RH | 70°C/75%RH |
| 006/01P1 | COAT-025 | PVA | $TiO_2$+Talc+$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | No noticeable change in color observed throughout the duration of the study. |
| 007/01P1 | COAT-013 | PVA+HPMC | $CaCO_3$+Talc+$Fe_2O_3$ | A small change in color was observed at the 21-day time-point where the tablets became slightly darker compared to earlier time points. | A slight darkening of tablets was observed at 7 days. The darkening was slightly more visible at 14 and 21 days. |
| 008/01P1 | COAT-015 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 21-day time-point where the tablets appear slightly darker compared to earlier time points. |
| 009/01P1 | COAT-014 | PVA | $CaCO_3$+Talc+$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 7-day time-point. A more significant change was observed at further time points. |

Color Code: Green = No change in appearance throughout stability study. Red = Change in appearance occurred.

Table 71: Color differences between the coated olmesartan tablet samples on accelerated stability versus $T_0$

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Color Difference - 50°C/30%RH | | | Color Difference 70°C/75%RH | | |
|---|---|---|---|---|---|---|---|
| | | T=7 Days | T=14 Days | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 006/01P1 | COAT-025 | 0.40 | 0.35 | 0.23 | 0.26 | 0.53 | 1.08 |
| 007/01P1 | COAT-013 | 0.69 | 0.47 | 0.44 | 0.83 | 1.29 | 1.99 |
| 008/01P1 | COAT-015 | 4.79 | 4.53 | 4.63 | 4.60 | 4.97 | 4.75 |
| 009/01P1 | COAT-014 | 0.55 | 0.60 | 0.72 | 2.19 | 4.49 | 6.31 |

Color Code: Green = $\Delta E^*_{00}$ values < 2 (acceptance criterion for colored coated tablets) Red = $\Delta E^*_{00}$ values > 2

Rosuvastatin Coated Tablets

The visual appearance of the coated rosuvastatin batches on accelerated stability are described in Table 72 and the color differences ($\Delta E^*_{00}$ values) between the coated rosuvastatin tablet samples versus $T_0$ presented in Table 73. Two examples of the photographs of the rosuvastatin stability samples are shown in Figure 40.

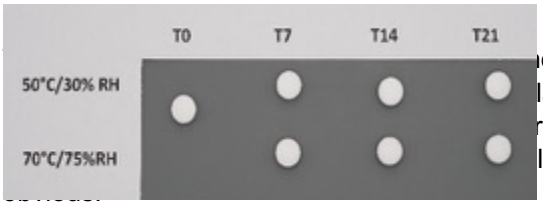
For Batch ENQ3822/AIRT/011/01P1, coated with the HPMC-based $TiO_2$ reference coat, COAT-017, there was no visible change in any of the stability samples both at 50°C/30%RH and 70°C/75%RH. For Batch ENQ3822/AIRT/010/01P1, coated with the PVA-based $TiO_2$ reference coat, COAT-018, and all the batches coated with the $TiO_2$-free coatings, there was no visible change in tablet appearance compared to $T_0$ at 50°C/30%RH. However, this was not the case at 70°C/75%RH. At this stability condition a color change could observed, whose initial appearance varied between batches and whose nature and intensity was different from batch-to-batch and from time-point to time-point. For example, a color difference was first observed for ENQ3822/AIRT/010/01P1 at the 21-day time-point with the tablets becoming slightly darker. In contrast, Batches ENQ3822/AIRT/016/01P1 and ENQ3822/AIRT/017/01P1 started to yellow at the 7-day time-point, with the tablets turning brown by the 21-day time-point.

The $\Delta E^*_{00}$ values agree with the visual data for the samples stored at 50°C/30%RH for 14 days and 21 days in that they are all ≤ 1 or close to 1, suggesting no perceptible color change from $T_0$. However, they are well above 2 for the samples stored for 7 days. The $\Delta E^*_{00}$ values for Batch ENQ3822/AIRT/011/01P1 at 70°C/75% RH storage also do not correspond to the visual data. For this batch, no noticeable visible change was observed at any time-point following storage at 70°C/75% RH. However, the $\Delta E^*_{00}$ values are well above 2 suggesting a color change should be noticeable at a glance. The differences between the visual and colorimetry data are currently under investigation.

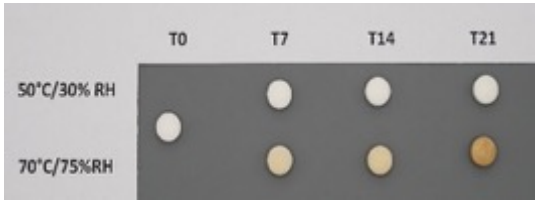
The highest $\Delta E^*_{00}$ values were obtained with Batches ENQ3822/AIRT/016/01P1 and ENQ3822/AIRT/017/01P1, which turned brown after 21 days of storage at 70°C/75%RH.

Figure 40: Photographs of Batch ENQ3822/AIRT/011/01P1 and ENQ3822/AIRT/016/01P1

Batch ENQ388/AIRT/011/01P1




Batch ENQ388/AIRT/016/01P1




…ce between the samples of both batches stored at …later time-points. There is also no difference between the …red at 70°C/75% RH and $T_0$, while the color difference …les stored under these conditions and $T_0$ are instantly …

Table 72: Description of the visual appearance of the coated rosuvastatin tablet batches following storage under accelerated conditions

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Film Former | Opacifier | Appearance | |
|---|---|---|---|---|---|
| | | | | 50°C/30%RH | 70°C/75%RH |
| 010/01P1 | COAT-018 | PVA | $TiO_2$+Talc | No noticeable change in color observed throughout study duration. | A small change in color was observed at the 21-day time point, where the tablets became slightly darker compared to the earlier time points. |
| 011/01P1 | COAT-017 | HPMC | $TiO_2$ | No noticeable change in color observed throughout study duration. | No noticeable change in color observed throughout the duration of the study. |
| 012/01P1 | COAT-020 | HPMC+HPC | Rice Starch+D | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 7-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |
| 013/01P1 | COAT-019 | HPMC | $CaCO_3$+D+E | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 7-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |
| 014/01P1 | COAT-010 | HPMC | Rice Starch+D | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 14-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |
| 015/02P1 | COAT-030 | HPMC | B+E | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 7-day time point and further time points, where a slight yellowing was observed. This was more pronounced in tablets removed from stability chambers at the 14 and 21-day time-points. |
| 016/01P1 | COAT-005 | HPMC | MgO | No noticeable change in color observed throughout the duration of the study | A yellowing was observed at the 7 and 14 day-time points. At 21 days a significant change in color occurred where the tablets became brown. |
| 017/01P1 | COAT-001 | HPMC+HPC | $MgCO_3$+A+B | No noticeable change in color observed throughout the duration of the study. | Yellowing was observed at the 7 and 14-day time points. At 21 days a significant change in color occurred where the tablets became light brown with visible darker spots. |
| 018/01P1 | COAT-034 | HPMC | Rice starch | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 14-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |
| 019/01P1 | COAT-023 | PVA | F+Talc | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 14-day time point and further time points, where the tablets became |

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Film Former | Opacifier | Appearance | |
|---|---|---|---|---|---|
| | | | | 50°C/30%RH | 70°C/75%RH |
| | | | | | slightly off-white compared to the $T_0$. |

Color Code: Green = No change in appearance throughout stability study  Red = Change in appearance

Table 73: Color differences between the coated rosuvastatin tablet samples on accelerated stability versus $T_0$

| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref. | Color Difference - 50°C/30%RH | | | Color Difference 70°C/75%RH | | |
|---|---|---|---|---|---|---|---|
| | | T=7 Days | T=14 Days | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 010/01P1 | COAT-018 | 3.69 | 0.56 | 0.35 | 5.78 | 3.89 | 5.59 |
| 011/01P1 | COAT-017 | 4.35 | 0.67 | 0.35 | 5.39 | 1.79 | 2.08 |
| 012/01P1 | COAT-020 | 4.25 | 0.31 | 0.43 | 6.96 | 5.23 | 5.92 |
| 013/01P1 | COAT-019 | 3.94 | 0.39 | 0.38 | 5.88 | 3.26 | 4.56 |
| 014/01P1 | COAT-010 | 4.24 | 0.44 | 0.38 | 6.21 | 4.04 | 4.52 |
| 015/02P1 | COAT-030 | 4.29 | 0.37 | 0.45 | 11.09 | 11.44 | 13.18 |
| 016/01P1 | COAT-005 | 3.96 | 1.42 | 1.85 | 13.73 | 16.37 | 21.69 |
| 017/01P1 | COAT-001 | 3.96 | 0.25 | 0.31 | 15.25 | 16.36 | 19.21 |
| 018/01P1 | COAT-034 | 3.96 | 0.51 | 0.33 | 8.04 | 6.95 | 7.32 |
| 019/01P1 | COAT-023 | 4.13 | 0.27 | 0.47 | 8.16 | 8.30 | 10.84 |

Color Code: Green = $\Delta E^*_{00}$ values ≤ 1 (acceptance criterion for white coated tablets).  Yellow = $\Delta E^*_{00}$ values 1-2  Red = $\Delta E^*_{00}$ values > 2

7-day samples at 50°C/30%RH not colored as results under investigation.

Prasugrel Coated Tablets

The visual appearance of the coated praugrel batches on accelerated stability are described in Table 74 and the color differences ($\Delta E^*_{00}$ values) between the coated prasugrel tablet samples versus $T_0$ presented in Table 75.

There was no visible color change for all samples stored at 50°C/30%RH. For Batches ENQ3822/AIRT/020/01P1, coated with the $TiO_2$ reference coat, COAT-026, and ENQ3822/AIRT/021/01P1, coated with the $TiO_2$-free coat, COAT-016, the colorimetry data agree with the visual results in that the $\Delta E^*_{00}$ values are ≤ 2, although there is a trend of increasing $\Delta E^*_{00}$ with exposure time. The $\Delta E^*_{00}$ values for Batch ENQ3822/AIRT/022/01P1, coated with the $TiO_2$-free batch, COAT-002, are higher and at the 14-day and 21-day time-points close to 2. However, in general, the colorimetry data agree with the visual for this batch. The $\Delta E^*_{00}$ values for Batch ENQ3822/AIRT/023/01P1, coated with the $TiO_2$-free COAT-030 and COAT-031 combination containing red iron oxide, are in well in excess of 2, suggesting a visible difference should be easily perceptible in contrast to the visual data. Experience obtained on working with iron oxide containing capsule shells [11] indicated that the color intensity of this metal oxide makes it difficult for the human eye to detect color variations and this may be the reason for difference in the findings between the visual and colorimetry data for Batch ENQ3822/AIRT/023/01P1.

At 70°C/75%RH significant visual differences were observed for all batches and $\Delta E^*_{00}$ values were correspondingly high. Examples of the visual changes observed can be seen in the photographs of Batches ENQ3822/AIRT/020/01P1 and ENQ3822/AIRT/023/01P1.

Figure 41: Photographs of Batches ENQ3822/AIRT/020/01P1 and ENQ3822/AIRT/023/01P1

Batch ENQ3822/AIRT/020/01P1 ENQ3822/AIRT/023/01P1

Table 74: Description of the visual appearance of the coated prasugrel tablet batches following storage under accelerated conditions

| Batch No. ENQ3822/AIRT/ | Consort Coat Ref. | Film Former | Opacifier | Appearance | |
|---|---|---|---|---|---|
| | | | | 50°C/30%RH | 70°C/75%RH |
| 020/01P1 | COAT-026 | HPMC | $TiO_2$+$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | Significant change in the color (darkening) of tablets was observed after 7 days. The change was more pronounced at further time points and some dark spots could be observed on the tablets. |
| 021/01P1 | COAT-016 | HPMC | Rice Starch+D +$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | A yellowing was observed at the 7 and 14 day- time points. At 21 days a significant change in color occurred where the tablets became light brown with visible darker spots. |
| 022/01P1 | COAT-002 | HPMC | Rice Starch+ A+B+D+Fe2O3 | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 14-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |
| 023/01P1 | COAT-030 & COAT-031 | HPMC | B+E+$Fe_2O_3$ | No noticeable change in color observed throughout the duration of the study. | A small change in color was observed at the 14-day time point and further time points, where the tablets became slightly off-white compared to the $T_0$. |

Color Code: Green = No change in appearance throughout stability study.

Table 75: Color differences between the coated prasugrel tablet samples on accelerated stability versus $T_0$

| Batch No. ENQ3822/AIRT/ | Consortium Coat Reference | Color Difference - 50°C/30%RH | | | Color Difference 70°C/75%RH | | |
|---|---|---|---|---|---|---|---|
| | | T=7 Days | T=14 Days | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 020/01P1 | COAT-026 | 0.52 | 0.76 | 0.78 | 13.33 | 14.89 | 15.66 |
| 021/01P1 | COAT-016 | 0.75 | 0.91 | 1.00 | 11.59 | 13.39 | 15.70 |
| 022/01P1 | COAT-002 | 1.38 | 1.67 | 2.11 | 15.61 | 16.72 | 18.35 |
| 023/01P1 | COAT-030 & COAT-031 | 2.18 | 3.49 | 4.50 | 17.76 | 19.79 | 19.85 |

Color Code: Green = $\Delta E^*_{00}$ values ≤ 2 (acceptance criterion for colored coated tablets). Red = $\Delta E^*_{00}$ values > 2

.

## 49. Coat Thickness

Digital optical microscopy measurements were made on one tablet from each stability sample plus $T_0$ and therefore 7 measurements per batch were available to provide more information on intra-batch coating thickness variation than previous work using this technique on the 23 batches of coated active cores (see 18 and 43). Since it was not always easy to clearly define the boundary between coating and core due to poor contrast in digital microscopy, some coating thickness measurements were approximate. The measurements for each stability sample were averaged to give the mean result and the minimum and maximum values for the 7 measurements determined. In order to compare the variation between batches, they were divided into 3 groups depending on whether the difference between the minimum and maximum average thickness was < 25 µm, between 25 µm to 50 µm or 50 µm to 75 µm.

The average coating thickness (land, belly, surface) of the coated nifedipine, coated rosuvastatin and coated prasugrel tablet samples from the accelerated stability are shown in Table 76, Table 78 and Table 79 respectively, together with the mean values for the $T_0$ samples. The average coating thickness (land, belly, surface and debossed image) for the samples from the accelerated stability study on the coated olmesartan tablets are shown in Table 77.

The overall lowest mean coating thickness of 20 µm was found for the $T_0$ sample of Batch ENQ3822/AIRT/014/01P1, a rosuvastatin batch coated with the $TiO_2$-free coat, COAT-010. Batch ENQ3822/AIRT/010/01P1, also a rosuvastatin batch coated with the PVA-based $TiO_2$ reference coat, COAT-018, had the largest average coat thickness at over 100 µm. However, the other samples from these batches had either had thicker or thinner coatings than the tablet measured from the respective $T_0$ sample.

If the level of variation in coating thickness was considered based on the minimum and maximum mean values, the coated nifedipine tablet batches all had relatively low variation of < 25 µm, while the rosuvastatin coated tablet samples had the greatest degree of variation, with a number of batches having average coating thickness variation in the 50 µm to 75 µm range. Olmesartan and prasugrel each had two batches with average coating thickness variation in the 25 µm to 50 µm range and two in the < 25 µm range.

Coating thickness per se is not an issue provided the coating is homogeneous, evenly spread and is thick enough to provide sufficient opacification and coverage to hide a colored core or any color differences or imperfections within the core tablet. Large amounts of variation in coating thickness can be an issue when coating quality is border-line, meaning that some tablets are perfectly coated, while others are not. However, low variation does not always indicate successful coating. For example, all of the nifedipine coated batches had low coating thickness variation. Despite this, the yellow color of the core was not completely hidden in the batches coated with the $TiO_2$-free coatings even at a 6% weight gain.

Table 76: Comparison of the coat thickness on the coated nifedipine tablet samples on accelerated stability

| Nifedipine 10 mg Retard Coated Tablets | | Average Coat Thickness (µm) | | | | | | | | | Variation in Mean Coat Thickness Between Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | Overall Range | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | $T_0$ | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | Min | Max | |
| 001/01P1 | COAT-024 | 38.0 | 40.7 | 32.3 | 43.3 | 39.7 | 39.0 | 39.0 | 32.3 | 43.3 | Max difference < 25 µm |
| 002/01P1 | COAT-001 | 46.0 | 53.7 | 46.0 | 42.7 | 64.0 | 47.0 | 52.0 | 42.7 | 64.0 | Max difference < 25 µm |
| 003/01P1 | COAT-033 | 34.0 | 46.0 | 38.3 | 40.3 | 49.3 | 38.0 | 42.7 | 34.0 | 49.3 | Max difference < 25 µm |
| 004/01P1 | COAT-004 | 34.3 | 40.0 | 42.7 | 50.3 | 43.0 | 36.0 | 49.3 | 34.3 | 50.3 | Max difference < 25 µm |
| 005/01P1 | COAT-023 | 35.0 | 38.3 | 29.7 | 41.7 | 41.0 | 43.3 | 47.3 | 29.7 | 47.3 | Max difference < 25 µm |

Table 77: Comparison of the coat thickness on the coated olmesartan tablet samples on accelerated stability

| Olmesartan 20 mg Coated Tablets | | Average Coat Thickness (µm) | | | | | | | | | Variation in Mean Coat Thickness Between Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | Overall Range | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | $T_0$ | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | Min | Max | |
| 006/01P1 | COAT-025 | 60.8 | 51.0 | 54.0 | 56.8 | 59.3 | 47.5 | 53.3 | 47.5 | 60.8 | Maxi difference < 25 µm |
| 007/01P1 | COAT-013 | 77.5 | 49.3 | 58.0 | 65.5 | 49.3 | 69.0 | 50.5 | 49.3 | 77.5 | Max difference – 25 - 50 µm |
| 008/01P1 | COAT-015 | 65.5 | 64.5 | 51.3 | 59.0 | 83.3 | 66.5 | 51.8 | 51.3 | 83.3 | Max difference – 25 - 50 µm |
| 009/01P1 | COAT-014 | 56.8 | 73.8 | 64.3 | 54.0 | 63.0 | 67.3 | 62.8 | 54.0 | 73.8 | Max difference < 25 µm |

Table 78: Comparison of the coat thickness on the coated rosuvastatin tablet samples on accelerated stability

| Rosuvastatin 10 mg Coated Tablets | | Average Coat Thickness (µm) | | | | | | | | | Variation in Mean Coat Thickness Between Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | Overall Range | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | $T_0$ | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | Min | Max | |
| 010/01P1 | COAT-018 | 103.7 | 36.0 | 63.7 | 48.3 | 48.3 | 43.0 | 55.3 | 36.0 | 103.7 | Max difference 50 – 75 µm |
| 011/01P1 | COAT-017 | 70.7 | 39.3 | 50.7 | 54.3 | 40.3 | 44.3 | 42.0 | 39.3 | 70.7 | Max difference 25 -50 µm |
| 012/01P1 | COAT-020 | 49.3 | 54.0 | 88.0 | 102.0 | 66.7 | 66.0 | 67.0 | 49.3 | 102.0 | Max difference 50 – 75 µm |
| 013/01P1 | COAT-019 | 33.3 | 45.0 | 78.0 | 71.7 | 49.7 | 50.3 | 61.0 | 33.3 | 78.0 | Max difference 25 -50 µm |
| 014/01P1 | COAT-010 | 20.0 | 58.7 | 81.0 | 69.0 | 74.7 | 46.7 | 69.0 | 20.0 | 81.0 | Max difference 50 – 75 µm |
| 015/02P1 | COAT-030 | 65.7 | 49.7 | 45.3 | 56.7 | 53.3 | 45.0 | 46.0 | 45.0 | 65.7 | Maximum difference < 25 µm |
| 016/01P1 | COAT-005 | 51.7 | 74.0 | 75.0 | 59.7 | 54.3 | 44.7 | 67.7 | 44.7 | 75.0 | Max difference 25 -50 µm |
| 017/01P1 | COAT-001 | 52.0 | 48.7 | 74.3 | 68.7 | 56.7 | 66.0 | 55.7 | 48.7 | 74.3 | Max difference 25 -50 µm |
| 018/01P1 | COAT-034 | 66.0 | 56.7 | 88.3 | 45.3 | 61.0 | 60.3 | 63.0 | 45.3 | 88.3 | Max difference 25 -50 µm |
| 019/01P1 | COAT-023 | 50.0 | 45.3 | 69.0 | 69.3 | 47.0 | 46.0 | 41.3 | 41.3 | 69.3 | Max difference 25 -50 µm |

Table 79: Comparison of the coat thickness on the coated prasugrel tablet samples on accelerated stability

| Prasugrel 10 mg Coated Tablets | | Average Coat Thickness (µm) | | | | | | | | | Variation in Mean Coat Thickness Between Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | Overall Range | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | $T_0$ | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | Min | Max | |
| 020/01P1 | COAT-026 | 53.3 | 73.7 | 57.7 | 43.7 | 44.3 | 52.3 | 41.3 | 41.3 | 73.7 | Max difference 25 -50 µm |
| 021/01P1 | COAT-016 | 57.0 | 68.3 | 74.3 | 69.7 | 66.7 | 59.3 | 60.7 | 57.0 | 74.3 | Max difference < 25 µm |
| 022/01P1 | COAT-002 | 63.3 | 63.7 | 52.3 | 66.0 | 56.0 | 71.3 | 49.3 | 49.3 | 71.3 | Max difference < 25 µm |
| 023/01P1 | COAT-030 & COAT-031 | 65.3 | 67.0 | 69.7 | 69.7 | 58.7 | 45.3 | 34.3 | 34.3 | 69.7 | Max difference 25 -50 µm |

## 50. XRPD Results

The results of the X-ray powder diffraction studies on the various accelerated stability samples versus $T_0$ are shown in Table 80, Table 81, Table 82 and Table 83. The samples from the nifedipine batches all showed a nifedipine pattern A and an elevated baseline which may be indicative of some disorder or amorphous content. This elevated baseline had been previously observed when the batches were first tested (see Figure 24). The samples on accelerated stability from the coated olmesartan, rosuvastatin and prasugrel tablets all displayed the characteristic olmesartan pattern A, rosuvastatin pattern A and prasugrel pattern A respectively.

In addition to the characteristic pattern A of the relevant API, many of the stability samples from the 23 coated batches showed additional peaks. Additional peaks had been previously seen in certain of the rosuvastatin and prasugrel coated tablet samples (see Table 39 and Table 40).

There appeared to be no trend in the appearance of these peaks and they were also present in certain of the $T_0$ samples including $T_0$ samples from the nifedipine batches and olmesartan batches. The cause of the additional peaks in the exposed and control batches is unknown and would require further investigation.

In summary, overall, there were no major differences in the XRPD results for any of the coated tablet samples on accelerated stability when compared to $T_0$.

Table 80: XRPD patterns of the coated nifedipine tablet samples on accelerated stability versus $T_0$

| **Batch No. ENQ3822/AIRT/** | **001/01** | **002/01** | **003/01** | **004/01** | **005/01** |
|---|---|---|---|---|---|
| **Consort. Cap Ref.** | **COAT-024** | **COAT-001** | **COAT-033** | **COAT-004** | **COAT-023** |
| **Film Former/Opacifier** | **HPMC/$TiO_2$** | **HPMC+HPC/ $MgCO_3$+A+B** | **HPMC/$CaCO_3$+D+F** | **HPMC/$CaCO_3$+C** | **PVA/F+Talc** |
| $T_0$ | Nifedipine Pattern A + additional peaks at 25.3 and 28.3°2θ | Nifedipine Pattern A + additional peak at 25.4°2θ | Nifedipine Pattern A + additional peaks at 31.7 sand 34.4°2θ | Nifedipine Pattern A | Nifedipine Pattern A |
| **Storage Conditions - 50°C/30%RH** | | | | | |
| 7 Days | Nifedipine Pattern A + additional peak at 25.3°2θ | Nifedipine Pattern A + additional peak at 25.4°2θ | Nifedipine Pattern A + additional peak at 31.7°2θ | Nifedipine Pattern A | Nifedipine Pattern A + additional peaks at 31.8°2θ |
| 14 Days | Nifedipine Pattern A | Nifedipine Pattern A + additional peak at 25.4°2θ | Nifedipine Pattern A | Nifedipine Pattern A | Nifedipine Pattern A + additional peak at 34.3°2θ |
| 21 Days | Nifedipine Pattern A + additional peak at 25.2°2θ | Nifedipine Pattern A | Nifedipine Pattern A | Nifedipine Pattern A + additional peaks at 31.7 and 36.2°2θ | Nifedipine Pattern A + additional peaks at 31.7, 34.4 and 36.2°2θ |
| **Storage Conditions - 70°C/75%RH** | | | | | |
| 7 Days | Nifedipine Pattern A + additional peak at 36.7°2θ | Nifedipine Pattern A + additional peak at 25.4°2θ | Nifedipine Pattern A | Nifedipine Pattern A + additional peak at 29.3°2θ | Nifedipine Pattern A + additional peak at 31.7°2θ |
| 14 Days | Nifedipine Pattern A + additional peaks at 25.2 and 37.7°2θ | Nifedipine Pattern A + additional peaks at 31.8, 33.1 and 38.8°2θ | Nifedipine Pattern A + additional peak at 34.6°2θ | Nifedipine Pattern A + additional peaks at 33.1 °2θ | Nifedipine Pattern A + additional peaks at 31.8, 33.2 and 36.3°2θ |
| 21 Days | Nifedipine Pattern A + additional peaks at 33.2 and 37.8°2θ | Nifedipine Pattern A | Nifedipine Pattern A + additional peaks at 33.2, 36.9 and 37.8°2θ | Nifedipine Pattern A + additional peaks at 33.2 °2θ | Nifedipine Pattern A + additional peaks at 31.7 and 36.3°2θ |

Table 81: XRPD patterns of the coated olmesartan tablet samples on accelerated stability versus $T_0$

| **Batch No. ENQ3822/AIRT/** | **006/01P1** | **007/01P1** | **008/01P1** | **009/01P1** |
|---|---|---|---|---|
| **Consort. Cap Ref.** | **COAT-025** | **COAT-013** | **COAT-015** | **COAT-014** |
| **Film Former/Opacifier** | **PVA/$TiO_2$+Talc+$Fe_2O_3$** | **PVA+HPMC/$CaCO_3$+Talc+$Fe_2O_3$** | **PVA/$CaCO_3$+Talc+$Fe_2O_3$** | **PVA/$CaCO_3$+Talc+$Fe_2O_3$** |
| $T_0$ | Olmesartan Pattern A | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ |
| **Storage Conditions - 50°C/30%RH** | | | | |
| 7 Days | Olmesartan Pattern A + additional peak at 12.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ |
| 14 Days | Olmesartan Pattern A + additional peak at 32.3°2θ | Olmesartan Pattern A + additional peaks at 29.4 and 30.2°2θ | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ |
| 21 Days | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ |
| **Storage Conditions - 70°C/75%RH** | | | | |
| 7 Days | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peaks at 29.4 and 37.6°2θ + peak shifting |
| 14 Days | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peaks at 29.4 and 34.0°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ |
| 21 Days | Olmesartan Pattern A | Olmesartan Pattern A + additional peak at 29.4°2θ | Olmesartan Pattern A + additional peaks at 29.4 and 34.0°2θ | Olmesartan Pattern A + additional peak at 29.4°2θ |

Table 82: XRPD patterns of the coated rosuvastatin tablet samples on accelerated stability versus $T_0$

| **Batch No. ENQ3822/AIRT/** | **010/01** | **011/01** | **012/01** | **013/01** | **014/01** |
|---|---|---|---|---|---|
| **Consort. Cap Ref.** | **COAT-018** | **COAT-017** | **COAT-020** | **COAT-019** | **COAT-010** |
| **Film Former/Opacifier** | **PVA/$TiO_2$+Talc** | **HPMC/$TiO_2$** | **HPMC+HPC/Rice Starch+D** | **HPMC/$CaCO_3$+D+E** | **HPMC/Rice Starch+D** |
| $T_0$ | Rosuvastatin Pattern A[a] | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| **Storage Conditions - 50°C/30%RH** | | | | | |
| 7 Days | Rosuvastatin Pattern A[a] | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| 14 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| 21 Days | Rosuvastatin Pattern A + additional peak at 9.4°2θ | Rosuvastatin Pattern A[a] | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| **Storage Conditions - 70°C/75%RH** | | | | | |
| 7 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 39.8°2θ | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| 14 Days | Rosuvastatin Pattern A[a] | Rosuvastatin Pattern A[a] | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| 21 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 29.4°2θ | Rosuvastatin Pattern A |
| **Batch No. ENQ3822/AIRT/** | **015/02** | **016/01** | **017/01** | **018/01** | **019/01** |
| **Consort. Cap Ref.** | **COAT-030** | **COAT-005** | **COAT-001** | **COAT-034** | **COAT-023** |
| **Film Former/Opacifier** | **HPMC/B+E** | **HPMC/MgO** | **HPMC+HPC/$MgCO_3$+A+B** | **HPMC/Rice Starch** | **PVA/+Talc** |
| $T_0$ | Rosuvastatin Pattern A + additional peak at 21.5°2θ | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |
| **Storage Conditions - 50°C/30%RH** | | | | | |
| 7 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A + additional peak at 25.6°2θ | Rosuvastatin Pattern A | Rosuvastatin Pattern A |
| 14 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |
| 21 Days | Rosuvastatin Pattern A + additional peak at 28.3°2θ | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |
| **Storage Conditions - 70°C/75%RH** | | | | | |
| 7 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |
| 14 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |

| | | | | | |
|---|---|---|---|---|---|
| 21 Days | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A | Rosuvastatin Pattern A |

[a]For these samples the peaks around 25.3°2θ and 25.6°2θ showed differing intensities compared to the reference sample.

Table 83: XRPD patterns of the coated prasugrel tablet samples on accelerated stability versus $T_0$

| **Batch No. ENQ3822/AIRT/** | **020/01** | **021/01** | **022/01** | **023/01** |
|---|---|---|---|---|
| **Consort. Cap Ref.** | **COAT-026** | **COAT-016** | **COAT-002** | **COAT-030/COAT-031** |
| **Film Former/Opacifier** | **HPMC/$TiO_2$+$Fe_2O_3$** | **HPMC/Rice Starch+D+$Fe_2O_3$** | **HPMC/Rice Starch+A+B+D+$Fe_2O_3$** | **HPMC/B+E+$Fe_2O_3$** |
| $T_0$ | Prasugrel Pattern A | Prasugrel Pattern A | Prasugrel Pattern A | Prasugrel Pattern A + additional peak at 21.7°2θ |
| **Storage Conditions - 50°C/30%RH** | | | | |
| 7 Days | Prasugrel Pattern A + additional peak at 25.3°2θ | Prasugrel Pattern A | Prasugrel Pattern A + additional peak at 20.0°2θ | Prasugrel Pattern A + additional peak at 37.8°2θ |
| 14 Days | Prasugrel Pattern A + additional peaks at 25.3 and 37.8°2θ | Prasugrel Pattern A | Prasugrel Pattern A | Prasugrel Pattern A + additional peak at 37.6°2θ |
| 21 Days | Prasugrel Pattern A + additional peak at 25.3°2θ | Prasugrel Pattern A + additional peak at 32.2°2θ | Prasugrel Pattern A | Prasugrel Pattern A + additional peak at 21.4°2θ |
| **Storage Conditions - 70°C/75%RH** | | | | |
| 7 Days | Prasugrel Pattern A + additional peak at 25.3°2θ | Prasugrel Pattern A + additional peak at 33.6°2θ and minus peaks at 13.5 and 25.5°2θ | Prasugrel Pattern A minus peak at 13.5 °2θ | Prasugrel Pattern A minus peaks at 13.5 and 25.5°2θ |
| 14 Days | Prasugrel Pattern A + additional peak at 25.3°2θ and minus peaks at 8.0, 13.5 and 25.5°2θ | Prasugrel Pattern A minus peaks at 8.0, 13.5 and 25.5°2θ | Prasugrel Pattern minus peaks at 8.0 and 13.5°2θ | Prasugrel Pattern A minus peaks at 8.0, 13.5 and 25.5°2θ |
| 21 Days | Prasugrel Pattern A + additional peak at 25.3°2θ and minus peaks at 8.0, 13.5 and 25.5°2θ | Prasugrel Pattern A + additional peak at 20.0°2θ and minus peaks at 8.0, 13.5 and 25.5°2θ | Prasugrel Pattern A + additional peak at 25.4°2θ and minus peaks at 8.0 and 13.5°2θ | Prasugrel Pattern A minus peaks at 8.0, 13.5 and 25.5°2θ |

## 51. Disintegration

Table 84, Table 85, Table 86 and Table 87 show the disintegration results for the coated nifedipine, olmesartan, rosuvastatin and prasugrel tablets on accelerated stability, respectively and provide detailed comments on each batch. These are summarized below:

Coated Nifedipine Batches

Overall, there was no significant change in disintegration times for the coated nifedipine batches stored at 50°C/30%RH and 70°C/75%RH for up to 21 days compared to $T_0$ except for ENQ3822/AIRT/004/01P1 whose $T_0$ sample had a prolonged disintegration time compared with the other batches. All batches except ENQ3822/AIRT/002/01P1 disintegrated within a slightly shorter time-scale than $T_0$ or those samples stored at 50°C/30%RH following storage at 70°C/75%RH.

In summary, the disintegration times of both the $TiO_2$ reference and the $TiO_2$-free reference batches were not adversely impacted by the accelerated stability storage.

Coated Olmesartan Batches

There was no significant change in disintegration times compared with $T_0$ for any of the batches either for samples stored at 50°C/30%RH or 70°C/75%RH. The disintegration times of both the $TiO_2$ reference and the $TiO_2$-free reference batches were not adversely impacted by the accelerated stability storage.

Coated Rosuvastatin Batches

In general, storage at 50°C/30%RH for up to 21 days did not impact significantly on disintegration time with either no change or a slight decrease compared with $T_0$ observed for all of the coated rosuvastatin batches.

Following storage at 70°C/75%RH, the disintegration times increased significantly for all batches except Batch ENQ3822/AIRT/014/01P1, whose disintegration times increased only slightly. The majority of the batches had disintegration times of > 15 min at the 7-day time-point and for some the disintegration time increased further at the 14-day and/or 21-day time-point. The disintegration times of ENQ3822/AIRT/018/01P1 samples increased to a lesser extent than those of the other batches, with all samples at 70°C/75% disintegrating within 9 to 11.5 min. Both Batch ENQ3822/AIRT/014/01P1 and ENQ3822/AIRT/018/01P1 contain rice starch as an opacifier and its presence may have facilitated disintegration. However, ENQ3822/AIRT/012/01P1 also contains rice starch but its disintegration times were approximately 18 min at the 7-day time-point and just over 25 min at the 21-day time-point, thus, indicating that disintegration times are affected by overall coating composition of the samples stored at 70°C/75%RH.

In summary, the disintegration times of both the $TiO_2$ reference and the $TiO_2$-free reference batches were not adversely impacted by storage at 50°C/30%RH for up to 21 days. However, this was not the case for the majority of batches stored at 70°C/75%RH including the two coated with the $TiO_2$ reference coatings.

Coated Prasugrel Batches

There was no significant change in disintegration times for the coated prasugrel batches stored at 50°C/30%RH for up to 21 days compared to $T_0$. In contrast, disintegration of all of the samples stored at 70°C/75%RH increased significantly from approximately 3 to 4 min at $T_0$ to between 14 to 16 min after 7 days storage at 70°C/75%RH increasing to between 17 min and 24 min at the 21-day time-point.

Table 84: Disintegration times for the coated nifedipine tablets on accelerated stability versus $T_0$

| Nifedipine 10 mg Retard Coated Tablets | | Disintegration (min:sec) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 001/01P1 | COAT-024 | 6:36 | 6:34 | 5:39 | 5:27 | 3:48 | 3:41 | 4:52 | The samples stored at 70°C/75%RH disintegrated slightly faster than $T_0$ and those stored at 50°C/30%RH. |
| 002/01P1 | COAT-001 | 7:11 | 6:29 | 5:44 | 5:50 | 5:26 | 6:08 | 7:49 | No significant change or trend in disintegration times compared with $T_0$. |
| 003/01P1 | COAT-033 | 7:12 | 6:25 | 6:30 | 5:47 | 2:24 | 2:54 | 2:37 | The samples stored at 70°C/75%RH disintegrated approx. 4 min faster than $T_0$ and those stored at 50°C/30%RH. |
| 004/01P1 | COAT-004 | 15:12 | 6:13 | 6:20 | 5:43 | 3:52 | 3:10 | 3:30 | The samples stored at 70°C/75%RH and 50°C/30%RH disintegrated considerably faster than $T_0$ and those stored at 70°C/75%RH disintegrated faster than those at 50°C/30%RH by approx. 2 to 3 min. |
| 005/01P1 | COAT-023 | 9:26 | 5:55 | 5:51 | 5:35 | 4:18 | 2:27 | 3:39 | The samples stored at 70°C/75%RH and 50°C/30%RH disintegrated considerably faster than $T_0$ and those stored at 70°C/75%RH disintegrated faster than those at 50°C/30%RH by approx. 2 to 3 min. |

Table 85: Disintegration times for the coated olmesartan tablets on accelerated stability versus $T_0$

| Olmesartan 20 mg Coated Tablets | | Disintegration (min:sec) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No: ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 006/01P1 | COAT-025 | 2:26 | 1:55 | 1:48 | 1:47 | 1:01 | 1:21 | 1:10 | No significant change in disintegration times compared with $T_0$ for any of the batches either for samples stored at 50°C/30%RH or 70°C/75%RH. |
| 007/01P1 | COAT-013 | 2:27 | 2:00 | 1:51 | 1:59 | 1:36 | 1:20 | 1:17 | |
| 008/01P1 | COAT-015 | 2:23 | 1:52 | 1:46 | 1:45 | 1:15 | 1:35 | 1:22 | |
| 009/01P1 | COAT-014 | 2:39 | 2:16 | 2:23 | 2:09 | 1:53 | 2:06 | 1:56 | |

Table 86: Disintegration times for the coated rosuvastatin tablets on accelerated stability versus $T_0$

| Rosuvastatin 10 mg Coated Tablets | | Disintegration (min:sec) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 010/01P1 | COAT-018 | 5:49 | 3:42 | 3:04 | 3:01 | 15:05 | 18:43 | 18:10 | For the two batches coated with the $TiO_2$ reference coats, there was a very slight decrease in disintegration times compared with $T_0$ for the samples stored at 50°C/30%RH. There was a significant increase in disintegration times for the samples stored at 70°C/75%RH. This increase was higher at 21 days than at 7 days for both batches. |
| 011/01P1 | COAT-017 | 6:50 | 3:37 | 3:44 | 3:48 | 17:27 | 16:47 | 19:14 | |
| 012/01P1 | COAT-020 | 5:33 | 4:06 | 4:05 | 4:32 | 18:04 | 21:54 | 25:05 | There was no significant change in the disintegration times of Batches ENQ3822/AIRT/012/01P1 and ENQ3822/AIRT/013/01P1 stored at 50°C/%RH compared with $T_0$. However, following storage at 70°C/75%RH, disintegration times increased significantly and for ENQ3822/AIRT/012/01P1, this prolongation increased with storage time. |
| 013/01P1 | COAT-019 | 6:33 | 6:27 | 7:06 | 6:28 | 20:36 | 22:14 | 21:50 | |
| 014/01P1 | COAT-010 | 4:29 | 2:32 | 2:30 | 2:28 | 6:23 | 5:43 | 6:00 | There was a slight decrease in disintegration times compared with $T_0$ for those samples stored at 50°C/30% RH and a slight increase for the samples stored at 70°C/75%RH. |
| 015/02P1 | COAT-030 | 9:28 | 7:06 | 7:59 | 7:44 | 19:49 | 23:03 | 23:58 | Disintegration times decreased slightly for Batches ENQ3822/AIRT/015/02P1 to ENQ3822/AIRT/019/01P1 compared to $T_0$ for the samples stored at 50°C/30%RH. However, those stored at 70°C/75%RH disintegrated significantly more slowly. The disintegration times of ENQ3822/AIRT/018/01P1 samples increased to a lesser extent than those from the other batches. For |
| 016/01P1 | COAT-005 | 7:15 | 4:30 | 4:12 | 4:41 | 21:19 | 31:58 | 28:15 | |
| 017/01P1 | COAT-001 | 6:07 | 3:35 | 3:29 | 3:57 | 18:38 | 27:08 | 24:35 | |
| 018/01P1 | COAT-034 | 5:22 | 2:40 | 2:52 | 2:40 | 9:22 | 11:17 | 9:42 | |
| 019/01P1 | COAT-023 | 5:22 | 3:10 | 3:35 | 3:26 | 16:16 | 26:58 | 25:02 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | the other batches there was also an increase in disintegration time at Day 14 compared with Day 7. |

Table 87: Disintegration times for the coated prasugrel tablets on accelerated stability versus $T_0$

| **Prasugrel 10 mg Coated Tablets** | | **Disintegration (min:sec)** | | | | | | | **Comments** |
|---|---|---|---|---|---|---|---|---|---|
| **Storage Condition** | | **NA** | **50°C/30%RH** | | | **70°C/75%RH** | | | |
| **Batch No: ENQ3822/AIRT/** | **Consortium Coat Ref** | **T=0** | **7 Days** | **14 Days** | **21 Days** | **7 Days** | **14 Days** | **21 Days** | |
| 020/01P1 | COAT-026 | 3:09 | 3:06 | 3:57 | 3:37 | 14:18 | 17:29 | 17:35 | At 50°C/30%RH there were no significant changes in disintegration times for Batches ENQ3822/AIRT/020/01P1 and ENQ3822/AIRT/021/01P1 and only a very minor increase in the time taken for the other two batches to disintegrate at the 21-day time-point compared to $T_0$. At 70°C/75%RH the disintegration times of all batches increased significantly at the 7-day time-point and were prolonged further as the storage time was extended to 14 days. The disintegration times of the $TiO_2$-free coated batches continued to increase up to the final time-point. |
| 021/01P1 | COAT-016 | 2:54 | 2:22 | 2:30 | 2:42 | 14:11 | 16:37 | 23:56 | |
| 022/01P1 | COAT-002 | 2:55 | 3:09 | 3:31 | 4:16 | 15:46 | 17:23 | 23:34 | |
| 023/01P1 | COAT-030 & COAT-031 | 3:49 | 4:09 | 4:14 | 5:29 | 15:54 | 17:32 | 23:53 | |

## 52. Assay and %Total Impurities

Coated Nifedipine Tablet Batches

Table 88 and Table 89 show respectively the average assay results and the %total impurities for the coated nifedipine tablet samples on accelerated stability versus $T_0$. Nifedipine is prone to photodegradation as shown in Section 0 but is relatively stable to heat and humidity as the results and literature data would suggest [17]. The average assay values did not change significantly at either of the accelerated stability conditions. However, a slight increase in impurities was observed for all batches except Batch ENQ3822/AIRT/004/01P1 following storage at 50°C/30%RH and for all batches following storage at 70°C/75%RH.

Coated Olmesartan Tablet Batches

Table 90 and Table 91 show respectively the average assay results and the %total impurities for the coated olmesartan tablet samples on accelerated stability versus $T_0$. Olmesartan is sensitive to moisture and this is evident from the results at 70°C/75%RH. Under the low humidity conditions of 50°C/30%RH, there was no significant change in the assay for any of the batches, while at 70°C/75%RH there was a major reduction in assay values which increased with storage time.

With respect to impurities, the results at 50°C/30%RH show that olmesartan degradation did occur under these conditions. This is especially evident from the results with Batch ENQ3822/AIRT/008/01P1 and ENQ3822/AIRT/009/01P1 coated with the $TiO_2$-free coats, COAT-015 and COAT-014 respectively. However, the impurity levels in Batch ENQ3822/AIRT/007/01P1, coated with $TiO_2$-free COAT-013, did not increase, while the $TiO_2$ reference coated batch, ENQ3822/AIRT/006/01P1, increased only slightly. Samples taken from Batches ENQ3822/AIRT/008/01 and ENQ3822/AIRT/009/01 appeared to have poorer coating quality than the other two lots (see Table 29) and this may have contributed to the higher levels of degradation observed, although differences in qualitative and quantitative composition between the coatings may also play a role.

High levels of degradation were observed in all samples stored at 70°C/75%RH which increased at each successive time-point. Batch ENQ3822/AIRT/006/01P1, coated with the $TiO_2$ reference coat, COAT-025, had the lowest level of degradants at 21 days under these storage conditions, whereas the batches coated with the $TiO_2$-free coating all had similarly high levels of degradation.

Based on the results at 70°C/75%RH, the rank order of coatings best able to protect the olmesartan from the effects of heat and humidity were the $TiO_2$ reference COAT-025 followed by the $TiO_2$-free coats, COAT-013, COAT-015 and COAT-014.

Table 88: Average assay values for the coated nifedipine batches on accelerated stability versus $T_0$

| Nifedipine 10 mg Retard Coated Tablets | | Average Assay (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 001/01P1 | COAT-024 | 99.2 | 98.3 | 98.2 | 98.1 | 97.9 | 97.9 | 97.6 | The average assay values did not change significantly for any of the batches stored at either 50°C/30%RH and 70°C/75%RH for up to 21 days. |
| 002/01P1 | COAT-001 | 98.4 | 98.7 | 98.5 | 98.6 | 98.4 | 98.3 | 99.0 | |
| 003/01P1 | COAT-033 | 98.9 | 98.7 | 99.1 | 98.7 | 98.5 | 97.7 | 98.4 | |
| 004/01P1 | COAT-004 | 98.4 | 99.2 | 99.0 | 99.6 | 99.2 | 98.9 | 98.6 | |
| 005/01P1 | COAT-023 | 98.0 | 98.7 | 98.1 | 98.0 | 98.4 | 97.2 | 97.8 | |

Table 89: %Total related impurities for the coated nifedipine batches on accelerated stability versus $T_0$

| Nifedipine 10 mg Retard Coated Tablets | | Total Impurities (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 001/01P1 | COAT-024 | 0.12 | 0.23 | 0.26 | 0.36 | 0.32 | 0.40 | 0.64 | At 50°C/30%RH there was a slight increase in the %total related impurities for all of the batches except Batch ENQ3822/AIRT/004/01P1. At 70°C/75%RH the increase was more pronounced and occurred with all batches. At 21 days Batch ENQ3822/AIRT/001/01P1 and ENQ3822/AIRT/005/01P1 had the highest related impurity levels which were stlll relatively low given the extreme conditions under which the samples had been stored. |
| 002/01P1 | COAT-001 | 0.15 | 0.21 | 0.35 | 0.20 | 0.25 | 0.36 | 0.39 | |
| 003/01P1 | COAT-033 | 0.13 | 0.19 | 0.22 | 0.23 | 0.25 | 0.33 | 0.33 | |
| 004/01P1 | COAT-004 | 0.13 | 0.15 | 0.17 | 0.13 | 0.26 | 0.24 | 0.33 | |
| 005/01P1 | COAT-023 | 0.12 | 0.23 | 0.31 | 0.28 | 0.52 | 0.69 | 0.67 | |

Table 90: Average assay values for the coated olmesartan batches on accelerated stability versus $T_0$

| Olmesartan 20 mg Coated Tablets | | Average Assay (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No: ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 006/01P1 | COAT-025 | 100.0 | 99.6 | 100.0 | 99.1 | 94.0 | 90.2 | 85.6 | The average assay values did not change significantly for any of the batches stored at 50°C/30%RH for up to 21 days. There was a progressive reduction in average assay values over the course of 21 days for all samples stored at 70°C/75%RH. The batch with the lowest average assay values at each time-point was Batch ENQ3822/AIRT/009/01P1. The average assay values for the other batches were similar to each other. |
| 007/01P1 | COAT-013 | 99.8 | 100.0 | 98.8 | 99.4 | 92.2 | 87.4 | 83.9 | |
| 008/01P1 | COAT-015 | 101.3 | 100.4 | 100.4 | 101.1 | 92.0 | 85.5 | 83.3 | |
| 009/01P1 | COAT-014 | 99.9 | 98.8 | 99.2 | 98.5 | 79.2 | 70.4 | 66.1 | |

Table 91: %Total related impurities for the coated olmesartan batches on accelerated stability versus $T_0$

| Olmesartan 20 mg Coated Tablets | | Total Impurities (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No: ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 006/01P1 | COAT-025 | 3.09 | 3.06 | 3.57 | 3.37 | 14.18 | 17.29 | 17.35 | Compared with $T_0$, there was a slight increase in %total related impurities in the samples stored at 50°C/30%RH at the 14-day and 21-day time-points Significant degradation was observed at all time-points in the samples stored at 70°C/75%RH with the values at 14 and 21 days being higher than that at 7 days. |
| 007/01P1 | COAT-013 | 2.54 | 2.22 | 2.30 | 2.42 | 14.11 | 16.37 | 23.56 | There was no increase in the %total related impurities in the samples stored at 50°C/30%RH. At 70°C/75%RH, the samples degraded significantly with the %total related impurities increasing at each time-point. |
| 008/01P1 | COAT-015 | 2.55 | 3.09 | 3.31 | 4.16 | 15.46 | 17.23 | 23.34 | For both batches there was a moderate increase in %total related impurities with time at 50°C/30%RH. At 70°C/75%RH degradation was significant and the %total related impurities increased with each time-point. |
| 009/01P1 | COAT-014 | 3.49 | 4.09 | 4.14 | 5.29 | 15.54 | 17.32 | 23.53 | |

Coated Rosuvastatin Tablet Batches

Table 92 and Table 93 show respectively the average assay results and the %total impurities for the coated rosuvastatin tablet samples on accelerated stability versus $T_0$. Rosuvastatin is sensitive to moisture and to acidic conditions [15]. The average assay results at 50°C/30%RH showed that there was no decrease in assay for any of the batches except for Batch ENQ3822/AIRT/013/01P1 coated with COAT-019 and Batch ENQ3822/AIRT/015/02P1 coated with COAT-030. The impurity results echo those of the assay with most batches showing a small gradual increase in %total impurities with time for the samples stored at 50°C/30%RH. In line with the assay results, the levels of degradants in Batch ENQ3822/AIRT/013/01P1 and Batch ENQ3822/AIRT/015/02P1 were significantly higher compared with $T_0$. Both of these coats contain an acidic component which may have contributed to the increased degradation observed under low humidity conditions compared to the other coatings.

At 70°C/75%RH all batches decreased in assay and had much higher levels of impurities compared with $T_0$. The best performing batch was ENQ3822/AIRT/016/01P1, coated with COAT-005, followed by ENQ3822/AIRT/017/01P1, coated with COAT-001. For these batches 2-3% drop in assay was only observed at the 14-day time-point for ENQ3822/AIRT/017/01P1 and at the 21-day time-point for ENQ3822/AIRT/016/01P1. These batches also had the lowest level of impurities at the 21-day time-point.

Again, Batch ENQ/AIRT/013/01P1 and Batch ENQ/AIRT/015/02P1 showed higher assay loss than the other rosuvastatin batches at all time-points and the level of impurities for the latter batch was much higher than the other rosuvastatin tablet lots.

The remaining batches showed a decrease in assay which was evident at the 7-day time-point and had decreased by around 9 to 11% at 21 days with levels of degradants which lay between 7% and 10% of label claim.

None of the coatings were able to protect the moisture-sensitive rosuvastatin completely at 70°C/75%. However, the $TiO_2$-free coats, COAT-001 and COAT-005, both of which contain magnesium opacifiers, gave the best results.

Coated Prasugrel Tablet Batches

Table 94 and Table 95 show respectively the average assay results and the %total impurities for the coated prasugrel tablet samples on accelerated stability versus $T_0$. Prasugrel is sensitive to alkaline conditions and moisture [16]. There was a gradual decrease in assay and increase in impurities with time for all samples stored at 50°C/30%RH. Taking both the assay and related impurity results into account, prasugrel degradation under these conditions does not appear to be significantly influenced by the coating used. Based on the 21-day time-point impurity results, use of COAT-002 gave marginally better results.

At 70°C/75%RH assay values fell below 1% for all batches at the 14-day time-point and, as a result, the related impurity testing was not carried out.

Table 92: Average assay values for the coated rosuvastatin batches on accelerated stability versus $T_0$

| Rosuvastatin Coated Tablets | | Average Assay (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 010/01P1 | COAT-018 | 102.5 | 103.0 | 102.3 | 102.7 | 98.5 | 97.2 | 91.8 | The average assay values for the two batches coated with the $TiO_2$ reference coatings and the $TiO_2$-free coated batch, ENQ3822/AIRT/012/01P1, followed a similar pattern. At 50°C/30%RH there was no significant change in average assay values over time. At 70°C/75%RH there was a steady decrease in assay with each time-point to reach 92 to 93% of label claim at 21 days. |
| 011/01P1 | COAT-017 | 101.8 | 102.1 | 102.5 | 100.5 | 98.2 | 96.6 | 92.1 | |
| 012/01P1 | COAT-020 | 102.1 | 102.5 | 102.3 | 104.2 | 100.0 | 96.5 | 92.9 | |
| 013/01P1 | COAT-019 | 102.2 | 100.0 | 101.0 | 97.2 | 86.5 | 81.7 | 75.9 | For the samples stored at 50°C/30%RH, a decrease in average assay values was first observed at 21 days. At 70°C/75%RH there was a large reduction in average assay at 7 days which decreased further with each successive time-point. |
| 014/01P1 | COAT-010 | 103.4 | 104.1 | 101.4 | 101.0 | 98.6 | 94.2 | 92.3 | At 50°C/30%RH there was no significant change in average assay values over time. At 70°C/75%RH there was a steady decrease in assay with each time-point to reach 92% of label claim at 21 days. |
| 015/02P1 | COAT-030 | 101.8 | 99.9 | 98.4 | 97.0 | 86.5 | 81.6 | 75.6 | For the samples stored at 50°C/30%RH, there was a slight progressive decrease in average assay values over time. At 70°C/75%RH there was a large reduction in average assay at 7 days which decreased further with each successive time-point. |
| 016/01P1 | COAT-005 | 99.6 | 101.3 | 101.9 | 100.8 | 102.0 | 100.0 | 97.8 | For Batches ENQ3822/AIRT/016/01P1 and ENQ3822/AIRT/017/01P1, there was no decrease in average assay values at 50°C/30%RH over 21 days. At 70°C/75%RH the average assay values first decreased at the 14-day time-point for ENQ3822/AIRT/017/01P1 and at the 21-day time- |
| 017/01P1 | COAT-001 | 100.8 | 101.5 | 103.2 | 101.7 | 101.6 | 97.8 | 97.8 | |

| Rosuvastatin Coated Tablets | | Average Assay (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| | | | | | | | | | point for ENQ3822/AIRT/016/01P1. |
| 018/01P1 | COAT-034 | 101.9 | 101.2 | 100.8 | 100.9 | 96.1 | 93.5 | 90.8 | At 50°C/30%RH there was no significant change in average assay values over time for these two batches. At 70°C/75%RH there was a steady decrease in assay with each time-point to reach approx. 91% of label claim at 21 days. |
| 019/01P1 | COAT-023 | 101.8 | 100.0 | 99.0 | 101.3 | 98.2 | 94.0 | 91.3 | |

Table 93: %Total related impurities for the coated rosuvastatin batches on accelerated stability versus $T_0$

| Rosuvastatin Coated Tablets | | Total Impurities (% LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 010/01P1 | COAT-018 | 0.64 | 0.72 | 0.76 | 0.79 | 3.83 | 6.17 | 8.40 | The %total related impurities for the batches coated with the $TiO_2$ reference coatings and the $TiO_2$-free coated batch, ENQ3822/AIRT/012/01P1, followed a similar trend for the samples stored under both accelerated stability conditions. Following storage at 50°C/30%RH there was a slight gradual increase in impurities with time. At 70°C/75%RH there was significant degradation at the 7-day time-point which increased 14 days and t21 days. |
| 011/01P1 | COAT-017 | 0.64 | 0.74 | 0.77 | 0.87 | 3.80 | 6.09 | 8.16 | |
| 012/01P1 | COAT-020 | 0.63 | 0.74 | 0.77 | 0.85 | 3.96 | 6.44 | 8.65 | |
| 013/01P1 | COAT-019 | 0.67 | 1.04 | 1.28 | 1.46 | 3.43 | 5.32 | 7.13 | Following storage at 50°C/30%RH, there was a moderate increase in %total related impurities with time. Degradation in the samples stored at 70°C/75%RH was higher and increased at each successive time-point. |
| 014/01P1 | COAT-010 | 0.62 | 0.69 | 0.76 | 0.83 | 3.81 | 6.21 | 8.67 | Following storage at 50°C/30%RH there was a slight gradual increase in impurities with time. At |

| Rosuvastatin Coated Tablets | | Total Impurities (% LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No. ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| | | | | | | | | | 70°C/75%RH there was significant degradation at the 7-day time-point which then increased at 14 days and then again at 21 days. |
| 015/02P1 | COAT-030 | 0.84 | 2.49 | 3.95 | 5.89 | 14.30 | 19.53 | 22.91 | High levels of related impurities occurred in samples stored under both accelerated stability conditions, with significantly increased quantities found in the 70°C/75%RH samples. For both stability conditions, the level of degradants increased as the study progressed. |
| 016/01P1 | COAT-005 | 0.59 | 0.64 | 0.65 | 0.74 | 1.49 | 2.84 | 4.39 | Following storage at 50°C/30%RH there was a slight gradual increase in impurities with time. At 70°C/75%RH there was significant degradation at the 7-day time-point which increased following exposure for 14 days and then again at 21 days. |
| 017/01P1 | COAT-001 | 0.60 | 0.70 | 0.74 | 0.82 | 2.34 | 3.65 | 5.36 | |
| 018/01P1 | COAT-034 | 0.63 | 0.74 | 0.77 | 0.89 | 4.80 | 7.45 | 9.91 | |
| 019/01P1 | COAT-023 | 0.63 | 0.73 | 0.77 | 0.88 | 3.26 | 5.52 | 7.37 | |

Table 94: Average assay values for the coated prasugrel batches on accelerated stability versus $T_0$

| Prasugrel 10 mg Coated Tablets | | Average Assay (%LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No: ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 020/01P1 | COAT-026 | 99.5 | 96.9 | 96.0 | 94.4 | 7.1 | 0.9 | N/A | The average assay results decreased at the 7-day time-point at 50°C/30%RH for all batches by approximately 2% to 3%. This decrease continued at the two later time-points to reach around 92 to 94% at 21 days. At 70°C/75%RH the average assay values plummeted to 5 to 7% at the 7-day time-point, with assay loss almost complete at the 14-day time-point. |
| 021/01P1 | COAT-016 | 99.6 | 96.7 | 94.9 | 94.5 | 5.4 | 0.1 | N/A | |
| 022/01P1 | COAT-002 | 98.8 | 96.8 | 95.1 | 94.2 | 6.5 | 0.3 | N/A | |
| 023/01P1 | COAT-030 & COAT-031 | 98.6 | 96.5 | 94.3 | 92.3 | 7.5 | 0.7 | N/A | |

Table 95: %Total related impurities for the coated prasugrel batches on accelerated stability versus $T_0$

| Prasugrel 10 mg Coated Tablets | | Total Impurities (% LC) | | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Storage Condition | | NA | 50°C/30%RH | | | 70°C/75%RH | | | |
| Batch No: ENQ3822/AIRT/ | Consortium Coat Ref | T=0 | 7 Days | 14 Days | 21 Days | 7 Days | 14 Days | 21 Days | |
| 020/01P1 | COAT-026 | 0.7 | 1.6 | 2.0 | 2.5 | Due to the severe degradation observed during the assay determinations, the %total related impurities were not determined for these samples. | | | The %total related impurities increased gradually with time in all batches after being subjected to 50°C/30%RH storage. The impurity levels were similar for all batches. |
| 021/01P1 | COAT-016 | 0.9 | 1.8 | 2.3 | 2.6 | | | | |
| 022/01P1 | COAT-002 | 0.7 | 1.3 | 1.7 | 2.1 | | | | |
| 023/01P1 | COAT-030 & COAT-031 | 0.6 | 1.5 | 2.1 | 2.5 | | | | |

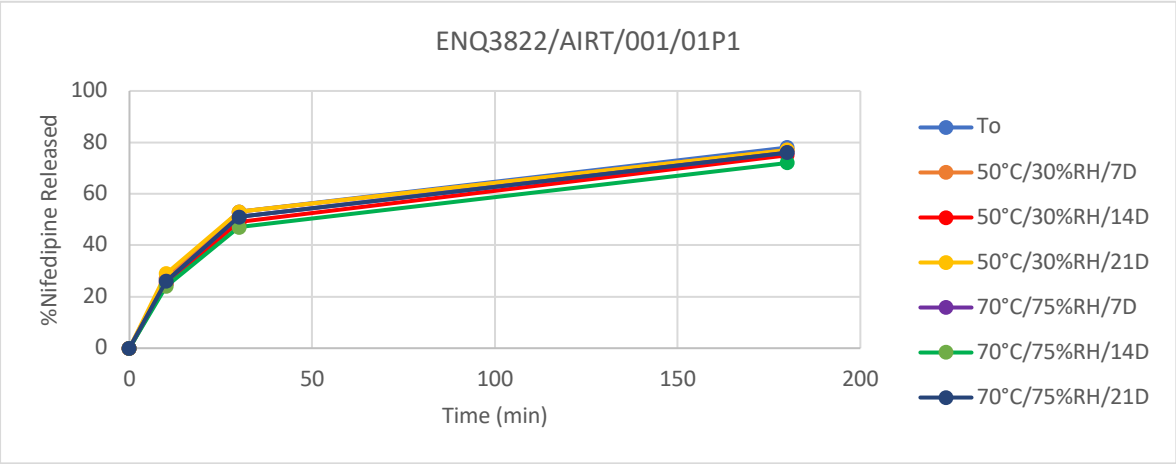
## 53. Dissolution

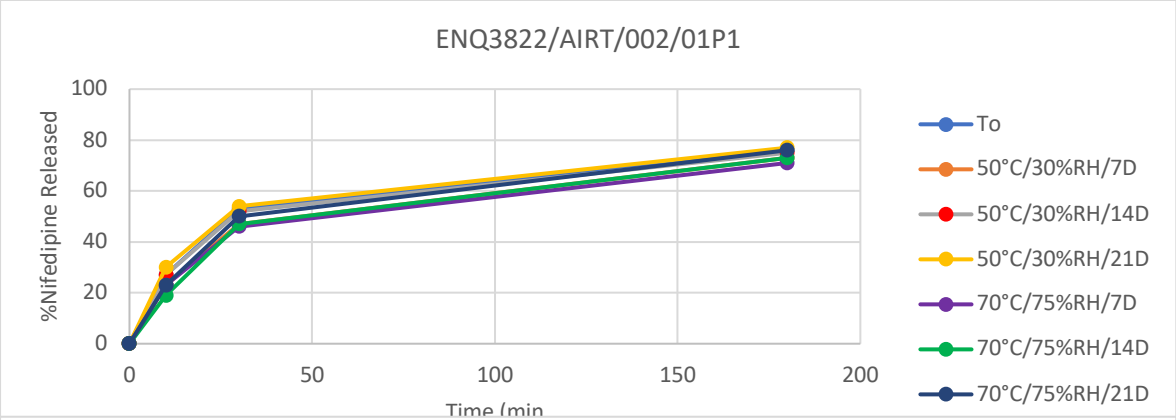
Nifedipine Coated Batches

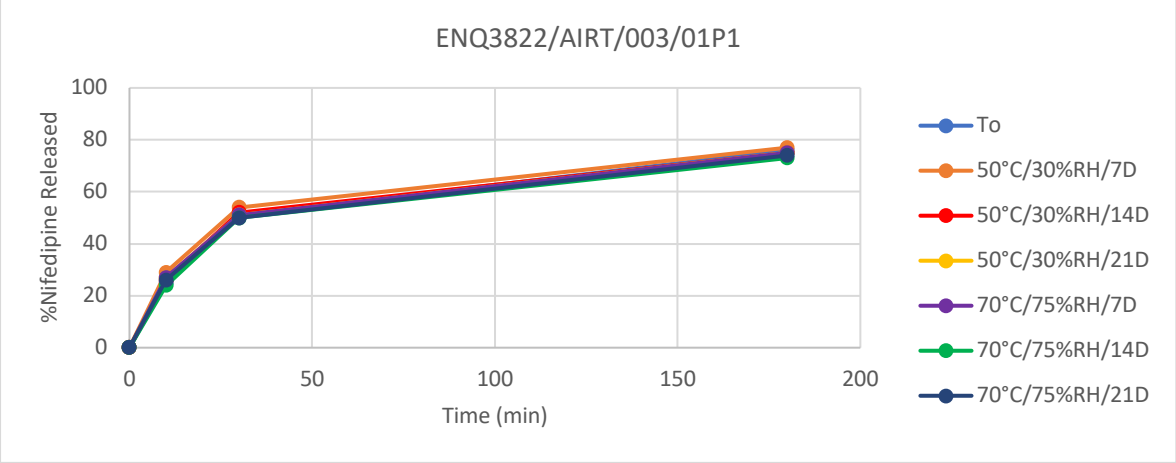
Figure 42 and Figure 43 show the dissolution data for nifedipine from the coated nifedipine batches. The graphical data for the batches from Runs 11 to 13 are shown below and overleaf for the batches from Runs 14 and 15.

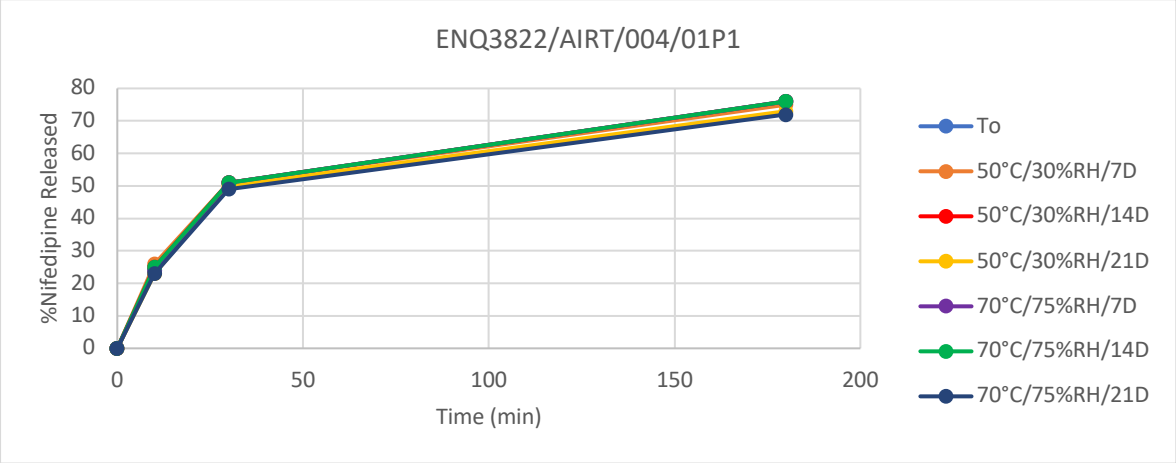
The data show that for all batches storage under either 50°C/30%RH or 70°C/75%RH did not impact on the dissolution profile or the total amount released and that any variations were minor and no trends were observed.

Figure 42: %Nifedipine released from the coated nifedipine batches on accelerated stability versus $T_0$




ENQ3822/AIRT/004/01P1
%Nifedipine Released
80
70
60
50
40
30
20
10
0
0
50
100
150
200
Time (min)
To
50°C/30%RH/7D
50°C/30%RH/14D
50°C/30%RH/21D
70°C/75%RH/7D
70°C/75%RH/14D
70°C/75%RH/21D

Olmesartan Coated Batches

Table 96 shows the dissolution data for olmesartan from the coated olmesartan tablet batches on accelerated stability versus $T_0$.

Table 96: %Olmesartan released from the coated olmesartan batches on accelerated stability versus $T_0$

| **Storage Condition** | | **NA** | **50°C/30%RH** | | | **70°C/75%RH** | | |
|---|---|---|---|---|---|---|---|---|
| **Batch No. ENQ3822/AIRT/** | **Diss Time (min(** | **$T_0$** | **7 D** | **14 D** | **21 D** | **7 D** | **14 D** | **21 D** |
| 006/01P1 | 0 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| COAT-025 | 15 | 93% | 92% | 92% | 91% | 83% | 73% | 68% |
| PVA/$TiO_2$+Talc+ $Fe_2O_3$ | | | | | | | | |
| 007/01P1 | 0 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| COAT-013 | 15 | 94% | 91% | 93% | 94% | 81% | 74% | 72% |
| PVA+HPMC/ $CaCO_3$+Talc+ $Fe_2O_3$ | | | | | | | | |
| 008/01P1 | 0 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| COAT-015 | 15 | 93% | 93% | 94% | 92% | 89% | 82% | 65% |
| PVA/$CaCO_3$+ Talc+$Fe_2O_3$ | | | | | | | | |
| 009/01P1 | 0 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| COAT-014 | 15 | 93% | 88% | 90% | 87% | 88% | 83% | 79% |
| PVA/$CaCO_3$+ Talc+$Fe_2O_3$ | | | | | | | | |

The data show that the amount released after 15 min from all samples stored at 50°C/30%RH lies close to the $T_0$ value. However, as would be expected based on the assay values (see Table 90), the %released from all batches stored at 70°C/75%RH was lower than $T_0$ and decreased at each time-point.

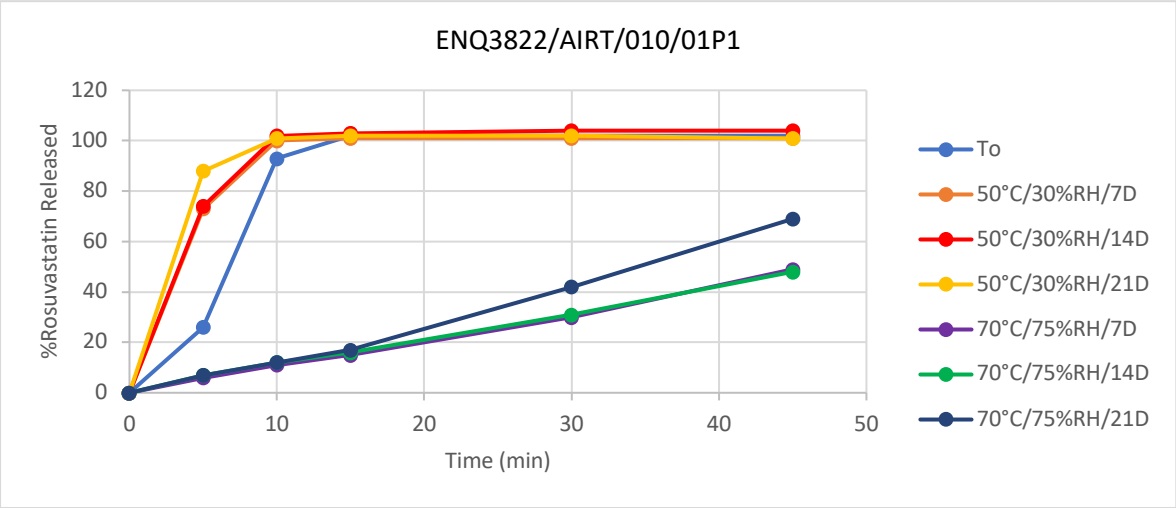
Rosuvastatin Coated Batches

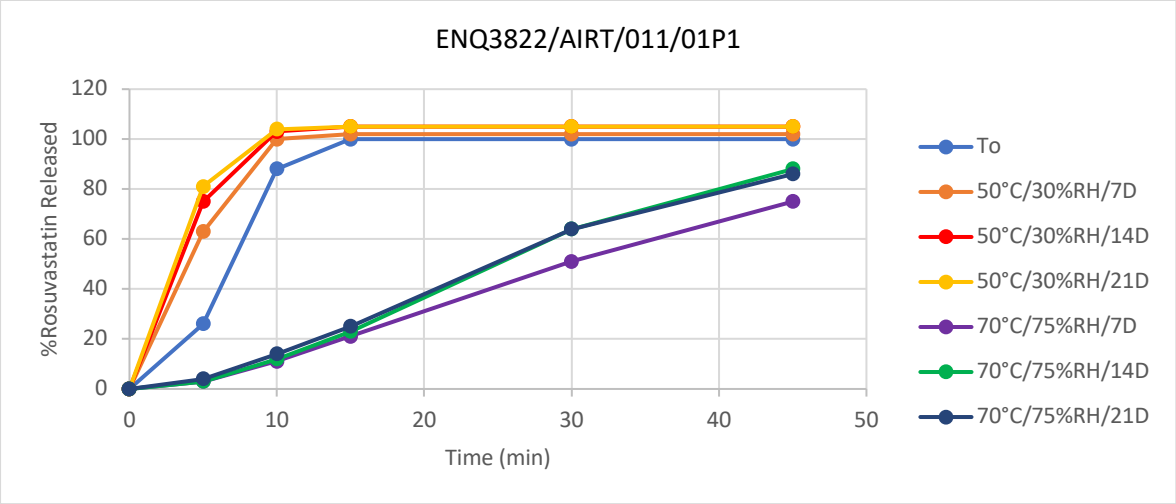
Figure 43 show the dissolution data for the coated rosuvastatin batches that were coated with the $TiO_2$ containing references coatings, COAT-017 and COAT-018, and placed on accelerated stability versus $T_0$.

For the samples of both batches stored at 50°C/30%RH, release at the 5-min time-point was much faster than for $T_0$ but otherwise the profiles shape and %recovery at the end of the dissolution test were similar. Therefore, storage under these accelerated conditions had not significantly impacted dissolution from the samples stored up to 21 days.

For the samples stored at 70°C/75%RH from both batches, there was both a change in the rate of release and the %recovery at the end of the 45-min period. For these samples release occurred in an almost linear manner over the 45-min dissolution test. Their profiles are very different from the rapid release over 10 min seen with $T_0$ and the samples stored at 50°C/30%RH. These changes in dissolution rate are likely to be at least in part due to the increase in disintegration times observed following the tablets' exposure to the high temperature/high humidity conditions (see Table 86).

Figure 43: %rosuvastatin released from the coated rosuvastatin batches on accelerated stability versus $T_0$ (Run 20 and Run 21)



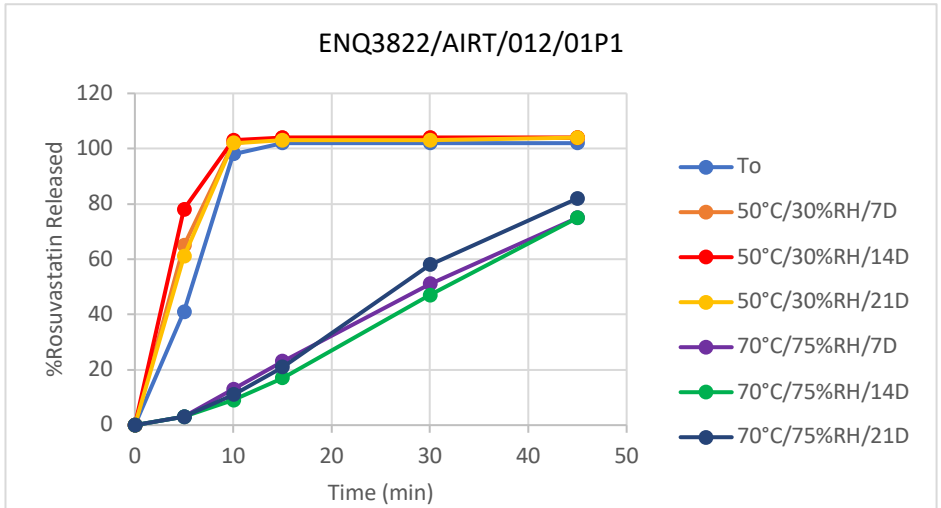



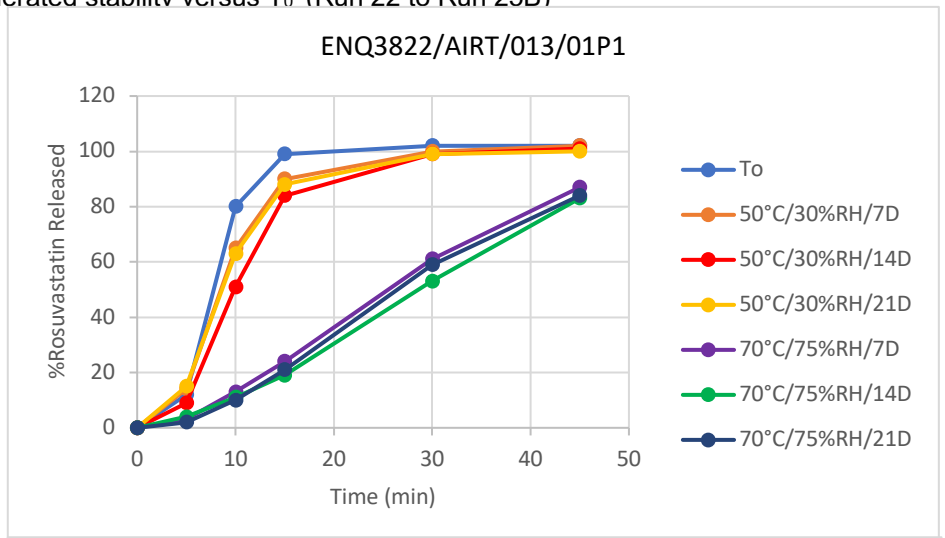

for release. However, only the %released by the 14 and 21-day samples of Batch ENQ3822/AIRT/011/01P1 came close to this value at 45 min. The %recoveries at the end of the dissolution test for Batch ENQ3822/AIRT/010/01P1 samples were lower still especially for the 7- and 14-day samples. This is likely due to their slower release rate.

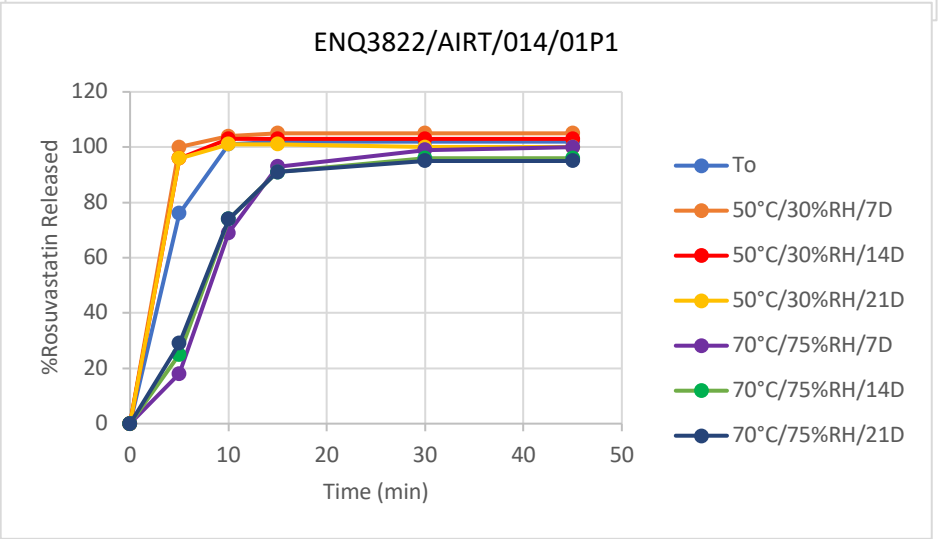
In summary, there was a change in dissolution profile and %recovery at 45 min for both $TiO_2$ reference coated batches stored at 70°C/75%RH. This could be attributed at least in part to their increased disintegration times. There was also no trend with increasing exposure time, with, in both cases, the 21-day sample releasing faster and to a greater extent than the 7-day samples.

Figure 44 and Figure 45 show the dissolution data for the coated rosuvastatin batches that were coated with the $TiO_2$-free coatings and placed on accelerated stability versus $T_0$. For the samples stored at 50°C/30%RH, the dissolution profiles were similar to the corresponding $T_0$ sample. All of the 50°C/30%RH samples for Batches ENQ/AIRT/012/01P1, ENQ3822/AIRT/014/01P1, ENQ3822/AIRT/016/01P1, ENQ/AIRT/017/01P1, ENQ3822/AIRT/018/01P1 and ENQ/AIRT/019/01P1 released the majority of the API slightly faster than the $T_0$ samples. The faster release was particularly noticeable at the 5-min time-point. This corresponded with the slightly faster disintegration times noted for these batches following 50°C/30%RH storage (see Table 86).

Figure 44: %rosuvastatin released from the coated rosuvastatin batches on accelerated stability versus $T_0$ (Run 22 to Run 25B)



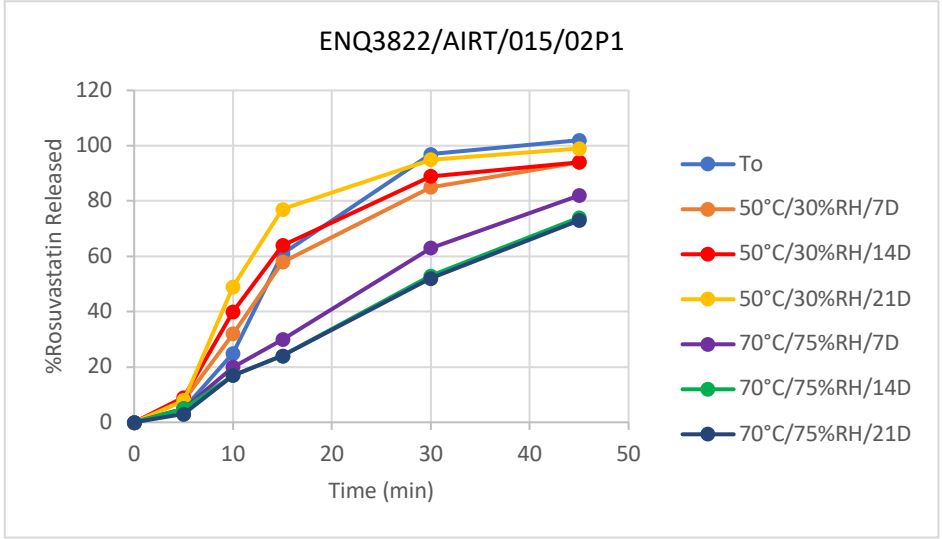
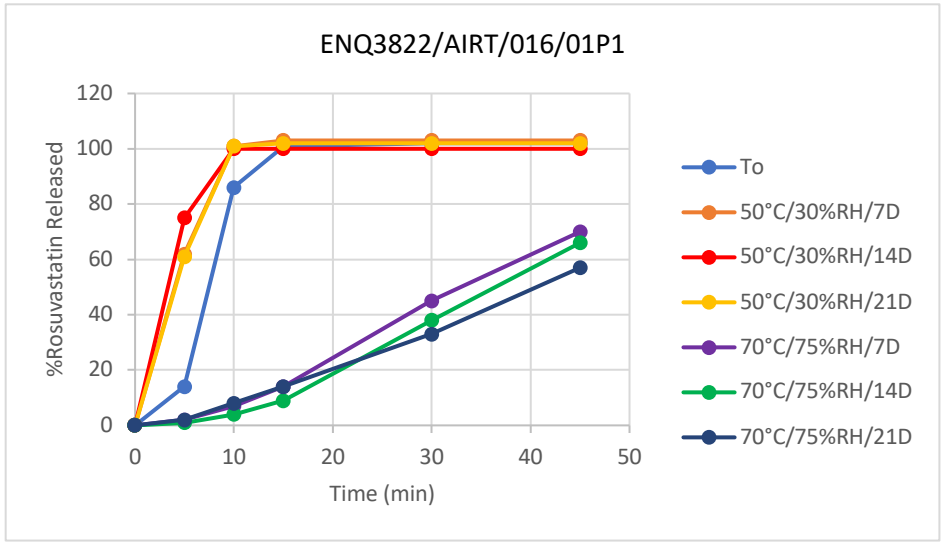
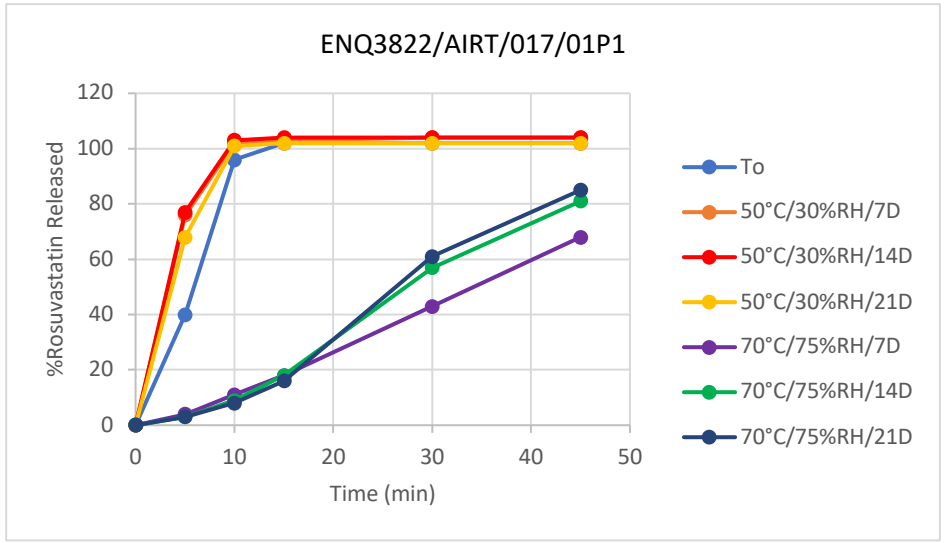
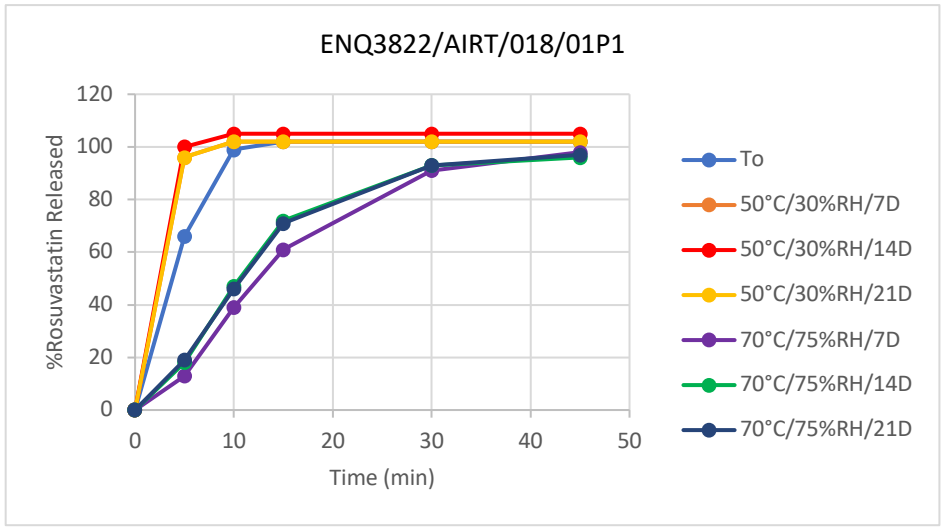
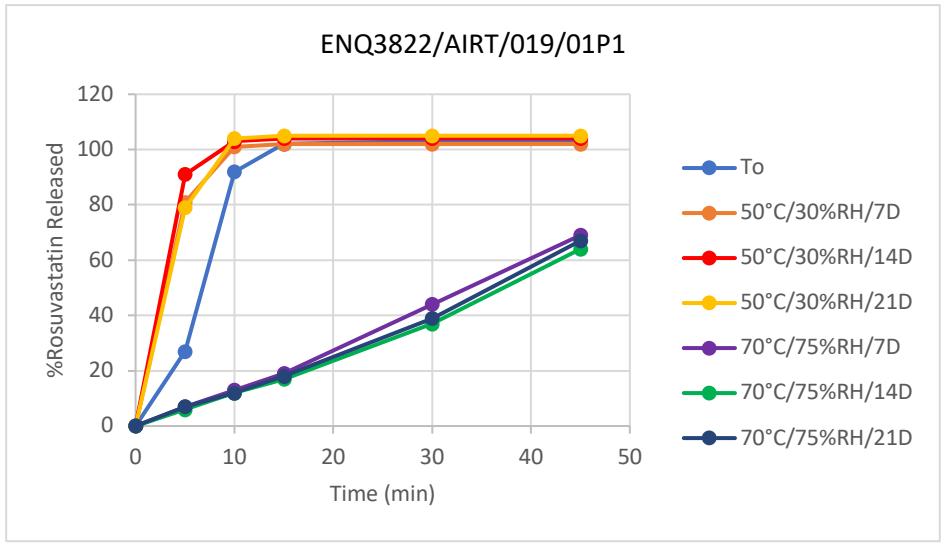



Figure 45: %rosuvastatin released from the coated rosuvastatin batches on accelerated stability versus $T_0$ (Run 26 to Run 29)



The samples from Batch ENQ3822/AIRT/015/02P1 had a slightly prolonged profile similar to the $T_0$ profile but different to the other lots, while the samples from Batch ENQ3822/AIRT/013/01P1 released slightly more slowly than the $T_0$ sample with release complete in 30 min as opposed to 15 min.

Overall, exposure to 50°C/30%RH for up to 21 days had little impact on the dissolution profiles of the $TiO_2$-free coated batches.

The same could not be said of storage at 70°C/75%RH. With the exception of Batches ENQ/AIRT/014/01P1 and ENQ/AIRT/018/01P1, storage under these conditions prolonged disintegration to over 16 minutes at the 7-day time-point and over 20 min at the 21-day time-point for all $TiO_2$-free coated batches (see Table 86). In addition, assay values had decreased for the majority of the stability samples but particularly for those from Batch ENQ3822/AIRT/015/02P1 and Batch ENQ3822/AIRT/013/01P1. These changes impacted on the dissolution profiles and %recovery at the end of the dissolution tests.

The stability samples from Batch ENQ/AIRT/014/01P1, whose disintegration times were only slightly longer than $T_0$, released more slowly than their 50°C/30%RH counterparts or the $T_0$ sample. However, release was complete within 30 min as opposed to 15 min with 95% or more API recovered. For Batch ENQ/AIRT/018/01P1 samples, whose disintegration times were around 5 to 6 mins slower than $T_0$, rosuvastatin release was slower but still complete at 45 min with over 95% recovery.

For the other batches whose samples displayed disintegration times above 16 min, the dissolution profile up to 45 min was almost linear and recovery ranged from 57% for the 21-day sample from Batch ENQ/AIRT/016/01P1 to 87% for the 7-day sample for Batch ENQ/AIRT/013/01P1. Batch ENQ/AIRT/016/01P1 also had the longest disintegration times of all of the 70°C/75%RH stability samples at all time-points.

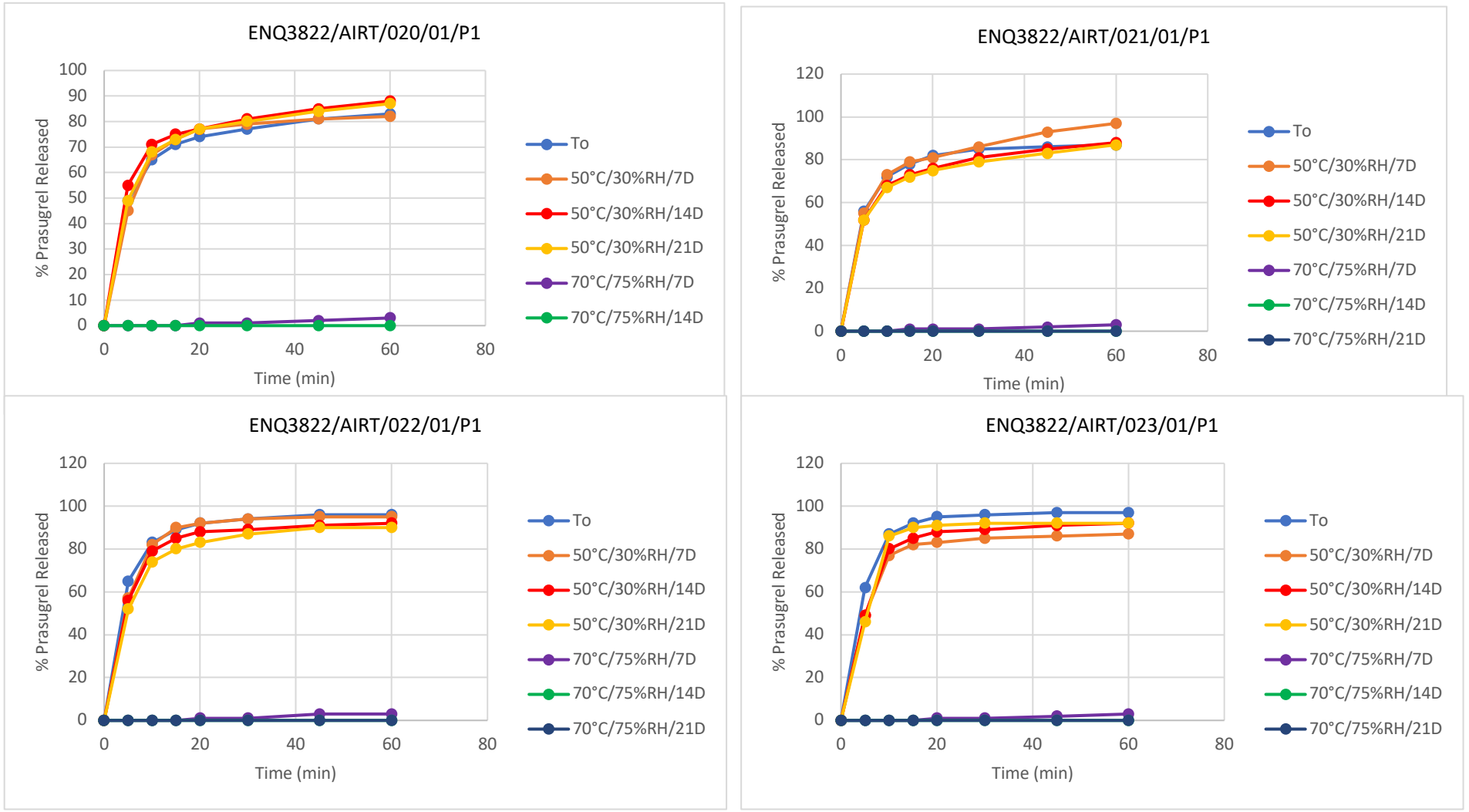
These results show that changes in the disintegration times are the driver for changes in dissolution profile, although loss of assay due to degradation will contribute to some extent.

Prasugrel Coated Tablets

Figure 46 shows the dissolution data for the coated prasugrel batches that were placed on accelerated stability versus $T_0$.

The data show that storage at 50°C/30% RH had minimal effect on prasugrel dissolution at all stability time-points as would be expected given the relatively small impact it had on the assay, related impurity and disintegration results. At 70°C/75%RH almost all of the prasugrel was severely degraded regardless of the coating used and hence the dissolution values were close to zero.

Figure 46: %prasugrel released from the coated prasugrel batches on accelerated stability versus $T_0$



## Section Summary and Conclusions

Coated Nifedipine Batches

Nifedipine is relatively stable to heat and humidity. Therefore, it is not surprising that following storage at 50°C/30%RH there was little change in the visual appearance, assay and impurity levels for all of the nifedipine coated batches. However, at 70°C/75%RH the appearance of the tablets was affected, particularly for the batch coated with COAT-004 which turned orange. There was also a slight increase in related impurities, although the assay levels remained similar. Disintegration and dissolution were not significantly affected by storage under the accelerated conditions for 21 days.

Coated Olmesartan Batches

Olmesartan is sensitive to moisture. At 50°C/30%RH the batch coated with COAT-013 darkened slightly at the 21-day time-point based on visual appearance data, while the $TiO_2$ reference coated batch and the other two $TiO_2$-free coated batches did not. At 70°C/75%RH only the batch coated with the $TiO_2$ reference coat did not change color based on both visual and colorimetry data.

The disintegration times of both the $TiO_2$ reference and the $TiO_2$-free reference batches were not adversely impacted by the accelerated stability storage. Following storage at 50°C/30%RH there was no significant change in assay values but there were small increases in related impurities. However, at 70°C/75%RH, the moisture-sensitive olmesartan degraded significantly in all batches and the level of impurities increased at each successive time-point. Batch ENQ3822/AIRT/006/01P1, coated with the $TiO_2$ reference coat, COAT-025, had the lowest level of degradants at 21 days under these storage conditions, whereas the batches coated with the $TiO_2$-free coatings all had similarly high levels of degradation.

Based on the results at 70°C/75%RH, the rank order of coatings best able to protect the olmesartan from the effects of heat and humidity were the $TiO_2$ reference COAT-025 followed by the $TiO_2$-free coats, COAT-013, COAT-015 and COAT-014.

Coated Rosuvastatin Batches

The batch coated with the HPMC-based $TiO_2$ reference coat, COAT-017, did not change visibly change in appearance either at 50°C/30%RH or 70°C/75%RH, while for the PVA-based $TiO_2$ reference coating, a change was only visible at 70°C/75%RH at the 21-day time-point.

The $TiO_2$-free coated batches remained unchanged in appearance following storage at 50°C/30%RH. However, at 70°C/75%RH the color of all the $TiO_2$-free coated batches altered. This color change varied between the batches both in terms of the time-point at which it first became perceptible, and its nature and intensity with some batches turning off-white, while others yellowed.

At 50°C/30%RH there was no significant change from $T_0$ in the assay, impurity, disintegration and dissolution results regardless of the type of coating used except for the batches coated with COAT-019 and COAT-030 which had reduced assay and increased impurity levels. Both of these coats contain an acidic component which may have contributed to the increased degradation of the acid-sensitive rosuvastatin observed under low humidity conditions compared to the other coatings.

At 70°C/75%RH all batches decreased in assay and had much higher levels of impurities compared with $T_0$. The best performing batch with respect to assay and related impurity levels was the batch coated with COAT-005, followed by the one coated with COAT-001. COAT-001 and COAT-005 both contain magnesium opacifiers.

Disintegration and dissolution from the coated rosuvastatin batches stored at 50°C/30%RH did not change significantly. However, following storage at 70°C/75%RH there was a significant increase in disintegration times, and changes in dissolution profile and %recovery at 45 min for both $TiO_2$ reference coated batches and the majority of the $TiO_2$-free coated batches. This could be attributed at least in part to the increased disintegration times as the batches coated with COAT-010 and COAT-034, whose disintegration times increased to a lesser extent, had similar, if somewhat slower dissolution profiles, to the corresponding $T_0$ samples. The %recovery at the 45-min time-point was also higher for these

batches. COAT-010 and COAT-034, both contain rice starch which may have facilitated the disintegration of the tablets.

Coated Prasugrel Batches

Prasugrel is sensitive to alkaline conditions and moisture. The coated prasugrel batches did not change in appearance following storage at 50°C/30%RH. There was a gradual decrease in assay and increase in impurities with time for all samples stored at 50°C/30%RH. Taking both the assay and related impurity results into account, prasugrel degradation under these conditions did not appear to be significantly influenced by the coating used. Based on the 21-day time-point impurity results, use of COAT-002 gave marginally better results.

However, at 70°C/75%RH an appearance change occurred in all batches, disintegration times increased significantly and assay values were < 1% by the 14-day time-point showing that prasugrel had degraded in all of the samples following storage at this condition.

# Extended Friability Results

## Introduction

Based on the results of the small-scale studies at a 3 kg batch size, several of the $TiO_2$-free coatings were taken forward to further studies at a 5 kg scale in which the impact of processing parameters was evaluated. The coating machine used was an O'Hara LabCoat MX Coater fitted with a 15-inch pan. The ability of the selected $TiO_2$-free and reference coating materials to coat yellow, round placebo cores (Batch ENQ3822/PIRT/001/01) was assessed under conditions of low tablet bed/exhaust temperature and high spray rate (wet conditions) and high tablet bed/exhaust temperature and low spray rate (dry conditions). The manufacture of the placebo batch is described in Section 0. In addition, to the various tests previously carried out on the coated placebo tablets at a 3 kg scale (see Section 12), these tablets were also subjected to extended friability testing. Friability was performed over an extended period of time in order to evaluate differences between the tablets coated with $TiO_2$-free coats and the $TiO_2$ reference coated tablets. This test is often carried out during development studies to evaluate the mechanical integrity of different tablet formulations and assess coat adhesion strength to indicate if there could be risks with downstream processes such as packaging and transportation.

Analytical testing of the batches from these studies is ongoing. However, the results of the extended friability studies are available for all batches manufactured under "dry conditions" and for certain batches manufactured under "wet conditions". These are reported as extended friability testing was not carried out on the batches at the 3 kg scale.

## Materials and Method

Extended friability testing was carried out on 20 to 21 tablets from each batch using a Copley FRV2000 friability tester rotating at 30 rpm. The tablet checks were performed after 1, 2, 3 and, for a limited number of batches, after 4 hours. The 4-hour assessment was only performed on the two $TiO_2$ containing reference coated batches and one $TiO_2$-free coated tablet batch that did not show any signs of damage after 3 hours. The tablets were weighed before starting the test and at the end of the test. Before the final weighing the tablets were dedusted with a brush. The friability of the tablet batches was calculated at the end of the test i.e. 3 hours for the majority of batches and at 4 hours for those batches tested for the longer time-period.

The acceptance criterion was ≤1 tablet damaged per sample (approx. 5% of sample).

## Results and Discussion

Table 97 provides a summary of the number of damaged tablets observed at each time-point and Table 98 the visual appearance descriptions and the friability results at the end of testing. Figure 47, Figure 48 and Figure 49 shows photographs of tablets coated with the $TiO_2$ reference coatings, the best performing $TiO_2$-free coating and three poorly performing $TiO_2$-free coatings in the extended friability test.

Extended friability testing proved discriminatory between the batches and coatings in that there was a clear rank order in the level of tablet damage at the different time-points. Only the following batches were subjected to testing for 4 hours instead of 3:

- Batches ENQ3822/PIRT/014/01 and ENQ3822/PIRT/014/02 coated with the HPMC-based $TiO_2$ reference coat, COAT-017 under "dry" and "wet" conditions
- ENQ3822/PIRT/020/01, coated with the PVA-based $TiO_2$ reference, COAT-018, under "dry" conditions
- ENQ3822/PIRT/022/01 coated with the $TiO_2$-free HPMC-based coat, COAT-034, under "dry" conditions

Table 97: No of damaged tablets at each time-point.

| Batch No. ENQ3822/PIRT/ | Wet or Dry[a] | Consort Coat Ref | Film Former/ Opacifier | No. Tabs Tested | No. of Tablets Damaged | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| 014/01 | Dry | COAT-017 | HPMC/ $TiO_2$ | 20 | 0 | 0 | 0 | 0 |
| 014/02 | Wet | | | 21 | 0 | 0 | 0 | 0 |
| 015/01 | Dry | COAT-006 | HPMC/ $CaCO_3$+D | 21 | 1 | 12 | 14 | - |
| 015/02 | Wet | | | 21 | 7 | 19 | 20 | - |
| 016/01 | Dry | COAT-010 | HPMC/ Rice Starch +D | 21 | 1 | 7 | 17 | - |
| 016/02) | Wet | | | 21 | 0 | 1 | 15 | - |
| 017/01 | Dry | COAT-023 | PVA/ F+Talc | 21 | 0 | 0 | 5 | - |
| 017/02 | Wet | | | 20 | 0 | 1 | 8 | - |
| 018/01 | Dry | COAT-032 | HPMC/ $CaCO_3$+H | 21 | 3 | 20 | 21 | - |
| 018/01 | Wet | | | 21 | 3 | 18 | 21 | - |
| 019/01 | Dry | COAT-002 | HPMC/ Rice Starch+ A+B+D+$Fe_2O_3$ | 21 | 0 | 7 | 19 | - |
| 019/02 | Wet | | | 21 | 0 | 0 | 9 | - |
| 020/01 | Dry | COAT-018 | PVA/ $TiO_2$+Talc | 21 | 0 | 0 | 7 | 19 |
| 021/01 | Dry | COAT-027 | HPMC/ $CaCO_3$+D | 21 | 2 | 18 | 20 | - |
| 022/01 | Dry | COAT-034 | HPMC/Rice Starch | 21 | 0 | 0 | 0 | 7 |
| 023/01 | Dry | COAT-030 | HPMC/B+E | 21 | 21 | 21 | 21 | - |
| 024/01 | Dry | COAT-013 | PVA+HPMC $CaCO_3$+Talc+$Fe_2O_3$ | 21 | 0 | 1 | 3 | - |

[a]Wet conditions = low tablet bed/exhaust temperature and high spray rate

[a]Dry conditions = high tablet bed/exhaust temperature and low spray rate

Table 98: Visual appearance and % friability

| Batch No. ENQ3822/PIRT/ | Wet or Dry[a] | Consort Coat Ref | Appearance | %Friability |
|---|---|---|---|---|
| 014/01 | Dry | COAT-017 | No noticeable damage to the tablets observed after 4 hours. | 0.4%[b] |
| 014/02 | Wet | | No damage to the tablets was observed after 4 hours. | 0.1%[b] |
| 015/01 | Dry | COAT-006 | Chipping on the edges of some tablets was observed after 2 hours. | 0.4% |
| 015/02 | Wet | | Chipping on the edges of some tablets was observed after 1 hour. | 1% |
| 016/01 | Dry | COAT-010 | Small damage to the edge of one tablet observed after 1 hour. Chipping on the edges of some tablets was observed after 2 hours. | 0.2% |
| 016/02 | Wet | | Chipping on the edges of some tablets was observed after 2 hours. | 0.1% |
| 017/01 | Dry | COAT-023 | Signs of chipping on the edges of some tablets was observed after 3 hours. | 0.1% |
| 017/02 | Wet | | Signs of chipping on the edges of some tablets was observed after 2 hours. | 0.4% |
| 018/01 | Dry | COAT-032 | Minor damage to the edges of some tablets was observed after 1 hour. Chipping on the edges of some tablets was observed after 2 hours. | 0.5% |
| 018/02 | Wet | | Signs of chipping of the edges of some tablets was observed after 1 hour. | 0.7% |
| 019/01 | Dry | COAT-002 | Chipping of the edges of some tablets was observed after 2 hours. | 1.3% |
| 019/02 | Wet | | Chipping of the edges of some tablets was observed after 3 hours. | 0.3% |
| 020/01 | Dry | COAT-018 | Minor chipping of the edges of some tablets was observed after 3 hours. Chipping of the edges observed after 4 hours. | 0.4%[b] |
| 021/01 | Dry | COAT-027 | Chipping of the edges of some tablets was observed after 1 hour. | 1.3% |
| 022/01 | Dry | COAT-034 | Minor chipping of the edges of some tablets was observed after 3 hours. Chipping of the edges observed after 4 hours | 0.3%[b] |
| 023/01 | Dry | COAT-030 | Wear to the coating o the edges observed after 1 hour. | 0.1% |
| 024/01 | Dry | COAT-013 | Chipping of the edges of some tablets was observed after 2 hours. | 0.1% |

[a]Wet conditions = low tablet bed/exhaust temperature and high spray rate

[b]Dry conditions = high tablet bed/exhaust temperature and low spray rate

[b]%Friability after 4 hours testing. For the other samples friability values were measured at 3 hours (end of testing).

Overall, the batches coated with COAT-017, the $TiO_2$ reference coating, gave the best results with 0 tablets damaged after 4 hours and low friability even when coated under two the extremes of coating conditions. The next best-performance was observed for the batch coated under “dry” conditions with COAT-034, also an HPMC-based coating containing rice starch as an opacifier. For this batch there was no tablet damage at the 3-hour time-point. However, after 4 hours one third of the tablets had defects. The worst results were found for Batch ENQ3822/PIRT/023/01, coated with the clear coat, COAT-030, processed under “dry” conditions. For this batch all of the tablets displayed damage in the form of “wear” to the tablet edges by the 1-hour time-point. Batches coated with HPMC-based $TiO_2$-free

coatings containing $CaCO_3$ as the opacifier performed worse in the extended friability test than those containing rice starch (see Figure 48). For almost all batches of the former, damage to 2 or more tablets could be observed by 1 hour and by three hours all or almost all tablets had been damaged. The exception was Batch ENQ3822/PIRT/015/01, coated with COAT-006 and processed under "dry" conditions, which showed a slightly improved performance with only one tablet damaged at the 1 hour time-point and two-thirds of the tablets damaged at 3 hours.

For batches coated with the PVA-based $TiO_2$-free and $TiO_2$ reference coatings, tablet damage was not observed or was minimal up to the 2-hour time-point after which it increased. Batch ENQ3822/PIRT/020/01, coated with the $TiO_2$ reference, COAT-018, was the only PVA-based coating tested at 4 hours. However, poor results were achieved after 4 hours with almost all of the tablets damaged. At the 3-hour time-point, the number of tablets damaged were similar (3 to 8) for all of the batches coated with PVA-based coatings and, based on the limited data-set, it would appear that the $TiO_2$-free coated tablet batches performed similarly to the batch coated with the $TiO_2$ reference batch in the extended friability tests.

For two of the coatings, processing conditions appear to impact on the extended friability results. For Batch ENQ3822/PIRT/019/01 and ENQ3822/PIRT/019/02, there was a reduced number of damaged tablets for the batch coated under low tablet bed/exhaust temperature and high spray rate conditions compared with the batch coated under "dry" conditions. %friability at the 3-hour time-point was also higher for the batch coated under "dry" conditions. These batches were coated with COAT-002.

In contrast for Batches ENQ3822/PIRT/015/01 and ENQ3822/PIRT/015/02, less tablet damage and reduced friability were observed for the former batch processed under "dry" conditions (high tablet bed/exhaust temperature and low spray rate).

In summary, the batches coated with the $TiO_2$ reference coating, COAT-017, showed clearly superior performance in the extended friability testing compared with all of the $TiO_2$ -free coatings and the PVA-based $TiO_2$ reference. The batch coated with the $TiO_2$-free coat, COAT-034, gave the next best performance in terms of mechanical robustness to tumbling. However, it should be noted that unlike the HPMC-based $TiO_2$ reference, COAT-034 failed to achieve complete opacification of the tablets and a yellow tinge is visible in the photographs.

For the PVA-based coatings, there was no clear difference in performance between the batches and the results were similar for the batches coated with the $TiO_2$ containing reference and those with the $TiO_2$-free PVA-based coatings. However, the batches coated with PVA-based coatings ($TiO_2$ reference and $TiO_2$-free) had lower levels of damage during testing than the majority of batches coated with HPMC-based $TiO_2$-free coatings.

Figure 47: Appearance of tablets coated with the HPMC-based $TiO_2$ reference versus the best performing $TiO_2$-free coat, COAT-034

ENQ3822/PIRT/014/01 (4 Hours - Dry)
HPMC-based $TiO_2$ Reference, COAT-017

ENQ3822/PIRT/022/01 (4 Hours, Dry)
HPMC-based COAT-034 (Rice Starch)

Figure 48: Appearance of tablets coated the $TiO_2$-free COAT-010 (Rice Starch+D) versus $TiO_2$-free COAT-027 ($CaCO_3$+D)

ENQ3822/PIRT/016/01 (3 hours - Dry)

HPMC-based COAT-010 (Rice Starch + D)

ENQ3822/PIRT/021/01 (3 hours - Dry)

HPMC-based COAT-027 ($CaCO_3$ + D)

Figure 49: Appearance of tablets coated with the PVA-based $TiO_2$ reference versus the HPMC-based $TiO_2$-free COAT-002

ENQ3822/PIRT/020/01 (3 hours - Dry)

PVA-based $TiO_2$ Reference, COAT-018

ENQ3822/PIRT/019/01 (3 hours - Dry)

HPMC-based $TiO_2$-free COAT-002 (Rice Starch+A+B+D+$Fe_2O_3$)

# Overall Discussion and Conclusion of Small-Scale Studies

## Selection of $TiO_2$ Coating Materials and Design of Evaluation

$TiO_2$ is a ubiquitous excipient in pharmaceuticals and its many important functional properties make it difficult to replace should it be banned in medicines (see Section 0). In order to conduct a comprehensive evaluation of potential alternatives to $TiO_2$, the $TiO_2$ Alternatives Consortium, worked together with experts from numerous coating material manufacturers, to identify $TiO_2$-free coating materials that were either commercially available or close to commercialization. From an initial documentation review of over 100 different $TiO_2$ coating materials, 29 were selected for initial evaluation against 5 $TiO_2$ containing reference coats.

Selection of the $TiO_2$-free coatings was based on a set of basic criteria e.g. availability at the start of the work and disclosed qualitative composition. The latter was important so that a selection could be made that included a variety of film-forming polymers, plasticizers, alternative opacifiers and coatings of two different colors – white and pink. Since some of these coating materials had similar compositions e.g., only difference was the absence/inclusion of colorants, following initial characterization, 20 $TiO_2$-free coating materials were selected for detailed evaluation in coating studies at small (3 kg) scale. The studies were based on a matrix designed by Consortium members. The coating studies were designed to present typical challenges to the power of the $TiO_2$-free coatings to provide surface coverage, opacification and protection. They included the coating of yellow round and oval placebo tablets to evaluate the ability of the coating materials to coat two of the most common tablet shapes. The coating studies also included the coating of tablet cores of APIs currently on the European market as film-coated tablets. These APIs have known sensitivities to factors of relevance to the replacement of $TiO_2$ in tablet coatings with $TiO_2$ alternatives. These active cores contained either nifedipine (prone to photodegradation), olmesartan (sensitive to moisture), rosuvastatin (sensitive to light, and potential for hygroscopicity and salt metathesis/disproportionation) and prasugrel (sensitive to alkali and potential for salt disproportionation).

In all of the coating suspension characterization, tablet coating and associated photostability and accelerated stability studies, the results on the $TiO_2$-free coats were compared to $TiO_2$ containing reference coatings and against a set of key performance indicators (KPIs). The KPIs and their rationale are described in Table 2 which is included again in this discussion section for the convenience of the reader (Table 99).

Table 99: Key performance indicators for the $TiO_2$-free coats and their rationale

| Key Performance Indicator | Rationale |
|---|---|
| **Manufacturability** in terms of the following:<br>• How easy it is to prepare and obtain an agglomerate-free suspension. Coating suspensions must be stable with little or no sedimentation.<br>• $TiO_2$-free coating suspension viscosity must enable pumping and spray rates typical for coating operations with no increased risk of spray line or spray gun blockage. However, it should also be sufficient to keep the coat constituents suspended during the coating process.<br>• Coating operations are not compromised or made more difficult or time-consuming by using $TiO_2$-free coating materials. | Manufacturability was selected as a KPI because a more difficult, time-consuming manufacturing process would add to the cost of manufacturing medicines, making them more expensive. It could also potentially result in a less robust process, increasing the risk of batch-to-batch variability in drug product quality. In turn, this could impact on the reliability of stable supplies to the market and potentially lead to medicine shortages. |
| **Acceptable coat appearance and coverage at ≤ 6% weight gain** (as evaluated by visual appearance, colorimetry, digital optical microscopy).<br><br>• The appearance of the coat obtained should be as good or better than the $TiO_2$-containing reference coat(s) with respect to visual elegance and underlying tablet surface coverage and opacity at coating %weight gains of ≤ 6%.<br>• The change in coating system should not impact the quality of any debossed image. | Appearance was chosen as a key performance indicator as consistent batch-to-batch color performance is important to ensure consistent product quality and patient confidence in their medicines. The quality of the debossed image is important for patient compliance, medication identification and to tackle counterfeiting.<br><br>**Rationale for selection of ≤6%w/w coating levels**<br>A weight gain of 6 %w/w is two to three times more than that typically required for $TiO_2$ coatings (2-3% w/w). However, in order to give $TiO_2$-free coatings the best chance of success, the acceptance criteria was a comparable coating to the $TiO_2$ reference coatings at a weight gain of ≤6% w/w. This is despite the increase in processing times and costs that are incurred with higher coating weight gains. |
| **Potential for wide color palette which enables a match with existing tablet colors** (as evaluated by visual appearance and colorimetry) so that existing tablet colors can be maintained. | The ability of the $TiO_2$-free coatings to enable a wide color palette and allow color matching was considered key to their performance, as it is important to differentiate between medicines to facilitate patient compliance and medication identification. The ability to match existing tablet coat colors is important if there were to be a requirement to replace $TiO_2$ coats in pre-existing products or when blinding products for clinical trial purposes. |
| **Mechanical strength of the coat and its adherence to the tablet surface (**as assessed by extended friability studies).<br><br>The mechanical strength of the coat should not be compromised by a change from $TiO_2$-containing coating systems to $TiO_2$-free ones. | This performance indicator was chosen as poor mechanical strength could lead to issues with coat adhesion to the tablet core creating coating and tablet core defects in downstream processes, such as packaging and transportation/shipment, which would impact product quality and could result in patient complaints. Dealing with the issues caused by poor mechanical coat strength would increase production costs as processes, such tablet sorting to remove defective tablets, are time-consuming. |

| | |
|---|---|
| **In vitro performance** (as assessed by disintegration, dissolution) | Dissolution is a critical quality attribute for solid dosage forms. The use of $TiO_2$-free coats should not compromise tablet disintegration and/or release of active compounds from the tablets. |
| **Photostability of the coat** (as assessed by visual appearance, colorimetry, coat thickness).<br><br>The appearance of the $TiO_2$-free coats should be as stable or more stable than the $TiO_2$-containing reference coats to conditions of extreme light exposure (2 x ICH Q1B requirements). | Photostability is a KPI because color fading/change on exposure to UV light could result in product not meeting its appearance specification which is typically a drug product critical quality attribute.<br><br>Light exposure could also potentially cause degradation or changes in the properties of the film coating, which can in turn affect the thickness of the coating. |
| **Ability of $TiO_2$-free coatings to protect light-sensitive actives against photodegradation** (as assessed by assay, related impurities, disintegration and dissolution on samples exposed to the equivalent of 2 x ICH Q1B conditions).<br><br>$TiO_2$-free coatings should provide equivalent or greater light exposure protection to photosensitive actives than $TiO_2$-containing systems. | $TiO_2$ has the ability to block ultra-violet (UV) light, thus, $TiO_2$ coatings can provide protection to light-sensitive actives and excipients. The loss of this protection through replacement of a $TiO_2$ containing coating with a $TiO_2$-free one could result in a loss of light-protection with consequences for product stability. Therefore, it is important that $TiO_2$ coatings provide equivalent or greater protection against photodegradation as $TiO_2$ containing ones. |
| **Chemical and physical stability of $TiO_2$-free coatings** (as assessed by tablet visual appearance, colorimetry and coat thickness on samples stored under accelerated stability conditions versus $T_0$ results).<br><br>The stability of $TiO_2$-free coats during accelerated studies should be equivalent or greater than $TiO_2$-containing ones. | The chemical and physical properties of the coat should not change on storage as this would result in the medicine failing its appearance specification, potentially reduced protection for light sensitive APIs, product recalls and, most importantly, a reduction in patient faith in their medicine. |
| **Ability of $TiO_2$-free coatings to protect susceptible APIs from chemical and physical instability during storage** (as measured by assay, related impurities, disintegration and dissolution on samples stored under accelerated stability conditions versus $T_0$ results).<br><br>The ability of $TiO_2$-free coats to protect susceptible APIs from degradation in accelerated stability studies should be equivalent or greater than $TiO_2$-containing ones. In addition, the properties of the $TiO_2$ alternative should not promote API or excipient instability. | $TiO_2$ is non-hygroscopic, chemically inert and its presence does not result in a strongly acidic or alkaline microenvironment. Therefore, its inclusion in coatings facilitates the protection of moisture-sensitive compounds and does not promote degradation of actives. Any $TiO_2$-free coating must also provide similar protection and not promote degradation. |

## Overview of Results

The results of the studies are grouped and discussed based mainly on the opacifier and the film-forming polymer they contain. It should be noted that a number of the $TiO_2$-free coats contain more than one opacifier and many, including the PVA-based $TiO_2$ reference coats and colored coatings, contain excipients which contribute to opacification but also have other functions e.g. talc and $Fe_2O_3$.

In vitro performance, as measured by disintegration and dissolution, has been excluded from the following discussion, as in general, the use of $TiO_2$-free coatings does not impact this KPI for the tablets assessed unless significant degradation has occurred. However, the use of $TiO_2$ -free coatings could impact on the dissolution of APIs from sustained release and enteric coating systems. Studies on these systems were outside the scope of the Consortium's work.

### 54. HPMC-based $TiO_2$-free Coatings Containing $CaCO_3$

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference, mechanical strength

The white $TiO_2$-free coatings studied in this category were COAT-004, COAT-006, COAT-019, COAT-027 and COAT-032. The colored $TiO_2$-free coatings in this category were COAT-011, COAT-021 and COAT-028. Only the white coatings in this group were taken forward into the coating studies.

In all cases no major issues were experienced during coating suspension preparation or coating.

COAT-006, COAT-027 and COAT-032 were used to coat the yellow round and oval tablets. In this study a higher %weight gain and coating thickness were required compared with the $TiO_2$ containing coatings to ensure the yellow color of the tablet cores' surface was completely hidden. The rank order of coating quality on the placebo round tablets for the HPMC-based $TiO_2$-free coatings was as follows: COAT-006 = COAT-027 > COAT-032. For the oval tablets, COAT-027 and COAT-032 failed to completely opacify the oval tablet surface based on visual inspection during manufacture even at a 6% weight gain, although COAT-006 was judged as providing surface coverage. However, visual descriptions of photographed samples from the batches judged the tablets coated with COAT-027, COAT-006 and COAT-032 to be fully coated at a 6% coating weight gain, although both COAT-006 and COAT-032 coated tablets were described as being off-white. This is in comparison with the HPMC-based $TiO_2$-containing reference, COAT-017, and the PVA-based reference coat, COAT-018, whose use resulted in white tablets at a 3% and 5% weight gain respectively. Therefore, the $TiO_2$-free coatings were less effective at opacification than the $TiO_2$ reference coatings for the placebo tablets.

COAT-032 resulted in an off-white coat at the 6% coating level on both round and oval placebo tablets. Therefore, it was not a color match for the $TiO_2$ reference coated tablets, whereas COAT-006 and COAT-027 were or came close to being a color match based on colorimetry data with $\Delta E^*_{00}$ values just under 1 or between 1 and 2. However, it should be noted that visual appearance data rated the oval tablet batch coated with COAT-006 to result in off-white tablets at a 6% coating weight gain.

COAT-019 was used to coat rosuvastatin cores and COAT-004 was used to coat nifedipine cores. Based on the colorimetry results, the COAT-019 coated rosuvastatin batch color matched the HPMC-based $TiO_2$ coated reference lot. However, it was difficult to determine when coverage was complete as the white coating was performed on white tablet cores. The suspensions of COAT-004 had lower %solids contents than the other HPMC-based $TiO_2$-free coating suspensions. COAT-004 failed to completely hide the surface of the nifedipine cores even at a 6% weight gain.

COAT-006, COAT-027 and COAT-032 proved inferior to the HPMC-based $TiO_2$ reference coating, COAT-017, with respect to mechanical strength as measured by extended friability testing with significant numbers of damaged tablets being observed at the 2-hour time-point.

KPIs: Stability of $TiO_2$-free coats to light and accelerated stability conditions and ability to protect sensitive actives

Neither COAT-004 or COAT-019 were able to protect nifedipine and rosuvastatin respectively against the harmful effects of light exposure. A change in the color of the tablet surface was noted visually and by colorimetry following exposure to extreme light conditions (2 x ICH Q1B) and there was a significant

reduction in assay and increase in related impurities for these batches. The $TiO_2$ reference batch used to coat the nifedipine cores (COAT-024) also failed to protect nifedipine from the effects of light. However, based on color difference data and assay and impurity data, it gave greater protection than COAT-004. Similarly, the $TiO_2$ reference coatings provided better protection to rosuvastatin than COAT-019.

Nifedipine is fairly stable to heat and humidity and therefore did not present a significant stability challenge. However, the results with COAT-004 were better than the other $TiO_2$-free coatings and the $TiO_2$ reference with respect to the %related impurity data. However, the coating itself underwent a significant color change at 70°C/75%RH.

The appearance of the rosuvastatin batch coated with COAT-019 underwent a slight color change on accelerated stability. However, coating of rosuvastatin tablets with COAT-019 had a detrimental effect on API stability especially at 70°C/75%RH. COAT-019 contains an acidic component which it is postulated promoted the degradation of this acid sensitive compound.

**Key Findings**: All of the white HPMC-based $TiO_2$-free coating containing $CaCO_3$ were inferior to the corresponding $TiO_2$ reference batch when compared against the KPIs. Overall, COAT-006 and COAT-027 performed best and COAT-004, the worst. However, the former two coats were not subjected to the same stability challenges as COAT-019 and COAT-004.

### 55. PVA-based $TiO_2$-free Coats Containing $CaCO_3$

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference, mechanical strength

The $TiO_2$-free coatings in this category were COAT-008, COAT-009, COAT-013, COAT-014 and COAT-015. The coating suspension preparation of these coatings went reasonably smoothly. However, only the three colored PVA-based coatings, COAT-013, COAT-014 and COAT-015 were used in the coating studies to coat olmesartan core tablets. These three $TiO_2$-free coatings were inferior to the $TiO_2$ reference pink coat as the pink color deepened with %weight gain. This is a major issue as the use of such coatings could potentially result in non-robust coating processes where coating efficiency and differences in coating parameters or material properties produces batch-to-batch differences in coating color. The batches coated with COAT-014 and COAT-015 also displayed a spray pattern on their surface, making tablet appearance unacceptable.

Only COAT-013 was tested in the extended friability studies. It performed similarly to the PVA-based $TiO_2$ reference but not as well as the HPMC-based $TiO_2$ reference.

KPIs: Stability of $TiO_2$-free coats to light and accelerated stability conditions and ability to protect sensitive actives

Overall COAT-013, COAT-014 and COAT-015 were stable to light in the photostability studies and the color variation on the bellyband of the exposed sample of the batch coated with COAT-015 was believed to be a coating issue as opposed to being due to light exposure. No significant color differences were found between the exposed and control samples of any of these $TiO_2$-free batches by colorimetry which supports this theory. However, visual appearance changes were observed for the samples on accelerated stability at 70°C/75%RH.

Olmesartan is sensitive to moisture but not light. Although neither the $TiO_2$-free nor the $TiO_2$ reference coatings could protect the API fully at 70°C/75% RH, the $TiO_2$-free coats were inferior in this regard, as shown by higher levels of related impurities. The assay value for the batch coated with COAT-014 was much lower than the others.

**Key Finding**: COAT-013, COAT-014 and COAT-015 performed poorly against the KPIs for manufacturability, tablet appearance and color matching to the $TiO_2$ reference. They were less effective at protecting olmesartan from the effects of moisture.

### 56. PEG-PVA Graft Copolymer $TiO_2$-free Coats Containing $CaCO_3$

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference

COAT-007 and COAT-012 were selected for initial evaluation. COAT-012 has the same qualitative composition to COAT-007 except for the presence of colorants such as $Fe_2O_3$, and both were prepared at 30% coating solids. Both were described as being easy to disperse, had low viscosity and the suspensions appeared homogeneous. However, for COAT-007 there was evidence of sedimentation at the end of coating and this may have been the cause of the low coat thickness and poor tablet surface coverage and opacification results at 6%w/w gain when COAT-007 was used to coat the round and oval placebo tablets. No settling was observed for COAT-012. It was only evaluated at a 500 g scale and it was not used for coating. The preparation at smaller scale and the presence of the colorant iron ions may have facilitated COAT-012 suspension.

COAT-007 was found to be stable to light on photostability based on colorimetry data. It was not tested on active cores or for the mechanical strength of the coating.

**Key Finding**: Based on the results, COAT-007, performed poorly against the KPIs for manufacturability, coated tablet visual appearance at 6% weight gain and color matching with the $TiO_2$ reference batch.

### 57. $TiO_2$-free Coatings Containing Other Divalent Metal Opacifiers

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference, mechanical strength

This group of $TiO_2$-free coatings included COAT-001, COAT-002, COAT-005, COAT-023 and COAT-033. COAT-033 also contains $CaCO_3$ and COAT-002 also contains rice starch.

COAT-005 containing MgO was hard to disperse and contained many agglomerates. It required screening to remove agglomerates which caused gun blockages and resulted in a failed batch. The suspension also had a high pH of 11 due to the presence of MgO and was creamy beige in color and not white. It was used to coat rosuvastatin core tablets. COAT-001 also had a high pH due to the presence of $MgCO_3$. However, its coating suspension was easy to manufacture, as was that of COAT-002 and COAT-023. COAT-033 had poor flow properties and contained clumps and although a coating suspension could be formed at small scale, its poor flow properties are likely to cause issues at larger scale. COAT-001, COAT-023 and COAT-033 were used to coat nifedipine core tablets, while COAT-002 was used to coat prasugrel cores. COAT-023 and COAT-001 were also used to coat rosuvastatin cores.

Neither COAT-001, COAT-023 nor COAT-033 were effective at coating the nifedipine batches even at a 6% weight gain and therefore there was no color match with the $TiO_2$ reference coated batch. However, there was a color match between the rosuvastatin batches coated with COAT-001 and the $TiO_2$ reference coated lot showing the greater challenge of covering and opacifying the surface of yellow tablet cores compared with white ones.

COAT-005 produced an off-white/cream coat on the rosuvastatin cores while coverage of the prasugrel tablet cores was achieved by COAT-002 at a 6% weight gain.

Only batches coated with COAT-023 and COAT-002 were subjected to extended friability testing. The performance of PVA-based COAT-023 was similar to that of the PVA $TiO_2$ reference but the HPMC-based COAT-002 coated tablets were less able to stand up to the rigors of the friability testing than the HPMC-based $TiO_2$ reference.

KPIs: Stability of $TiO_2$-free coats to light and accelerated stability conditions and ability to protect sensitive actives

COAT-001, COAT-023 and COAT-033 coated nifedipine tablets changed color to a greater extent than the $TiO_2$ coated reference batch in response to extreme light exposure based on both visual and colorimetry data. There was also a greater decrease in assay for the batches coated with the $TiO_2$-free coatings. These coatings underwent a color change on accelerated stability at 70°C/75%RH. However, COAT-033 and COAT-001 were better able to protect nifedipine against the effects of heat and moisture than the $TiO_2$ reference coating or COAT-023.

The rosuvastatin tablets coated with COAT-005 underwent a significant color change on photostability, turning yellow. Those coated with COAT-001 and COAT-023 also changed color but to a lesser extent.

However, based on colorimetry data only the exposed COAT-023 tablet sample was a match for the control. This coat was the only $TiO_2$-free coating to protect rosuvastatin from photodegradation to a similar extent to the $TiO_2$-reference coatings.

On accelerated stability COAT-033 and COAT-001 coated rosuvastatin batches underwent a significant color change at 70°C/75%RH, turning brown at the 21-day time-point. However, based in the rosuvastatin assay and related impurity results, they were more effective at protecting the API from the effects of moisture than the $TiO_2$ reference coatings.

COAT-002 coated prasugrel tablets changed color as a result of photostability. However, assay values and impurity levels for the exposed batch were similar to the $TiO_2$ reference. The batch was not significantly affected by storage at 50°C/30%RH for 3 weeks. However, like all the other coated prasugrel lots, degradation was almost total at 70°C/75%RH.

**Key Findings**: None of the $TiO_2$-free coatings were equivalent to $TiO_2$ coatings for all of the KPIs, although some like COAT-023 and COAT-019 were equivalent or almost equivalent to $TiO_2$ reference coatings for a very limited number of KPIs e.g. COAT-023 protected rosuvastatin from photodegradation. COAT-001 and COAT-005 seemed better at protecting the acid-sensitive rosuvastatin from the effects of heat and moisture at 70°C/75%RH based on assay and related impurity results, perhaps because of their alkaline nature. However, the coatings themselves turned brown following storage under these conditions.

The effectiveness of the coating with $TiO_2$-free coating materials may depend on the extent of the coating and opacification challenge e.g. COAT-001 was successful in coating and color matching with the corresponding white rosuvastatin $TiO_2$ reference but did not produce enough coverage/opacification to hide the yellow color of the nifedipine cores.

### 58. $TiO_2$-free Coatings Containing Rice Starch

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference, mechanical strength

This group contained the $TiO_2$-free COAT-010, COAT-016, COAT-020, COAT-022 and COAT-034. COAT-022 was only ever tested for viscosity and coating suspension characteristics. No issues were found during suspension preparation. Some difficulties were experienced dispersing COAT-020 which was used to coat rosuvastatin cores. No issues were experienced preparing suspensions of COAT-010, COAT-016 and COAT-034. COAT-010, COAT-020 and COAT-034 were used to coat rosuvastatin cores, while COAT-016 were used to coat prasugrel cores.

It was difficult to ascertain when surface coverage by COAT-010, COAT-020 and COAT-034 on the rosuvastatin cores was complete due to a white coating being applied to a white core tablet. However, COAT-010 and COAT-020 met the criterion for a color match with the HPMC-based $TiO_2$ reference batch based on colorimetry data, while the batch coated with COAT-034 did not.

The coating of the prasugrel cores with COAT-016 was unsatisfactory as it resulted in a spray pattern at all coating levels.

COAT-010 and COAT-034 were assessed for mechanical strength in the extended friability study with COAT-034 being the best performing $TiO_2$-free coat, only second to the HPMC-based $TiO_2$ reference. Tablets coated with COAT-010 had an inferior performance in this test.

KPIs: Stability of $TiO_2$-free coats to light and accelerated stability conditions and ability to protect sensitive actives

The rosuvastatin cores coated with COAT-010 and COAT-020 underwent a slight color change following light exposure during photostability. However, those coated with COAT-034 turned yellow. The visual results were confirmed by high color difference values by colorimetry. These coatings were inferior to both $TiO_2$ reference coatings in protecting rosuvastatin against photodegradation.

The COAT-016 coated prasugrel tablets changed color slightly on photostability but the assay and related impurity levels remained similar to that of the $TiO_2$ reference batch. This reflects in part that prasugrel is not particularly sensitive to light in the solid state. The visual appearance, assay and related

impurity results for the COAT-016 coated batch did not change on accelerated stability at 50°C/30%RH. However, like the $TiO_2$ reference coat, it could not protect prasugrel against degradation at 70°C/75%RH.

**Key Findings**: The $TiO_2$-free coatings containing rice starch were inferior to the $TiO_2$ reference coatings in terms of their ability to protect sensitive APIs and in coating the colored prasugrel core tablets. However, COAT-034 was the best $TiO_2$-free coating of those tested with respect to mechanical strength as evaluated by extended friability testing.

### 59. $TiO_2$-free Coatings Containing Other Opacifiers

KPIs: Manufacturability, appearance, color matching with the $TiO_2$ reference, mechanical strength

This group contained COAT-003, COAT-029, COAT-030 and COAT-031. COAT-003 and COAT-030 are described by their manufacturers as clear coats, although some of their constituents will confer some opacification. Only viscosity was ever measured on COAT-029 and viscosity and coating suspension characterisation was carried out on COAT-003. The former was due to the Consortium's plan to focus on the clear $TiO_2$-free coating COAT-030 alone and in combination with the colorant admix, COAT-031.

COAT-003, another clear coating, was prepared without difficulties. However, difficulties were encountered when preparing coating suspensions of COAT-030 alone or in combination with COAT-031-The issue was with the dispersal of COAT-030, and not COAT-031, and the formation of many agglomerates. Sieving of the suspensions was required to prevent the spray gun blockages which occurred the first time COAT-030 was used. COAT-030 alone was used to coat rosuvastatin cores and the COAT-030/COAT-031 combination, prasugrel cores. Again, it was difficult to ascertain when surface coverage of the rosuvastatin cores by COAT-030 was complete due to a white coating suspension being applied to a white core tablet. However, coating with COAT-030 did not result in a color match with the $TiO_2$ reference.

Coating of the prasugrel cores with the COAT-030/031 combination did not produce an acceptable coat as a spray pattern was visible at all coating levels. The COAT-030/COAT-031 combination produced red tablets, not pink, and therefore could not be compared with the $TiO_2$ reference coating.

COAT-030 was the worst performing coating in the extended friability test with all tablets tested damaged after 1 hour. In contrast, the HPMC-based $TiO_2$ reference coated tablets showed no tablet damage after 4 hours of testing.

KPIs: Stability of $TiO_2$-free coats to light and accelerated stability conditions and ability to protect sensitive actives

The exposed rosuvastatin tablets coated with COAT-030 turned yellow as a result of photostability and the color of the COAT-030/COAT-031 coated prasugrel tablets also altered. However, in the former case the stability of the API was adversely affected showing that COAT-030 could not protect the light sensitive API. Prasugrel is not particularly light sensitive in the solid state so that the assay and impurity levels in the tablets coated with COAT-030/COAT-031 remained similar to the exposed sample of the batch coated with the $TiO_2$ reference. COAT-030 was also inferior at protecting rosuvastatin in the accelerated stability study in comparison to both the $TiO_2$ reference coatings and the other $TiO_2$-free coats.

**Key Findings**: COAT-030, either alone or in combination with COAT-031, performed very poorly in the coating studies, not only in comparison to the $TiO_2$ reference coatings but also the other $TiO_2$-free coatings.

### 60. Colored $TiO_2$-free Coatings versus White $TiO_2$-free Coatings

All of the $TiO_2$-free colored coatings contained $Fe_2O_3$ and this will contribute to the opacification properties of the coats. However, in general, all of the $TiO_2$-free colored coatings were inferior to the $TiO_2$-free white coatings. A spray pattern was observed on many of the tablet batches coated with colored $TiO_2$-free coatings and the color changed with %weight gain, the latter being a potentially

serious issue for the development of robust coating processes. The poor performance of the colored $TiO_2$-free coatings significantly reduces the potential color palette of coatings available for use, should $TiO_2$ be banned for use in medicines.

### Comments on Methodologies

#### 61. Visual Appearance and Colorimetry

The perception of differences in appearance and color by the human eye is subjective and depends on the lighting conditions. For this reason, lighting, background and camera settings were standardized for comparative visual appearance studies and also colorimetry was used. Color differences were calculated using the $\Delta E^*_{00}$ equation which is the most accurate color difference equation currently in use. In most cases there was relatively good agreement between the visual appearance and colorimetry results. However, in three cases in the accelerated stability study, there were significant differences. The first case concerned the comparison between the coated nifedipine batches on stability at 50°C/30%RH versus $T_0$. Visual appearance data suggested no changes had occurred, while colorimetry data indicated that a color difference should be noticeable at a glance. The same results were found on repeating the photography work and no errors or issues were found when the colorimetry data were reviewed. In order to investigate the difference further, the individual L* a* b* chroma and hue angle were compared in more detail. The L* values between the 50°C/30%RH samples and the $T_0$ control were found to be the main driver for the high $\Delta E^*_{00}$ values. Therefore, whilst the nifedipine colorimetry results suggest an obvious visual difference ($\Delta E^*_{00}$ >2), this difference is mainly due to a measured change in the lightness value which is not always perceptible in standard photographs and visual observations and, thus, is thought to be of little practical relevance.

The second and third case of significant discrepancy between the visual and colorimetry data occurred with the 7-day rosuvastatin stability samples stored at 50°C/30%RH and the $TiO_2$ reference coated prasugrel batch. Again, visual appearance data indicated no change in appearance for both sets of samples versus $T_0$, whilst colorimetry data suggested a significant difference. In the former case the colorimetry results were out of line with those of the 14-day and 21-day samples which agreed with the visual data that no appearance change had occurred. Both the visual and colorimetry data were re-checked and no errors, issues or omissions could be identified. These differences are currently being investigated further.

## Overall Conclusion

All of the 20 $TiO_2$-free coatings studied in detail in the course of the Consortium's work were inferior to the $TiO_2$ reference coats based on the entire set of KPIs identified at the start of the project. Some performed well when assessed against certain criteria but not others. Many did not achieve surface coverage and opacification at a 6% weight gain and those, which did, required significantly more coating than the $TiO_2$ reference coats. The performance of the colored $TiO_2$-free coatings and the clear COAT-030 was in general poorer than that of the white $TiO_2$-free coatings. Therefore, none of the $TiO_2$-free coatings could match the properties of $TiO_2$ which will result in longer and potentially less robust coating processes, and may also impact on the stability and shelf-life of products and, thus, impact overall quality. There is also a risk to patient adherence due to color changes seen in some $TiO_2$-free coatings, and to patient safety due to the limted color palette available to distinguish between different products/strengths.

## Studies Outside the Scope of the Consortium's Work Plan

The following factors/aspects were outside the scope of the consortium's work plan and have not been evaluated.

Tablet sizes and shapes other than those evaluated

Studies were conducted on round and oval tablets based on the fact that they are the most common tablet shapes. No work was carried out on other tablet shapes which may be more prone to mechanical damage and require coatings of optimal mechanical strength to protect them against the rigors of blister packing and transportation. The largest round tablet core coated in these studies was the placebo tablet at 9 mm in diameter while the smallest round tablet was the 5 mm nifedipine core. The largest oval tablet was also the placebo at 15 x 7 mm, while the smallest was the prasugrel core at 10 mm x 5.1 mm. The sizes were selected to provide a range of small and mid-range sized tablets. However, the ability of the $TiO_2$-free coatings to coat very small and very large tablets was not assessed.

Long-term stability of the coated tablet batches

Although typical development studies, the photostability and accelerated stability results may not be representative of those from long-term stability studies. Long-term stability was not generated on the $TiO_2$-free coated tablet batches due to the relatively short time-frame of the project.

Extensive color matching studies involving commercial products

The Consortium's work program included a limited amount of color comparison between the $TiO_2$-free coated tablets and the $TiO_2$ reference coated tablets with disappointing results. Only white and pink/red $TiO_2$-free coatings were evaluated. If $TiO_2$ is banned in medicines, it would require suitable $TiO_2$-free coatings in a variety of colors to be available, and extensive color matching studies to determine whether it was possible to color match reformulated $TiO_2$-free products with existing commercial products.

Sustained-release matrix tablets and enteric coated tablets

The coating of nifedipine retard tablets with $TiO_2$-free systems was evaluated and found not to affect their in vitro performance. However, these tablets disintegrate and release the majority of their contents over a relatively short time-frame (3 hours). Products with longer dissolution profiles and sustained-release matrix tablets were not assessed. Dissolution from these more prolonged release systems is more likely to be affected by thicker coatings and impacted by changes in coat composition.

The $TiO_2$-free coating systems were not evaluated for coating tablets which required an enteric coating. Many of the $TiO_2$-free coating suspensions have higher pH than $TiO_2$ containing systems and some much higher pH (see Section 3) and therefore are unlikely to be suitable for use with enteric coatings, thus, further limiting the possibilities of successful development of $TiO_2$-free tablets.

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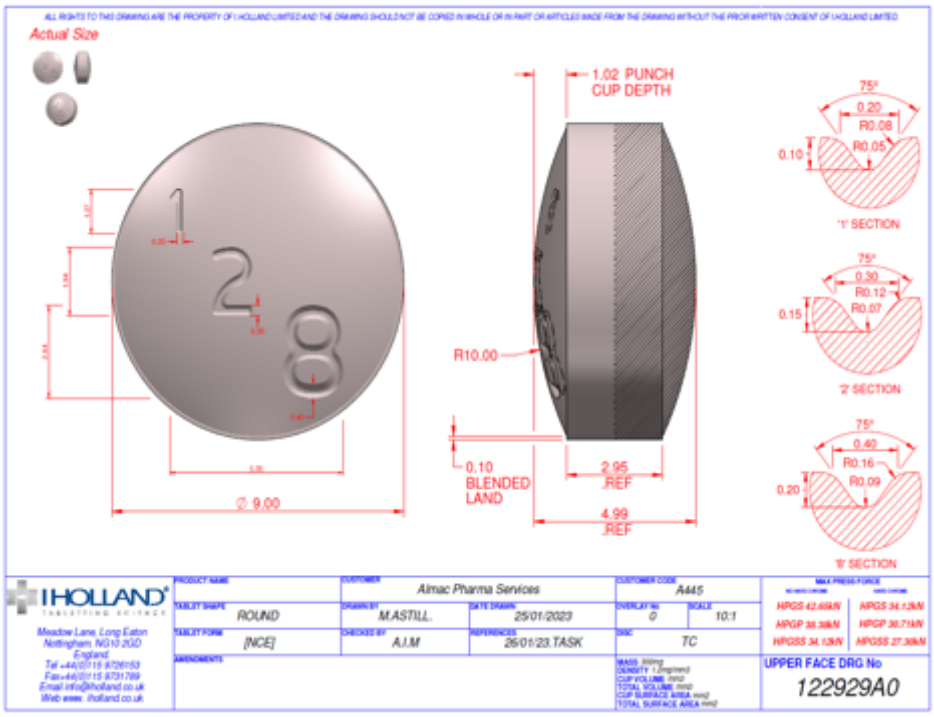
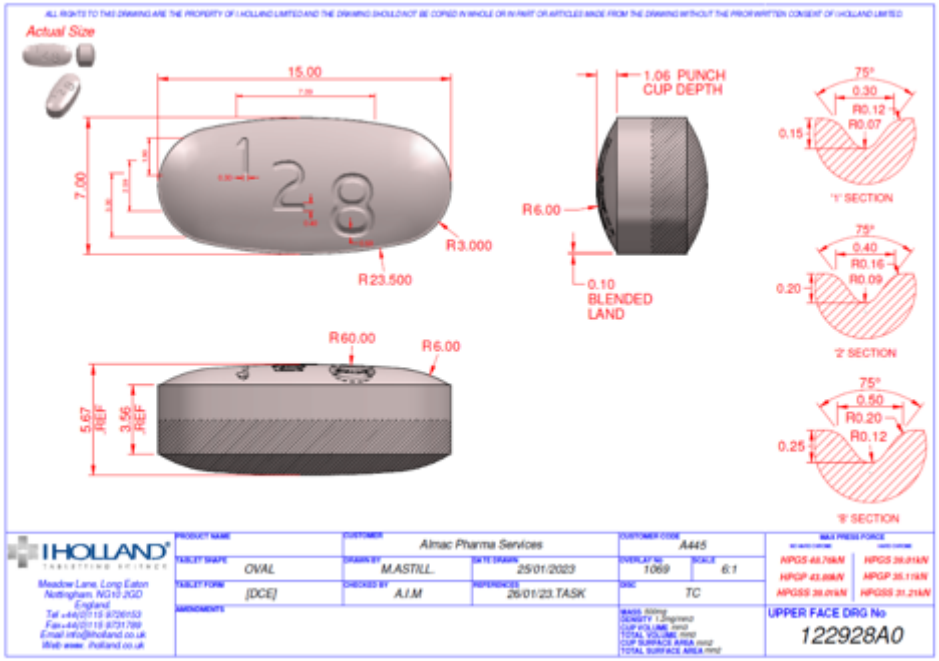
# Appendix B: Tooling Used for Placebo Tablet Manufacture

The tooling drawings for the round placebo and the oval tablets manufactured as described in Section 0 are shown below.

Tooling for Round Placebo Tablets



**ANNEX 3**

# Alternatives to Titanium Dioxide in Hard Capsules

Technical Evaluation of Titanium Dioxide-free Capsules

Report for the European Medicines Agency

08 February 2024

| Written By | Affiliation | Signature | Date |
|---|---|---|---|
| Louise Rosenmayr-Templeton | Consultant,<br>Tower Pharma Consulting | | 08 Feb 2024 |
| **Approved By** | | | |
| Jon Beaman | Executive Director, Pfizer | | |

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## List of Tables

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## List of Abbreviations

| | |
|---|---|
| °C | Degrees Celsius |
| API | Active Pharmaceutical Ingredient |
| AQL | Acceptable Quality Level |
| $CaCO_3$ | Calcium Carbonate |
| Cap | Capsule |
| Consort | Consortium |
| Diss | Dissolution |
| EC | European Commission |
| EMA | European Medicines Agency |
| $Fe_2O_3$ | Iron Oxide Red |
| G | Gram |
| HDPE | High Density Polyethylene |
| hr | Hour |
| HPC | Hydroxypropylcellulose |
| HPMC | Hydroxypropylmethylcellulose (hypromellose) |
| IPC | In-Process Control |
| Kg | Kilogram |
| %LC | %Label Claim |
| Lab | Laboratory |
| LOQ | Limit of Quantification |
| m3/hr | Metres-Cubed Per Hour |
| mbar | Millibar |
| mg | Milligram |
| min | Minute |
| mm | Millimetre |
| NaPOx | Sodium Phosphates |
| NA | Not applicable |
| PE | Polyethylene |
| Ph.Eur | European Pharmacopoeia |
| Ref | Reference |
| %RH | % Relative Humidity |
| RPM | Revolutions Per Minute |
| RT | Retention Time |
| RRT | Relative Retention Time |
| sec | Seconds |
| $TiO_2$ | Titanium Dioxide |
| USP | United States Pharmacopeia |
| vs | Versus |
| Aw | Water Activity |
| w/w | Weight for Weight |

# Executive Summary

Titanium dioxide ($TiO_2$) is a commonly used opacifier and colorant in pharmaceuticals and is estimated to be present in at least 100,000 human medicinal products and 1600 veterinary medicinal products in the European Union. On 14 January 2022, the European Commission (EC) banned $TiO_2$ as a food additive based on safety concerns and, as a result, E171 was removed from the permitted food additives list. At present $TiO_2$ is still allowed for use in medicines per EC Regulation 2022/63. However, given the impact of a potential $TiO_2$ ban on medicine availability, and in response to a request from the EC to the pharmaceutical industry, the $TiO_2$ Alternatives Consortium was formed to conduct a comprehensive evaluation of potential alternatives to $TiO_2$ in medicines.

This report concerns the studies to evaluate 13 $TiO_2$-free hard capsule shells and compare them with 4 $TiO_2$ reference capsule shells. The studies were conducted mainly at small scale.The $TiO_2$-free and $TiO_2$ reference white and colored (red/orange/pink) capsule shells were evaluated empty, as well filled with three different active blends, against a set of key performance factors important for their use in medicinal products and in over-encapsulation to blind products for use in clinical trials. A summary of the experimental findings against these key performance parameters is included in Table 100 overleaf for the gelatin capsule shells and Table 101 for the hypromellose (hydroxypropylmethylcellulose (HPMC)) capsule shells.

Overall Conclusions

The results show that for white capsule shells, all of the $TiO_2$-free capsule shells have inferior properties to $TiO_2$ containing reference shells in terms of opacity and ability to camouflage the capsule shell contents. In some cases, they had reduced mechanical integrity than the $TiO_2$-containing counterparts. The gelatin-based $TiO_2$-free capsule shell, CAP-002's opacity varied significantly in response to changes in relative humidity. Therefore, none of the white $TiO_2$-free capsule shells evaluated were considered suitable replacements for $TiO_2$ containing capsule shells.

The red/orange $TiO_2$-free capsules containing the colorant, $Fe_2O_3$, performed well in the battery of tests. The capsule shells are opaque and therefore capable of camouflaging any color differences in the capsule contents. $Fe_2O_3$ is not an opacifier per se but imparts opacification through its intense red color. The intensity of color makes it difficult for the human eye to detect color changes in the capsule shell e.g., following accelerated stability storage, even though colorimetry data showed that changes had occurred. However, exact color matching for the purposes of reformulating an existing product as $TiO_2$-free may be difficult as CAP-014, the $TiO_2$ reference and the $TiO_2$-free CAP-001 from the same supplier, product line and tradename had color difference values of above 2.

This pink semi-translucent capsule shell was the only non-red/orange colored capsule shell evaluated. It does not contain $Fe_2O_3$. Its pink color bleached to white in the photostability studies and it was found to be very brittle. In addition, its semi-transparency would not hide the color and appearance of its contents. For the above reasons it is not considered a replacement for $TiO_2$ containing pink capsule shells. $TiO_2$-free capsule shells of other colors were not evaluated as part of the Consortium's work due to lack of availability at the start of the project.

Based on the results, only $TiO_2$-free red/orange capsule containing $Fe_2O_3$ could be suitable replacements for $TiO_2$ containing capsules. If $TiO_2$ was banned in medicines, this would severely restrict the color palette available for new medicines or reformulating commercially available ones to be $TiO_2$-free, with a down-stream impact on the ability to identify medicines and prevent counterfeiting. In addition to a reduced color palette caused by the darker colors imparted by iron oxides to the capsule shell, finding an imprinting ink with sufficient contrast to the capsule shell color will be difficult because the lighter ink colors, e.g. white ink, contains $TiO_2$. The daily intake of iron oxide (E172) is restricted by authorities such as the World Health Organization, the FDA and the Japanese authorities for safety reasons. These limits translate approximately to the equivalent of 3 x Size 0 capsules per day. Based on these limitations, $Fe_2O_3$ would not be a suitable replacement for $TiO_2$ as it would not have global regulatory acceptability and could not be used in medicines developed for global markets especially those involving multiple dosing or chronic use.

Table 100: Summary of the study findings for $TiO_2$-free versus $TiO_2$ containing reference gelatin capsule shells

| Key Performance Indicator | Parameter(s) Assessed | Acceptance Criteria | Consortium Capsule Shell Reference | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 013[a] | 002 | 005 | 006 | 012 | 017[b] | 010 |
| **Empty Capsule Shells** | | | **Acceptance Criteria Met (Yes/No)** | | | | | | |
| Appearance & Opacity | Visual appearance | Opaqueness similar to $TiO_2$ reference | NA | No | No | No | No | NA | Yes |
| | Colorimetry | $\Delta E^*_{00}$ values ≤ 1 (white capsules)<br>$\Delta E^*_{00}$ values < 2 (colored capsules) | NA | No | No | No | No | NA | NA |
| Color match to gelatin- based $TiO_2$ reference | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | NA |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | NA | No | No | No | No | NA | NA |
| Capsule shell stability-to light 2 x ICH Q1B | Visual appearance | No visible difference exposed vs control | No | No | Yes | No | No | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | No | No | Yes | Yes | No | Yes | Yes |
| Capsule shell stability to %relative humidity | Visual appearance | No visible difference conditioned vs control | Yes | No | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Mechanical Robustness | % Brittle shells | ≤ 4 % brittle capsule shells at ≥33%RH | Yes | Yes | No | Yes | No | Yes | Yes |
| Manufacturability | In-process data | In-process controls within specification<br>No manufacturing issues | NA | Yes | Yes | Yes | Yes | NA | NA |
| **Blend Filled Capsules – Benserazide HCl/Levodopa, Fluvastatin or Loperamide** | | | | | | | | | |
| Appearance & Opacity & Color Match with $TiO_2$ reference batches | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | Ni | No | NA | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | NA | No | No | No | No | NA | NA |
| Mechanical Robustness ($T_0$) Lab Storage | % Brittle shells | ≤ 5% brittle capsule shells | Yes | Yes | Yes | No | Yes | No | Yes |
| In vitro performance ($T_0$) | Disintegration/Dissolution | No difference to $TiO_2$ reference batches | NA | Yes | Yes | Yes | Yes | NA | Yes |
| Capsule shell stability-to light -2 x ICH Q1B | Visual Appearance | No visible difference exposed vs control | Yes | Yes | No | Yes | No | Yes | Yes |
| Ability to protect actives against photodegradation (2 x ICH Q1B) | Assay & Related Impurities | No difference exposed versus control | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection | | | | | | |
| In vitro performance (2 x ICH Q1B) | Disintegration/Dissolution | No difference exposed versus control | Slower profiles obtained for fluvastatin capsule batches | | | | | | |
| Capsule shell stability-to accelerated stability conditions 5°C; 50°C/50% RH versus $T_0$ | Visual Appearance | No difference to $T_0$ | Yes | No | No | No | No | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | Yes | No | No | No | No | No | No |
| | % Brittle shells | ≤ 5% brittle capsule shells | Results variable for $TiO_2$ references but no trend $TiO_2$-free capsules consistently met criteria. | | | | | | |
| Ability to protect actives on stability: 5°C; 50°C/50% RH versus $T_0$ | Assay & Related Impurities, | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection. | | | | | | |
| In vitro performance on stability: 5°C; 50°C/50% RH versus $T_0$ | Disintegration/Dissolution | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to disintegration and dissolution. | | | | | | |

[a]White $TiO_2$ reference gelatin capsule shell [b]Red $TiO_2$ reference gelatin capsule shell NA = Not applicable

Color Code: Green = Meets acceptance criteria; Yellow = Slight change or $\Delta E^*_{00}$ = 1-2; Red = Does not meet acceptance criteria

Table 101: Summary of the study findings for $TiO_2$-free versus $TiO_2$ containing HPMC capsule shells

| Key Performance Indicator | Parameter(s) Assessed | Acceptance Criteria | Consortium Capsule Shell Reference | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 016[a] | 003 | 004 | 007 | 009 | 014[b] | 001 | 008 | 015 | 011 |
| Appearance & Opacity | Visual appearance | Opaqueness similar to $TiO_2$ reference | NA | No | No | No | No | NA | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ values ≤ 1 (white capsules)<br>$\Delta E^*_{00}$ values < 2 (colored capsules) | NA | No | No | No | No | NA | No | NA | NA | NA |
| Color match to gelatin- based $TiO_2$ reference | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | No | NA | NA | NA |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | NA | No | No | No | No | NA | No | NA | NA | NA |
| Capsule shell stability-to light 2 x ICH Q1B | Visual appearance | No visible difference exposed vs control | No | No | No | No | No | Yes | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | No | No | No | No | No | Yes | Yes | Yes | Yes | No |
| Capsule shell stability to %relative humidity | Visual appearance | No visible difference exposed vs control | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Mechanical Robustness | % Brittle shells | ≤ 4 % brittle capsule shells at ≥33%RH | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| Manufacturability | In-process data | In-process controls within specification<br>No manufacturing issues | NA | Yes | Yes | Yes | Yes | NA | NA | NA | NA | NA |
| Appearance & Opacity & Color Match with $TiO_2$ reference batches | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | NA | No | No | No | No | NA | No | NA | NA | NA |
| Mechanical Robustness ($T_0$) | % Brittle shells | ≤ 5% brittle capsule shells | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | No |
| In vitro performance ($T_0$) | Disintegration Dissolution | No difference to $TiO_2$ reference batches | NA | Yes | Yes | Yes | Yes | NA | Yes | Yes | Yes | Yes |
| Capsule shell stability-to light 2 x ICH Q1B | Visual Appearance | No visible difference exposed vs control | Yes /No | Yes | No | No | Yes | Yes | Yes | Yes | Yes | No |
| Ability to protect actives against photodegradation (2 x ICH Q1B) | Assay & Related Impurities | No difference exposed vs control | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection. | | | | | | | | | |
| In vitro performance (2 x ICH Q1B) | Disintegration Dissolution | No difference exposed vs control | Slower profiles obtained for fluvastatin capsule batches except for CAP-014. Slowdown may be related to significant API degradation. | | | | | | | | | |
| Capsule shell stability- 5°C; 60°C/30% RH versus $T_0$ | Visual Appearance | No difference to $T_0$ | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | No | No | No | No | No | No | No | No | No | No |
| | % Brittle shells | ≤ 5% brittle capsule shells | Variable results but CAP-003, CAP-007, CAP-009, CAP-011 very brittle. | | | | | | | | | |
| Ability to protect actives on stability 5°C; 60°C/30% RH versus $T_0$ | Assay & Related Impurities | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to assay and degradation | | | | | | | | | |
| In vitro performance following stability: 5°C; 60°C/30% RH versus $T_0$ | Disintegration Dissolution | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to disintegration and dissolution. | | | | | | | | | |

[a]White $TiO_2$ reference gelatin capsule shell [b]Red $TiO_2$ reference gelatin capsule shell, NA= Not applicable

Color Code: Green = Meets acceptance criteria; Yellow = Slight change or $\Delta E^*_{00}$ = 1-2; Red = Does not meet acceptance criteria, Yes/No = variable results

# Introduction

Titanium Dioxide ($TiO_2$) is a commonly used pharmaceutical excipient used in most solid dosage forms and is estimated to be present in at least 100 000 human medicinal products and 1600 veterinary medicinal products in the European Union [1]. Until recently it was listed under the European food additive list as E171. It is typically used as a colorant and opacifier in tablet coatings and capsules shells [2]. However, there have been recent concerns about its safety when administered orally [3].

On 14 January 2022, the European Commission (EC) banned $TiO_2$ as a food additive, with the result that Annexes II and III to Regulation (EC) No. 1333/2008 of the European Parliament and European Council were amended and E171 removed from the permitted food additives list [4].

At present $TiO_2$ is still allowed for use in medicines per EC Regulation 2022/63. However, given its presence in numerous medicines on the European market and the impact of a potential ban on medicine availability, the EC has carried out the following:

- Requested that the European Medicines Agency (EMA) conduct a re-evaluation of the impact in preparation for a Commission review by 01 April 2024,
- Stated that it is critical for the pharmaceutical industry to work towards identifying alternatives to $TiO_2$ addressing quality, safety and efficacy.

In order to carry out a thorough evaluation of $TiO_2$-free coating systems and $TiO_2$-free capsules, a consortium was formed between various pharmaceutical companies (listed in Appendix A). This consortium was to work collaboratively with color mixture and capsule shell suppliers to identify potential $TiO_2$-free alternatives which would not impact the quality, safety and efficacy of medicinal products as outlined in the EC Regulation 2022/63.

This report concerns the experimental work and results from studies comparing commercially/close to commercialization $TiO_2$-free hard capsule shells with $TiO_2$ containing ones.

# $TiO_2$ – Uses and Excipient Properties

Titanium dioxide, in the high purity form used in foods and pharmaceuticals (E171), has a dual role as opacifier and colorant in tablet coatings and hard capsule shells. It is also used for similar reasons in soft gel capsules, sprinkles, suspensions and other pharmaceutical products [1][2]. ]However, its use in these products is outside of the scope of the Consortium's work.

$TiO_2$ has many useful physicochemical properties that make it an excellent opacifier and colorant for pharmaceutical products. It exists in a number of crystalline forms but only the rutile and anatase polymorphs are of commercial relevance [5][6]. $TiO_2$ has very high heat stability, both in terms of chemical stability and conversion to other crystalline forms (anatase to rutile conversion occurs at 915°C). The anatase polymorph changes color from white to grey under high energy conditions and this has been exploited for laser printing of tablets and capsules [6][8].

$TiO_2$ has a high refractive index (2.55 for anatase and 2.72 for rutile). The anatase polymorph is used mainly in pharmaceuticals as it is less hard and abrasive and results in a more lustrous finish [1][5]. The ability of $TiO_2$ to scatter visible light means that it confers a vivid, opaque, white color to tablet coatings and capsule shells, and in combination with other colorants, opacifies colored capsule shells and tablet coatings. It therefore significantly broadens the range of colors which can be obtained. This makes for elegant solid dosage forms, facilitates medicine identification, while conversely hindering counterfeiting, and prevents batch-to-batch color variations which may raise patient concerns and negatively impact on patient adherence to therapy. Its ability to opacify capsule shells enables the use of over-encapsulation as a commonly used and effective method of blinding investigational medicines for clinical trials. Titanium dioxide is also both tasteless and odourless, an important property given the role of coatings and capsules to mask taste, and $TiO_2$'s presence in the outer layers of the dosage form [1][5]

$TiO_2$ absorbs ultra-violet light [9] and this together with its scattering of visible light, plays an important role in protecting photo-sensitive drugs in solid dosage forms from degradation [5][6]. In addition, it is

chemically inert at the temperatures and conditions used during manufacturing processes and dosage form storage. $TiO_2$ is very poorly water-soluble and non-hygroscopic. Its non-hygroscopicity means that its presence in tablet coatings and capsule shells does not impact adversely on moisture-sensitive compounds. In addition, its presence neither hydrates nor dehydrates coatings or capsule shells which can lead to cracking or softening. It also does not result in an extreme acidic or alkaline microenvironment within the coat or capsule shell which could impact on acid or alkaline instable drugs or result in physical form conversion e.g., salt to base.

Overall, from a formulation perspective, $TiO_2$'s has numerous useful functional properties when it is incorporated into tablet coatings and capsule shells [5][6] and has been only rarely associated with instability of active compounds [10].

From a processing perspective, the $TiO_2$ used in pharmaceuticals has a particle size of around 200 nm but forms larger aggregates which facilitate particle flow and easy processing [5]. In addition, at the $TiO_2$ concentration used in coating suspensions (10%w/w - 30% w/w), the suspensions formed with coating polymers, plasticisers and other coating ingredients are of suitable viscosity to flow, be pumped and sprayed. Similarly at the 5%w/w concentration typically found in capsules, there is no interference with capsule formation.

Any $TiO_2$-free coating or capsule shell needs to possess many of the functional excipient properties of $TiO_2$ in a comparable way. To date very little has been published on comparing $TiO_2$-free systems with $TiO_2$ ones [6] [11]. However, both $TiO_2$-free ready-to-use admixes for the preparation of coating suspensions for tablet coating and $TiO_2$-free hard capsule shells are available from a variety of vendors.

## Objectives of Evaluation

As previously stated, this report deals with the data generated by the $TiO_2$ Alternatives Consortium comparing $TiO_2$-free hard capsule shells with $TiO_2$ containing ones. The study details and results from comparative work on $TiO_2$-free coating systems for tablet coating versus $TiO_2$-containing ones are recorded in a separate technical report [12].

The objectives of the study on capsules were as follows:

- To conduct a comprehensive evaluation of $TiO_2$-free hard capsules shells manufactured from the typical shell-forming materials, gelatin or hydroxypropylmethylcellulose (hypromellose), which were commercially available or close to being commercially available at the start of the study, February 2023. In all experiments the results were compared to samples of $TiO_2$-containing hard capsules treated in the same manner.
- To assess and compare the appearance and mechanical integrity of $TiO_2$-free capsule shells with $TiO_2$ containing ones, both prior to any conditioning (storage under ambient conditions) and following exposure to various environmental conditions (various % relative humidities or light exposure equivalent to twice that recommended for photostability studies in ICH Q1B).
- To encapsulate three different drug-excipient blends at a batch size of approximately 5000 capsules per blend. Each blend contains an active or active combination already on the European market encapsulated in gelatin-based capsules. The active compounds were selected due to their known sensitivity to certain conditions of relevance to the replacement of $TiO_2$ in the capsule shells e.g., light, moisture. The encapsulated products were assessed for appearance (visual and colorimetry), brittleness, assay, impurities, disintegration and dissolution.
- To conduct accelerated and photostability on the encapsulated product.
- To assess the manufacturing performance of the $TiO_2$-free capsule shells selected from the small-scale studies at an scale of approximately 40,000 empty capsules.

Key performance parameters for capsule evaluation included the following:

- Capsule appearance with regard to opacity – both visual appearance nd by colorimetry
- Color matching with the $TiO_2$ reference capsule shells

- Brittleness of capsule as measured by brittleness tests and observations during manufacture and stability
- In vitro performance by disintegration and dissolution times of encapsulated blends at $T_0$ and on accelerated and photostability stability
- Stability of capsule shell during accelerated and photostability studies as measured by capsule appearance and in the case of accelerated stability also capsule shell brittleness
- Ability to protect susceptible actives from chemical instability during accelerated and photostability stability studies as measured by assay, impurities, disintegration and dissolution.

All experimental work was carried out at Almac Pharma Services, Craigavon, UK on behalf of the Consortium with certain experiments outsourced to Reading Scientific Services Ltd., Reading, UK.

# Selection of Capsules for Evaluation

## Rationale for Selection

In total 17 different capsule shells of Size 0 were selected for evaluation, 13 of which were $TiO_2$-free and four which contained $TiO_2$. All of the results obtained with the $TiO_2$-free capsules were evaluated against the $TiO_2$-containing controls. In the study both $TiO_2$-free white and colored capsule shells were studied (red or orange or pink) and compared to either the white $TiO_2$-containing capsule shell control or the red ones. The selection was based on a number of criteria including the following:

- Capsule wall material constituents are either compendial or supported by an adequate safety package
- Capsule wall constituents are suitable for pediatric formulations for children of ≥ 2 years.
- Samples of capsule shells were available for Consortium evaluation before March 2023
- Capsules are available in white or orange/red/pink or both. These colors were chosen as they are typical colors offered by capsule shell suppliers and therefore are representative of capsule shells that are/will be commercially available in the near future. These colors are also were likely to be available “off-the-shelf” without further color development, an important consideration given the Consortium’s timelines.
- The composition of the capsule wall material is disclosed so that capsules can be chosen to enable evaluation of a variety of substitutes for $TiO_2$ in combination with either gelatin or hydroxypropylmethylcellulose (hypromellose) (HPMC) and/or gelling agents.
- Incorporation of capsules from a variety of vendors.
- It was not feasible to carry out a comprehensive study comparing $TiO_2$-free capsules versus $TiO_2$ containing ones on all capsule sizes. Size 0 capsules were selected for the evaluation based on availability, the fact that they are commonly used especially for clinical trials and their larger size makes them more sensitive to stress than smaller capsule sizes. Therefore, they represent a worst-case scenario for smaller capsules.

## Anonymization of Capsule Shell Details and Grouping for Analysis Purposes

Table 102 provides details of the capsule shells selected for evaluation. For confidentiality purposes the trade name and description, the vendor and full details of the composition of the capsule shells are not disclosed. Each $TiO_2$-free and $TiO_2$ containing capsule shell studied was given a Consortium Capsule Shell Reference (CAP-001 to CAP-017). Some of the alternative opacifiers have been disclosed, while others have been given an identifier letter. Red iron oxide ($Fe_2O_3$) is not an opacifier per se but contributes to opacification through its colorant properties.

With respect to analysis, the capsule shells are often grouped in tables and graphs based on whether they are composed of gelatin or hypromellose (described hereafter as hydroxypropylmethylcellulose (HPMC)). In some cases they have been grouped on whether they are white or colored capsule shells. In Sections 7, 8 and 9 the capsules filled with active ingredient (API) blends are grouped for analysis on the basis of the API blend used.

Table 102: Capsules selected for evaluation

| Consortium Capsule Shell Reference | $TiO_2$ Free (Y/N) | Color | Shell Former | Gelling Agent | Opacifier(s)[c] |
|---|---|---|---|---|---|
| CAP-001 | Y | Orange | HPMC | NA | $Fe_2O_3$ |
| CAP-002 | Y | White[d] | Gelatin | NA | Sodium Phosphates (NaPOx) |
| CAP-003 | Y | White | HPMC | NA | Calcium Carbonate ($CaCO_3$) |
| CAP-004 | Y | White | HPMC | NA | $CaCO_3$ |
| CAP-005 | Y | White | Gelatin | NA | $CaCO_3$ |
| CAP-006 | Y | White | Gelatin | NA | $CaCO_3$ + A |
| CAP-007 | Y | White | HPMC | NA | $CaCO_3$ + A |
| CAP-008 | Y | Red | HPMC | Carrageenan | $Fe_2O_3$ |
| CAP-009 | Y | White | HPMC | Carrageenan | $CaCO_3$ + A |
| CAP-010 | Y | Red | Gelatin | NA | $Fe_2O_3$ |
| CAP-011 | Y | Pink | HPMC | Carrageenan | $CaCO_3$ + B +C+D |
| CAP-012 | Y | White | Gelatin | NA | $CaCO_3$ + B+D |
| CAP-013[a] | N | White | Gelatin | NA | $TiO_2$ |
| CAP-014[b] | N | Orange | HPMC | NA | $TiO_2$ + $Fe_2O_3$ |
| CAP-015 | Y | Red | HPMC | NA | $Fe_2O_3$ |
| CAP-016[a] | N | White | HPMC | NA | $TiO_2$ |
| CAP-017[b] | N | Red | Gelatin | NA | $TiO_2$ |

[a]White $TiO_2$-containing reference capsule shells

[b]Red $TiO_2$-containing reference capsule shells

[c]Iron oxide red's main role is as a colorant. It is not an opacifier per se. However, it imparts opacity to the capsule shells through its colorant properties

[d]The consortium requested a colored version (red) of the gelatin, $TiO_2$-free capsule from the supplier. This requested capsule shell was not received for evaluation.

All capsule shell constituents are pharmacopoeial and/or food grade.

# Experimental Part 1: Mechanical Integrity of Empty Capsule Shells

## Materials

The 13 $TiO_2$-free capsule shells and 4 $TiO_2$-containing capsule shells listed in Table 102 were assessed for their mechanical integrity pre-exposure (storage under ambient conditions) and following exposure to light and various humidities.

## Methodology

### 62. Assessment Prior to Conditioning

The appearance of each capsule shell type prior to conditioning was assessed visually and by colorimetry.

### 63. Assessment Following Exposure to Light

Two samples of each capsule shell type were placed in clear containers (approximately 50 capsules per sample). One container of each capsule shell type was wrapped in aluminium foil to act as a "dark control". All samples were then exposed to light providing an overall illumination corresponding to twice that required by ICH Q1B (1 x ICH Q1B is equivalent to not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter). The exposed and control samples were assessed visually and by colorimetry.

### 64. Evaluation Following Exposure to Various Humidities

Samples of each capsule shell type were evaluated following exposure to various humidities in laboratory stability chambers. Dry conditions were achieved by placing the samples in a desiccator. The samples were exposed to the various humidities for not less than 72 hours. Capsule shells stored in the laboratory (humidity not controlled, ~50%RH), which had not undergone conditioning, were used as a reference for comparison to the shells that had undergone exposure to the various humidities.

### 65. Analytical Methods Used for Testing the Empty Capsule Shells

Table 103 shows the analytical tests carried on the $TiO_2$-free and $TiO_2$ reference empty capsule shells.

Table 103: Summary of analytical testing carried out to assess capsule shell mechanical integrity.

| Testing | Parameter | Methodology |
|---|---|---|
| Evaluation prior to conditioning | Visual appearance | Photography |
| | Appearance | Colorimetry |
| Photostability Assessment | Visual appearance | Photography |
| | Appearance | Colorimetry |
| Assessment following conditioning at various humidities with unconditioned samples as a control | Visual appearance | Photography |
| | Appearance | Colorimetry |
| | Water activity | Analyses performed immediately upon the removal from the chambers. |
| | Loss on drying | |
| | Brittleness on the empty capsule shells. | |

## 66. Analytical Methodology - Photography

Photographs of the pre-conditioned and conditioned capsule shells were carried out in the course of the colorimetry testing (see Section 67). The samples and corresponding controls from the photostability study were photographed side-by-side.The camera used was a Nikon DX, in auto mode, focus 45-35mm. The distance from camera to sample was approximately 28-30cm.

## 67. Analytical Methodology - Colorimetry

The color of the empty capsule shells prior to conditioning, from the photostability and conditioning under a range of humidities was carried out using DigiEye equipment. The DigiEye equipment consists of a D65 a with Nikon\Nikkor Z f/4-6.3 VR Lens for image capture.

For each sample 20 capsule shells were placed into the custom capsule holder The holder was then placed centrally in the DigiEye equipment and photographed using a calibrated camera with a 105mm focal length zoomed to the size of the holder. The average results for the L*, a*, b*, chroma and hue angle values were then calculated from the twenty total samples. These values are measurements of the following:

L* Lightness on a scale of 0 black/total absorption to 100 white/total reflection.

a* Red/green value from negative 100 as green to positive 100 as red values.

b* Yellow/blue value from negative 100 as blue to positive 100 as yellow values.

C Specifies chroma which describes the vividness of color.

h h specifies hue angle which is how the color is perceived.

$\Delta E^*_{00}$ Total color difference value.

The $\Delta E^*_{00}$ values for the photostability samples and corresponding controls and for conditioned samples and corresponding controls were calculated using the Delta E 2000 equation [13]:

$$\Delta E^*_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H}}$$

This International Commission on Illumination (CIE) equation provides the most accurate color difference values currently available. It was also used to compare the color of white TiO2-free capsule shells and the corresponding white TiO2-containing reference prior to conditioning.

The $\Delta E^*_{00}$ were interpreted as follows [14]:

White Capsules

For white capsules the TiO2-free capsule shells were determined to match the color of the corresponding $TiO_2$ reference if $\Delta E^*00 \leq 1.0$. $\Delta E^*_{00} \leq 1.0$ is considered to mean a color difference which is not perceptible to the eye. A $\Delta E^* > 1.0$ was considered to be noticeable to a patient.

Colored Capsules

For colored capsules, the $\Delta E^*_{00}$ values were interpreted as follows:

$\Delta E^*_{00} \leq 1.0$ Color difference not perceptible to the human eye

$\Delta E^*_{00}$ 1 - 2 Color difference perceptible through close observation.

$\Delta E^*_{00} > 2$ Color difference noticeable at a glance

The acceptance criterion for color matching of colored $TiO_2$-free capsule shells to the corresponding $TiO_2$ reference was $\Delta E^*_{00} < 2$.

Rationale for Differences in Acceptance Criteria for White and Colored Capsules

White is a color associated in many cultures with cleanliness, purity and the health professions. White surfaces reflect light back to the human eye, while colored surfaces reflect only a portion. Therefore, surface imperfections and color differences are more perceptible to the human eye when two white objects are being compared than the comparison is made between two colored objects.

## 68. Analytical Methodology – Brittleness

The brittleness assessment is an analytical procedure commonly employed by hard capsule shell manufacturers to assess the mechanical integrity of a capsule shell. This method can also be used to simulate de-blistering of a product. It was carried out individually on 50 empty capsules of each shell type according to the following protocol:

One closed capsule was placed on a hard, flat surface and a 10 cm tube placed over it. A calibrated 100 g weight was released from the top of the tube onto the capsule. If the capsule shell film cracked or shattered, then the capsule was considered brittle. If it only deformed, then it was assessed as non-brittle. The % of brittle capsule shells in the 50 capsule shell sample was calculated. The analysis was conducted immediately after removal of the sample from the humidity chambers.

The acceptance criterion was set at ≤ 4% brittle capsules at %RH ≥ 33%RH. This acceptance criterion was set based on a review of documentation from a variety of capsule manufacturers and equates to a maximum of 2 brittle capsules per 50 sampled.

Table 104: Acceptance criteria for % brittle capsule shells

| Storage Conditions | Evaluation | |
|---|---|---|
| | Satisfactory | Not Satisfactory |
| Desiccated (0% RH) | NA | NA |
| 11% RH | | |
| 23% RH | | |
| 33% RH | ≤4% (NMT 2 brittle capsules per 50) | >4% (> 2 brittle capsules per 50) |
| Laboratory storage[a] | ≤4% (NMT 2 brittle capsules per 50) | >4% (> 2 brittle capsules per 50) |
| 53% RH | ≤4% (NMT 2 brittle capsules per 50) | >4% (> 2 brittle capsules per 50) |

[a]The RH in the Almac laboratory is not controlled but is approximately 50% RH.

## 69. Analytical Methodology – Loss on Drying and Water Activity

Loss on drying was conducted using a calibrated Halogen Moisture Analyser and aluminium sample pans. Water activity was measured using an Aqualab 4TE water activity analyser which was calibrated on each day of analysis with 0.250 $a_W$ and a 0.984 $a_W$ standards.

For both the loss on drying and water activity measurements, the conditioned samples were analyzed immediately after removal of the samples from the humidity chambers. If this was not possible for the water activity samples, the capsules were covered with a lid to minimize water transfer.

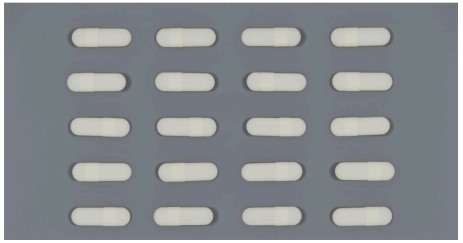
## Results and Discussion

### 70. Capsule Shell Appearance – Pre-conditioning

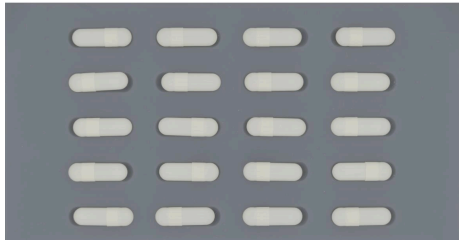
Figure 50 and Figure 51 show respectively the photographs of the gelatin and HPMC-based capsule shells stored at laboratory ambient temperature. For the white gelatin capsule shells, the photographs show that CAP-005 and CAP-006, which both contain $CaCO_3$ as the only opacifier, are more translucent and much less opaque than either the CAP-013, the $TiO_2$-containing reference or CAP-002 and CAP-012 which contain more than one opacifier. Similarly, the white $CaCO_3$ containing $TiO_2$-free HPMC capsule shells (CAP- 003, CAP-004, CAP-007 and CAP-009) are more translucent than the $TiO_2$ reference. The visual evaluation of the red/orange colored gelatin and HPMC capsule shells would suggest that a similar level of opaqueness and coloring can be achieved with red iron oxide without the addition of $TiO_2$. However, the pink capsules, CAP-011, containing $CaCO_3$ plus other opacifiers are more translucent compared with the other colored capsule shells which use iron oxides for color and opacity.

Figure 50: $TiO_2$-free and $TiO_2$ containing gelatin capsule shells

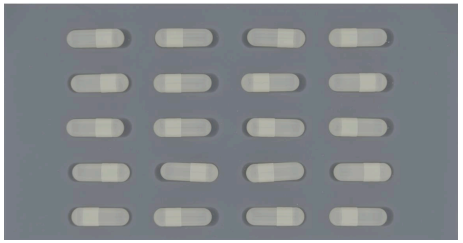
CAP-013-$TiO_2$ reference



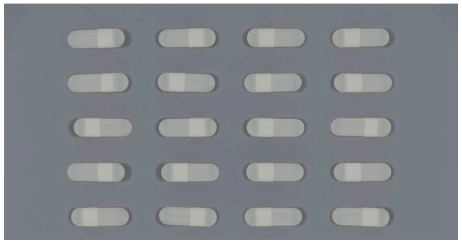
CAP-002



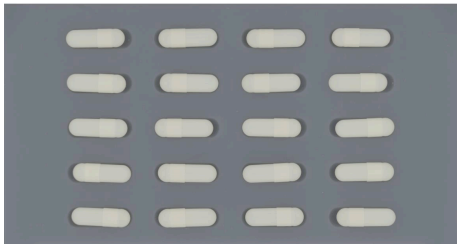
CAP-005



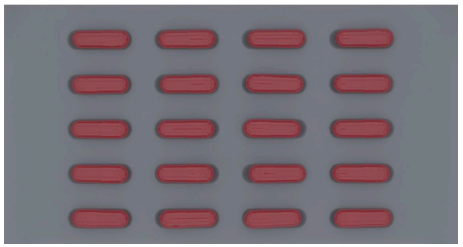
CAP-006



CAP-012



CAP-017-$TiO_2$ reference



CAP-010

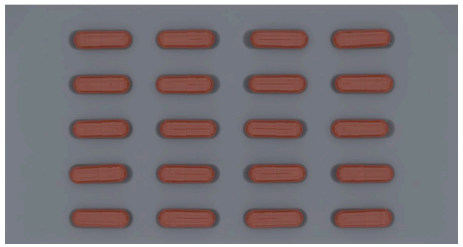
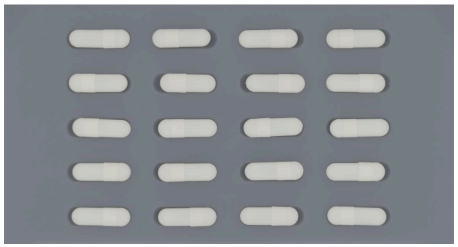
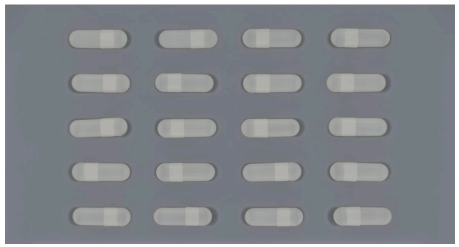


Figure 51: $TiO_2$-free and $TiO_2$ containing HPMC capsule shell

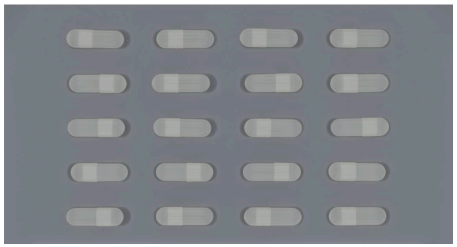
CAP-016-$TiO_2$ reference



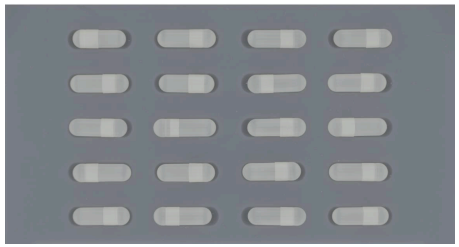
CAP-003



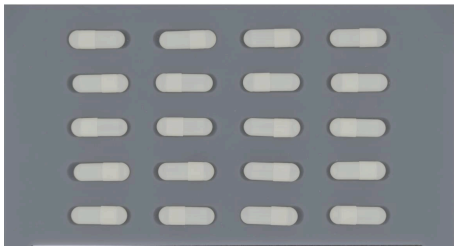
CAP-004



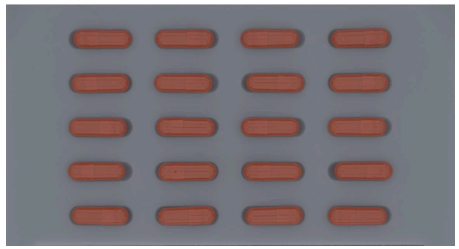
CAP-007



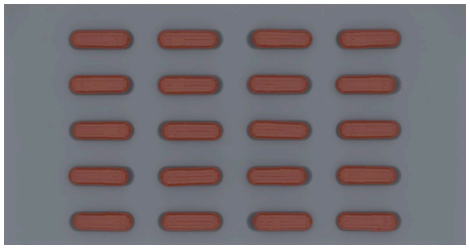
CAP-009



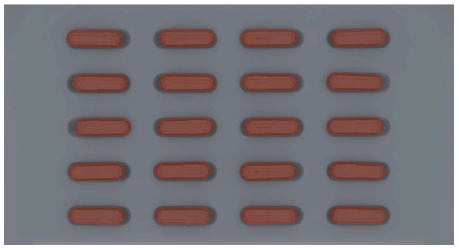
CAP-014-$TiO_2$ reference



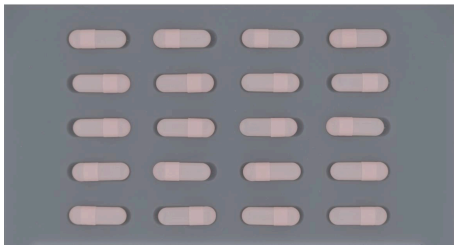
CAP-001



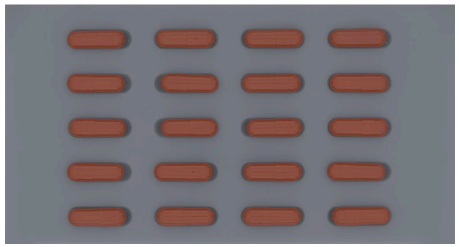
CAP-008



CAP-011



CAP-015



## 71. Colorimetry – Pre-conditioning

In order to evaluate the differences in color and opacity further between the $TiO_2$-free capsule shells and the corresponding $TiO_2$ reference, colorimetry was carried out on samples from each of the 17 different capsule shells as described in Section 67. This comparison is shown in les.

Figure 52 for the white gelatin capsule shells. Figure 53 shows the comparison for the HPMC-based capsules.

Figure 52: Colorimetry data – white gelatin $TiO_2$-free versus $TiO_2$ containing capsule shells

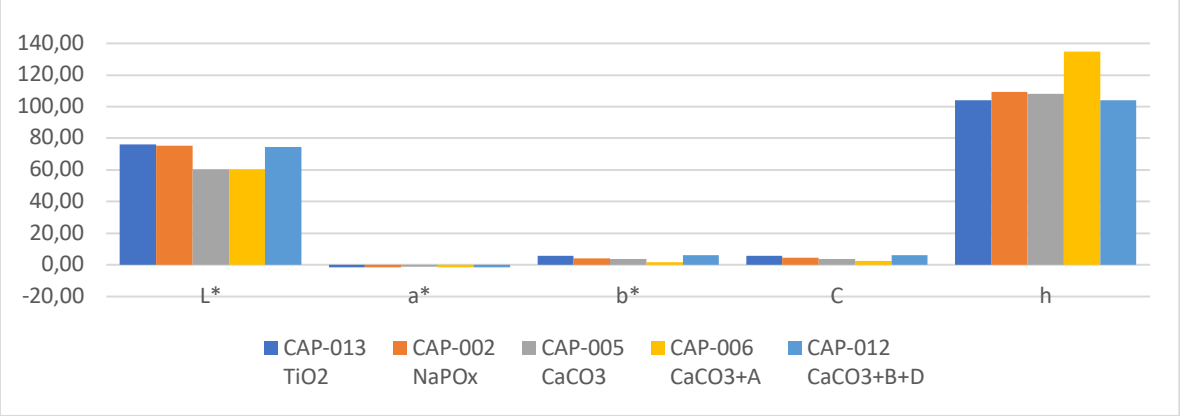
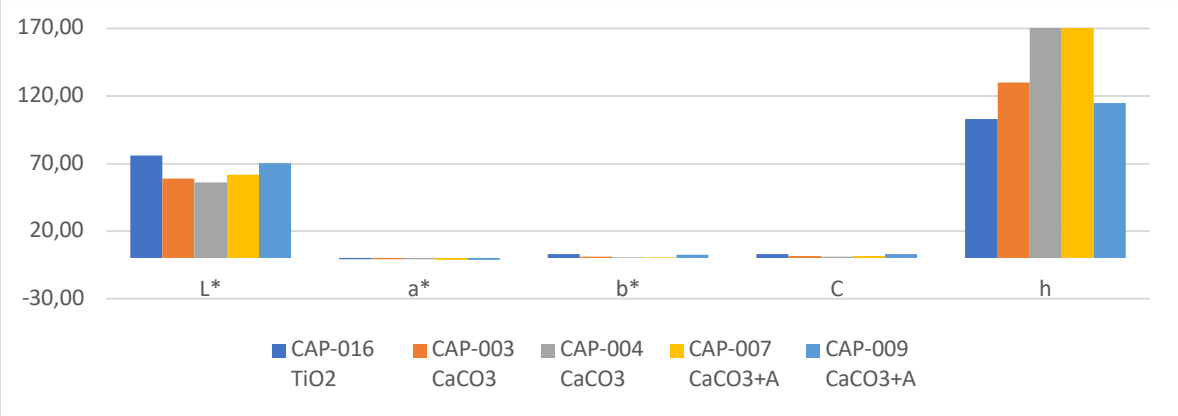


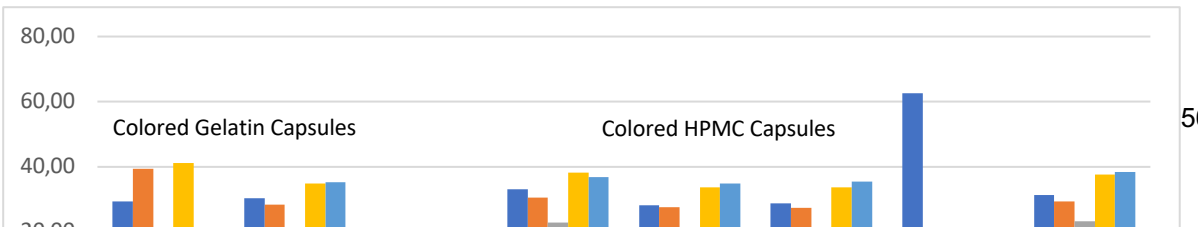

Figure 53: Colorimetry data – white HPMC $TiO_2$-free versus $TiO_2$ containing capsule shells




For both white gelatin and HPMC capsule shells the values of a*, b* and chroma are low and only vary slightly. The L* values at approximately 76 are highest for both the gelatin and HPMC $TiO_2$ reference capsule shells as would be expected given their opacity. For the gelatin capsule shells, CAP-002 and CAP-012, have the next highest L* values at 75 and 74 which are only slightly lower and align with their visual appearance. The $CaCO_3$ containing capsule shells, CAP-005 and CAP-006, have significantly lower L* values at around 60. With respect to the hue angle, only CAP-006, which contains $CaCO_3$ plus opacifier A, is significantly higher (135) than that of the other capsules (104-109). For the HPMC capsule shells, CAP-009 had the next highest L* value at approx. 70 compared with 76 for the $TiO_2$ reference. while the other capsule shells had L* values varying between 56 and 62. This again fits with the appearance data as CAP-003, CAP-004 and CAP-007 are more translucent and not so opaque as CAP-016 or CAP-009. In general, the $TiO_2$-free HPMC capsule shells have a higher hue angle than their gelatin counterparts. CAP-004 and CAP-007 have the highest hue angle at 170, while the hue angles of the $TiO_2$ reference gelatin and HPMC capsule shells are very similar (104 and 103 respectively).

Figure 54 shows the comparison of the colorimetry data for colored $TiO_2$-free capsule shells versus the $TiO_2$ references.

Figure 54: Colorimetry data – Colored gelatin & HPMC $TiO_2$-free versus $TiO_2$ containing capsule shells



CAP-017 and CAP-014 are the $TiO_2$ reference capsules.

As expected for the colored capsules, the L* values are lower and the a* and b* values higher than those for the white capsules in Figure 52 and Figure 53. The highest L* value and lowest a* and b* values are found for CAP-O11, which is a pink capsule. Table 105 shows the $\Delta E^*_{00}$ values for both the white gelatin and white HPMC $TiO_2$-free capsule shells compared to their corresponding $TiO_2$ containing reference.

Table 105: $\Delta E^*_{00}$ values for white gelatin and HPMC $TiO_2$-free capsules shells versus the $TiO_2$ reference.

| **Gelatin Capsule Shells** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Consort. Cap Shell Ref.** | **Opacifier** | **L*** | **a*** | **b*** | **C** | **h** | **$\Delta E^*_{00}$** |
| CAP-013[a] | $TiO_2$ | 76.17 | 1.42 | 5.61 | 5.78 | 104.20 | NA |
| CAP-002 | NaPOx | 75.39 | -1.47 | 4.21 | 4.46 | 109.28 | 1.34 |
| CAP-005 | $CaCO_3$ | 60.41 | -1.16 | 3.55 | 3.74 | 108.08 | 12.56 |
| CAP-006 | $CaCO_3$+A | 60.41 | -1.65 | 1.65 | 2.33 | 134.94 | 12.92 |
| CAP-012 | $CaCO_3$+B+D | 74.37 | -1.48 | 5.98 | 6.16 | 103.91 | 1.38 |
| **HPMC Capsule Shells** | | | | | | | |
| **Consort. Cap Shell Ref.** | **Opacifier** | **L*** | **a*** | **b*** | **C** | **h** | **$\Delta E^*_{00}$** |
| CAP-016[a] | $TiO_2$ | 75.98 | -0.66 | 2.87 | 2.95 | 102.92 | NA |
| CAP-003 | $CaCO_3$ | 59.08 | -0.89 | 1.06 | 1.38 | 130.10 | 13.59 |
| CAP-004 | $CaCO_3$ | 55.93 | -0.99 | 0.17 | 1.00 | 170.41 | 16.50 |
| CAP-007 | $CaCO_3$+A | 61.50 | -1.45 | 0.24 | 1.47 | 170.47 | 11.70 |
| CAP-009 | $CaCO_3$+A | 70.03 | -1.26 | 2.71 | 2.99 | 114.92 | 4.54 |

Color Code: Green = $\Delta E^*_{00} \leq 1$, Yellow = $\Delta E^*_{00}$ = 1-2 and Red = $\Delta E^*_{00} > 2$

[a]$TiO_2$ reference capsules

The $\Delta E^*_{00}$ values in Table 105 show that none of the $TiO_2$-free capsule shells had $\Delta E^*_{00} < 1$ compared to the HPMC $TiO_2$ reference (CAP-013), and only CAP-002 containing NaPOx and CAP-012 containing both $CaCO_3$ + B + D had $\Delta E^*_{00}$ values less than 2 (1.34 and 1.38). This means that there is a color/opacity difference between these capsules and the $TiO_2$ reference. However, it can only be perceived on close observation. As expected, none of the HPMC $TiO_2$-free capsules had a $\Delta E^*_{00} < 2$ when compared to the $TiO_2$-containing HPMC reference (CAP-016) and it is immediately obvious that the capsule shells differ visually from it.

The colored capsule shells are described by their manufacturers as being of different colors or shades of color e.g., red, orange and pink. Therefore, only a limited $\Delta E^*_{00}$ comparison was carried out on CAP-001 versus the corresponding $TiO_2$ reference capsule shell, CAP-014. These two capsule shells are both HPMC-based, sourced from the same vendor and product line and are described by the vendor as

having the same shade of orange. However, the $\Delta E^*_{00}$ color difference value was 4.37. This result shows how difficult it can be to color match $TiO_2$-free and $TiO_2$ containing capsule shells even for experienced capsule manufacturers. All other $\Delta E^*_{00}$ comparisons between the red/orange capsules and the corresponding $TiO_2$ reference shell yielded values > 2. However, based on the manufacturers' descriptions of the color or shade of color, a color match was not anticipated.

### 72. Conclusions from the Work on Capsule Shells Prior to Conditioning

Based on visual appearance and colorimetry, none of the white $TiO_2$-free capsules were as opaque as the corresponding $TiO_2$ containing reference capsule shells, making them less suitable for camouflaging underlying color differences in capsule contents or as use for blinding medication for clinical trial purposes. CAP-002 and CAP-012 were the most opaque of the $TiO_2$-free capsule shells. However, neither of these were an exact color match to CAP-013, the gelatin-based $TiO_2$ reference, with $\Delta E^*_{00}$ between 1-2 meaning that differences would be perceptible on close inspection.

All of the red/orange $TiO_2$-free capsule shells were visually opaque. CAP-011, the pink $TiO_2$-free capsule shell was not. However, the most similar in terms of description, composition and vendor and product line were not a color match based on colorimetry data.

### 73. Colorimetry and Photography – Photostability Empty Capsule Shells

Table 106 shows the visual appearance description and colorimetry data comparison for the exposed and dark control samples of the $TiO_2$-free and $TiO_2$ containing gelatin capsule shells. Table 107 shows the same data for the HPMC capsule shells. For the white gelatin capsule shells, the $TiO_2$ reference, CAP-013, and the $TiO_2$-free capsule shells, CAP-002 and CAP-012, showed a slight change in color following exposure and this was in alignment with their $\Delta E^*_{00}$ between 1 and 2. CAP-005 and CAP-006, which contain $CaCO_3$, had $\Delta E^*_{00}$ which is considered to mean that the color difference between the light exposed capsule shells and the control is imperceptible. However, for CAP-006 with a $\Delta E^*_{00}$ value of 0.83, a visible difference could be detected with the light exposed capsule being more translucent.

In order to investigate these differences further, the $\Delta L^*$, $\Delta a^*$, $\Delta b^*$, $\Delta C^*$ and $\Delta h^*$ for all of the gelatin capsules were plotted as shown in Figure 55. These data show that for most of the light exposed gelatin capsule shells the values of L*, a*, b*, chroma and hue angle decrease to varying extents or increase only slightly compared with the dark controls.

The hue angle for CAP-006 increased significantly following light exposure, while the other color parameters remain similar or slightly less than for its control. This change in hue angle may explain why the capsule shell appears more transparent than its corresponding control, despite having a $\Delta E^*_{00} < 1$.

No visible difference could be detected between the exposed and control samples for the gelatin $TiO_2$ red reference, CAP-017 and the $TiO_2$-free alternative capsule shell, CAP-010.

Table 106: Colorimetry and visual appearance results on gelatin-based capsule shells from the photostability study

| Consort. Cap Shell Reference | RSSL Ref. P23- | Treatment | Opacifier | L* | a* | b* | C | h | ΔE*$_{00}$ | Visual Comparison Exposed vs Dark Control |
|---|---|---|---|---|---|---|---|---|---|---|
| CAP-013 (White $TiO_2$ reference) | 05586-102 | Exposed | $TiO_2$ | 75.24 | -1.04 | 3.99 | 4.12 | 104.56 | 1.58 | Exposed sample has a slightly lighter color |
| | 05586-103 | Dark Control | | 76.43 | -1.43 | 5.46 | 5.65 | 104.70 | | |
| CAP-002 | 05586-32 | Exposed | NaPOx | 77.59 | -0.89 | 3.39 | 3.51 | 104.62 | 1.27 | Exposed sample has a slightly lighter color |
| | 05586-33 | Dark Control | | 76.51 | -1.33 | 4.31 | 4.51 | 107.10 | | |
| CAP-005 | 05586-46 | Exposed | $CaCO_3$ | 60.84 | -0.95 | 3.33 | 3.46 | 105.96 | 0.65 | No visible difference |
| | 05586-47 | Dark Control | | 60.55 | -1.14 | 3.66 | 3.83 | 107.35 | | |
| CAP-006 | 05586-39 | Exposed | $CaCO_3$+A | 60.71 | -1.44 | 0.96 | 1.73 | 146.40 | 0.83 | Exposed sample more transparent |
| | 05586-40 | Dark Control | | 60.38 | -1.59 | 1.73 | 2.36 | 132.60 | | |
| CAP-012 | 05586-53 | Exposed | $CaCO_3$ +B+D | 74.60 | -1.09 | 4.43 | 4.56 | 103.89 | 1.42 | Exposed sample has a slightly lighter color |
| | 05586-54 | Dark Control | | 74.46 | -1.46 | 6.05 | 6.23 | 103.58 | | |
| CAP-017 (Red $TiO_2$ reference) | 05586-110 | Exposed | $TiO_2$ | 28.85 | 37.59 | 11.02 | 39.17 | 16.34 | 1.08 | No visible difference |
| | 05586-111 | Dark Control | | 29.71 | 39.76 | 11.77 | 41.47 | 16.49 | | |
| CAP-010 | 05586-60 | Exposed | $Fe_2O_3$ | 30.28 | 28.09 | 19.59 | 34.25 | 34.88 | 0.36 | No visible difference |
| | 05586-61 | Dark Control | | 30.40 | 28.41 | 20.00 | 34.75 | 35.15 | | |

Color code: Green = ΔE* ≤ 1 (no perceptible color difference), Yellow = ΔE* 1-2 (color difference perceptible on close inspection), Red = ΔE* > 2 (color difference easily perceptible).

Acceptance criteria> White capsules ΔE* ≤ 1, ΔE* < 2 Colored capsules

Iron oxide is a colorant and not an opacifier per se.

Table 107: Colorimetry and visual appearance results on HPMC-based capsule shells from the photostability study

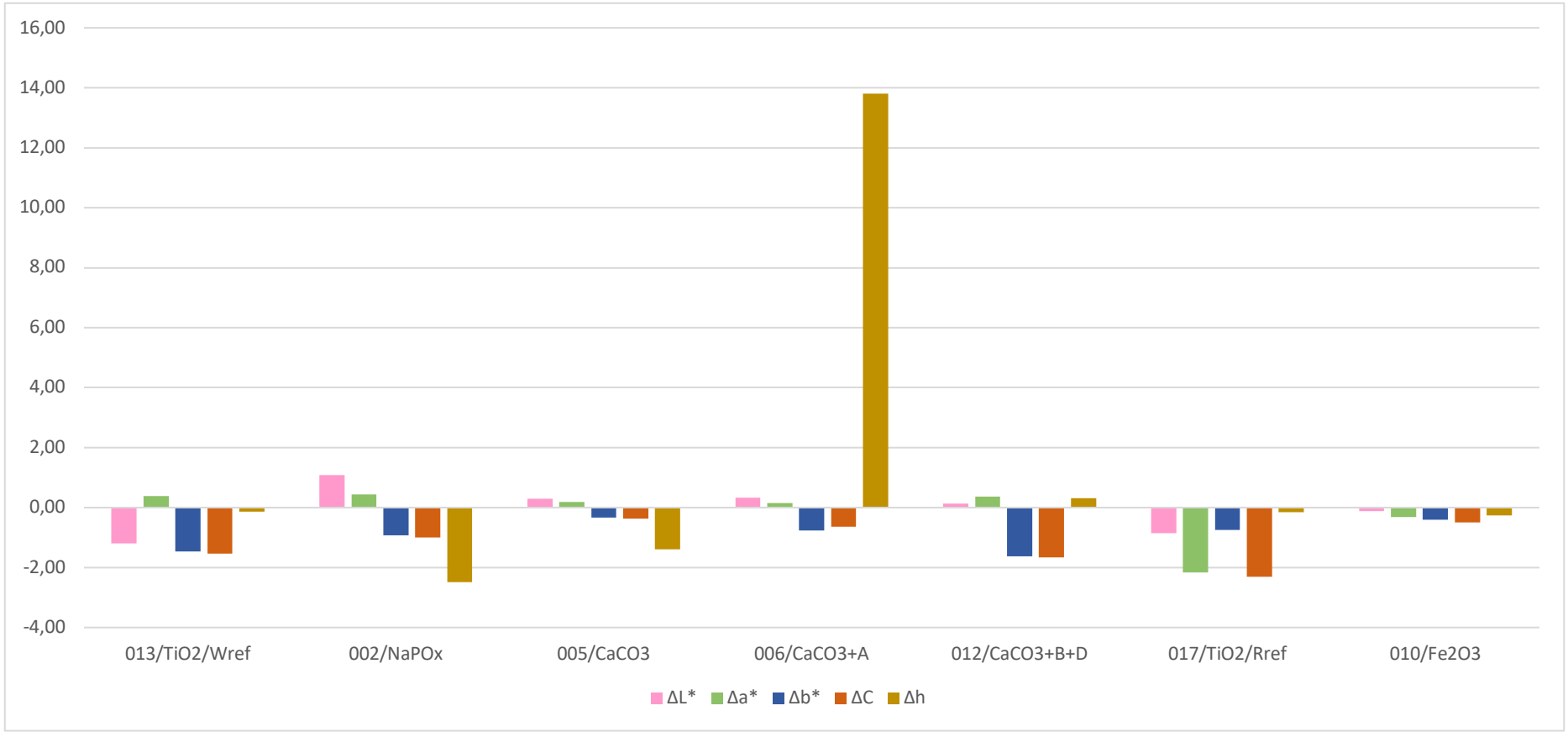
| Consort. Cap Shell Ref. | RSSL Ref. No. P23- | Treatment | Opacifier | L* | a* | b* | C | h | ΔE*$_{00}$ | Visual Comparison Exposed vs Control |
|---|---|---|---|---|---|---|---|---|---|---|
| CAP-016 (White $TiO_2$ reference) | 05586-108 | Exposed | $TiO_2$ | 77.34 | -0.82 | 0.24 | 0.85 | 163.86 | 2.90 | Exposed sample has a slightly lighter color |
| | 05586-109 | Dark Control | | 75.52 | -0.65 | 2.93 | 3.00 | 102.46 | | |
| CAP-003 | 05586-67 | Exposed | $CaCO_3$ | 60.27 | -1.01 | -2.01 | 2.25 | 243.31 | 2.87 | Exposed sample is more transparent |
| | 05586-68 | Dark Control | | 59.01 | -0.82 | 0.68 | 1.07 | 140.31 | | |
| CAP-004 | 05586-88 | Exposed | $CaCO_3$ | 56.95 | -0.99 | -2.61 | 2.79 | 249.28 | 2.69 | Exposed sample has a slightly lighter color |
| | 05586-89 | Dark Control | | 55.86 | -0.89 | 0.02 | 0.89 | 179.02 | | |
| CAP-007 | 05586-81 | Exposed | $CaCO_3$+A | 62.49 | -1.32 | -2.97 | 3.24 | 246.07 | 3.26 | Exposed sample has a slightly lighter color |
| | 05586-82 | Dark Control | | 60.94 | -1.33 | 0.16 | 1.34 | 173.28 | | |
| CAP-009 | 05586-74 | Exposed | $CaCO_3$+A | 71.01 | -1.14 | -0.21 | 1.16 | 190.33 | 2.95 | Exposed sample has a slightly lighter color |
| | 05586-75 | Dark Control | | 69.99 | -1.17 | 2.79 | 3.03 | 112.81 | | |
| CAP-014 (Red $TiO_2$ reference) | 05586-104 | Exposed | $TiO_2$+$Fe_2O_3$ | 32.90 | 30.54 | 22.57 | 37.98 | 36.47 | 0.30 | No visible difference |
| | 05586-105 | Dark Control | | 32.85 | 30.44 | 22.69 | 37.97 | 36.70 | | |
| CAP-001 | 05586-18 | Exposed | $Fe_2O_3$ | 28.01 | 27.61 | 19.12 | 33.58 | 34.71 | 0.29 | No visible difference |
| | 05586-19 | Dark Control | | 27.96 | 27.61 | 18.96 | 33.49 | 34.48 | | |
| CAP-008 | 05586-25 | Exposed | $Fe_2O_3$ | 28.80 | 27.33 | 19.30 | 33.46 | 35.22 | 0.22 | No visible difference |
| | 05586-26 | Dark Control | | 28.74 | 27.40 | 19.46 | 33.61 | 35.38 | | |
| CAP-011 | 05586-95 | Exposed | $CaCO_3$+B+C+D | 66.10 | 3.14 | -1.21 | 3.36 | 338.98 | 7.17 | Significant change in color. Exposed sample now presents as white instead of pink |
| | 05586-96 | Dark Control | | 62.47 | 8.75 | 1.79 | 8.94 | 11.54 | | |
| CAP-015 | 05586-106 | Exposed | $Fe_2O_3$ | 31.16 | 29.37 | 22.90 | 37.24 | 37.94 | 0.31 | No visible difference |
| | 05586-107 | Dark Control | | 31.22 | 29.58 | 23.04 | 37.49 | 37.92 | | |

Color code: Green = ΔE* ≤ 1 (no perceptible color difference), Yellow = ΔE* 1-2 (color difference perceptible on close inspection), Red = ΔE* > 2 (color difference easily perceptible).

Acceptance criteria> White capsules ΔE* ≤ 1, ΔE* < 2 Colored capsules

Iron oxide is a colorant and not an opacifier per se.

Figure 55: Changes in color parameters following light exposure - gelatin capsule shells



The appearance of all white HPMC light-exposed capsules including the HPMC-$TiO_2$ reference, CAP-016, changed noticeably following light exposure and had $\Delta E^*_{00}$ values > 2. No visible difference was observed between any of colored HPMC capsules with the exception of CAP-011. In addition, for these red/orange capsules all of the $\Delta E^*_{00}$ values were < 0.5.

CAP-011 contains $CaCO_3$ and a number of other opacifiers. Following light exposure, it turned from pink to white as can be seen from Figure 56 below.

Figure 56: CAP-011 photostability sample (left) versus control (right)

## 74. Conclusions from the Work on the Photostability of the Empty Capsule Shells

Based on the visual and colorimetric data, the appearance of the gelatin-based white $TiO_2$-free and $TiO_2$ reference capsule shells changed only slightly or not at all following extreme light exposure. In contrast, the appearance of the white HPMC-based capsule shells, changed noticeably. This change was observed for both the white $TiO_2$-free and the $TiO_2$ reference capsule shells. Since many of the same opacifiers were used in both the white gelatin-based and HPMC-based capsule shells, this would suggest that this change is related to HPMC being used as the shell former

For both red/orange gelatin-based and HPMC-based $TiO_2$-free and $TiO_2$ reference capsule shells, there was no change in appearance and all $\Delta E^*_{00}$ values were close to 1 or <1. These $TiO_2$-free red/orange capsule shells contain red iron oxide and it is believed it is the presence of this intensely colored material that is responsible for preventing perceptible color change on extreme light exposure. The pink CAP-011, $TiO_2$-free capsule's bleached appearance following the photostability reflects how challenging it will be to produce colored $TiO_2$-free capsule shells without the use of $Fe_2O_3$.

## 75. Colorimetry – Capsules Conditioned at Different Humidities

Table 108, Table 109, Table 110 and Table 111 show the colorimetry data from the capsule shells stored at different humidities compared with those stored at ambient temperature in the laboratory.

Table 108: Colorimetry results on white gelatin capsule shells from the conditioning studies

| Consort. Cap Shell Ref. | RSSL Ref.P23-05586- | Condition | L* | a* | b* | C | h | ΔE*$_{00}$ |
|---|---|---|---|---|---|---|---|---|
| CAP-013 (White $TiO_2$ reference) | 13 | Ambient | 76.17 | -1.42 | 5.61 | 5.78 | 104.20 | NA |
| | 119 | Desiccated | 76.50 | -1.45 | 6.51 | 6.67 | 102.57 | 0.82 |
| | 124 | 11% RH | 76.54 | -1.47 | 6.53 | 6.69 | 102.67 | 0.85 |
| | 131 | 23% RH | 76.55 | -1.43 | 6.43 | 6.58 | 102.57 | 0.79 |
| | 112 | 33% RH | 76.54 | -1.49 | 5.83 | 6.01 | 104.35 | 0.43 |
| | 113 | 53% RH | 76.42 | -1.49 | 5.75 | 5.94 | 104.52 | 0.37 |
| CAP-002 | 2 | Ambient | 75.39 | -1.47 | 4.21 | 4.46 | 109.28 | NA |
| | 34 | Desiccated | 78.04 | -1.31 | 5.10 | 5.27 | 104.39 | 2.09 |
| | 35 | 11% RH | 77.23 | -1.35 | 4.88 | 5.06 | 105.46 | 1.49 |
| | 36 | 23% RH | 76.73 | -1.43 | 4.80 | 5.01 | 106.56 | 1.12 |
| | 37 | 33% RH | 76.08 | -1.49 | 4.58 | 4.81 | 108.02 | 0.68 |
| | 38 | 53% RH | 74.11 | -1.72 | 4.28 | 4.61 | 111.93 | 1.04 |
| CAP-005 | 5 | Ambient | 60.41 | -1.16 | 3.55 | 3.74 | 108.08 | NA |
| | 48 | Desiccated | 61.16 | -1.10 | 3.56 | 3.72 | 107.21 | 0.68 |
| | 49 | 11% RH | 61.06 | -1.08 | 3.40 | 3.57 | 107.59 | 0.66 |
| | 50 | 23% RH | 60.74 | -1.08 | 3.41 | 3.58 | 107.50 | 0.42 |
| | 51 | 33% RH | 60.83 | -1.04 | 3.26 | 3.43 | 107.69 | 0.55 |
| | 52 | 53% RH | 60.64 | -1.05 | 3.22 | 3.38 | 108.09 | 0.51 |
| CAP-006 | 6 | Ambient | 60.41 | -1.65 | 1.65 | 2.33 | 134.94 | NA |
| | 41 | Desiccated | 60.96 | -1.54 | 1.50 | 2.15 | 135.63 | 0.58 |
| | 42 | 11% RH | 60.77 | -1.48 | 1.45 | 2.07 | 135.57 | 0.51 |
| | 43 | 23% RH | 60.80 | -1.54 | 1.55 | 2.19 | 134.76 | 0.47 |
| | 44 | 33% RH | 60.67 | -1.53 | 1.45 | 2.10 | 136.46 | 0.47 |
| | 45 | 53% RH | 60.82 | -1.53 | 1.54 | 2.18 | 134.85 | 0.51 |
| CAP-012 | 12 | Ambient | 74.37 | -1.48 | 5.98 | 6.16 | 103.91 | NA |
| | 55 | Desiccated | 74.82 | -1.53 | 6.33 | 6.51 | 103.58 | 0.53 |
| | 56 | 11% RH | 74.73 | -1.53 | 6.35 | 6.53 | 103.51 | 0.49 |
| | 57 | 23% RH | 74.67 | -1.51 | 6.26 | 6.44 | 103.59 | 0.45 |
| | 58 | 33% RH | 74.74 | -1.49 | 6.05 | 6.23 | 103.86 | 0.38 |
| | 59 | 53% RH | 74.71 | -1.50 | 6.06 | 6.24 | 103.91 | 0.40 |

Color code: Green = ΔE* ≤ 1 (no perceptible color difference), Yellow = ΔE* 1-2 (color difference perceptible on close inspection), Red = ΔE* > 2 (color difference easily perceptible).

Acceptance criteria> White capsules ΔE* ≤1,

With the exception of CAP-002, the $\Delta E^*_{00}$ values calculated for capsule shells stored under the different relative humidity conditions compared with those at laboratory ambient are all < 1. In general, both $\Delta E^*$ values were highest under desiccated conditions and reduced as the % RH increases. Therefore, relative humidity does not significantly influence both $\Delta E^*_{00}$ values for CAP-013, the $TiO_2$ reference gelatin capsule or the $TiO_2$-free capsule shells, CAP-005, CAP-006 and CAP-012. However, the $\Delta E^*_{00}$ values and the appearance of CAP-002 varied significantly with the relative humidity and were highest at > 2 under desiccated conditions (see Figure 57). For this capsule the $\Delta E^*_{00}$ values compared with ambient storage are only < 1 for the sample stored a 33% RH.

Figure 57: CAP-002 stored under desiccated conditions (left) versus ambient (right)

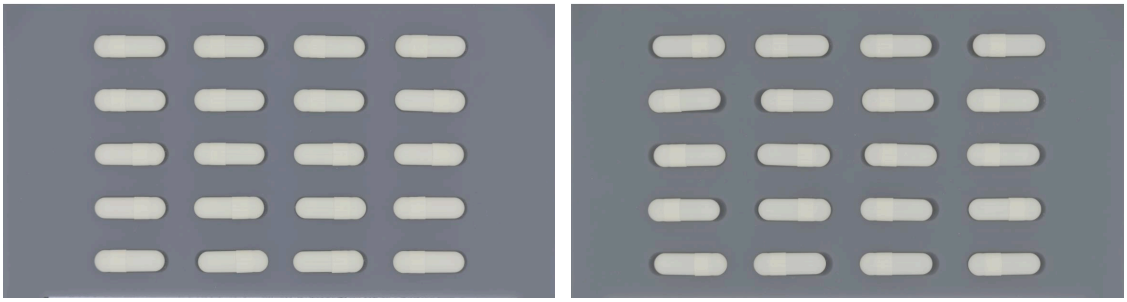


Table 109: Colorimetry results on colored gelatin capsule shells from the conditioning studies

| Consort. Capsule Shell Ref. | RSSL Ref. P23-05586- | Condition | L* | a* | b* | C | h | $\Delta E^*_{00}$ |
|---|---|---|---|---|---|---|---|---|
| CAP-017 (Red $TiO_2$ reference) | 17 | Ambient | 29.28 | 39.35 | 11.69 | 41.05 | 16.54 | NA |
| | 123 | Desiccated | 29.16 | 37.84 | 12.18 | 39.75 | 17.84 | 0.80 |
| | 127 | 11% RH | 29.36 | 38.47 | 12.49 | 40.45 | 17.98 | 0.73 |
| | 133 | 23% RH | 29.30 | 38.68 | 12.59 | 40.68 | 18.03 | 0.74 |
| | 136 | 33% RH | 29.45 | 39.07 | 12.75 | 41.10 | 18.08 | 0.74 |
| | 118 | 53% RH | 29.53 | 39.19 | 11.97 | 40.98 | 16.99 | 0.39 |
| CAP-010 | 10 | Ambient | 30.41 | 28.42 | 20.09 | 34.80 | 35.25 | NA |
| | 62 | Desiccated | 30.26 | 28.86 | 18.88 | 34.49 | 33.19 | 0.95 |
| | 63 | 11% RH | 30.37 | 28.96 | 18.99 | 34.63 | 33.26 | 0.91 |
| | 64 | 23% RH | 30.40 | 29.06 | 19.11 | 34.78 | 33.32 | 0.88 |
| | 65 | 33% RH | 30.36 | 29.14 | 19.21 | 34.90 | 33.39 | 0.86 |
| | 66 | 53% RH | 30.46 | 29.22 | 19.36 | 35.05 | 33.53 | 0.81 |

Color code: Green = $\Delta E^* \leq 1$ (no perceptible color difference), Yellow = $\Delta E^*$ 1-2 (color difference perceptible on close inspection), Red = $\Delta E^* > 2$ (color difference easily perceptible).

Acceptance criteria for the colored capsules $\Delta E^* < 2$

The $\Delta E^*_{00}$ values of the red $TiO_2$ reference, CAP-017, and the $TiO_2$-free CAP-010 were <1. For conditioning at 53% RH the difference between the colorimetry data for the conditioned reference sample and the ambient stored control falls to around 0.4. However, for CAP-010 the values remain close to 1. Overall, relative humidity does not have a significant effect on the appearance of the colored gelatin capsules as the color of the red iron oxide is so intense.

Table 110: Colorimetry results on white HPMC capsule shells from the conditioning studies

| Consort. Cap Shell Reference | RSSL Ref. P23-05586- | Condition | L* | a* | b* | C | H | ΔE*00 |
|---|---|---|---|---|---|---|---|---|
| CAP-016 (White TiO2 reference) | 16 | Ambient | 75.98 | -0.66 | 2.87 | 2.95 | 102.92 | NA |
| | 122 | Desiccated | 76.37 | -0.65 | 3.69 | 3.75 | 100.04 | 0.85 |
| | 128 | 11% RH | 76.07 | -0.58 | 3.69 | 3.74 | 98.87 | 0.84 |
| | 132 | 23% RH | 76.13 | -0.64 | 4.01 | 4.06 | 99.09 | 1.06 |
| | 135 | 33% RH | 76.24 | -0.63 | 3.60 | 3.65 | 99.93 | 0.77 |
| | 117 | 53% RH | 75.94 | -0.76 | 3.20 | 3.29 | 103.28 | 0.48 |
| CAP-003 | 3 | Ambient | 59.08 | -0.89 | 1.06 | 1.38 | 130.10 | NA |
| | 69 | Desiccated | 59.14 | -0.81 | 0.74 | 1.10 | 137.59 | 0.50 |
| | 70 | 11% RH | 59.13 | -0.81 | 0.88 | 1.20 | 132.58 | 0.44 |
| | 71 | 23% RH | 59.19 | -0.83 | 0.98 | 1.28 | 130.25 | 0.33 |
| | 72 | 33% RH | 59.14 | -0.79 | 0.93 | 1.22 | 130.41 | 0.38 |
| | 73 | 53% RH | 59.32 | -0.80 | 1.28 | 1.51 | 121.98 | 0.50 |
| CAP-004 | 4 | Ambient | 55.93 | -0.99 | 0.17 | 1.00 | 170.41 | NA |
| | 90 | Desiccated | 56.58 | -0.90 | -0.17 | 0.92 | 190.54 | 0.73 |
| | 91 | 11% RH | 56.12 | -0.85 | 0.06 | 0.85 | 175.72 | 0.37 |
| | 92 | 23% RH | 56.13 | -0.85 | 0.10 | 0.86 | 172.97 | 0.39 |
| | 93 | 33% RH | 56.16 | -0.83 | -0.06 | 0.84 | 184.13 | 0.44 |
| | 94 | 53% RH | 56.02 | -0.81 | 0.08 | 0.81 | 174.26 | 0.42 |
| CAP-007 | 7 | Ambient | 61.50 | -1.45 | 0.24 | 1.47 | 170.47 | NA |
| | 83 | Desiccated | 61.59 | -1.26 | 0.07 | 1.26 | 177.00 | 0.65 |
| | 84 | 11% RH | 61.54 | -1.26 | 0.20 | 1.28 | 170.81 | 0.66 |
| | 85 | 23% RH | 61.61 | -1.29 | 0.16 | 1.30 | 172.90 | 0.50 |
| | 86 | 33% RH | 61.63 | -1.27 | 0.07 | 1.28 | 176.66 | 0.64 |
| | 87 | 53% RH | 61.75 | -1.28 | 0.08 | 1.28 | 176.37 | 0.54 |
| CAP-009 | 9 | Ambient | 70.03 | -1.26 | 2.71 | 2.99 | 114.92 | NA |
| | 76 | Desiccated | 70.35 | -1.12 | 2.96 | 3.17 | 110.64 | 0.50 |
| | 77 | 11% RH | 70.35 | -1.12 | 3.14 | 3.34 | 109.66 | 0.61 |
| | 78 | 23% RH | 70.21 | -1.14 | 3.19 | 3.39 | 109.68 | 0.58 |
| | 79 | 33% RH | 70.29 | -1.12 | 3.08 | 3.28 | 110.06 | 0.58 |
| | 80 | 53% RH | 70.40 | -1.13 | 3.26 | 3.45 | 109.07 | 0.66 |

Color code: Green = ΔE* ≤ 1 (no perceptible color difference), Yellow = ΔE* 1-2 (color difference perceptible on close inspection), Red = ΔE* > 2 (color difference easily perceptible).

Acceptance Criteria for white capsules ΔE* ≤1

Based on the ΔE*00 values, relative humidity does not significantly influence the appearance of both CAP-016, the TiO2 white reference sample or the TiO2-free capsule shells, CAP-003, CAP-004, CAP-

007 and CAP-009. The majority of the ΔE* values are less than 1. The $TiO_2$ reference has a $\Delta E^*_{00}$ value slightly greater than 1 for the sample stored at 23% RH only. The $\Delta E^*_{00}$ values for this capsule shell, CAP-016, remain close to 1 at most of the %relative humidities studied. However, it falls to < 0.5 for the sample stored at 53%RH. The ΔE* values for CAP-003, CAP-007 and CAP-009 remain fairly constant across the humidity range, while CAP-004 has a slightly higher $\Delta E^*_{00}$ value (but still < 1) under desiccated conditions than at the other humidity conditions. The $\Delta E^*_{00}$ values for this capsule shell fairly remain constant for the samples conditioned at 11% RH to 53% RH.

The $\Delta E^*_{00}$ values for the HPMC colored capsules stored under the various relative conditions compared with ambient storage are shown in Table 111. Based on the $\Delta E^*_{00}$ values the colorimetry data indicate that no color difference can be detected between the conditioned capsules and the corresponding ambient stored controls. The $\Delta E^*_{00}$ values remain fairly constant across the range of relative humidities studied.

Table 111: Colorimetry results on colored HPMC capsule shells from the conditioning studies

| Consort. Capsule Shell Ref. | RSSL Ref. P23-05586- | Condition | L* | a* | b* | C | h | $\Delta E^*_{00}$ |
|---|---|---|---|---|---|---|---|---|
| CAP-014 (Red $TiO_2$ reference) | 14 | Ambient | 33.06 | 30.57 | 22.92 | 38.21 | 36.86 | NA |
| | 120 | Desiccated | 33.06 | 31.30 | 22.54 | 38.58 | 35.76 | 0.59 |
| | 125 | 11% RH | 33.14 | 31.34 | 22.57 | 38.62 | 35.75 | 0.61 |
| | 129 | 23% RH | 32.97 | 31.25 | 22.53 | 38.52 | 35.79 | 0.60 |
| | 114 | 33% RH | 33.10 | 31.36 | 22.24 | 38.44 | 35.34 | 0.75 |
| | 115 | 53% RH | 32.98 | 31.40 | 22.24 | 38.47 | 35.31 | 0.77 |
| CAP-001 | 1 | Ambient | 28.06 | 27.63 | 19.17 | 33.63 | 34.76 | NA |
| | 20 | Desiccated | 28.20 | 28.69 | 18.85 | 34.33 | 33.31 | 0.74 |
| | 21 | 11% RH | 28.09 | 28.61 | 18.76 | 34.22 | 33.25 | 0.73 |
| | 22 | 23% RH | 28.02 | 28.58 | 18.76 | 34.19 | 33.28 | 0.71 |
| | 23 | 33% RH | 28.11 | 28.63 | 18.80 | 34.25 | 33.29 | 0.73 |
| | 24 | 53% RH | 28.04 | 28.59 | 18.75 | 34.19 | 33.25 | 0.73 |
| CAP-008 | 8 | Ambient | 28.79 | 27.42 | 19.53 | 33.67 | 35.46 | NA |
| | 27 | Desiccated | 28.88 | 28.32 | 19.04 | 34.13 | 33.92 | 0.73 |
| | 28 | 11% RH | 28.77 | 28.30 | 18.98 | 34.08 | 33.84 | 0.75 |
| | 29 | 23% RH | 28.73 | 28.28 | 18.99 | 34.07 | 33.89 | 0.75 |
| | 30 | 33% RH | 28.61 | 28.26 | 18.92 | 34.01 | 33.80 | 0.78 |
| | 31 | 53% RH | 28.52 | 28.25 | 18.92 | 34.00 | 33.82 | 0.79 |
| CAP-011 | 11 | Ambient | 62.50 | 8.78 | 1.65 | 8.93 | 10.65 | NA |
| | 97 | Desiccated | 62.96 | 9.11 | 1.90 | 9.31 | 11.80 | 0.64 |
| | 98 | 11% RH | 62.87 | 9.01 | 1.74 | 9.18 | 10.89 | 0.57 |
| | 99 | 23% RH | 62.92 | 9.10 | 1.83 | 9.28 | 11.38 | 0.63 |
| | 100 | 33% RH | 62.92 | 9.26 | 1.75 | 9.43 | 10.70 | 0.71 |
| | 101 | 53% RH | 62.76 | 9.13 | 1.63 | 9.27 | 10.11 | 0.57 |
| CAP-015 | 15 | Ambient | 31.33 | 29.42 | 23.27 | 37.51 | 38.34 | NA |

| Consort. Capsule Shell Ref. | RSSL Ref. P23-05586- | Condition | L* | a* | b* | C | h | ΔE*00 |
|---|---|---|---|---|---|---|---|---|
| | 121 | Desiccated | 31.31 | 30.25 | 22.58 | 37.75 | 36.74 | 0.80 |
| | 126 | 11% RH | 31.16 | 30.20 | 22.53 | 37.67 | 36.72 | 0.81 |
| | 130 | 23% RH | 31.30 | 30.30 | 22.67 | 37.85 | 36.80 | 0.78 |
| | 134 | 33% RH | 31.44 | 30.39 | 22.78 | 37.98 | 36.86 | 0.76 |
| | 116 | 53% RH | 31.25 | 30.41 | 22.35 | 37.74 | 36.31 | 0.99 |

Color code: Green = ΔE* ≤ 1 (no perceptible color difference), Yellow = ΔE* 1-2 (color difference perceptible on close inspection), Red = ΔE* > 2 (color difference easily perceptible).

Acceptance criteria: for colored capsule shells: ΔE*< 2.

## 76. Additional Conditioning Experiments Performed on CAP-002

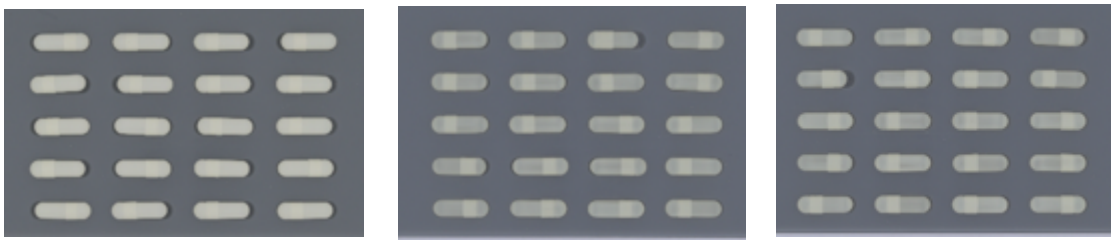
In order to investigate the color change in capsule shell, CAP-002, at a higher humidity than previously tested, a sample of CAP-002 was conditioned at 23°C at 75%RH for 18 hours and then exposed to room conditions for 7 hours. The results are shown in Table 112 and Figure 58.

Table 112: Colorimetry data on CAP-002.

| Storage Conditions | L* | a* | b* | C | h | ΔE*00 |
|---|---|---|---|---|---|---|
| Ambient | 75.39 | -1.47 | 4.21 | 4.46 | 109.28 | NA |
| Ambient + 23°C/75%RH for 18 hr | 65.00 | -1.73 | 1.74 | 2.46 | 134.74 | 8.33 |
| Ambient + 23°C/75%RH for 18 hr + room conditions for 7 hr | 66.45 | -1.76 | 1.76 | 2.49 | 134.92 | 7.22 |

Figure 58: Appearance of CAP-002 after additional conditioning experiments

Ambient + 23°C/75%RH for 18 hr +23°C/75%RH 18 hr + room temp for 7hr



The data show following conditioning at 75%RH the capsules become even more translucent compared to ambient or lower humidities (see Table 108 and Figure 58). This change in opacity is not reversed after exposure to room conditions for 7 hours. Since 75%RH is encountered in many countries, both patients and physicians would notice the change in capsule shell appearance with changing humidity. In addition, CAP-002 is not suitable for the blinding of clinical trial supplies and its appearance would fail specification under accelerated ICH stability conditions. It is therefore not a potential replacement for $TiO_2$ containing white capsules.

## 77. Capsule Brittleness

The results of the brittleness testing are shown Table 113.

Table 113: Comparison capsule shell brittleness – $TiO_2$-free capsule shells versus $TiO_2$ reference

| Cap Shell Type | Consort. Cap Ref No. | Opacifier[a] | % Brittle Capsule Shells | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0%RH | 11%RH | 23%RH | 33%RH | Lab[b] Storage | 53%RH |
| Gelatin | CAP-013 | $TiO_2$ | 6 | 0 | 0 | 0 | 0 | 0 |
| | CAP-002 | NaPOx | 30 | 12 | 4 | 0 | 2 | 2 |
| | CAP-005 | CaCO3 | 100 | 88 | 4 | 0 | 0 | 0 |
| | CAP-006 | $CaCO_3$+A | 100 | 100 | 36 | 6 | 0 | 2 |
| | CAP-012 | $CaCO_3$ +B+D | 100 | 100 | 34 | 6 | 0 | 0 |
| | CAP-017 | $TiO_2$ | 38 | 14 | 18 | 4 | 0 | 4 |
| | CAP-010 | $Fe_2O_3$ | 76 | 18 | 0 | 0 | 0 | 0 |
| HPMC | CAP-016 | $TiO_2$ | 6 | 2 | 2 | 2 | 2 | 0 |
| | CAP-003 | $CaCO_3$ | 84 | 42 | 18 | 6 | 2 | 6 |
| | CAP-004 | $CaCO_3$ | 50 | 8 | 0 | 0 | 0 | 2 |
| | CAP-007 | $CaCO_3$+A | 26 | 0 | 2 | 0 | 2 | 0 |
| | CAP-009 | $CaCO_3$+A | 90 | 70 | 54 | 4 | 0 | 0 |
| | CAP-014 | $TiO_2$+$Fe_2O_3$ | 4 | 10 | 4 | 0 | 4 | 0 |
| | CAP-001 | $Fe_2O_3$ | 20 | 4 | 0 | 0 | 0 | 0 |
| | CAP-008 | $Fe_2O_3$ | 38 | 14 | 4 | 0 | 0 | 0 |
| | CAP-011 | $CaCO_3$ +B+C+D | 100 | 92 | 38 | 38 | 52 | 16 |
| | CAP-015 | $Fe_2O_3$ | 8 | 0 | 0 | 0 | 0 | 0 |

Color Code:

Green - ≤ 4% capsules brittle at %RH ≥33%RH

Red - > 4 % capsules brittle at %RH ≥33%RH

[a]$Fe_2O_3$ is not an opacifier per se but contributes to opacification.

[b]The humidity in the laboratory was not controlled but was approximately 50 %RH

Overall, CAP-013, the white gelatin $TiO_2$ reference, performs the best across the range of relative humidities. Under desiccated conditions, 6 % of the capsule shells were brittle. However, at the other relative humidities and during ambient laboratory storage this percentage sank to 0%. The white $TiO_2$-free gelatin capsule shells are more brittle than their $TiO_2$ containing counterpart at very low humidities. CAP-002 and CAP-005 show low levels of brittleness at humidities -≥ 23% RH and under laboratory storage. CAP-005 had 0% brittleness following storage at 33% RH, 53% RH and laboratory conditions and CAP-002 had 0% brittleness at 33%RH. CAP-006 and CAP-012 only met the criteria for acceptable brittleness following laboratory storage or at 53% RH.

The $TiO_2$-containing red gelatin reference (CAP-017) performed worse than its $TiO_2$-free counterpart, CAP-010. CAP-017 only had ≤4% brittle capsule shells when stored under laboratory ambient conditions and at %RH ≥33, whereas 0% of CAP-010 capsule shells were brittle at relative humidities ≥ 23%RH as well as following laboratory storage.

The white HPMC $TiO_2$ containing reference, CAP-016, performed worse than its gelatin-based counterpart in that 2% of capsule shells failed the brittleness test following storage at 11% RH, 23% RH, 33 %RH storage and in the laboratory. CAP-003 and CAP-009 were very brittle after storage under desiccation, at 11% RH and at 23% RH. Following storage at higher % RH and under laboratory conditions. the % of damaged capsules dramatically reduced, although with CAP-003 it never reached 0% and was still 6% at 33%RH and 53%RH.

CAP-007 and CAP-004 were very brittle after storage under desiccation with 26% and 50% of the capsules failing the test respectively. The %damaged capsule shells reduced for samples conditioned at higher % relative humidities and CAP-004 and CAP-007 met the acceptance criteria of ≤4% brittle capsules for samples stored at ≥33%RH.

The red HPMC $TiO_2$ reference, CAP-014, had variable performance in the brittleness test. Overall, the % broken/damaged capsule shells were low but was only 0% for the 33%RH and 53%RH stored samples. The $TiO_2$-free capsule shell, CAP-015, fared better in the test with 0% brittle capsule shells found for all conditioned samples except the desiccated sample. The % brittle for the other two red/orange $TiO_2$-free HPMC capsule shells, CAP-001 and CAP-008, was also 0% for samples stored at % relative humidities ≥23% and ≥33%RH respectively and in the laboratory. The pink CAP-011, which contained$CaCO_3$, plus other opacifiers was extremely brittle. Even the sample stored at 53%RH was much more brittle than any of the other capsules ($TiO_2$ and $TiO_2$-free) and the samples did not meet the acceptance criteria of ≤4% brittle capsules at ≥33% RH.

Overall if the %capsule shell brittleness results across the range of conditions are taken into account, CAP-013, the gelatin $TiO_2$ containing reference, performed best, followed by CAP-015, a HPMC colored $TiO_2$-free capsule shell and then CAP-016, the HPMC $TiO_2$ containing reference. The most brittle capsule was CAP-011 (a colored HPMC $TiO_2$-free capsule), followed by CAP-006 and CAP-003], both white $TiO_2$-capsule shells.

If the % capsule brittleness following storage at ≥33% RH humidity, the following capsule shells had acceptable performance (≤4% brittle capsules):

- CAP-013 - white, gelatin, $TiO_2$ reference capsule
- CAP-002 and CAP-005 - $TiO_2$-free white, gelatin capsules
- CAP-010 - colored, gelatin $TiO_2$-free capsule
- CAP-016 - white, HPMC, $TiO_2$ reference capsule
- CAP-004 and CAP-007 - $TiO_2$-free white, HPMC capsules
- CAP-014 - colored, HPMC, $TiO_2$ reference capsule
- CAP-001, CAP-008 and CAP-015 - $TiO_2$-free colored, HPMC capsules
- CAP-017, the red, gelatin $TiO_2$ containing reference
- CAP-009, a HPMC $TiO_2$-free white capsule shell

Therefore, it can be concluded that some but not all of the $TiO_2$-free capsules had acceptable mechanical integrity after conditioning at %RH relevant to pharmaceutical manufacture and storage and to global climatic conditions. The integrity testing did show that many of the $TiO_2$-free capsules at lower humidity exposure were not similar to the $TiO_2$ capsules even though acceptance criteria were not applied to those conditions. Capsule shell mechanical integrity of $TiO_2$ containing versus $TiO_2$-free systems was also evaluated in the course of larger scale capsule filling trials (see Section 0).

### 78. %Water Content and Water Activity

The results for %water content of the gelatin and HPMC capsule shells are shown in Figure 59 and Figure 60 respectively. As expected, the capsule shell water content increases as environmental humidity increases for all of the capsules, with $TiO_2$-free capsule shells displaying a similar trend to the reference capsule shells. The data show that the $CaCO_3$ containing capsules have lower moisture content than those employing $TiO_2$ or iron oxides as opacifiers. The exception is CAP-009, the capsule shell containing $CaCO_3$ + A. The moisture content of CAP-002 at 53%RH is above 16% and therefore outside the proposed draft pharmacopoeial moisture limits for gelatin capsules. This may be related to the inclusion of sodium phosphate salts in this capsule shell [15].

The % water content for the HPMC capsules ranged from 1.79% - 2.36% for the desiccated samples to 6.45% to 6.98% for those stored at 53%RH. It is therefore much lower than that of the gelatin-based capsule shells and falls with the limits for the proposed draft USP monograph for hard capsules [15]. Both $TiO_2$-free and reference capsule shells showed a similar upward trend in moisture content.

The variation in % water content for the different HPMC-based capsule types was higher than for the gelatin capsules at 23 % RH and for the laboratory stored capsules. This probably reflects the > 1.0% increase in % water content between 11%RH and 23%RH storage for some (CAP-003, CAP-004, CAP-007, CAP-008 and CAP-009) but not all of the capsule shells whose moisture content increased to a lesser extent. Therefore, the variation in capsule shell % water content is higher at 23%RH than at other % relative humidities. For the laboratory stored capsules, the variation is affected by an outlier, CAP-008, which is a red iron oxide containing $TiO_2$-free capsule. This may reflect minor variations in laboratory environmental and storage conditions.

In summary the data show that the % water content of the $TiO_2$ containing and $TiO_2$-free HPMC capsules does not greatly differ and there is no trend to suggest that it varies to a different extent with changing %relative humidity.

### 79. Conclusions from the Studies on Capsule Shells Conditioned at Different Humidities

Based on visual appearance and colorimetry data, the appearance of all of the $TiO_2$-free capsule shells except CAP-002, and the $TiO_2$ reference shells did not alter perceptibly in response to conditioning at different %humidities. The opaqueness of the $TiO_2$-free capsule shell, CAP-002, was significantly affected by the relative humidity of the environment in which it is stored and these changes persist for some time even after the humidity conditions has changed again. This is a significant drawback to its use in medicinal products and for over-encapsulation in clinical product blinding for clinical trials.

The $TiO_2$-free capsule shells, CAP-003, CAP-005, CAP-012 and CAP-011 proved unacceptably brittle in a commonly used capsule shell brittleness test, and were far more brittle than the corresponding $TiO_2$ reference capsule shells. These results could not be attributed to their water content or activity which was not significantly different from either the $TiO_2$ references and the other $TiO_2$-free capsule shell batches.

Figure 59: % Water content versus %RH for $TiO_2$ -containing and $TiO_2$-free gelatin capsules

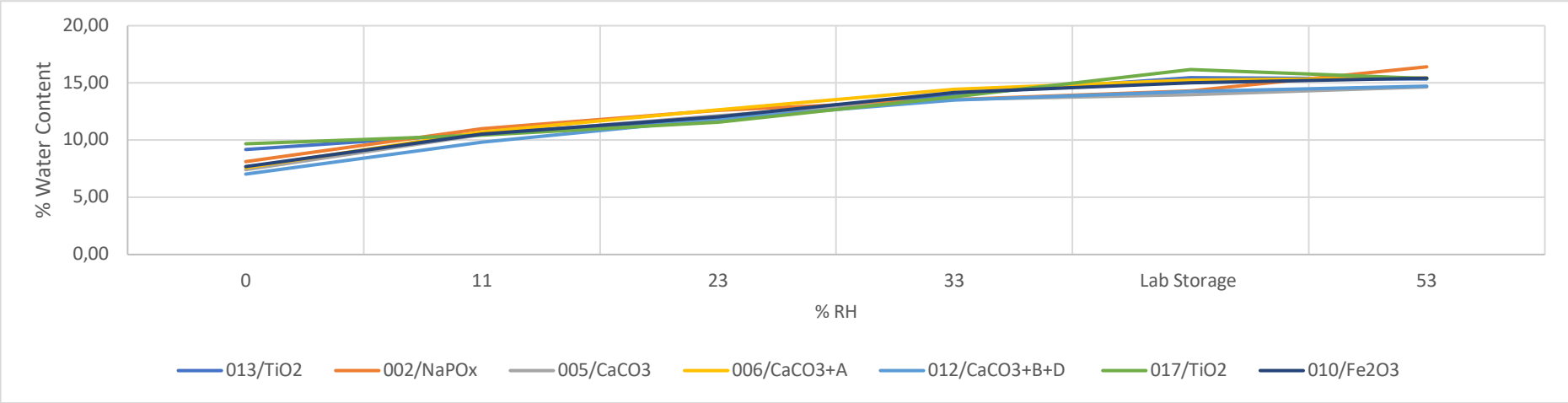
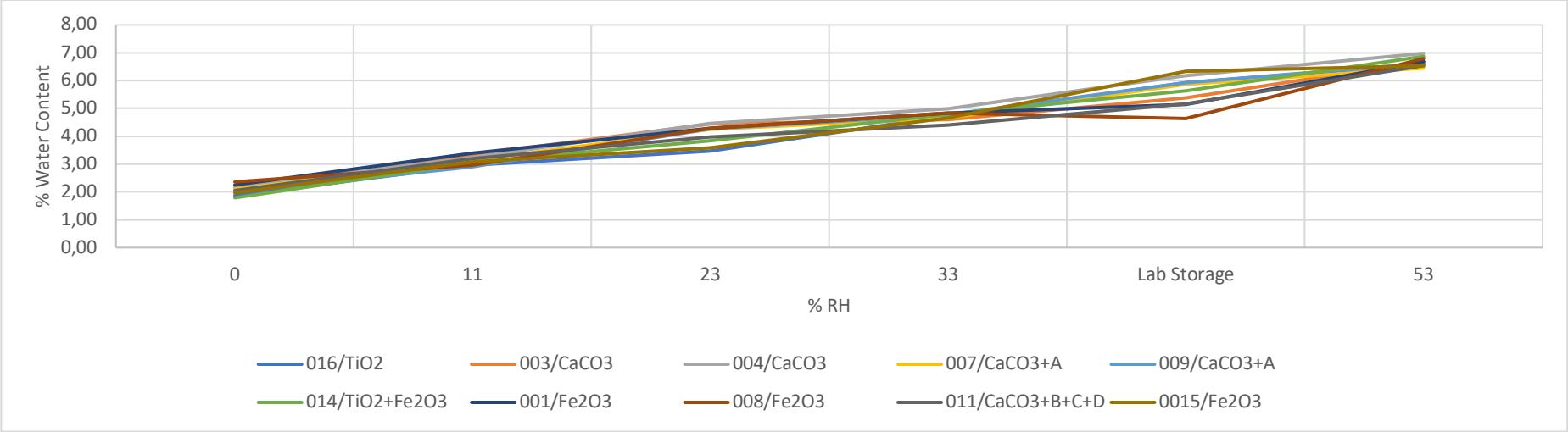


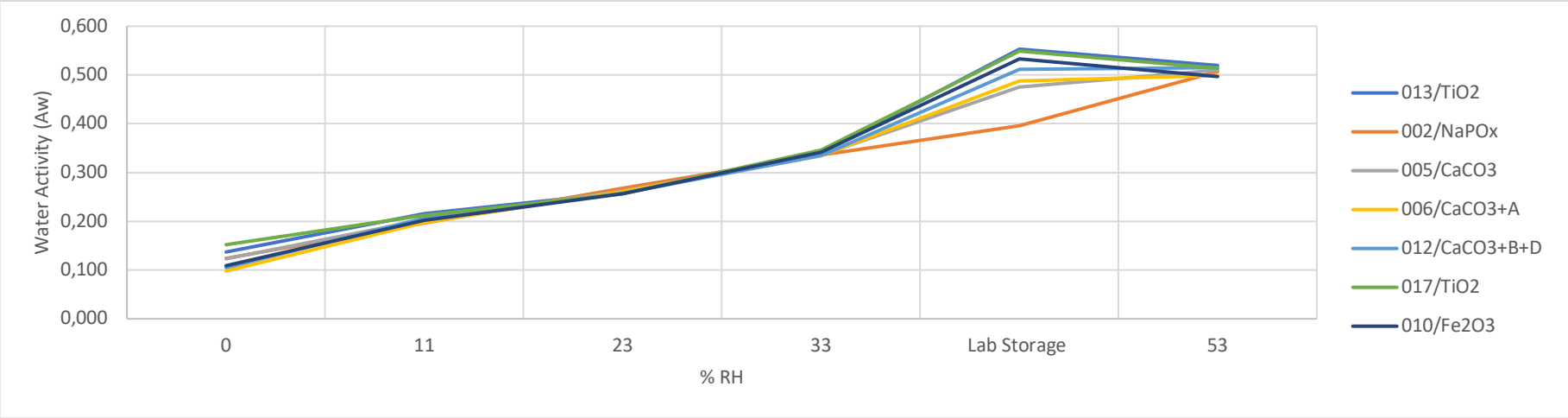

Figure 60: % Water content versus %RH for $TiO_2$ -containing and $TiO_2$-free HPMC capsules



The results for water activity of the gelatin and HPMC capsule shells are shown in Figure 61 and

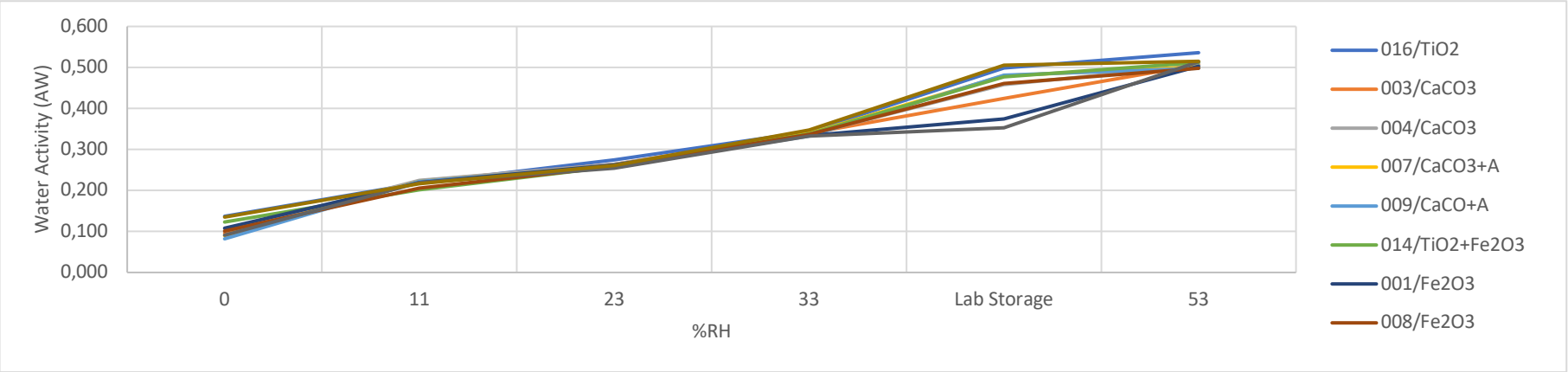
Figure 62 respectively. Water activity increased with % relative humidity for both gelatin and HPMC capsule shells. Both $TiO_2$ containing and $TiO_2$-free capsule shells followed a similar trend. The water activity of the gelatin-based capsule shells ranged from a minimum of 0.098Aw to a maximum of 0.553Aw across the %RHs tested. The water activity of the HPMC-based capsule shells ranged from a minimum of 0.082Aw to a maximum of 0.536Aw. Variation in water activity between individual capsule shell types was greatest for the capsules stored at 0%RH and under laboratory conditions. The latter may reflect minor variations in the laboratory storage conditions. At the other % RH conditions, variation was relatively low.

Figure 61: Water activity versus %RH for $TiO_2$ -containing and $TiO_2$-free gelatin capsule shells




der those

Figure 62: % Water activity versus %RH for $TiO_2$ -containing and $TiO_2$-free HPMC capsule shells




The water activity meter was calibrated at a lower limit of 0.25 Aw and therefore the results at 0%RH and 11% RH are only an indication of the trend in water activity under those conditions.

# Experimental Part 2: Encapsulation of Active Blends

## Experimental Objectives and Rationale

In Section 0 the properties of the selected $TiO_2$-free capsules were evaluated as empty capsule shells and the results compared to the empty $TiO_2$-containing reference capsule shells. In Sections 0, 0 and 0 the same 17 capsule shells are evaluated and compared following filling with powder blends containing selected APIs. Section 0 describes the results of 19 small-scale encapsulation trials, both in terms of in-process results and analytical testing of the batches, and the packing of these batches for accelerated stability (see Section 0). The results of initial batch testing serve as $T_0$ testing for the aforementioned stability studies.

## Selection of Active Blends

Three blends of actives were selected for encapsulation so that the impact of the capsule shell composition in combination with these blends could be assessed, both in terms of stability of the capsule shell itself and also the stability active compound. All of the compounds are already formulated and encapsulated in $TiO_2$ containing gelatin capsule shells in medicinal products authorized in the EU. The marketed products are encapsulated in smaller capsules than the Size 0 chosen for the Consortium studies. The marketed capsules are therefore very different to the encapsulated products described in this report.

The compounds were selected due to their known instability under certain conditions e.g., light, moisture etc. Therefore, their stability may be compromised as a result of a change in capsule shell composition. Details of the active blends, their sourcing, batch numbers and the rationale for selection are shown in Table 114.

Table 114: Active Blend Details

| Active Blend | Appearance Specification | Rationale for Selection | Almac Item Code & Batch No. | Manufacturer/Supplier |
|---|---|---|---|---|
| Loperamide Hydrochloride 0.7% Blend | White to off white powder | Sensitive to alkali and potential disproportion risk | Code CFDHB2004<br><br>Batch No. G172477 | Teva Pharmaceutical S.L.U., Poligono, Malpica, C/C No. 4, 50016, Zaragoza, Spain. |
| Fluvastatin sodium 13.8% Blend | Off-white to yellowish powder with small agglomerates | Potential for photodegradation and moisture sensitivity | Code CFDHB2003<br><br>Batch No. G172476 | Teva Pharmaceutical Works Co. Ltd., Pallagi Street 13, Debrecen 4042, Hungary |
| Benserazide HCl 9.5% / Levodopa 33.33% Blend | White to brownish powder | Potential for photodegradation and moisture sensitivity | Code CFDHB2005<br><br>Batch No. G175016 | Hemofarm, A.D., Hajduk Veljkova b.b., 15000 Šabac, Serbia. |

## Encapsulation of Active Blends

### 80. Encapsulation and In-process Testing

Details of each encapsulation run are given in Table 115. The active blends and capsule combinations were selected to provide two $TiO_2$-containing controls for each active, $TiO_2$-free capsule shells based on either gelatin or HPMC containing various opacifiers or iron oxide red. Iron oxide red is a colorant and not an opacifier per se but imparts opacity to the capsule shells through its colorant properties.

The active blends were encapsulated using a MG2 Labby encapsulation machine fitted with Size 0 capsule transport parts. The target batch size was approximately 5000 capsules to afford a sufficient quantity of capsules for analysis and stability.

Prior to each of the encapsulation runs, 100 empty capsules were weighed and used to calculate the mean empty capsule weight. This was then used to calculate the target gross weight and the individual and average weight limits. The net fill weights depended on the blend (see Table 116) and were maintained within the target control/ action limits throughout each filling run. IPC checks were performed at the beginning of each run and then every 20 min thereafter. These IPC tests were weight checks, closure length and capsule appearance. In addition, capsule disintegration time was evaluated at set-up and after completion of the run (n=6 capsules in purified water (temperature 37 ±1°C, with discs). At the end of each run an acceptable quality limit (AQL) check was carried out and any defects in the capsules together with the relative frequency noted.

Table 115: Details of the encapsulation trials

| Trial | Filled Capsule[a] | Bulk Cap Batch No. ENQ3860/AIRC/ | Packed Cap Batch No. ENQ3860/AIRC/ | Consortium Capsule Shell Reference | Shell Former | Opacifier[b] | Active Blend |
|---|---|---|---|---|---|---|---|
| 1 | 14.25 mg Benserazide HCl 50mg Levodopa | 001/01 | 001/01/P1 | CAP-016a | HPMC | $TiO_2$ | Benserazide HCL Levodopa |
| 2 | 14.25 mg Benserazide HCl 50mg Levodopa | 002/01 | 002/01/P1 | CAP-017b | Gelatin | $TiO_2$ | Benserazide HCL Levodopa |
| 3 | 14.25 mg Benserazide HCl 50mg Levodopa | 003/01 | 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | Benserazide HCL Levodopa |
| 4 | 14.25 mg Benserazide HCl 50mg Levodopa | 004/01 | 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | Benserazide HCL Levodopa |
| 5 | 14.25 mg Benserazide HCl 50mg Levodopa | 005/01 | 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | Benserazide HCL Levodopa |
| 6 | 14.25 mg Benserazide HCl 50mg Levodopa | 006/01 | 006/01/P1 | CAP-006 | Gelatin | CaCO3 + A | Benserazide HCL Levodopa |
| 7 | 20 mg Fluvastatin | 007/01 | 007/01/P1 | CAP-016a | HPMC | $TiO_2$ | Fluvastatin Na |
| 8 | 20 mg Fluvastatin | 008/01 | 008/01/P1 | CAP-014c | HPMC | $TiO_2$ + $Fe_2O_3$ | Fluvastatin Na |
| 9 | 20 mg Fluvastatin | 009/01 | 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | Fluvastatin Na |
| 10 | 20 mg Fluvastatin | 010/01 | 010/01/P1 | CAP-009 | HPMC | $CaCO_3$ + A | Fluvastatin Na |
| 11 | 20 mg Fluvastatin | 011/01 | 011/01/P1 | CAP-007 | HPMC | $CaCO_3$ + A | Fluvastatin Na |
| 12 | 20 mg Fluvastatin | 012/01 | 012/01/P1 | CAP-002 | Gelatin | NaPOx | Fluvastatin Na |
| 13 | 20 mg Fluvastatin | 013/01 | 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | Fluvastatin Na |
| 14 | 20 mg Fluvastatin | 014/01 | 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$ + B +D | Fluvastatin Na |
| 15 | 2 mg Loperamide | 015/01 | 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | Loperamide HCl |

| Trial | Filled Capsule[a] | Bulk Cap Batch No. ENQ3860/AIRC/ | Packed Cap Batch No. ENQ3860/AIRC/ | Consortium Capsule Shell Reference | Shell Former | Opacifier[b] | Active Blend |
|---|---|---|---|---|---|---|---|
| 16 | 2 mg Loperamide | 016/01 | 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | Loperamide HCl |
| 17 | 2 mg Loperamide | 017/01 | 017/01/P1 | CAP-011 | HPMC | $CaCO_3$ | Loperamide HCl |
| 18 | 2 mg Loperamide | 018/01 | 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | Loperamide HCl |
| 19 | 2 mg Loperamide | 019/01 | 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | Loperamide HCl |

[a]Quantities of actives in the filled capsules expressed as base except for benserazide. 12.5 mg benserazide = 14.25 mg benserazide HCL

[b]Red iron oxide is not an opacifier per se but contributes to opacification

CAP-016 = White HPMC reference capsule

CAP-017 = Red gelatin reference capsule

CAP-014 = Red HPMC reference capsule

CAP-013 = White gelatin reference capsule

Details of the IPC net fill weight and associated limits and capsule closure lengths specifications are given in Table 116.

Table 116: IPC net fill weight and limits for the encapsulation trials

| Active Blend | Benserazide HCL/Levodopa | Fluvastatin Na | Loperamide HCl |
|---|---|---|---|
| Target fill weight (mg) | 150 | 153 | 272 |
| Individual fill weight limits (mg) | 135 – 165 | 138 – 168 | 245 – 299 |
| Average fill weight control limits (mg) | 147.5 – 152.5 | 150.5 – 155.5 | 267.5 – 276.5 |
| Average fill weight action limits (mg) | 145.0 – 155.0 | 147.9 – 158.1 | 263.0 – 281.0 |
| % RSD | <3.3 | <3.3 | <3.3 |
| Target capsule closure length (mm)[a] | 21.4 - 21.8 | 21.4 - 21.8 | 21.4 - 21.8 |

[a]The closure length specification was set to cover the range recommended by the capsule shell manufacturers.

## 81. Sampling and Stability Packing

194 filled capsules were sampled from each of the 19 encapsulation batches listed in Section 80 (Table 115) at the completion of the encapsulation run. They were stored in polypropylene tubs under ambient conditions and protected from light in the analytical laboratory and used for appearance evaluation, colorimetry studies and photostability studies. The remainder of the encapsulated batches were packed in in 120mL, HDPE bottles and sealed with a 45mm screw cap, with safeguard plus SG+770 liner (foil) (64 capsules per bottle). Both induction sealed bottles and non-induction sealed bottles were produced and used for accelerated stability studies (see Section 0 for further details). The $T_0$ results from the stability studies on assay, related impurities, disintegration and dissolution from the packed capsules are also included in this section of the report. They were also used as the dark controls in the photostability study (see Section 0).

## 82. Analytical Testing

The filled capsules were subjected to the testing as described in Table 117.

Table 117: Testing of the filled capsules from the 19 encapsulations trials

| Attribute | Methodology |
|---|---|
| **Samples from all 19 encapsuled batches** | |
| Appearance – Visual | Photography |
| Appearance – Colorimetry | DigiEye |
| Brittleness | In-house test |
| Disintegration | USP <701> |
| **14.25mg Benserazide HCL/50mg Levodopa capsules only[a]** | |
| Assay | HPLC |
| Related substances | UPLC |
| Dissolution | USP Type 2 Apparatus (Paddle) HPLC |
| **20 mg Fluvastatin capsules only[a]** | |
| Assay | HPLC |
| Related substances | HPLC ( |
| Dissolution | USP Type 2 Apparatus (Paddle) HPLC |
| **2 mg Loperamide capsules only[a]** | **2 mg Loperamide capsules only** |
| Assay | HPLC |
| Related substances | HPLC |
| Dissolution | USP Type 1 Apparatus (Basket) HPLC |

[a]Analytical methods supplied by the blend manufacturers. Each dissolution test carried out on 6 filled capsules.

## 83. Analytical Methodology - Visual Appearance Filled Capsules

10 capsules from each batch were examined for appearance and any physical defects. Both the capsule shell and contents were examined.

## 84. Analytical Methodology – Colorimetry Filled Capsules

Colorimetry was carried out using DigiEye equipment as described in Section 67. The L*, a*, b*, chroma and hue angle values were reported as were the $\Delta E^*_{00}$ values for color differences between the $TiO_2$-free and $TiO_2$ containing blend-filled capsule using the Delta E 2000 equation [13]:

$$\Delta E^*_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H}}$$

The $\Delta E^*_{00}$ values were interpreted as described in Section 67.

## 85. Analytical Methodology - Brittleness Test Filled Capsules

20 capsules from each batch of packed capsules were tested for brittleness by rolling and pinching the capsule while applying enough force to deform it. If the shell film cracked or shattered the capsule was considered brittle. If the shell only deformed and there was no breach in the film, the capsule was considered not brittle. The acceptance criterion was set at ≤ 5% (1 brittle capsule in 20). This brittleness method differed from the one used for empty capsule testing and was employed to enable safe handling of the filled capsules and limit analyst exposure to the blend in cases where brittleness was observed.

### 86. Analytical Methodology -Disintegration Filled Capsules

6 capsules for each sample were tested for disintegration using Apparatus A and disks. Purified water was used as an immersion fluid as per USP<701>.

## Results and Discussion

### 87. Manufacturing Results

Encapsulation of the active blends into the $TiO_2$-free capsule shells and the corresponding $TiO_2$ references was beset with recurring manufacturing difficulties due to the capsule shells jamming and/or capsule shell double-feeding in the Labby equipment. These issues did not appear to be related to the blend or capsule shell type involved, although fewer problems occurred with the gelatin-based capsule shells compared with the HPMC ones for both $TiO_2$-free capsule shells and the $TiO_2$ containing references. The manufacturing difficulties resulted in large numbers of crushed capsules which, in turn, caused a build-up of blend within the capsule filler and on a number of occasions the equipment had to be stripped down, cleaned and set-up again. Engineers from the equipment supplier identified that the problems were related to an issue with the vacuum resulting in defective capsule feeding especially for the heavier HPMC capsule shells.

Since the defective vacuum could not be sorted immediately, a decision was taken to continue with the encapsulation runs as all of the capsules were individually check-weighed and, based on AQL inspections following check-weighing, very small numbers of defective capsules were found in the final encapsulated product (no critical defects, maximum of 5 capsules with major defects (Trial 8 and 17) and a maximum of 6 capsules with minor defects (Trial 9). Using this approach sufficient capsules (approx. 4700) were manufactured for the planned stability trials from each of encapsulation runs conducted prior to the equipment issue being rectified.

As a result of the equipment issues, no conclusion can be drawn over the processability of the various $TiO_2$ -free capsules compared with the corresponding $TiO_2$ references as it is not possible to discern whether capsule shell composition was also a factor in the manufacturing issues observed. The processability of $TiO_2$-free capsule shells versus $TiO_2$ references was evaluated during larger scale encapsulation runs (see Section 0).

### 88. Visual Appearance Results

The physical description of the appearance of the capsule shells and their contents are shown in Table 118, Table 119 and Table 120 for the benserazide HCl/levodopa capsules, the loperamide capsules and fluvastatin capsules respectively. Overall, the descriptive results of the capsule shells match the photographs in Figure 50 and Figure 51. However, one cut capsule was observed in the samples from Trial 8, Trial 9 and Trial 14 (fluvastatin). The cut capsule from Trial 8 was leaking. During the manufacture of Trial 8, 4 failed appearance checks were noted with cracked/broken capsules. This may have been due to the aforementioned equipment issues as outlined in Section 87.

Table 118: Visual appearance of the benserazide HCl/levodopa capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | Appearance Shell | Appearance Content |
|---|---|---|---|---|---|---|
| 1 | 001/01 | CAP-016 | HPMC | $TiO_2$ | Off white, no physical defects, opaque. | White powder, small amount of clumping, free from contaminants. |
| 2 | 002/01 | CAP-017 | Gelatin | $TiO_2$ | Dark Red, no physical defects, opaque. | White powder, small amount of clumping, free from contaminants. |
| 3 | 003/01 | CAP-015 | HPMC | $Fe_2O_3$ | Dark Red, no physical defects, opaque. | White powder, small amount of clumping, free from contaminants. |
| 4 | 004/01 | CAP-004 | HPMC | $CaCO_3$ | Off white, slightly translucent, no physical defects | White powder, small amount of clumping, free from contaminants. |
| 5 | 005/01 | CAP-008 | HPMC | $Fe_2O_3$ | Dark Red, no physical defects. opaque. | White powder, small amount of clumping, free from contaminants. |
| 6 | 006/01 | CAP-006 | Gelatin | CaCO3 + A | Off white, slightly translucent, no physical defects | White powder, small amount of clumping, free from contaminants. |

Red iron oxide is not an opacifier per se but contributes to opacification.

Table 119: Visual appearance of the loperamide capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | Appearance Shell | Appearance Content |
|---|---|---|---|---|---|---|
| 15 | 015/01 | CAP-013 | Gelatin | $TiO_2$ | Off white, no physical defects | White powder, small amount of clumping, free from contaminants. |
| 16 | 016/01 | CAP-017 | Gelatin | $TiO_2$ | Dark red, no physical defects | White powder, small amount of clumping, free from contaminants |
| 17 | 017/01 | CAP-011 | HPMC | $CaCO_3$ | Light pink, no physical defects | White powder, small amount of clumping, free from contaminants |
| 18 | 018/01 | CAP-001 | HPMC | $Fe_2O_3$ | Dark red, slightly dusty, no other visual defects. | White powder, small amount of clumping, free from contaminants. |
| 19 | 019/01 | CAP-010 | Gelatin | $Fe_2O_3$ | Dark red, no physical defects | White powder, small amount of clumping, free from contaminants. |

Red iron oxide is not an opacifier per se but contributes to opacification.

Table 120: Visual appearance of the fluvastatin capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | Appearance Shell | Appearance Content |
|---|---|---|---|---|---|---|
| 7 | 007/01 | CAP-016 | HPMC | $TiO_2$ | Off white, no physical defects, opaque. | White powder, small amount of clumping, free from contaminants. |
| 8 | 008/01 | CAP-014 | HPMC | $TiO_2$ + $Fe_2O_3$ | Red, one capsule roughly cut and leaking. Remaining capsule shells free from physical defects. Very slightly translucent. | White powder, small amount of clumping, free from contaminants. |
| 9 | 009/01 | CAP-003 | HPMC | $CaCO_3$ | Off white, one capsule roughly cut. Remaining capsule shells free from physical defects, slightly translucent. | White powder, small amount of clumping, free from contaminants |
| 10 | 010/01 | CAP-009 | HPMC | $CaCO_3$ + A | Off white, no physical defects, slightly transparent. | White powder, small amount of clumping, free from contaminants. |
| 11 | 011/01 | CAP-007 | HPMC | $CaCO_3$ + A | Off white, no physical defects. translucent. | White powder, small amount of clumping, free from contaminants. |
| 12 | 012/01 | CAP-002 | Gelatin | NaPOx | Off white, no physical defects, opaque. | White powder, small amount of clumping, free from contaminants. |
| 13 | 013/01 | CAP-005 | Gelatin | $CaCO_3$ | Off white, no physical defects, translucent. | White powder, small amount of clumping, free from contaminants. |
| 14 | 014/01 | CAP-012 | Gelatin | $CaCO_3$ + B +D | Off white, one capsule roughly cut. Remaining capsule shells free from physical defects, opaque | White powder, small amount of clumping, free from contaminants. |

## 89. Colorimetry

Benserazide HCL/Levodopa Capsules

Table 121 shows the colorimetry results for the benserazide HCl/levodopa capsules. In Trials 1 to 6, two filled white $TiO_2$.free capsule shells, one based on HPMC (Batch No. ENQ3860/AIRC/004/01) and one based on gelatin (ENQ3860/AIRC/006/01), were assessed against an HPMC-based $TiO_2$ reference (Batch No. ENQ3860/AIRC/001/01). Similarly, two filled $TiO_2$-free colored capsule shells, both based on HPMC (Batch Nos. ENQ3860/AIRC/003/01 and ENQ3860/AIRC/005/01) were compared against the gelatin-based $TiO_2$-containing reference (Batch No. ENQ3860/AIRC/002/01). Table 121 shows clearly the difference in the colorimetry data for the white and colored capsules, with the white capsules having much higher L* and hue angle values and significantly lower a*, b* and chroma values than the colored capsules. However, when the $\Delta E^*_{00}$ values were calculated for the white and colored capsules against their respective control, all values were > 2 (see Table 121), indicating a significant color difference which would be easily noticed by a patient.

Loperamide Capsules

The colorimetry data for the loperamide capsules are shown in Table 122.

The encapsulation trials using the loperamide blend focused mainly on comparing the filled colored capsule shells against the gelatin-based $TiO_2$ reference (Batch No. ENQ3860/AIRC/016/01). Of the colored capsules evaluated, two were red and one pink. The loperamide blend was also filled into the gelatin-based $TiO_2$ white reference capsule shell (Batch No. ENQ3860/AIRC/015/01) for comparison. The colorimetry data clearly show the differences between the white, pink and red capsules with the values for L*, a*, b* and chroma for the pink capsule shell lying closer to those of the white $TiO_2$ reference than the red capsules. However, the hue angle of $TiO_2$ white reference is drastically different from all of the other batches. A comparison of the color differences was conducted for the $TiO_2$-free red capsules against the red reference (see Table 122). The $\Delta E^*_{00}$ values were high at above 3.

Table 121: Colorimetry data on the benserazide HCl/levodopa capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | RSSL Ref. P23-06656 | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | L* | a* | b* | C | h | ΔE*00 ($TiO_2$ vs ref) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 001/01 | 14 | CAP-016 | HPMC | $TiO_2$ | 77.77 | -0.31 | 4.95 | 4.96 | 93.70 | NA |
| 2 | 002/01 | 15 | CAP-017 | Gelatin | $TiO_2$ | 30.43 | 40.16 | 12.77 | 42.14 | 17.63 | NA |
| 3 | 003/01 | 16 | CAP-015 | HPMC | $Fe_2O_3$ | 31.50 | 30.19 | 22.12 | 37.43 | 36.23 | 8.73[a] |
| 4 | 004/01 | 17 | CAP-004 | HPMC | $CaCO_3$ | 71.18 | -0.08 | 6.59 | 6.59 | 90.92 | 5.13[b] |
| 5 | 005/01 | 18 | CAP-008 | HPMC | $Fe_2O_3$ | 29.25 | 27.74 | 18.25 | 33.21 | 33.35 | 7.69[a] |
| 6 | 006/01 | 19 | CAP-006 | Gelatin | CaCO3 + A | 73.94 | -0.68 | 6.28 | 6.31 | 96.31 | 3.08[b] |

[a]Comparison with red $TiO_2$ reference Batch No. ENQ3860/AIRC/002/01.

[b]Comparison with white $TiO_2$ reference Batch No. ENQ3860/AIRC/001/01.

Red iron oxide is not an opacifier per se but contributes to opacification.

Table 122: Colorimetry data on the loperamide capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | RSSL Ref. P23-06656 | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | L* | a* | b* | C | h | ΔE*$_{00}$ ($TiO_2$ vs ref) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 015/01 | 7 | CAP-013 | Gelatin | $TiO_2$ | 80.45 | -0.99 | 4.20 | 4.32 | 103.32 | NA |
| 16 | 016/01 | 10 | CAP-017 | Gelatin | $TiO_2$ | 30.41 | 40.81 | 13.19 | 42.89 | 17.91 | NA |
| 17 | 017/01 | 11 | CAP-011 | HPMC | $CaCO_3$ | 70.86 | 12.24 | 5.59 | 13.45 | 24.53 | NA |
| 18 | 018/01 | 12 | CAP-001 | HPMC | $Fe_2O_3$ | 29.07 | 27.96 | 17.51 | 32.99 | 32.05 | 7.27[a] |
| 19 | 019/01 | 13 | CAP-010 | Gelatin | $Fe_2O_3$ | 31.55 | 29.80 | 19.66 | 35.70 | 33.42 | 7.52[a] |

[a]Comparison with red $TiO_2$ reference Batch No. ENQ3860/AIRC/016/01.

Red iron oxide is not an opacifier per se but contributes to opacification.

The colorimetry data for the fluvastatin capsules are shown in Table 123.

Encapsulation trials 7 to 14 focused on the comparison of $TiO_2$-free white capsules with the HPMC-based $TiO_2$ reference (Batch No. ENQ3860/AIRC/007/01). The capsule shells of three of the $TiO_2$-free capsule batches were HPMC-based (Batch Nos. ENQ3860/AIRC/009/01, ENQ3860/AIRC/010/01 and ENQ3860/AIRC/011/01) and the other three white capsules batches (Batch Nos. ENQ3860/AIRC/012/01, ENQ3860/AIRC/013/01 and ENQ3860/AIRC/014/01) were gelatin-based. The only colored capsule shell used was the HPMC-based $TiO_2$ reference, CAP-014 (Batch No. ENQ3860/AIRC/008/01).

Table 123 shows that again that all colorimetry data for the white capsules show a similar pattern with high L* and hue angle values and low a*, b* and chroma values. However, there are differences in the values obtained e.g., only the batches encapsulated in CAP-002 (Batch No. ENQ3860/AIRC/012/01) and CAP-012 shells (Batch No. ENQ3860/AIRC/014/01) have L* values close to the $TiO_2$ reference (78.49). This is despite both of these capsules being gelatin-based and the control being HPMC-based.

With respect to the hue angle, the batches encapsulated in CAP-003 (Batch No. ENQ3860/AIRC/009/01), CAP-005 (Batch No. ENQ3860/AIRC/013/01) and CAP-012 (Batch No. ENQ3860/AIRC/014/01) lie closest to the $TiO_2$ reference value of 96.38. Table 123 shows the $\Delta E^*_{00}$ values calculated from the colorimetry data on the white capsules using Batch No. ENQ3860/AIRC/007/01 as a control. The $\Delta E^*_{00}$ values were all greater than 2 except between Batch No. ENQ3860/AIRC/012/01 (CAP-002) and the $TiO_2$ reference batch (CAP-013) at 1.76. The color difference value would suggest that a difference between CAP-002 and CAP-013 would be perceptible on close inspection. The result also fits with visual similarity in opacity observed between the empty CAP-002 and CAP-013 (see Figure 50) and is in line with colorimetry results on the empty capsule shells (see Table 105).

All of these capsules are filled with blends of API described as white (see Section 88). These have the potential to influence the perceived capsule color especially where the capsule shells are partially translucent. However, based on the colorimetry data generated none of the encapsulated batches is color matched with the white $TiO_2$ reference batch (acceptance criterion for white capsules $\Delta E^*_{00} \leq 1$).

Table 123: Colorimetry data on the fluvastatin capsule batches

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | RSSL Ref. P23-06656- | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | L* | a* | b* | C | h | ΔE*00 ($TiO_2$ vs ref) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 007/01 | 5 | CAP-016 | HPMC | $TiO_2$ | 78.49 | -0.69 | 6.16 | 6.20 | 96.38 | NA[a] |
| 8 | 008/01 | 8 | CAP-014 | HPMC | $TiO_2$ + Fe2O3 | 36.23 | 32.80 | 23.00 | 40.06 | 35.04 | NA |
| 9 | 009/01 | 1 | CAP-003 | HPMC | $CaCO_3$ | 73.34 | -0.99 | 9.03 | 9.09 | 96.34 | 4.50 |
| 10 | 010/01 | 9 | CAP-009 | HPMC | $CaCO_3$ + A | 75.64 | -0.48 | 9.72 | 9.73 | 92.88 | 3.46 |
| 11 | 011/01 | 6 | CAP-007 | HPMC | $CaCO_3$ + A | 74.52 | -1.22 | 8.97 | 9.06 | 97.80 | 3.68 |
| 12 | 012/01 | 2 | CAP-002 | Gelatin | NaPOx | 79.67 | -1.34 | 7.85 | 7.96 | 99.72 | 1.76 |
| 13 | 013/01 | 3 | CAP-005 | Gelatin | $CaCO_3$ | 76.22 | -1.46 | 12.05 | 12.14 | 96.93 | 4.67 |
| 14 | 014/01 | 4 | CAP-012 | Gelatin | $CaCO_3$ + B +D | 78.34 | -1.30 | 11.16 | 11.24 | 96.69 | 3.70 |

[a]This batch was used as the $TiO_2$ reference for the $\Delta E^*_{00}$ color difference comparison

## 90. Capsule Brittleness

The % capsule brittleness results for the encapsulated batches are shown in Table 124, Table 125 and Table 126 for the benserazide HCl/levodopa capsules, the loperamide capsules and fluvastatin capsules respectively. The tables also contain a comparison of the % capsule found to be brittle during testing of the empty capsules stored under laboratory conditions (see Table 113).

Table 124: % Capsule brittle for the benserazide HCl/levodopa capsule batches

| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | % Brittle Filled Caps | % Brittle Empty Caps[a] (Lab Storage) |
|---|---|---|---|---|---|---|
| 1 | 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 0% | 2% |
| 2 | 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 0% | 0% |
| 3 | 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 5% | 0% |
| 4 | 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 0% | 0% |
| 5 | 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 5% | 0% |
| 6 | 006/01/P1 | CAP-006 | Gelatin | CaCO3 + A | 10% | 0% |

Color Code:

Green=% Brittle capsules ≤5%

Red = % Brittle capsules > 5%

[a]Test carried out on 50 capsules using a different technique (see Section 68).

Table 125: % Capsule brittle for the loperamide capsule batches

| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | % Brittle Filled Caps | % Brittle Empty Caps[a] (Lab Storage) |
|---|---|---|---|---|---|---|
| 15 | 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 0% | 0% |
| 16 | 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 15% | 0% |
| 17 | 017/01/P1 | CAP-011 | HPMC | $CaCO_3$ | 60% | 52% |
| 18 | 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | 0% | 0% |
| 19 | 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | 0% | 0% |

Color Code:

Green=% Brittle capsules ≤5%

Red = % Brittle capsules > 5%

[a]Test carried out on 50 capsules using a different technique (see Section 68).

Table 126: % Capsule brittle for the fluvastatin capsule batches

| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | % Brittle Filled Caps | % Brittle Empty Caps[a] (Lab Storage* |
|---|---|---|---|---|---|---|
| 7 | 007/01/P1 | CAP-016 | HPMC | $TiO_2$ | 5% | 2% |
| 8 | 008/01/P1 | CAP-014 | HPMC | $TiO_2$ + Fe2O3 | 5% | 4% |
| 9 | 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 0% | 2% |
| 10 | 010/01/P1 | CAP-009 | HPMC | $CaCO_3$ + A | 65% | 0% |
| 11 | 011/01/P1 | CAP-007 | HPMC | $CaCO_3$ + A | 5% | 2% |
| 12 | 012/01/P1 | CAP-002 | Gelatin | NaPOx | 0% | 2% |
| 13 | 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 0% | 0% |
| 14 | 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$ + B +D | 0% | 0% |

Color Code:

Green=% Brittle capsules ≤5%

Red = % Brittle capsules > 5%

[a]Test carried out on 50 capsules using a different technique (see Section 68).

The majority of the filled capsule batches met the % brittle capsules acceptance criterion of ≤ 5% under laboratory storage conditions. Batch ENQ3860/AIRC/017/01/P1 encapsulated in CAP-011, a pink HPMC capsule shell containing $CaCO_3$ and three other opacifiers did not. CAP-011 was also shown to be very brittle when capsule shell brittleness was evaluated on empty capsules (see Table 113). Therefore, the brittleness of the filled capsules reflects the brittleness of the shell itself prior to filling.

Batch ENQ3860/AIRC/010/01/P1 had 65% brittle capsules. It was encapsulated in CAP-009, a HPMC-based shell containing $CaCO_3$ + A as opacifiers. When evaluated as an empty capsule, 0% brittle capsules were found following laboratory storage. In addition, the empty CAP-009 capsule shells only showed significant brittleness at ≤ 23% RH. During manufacture of Batch ENQ3860/AIRC/010/01/P1, only 1 failed appearance check was observed (cracked capsule) with no AQL defects. Therefore, the manufacturing issues, as described in Section 87 do not explain why the filled capsules should be so brittle, as a higher number of defective capsules were detected in some other filled capsule lots which performed better in the brittleness test e.g., Batch ENQ3860/AIRC/008/01/P1.

Batch ENQ3860/AIRC/016/01/P1 and ENQ3860/AIRC/006/01/P1 were just outside the ≤ 5% acceptance criterion for capsules brittle under laboratory conditions. Batch ENQ3860/AIRC/016/01/P1 contains the red $TiO_2$ gelatin reference capsule shell (CAP-017) and had 15% brittle capsules. However, this capsule shell was also used for Batch ENQ3860/AIRC/002/01/P1 where no brittle capsules were found during testing. In addition, when CAP-017 was tested as empty capsules, 0 % brittle capsules were found for those stored under laboratory conditions and only 4% were found to be brittle following 33%RH and 53%RH storage (see Table 113). Batch ENQ3860/AIRC/006/01/P1 had 10% brittle capsules. The gelatin capsule shell used was CAP-006 which contains $CaCO_3$ + A. Again, CAP-006 had 0% brittle capsules under laboratory storage and 2% at 53%RH when tested as empty capsule shells.

The reasons for the differences between the brittleness results obtained on the filled and empty capsules are likely to be the result of method differences. The method for testing the filled capsules was introduced to minimise analyst exposure to the powder blends should the capsules fracture. It is more operator dependent than the empty capsule shell methodology as it relies on the analyst to apply the same

pressure to the capsule shell each time. The number of capsules tested is also different (see Section 68).

All of the filled capsule batches were tested again for brittleness in the course of accelerated stability studies and therefore a fuller understanding of the brittleness of the various capsule shells was obtained (see Sections 0).

### 91. Assay and Related Impurities Results

The average assay and total related impurity results for the encapsulated batches are shown in Table 127, Table 128 and Table 129 for the benserazide HCl/levodopa capsules, the loperamide capsules and fluvastatin capsules respectively. The assay results for the benserazide HCl/levodopa capsule batches ranged between 96.1% label to 101.8 % label claim for benserazide HCl and 97.2% to 102.0% label claim for levodopa. The impurity results ranged from 2.13% LC to 2.33% label claim for five out of the six batches. The total related impurities for Batch ENQ3860/AIRC/002/01/P1 were slightly higher at 3.00% and the impurity profiles had an additional peak at approximately 4.4 min in both duplicate sample preparations at a level of around 0.4%. This batch is encapsulated in the gelatin $TiO_2$ red reference capsule (CAP-017). Further experimentation attributed this peak to the capsule shell and therefore this peak was later excluded from the %total related impurity calculation.

The assay results for the loperamide capsule batches ranged from 95.3% to 98.6% label claim and were within expectation. No related impurities were detected in any of the batches.

The average assay for the fluvastatin capsule batches ranged from 93.2% to 95.1 % label claim while the total impurities varied from 0.18% LC to 0.27% LC.

There was no trend observed in the assay and impurity results that would suggest a difference between $TiO_2$-free capsules and the corresponding reference capsules. The slight variation in assay results between batches may be the result of minor variation in capsule fill weights. In all cases the obtained results are within expectation and no significant issues were observed.

### 92. Disintegration Results

The disintegration results for the encapsulated batches are shown in Figure 63, Figure 64 and Figure 65 for the benserazide HCl/levodopa capsules, the loperamide capsules and fluvastatin capsules respectively.

The capsules from all batches disintegrated within 6 minutes. A difference in disintegration times was observed between the gelatin and HPMC- based capsule shells with the gelatin-based capsules in general disintegrating significantly quicker than the HPMC ones. This was regardless of which API blend was encapsulated. The exception was Batch ENQ3860/AIRC/019/01/P1 which had a disintegration time close to 6 min. The capsule shell used to manufacture this batch was CAP-010, a gelatin-based $TiO_2$-free capsule shell containing iron oxide red.

Table 127: Assay and impurity results for the benserazide HCl/levodopa capsule batches

| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Assay Benserazide HCl (%LC) | Assay Levodopa (%LC) | Total Related Impurities (%LC) |
|---|---|---|---|---|---|---|---|
| 1 | 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 96.1 | 97.2 | 2.21 |
| 2 | 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 101.8 | 102.0 | 2.59 (3.00)[a] |
| 3 | 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 98.9 | 100.0 | 2.33 |
| 4 | 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 98.2 | 99.4 | 2.13 |
| 5 | 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 98.9 | 99.0 | 2.21 |
| 6 | 006/01/P1 | CAP-006 | Gelatin | CaCO3 + A | 99.6 | 99.4 | 2.30 |

[a]Result re-calculated to remove peak related to the capsule shell.

Table 128: Assay and impurity results for the loperamide capsule batches

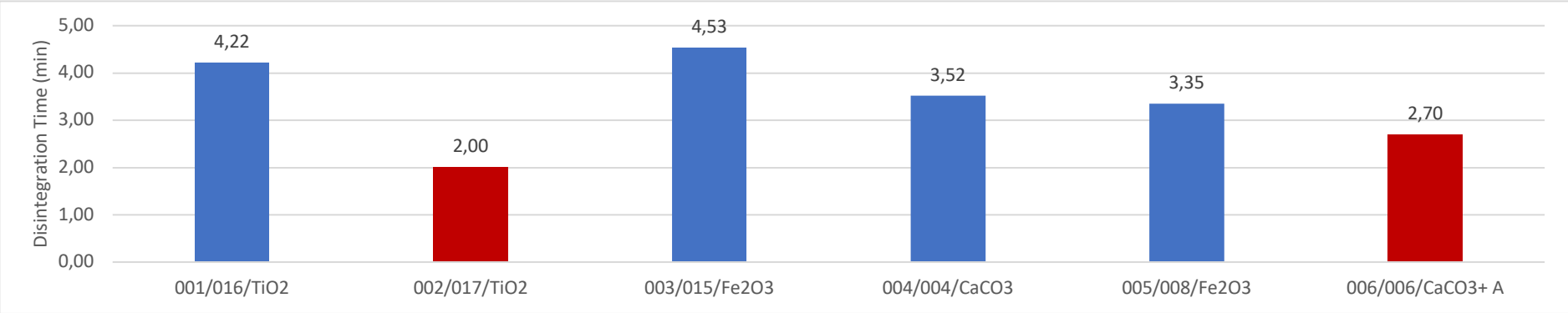
| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Average Loperamide Assay (%LC) | Total Related Impurities (%LC) |
|---|---|---|---|---|---|---|
| 15 | 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 97.2 | ND |
| 16 | 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 98.4 | ND |
| 17 | 017/01/P1 | CAP-011 | HPMC | $CaCO_3$ | 98.6 | ND |
| 18 | 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | 95.3 | ND |
| 19 | 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | 97.6 | ND |

ND = Not detected

Table 129: Assay and related impurity results for the fluvastatin capsule batches

| Trial | Packed Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Average Fluvastatin Assay (%LC) | Total Related Impurities (%LC) |
|---|---|---|---|---|---|---|
| 7 | 007/01/P1 | CAP-016[a] | HPMC | $TiO_2$ | 94.9 | 0.18 |
| 8 | 008/01/P1 | CAP-014[c] | HPMC | $TiO_2$ + $Fe_2O_3$ | 95.0 | 0.27 |
| 9 | 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 95.1 | 0.25 |
| 10 | 010/01/P1 | CAP-009 | HPMC | $CaCO_3$ + A | 93.2 | 0.26 |
| 11 | 011/01/P1 | CAP-007 | HPMC | $CaCO_3$ + A | 94.2 | 0.20 |
| 12 | 012/01/P1 | CAP-002 | Gelatin | NaPOx | 94.9 | 0.20 |
| 13 | 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 94.8 | 0.20 |
| 14 | 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$ + B +D | 93.8 | 0.21 |

Figure 63: Disintegration time results for the benserazide HCl/levodopa capsule batches

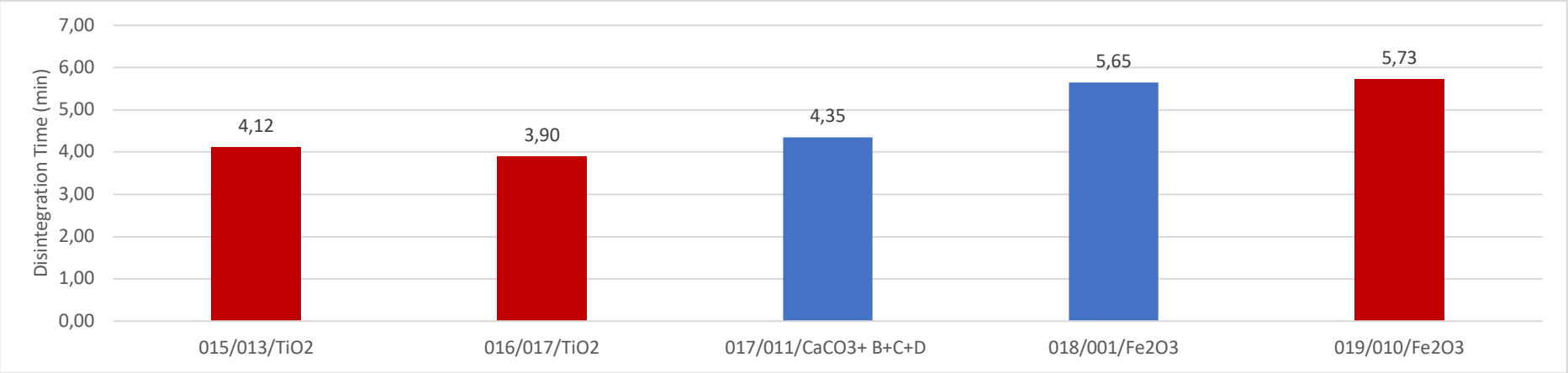



Data description: Batch Identifier/Consortium Capsule Shell Ref/Opacifier,

Color Code: Blue – HPMC-based, Red – Gelatin-based

Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

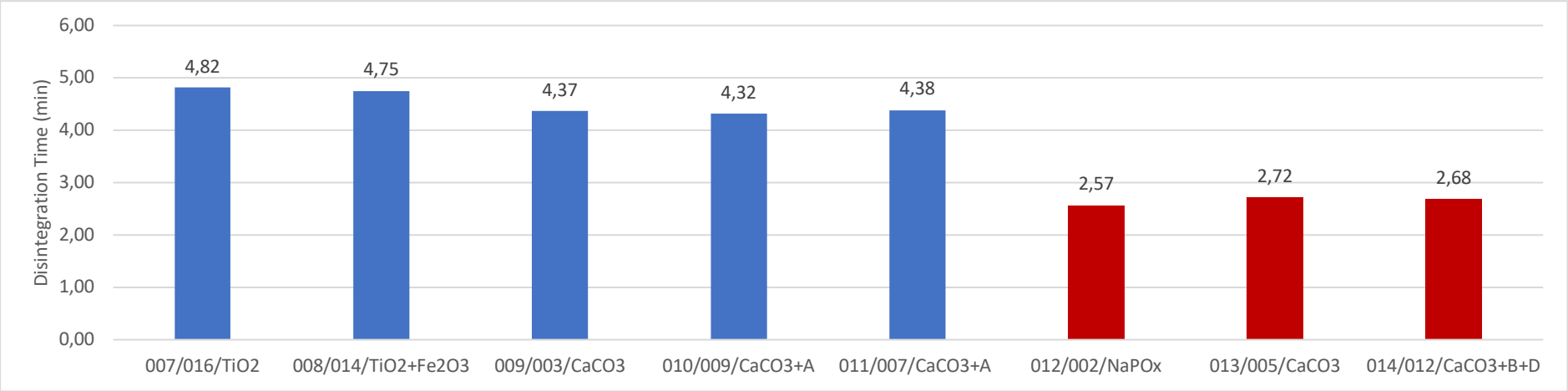
Figure 64: Disintegration time results for the loperamide capsule batches




Data description: Batch Identifier/Consortium Capsule Shell Ref/Opacifier, Color Code: Blue – HPMC-based, Red – Gelatin-based

Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

Figure 65: Disintegration time results for the fluvastatin capsule batches




Data description: Batch Identifier/Consortium Capsule Shell Ref/Opacifier

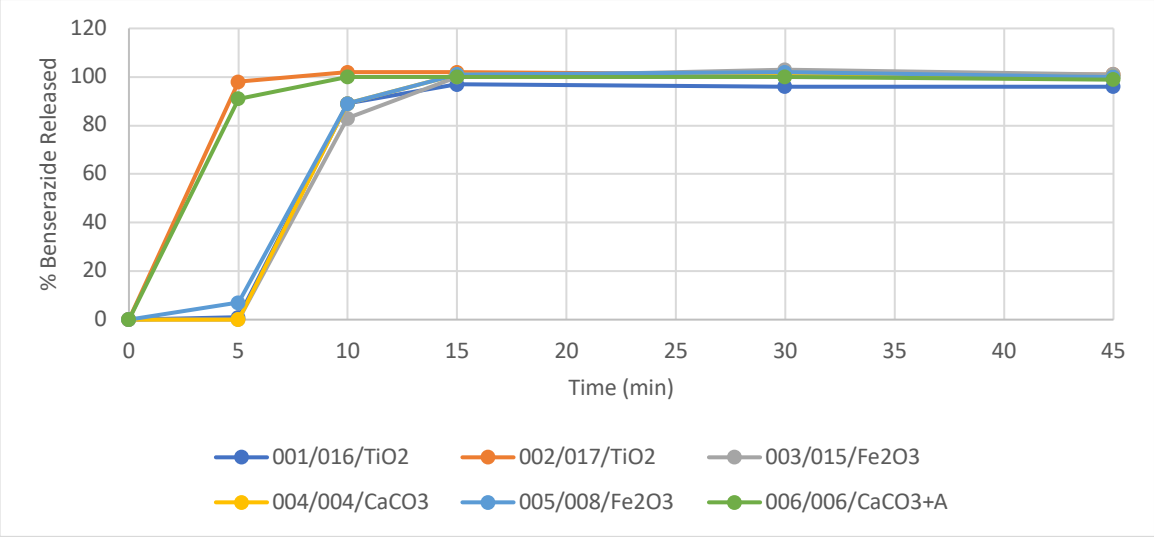
Color Code: Blue – HPMC-based, Red – Gelatin-based

Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

## 93. Dissolution Results

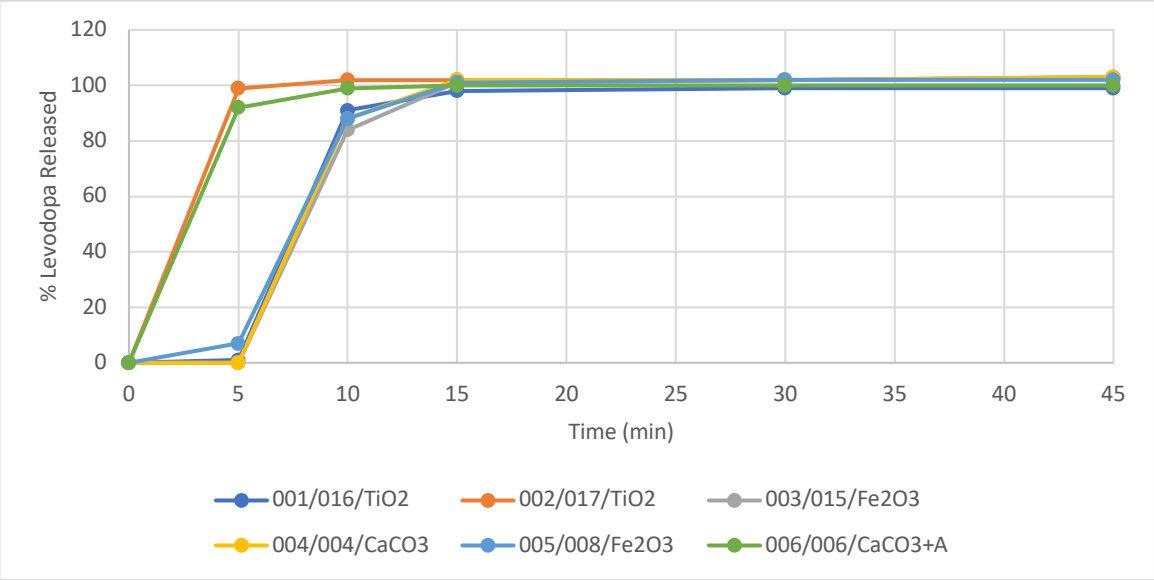
The dissolution results for the benserazide HCl/levodopa capsule batches are shown in Figure 66 and Figure 67.

Figure 66: Dissolution of benserazide from benserazide HCl/levodopa capsule batches




Legend: Batch Identifier/Consortium Capsule Shell Ref/Opacifier

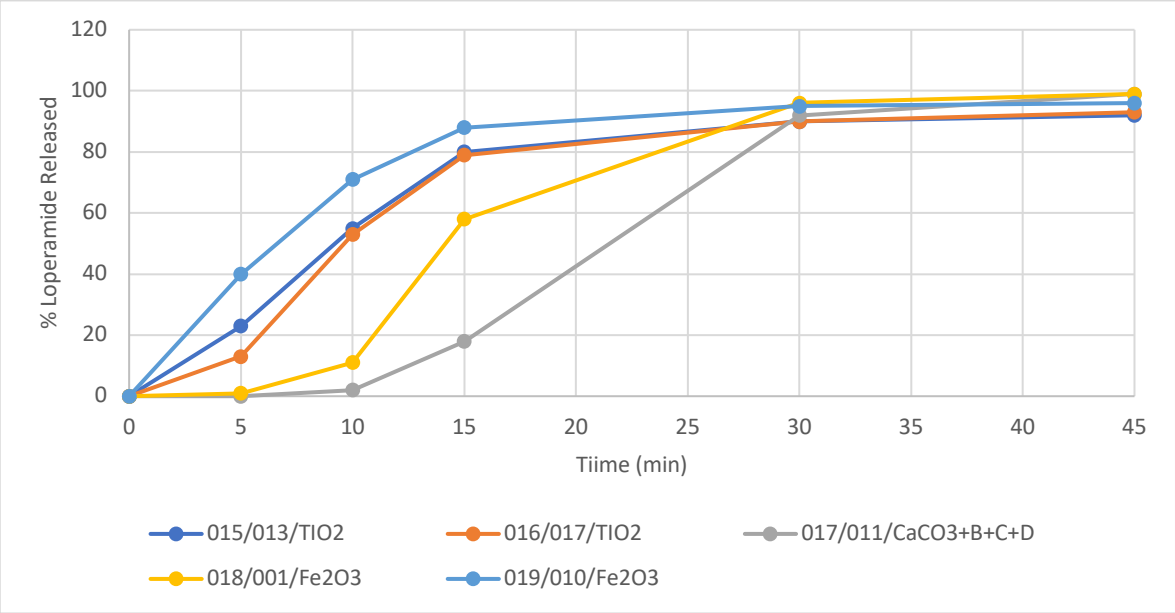
Figure 67: Dissolution of levodopa from benserazide HCl/levodopa capsule batches




Legend: Batch Identifier/Consortium Capsule Shell Ref/Opacifier

The dissolution data for the benserazide HCl/levodopa capsule batches show that both compounds were completely released and dissolved after 15 min from all of the capsule batches regardless of whether the capsule shells were $TiO_2$-free or not. In line with the disintegration data, the gelatin-based capsule batches (ENQ3860/AIRC/002/01/P1 and ENQ3860/AIRC/006/01/P1) released faster than the HPMC-based ones with over 90% of both compounds released in 5 min.

Figure 68: Dissolution from the loperamide batches

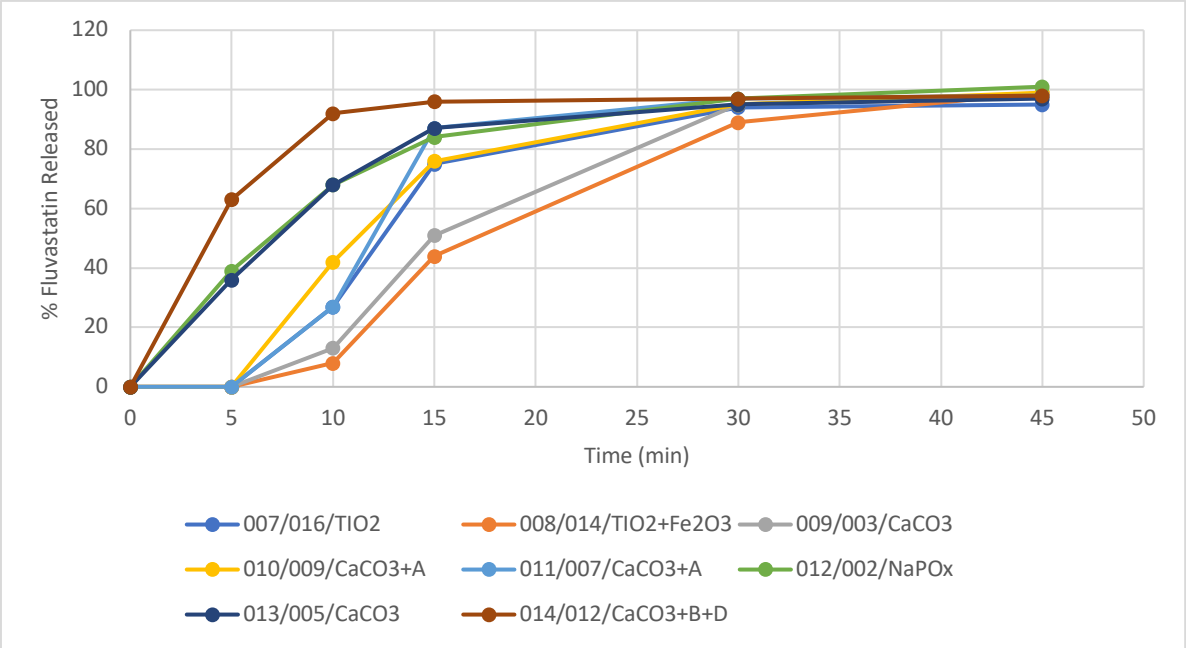



Legend: Batch identifier/Consortium Capsule Shell Ref/Opacifier

The dissolution data for $TiO_2$-free and $TiO_2$ reference capsule batches show that at least 90% of the loperamide is released from all of the batches within 30 min. There is some variation in the release rate between the various batches within this period. The gelatin-based capsule shell batches ENQ3860/AIRC/015/01/P1, ENQ3860/AIRC/016/01/P1 and ENQ3860/AIRC/019/01/P1) released over 75% of loperamide in the first 15 min. Their release rate was considerably faster than the two batches based on HPMC, ENQ3860/AIRC/017/01/P1 and ENQ3860/AIRC/018/01/P1. ENQ3860/AIRC/017/01/P1, which contains the pink HPMC-based CAP-011 had the slowest initial release rate with only 18% of the loperamide being released at the 15 min time point. However, at the 30-minute time-point, the % release was over 90%.

Figure 69 shows the dissolution results for the fluvastatin capsule batches.

Figure 69: Dissolution from the fluvastatin batches




All of the batches released almost all of the API within 30 minutes. Again, the three batches encapsulated in gelatin-based capsules released at a faster rate than the HPMC-based ones with over 75% API dissolving within the first 15 min.

## Section Summary and Conclusions

Capsule Shell Manufacture

The 19 encapsulation trials were successful in that sufficient filled capsules of acceptable quality were produced from each batch to conduct analytical testing and the photostability and accelerated stability studies. However, due to equipment issues, it was not possible to draw conclusions on the manufacturability of the $TiO_2$-free capsule shells based on the small-scale lots and further studies to investigate this were done at larger scale on empty capsule shells (see Section 0).

Filled Capsule Shell Appearance

None of the white $TiO_2$-free filled capsules were a color match for the $TiO_2$ reference capsules. CAP-002 was the closest match with a $\Delta E^*_{00}$ value of between 1-2 suggesting that a difference in color would only be perceived on close inspection. The colored capsules except CAP-004 and CAP-001 were described by different colors by their manufacturers and, therefore although a $\Delta E^*_{00}$ values were calculated between these $TiO_2$-free capsules and the corresponding controls, the results were not taken into account in the overall evaluation of these $TiO_2$-free capsule shells. The orange $TiO_2$-free capsule, CAP-001, was compared with the orange $TiO_2$ reference, CAP-014 (same shell former, product line, manufacturer). However, no color match was found.

Capsule Brittleness

All $TiO_2$-free filled capsules batches met the filled capsule brittleness test criterion of ≤ 5% brittle capsules except for those using CAP-006, CAP-009 and CAP-011. CAP-006 and CAP-011 had also been shown to be brittle as empty capsule shells at ≥33%RH using a different method of evaluation. CAP-017, the red gelatin-based $TiO_2$ reference was found to be brittle when filled with loperamide blend but not when filled with benserazide HCL/levodopa blend. These differences and also those between filled and empty capsules for CAP-009, may reflect the manual nature of the filled capsule brittleness test which is likely to be more operator-dependent than the test applied to the empty capsules, the former being introduced to minimise exposure to the powder blends.

In vitro Performance

The disintegration times of all the $TiO_2$-free encapsulated batches were not significantly different from those using the corresponding $TiO_2$ reference. All batches released around over 85 % of the API in 30 minutes regardless of capsule shell film material.

# Experimental Part 3: Photostability Study

## Protocol

Filled capsules from the 19 encapsulation runs (Section 0) plus the three active blends were subjected to photostability testing. The conditions used were equivalent to 2 x ICH Q1B cycles where 1 x ICH Q1B cycle equals light not less than 1.2 million lux hours; UV - not less than 200 Wh/m$^2$.

102 capsules from each of the 19 filled capsule batches were placed in clear borosilicate petri dishes and exposed to 2 x ICH conditions in a stability chamber. The stability chamber was cooled to maintain the temperature between approximately 10°C to 30°C. Following photoexposure, the samples were stored under laboratory conditions (15°C to 25°C, humidity monitored).

These photoexposed samples were compared to the corresponding $T_0$ samples, described in Section 0 of this report, stored protected from light. In Section 0, the $T_0$ samples are referred to as the control samples when discussing the photostability results.

With respect to the three active blends, 13 g Loperamide Hydrochloride 0.7% blend (Batch No. G172477), 4 g Fluvastatin sodium 13.8% blend (Batch No. G172476) and 8 g Benserazide HCl 9.5%/ Levodopa 33.33% blend (Batch No. G175016) were each placed in in clear borosilicate petri dishes and exposed to 2 x ICH conditions in a stability chamber. The same quantities of each blend were placed in clear borosilicate petri dishes wrapped in aluminium foil and placed in the stability chamber to act as dark control samples. The samples were tested as described in Table 130.

Table 130: Analytical testing of the samples placed on photostability

| **Benserazide HCl/Levodopa capsules & active blend** | | **Performed On** | |
|---|---|---|---|
| **Attribute** | **Methodology** | **Capsules*** | **Blend** |
| Appearance** | Visual | Yes | Yes |
| Assay | HPLC | Yes | Yes |
| Related substances | HPLC | Yes | Yes |
| Disintegration | USP <701> | Yes | Not required |
| Dissolution | USP Apparatus Type II (Paddles) | Yes | Not required |
| **Fluvastatin capsules & active blend** | | **Performed On** | |
| **Attribute** | **Methodology** | **Capsules** | **Blend** |
| Appearance | Visual | Yes | Yes |
| Assay | HPLC | Yes | Yes |
| Related substances | HPLC | Yes | Yes |
| Disintegration | USP <701> | Yes | Not required |
| Dissolution | USP Apparatus Type II (Paddles) | Yes | Not required |
| **Loperamide capsules & active blend** | | **Performed On** | |
| **Attribute** | **Methodology** | **Capsules** | **Blend** |
| Appearance | Visual | Yes | Yes |
| Assay | HPLC | Yes | Yes |
| Related substances | HPLC | Yes | Yes |
| Disintegration | USP <701> | Yes | Not required |
| Dissolution | USP Apparatus Type I (Baskets) | Yes | Not required |

*The photostability results from the capsules were compared to non-exposed control samples ($T_0$) samples from the accelerated stability study studies (see Section 0 and Section 0).

**10 capsules per used per batch for the appearance testing and 1 to 2 g of blend.

The methodology used is as described in Section 0.

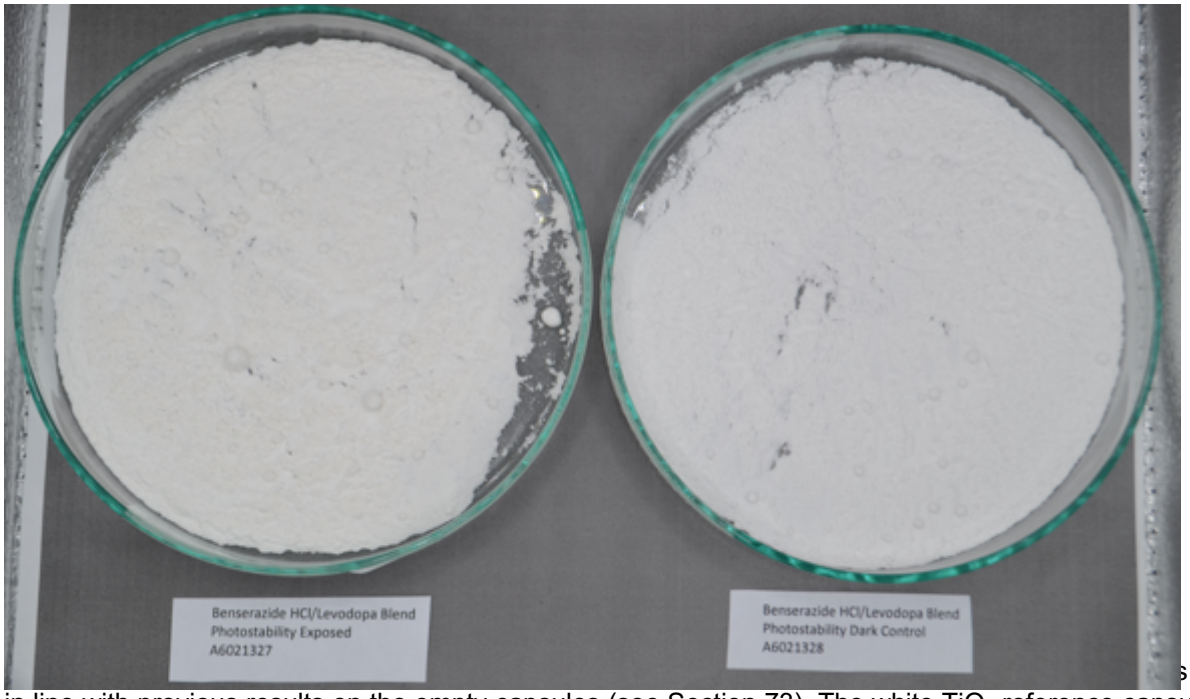
## Results and Discussion

### 94. Visual Appearance

Benserazide HCl/Levodopa Capsules

The visual appearance results for the photoexposed benserazide HCl/levodopa capsules versus the control samples are shown in Table 131. The appearance of the capsule contents did not change following photoexposure compared with that of the control samples, regardless of whether the capsule shells contained titanium dioxide or not. This is in line with the results for the benserazide HCl/levodopa blend which did not change in appearance following storage under extreme light exposure as shown in Figure 70. This is despite both component APIs having the potential for photodegradation.

Figure 70: Appearance of the benserazide HCl/levodopa powder blend before and after exposure




s is in line with previous results on the empty capsules (see Section 73). The white $TiO_2$ reference capsule shell, CAP-016, and the $TiO_2$-free CAP-004, which were used to encapsulate Batches ENQ3860/AIRC/001/01 and ENQ3860/AIRC/004/01, changed slightly in color following extreme light exposure. Again, similar results were found with these capsule shells when the empty capsules were tested. However, there was no change in the appearance of the $TiO_2$-free capsule shell, CAP-006, on photo-exposure. This was not the case for the same capsule shell exposed when empty, where a visual difference between the exposed sample and control was observed (see Table 106). This capsule shell is only semi-opaque (see Figure 50) and the colorimetry data showed that the $\Delta E_{00}$ values were just less than 1, suggesting that the exposed and control samples were very similar in color, despite a visual change being perceived.

Table 131: Visual appearance of the photoexposed benserazide HCl/levodopa capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample |
|---|---|---|---|---|---|---|
| NA | Blend Batch No. G175016 | NA | NA | NA | White powder, small amount of clumping, free from contamination. No visual difference between photostability exposed and dark control. | White powder, small amount of clumping, free from contamination. |
| 1 | 001/01 | CAP-016 | HPMC | $TiO_2$ | **Capsule shells**: white, no physical defects, Size 0, opaque. Slightly brighter white compared to the control. | **Capsule Shells**: Off white, no physical defects, Size 0. Opaque. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants |
| 2 | 002/01 | CAP-017 | Gelatin | $TiO_2$ | **Capsule shells**: dark red, no physical defects, Size 0, opaque. No observable difference compared to the control. | **Capsule Shells**: Dark Red, no physical defects, Size 0. Opaque. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants. |
| 3 | 003/01 | CAP-015 | HPMC | $Fe_2O_3$ | **Capsule shells**: dark red, no physical defects, Size 0, opaque. No visual difference compared to the control. | **Capsule Shells**: Dark Red, no physical defects, Size 0. Opaque. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants. |
| 4 | 004/01 | CAP-004 | HPMC | $CaCO_3$ | **Capsule shells**: off white, slightly transparent, no physical defects, Size 0. Slightly brighter white in comparison to the control. | **Capsule Shells**: Off white, slightly translucent, no physical defects, Size 0. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants. |

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample |
|---|---|---|---|---|---|---|
| 5 | 005/01 | CAP-008 | HPMC | $Fe_2O_3$ | **Capsule shells**: dark red, no physical defects, Size 0, opaque. No visual difference compared to the control. | **Capsule Shells**: Dark Red, no physical defects, Size 0. Opaque. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants. |
| 6 | 006/01 | CAP-006 | Gelatin | $CaCO_3$ + A | **Capsule shells**: off white, slightly translucent, no physical defects, Size 0. No visual difference compared to the control. | **Capsule Shells**: Off white, slightly translucent, no physical defects, Size 0. |
| | | | | | **Contents**: White powder, small amount of clumping, free from contaminants. No visual difference observed compared to the control. | **Contents**: White powder, small amount of clumping, free from contaminants. |

Color Code:

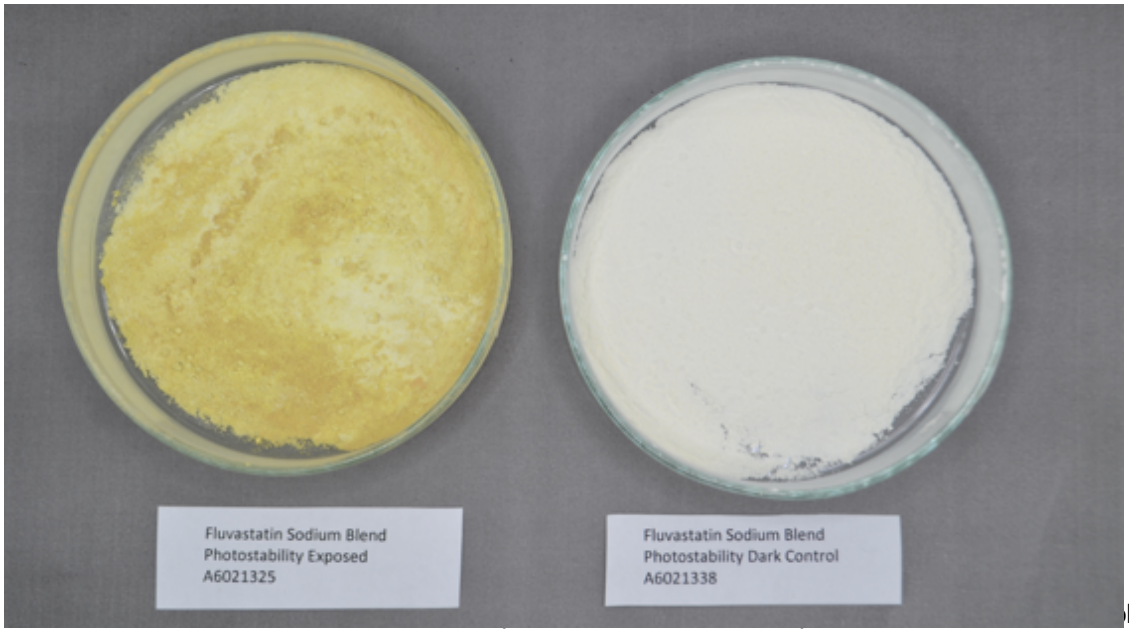
Green = No visible change in appearance between exposed sample and control

Red = Visible change in appearance between exposed sample and control

Fluvastatin Capsules

Table 132 shows the visual appearance results for the light-exposed fluvastatin capsules versus the corresponding controls. Fluvastatin is photo-sensitive and the blend turned yellow following exposure to the equivalent of 2 x ICH Q1B conditions. Figure 71 shows the transformation

Figure 71: Appearance of the exposed fluvastatin powder blend versus the control




loration following exposure. Figure 72 shows an example of the color change in the capsule contents following light exposure.

Figure 72: Appearance of the capsule contents from Batch ENQ3860/AIRC/010/01 (CAP-009) exposed to light versus the control

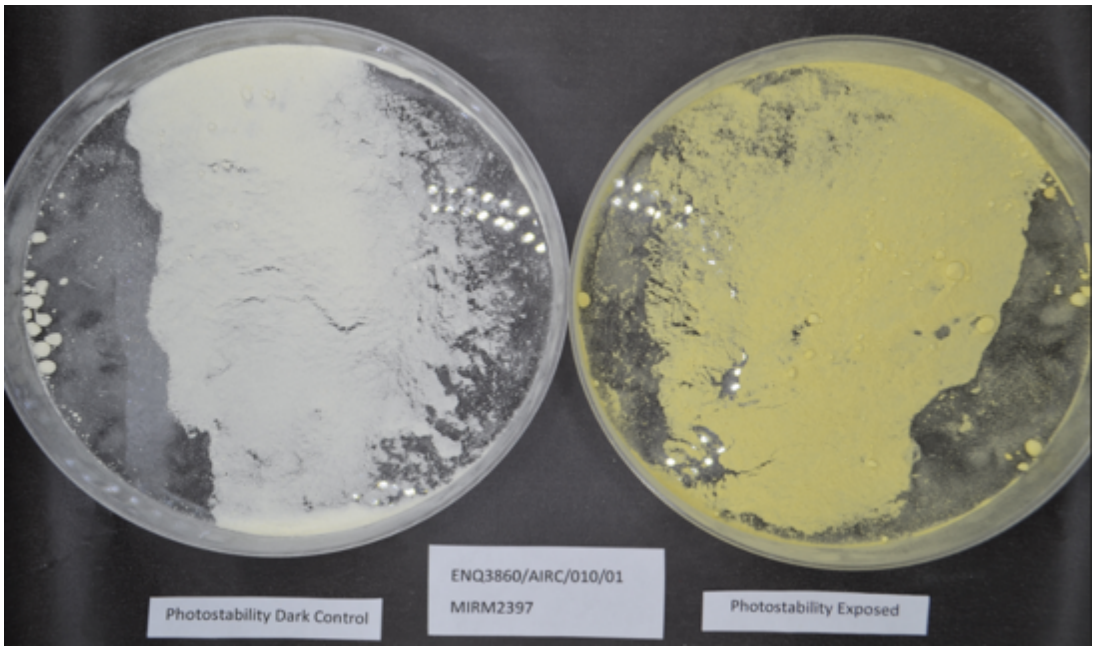


Table 132: Visual appearance of the photoexposed fluvastatin capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample |
|---|---|---|---|---|---|---|
| NA | Blend Batch No. G172476 | NA | NA | NA | Yellow powder, small amount of clumping, free from contamination. Obvious change from white to yellow compared to photostability dark control blend. | White powder, small amount of clamping, free from contamination. |
| 7 | 007/01 | CAP-016 | HPMC | $TiO_2$ | Capsule Shells: Off white, no physical defects, Size 0, opaque. No visual difference to control sample. | Capsule Shells: Off white, no physical defects, Size 0. Opaque. |
| | | | | | Contents: Yellow, small amount of clumping, free from contaminants. Obvious color change from white to yellow observed in comparison to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 8 | 008/01 | CAP-014 | HPMC | $TiO_2$+$Fe_2O_3$ | Capsule Shells: Red, one roughly cut capsule, remaining ones with no physical defects, Size 0, very slightly translucent. No visual difference between photostability exposed and control sample. | Capsule Shells: Red, one capsule roughly cut and leaking. Remaining capsule shells free from physical defects. Size 0. Very slightly translucent. |
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color difference from white to yellow when compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 9 | 009/01 | CAP-003 | HPMC | $CaCO_3$ | Capsule Shells: Yellow, no physical defects, Sz 0, slightly translucent. Obvious color change white to yellow when compared to the control sample. | Capsule Shells: Off white, one capsule roughly cut. Remaining capsule shells free from physical defects. Size 0. Slightly translucent. |
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color change from white to yellow compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample |
|---|---|---|---|---|---|---|
| 10 | 010/01 | CAP-009 | HPMC | $CaCO_3$+A | Capsule shells: Off white, no physical defects, Size 0, slightly translucent. No visual difference compared to the control sample. | Capsule Shells: Off white, no physical defects, Size 0. Slightly transparent. |
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color change from white to yellow when compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 11 | 011/01 | CAP-007 | HPMC | $CaCO_3$+A | Capsule shells: light yellow, no physical defects, Size 0, translucent. Obvious color change from white to light yellow compared to the control sample. | Capsule Shells: Off white, no physical defects, Size 0. Translucent. |
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color change from white to yellow compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 12 | 012/01 | CAP-002 | Gelatin | NaPOx | Capsule shells: off white, no physical defects, Size 0, opaque. No visual difference compared to the control sample. | Capsule Shells: Off white, no physical defects, Size 0. Opaque. |
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color change from white to yellow compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 13 | 013/01 | CAP-005 | Gelatin | $CaCO_3$ | Capsule shells: light yellow, one capsule with an indented end, remaining with no physical defects. Obvious color change from off white to yellow compared to the control sample. | Capsule Shells: Off white, no physical defects, Size 0. Translucent. |

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample |
|---|---|---|---|---|---|---|
| | | | | | Contents: Yellow powder, small amount of clumping, free from contamination. Obvious color change from white to yellow compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 14 | 014/01 | CAP-012 | Gelatin | $CaCO_3$+B+D | Capsule shells: off white, no physical defects, Sz 0, opaque. Slightly darker white than the control sample. | Capsule Shells: Off white, one capsule roughly cut. Remaining capsule shells free from physical defects. Size 0. Opaque |
| | | | | | Contents: Yellow powder, small amount of clamping, free from contamination. Obvious color change from white to yellow compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |

Color Code:

Green = No visible change in appearance between exposed sample and control

Red = Visible change in appearance between exposed sample and control

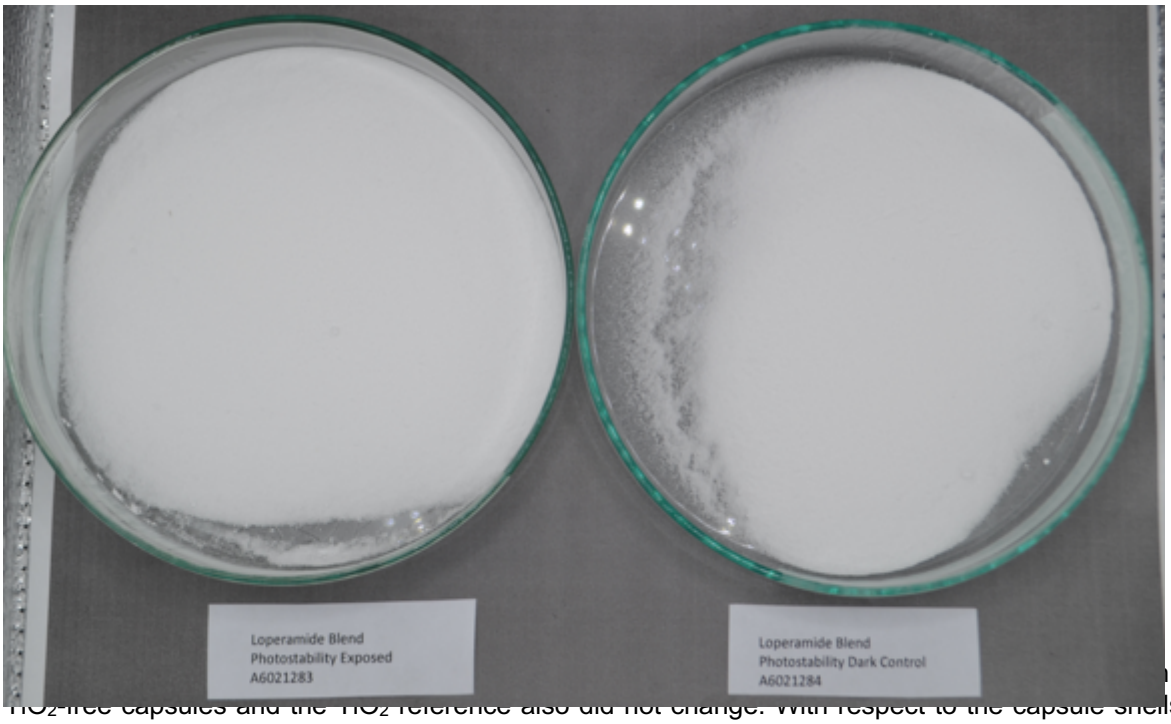
Both the white and colored HPMC-based capsule shells (CAP-016 and CAP-014), used to encapsulate Batch ENQ3860/AIRC/007/01 and Batch ENQ3860/AIRC/008/01 respectively, were not perceived to change color following the extreme light exposure. The white $TiO_2$-free gelatin-based capsule shell, CAP-002, and the $TiO_2$-free HPMC-based, CAP-009, also did not undergo a perceptible color change. These shells were used for Batch ENQ3860/AIRC/012/01 and Batch ENQ3860/AIRC/010/01 respectively. All of these capsule shells are opaque or relatively opaque (see Figure 50 and Figure 51). Despite their opacity and lack of color change in the shells themselves, none of these capsules could prevent discoloration of their contents, meaning that light was still able to penetrate through the shells.

The lack of color change in CAP-002, CAP-009 and CAP-016 differs from the visual appearance for the empty capsules (see Table 106 and Table 107) as all of these empty capsule shells were perceived to change color on exposure to 2x ICH Q1B conditions. This difference may be due to the adsorption of the light by the capsule contents during exposure, thus, protecting the shell. Alternatively, the subjective nature of visual assessment evaluation despite standardized testing conditions, may have resulted in the difference in the results. The visual appearance results for CAP-014 are in agreement with those of the empty capsules (see Table 107). The other $TiO_2$-free capsule shells used to encapsulate fluvastatin changed color following storage in the photostability cabinets. These results are in line with those on the empty capsules (see Table 106 and Table 107).

Loperamide Capsules

Table 133 shows the visual appearance results for the light-exposed loperamide capsules versus the corresponding controls and Figure 73 shows the appearance of the powder blend after exposure versus the control.

Figure 73: Appearance of the loperamide powder blend after exposure versus the control




the $TiO_2$-free capsules and the $TiO_2$ reference also did not change. With respect to the capsule shells, a visible difference following exposure only occurred with CAP-011, the pink capsule, used to encapsulate Batch ENQ3860/AIRC/017/01. This is in line with the results on this empty capsule (see Table 107).

CAP-017, the gelatin-based $TiO_2$ reference and the HPMC-based $TiO_2$-free CAP-001, are colored capsules. They were shown not to change color in the course of the photostability study. This is in agreement with the results for these capsule shells when empty (see Table 106 and Table 107). CAP-013, which is the white, gelatin-based $TiO_2$ reference, also did not change color as a result of the extreme light exposure. However, when tested as an empty capsule in a similar photostability study, a visual difference could be observed between the exposed and control samples.

Table 133: Visual appearance of the photoexposed loperamide capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample ($T_0$) |
|---|---|---|---|---|---|---|
| NA | Blend Batch No. G172477 | NA | NA | NA | White powder, free from visual defects or contaminants. No visual difference observed compared to dark control. | White powder, free from visual defects and contaminants. |
| 15 | 015/01 | CAP-013 | Gelatin | $TiO_2$ | Capsule shells: off white, no physical defects, Size 0, no visual difference observed compared to the control sample. | Capsule Shells: Off whit e, no physical defects, Size 0. |
| | | | | | Contents: white powder, small amount of clumping, free from contaminants, no visual difference compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 16 | 016/01 | CAP-017 | Gelatin | $TiO_2$ | Capsule shells: dark red, no physical defects, Size 0, no visual difference observed compared to the control sample. | Capsule Shells: Dark red, no physical defects, Size 0. |
| | | | | | Contents: white powder, small amount of clumping, free from contaminants, no visual difference compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 17 | 017/01 | CAP-011 | HPMC | $CaCO_3$+$Fe_2O_3$ | Capsule shells: very light pink, no physical defects, Size 0, lighter in color in comparison to the control sample. | Capsule Shell: Light pink, no physical defects, Size 0. |
| | | | | | Contents: white powder, small amount of clumping, free from contaminants, no visual difference compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |
| 18 | 018/01 | CAP-001 | HPMC | $Fe_2O_3$ | Capsule shells: dark red, slightly dusty, Size 0, no other visual defects. No visual difference compared to the control sample. | Capsule Shells: Dark red, slightly dusty, Size 0, no other visual defects. |
| | | | | | Contents: white powder, small amount of clumping, free from contaminants, no visual difference compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. No. | Shell Former | Opacifier | Appearance of Photoexposed Sample | Appearance of Control Sample ($T_0$) |
|---|---|---|---|---|---|---|
| 19 | 019/01 | CAP-010 | Gelatin | $Fe_2O_3$ | Capsule shells: dark red, no visual defects, Sz 0. No visual difference observed compared to the control sample. | Capsule Shells: Dark red, no physical defects, Size 0. |
| | | | | | Contents: white powder, small amount of clumping, free from contaminants, no visual difference compared to the control sample. | Contents: White powder, small amount of clumping, free from contaminants. |

Color Code:

Green = No visible change in appearance between exposed sample and control

Red = Visible change in appearance between exposed sample and control

## 95. Assay and Related Impurities

Benserazide HCL/Levodopa Capsules

Table 134 shows the mean assay and total related impurities results for the benserazide HCl/levodopa capsules. Following extreme light exposure, the average assay for both benserazide HCl and levodopa in the filled capsules did not alter significantly compared with the corresponding control samples for any of the batches tested. The average assay results for the exposed levodopa blend were also almost unchanged compared with the control sample. The benserazide HCl assay for the blend was significantly higher for the light exposed sample at 99.1 %w/w compared with 95.3% w/w for the control.

The total impurities increased slightly for all of the light-exposed samples compared with controls (range 0.04%LC to 0.37%LC). This increase was not considered significant. The largest impurity increase of 0.37% was observed for batch ENQ3860/AIRC/005/01 which contained CAP-008, a colored $TiO_2$-free capsule containing red iron oxide. The additional peaks due to capsule shell interference were observed in Batch ENQ3860/AIRC/002/01 and were excluded from the sum of total related impurities.

Fluvastatin Capsules

Table 135 shows the average assay and total related impurities results for the fluvastatin capsule batches and blend and the corresponding control samples. Exposure to the extreme light conditions resulted in a decrease in assay and increase in related impurities for all of the capsule batches and the blend compared to the controls. The lowest assay (74.4% w/w) and greatest increase in related impurities (5.52%w/w) were observed for the blend. This would suggest that encapsulation does result in partial protection against light. The highest assay (85.9% LC) and lowest related impurities results (2.84%) were obtained with Batch ENQ3860/AIRC/008/01 whose HPMC-based capsule shell contains both titanium dioxide and iron red oxide. The combination of the $TiO_2$ and the red colorant probably contributed to the improved protection. The batches encapsulated in the more opaque gelatin-based $TiO_2$-free capsules (CAP-002 and CAP-012) contained lower quantities of related impurities than the $TiO_2$-free HPMC-based capsules and the HPMC-based $TiO_2$ reference. This would suggest that they offer improved light protection. Nevertheless, none of the capsule shells tested could fully protect fluvastatin from photodegradation.

Loperamide Capsules

Table 136 shows the average assay and total related impurities results for the loperamide capsule batches and blend and the corresponding control samples. The data show that there was some variation in the assay values determined for the light exposed samples and the controls, with some exposed samples being slightly higher than the corresponding control, while others were slightly lower. The largest difference of 2.7%LC was for Batch for ENQ3860/AIRC/019/01, which was encapsulated in CAP-010, a gelatin $TiO_2$-free capsule. No impurities were reported for the control samples. However, all light-exposed batches showed some degradation. The blend had the highest total degradation with 0.33%w/w related impurities, while the encapsulated batches contained much lower levels showing that encapsulation provided partial protection from the environmental conditions. The highest level of related impurities in the exposed capsule batches was for ENQ3860/AIRC/019/01 at 0.13%w/w, while the lowest level was found in Batch ENQ3860/AIRC/016/01, which was encapsulated in the red gelatin-based $TiO_2$ reference capsule, CAP-017. In this batch the %w/w of related impurities lay below the limit of quantification (0.05%). CAP-017 contains both $TiO_2$ and red iron oxide. This might suggest that this combination was more effective at protecting the loperamide against degradation, than red iron oxide on its own in the other colored capsules or $TiO_2$ alone in the white, gelatin-based $TiO_2$ reference, CAP-013.

Table 134: Mean assay and total related impurities for the exposed benserazide HCl/levodopa capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. Shell Former & Opacifier | Assay Benserazide HCL | | | | Assay Levodopa | | | | Total impurities (%LC) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exposed | | Control ($T_0$) | | Exposed | | Control($T_0$) | | Exp | Control ($T_0$) |
| | | | %LC | mg/cap | %LC | mg/cap | %LC | mg/cap | %LC | mg/cap | %LC | %LC |
| NA | Blend Batch G175016 | NA | 99.1[a] | NA | 95.3[a] | NA | 97.9[a] | NA | 98.0[a] | NA | 2.69 | 2.56 |
| 1 | 001/01 | CAP-016 (HPMC/$TiO_2$) | 98.2 | 14.0 | 96.1 | 13.7 | 99.2 | 49.6 | 97.2 | 48.6 | 2.35 | 2.21 |
| 2 | 002/01 | CAP-017 Gelatin/$TiO_2$ | 101.1 | 14.4 | 101.8 | 14.5 | 101.4 | 50.7 | 102.0 | 51.0 | 2.62[b] | 2.40[b] |
| 3 | 003/01 | CAP-015 HPMC/$Fe_2O_3$ | 100.4 | 14.3 | 98.9 | 14.1 | 101.2 | 50.6 | 100.0 | 50.0 | 2.37 | 2.33 |
| 4 | 004/01 | CAP-004 HPMC/$CaCO_3$ | 101.1 | 14.4 | 98.2 | 14.0 | 100.4 | 50.2 | 99.4 | 49.7 | 2.42 | 2.11 |
| 5 | 005/01 | CAP-008 HPMC/$Fe_2O_3$ | 101.1 | 14.4 | 98.9 | 14.1 | 100.6 | 50.3 | 99.0 | 49.5 | 2.59 | 2.22 |
| 6 | 006/01 | CAP-006 Gelatin/$CaCO_3$ + A | 100.4 | 14.3 | 99.6 | 14.2 | 99.4 | 49.7 | 99.4 | 49.7 | 2.54 | 2.30 |

[a]%w/w

[b]Impurity peaks related to capsule shell not included in total calculations ( ~ RRT 10.12 & ~ RRT 1.30)

Table 135: Mean assay and total related impurities for the exposed fluvastatin capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consortium Cap Shell Reference | Shell Former | Opacifier | Average Fluvastatin assay (%LC) | | Total impurities (%LC) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Exposed | Control ($T_0$) | Exposed | Control ($T_0$) |
| NA | Blend Batch G172476 | NA | NA | NA | 74.4[a] | 92.2[a] | 5.52 | 0.11 |
| 7 | 007/01 | CAP-016[a] | HPMC | $TiO_2$ | 84.7 | 94.9 | 4.09 | 0.13 |
| 8 | 008/01 | CAP-014 | HPMC | $TiO_2$+$Fe_2O_3$ | 85.9 | 95.0 | 2.84 | 0.14 |
| 9 | 009/01 | CAP-003 | HPMC | $CaCO_3$ | 82.7 | 95.1 | 5.03 | 0.14 |
| 10 | 010/01 | CAP-009 | HPMC | $CaCO_3$+A | 82.0 | 93.2 | 4.39 | 0.14 |
| 11 | 011/01 | CAP-007 | HPMC | $CaCO_3$+A | 79.8 | 94.2 | 5.32 | 0.14 |
| 12 | 012/01 | CAP-002 | Gelatin | NaPOx | 85.5 | 94.9 | 3.62 | 0.14 |
| 13 | 013/01 | CAP-005 | Gelatin | $CaCO_3$ | 84.8 | 94.8 | 4.69 | 0.14 |
| 14 | 014/01 | CAP-012 | Gelatin | $CaCO_3$+B+D | 84.6 | 93.8 | 3.68 | 0.15 |

[a]% w/w

Table 136: Mean assay and total related impurities for the exposed loperamide capsule batches and blend versus the corresponding controls

| Trial | Bulk Capsule Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref. | Shell Former | Opacifier | Average Loperamide Assay (%w/w) | | Total impurities (%LC) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Exposed | Control ($T_0$) | Exposed | Control ($T_0$) |
| NA | Blend Batch No. G172477 | NA | NA | NA | 97.7 | 98.2 | 0.33 | <LOQ[a] |
| 15 | 015/01 | CAP-013 | Gelatin | $TiO_2$ | 96.2 | 97.2 | 0.11 | ND |
| 16 | 016/01 | CAP-017 | Gelatin | $TiO_2$ | 99.2 | 98.4 | <LOQ* | ND |
| 17 | 017/01 | CAP-011 | HPMC | $CaCO_3$+B+C+D | 99.2 | 98.6 | 0.12 | ND |
| 18 | 018/01 | CAP-001 | HPMC | $Fe_2O_3$ | 96.0 | 95.3 | 0.09 | ND |
| 19 | 019/01 | CAP-010 | Gelatin | $Fe_2O_3$ | 94.9 | 97.6 | 0.13 | ND |

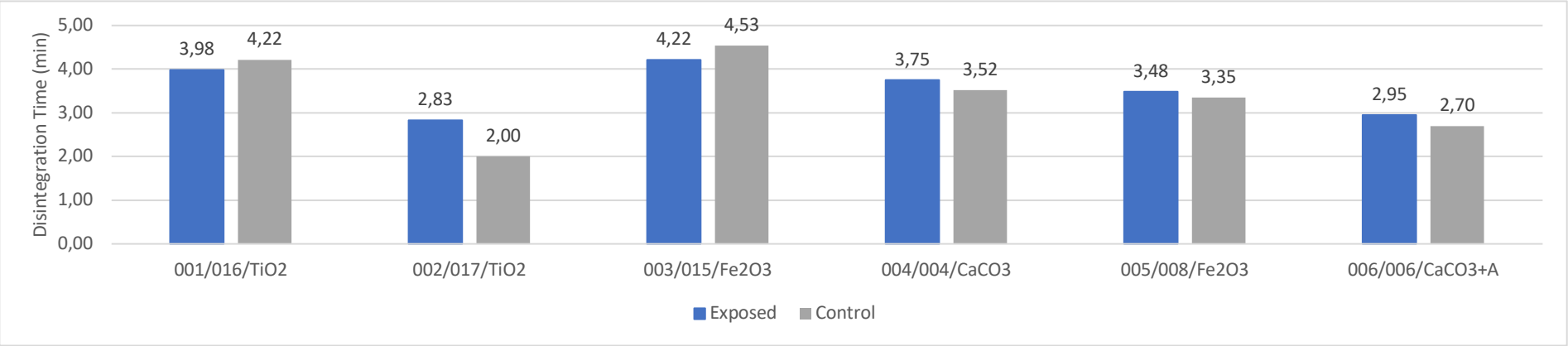
[a]LOQ = Limit of Quantification (0.05%)

## 96. Disintegration Results

The disintegration results for the exposed encapsulated batches and corresponding controls are shown in Figure 74, Figure 75 and Figure 76 for the benserazide HCl/levodopa capsules, the fluvastatin capsules and loperamide capsules respectively.

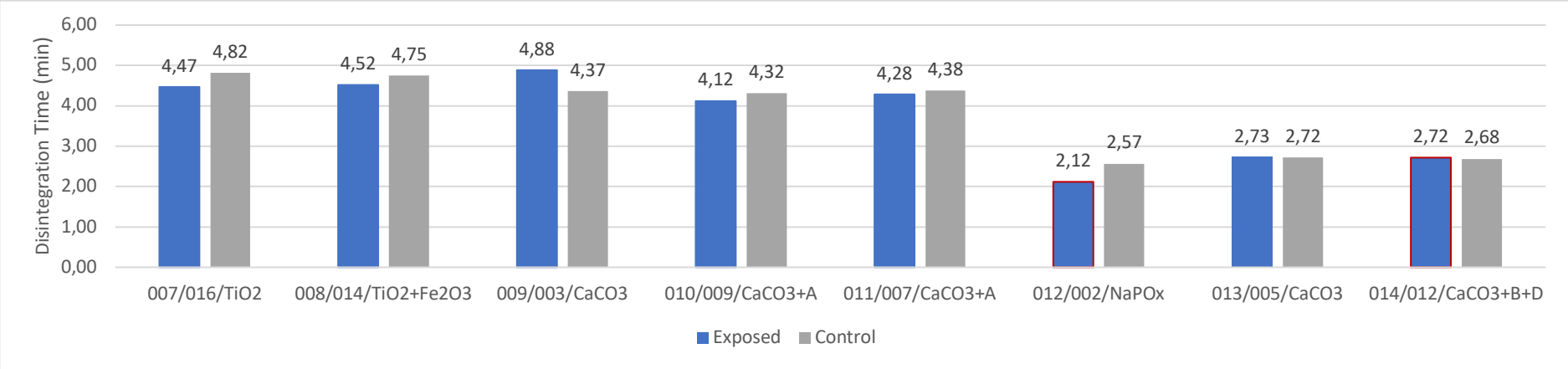
The capsules from all batches disintegrated within 6 minutes and there was minimal difference between the disintegration time for the exposed capsules and the corresponding controls. In most cases the difference was < 30s. A difference of around 50s in disintegration time between exposed and control samples was found for Batch ENQ3860/AIRC/002/01 which contained benserazide HCl/levodopa blend encapsulated in the gelatin-based $TiO_2$ red reference, CAP-017, Batch ENQ3860/AIRC/016/01, which contained loperamide blend in CAP-017 and Batch ENQ3860/AIRC/015/01 which contained loperamide blend in CAP-013, the white gelatin-based $TiO_2$ reference. None of these differences in disintegration time are significant.

Figure 74: Disintegration time for the exposed benserazide HCl/levodopa capsule batches versus the corresponding controls




Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

Figure 75: Disintegration time for the exposed fluvastatin capsule batches versus the corresponding controls

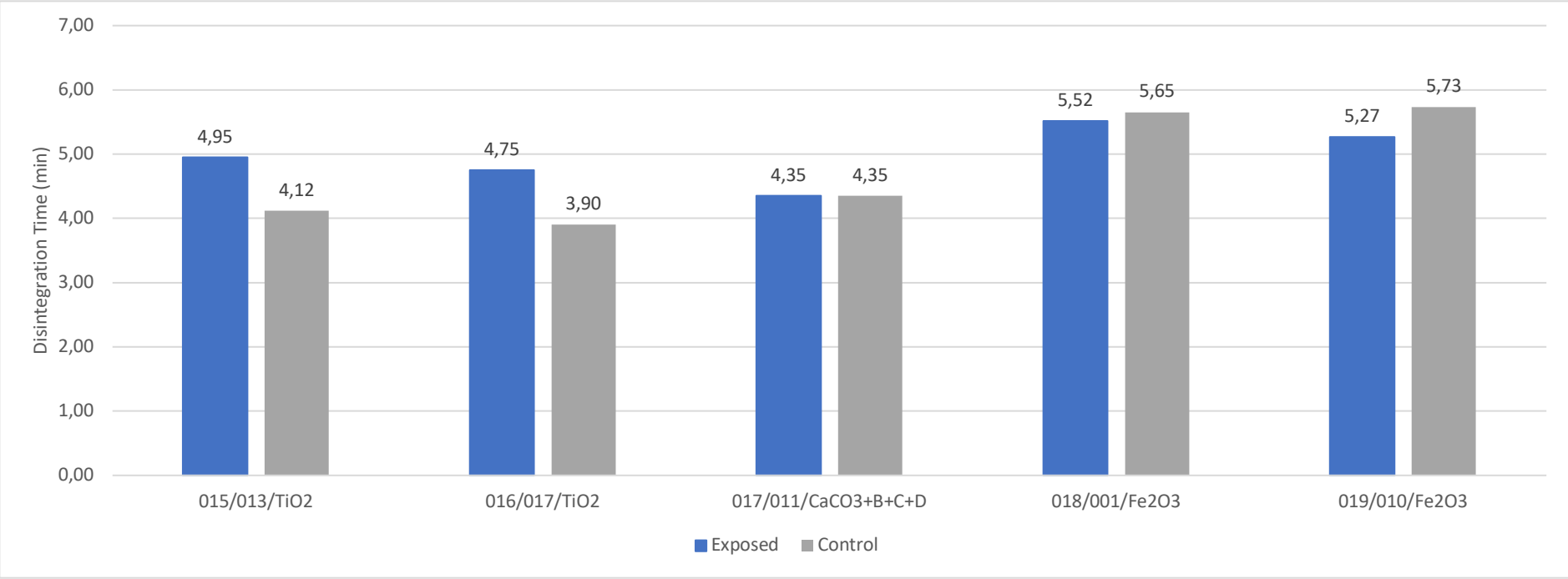



`

Data description: Batch Identifier/Consortium Capsule Shell Ref/Opacifier

Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

Figure 76: Disintegration time for the exposed loperamide capsule batches versus the corresponding controls




Disintegration time in minutes and seconds displayed as minutes and fractions of minutes.

## 97. Dissolution Results

Benserazide HCl/Levodopa Capsules

Figure 77 and Figure 78 compare the dissolution data for the exposed capsule batches versus the corresponding controls. Exposure to 2 x ICH Q1B conditions, did not result in as significant change in the %release of either benserazide or levodopa at the 10 min or 15 min time-points for any of the batches. At the 5 min-time-point, the exposed samples of Batch ENQ3860/AIRC/004/01 and ENQ3860/AIRC/005/01 released significantly more of both APIs than the control samples.

Fluvastatin Capsules

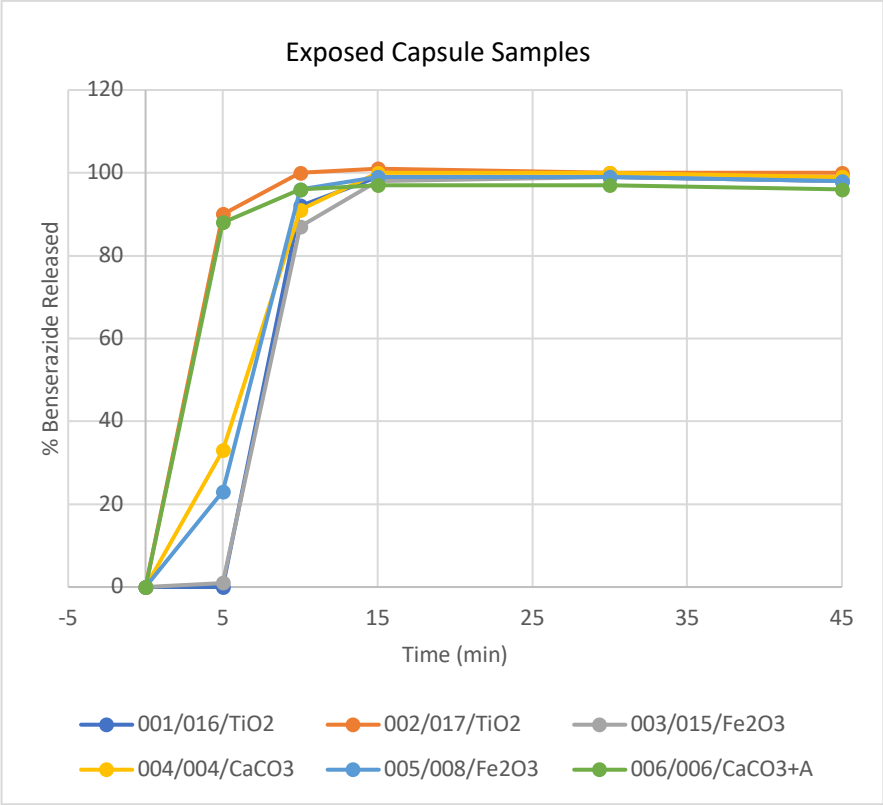
Figure 79 shows the dissolution data for the fluvastatin exposed capsules compared to the corresponding controls. The majority of the light exposed samples had slower dissolution compared to the controls but to different extents. This slowdown could not be attributed to an increase in disintegration times as the exposed samples disintegrated in a very similar time-frame to the control samples. In order to illustrate this in detail, Figure 79 includes graphs of the exposed sample versus control for the two batches encapsulated in $TiO_2$ reference capsule shells, the batches encapsulated in HPMC-based capsules and the batches encapsulated in gelatin-based capsule shells.

The light-exposed Batch ENQ3860/AIRC/007/01, encapsulated in the white HPMC-based $TiO_2$ reference capsule shell, released significantly more slowly than the control. However, the % fluvastatin released from the exposed Batch ENQ3860/AIRC/008/01, containing the colored HPMC-based $TiO_2$ reference, CAP-014, changed only minimally compared to the control across all time-points except at 15 mins when it was approximately 10% higher. Interestingly, this capsule shell did not visibly change color on light exposure and the batch had the lowest level of total related impurities of all the light exposed fluvastatin samples.

The light exposed samples encapsulated in $TiO_2$-free HPMC-based capsule shells all had slower dissolution than the corresponding controls. The least difference was observed for the exposed sample from Batch ENQ3860/AIRC/010/01, encapsulated in the $TiO_2$-free, CAP-009, whose dissolution profile only deviated by approximately 10% from that of the control at the 30-min and 45-min time-points. CAP-009 is one of the most opaque HPMC-based $TiO_2$-free capsule shells and the capsule shell appearance did not visibly change in the course of the photostability study. Despite this, like the other capsule shells, it did not prevent fluvastatin degradation as a result of the extreme light exposure.

The dissolution profile of the exposed sample encapsulated in the gelatin-based $TiO_2$-free, CAP-002 (Batch ENQ3860/AIRC/012/01), did not change significantly compared with the control at the 5-min and 10-min time-points. However, at later time-points, dissolution from the exposed sample was slower than the control. Again, it is one of the more opaque capsule shells and its appearance did not change on light exposure, although its opacity has been shown to change with %RH (Section 75). The other batches encapsulated in gelatin $TiO_2$-free capsules had slower dissolution than the control at all time-points.

Figure 77: Mean benserazide dissolution from the benserazide HCl/levodopa light-exposed capsules



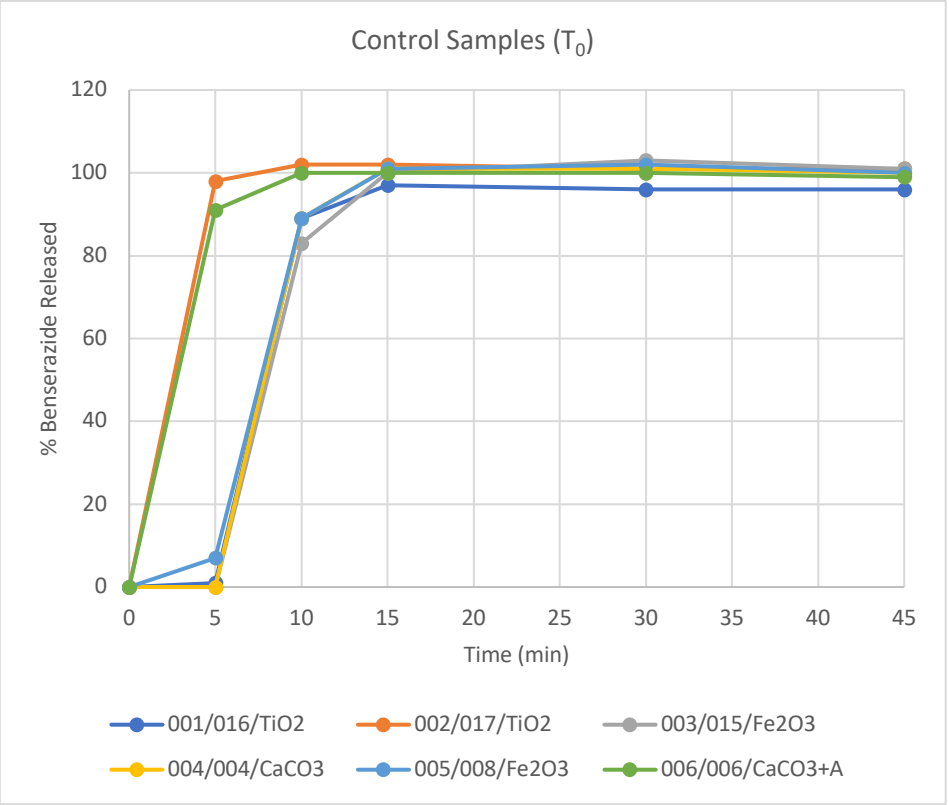
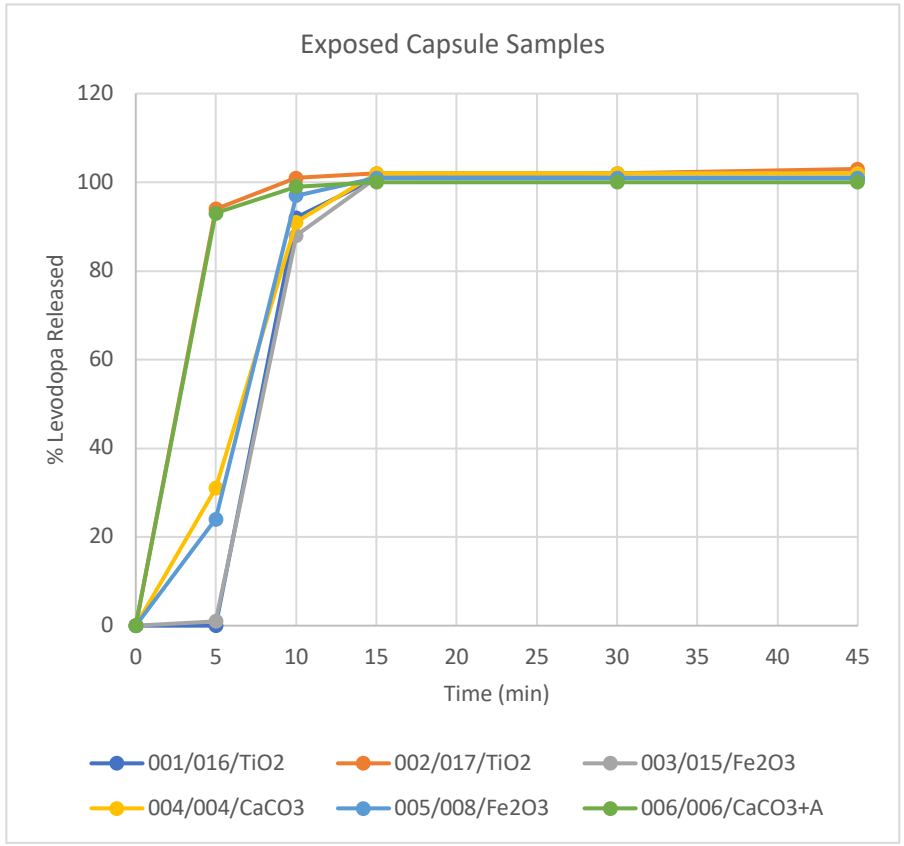



Figure 78: Mean levodopa dissolution from the benserazide HCl/levodopa light-exposed capsules



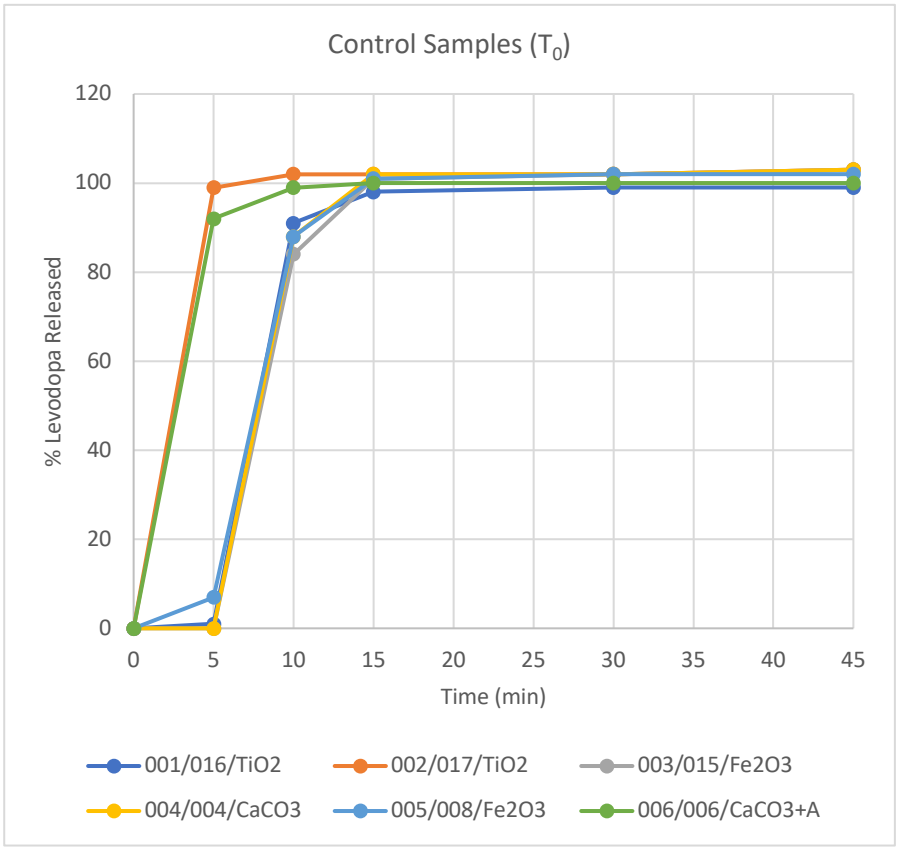
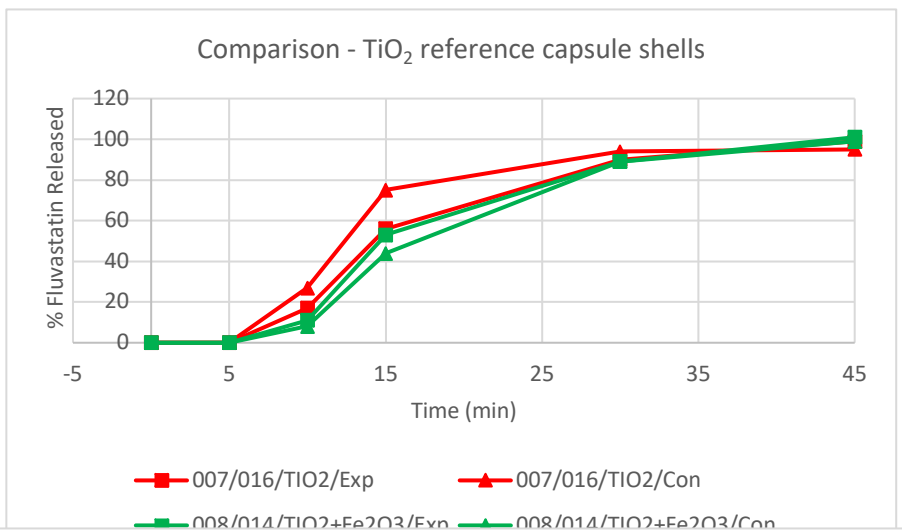
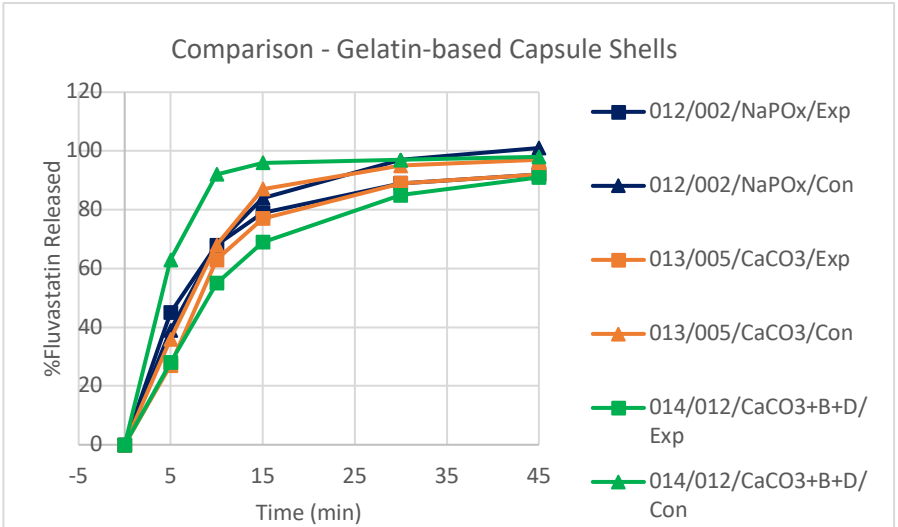
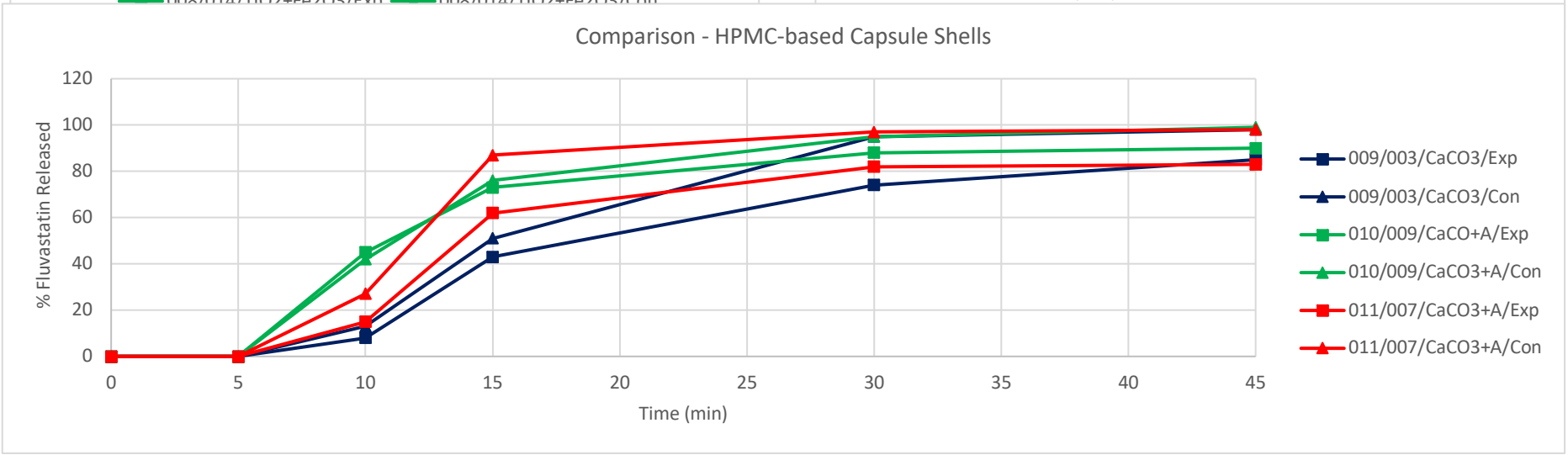



Figure 79: Mean fluvastatin dissolution from the fluvastatin light-exposed capsules versus controls



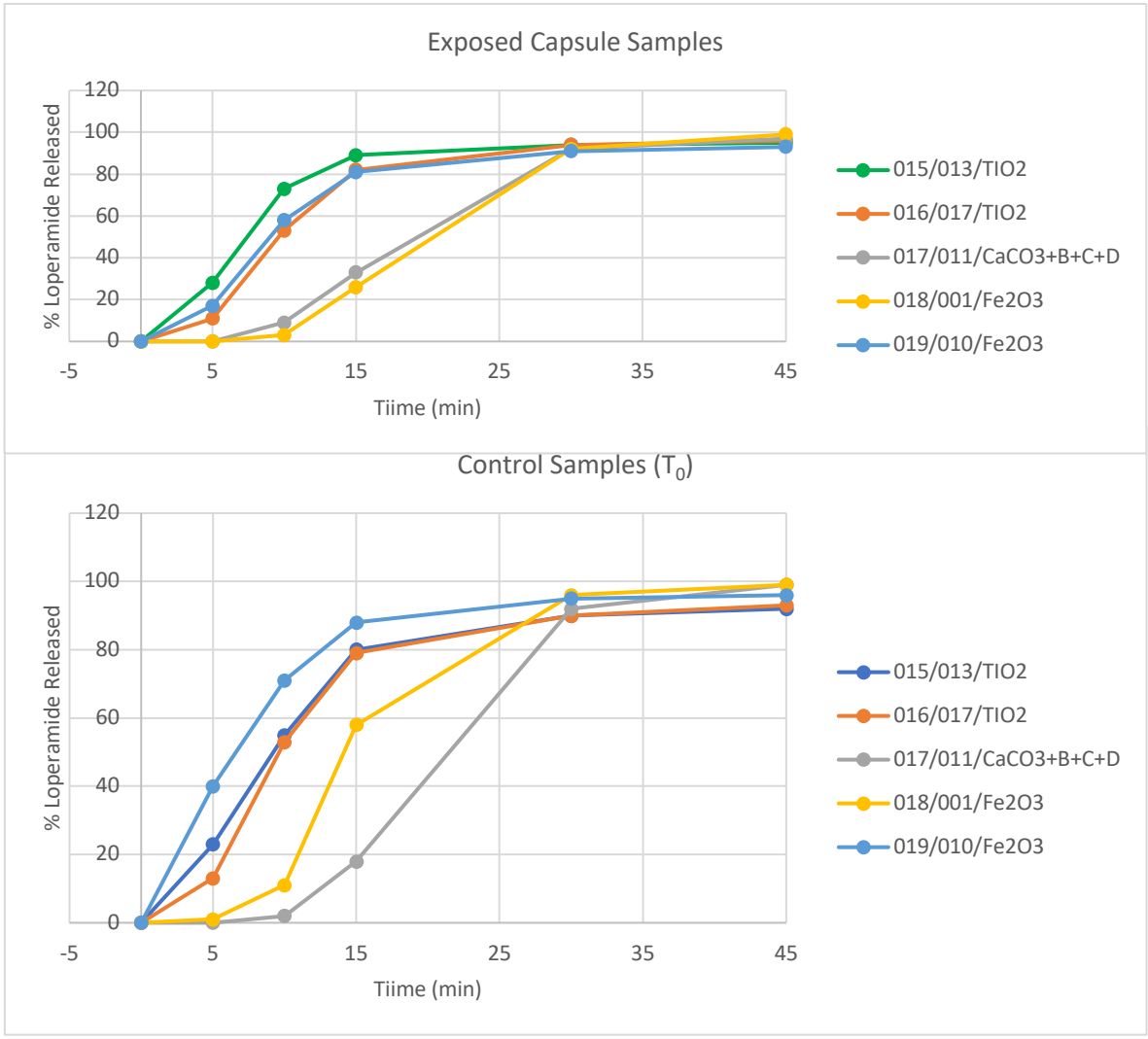



Loperamide Capsules

The dissolution data for the exposed loperamide batch samples versus the corresponding controls are shown in Figure 80.

Figure 80: Mean dissolution data for loperamide from the loperamide light-exposed capsules versus controls




Only Batch ENQ3860/AIRC/016/01, which is encapsulated in the red gelatin-based $TiO_2$ reference, CAP-017, maintained its dissolution profile after photo-exposure compared with the control. The exposed sample of Batch ENQ3860/AIRC/015/01 which contains the white gelatin-based $TiO_2$ reference capsule shell, released the API faster than the control sample. The release of loperamide from the

exposed sample of Batch ENQ3860/AIRC/019/01 was slightly slower than the control. Nevertheless, all of the aforementioned exposed and control samples released > 80% of the API within 15 min.

For the two remaining two batches, Batch ENQ3860/AIRC/017/01 and Batch ENQ3860/AIRC/018/01, release was >90% by the 30-minute time-point for the light-exposed samples. This is in agreement with the corresponding control sample results. At the earlier time-points, light exposure increased the dissolution rate of Batch ENQ3860/AIRC/017/01 which is encapsulated in the pink, $TiO_2$-free capsule shell and slowed it down for Batch ENQ3860/AIRC/018/01

## Section Summary and Conclusions

Benserazide HCl/Levodopa Capsule Batches

- Appearance

The blend and none of the contents of the capsule batches changed in appearance as a result of light exposure. All of the colored capsule shells did not visibly change in appearance following light exposure, while both the white $TiO_2$-free, CAP-004, and the $TiO_2$ reference, CAP-016 did. Both of these capsule shells are HPMC-based. The gelatin-based white $TiO_2$-free capsule shell, CAP-006, did not visibly change in color. However, a visible change in this capsule shell was observed during the photostability study on empty capsules.

- Assay and related impurities

Light exposure did not impact on the stability of the blend or the capsule contents of all the batches.

- In vitro performance

Disintegration and dissolution were not affected by light-exposure.

Fluvastatin Capsule Batches

- Appearance – Capsule Shells

Capsule shells, CAP-016, CAP-014, CAP-002 and CAP-009 did not change visibly change in appearance on light exposure. With the exception of CAP-014, these results differ from those obtained on the empty capsule shells (see Section 73).

- Assay, Impurities and Capsule Contents Appearance

Fluvastatin degraded under the conditions used for the photostability study (2 x ICH Q1B) as seen from the blend results. None of the capsule shells were able to protect the API fully from the effects of the extreme light exposure.

- In vitro performance

Disintegration times were not affected by light exposure for either the batches encapsulated in $TiO_2$-free or the $TiO_2$ reference capsule shells. Light exposure slowed the dissolution of fluvastatin from the exposed samples from the majority of batches compared with control. The exception was the samples encapsulated in CAP-014. This slowdown in dissolution may in part be due to the decreased API content in these batches due to degradation.

Loperamide Capsule Batches

- Appearance

The blend and capsule contents did not change in appearance for any of the batches tested. Capsule shell, CAP-011, a HPMC $TiO_2$-free capsule used for Batch ENQ3860/AIRC/017/01 changed color as was expected based on the photostability results on empty capsule shells (see Figure 56).

- Assay and Impurities

Overall, the results for the exposed sample were in line with that of the control samples. With regard to degradation, encapsulation could only reduce this, with the best results being obtained for CAP-017, the red gelatin $TiO_2$ reference.

- In vitro performance

Light exposure had no major impact on disintegration or dissolution of loperamide from the batches encapsulated $TiO_2$-free or $TiO_2$ reference capsules. The $TiO_2$-free capsules, CAP-011 and CAP-001 still gave the slowest profiles, as they did for the control samples.

Overall, if an API degrades in response to the extreme light conditions used in this study, encapsulation in either the $TiO_2$-free or $TiO_2$ containing capsule shells will only protect it to a certain extent.

# Experimental Part 4: Accelerated Stability Studies

## Protocol

Filled capsules from the 19 encapsulation runs (see Section 0, Table 115) were packed into 120 mL HDPE bottles with an induction seal and capped with an HDPE cap. They were subjected to accelerated testing over 21 days in three separate stability studies (one study per active blend type). During stability storage the packed capsules were stored in the bottles open with the induction seal removed. Details of the packed capsules are shown in Table 115 and the time-points and storage conditions are shown in Table 137.

Table 137: Accelerated stability storage conditions and time-points

| Storage Condition | $T_0$ | T = 7 Days | T = 14 Days | T = 21 Days |
|---|---|---|---|---|
| 5°C | X | o | o | X |
| 50°C/50%RH Gelatin-based Capsules Only | | X | x | x |
| 60°C/30%RH HPMC-based Capsules Only | | X | X | X |
| 70°C/75%RH* | | o* | NA | NA |

**X** = Scheduled testing

O = Optional testing if requested by the Consortium

*o = Both gelatin and HPMC-based capsules were stored at 70°C/75%RH and checked for appearance immediately after removal from the stability chamber. Only the HPMC capsules were retained in the 70°C/75%RH chamber after 7 days with testing only conducted at the Consortium's request. Upon further review of the other conditions the Consortium decided not to analyze any product exposed to the 70°C/75%RH conditions.

After removal of the samples from the stability chamber they were stored at 5°C in closed bottles. The tests carried out on the capsule samples are shown in Table 138.

The blends were tested for appearance, assay and related impurities. The analytical methodology was as described in Section 0.

Table 138: Analytical testing of the accelerated stability samples

| Attribute | Methodology |
|---|---|
| **All stability samples which underwent scheduled testing*** | |
| Visual assessment[a] | Photography |
| Appearance - Colorimetry | DigiEye |
| Brittleness[a] | In-house test ( |
| Disintegration | USP <701> |
| **14.25mg Benserazide HCl/50mg Levodopa capsules only** | |
| Assay | HPLC |
| Related Impurities | UPLC |
| Dissolution | USP Type 2 Apparatus (Paddles)/HPLC |
| **20 mg Fluvastatin capsules only** | |
| Assay | HPLC ( |
| Related substances | HPLC |
| Dissolution | USP Type 2 Apparatus (Paddles)/HPLC |
| **2 mg Loperamide capsules only** | |
| Assay | HPLC (ADM/23/040) |
| Related substances | HPLC (ADM/23/041) |

[a]Assessment performed at each time-point immediately after removal of the samples from the chamber and compared with a representative $T_0$ sample stored at room temperature.

## Results and Discussion

### 98. Visual Appearance

Samples stored at 70°C/75%RH

Initially it was planned to evaluate the appearance of $TiO_2$-free and $TiO_2$ reference capsule batches following storage at 70°C/75%RH. However, it was noted that all of the gelatin capsules had melted by the 7-Day time-point. The HPMC capsule batches were retained in the chamber. However, upon review of the results generated under the other conditions, the Consortium decided not to analyze any product exposed to the 70°C/75%RH conditions.

Benserazide HCl/Levodopa Capsules

Table 139 shows the visual appearance assessment of the benserazide/levodopa capsule batches placed on accelerated stability. None of the capsule shells stored at 5°C for 21 days changed color compared with the $T_0$ samples. However, the capsule contents appeared to be a brighter white than the $T_0$ control.

Table 139: Visual appearance of the benserazide HCl/levodopa capsule batches on accelerated stability versus the corresponding $T_0$ samples

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Ref** | **T= 0** | **T=7 Days** | **T=14 Days** | **T= 21 Days** | **T=21 Days** |
| 001/01/P1[a] | CAP-016 | Capsule Shells: Off white, no physical defects, Size 0. Opaque. | Capsule Shells: Color changed to slightly less white. | Capsule Shells: Compared to T=0 sample, the samples looked less white. | Capsule Shells: Compared to T=0 the sample were less white. | Capsule Shells: Compared to T=0, both appeared same color. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from white to white-peach observed in comparison to T=0. | Contents: Color change from white to peach observed in comparison to T=0. | Contents: Color change from white to peach observed in comparison to T=0. | Contents: Color of white brighter than that at T=0. |
| 002/01/P1[b] | CAP-017 | Capsule Shells: Dark Red, no physical defects, Size 0. Opaque. | Capsule Shells: No change from T=0.. | Capsule Shells: No change from T=0. | Capsule Shells: No change from T=0. | Capsule Shells: No change from T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from white to light peach in comparison to T=0. | Contents: Color change from white to dark peach in comparison to T=0. | Contents: Color change from white to dark peach and much more clumping in comparison to T=0. | Contents:<br>White color is brighter in comparison to T=0. |
| 003/01/P1[a] | CAP-015 | Capsule Shells: Dark Red, no physical defects, Size 0. Opaque. | Capsule Shells:<br>No change from T=0. | Capsule Shells:<br>No change from T=0.. | Capsule Shells: Compared to T=0, both samples appear the same.<br>However, small bits of powder were observed on samples, making them to appear less glossy than T=0. | Capsule Shells: No change from T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Slight difference in color from white to off-white in comparison to T=0. | Contents: Color change from white to light pink-peach in comparison with T=0. | Contents: Color change from white to light pink-peach in comparison with T=0. | Contents: White color brighter in comparison with T=0. |
| 004/01/P1[a] | CAP-004 | Capsule Shells: Off white, slightly translucent, no physical defects, Size 0. | Capsule Shells: Color compared to T=0 has changed to slightly more beige. | Capsule Shells: The color of the samples was slightly more beige compared to T=0. | Capsule Shells: Compared to T=0, the samples were more beige. | Capsule Shells: T=21 Days and T=0 samples appeared the same. |
| | | Contents: White powder, | Contents: Color change | Contents: Color change | Contents: Color change | Contents: White color |

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Ref** | **T= 0** | **T=7 Days** | **T=14 Days** | **T= 21 Days** | **T=21 Days** |
| | | small amount of clumping, free from contaminants. | from white to white-peach observed in comparison to T=0. | from white to light pink-peach in comparison to T=0. | from white to light pink-peach in comparison to T=0. | brighter in comparison. |
| 005/01/P1[a] | CAP-008 | Capsule Shells: Dark red, no physical defects, Size 0. Opaque. | Capsule Shells: No change in appearance from T=0. | Capsule Shells: No change in appearance from T=0. | Capsule Shells: No change in appearance from T=0. | Capsule Shells: White powder particles on capsule shell surface. No color change from T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Slight color change from white to off-white in comparison to T=0. | Contents: Color change observed from white to a light pink-peach compared to T=0. | Contents: Color change observed from white to a light pink-peach, and a little more clumping was observed in comparison with T=0. | Contents: A brighter white color observed in comparison to T=0. |
| 006/01/P1[b] | CAP-006 | Capsule Shells: Off white, slightly translucent, no physical defects, Size 0. | Capsule Shells: Compared to the T=0 sample the color changed from white to pale beige. | Capsule Shells: Compared to T=0, the sample had turned beige. | Capsule Shells: Compared to T=0 sample, the color changed to translucent brown from translucent white. The powder inside of the capsule was not free flowing, but stuck in one place. This was not the case for the T=0 sample. | Capsule Shells: No change in color compared to T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from white to light peach in comparison to T=0. | Contents: Color change from white to light peach and more clumping observed in comparison to T=0. | Contents: Color change from white to dark peach compared to T=0. Some clumping of powder. | Contents: Color of powder white brighter than powder at T=0. |

Color Code:

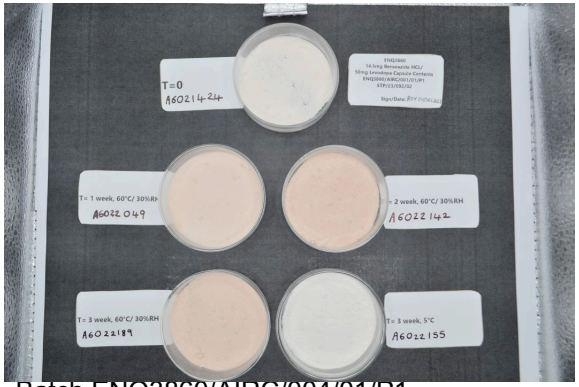
Green = No visible change in appearance between stability sample and $T_0$ control

[a]HPMC-based capsules were placed on stability at 60°C/30%RH and 5°C

[b]Gelatin based capsules were placed on stability at 50°C/50%RH and 5°C

Figure 81: Appearance of benserazide HCl/levodopa capsule contents following storage under the various storage conditions and time-points

Batch ENQ3860/AIRC/001/01/P1




Batch ENQ3860/AIRC/002/01/P1


Batch ENQ3860/AIRC/003/01/P1


Batch ENQ3860/AIRC/004/01/P1


Batch ENQ3860/AIRC/005/01/P1


Batch ENQ3860/AIRC/006/01/P1

The HPMC-based $TiO_2$-free colored capsule shells stored at 60°C/30%RH (CAP-008 and CAP-015) and the colored gelatin $TiO_2$ reference (CAP-017) stored at 50°C/50%RH did not change in appearance in comparison with the control capsules. All of the white capsule shells changed appearance on storage at the higher temperatures. The $TiO_2$ reference capsule, CAP-016, became less white, while the $TiO_2$-free CAP-004 and CAP-006 became more beige.

Figure 81 shows photographs of the benserazide HCl/levodopa capsule contents following storage at the various storage conditions and time-points. The contents of all of the benserazide HCl/levodopa capsule batches changed color from white to peach on storage at either 60°C/30%RH or 50°C/50%RH. The color increased in intensity over the 3-week period. CAP-004 and CAP-006 are semi-opaque and therefore the change in the capsule shell color was at least in part caused by the change in the color of the contents. The contents of the capsules were darkest with Batch ENQ3860/AIRC/002/01/P1 and Batch ENQ3860/AIRC/006/01/P1 at T = 21 days. Both of these batches are encapsulated in gelatin capsules and were stored at 50°C/50%RH. The darker color of the two batches stored under the higher %RH conditions may reflect the moisture sensitivity of the APIs involved.

In summary, no visual differences could be detected between the colored capsule shells placed on accelerated stability and the corresponding $T_0$ controls. However, none of the capsules were capable of protecting the benserazide HCl/levodopa blend from discoloring during storage in the non-sealed bottles at higher temperatures.

Fluvastatin Capsules

Table 140 shows the visual comparison of the fluvastatin capsule batches on accelerated stability versus the corresponding $T_0$ samples. After storage at 5°C for three weeks only the white $TiO_2$ reference capsule shell, CAP-016, and the colored $TiO_2$ reference capsule shell, CAP-014 had not visually changed in appearance. All of the $TiO_2$-free capsules had turned yellowish white to yellow. At the higher temperature storage conditions, all of the capsule shells with the exception of CAP-014, had changed color including the white $TiO_2$ reference, CAP-016.

At all storage conditions and time-points except the control sample, the capsule contents of every fluvastatin encapsulated batch had turned yellow as a result of exposure to moisture in the unsealed containers. This included the samples stored at 5°C, although they were less intensely colored than those stored at the higher storage temperatures. After 3 weeks the blend encapsulated in HPMC-based capsules at 60°C/30%RH were more intensely yellow than the batches encapsulated in gelatin-based capsules stored at 50°C/50%RH.

Loperamide Capsules

Table 141 shows the visual comparison of the loperamide capsule batches placed on accelerated stability versus the corresponding $T_0$ samples. None of the capsule shells or their contents changed color as a result of storage at 5°C or the higher temperature storage conditions. There was no differentiation between the batches encapsulated in $TiO_2$-free capsules and the $TiO_2$ reference samples.

Table 140: Visual appearance of the fluvastatin capsule batches on accelerated stability versus the corresponding $T_0$ samples

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Ref** | **T=0** | **T=7 Days** | **T=14 Days** | **T=21 Days** | **T=21 Days** |
| 007/01/P1[a] | CAP-016 | Capsule Shells: Off white, no physical defects, Size 0. Opaque. | Capsule Shells: T1 week sample turned yellowish compared to T=0, which looked more white. | Capsule Shells: Compared to T=0 sample the color was slightly yellowish compared to white. | Capsule Shells: Compared to T=0 samples, these samples had a tint of yellow, where T=0 appear white. | Capsule Shells: Both T=0 and T3 week samples appeared to be the same color. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color became a stronger yellow compared to off-white at T=0. | Contents: Color change from off-white to yellow and more clumping compared to T=0. | Contents: Color change from off-white to yellow compared to T=0. | Contents: Color change from off-white to pale yellow and more clumping compared to T=0. |
| 008/01/P1[a] | CAP-014 | Capsule Shells: Red, one capsule roughly cut and leaking. Remaining capsule shells free from physical defects. Size 0. Very slightly translucent. | Capsule Shells: No color change from T=0. | Capsule Shells: Deformed cap present on some capsules. No color change from T=0. | Capsule Shells: No color change from T=0. | Capsule Shells: No color change from T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from off-white to pale yellow compared to T=0. Some large clumps of powder. | Contents: Color change from off-white to yellow compared to T=0. | Contents: Some large clumps of powder present. Color change from an off-white to yellow compared to T=0. | Contents: Color change very slight from an off-white to a white-yellow compared to T=0. Some large lumps of powder. |
| 009/01/P1[a] | CAP-003 | Capsule Shells: Off white, one capsule roughly cut. Remaining capsule shells free from physical defects. Size 0. Slightly translucent. | Capsule Shells: One of the 10 capsules appeared deformed, the cap of the capsule was flattened. Another cap of the capsule had a crack over it. Sample had turned yellowish, whereas T=0 sample appeared off white. | Capsule Shells: Compared to T=0 sample, the color had turned yellowish white where T= 0 samples appeared off-white. | Capsule Shells: Compared to T=0 samples, the samples were light yellow translucent, whereas T=0 samples were off white translucent. | Capsule Shells: Samples were yellowish white compared to off white, translucent. |

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Ref** | **T=0** | **T=7 Days** | **T=14 Days** | **T=21 Days** | **T=21 Days** |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from an off-white to a yellow-colored powder compared to T=0. Clumping present. | Contents: Large clumps present. Color change from off-white to yellow compared to T=0. | Contents: Some clumps present. Color change from off-white to yellow compared to T=0. | Contents: Large clumps present. Color change from off-white to pale yellow compared to T=0. |
| 010/01/P1[a] | CAP-009 | Capsule Shells: Off white, no physical defects, Size 0. Slightly transparent. | Capsule Shells: Samples turned yellowish whereas T=0 sample was off-white. | Capsule Shells: Compared to T=0, the samples were slightly yellow whereas T=0 appeared off white, slightly translucent. | Capsule Shells: Samples were more yellowish compared to T=0. | Capsule Shells: Compared to T=0 the capsules were slightly yellowish white, whereas T=0 were white, slightly translucent. |
| | | Contents: Contents: White powder, small amount of clumping, free from contaminants. | Contents: Some large clumps present. Color change from off-white to yellow compared to T=0. | Contents: Some clumps present. Color change from off-white to yellow compared to T=0. | Contents: Obvious color change from off-white to vibrant yellow compared to T=0. | Contents: Some clumps present. Slight change in color from off-white to a pale yellow compared to T=0. |
| 011/01/P1[a] | CAP-007 | Capsule Shells: Capsule Shell: Off white, no physical defects, Size 0. Translucent. | Capsule Shells: Samples turned yellowish where T=0 sample were off white. | Capsule Shells: Compared to T=0, the samples were slightly yellow whereas T=0 appeared off white, translucent. | Capsule Shells: Compared to T=0 samples, the samples were light yellow translucent, whereas T=0 samples were white translucent. | Capsule Shells: Compared to T=0 capsules were slightly yellowish white, whereas T=0 samples were white translucent. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Large clumps present Color change from off-white to yellow and more clumping compared to T=0. | Contents: Color change from off-white to yellow and more clumping than in T=0. | Contents: Color change from off-white to a vibrant yellow and more clumping compared with T=0. | Contents: Color change from off-white to pale yellow and more clumping compared with T=0. |
| 012/01/P1[b] | CAP-002 | Capsule Shells: Off white, no physical defects, Size 0. | Capsule Shells: Sample changed to yellowish white from white compared to T=0. | Capsule Shells: Compared to T=0 sample, the color had turned yellowish white. | Capsule Shells: Compared to T=0 samples, capsules were slightly yellowish. | Capsule Shells: Compared to T=0, the color had turned yellowish. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from off-white to pale yellow compared with T=0. | Contents: Color change from off-white to pale yellow compared with T=0. | Contents: Color change from off-white to pale yellow compared to T=0. | Contents: Color change from off-white to very pale yellow. |

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Ref** | **T=0** | **T=7 Days** | **T=14 Days** | **T=21 Days** | **T=21 Days** |
| 013/01/P1[b] | CAP-005 | Capsule Shells: Off white, no physical defects, Size 0. Translucent. | Capsule Shells: Samples changed color to pale yellow from off white capsules at T=0. | Capsule Shells: Compared to T=0 sample, the color had turned yellowish white translucent compared to white translucent. | Capsule Shells: Compared to T=0 samples, capsules were slightly yellowish. | Capsule Shells: Compared to T=0, the color has turned yellowish white. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Some large clumps of powder present. Color change from off-white to pale yellow and more clumping observed compared to T=0. | Contents: Color change from off-white to pale yellow and more clumping observed compared to T=0. | Contents: Color change from off-white to pale yellow and more clumping compared to T=0. | Contents: Color change from off-white to pale yellow compared to T=0. |
| 014/01/P1[b] | CAP-012 | Capsule Shells: Off white, one capsule roughly cut. Remaining capsule shells free from physical defects. Size 0. Opaque. | Capsule Shells: Samples changed color to yellowish white from white compared to T=0. | Capsule Shells: Compared to T=0 sample, the color had turned yellowish white. | Capsule Shells: Compared to T=0 capsules, samples were slightly yellowish white. | Capsule Shells: Compared to T=0 capsules are slightly yellowish white, whereas T=0 samples are opaque white color. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: Color change from off-white to pale yellow and more clumping observed compared to T=0. | Contents: Color change from off-white to pale yellow and more clumping observed compared to T=0. | Contents: Color change from off-white to pale yellow and more clumping observed compared to T=0. | Contents: Color change from off-white to pale yellow compared to T=0. |

Color Code:

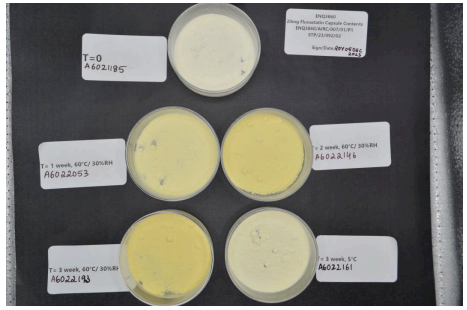
Green = No visible change in appearance between exposed sample and control

[a]HPMC-based capsule were placed on stability at 60°C/30%RH and 5°C

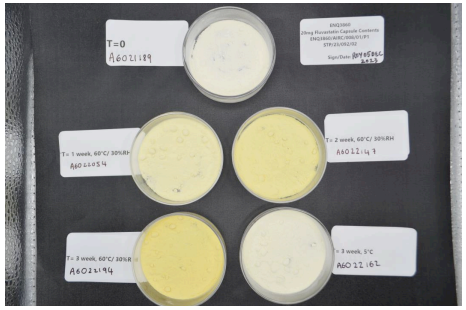
[b]Gelatin based capsules were placed on stability at 50°C/50%RH and 5°C

Figure 82: Appearance of fluvastatin capsule contents following storage under the various storage conditions and time-points

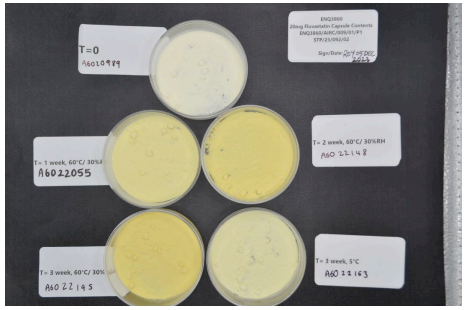
Batch ENQ3860/AIRC/007/01/P1



Batch ENQ3860/AIRC/008/01/P1

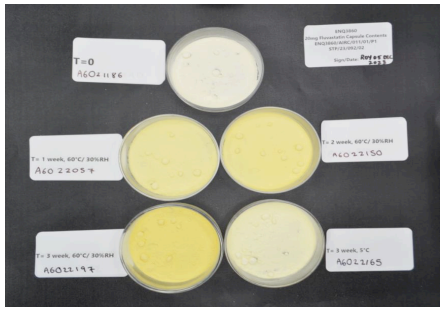


Batch ENQ3860/AIRC/009/01/P1

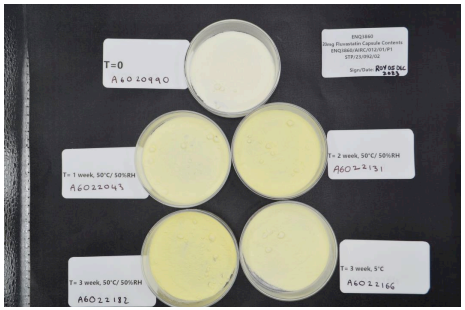


Batch ENQ3860/AIRC/010/01/P1

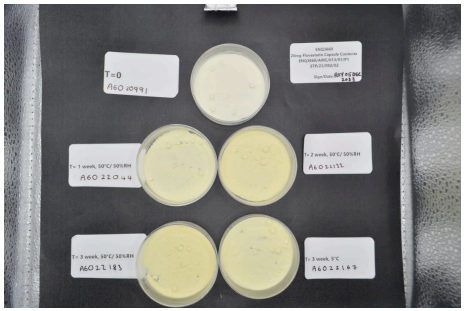
Batch ENQ3860/AIRC/011/01/P1



Batch ENQ3860/AIRC/012/01/P1



Batch ENQ3860/AIRC/013/01/P1



Batch ENQ3860/AIRC/014//01/P1

Table 141: Visual appearance of the loperamide capsule batches on accelerated stability versus the corresponding $T_0$ samples

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Shell Ref** | **T=0** | **T=7 Days** | **T=14 Days** | **T=21 Days** | **T=21 Days** |
| 015/01/P1[b] | CAP-013 | Capsule Shells: Off white, no physical defects, Size 0. | Capsule Shells: No difference to T=0 in color observed, both appeared white. | Capsule Shells: No difference in color compared to T=0 sample. | Capsule Shells: Compared to T=0 sample, both appeared to be the same. | Capsule Shells: Compared to T=0 sample, both appear to be the same. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. |
| 016/01/P1[b] | CAP-017 | Capsule Shells: Dark red, no physical defects, Size 0. | Capsule Shells: No change from T=0. | Capsule Shells: No change from T=0. | Capsule Shells: No change from T=0. | Capsule Shells: No change from T=0. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: No change observed from T=0. | Contents: No change observed from T=0. | Contents: No change observed from T=0. | Contents: No change observed from T=0. |
| 017/01/P1[a] | CAP-011 | Capsule Shells: Light pink, no physical defects, Size 0. | Capsule Shells: No color difference observed compared to T=0. | Capsule Shells: Compared to T=0, both samples appear the same color. | Capsule Shells: Both T=0 and T= 3week samples appeared the same. | Capsule Shells: Compared to T=0, both samples appear the same color. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. |
| 018/01/P1[a] | CAP-001 | Capsule Shells: Dark red, slightly dusty, Size 0, no other visual defects. | Capsule Shells: Powder present on surface of all capsules. No change from T=0. | Capsule Shells: No color change from T=0. Larger amount of powder particles present on surface compared to T=0 | Capsule Shells: No color change from T=0. White powder particles over the capsule shell surface. | Capsule Shells: No color change from T=0. White powder particles found over the capsule shell. |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. |
| 019/01/P1[b] | CAP-010 | Capsule Shells: Dark red, no physical defects, Size 0. | Capsule Shells: No change in color from T=0. | Capsule Shells: No difference in color compared to T=0 sample. | Capsule Shells: No difference in color compared to T=0 sample. | Capsule Shells: No difference in color compared to T=0 sample. |

| Storage Conditions | | Ambient | 50°C/50%RH or 60°C/30%RH | | | 5°C |
|---|---|---|---|---|---|---|
| **Batch No. ENQ3860/AIRC/** | **Consort. Cap Shell Ref** | **T=0** | **T=7 Days** | **T=14 Days** | **T=21 Days** | **T=21 Days** |
| | | | | Powder particles observed over the capsules. | | |
| | | Contents: White powder, small amount of clumping, free from contaminants. | Contents: No change observed in comparison to T=0. No change observed in comparison to T=0 | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. | Contents: No change observed in comparison to T=0. |

Color Code:

Green = No visible change in appearance between exposed sample and control

[a]HPMC-based capsule were placed on stability at 60°C/30%RH and 5°C

[b]Gelatin based capsules were placed on stability at 50°C/50%RH and 5°C

## 99. Colorimetry Results

Benserazide HCl/Levodopa Capsules

Table 142 shows the color difference values ($\Delta E^*_{00}$) for the benserazide/levodopa capsule batches on accelerated stability versus the $T_0$ capsule data. The colorimetry data for the $T_0$ values are reported in Section 89, Table 121 of this report. In Table 142 the $\Delta E^*_{00}$ values which meet the acceptance criteria for white capsules ($\Delta E^* \leq 1$) and for colored capsules ($\Delta E^*_{00} < 2$) have been colored green. Only the white $TiO_2$ reference capsule shell, CAP-016, met the criteria when stored at 5°C for 3 weeks. At the 60°C/30% RH storage conditions, none of the Batch ENQ3860/AIRC/001/01/P1 samples met the acceptance criterion.

For the two batches encapsulated in white $TiO_2$-free capsules (Batches ENQ3860/AIRC/004/01/P1 and ENQ3860/AIRC/006/01/P1), none of the stability samples met the criteria of $\Delta E^*_{00} \leq 1$. In addition, all of the $\Delta E^*$ values were higher than those for the white $TiO_2$ reference showing that the color difference between the $TiO_2$-free samples and the corresponding ambient-stored $T_0$ samples was greater. Both CAP-004 and CAP-006 are only semi-opaque and therefore less likely to be effective at hiding the change in capsule contents color on storage at the accelerated conditions (see Table 139 and Figure 81) than the opaque $TiO_2$ reference capsule, CAP-016.

None of the colored capsule batch stability samples (Batches ENQ3860/AIRC/002/01/P1, ENQ3860/AIRC/003/01/P1 and ENQ3860/AIRC/005/01/P1) met the acceptance criteria for $\Delta E^*_{00} < 2$. This is in contrast to the results of the visual assessment which showed the capsule shells did not visually change in appearance, while the contents did (see Table 139).

Fluvastatin Capsules

Table 143 shows the color difference values ($\Delta E^*_{00}$) for the fluvastatin capsule batches on accelerated stability versus the $T_0$ capsule data. The colorimetry data for the $T_0$ values are reported in Section 89, Table 123 of this report. None of the batches encapsulated in the white TiO2-free capsules or the white TiO2 reference met the color difference criteria of $\Delta E^*_{00} \leq 1$, although overall the $\Delta E^*$values for Batch ENQ3860/AIRC/007/01/P1, encapsulated in the $TiO_2$ white reference capsule shell, CAP-016, were lower than for the other TiO2-free capsule batches. In part, this may reflect that the more opaque $TiO_2$ containing capsule shell is better at camouflaging the color change in the capsule contents on accelerated stability (see Table 140 and Figure 82) than the more translucent $TiO_2$-free capsule shells.

The $\Delta E^*_{00}$ values for Batch ENQ3860/AIRC/008/01/P1, encapsulated in the red HPMC-based $TiO_2$ reference, CAP-014, were all > 2. This is in contrast to the visual appearance evaluation of the capsule shell that detected no perceptible difference in the appearance of the accelerated stability samples versus the $T_0$ sample.

Table 142: Color difference $\Delta E^*_{00}$ values for benserazide HCl/levodopa capsules compared with $T_0$ results (ambient storage)

| Storage Conditions | | | | 5°C vs T=0 | 50°C/50%RH vs T0 (gelatin capsules 60°C/30%RH vs T0 (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 0.57 | 1.83 | 2.28 | 2.63 |
| 002/01/P1 | CAP-017[a] | Gelatin | $TiO_2$ | 2.78 | 2.54 | 2.53 | 2.55 |
| 003/01/P1 | CAP-015[a] | HPMC | $Fe_2O_3$ | 3.40 | 2.87 | 2.94 | 2.99 |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 1.81 | 3.62 | 4.73 | 5.09 |
| 005/01/P1 | CAP-008[a] | HPMC | $Fe_2O_3$ | 2.76 | 2.68 | 2.70 | 2.91 |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 1.91 | 7.40 | 10.36 | 12.00 |

Color Code: Green = Meets acceptance criteria of $\Delta E^* \leq 1$ for white capsules and $\Delta E^* < 2$ for colored capsules

$T_o$ = Capsules stored under laboratory ambient conditions

[a]Colored capsule shell

Table 143: Color difference $\Delta E^*_{00}$ values of fluvastatin capsules compared with $T_0$ results (ambient storage)

| Storage Conditions | | | | 5°C vs T=0 | 50°C/50%RH vs T0 (gelatin capsules 60°C/30%RH vs T0 (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 007/01/P1 | CAP-016 | HPMC | $TiO_2$ | 1.44 | 2.64 | 2.35 | 2.58 |
| 008/01/P1 | CAP-014[a] | HPMC | $TiO_2$ | 3.54 | 4.71 | 3.07 | 3.75 |
| 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 3.75 | 10.04 | 7.71 | 8.35 |
| 010/01/P1 | CAP-009 | HPMC | $CaCO_3$+A | 2.25 | 5.11 | 5.12 | 5.25 |
| 011/01/P1 | CAP-007 | HPMC | $CaCO_3$+A | 2.27 | 7.38 | 6.39 | 7.30 |
| 012/01/P1 | CAP-002 | Gelatin | NaPOx | 5.12 | 6.17 | 4.32 | 4.98 |
| 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 3.04 | 7.58 | 5.30 | 7.19 |
| 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$+B+D | 1.95 | 3.79 | 3.16 | 3.62 |

Color Code: Green = Meets acceptance criteria of $\Delta E^* \leq 1$ for white capsules and $\Delta E^* < 2$ for colored capsules

$T_o$ = Capsules stored under laboratory ambient conditions

[a]Colored capsule

Loperamide Capsules

Table 144 shows the color difference values ($\Delta E^*_{00}$) for the loperamide capsule batches on accelerated stability versus the $T_0$ capsule data. The colorimetry data for the $T_0$ values are reported in Section 89, Table 122 of this report. Batch ENQ3860/AIRC/015/01/P1 contained the only white capsule shell used to encapsulate the loperamide blend, CAP-013, the gelatin-based $TiO_2$ reference. The appearance of this batch met the acceptance criteria of $\Delta E^*_{00}$ values of ≤ 1 at all storage conditions and time-points. This result in in line with the visual appearance data (see Table 141).

Batch ENQ3860/AIRC/017/01/P1, encapsulated in the pink CAP-011, had ΔE*values of ≤ 1 following storage at 5°C for 21 days and 1 week at 60°C/30%RH. Thereafter the values were between 1 and 2 suggesting that a difference could be detected on close observation. However, the visual assessment showed no change in appearance following removal from the stability chambers (see Table 141). The color of the remaining batches encapsulated in red/orange capsule shells, containing red iron oxide as the colorant and a contributor to the opacification. all had ΔE*values > 2 which is not in line with the visual data.

Discrepancy between Visual and Colorimetry Data for Batches Encapsulated in the Red/Orange Capsule Shells

In order to investigate why ΔE*values for the batches encapsulated in the red/orange capsule shells suggested a perceptible color change on accelerated stability while the visual assessment did not detect one, ΔL*, Δa*, Δb*, Δchroma and Δhue angle were calculated and shown in Table 145.

Overall, the L* values and a* values of the colored capsules increased slightly after 21 days under the accelerated conditions meaning that the capsules were lighter and redder in color than the T0 samples. The exception was Batch ENQ3860/AIRC/017/01/P1, which was encapsulated in the pink $TiO_2$-free CAP-011. Its L* and a* values reduced slightly on exposure to 60°C/30%RH.

The b*values increased by between 1 to 4 units for all lots except those encapsulated in the gelatin-based $TiO_2$ reference capsule shell, CAP-017 (Batches ENQ3860/AIRC/002/01/P1 and ENQ3860/AIRC/016/01/P1), indicating that the capsules had become more yellow following storage under the accelerated conditions for 21 days. The Chroma values also all increased following exposure to the accelerated conditions suggesting that the color of the capsules had become more intense. The color intensity of Batch ENQ3860/AIRC/017/01/P1, encapsulated in the pink CAP-011, increased only marginally. However, it had the largest difference in hue angle of 6.12, while the other batches had hue angles differences which ranged from minus 1,87 to plus 2.51. The larger hue angle difference for Batch ENQ3860/AIRC/017/01/P1, compared with the other colored capsule batches, may be the reason why visual changes in appearance were observed for it but not found for the other batches.

Table 144: Color difference $\Delta E^*_{00}$ values of loperamide capsules compared with $T_0$ results (ambient storage)

| Storage Conditions | | | | 5°C vs T=0 | 50°C/50%RH vs T0 (gelatin capsules 60°C/30%RH vs T0 (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell | Shell Former | Opacifier | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ | $\Delta E^*_{00}$ |
| 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 0.49 | 0.37 | 0.58 | 0.77 |
| 016/01/P1 | CAP-017[a] | Gelatin | $TiO_2$ | 3.07 | 2.63 | 2.81 | 3.15 |
| 017/01/P1 | CAP-011[a] | HPMC | $CaCO_3$+B+C+D | 1.00 | 0.89 | 1.30 | 1.41 |
| 018/01/P1 | CAP-001[a] | HPMC | $Fe_2O_3$ | 2.87 | 2.86 | 2.85 | 2.65 |
| 019/01/P1 | CAP-010[a] | Gelatin | $Fe_2O_3$ | 3.17 | 2.76 | 2.67 | 3.19 |

Color Code: Green = Meets acceptance criteria of $\Delta E^* \leq 1$ for white capsules and $\Delta E^* < 2$ for colored capsules

$T_o$ = Capsules stored under laboratory ambient conditions

[a]Colored capsule

Table 145: ΔL*, Δa*, Δb*, Δchroma and Δhue angle values for the colored capsule batches (T= 21 days under accelerated conditions vs $T_0$ ambient)

| Active and Trial No. | Batch No.* ENQ3860/AIRC/ | Storage | L* | a* | b* | C | h | ΔL* | Δa* | Δb* | ΔC | Δh |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Benserazide HCl/ Levodopa Capsules Trial 2 | 002/01 | Ambient | 30.43 | 40.17 | 12.77 | 42.15 | 17.63 | | | | | |
| Benserazide HCl/ Levodopa Capsules Trial 2 | 002/01/P1 | 50°C/50%RH, 21 Days | 33.29 | 42.75 | 12.07 | 44.42 | 15.76 | 2.86 | 2.58 | -0.70 | 2.27 | -1.87 |
| Benserazide HCl/ Levodopa Capsules Trial 3 | 003/01 | Ambient | 31.5 | 30.19 | 22.12 | 37.43 | 36.23 | | | | | |
| Benserazide HCl/ Levodopa Capsules Trial 3 | 003/01/P1 | 60°C/30%RH, 21 Days | 34.83 | 31.55 | 24.63 | 40.03 | 37.98 | 3.33 | 1.36 | 2.51 | 2.60 | 1.75 |
| Benserazide HCl/ Levodopa Capsules Trial 5 | 005/01 | Ambient | 29.25 | 27.74 | 18.25 | 33.2 | 33.34 | | | | | |
| Benserazide HCl/ Levodopa Capsules Trial 5 | 005/01/P1 | 60°C/30%RH, 21 Days | 32.57 | 28.80 | 20.55 | 35.38 | 35.51 | 3.32 | 1.06 | 2.30 | 2.18 | 2.17 |
| Fluvastatin Capsules Trial 8 | 008/01 | Ambient | 36.24 | 32.80 | 23.00 | 40.06 | 35.04 | | | | | |
| Fluvastatin Active Capsules Trial 8 | 008/01/P1 | 60°C/30%RH, 21 Days | 40.02 | 34.87 | 26.80 | 43.98 | 37.55 | 3.78 | 2.07 | 3.80 | 3.92 | 2.51 |
| Loperamide Capsules Trial 16 | 016/01 | Ambient | 30.41 | 40.82 | 13.19 | 42.90 | 17.91 | | | | | |
| Loperamide Capsules Trial 16 | 016/01/P1 | 50°C/50%RH, 21 Days | 33.83 | 45.23 | 13.24 | 47.12 | 16.32 | 3.42 | 4.41 | 0.05 | 4.22 | -1.59 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loperamide Trial 17 | Capsules | 017/01 | Ambient | 70.87 | 12.24 | 5.59 | 13.46 | 24.55 | | | | | |
| Loperamide Trial 17 | Capsules | 017/01/P1 | 60°C/30%RH, 21 Days | 70.76 | 11.75 | 6.97 | 13.66 | 30.67 | -0.11 | -0.49 | 1.38 | 0.20 | 6.12 |
| Loperamide Trial 18 | Capsules | 018/01 | Ambient | 29.08 | 27.96 | 17.50 | 32.99 | 32.04 | | | | | |
| Loperamide Trial 18 | Capsules | 018/01/P1 | 60°C/30%RH, 21 Days | 32.33 | 28.55 | 18.69 | 34.13 | 33.21 | 3.25 | 0.59 | 1.19 | 1.14 | 1.17 |
| Loperamide Trial 19 | Capsules | 019/01 | Ambient | 31.56 | 29.80 | 19.66 | 35.70 | 33.42 | | | | | |
| Loperamide Trial 19 | Capsules | 019/01/P1 | 50°C/50%RH, 21 Days | 35.24 | 31.67 | 21.73 | 38.41 | 34.46 | 3.68 | 1.87 | 2.07 | 2.71 | 1.04 |

*Ambient testing was carried out on bulk capsules stored at ambient laboratory conditions.

## 100. Assay and Related Impurities

Benserazide/Levodopa Capsules

Table 146 and Table 147 show the mean assay results for benserazide and levodopa in the benserazide HCl/levodopa capsule batches respectively, while Table 148 shows the % total related impurities in the batches. The results show that there is some variation in the mean assay values for benserazide in all batches encapsulated in HPMC-based capsules at $T_0$, 5°C and 60°C/30 %RH at the different time-points. The four batches had slightly lower assay values than $T_0$ storage at 60°C/30 %RH for 21 days. However, only Batch ENQ3860/AIRC/004/01/P1 and ENQ3860/AIRC/005/01/P1 had slightly increased %total impurities compared to $T_0$ at the 21-day time-point under accelerated conditions.

Batches ENQ3860/AIRC/002/01/P1 and ENQ3860/AIRC/006/01/P1 were encapsulated in gelatin-based capsules. For both batches, the assay values for the samples stored at 5°C for 21 days were close to the corresponding $T_0$ results. However, there was a significant reduction in benserazide assay at 50°C/50%RH which increased with storage time. These batches had slightly higher %total related impurities than the other batches at $T_0$ and following storage at 5°C for 21 days. However, the levels increased significantly for the samples stored at 50°C/50%RH and increased with storage time.

In contrast, for all batches the levodopa assay results remained similar to the $T_0$ results and no trend was observed linking capsule shell type or opacifier/colorant used to the assay levels.

Benserazide is moisture-sensitive and the gelatin-based capsule batches were exposed to higher relative humidity levels during the study compared with the batches encapsulated in HPMC-based capsule shells (50%RH versus 30%RH) and this may have contributed to the increased degradation levels seen with these batches.

Table 146: Mean assay values for benserazide in the benserazide HCl/levodopa capsule batches on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Mean Assay Benserazide (%LC) | Mean Assay Benserazide (%LC) | Mean Assay Benserazide (%LC) | Mean Assay Benserazide (%LC) | Mean Assay Benserazide (%LC) |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 96.1 | 94.7 | 98.2 | 95.4 | 94.7 |
| 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 101.8 | 98.2 | 89.1 | 77.2 | 63.9 |
| 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 98.9 | 99.6 | 94.0 | 96.1 | 96.8 |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 98.2 | 99.6 | 96.8 | 99.6 | 95.4 |
| 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 98.9 | 99.6 | 94.0 | 94.7 | 96.8 |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 99.6 | 101.1 | 86.3 | 76.5 | 63.2 |

Color Code: Red = A significant change in assay compared to $T_0$ ((>5%)

Table 147: Mean assay values for levodopa in the benserazide HCl/levodopa capsule batches on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Mean Assay Levodopa (%LC) | Mean Assay Levodopa (%LC) | Mean Assay Levodopa (%LC) | Mean Assay Levodopa (%LC) | Mean Assay Levodopa (%LC) |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 97.2 | 95.8 | 97.2 | 97.4 | 97.6 |
| 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 102.0 | 99.6 | 100.2 | 99.2 | 101.6 |
| 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 100.0 | 100.8 | 98.6 | 99.8 | 99.8 |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 99.4 | 100.2 | 98.6 | 101.2 | 99.0 |
| 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 99.0 | 100.6 | 98.6 | 98.6 | 99.8 |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 99.4 | 99.0 | 95.4 | 98.6 | 99.0 |

Color Code: Red = A significant decrease in assay compared to $T_0$ ((>5%)

Table 148: %Total related impurities in benserazide HCl/levodopa capsule batches on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Total Impurities (%LC) | Total Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 2.21 | 1.64 | 2.12 | 1.98 | 2.12 |
| 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 2.59 | 2.15 | 10.31 | 19.71 | 27.24 |
| 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 2.33 | 1.64 | 2.18 | 2.04 | 2.15 |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 2.13 | 2.12 | 2.24 | 2.30 | 2.42 |
| 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 2.21 | 2.12 | 2.93 | 2.72 | 2.41 |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 2.30 | 2.42 | 11.22 | 19.62 | 27.01 |

Color Code: Red = A significant change in %total related impurities compared to $T_0$

Fluvastatin Capsules

Table 149 shows the mean assay results for fluvastatin in the fluvastatin capsule batches on accelerated stability versus the $T_0$ results, while Table 150 shows the % total related impurities in the batches.

For all the capsule batches stored at 5°C for 21 days, there was a slight decrease in mean assay. There was a more noticeable increase in %total impurities for all of the batches following storage at 5°C except Batch ENQ3860/AIRC/008/01/P1, which was encapsulated in the $TiO_2$ red reference capsule shell, CAP-014. This increase was highest for the batches encapsulated in the gelatin-based $TiO_2$-free capsule shells. For these batches, the % total impurities were at least twice that of the corresponding $T_0$ sample.

Under accelerated conditions, the decrease in assay and increase in %total related impurities was far greater than at 5°C. The five batches, encapsulated in HPMC-based capsule shells, degraded to a greater extent following storage at 60°/30%RH than the three batches encapsulated in the gelatin-based capsule shells which had been stored at 50°C. However, the increase in %total related degradant was still high for these latter batches.

Loperamide Capsules

Table 151 shows the mean assay results for loperamide in the loperamide capsule batches on accelerated stability versus the $T_0$ results, while Table 152 shows the % total related impurities in the batches. There was slight variation in the assay results. However, there were no significant changes compared to $T_0$ and no trend could be observed. For most of the samples, either no related impurities were detected or they were below the limit of quantification. However, for Batch ENQ3860/AIRC/017/01/P1 and Batch ENQ3860/AIRC/018/01/P1, encapsulated in HPMC-based $TiO_2$-free capsule shells, small levels of related impurities could be detected on storage at 60°C/30%RH, which increased slightly with storage time. This slight increase would be expected since the HPMC capsule allow for greater ingress of oxygen when compared to gelatin. The impurities that increase was the loperamide n-oxide impurity. The increase impurity is not expected to be related to the use of $TiO_2$ alternatives.

Table 149: Mean assay values for fluvastatin in the fluvastatin capsule batches on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Mean Assay Fluvastatin (%LC) | Mean Assay Fluvastatin (%LC) | Mean Assay Fluvastatin (%LC) | Mean Assay Fluvastatin (%LC) | Mean Assay Fluvastatin (%LC) |
| 007/01/P1 | CAP-016a | HPMC | $TiO_2$ | 94.9 | 93.3 | 91.0 | 89.6 | 87.9 |
| 008/01/P1 | CAP-014c | HPMC | $TiO_2$ | 95.0 | 93.9 | 91.1 | 89.7 | 85.4 |
| 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 95.1 | 91.6 | 89.8 | 87.9 | 86.9 |
| 010/01/P1 | CAP-009 | HPMC | $CaCO_3$+A | 93.2 | 93.7 | 90.2 | 87.9 | 85.0 |
| 011/01/P1 | CAP-007 | HPMC | $CaCO_3$+A | 94.2 | 92.3 | 88.5 | 89.0 | 86.9 |
| 012/01/P1 | CAP-002 | Gelatin | NaPOx | 94.9 | 93.7 | 95.8 | 94.6 | 93.9 |
| 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 94.8 | 93.9 | 93.9 | 91.3 | 91.8 |
| 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$+B+D | 93.8 | 90.8 | 94.0 | 94.5 | 94.0 |

Color Code: Red = A significant decrease in assay compared to $T_0$ (>5%)

Table 150: %Total related impurities in fluvastatin capsule batches on accelerated stability

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T= 7 Days | T=14 Days | T=21 Days |
| | | | | Total Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) |
| 007/01/P1 | CAP-016a | HPMC | $TiO_2$ | 0.18 | 0.29 | 1.75 | 3.10 | 4.43 |
| 008/01/P1 | CAP-014c | HPMC | $TiO_2$ | 0.27 | 0.23 | 2.29 | 3.99 | 4.82 |
| 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 0.25 | 0.49 | 2.18 | 4.11 | 5.24 |
| 010/01/P1 | CAP-009 | HPMC | $CaCO_3$+A | 0.26 | 0.36 | 2.34 | 4.29 | 5.42 |
| 011/01/P1 | CAP-007 | HPMC | $CaCO_3$+A | 0.20 | 0.38 | 1.74 | 3.33 | 4.47 |
| 012/01/P1 | CAP-002 | Gelatin | NaPOx | 0.20 | 0.59 | 1.48 | 2.37 | 3.09 |
| 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 0.20 | 0.72 | 1.60 | 2.53 | 3.31 |
| 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$+B+D | 0.21 | 0.54 | 1.24 | 1.70 | 2.38 |

Color Code: Red = A significant change in %total related impurities compared to $T_0$

Table 151: Mean assay values for loperamide in the loperamide capsule batches on accelerated stability

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Mean Assay Loperamide (%w/w) | Mean Assay Loperamide (%w/w) | Mean Assay Loperamide (%w/w) | Mean Assay Loperamide (%w/w) | Mean Assay Loperamide (%w/w) |
| 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 97.2 | 97.7 | 98.4 | 97.6 | 99.1 |
| 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 98.4 | 96.6 | 99.0 | 99.0 | 100.2 |
| 017/01/P1 | CAP-011 | HPMC | $CaCO_3$+B+C+D | 98.6 | 96.3 | 98.5 | 98.5 | 96.2 |
| 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | 95.3 | 93.6 | 96.1 | 95.0 | 94.2 |
| 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | 97.6 | 96.6 | 98.0 | 97.8 | 97.0 |

Color Code: Red = A significant decrease in assay compared to $T_0$ (>5%)

Table 152: %Total related impurities in loperamide capsule batches on accelerated stability

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) | Total Related Impurities (%LC) |
| 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | ND | <LOQ | <LOQ | <LOQ | <LOQ |
| 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | ND | ND | <LOQ | ND | <LOQ |
| 017/01/P1 | CAP-011 | HPMC | $CaCO_3$+B+C+D | ND | ND | 0.08 | 0.10 | 0.12 |
| 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | ND | <LOQ | 0.06 | 0.07 | 0.09 |
| 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | ND | ND | 0.06 | ND | ND |

Color Code: Red = A significant change in %total related impurities compared to $T_0$

## 101. Capsule Brittleness Changes on Accelerated Stability

Benserazide HCl/Levodopa Capsules

Table 153 compares the %brittle capsules in a sample of 20 capsules from the benserazide HCl/levodopa capsule batches on accelerated stability versus the $T_0$ results. The acceptance criterion was ≤5% brittle capsules (1 brittle capsule in 20). Only the $T_0$ sample from Batch No. ENQ3860/AIRC/006/01 had more than 5% brittle capsules. However, all of the samples on stability from this batch met the acceptance criteria.

Fluvastatin Capsules

Table 154 compares the %brittle capsules in a sample of 20 capsules from the fluvastatin capsule batches on accelerated stability versus the $T_0$ results. All batches encapsulated in $TiO_2$-free and $TiO_2$ reference capsule shells had ≤5% brittle capsules after storage at 5°C for 21 days.

The 14-day sample of Batch ENQ3860/AIRC/007/01/P1 encapsulated in the white HPMC-based $TiO_2$ reference, CAP-016, stored at 60°C/%30RH, did not meet the acceptance criterion. However, all other samples lay within the ≤5% limit. Batch ENQ3860/AIRC/008/01/P1 encapsulated in the HPMC-based $TiO_2$ red reference capsule met the criteria at all time-points and storage conditions.

The results for the batches encapsulated in the $TiO_2$-free capsule shells depended on the shell used. The gelatin-based $TiO_2$-free capsule shells, CAP-002 and CAP-005 were not brittle either at $T_0$ or after storage at 50°C/50%RH. The other gelatin-based capsule shell, CAP-012, did not meet the acceptance criterion after 14-day and 21-day storage at 50°C/50%RH.

The HPMC-based $TiO_2$-free capsule shells were very brittle following 60°C/30% %RH storage. This was especially the case for the samples after 14-days storage at the accelerated conditions. Overall, CAP-009 was the most brittle of all, followed by CAP-003 and CAP-007.

Loperamide Capsules

Table 155 compares the %brittle capsules in a sample of 20 capsules from the loperamide capsule batches on accelerated stability versus the $T_0$ results. CAP-017 proved very brittle under all storage conditions, while the other two $TiO_2$-free capsule shells, CAP-001 and CAP-010 were not brittle under any of the conditions tested.

Table 153: %Capsule brittleness for benserazide HCl/levodopa capsules on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | %Brittle Caps | %Brittle Caps | %Brittle Caps | %Brittle Caps | %Brittle Caps |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 0% | 0% | 5% | 0% | 0% |
| 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 0% | 0% | 5% | 0% | 0% |
| 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 5% | 0% | 0% | 0% | 0% |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 0% | 5% | 5% | 0% | 0% |
| 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 5% | 0% | 0% | 0% | 5% |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 10% | 0% | 0% | 5% | 0% |

Color Code: Red = % brittle capsules > 5%

Table 154: %Capsule brittleness for fluvastatin capsules on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | % Brittle Caps | % Brittle Caps | % Brittle Caps | % Brittle Caps | % Brittle Caps |
| 007/01/P1 | CAP-016[a] | HPMC | $TiO_2$ | 5% | 0% | 0% | 15% | 0% |
| 008/01/P1 | CAP-014[c] | HPMC | $TiO_2$ | 5% | 0% | 0% | 0% | 0% |
| 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 0% | 0% | 0% | 35% | 95% |
| 010/01/P1 | CAP-009 | HPMC | $CaCO_3$+A | 65% | 5% | 55% | 100% | 100% |
| 011/01/P1 | CAP-007 | HPMC | $CaCO_3$+A | 5% | 0% | 10% | 20% | 20% |
| 012/01/P1 | CAP-002 | Gelatin | NaPOx | 0% | 0% | 0% | 0% | 0% |
| 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 0% | 0% | 0% | 0% | 0% |
| 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$+B+D | 0% | 0% | 5% | 20% | 10% |

Color Code: Red = % brittle capsules > 5%

Table 155: %Capsule brittleness for loperamide capsules on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | % Brittle Cap | % Brittle Cap | % Brittle Cap | % Brittle Cap | % Brittle Cap |
| 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 0% | 0% | 25% | 15% | 0% |
| 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 15% | 0% | 15% | 10% | 0% |
| 017/01/P1 | CAP-011 | HPMC | $CaCO_3$+B+C+D | 60% | 30% | 70% | 100% | 100% |
| 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | 0% | 0% | 0% | 0% | 0% |
| 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | 0% | 0% | 0% | 0% | 0% |

Color Code: Red = % brittle capsules > 5%

The results for the two $TiO_2$ reference capsule shells were variable. No brittle capsules were found in the samples of Batch ENQ3860/AIRC/015/01/P1 encapsulated in CAP-013, the white gelatin-based $TiO_2$ reference, stored at ambient, 5°C and after 21 days at 50°C/50%RH. However, this batch failed the acceptance criterion at the 7-day and 14-day time-point following storage under the accelerated stability conditions. Similarly, Batch ENQ3860/AIRC/016/01/P1, encapsulated in CAP-017, had 0% brittle capsules at the 21-day time-point at 50°C/50%RH. However, there was > 5% brittle capsules in the samples stored for 7 and 14 days under the same conditions.

## 102. Disintegration Results

Benserazide HCl/Levodopa Capsules

Table 156 shows the disintegration times for the benserazide HCl/levodopa capsule batches in the accelerated stability study compared with $T_0$. All of the capsule batches disintegrated between 2 and 5 minutes and no trends in disintegration times were observed following storage at 5°C or 60°C/30%RH for the HPMC-based capsule shells or 50°C/50%RH for the gelatin-based ones.

Fluvastatin Capsules

Table 157 shows the disintegration times for the fluvastatin capsule batches in the accelerated stability study compared with $T_0$. The HPMC-based capsule batches disintegrated between 3.5 min and 6.5 min. The gelatin-based capsule batches disintegrated between 2 min and 3.5 min. For both types of capsules, there was no trends in the disintegration times data for the batches stored at 5°C or 60°C/30%RH for the HPMC-based capsule shells or 50°C/50%RH for the gelatin-based ones.

Loperamide Capsules

Table 158 shows the disintegration times for the loperamide capsule batches in the accelerated stability study compared with $T_0$. Most of the capsule batch samples disintegrated between 2.5 min and 6 min. The 14-day sample of Batch ENQ3860/AIRC/018/01/P1 stored at 60°C/30%RH disintegrated more slowly than the other samples with a disintegration time of 7 min 25 seconds. However, there was no trend in the data with the 21-day sample disintegrating in 5 min 35 seconds.

Table 156: Disintegration time for benserazide HCl/levodopa capsules on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) |
| 001/01/P1 | CAP-016 | HPMC | $TiO_2$ | 04:13 | 03:41 | 04:05 | 04:40 | 03:18 |
| 002/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 02:00 | 03:18 | 02:30 | 03:11 | 02:22 |
| 003/01/P1 | CAP-015 | HPMC | $Fe_2O_3$ | 04:32 | 04:27 | 04:21 | 04:18 | 05:18 |
| 004/01/P1 | CAP-004 | HPMC | $CaCO_3$ | 03:31 | 02:46 | 04:05 | 03:53 | 03:53 |
| 005/01/P1 | CAP-008 | HPMC | $Fe_2O_3$ | 03:21 | 04:28 | 04:03 | 03:55 | 04:35 |
| 006/01/P1 | CAP-006 | Gelatin | $CaCO_3$+A | 02:42 | 02:08 | 02:30 | 02:56 | 02:51 |

Table 157: Disintegration time for fluvastatin capsules on accelerated stability vs $T_0$ (ambient storage)

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell Ref | Shell Former | Opacifier | T= 0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) |
| 007/01/P1 | CAP-016a | HPMC | $TiO_2$ | 04:49 | 05:04 | 05:21 | 04:58 | 04:46 |
| 008/01/P1 | CAP-014c | HPMC | $TiO_2$ | 04:45 | 04:25 | 06:16 | 04:14 | 05:07 |
| 009/01/P1 | CAP-003 | HPMC | $CaCO_3$ | 04:22 | 03:49 | 05:10 | 04:51 | 04:33 |
| 010/01/P1 | CAP-009 | HPMC | $CaCO_3$+A | 04:19 | 03:54 | 03:30 | 04:34 | 03:47 |
| 011/01/P1 | CAP-007 | HPMC | $CaCO_3$+A | 04:23 | 05:03 | 03:37 | 05:44 | 03:54 |
| 012/01/P1 | CAP-002 | Gelatin | NaPOx | 02:34 | 02:29 | 02:22 | 02:51 | 02:10 |
| 013/01/P1 | CAP-005 | Gelatin | $CaCO_3$ | 02:43 | 03:10 | 02:37 | 02:22 | 02:57 |
| 014/01/P1 | CAP-012 | Gelatin | $CaCO_3$+B+D | 02:41 | 02:25 | 02:35 | 02:11 | 02:30 |

Table 158: Disintegration time for loperamide capsules on accelerated stability

| Storage Conditions | | | | Ambient | 5°C | 50°C/50%RH (gelatin capsules 60°C/30%RH (HPMC capsules) | | |
|---|---|---|---|---|---|---|---|---|
| Batch No. ENQ3860/AIRC/ | Consort. Cap Shell | Shell Former | Opacifier | T=0 | T=21 Days | T=7 Days | T=14 Days | T=21 Days |
| | | | | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) | Disintegration (min:sec) |
| 015/01/P1 | CAP-013 | Gelatin | $TiO_2$ | 04:07 | 04:37 | 04:12 | 05:22 | 03:38 |
| 016/01/P1 | CAP-017 | Gelatin | $TiO_2$ | 03:54 | 03:02 | 02:44 | 04:48 | 04:58 |
| 017/01/P1 | CAP-011 | HPMC | $CaCO_3$+B+C+D | 04:21 | 03:22 | 03:12 | 04:39 | 03:41 |
| 018/01/P1 | CAP-001 | HPMC | $Fe_2O_3$ | 05:39 | 05:18 | 04:20 | 07:25 | 05:35 |
| 019/01/P1 | CAP-010 | Gelatin | $Fe_2O_3$ | 05:44 | 04:17 | 04:34 | 04:14 | 04:44 |

## 103. Results – Dissolution

Benserazide HCL/Levodopa Capsules

Figure 83 and Figure 84 show the dissolution of benserazide in %LC from benserazide HCl/levodopa capsule batches on accelerated stability versus the $T_0$ results. The results show that there was minimal change in the dissolution profiles of the stability samples for the batches, encapsulated in HPMC-based capsule shells (see Figure 83), with > 85% of the benserazide released within 10 min. For the batches encapsulated in $TiO_2$-free HPMC-based capsule shells, ENQ3860/AIRC/003/01/P1, ENQ3860/AIRC/004/01/P1 and ENQ3860/AIRC/005/01/P1, dissolution at the 5 min time-point was greater than the $T_0$ sample or very similar. For the sample of Batch ENQ3860/AIRC/003/01/P1 stored at 5°C for 21 days, release only reached 88% at the 15-min time-point and then declined slightly at later time-points. This is in contrast to the $T_0$ sample of the same batch and also all samples stored at 60°C/30%RH from which over 95% of benserazide was recovered. The assay and related impurity results for the 5°C/21 Day sample were similar to $T_0$ and therefore this result cannot be explained by a decrease in assay or increase in degradation. For Batch ENQ3860/AIRC/004/01/P1, encapsulated in the HPMC-based $TiO_2$ reference, the dissolution profiles were similar for all of the samples tested. In conclusion, the accelerated stability storage had little or no effect on the dissolution of benserazide from the batches encapsulated in HPMC-based capsule shells.

For ENQ3860/AIRC/002/01/P1 and ENQ3860/AIRC/006/01/P1, encapsulated in gelatin-based capsule shells, the benserazide dissolution rate remained the same for all samples. However, the total percentage released was significantly less for samples stored at 50°C/50%RH and decreased with storage time under this condition. The benserazide in these batches degraded significantly at 50°C/50%RH, with reduced assay and increased levels of related impurities. This decrease was greater the longer the samples had been stored under accelerated conditions. Therefore, the degradation is likely to account for the reduced benserazide recovery on dissolution.

Figure 85 and Figure 86 show the dissolution of levodopa in %LC from benserazide HCl/levodopa capsule batches on accelerated stability versus the $T_0$ results. The results show that for all batches, the dissolution profile for levodopa did not alter significantly compared with $T_0$ during the stability study. As for benserazide, Batches ENQ3860/AIRC/003/01/P1, ENQ3860/AIRC/004/01/P1 and ENQ3860/AIRC/005/01/P1, released levodopa faster or at a similar level to the $T_o$ samples at the 5 min time-point. However, at the 10-min time-point, the % released was similar to the $T_0$ sample.

r

Figure 83: Benserazide dissolution profiles from benserazide HCl/levodopa capsule batches (HPMC-based capsules)

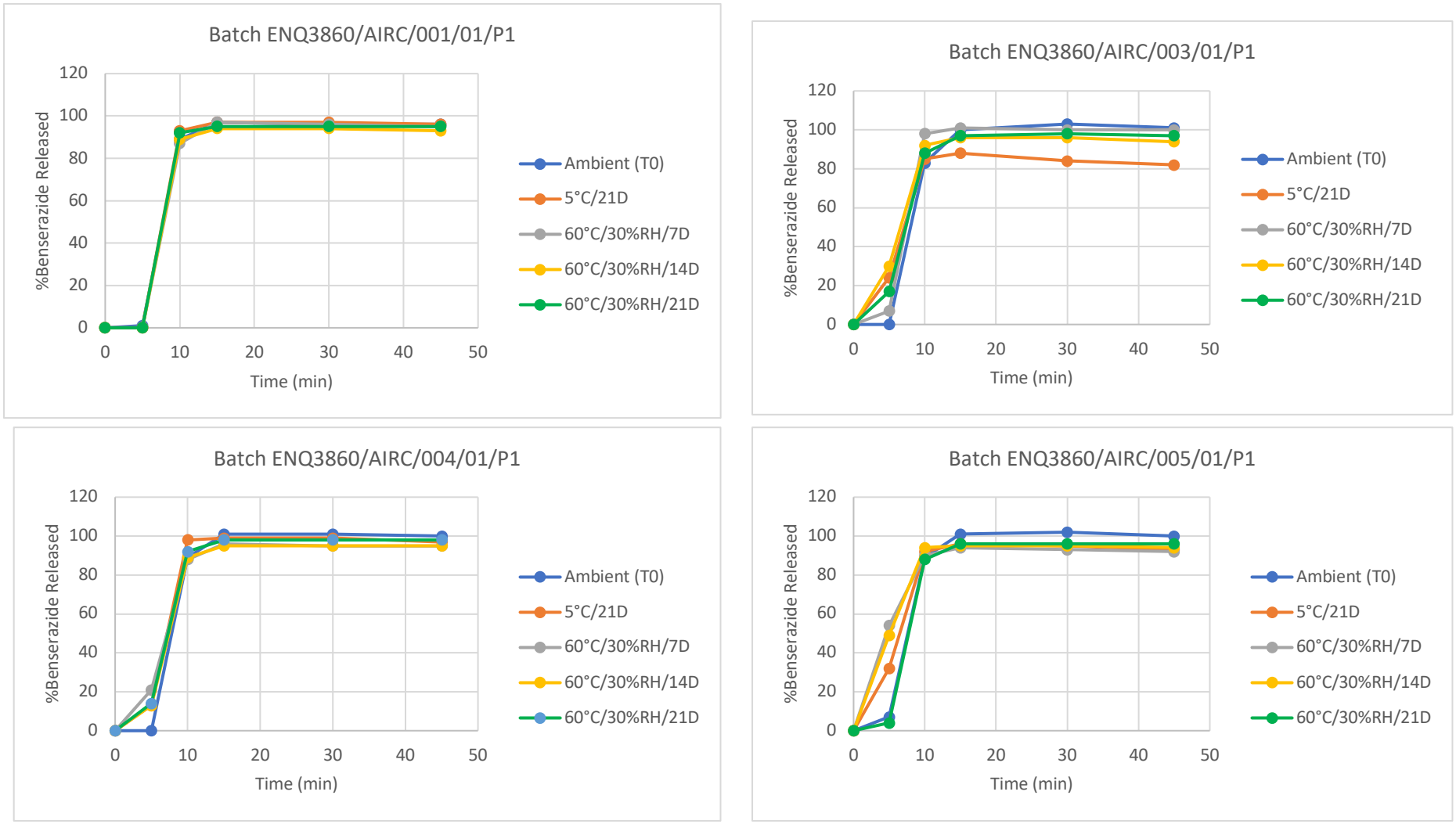
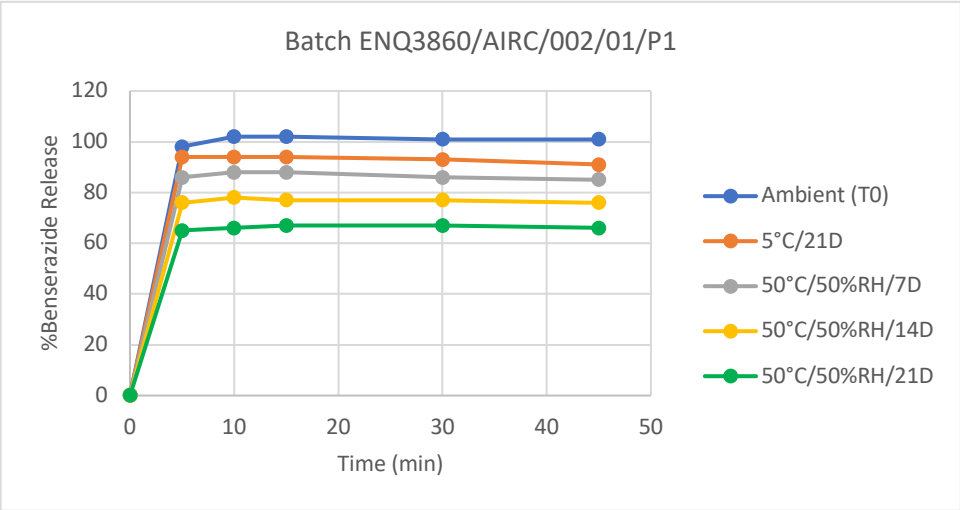



Figure 84: Benserazide dissolution profiles from benserazide HCl/levodopa capsule batches (gelatin-based capsules)


Batch ENQ3860/AIRC/002/01/P1
%Benserazide Release
120
100
80
60
40
20
0
0
10
20
30
40
50
Time (min)
Ambient (T0)
5°C/21D
50°C/50%RH/7D
50°C/50%RH/14D
50°C/50%RH/21D

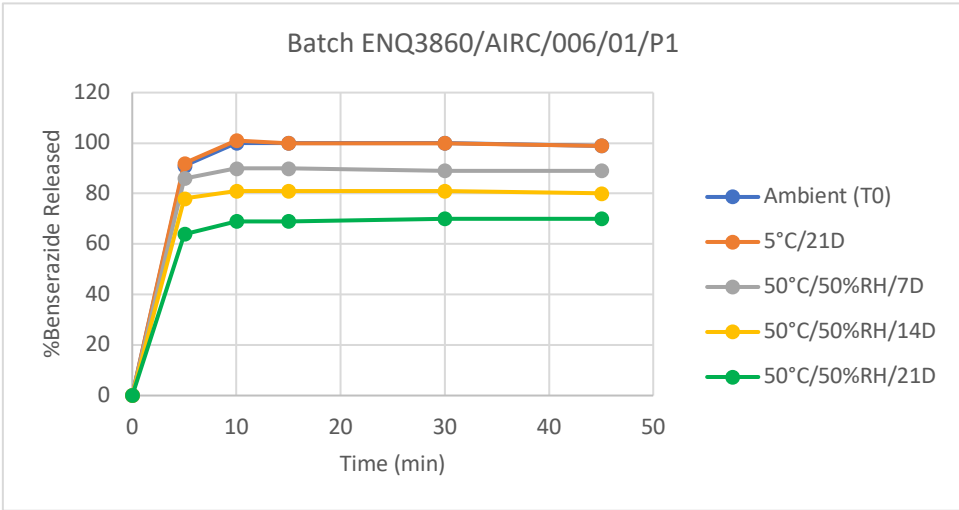

Batch ENQ3860/AIRC/006/01/P1
%Benserazide Released
120
100
80
60
40
20
0
0
10
20
30
40
50
Time (min)
Ambient (T0)
5°C/21D
50°C/50%RH/7D
50°C/50%RH/14D
50°C/50%RH/21D

Figure 85: Levodopa dissolution profiles from benserazide HCl/levodopa capsule batches (HPMC-based capsules)

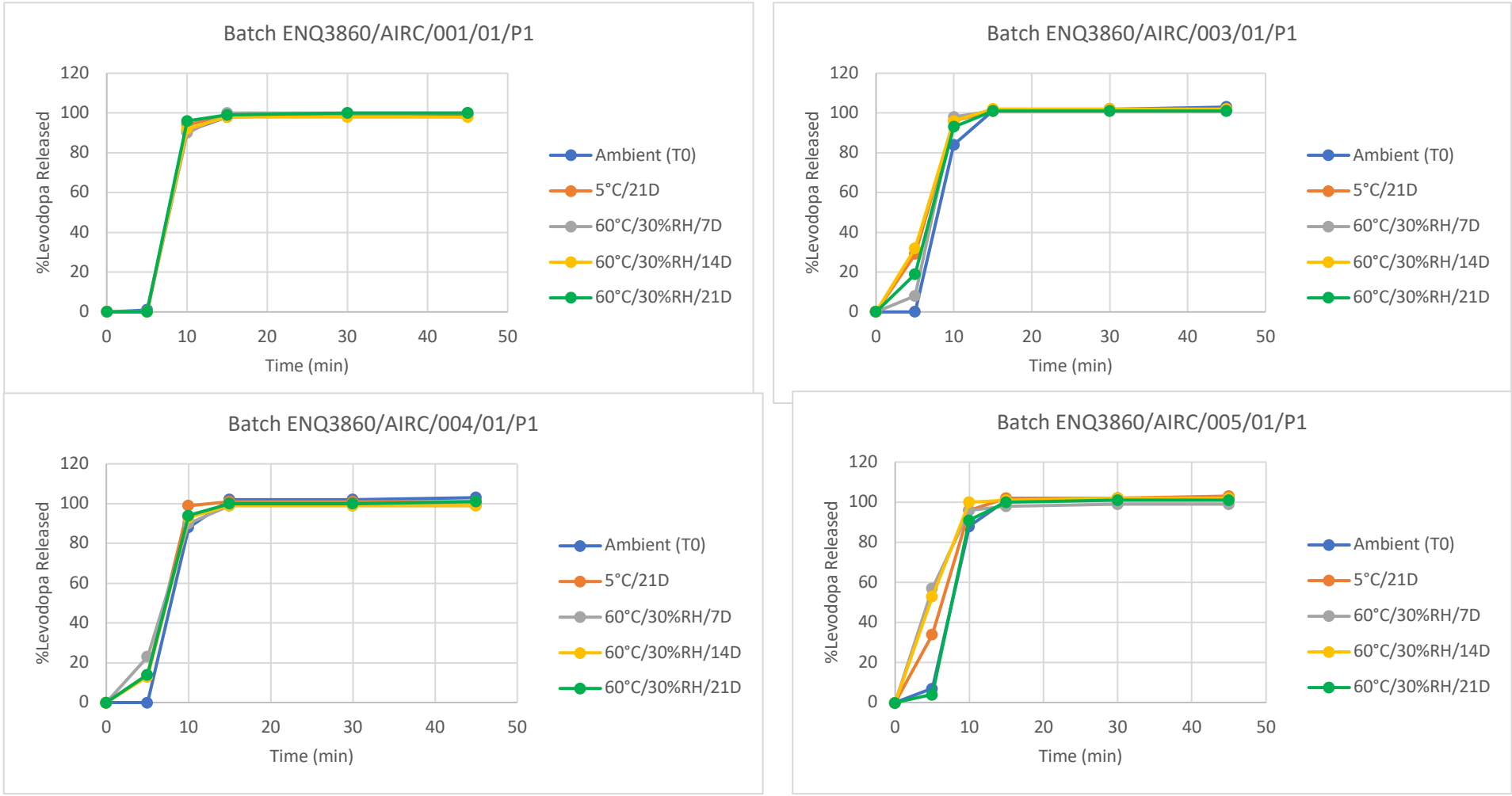
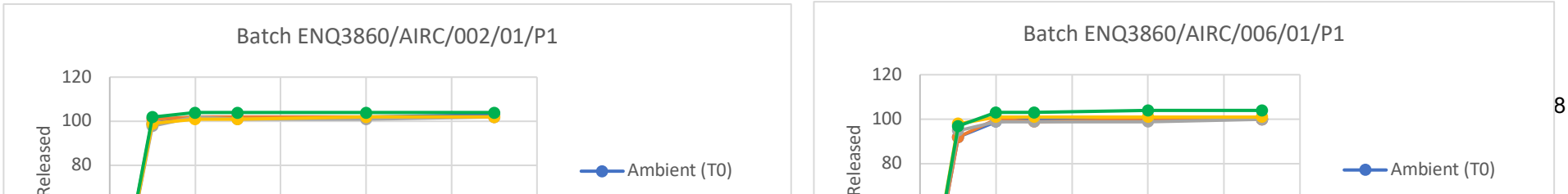



Figure 86: Levodopa dissolution profiles from benserazide HCl/levodopa capsule batches (gelatin-based capsules)



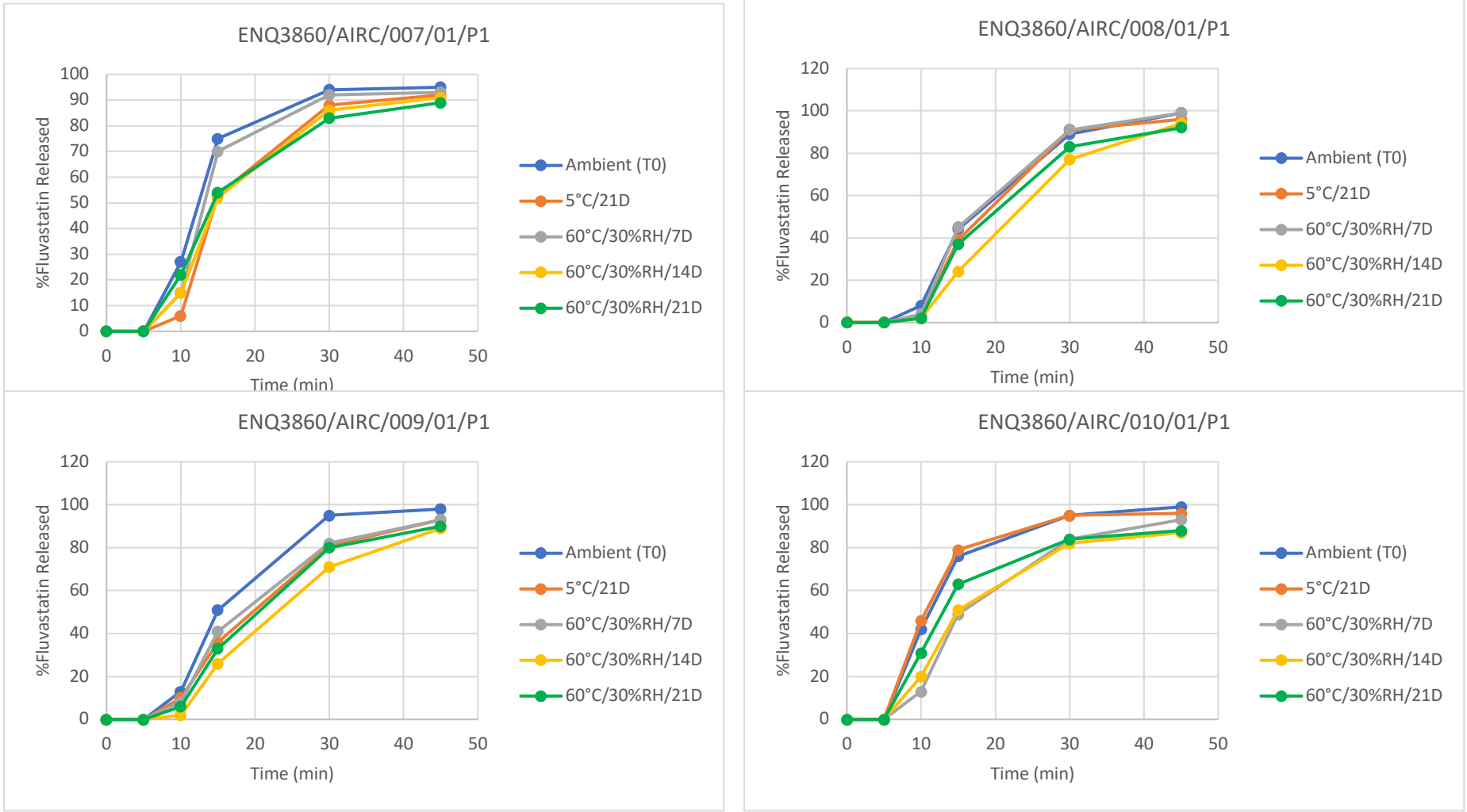
Fluvastatin Capsules

Figure 87 and

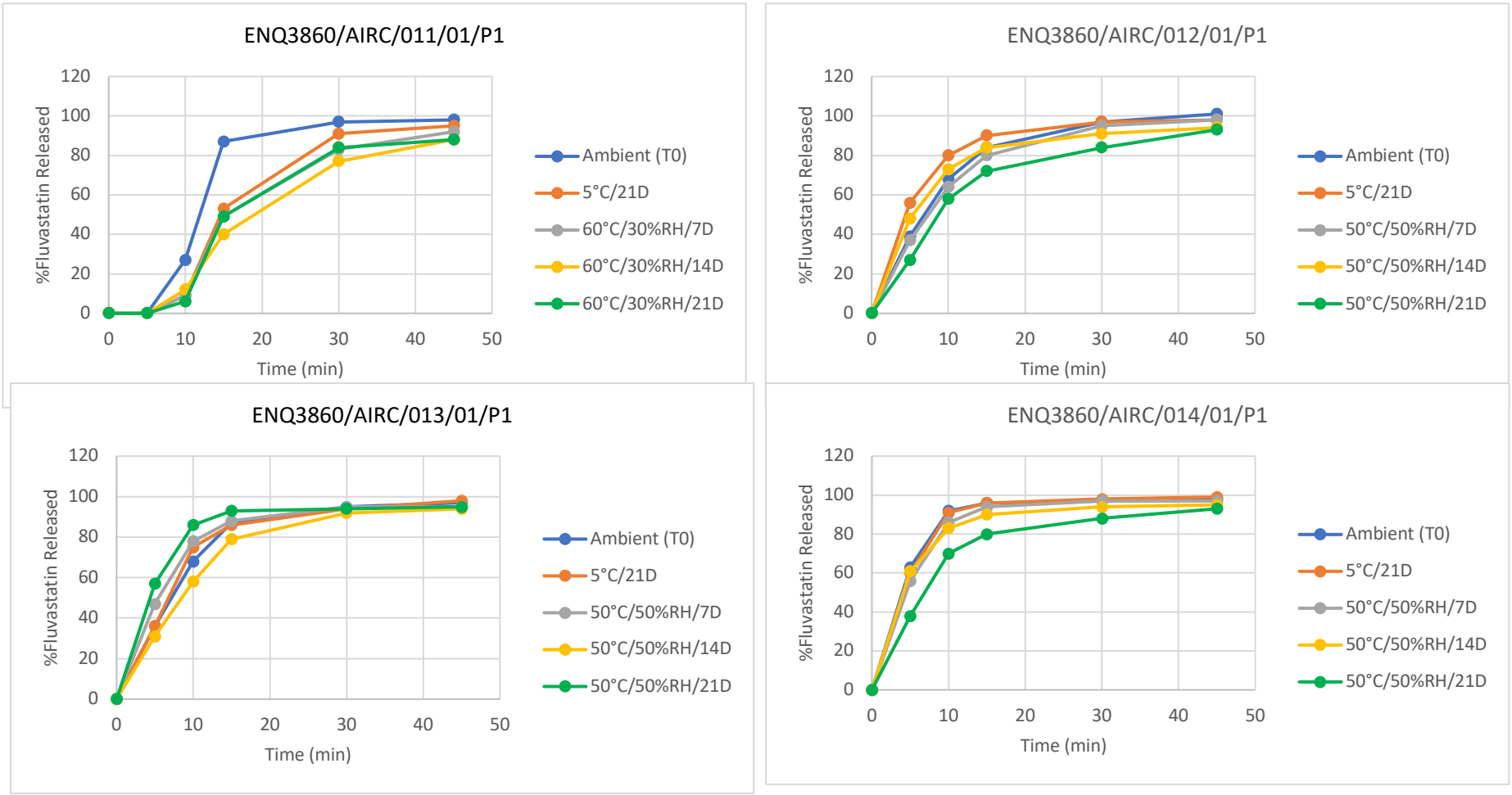
`Figure 88 show the dissolution of fluvastatin in %LC from fluvastatin capsule batches for the batches encapsulated in HPMC-based capsule shells (Batches ENQ3860/AIRC/007/01/P1 to ENQ3860/AIRC/011/01/P1), there was a decrease in the dissolution rate and the final % recovered for the samples stored at 60°C/30%RH compared with the $T_0$ results. The reduction in final recoveries is in line with the reduced assay values for these batches (see Table 149). For Batches ENQ3860/AIRC/007/01/P1, ENQ3860/AIRC/009/01/P1 and ENQ3860/AIRC/011/01/P1, the slowdown in dissolution was also observed for the stability samples stored at 5°C for 21 days.

For the batches encapsulated in the gelatin-based capsule shells (ENQ3860/AIRC/012/01/P1 to ENQ3860/AIRC/014/01/P1), there was variation in the dissolution rates with some samples slower than $T_0$ and others faster. For ENQ3860/AIRC/012/01/P1 and ENQ3860/AIRC/014/01/P1, only the stability sample stored at 50°C/50%RH for 21 days had significantly slowly dissolution than the $T_0$ sample. For Batch ENQ3860/AIRC/013/01/P1, the 7-day and 21-day sample stored at 50°C/50%RH had significantly faster dissolution profiles than $T_0$. The higher final %recoveries for these three batches reflect that their assay values were not significantly impacted by the stability storage conditions used (see Table 149).

Figure 87: Fluvastatin dissolution profiles from fluvastatin capsule batches (Trials 7 to 10)



`Figure 88: Fluvastatin dissolution profiles from fluvastatin capsule batches (Trials 11 to 14)




Batch ENQ3860/AIRC/011/01/P1 was encapsulated in a HPMC-based capsule shell, the other batches were encapsulated in gelatin-based capsule shells.

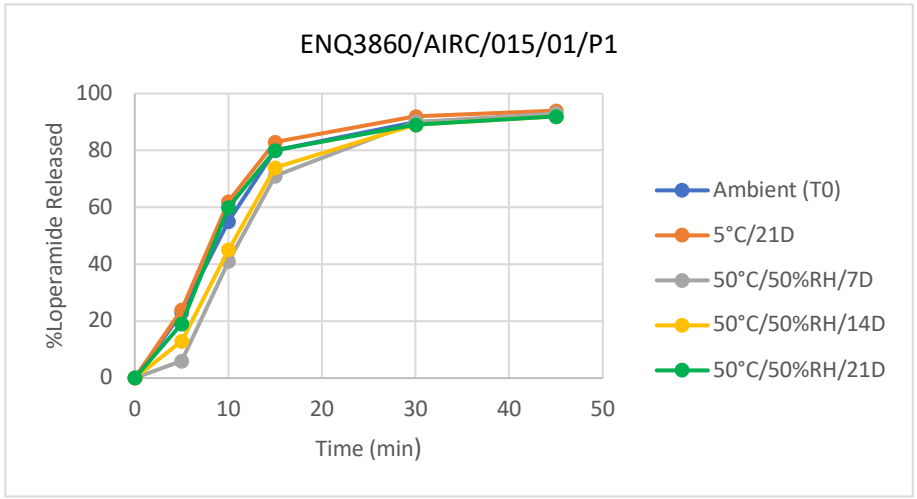
Loperamide Capsules

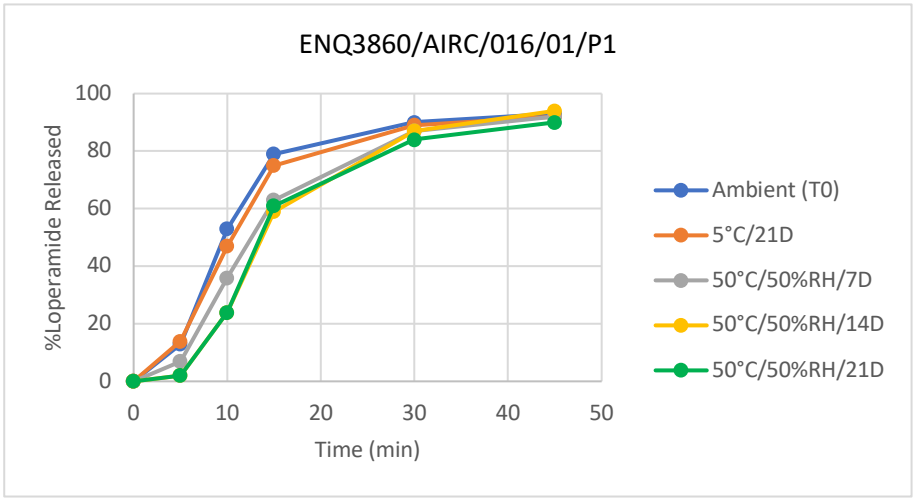
Figure 89 and Figure 90 show the dissolution of loperamide in %LC from loperamide capsule batches on accelerated stability versus the $T_0$ results.

The results show that for the batches encapsulated in gelatin-based capsule shells, the dissolution rate slowed slightly for the stability samples stored at 50°C/50%RH compared to $T_0$. This slowdown was most pronounced for Batch ENQ3860/AIRC/016/01/P1, which was encapsulated in the $TiO_2$ red reference capsule shell. The dissolution of the stability samples stored at 5°C were either similar or slightly faster or slower than those of the $T_0$ samples. The final %recovery was similar for all batches and in line with the assay results.

Loperamide release also slowed from the stability samples from Batch ENQ3860/AIRC/018/01/P1 which was encapsulated the HPMC-based $TiO_2$-free capsule shell, CAP-001. However, loperamide release from the stability samples of Batch ENQ3860/AIRC/017/01/P1 was highly variable. This batch contained the pink $TiO_2$-free capsule, CAP-011. All of the stability samples released loperamide faster than the $T_0$ sample except for the sample stored for 7 days at 60°C/30%RH. CAP-011 had been previously shown to be very brittle under all of the conditions tested (see Table 125) and this may have contributed to the variable release from the stability samples.

Figure 89: Loperamide dissolution profiles from loperamide capsule batches (gelatin-based capsules)



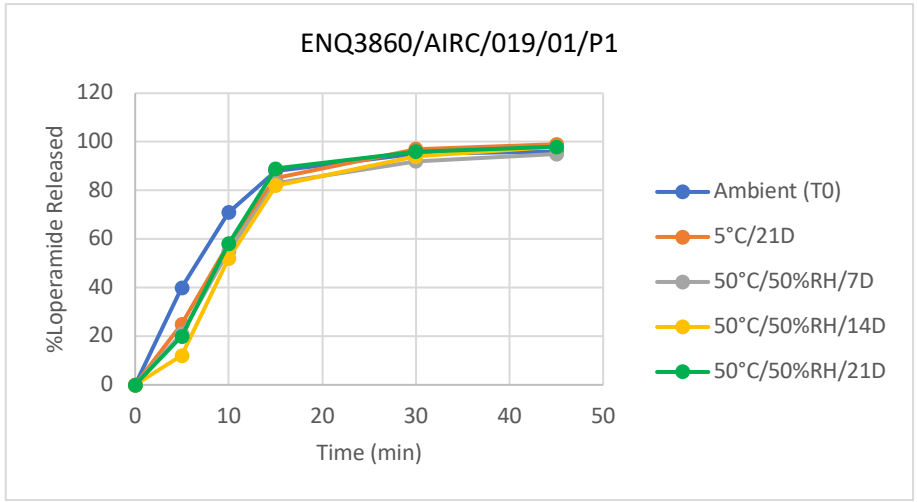
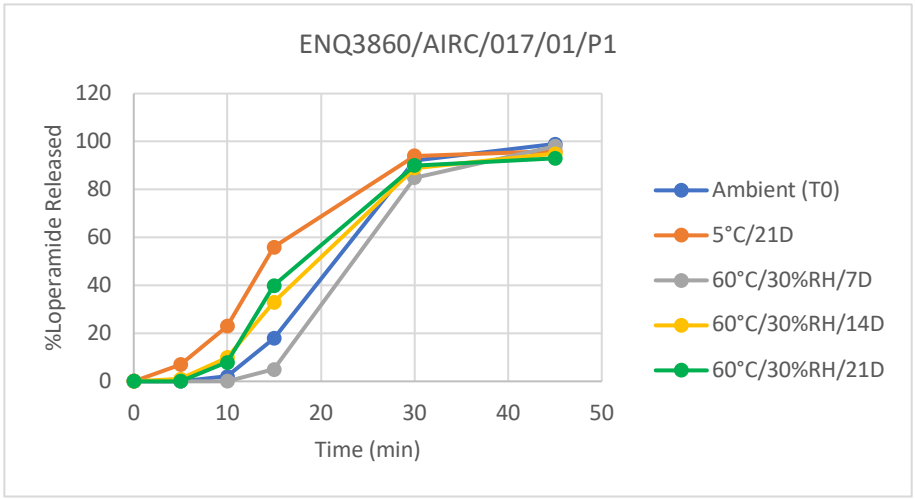
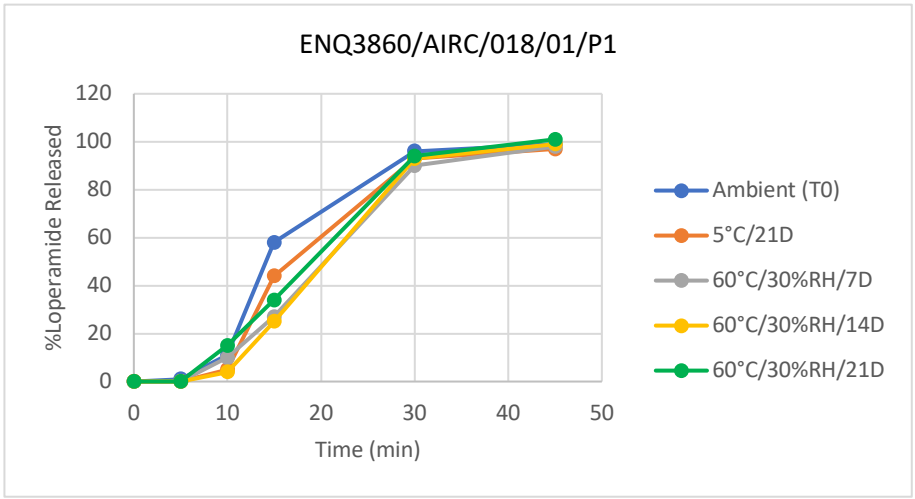



Figure 90: Loperamide dissolution profiles from loperamide capsule batches (HPMC-based capsules)



## Section Summary and Conclusions

Benserazide HCl/Levodopa Capsule Batches

- Appearance -Capsule shells

The visual appearance of the white capsule shells altered on exposure to the higher temperature conditions but not under refrigerated storage. The colored capsule shells did not visually change in appearance. However, colorimetry data suggested that the white $TiO_2$ reference capsule, CAP-016 changed to a lesser extent after 1 week at 60°C/30%RH than the $TiO_2$-free ones and the colored capsules. It was also the only capsule shell to meet the color difference criterion after 21 days storage at 5°C.

- Capsule Brittleness

None of the capsules on accelerated stability were brittle at any time-point

- Assay and Related Impurities and Capsule Contents Appearance

Benserazide assay and impurities changed to a greater extent when encapsulated in gelatin capsules stored at 50°C/50%RH, probably as a result of its sensitivity to high % relative humidity. This was regardless of whether the capsule shell contained $TiO_2$ or not.

- In vitro performance

Disintegration and dissolution profile shape were not significantly affected by storage under the accelerated stability conditions. Total recovery at the final dissolution test time-point was reduced for gelatin-based capsule batches which had significant degradant levels.

Fluvastatin Capsule Batches

- Appearance – Capsule Shells

The visual appearance of all of the capsule shells changed at the higher temperatures except for the colored $TiO_2$ reference capsule, CAP-014. There was no change in the appearance of any capsule shells after refrigerated storage.

- Capsule Brittleness

There was some variation in the capsule brittleness results perhaps reflecting the manual operator-dependent method used to assess this property. However, the TiO2-free capsules, CAP-003, CAP-007 and CAP-009 did not meet the acceptance criterion on more than two occasions.

- Assay, Related Impurities and Capsule Contents Appearance

Degradation occurred at 5°C to a greater extent in the batches stored in gelatin capsules and in contrast to a greater extent in the HPMC-based capsule batches at 60°C/30%RH than the gelatin ones stored at 50°C/50%RH. There appeared to be no major difference between batches encapsulated in $TiO_2$-free and $TiO_2$ reference capsule shells.

- In vitro performance

Disintegration and dissolution profile shape were not significantly affected by storage under the accelerated stability conditions. However, there was a tendency for the HPMC-based capsule batches to release more slowly than at $T_o$. Total recovery at the final dissolution test time-point was reduced for batches with significant degradation.

Loperamide Capsule Batches

- Appearance

The capsule shell and capsule contents did not change in appearance for any of the batches tested. However, colorimetry data showed that there was no color difference between the samples encapsulated in CAP-013, the white $TiO_2$ reference and the pink $TiO_2$-free CAP-011.

- Capsule Brittleness

There was some variation in the results for brittleness for the two batches encapsulated in the $TiO_2$ reference batches but no trend in the results. CAP-011 proved again to be the most brittle capsule shell of all.

- Assay and Related Impurities

There was no significant change in assay or impurity levels for any of the batches in the accelerated stability study. A difference in impurity level was noticed between the gelatin and HPMC capsule shells over the storage conditions.

- In vitro performance

There was no significant change or trend in the disintegration or dissolution data for any of the batches.

Overall, the results depended on API stability under the selected storage conditions and there was no significant difference between the $TiO_2$ and $TiO_2$-free batches with regard to assay, impurities, disintegration and dissolution. Capsule shell appearance and the changes observed on accelerated stability and capsule shell brittleness were affected by the capsule shell used. However, in cases where the same capsule shell was used for two different blends, there was some variation in the results showing that the API blend used also had some influence.

# Experimental Part 5: Manufacturability

## Experimental Objectives and Rationale

Section 0 of this report concerns the 19 small-scale encapsulation trials in which the 13 selected $TiO_2$-free and 4 $TiO_2$ reference capsule shells were filled with various active blends using a MG2 Labby encapsulation machine at a batch size of approximately 5000 capsules. These trials yielded adequate product in terms of quality and quantity of filled capsules for analytical testing (see Section 0), a photostability study (see Section 0) and accelerated stability studies (see Section 0). However, equipment issues due to a faulty vacuum, made it difficult to evaluate the manufacturability of the $TiO_2$-free capsules versus the $TiO_2$ references as it was impossible to differentiate whether the observed manufacturing issues were solely machine-related or were at least in part influenced by capsule shell composition.

In order to evaluate the manufacturability of the TiO2-free capsule shells further and at larger scale, 8 trials on empty capsule shells were conducted using a commercial scale Zanasi AZ40E encapsulation machine at a batch size of approximately 40,000 capsules. 4 $TiO_2$-free gelatin-based capsule shells, and 4 $TiO_2$-free HPMC-based capsule shells were selected for these studies based on the results of the active filling trials (see Section 0) and initial results from the accelerated stability studies (see Section 0). For both types of capsule shell, equipment set-up was carried out using $TiO_2$-containing capsule shells of the corresponding shell type.

The objectives of the trials were two-fold:

- To perform the encapsulation runs using the selected empty capsule shell types to assess the performance of each batch during manufacture.
- To perform appearance checks and an AQL inspection for each batch of capsules to determine the prevalence of any defects.

## Materials, Equipment and Methodology

The $TiO_2$-free capsule shells selected for the study and the reference capsule shells used for equipment set-up are shown in Table 159.

The key equipment used is listed below:

- Zanasi IMA AZ40E encapsulation machine
- AZ40 Encapsulation deduster
- Size 0 change parts for Zanasi IMA AZ40E encapsulation machine

Table 159: Capsule shells used in the manufacturability trials.

| Trial No. | Consort Capsule Ref | Shell Former | Color | Batch No. |
|---|---|---|---|---|
| HPMC $TiO_2$ reference | CAP-014 | HPMC | Red | G185009 |
| Gelatin $TiO_2$ reference | CAP-013 | Gelatin | White | G185007 |
| 1 | CAP-003 | HPMC | White | G185005 |
| 2 | CAP-009 | HPMC | White | G184997 |
| 3 | CAP-007 | HPMC | White | G185017 |
| 4 | CAP-004 | HPMC | White | G185012 |
| 5 | CAP-002 | Gelatin | White | G184998 |
| 6 | CAP-006 | Gelatin | White | G185019 |
| 7 | CAP-005 | Gelatin | White | G185014 |
| 8 | CAP-012 | Gelatin | White | G184993 |

CAP-014 and CAP-013 were used for the equipment set-up of trials involving HPMC and gelatin capsule shells, respectively. This was carried out in order to determine the optimized machine settings in advance of the trials with the $TiO_2$-free capsule shells. It also ensured that Trials 1 to 4 for the $TiO_2$-free HPMC-based capsule shells and Trials 5 to 8 for the $TiO_2$-free gelatin-based capsule shells were conducted under the same conditions and therefore could be compared.

The equipment was operated at a speed of 30,000 capsules per hour to ensure a run time of greater than 1 hour. The Zanasi was operated at 75% of its theoretical operational speed of 40,000 capsules per hour, which is typical of higher speed manufacturing conditions for this equipment. In order to mimic common manufacturing steps, the capsule shells were dedusted, passed through a metal detector and then collected in double-lined polyethylene bags, following their exit from the encapsulation machine.

During encapsulation the following in process checks were performed at the start, at 10 minutes intervals and at the end of each encapsulation run:

- Closure Lengths – Target closure length of 21.4 – 21.8 mm.
- Capsule Appearance

During each of the encapsulation trials an AQL check was performed every 60 min. A proportion of capsules was assessed for defects to assess the prevalence of any issues detected across the batch. Based on the batch size of 40,000 capsules, a minimum of 1250 capsules were assessed for critical defects, 500 for major defects and 500 minor defects, across the batch.

The temperature and humidity conditions of the room were also monitored throughout the manufacturability trials. The acceptable temperature and humidity ranges for the manufacturing room were set at 15°C to 25°C and the relative humidity of 25 – 55 %RH. However, no adverse trends were noted in the room temperature or humidity throughout the manufacturing period and temperature and humidity varied between 19°C to 20°C and 26% to 40%RH.

## Results and Discussion

For Trials 1 to 4 machine set-up was successfully completed using the HPMC-based, $TiO_2$ reference, CAP-014, with no issues reported. The results are shown in Table 160. For Trials 5 to 8 machine set-up was performed with no issues using the gelatin-based, $TiO_2$ reference, CAP-013, The results are shown in Table 161.

Table 160: Results from Trials 1 to 4 - HPMC-based $TiO_2$-free capsule shells

| Trial No. | Consort. Cap Ref. | Appearance Checks | Closure Length (mm) | AQL Checks | % Yield[a] | Comments/Minor Issues |
|---|---|---|---|---|---|---|
| 1 | CAP-003 | No defects observed | 21.6 - 21.7 | No critical, major or minor defects observed | 97.9% | None |
| 2 | CAP-009 | No defects observed | 21.6 - 21.8 | No critical, major or minor defects observed | 99.8% | 7 mashed capsules blocked the individual feed chutes of the capsule feeding system. This led to blockages and contributed to capsule misfeeding, resulting in ~50–100 crushed/ damaged capsules on the floor of the encapsulation machine at the end of the operation No obvious source of the dented capsules could be found as the capsules were transferred straight from the supplier container/ bag to the capsule hopper and no manual weighing or scooping of the capsules was carried out. It was therefore concluded that the dented capsule shells were not introduced during manufacture. |
| 3 | CAP-007 | No defects observed | 21.5 - 21.8 | No critical, major or minor defects observed | 98.0% | A small number (approx. 30) of crushed/ dented capsules were found on the floor of the encapsulation machine. These originated from the capsule feeding station. The cause of the capsule misfeeding could not be determined. |
| 4 | CAP-004 | No defects observed | 21.6 - 21.8 | No critical, major or minor defects observed | 98.8% | At the end of the trial 20 damaged (crushed/ dented) capsules shells were noted on the encapsulation machine floor. These originated from the capsule feeding station and no specific cause was identified for the capsule shell misfeeding.<br>Approx. 250 reject (unopened) capsule shells were found in the reject capsule shell station. No clear cause was identified for the unopened capsule shells. Since the trial was conducted under set-up conditions based on CAP-014 and no changes were made to the equipment set-up during manufacture of any of the trials, it was concluded that the unopened capsules are a result of the inherent properties of CAP-004 which may require a higher level of vacuum compared to the other capsule shells to consistently separate. Therefore, it is likely that further optimization of the machine set-up would reduce the occurrence of unopened capsules when using this capsule shell. |

[a]Based on acceptable closed capsule shells out of a total of 40,000.

Table 161: Results from Trials 5 to 8 - Gelatin-based $TiO_2$-free capsule shells

| Trial No. | Consort. Cap Ref. | Appearance Checks | Closure Length (mm) | AQL Checks | % Yield[a] | Comments/Minor Issues |
|---|---|---|---|---|---|---|
| 5 | CAP-002 | No defects observed | 21.6 - 21.7 | No critical, major or minor defects observed | 99.6% | No issues |
| 6 | CAP-006 | No defects observed | 21.5 - 21.7 | No critical, major or minor defects observed | 102.3%[b] | 2 damaged/ indented capsule shells (indent on capsule side) were noted being ejected at the feed station due to mis-feeding and, therefore, were not introduced into the final product. |
| 7 | CAP-005 | No defects observed | 21.4 - 21.7 | No critical, major or minor defects observed | 98.6% | None |
| 8 | CAP-012 | No defects observed | 21.4 - 21.7 | No critical, major or minor defects observed | 96.6% | 2 damaged/ indented capsule shells (indented on bottom of capsule) were noted. Again, these capsule shells were ejected at the feed station and, therefore, were not introduced to the final product. |

[a]Based on acceptable closed capsule shells out of a total of 40,000.

[b]A full line clearance of the equipment was performed following Trial 5 which preceded Trial 6. Therefore, the >100% yield was due to using the input quantity of shells (40,000) based on an average weight, as well as calculating the final quantity of capsule shells based on the weight of acceptable shells (3.883 kg) and the average capsule weight of 100 closed capsule shells (94.9 mg).

Overall, the performance of all of the $TiO_2$-free capsule shells was acceptable in the 8 manufacturability trials, with no defects being observed in the final accepted capsule shells for any of the capsule shells. In addition, no significant differences in performance were observed between the gelatin based capsule shells and the HPMC based capsule shells.

During Trials 2, 3, 4, 6 and 8, minor amounts of damaged capsules/capsule shell mis-feeding were observed. The misfeeding issue relating to Trial 2 was different to the those observed in the other trials in that the capsule shells were damaged prior to reaching the capsule feeding station. Since no clear cause could be identified, it suggests that the capsules were damaged prior to the encapsulation operation. In addition, in Trial 4 a significant quantity (~250) of capsules shells were rejected by the equipment due to failure of the vacuum to open these capsule shells.

The Zanasi capsule filler was set-up using either the HPMC-based CAP-014 (Trials 1 to 4) or the gelatin-based CAP-013 (Trials 5 to 8). Therefore, the machine-settings were optimized for the properties of these $TiO_2$ containing capsule shells. This enabled a comparison of the machine's handling of all HPMC-based $TiO_2$-free capsule shells under the same set-up parameters and likewise, the performance of all gelatin-based $TiO_2$-free capsule shells could be compared. However, the equipment set-up was not optimized for the material/physical properties of each individual $TiO_2$-free capsule shell which will be influenced by their composition. The $TiO_2$-free capsule shell properties will be different not only to the $TiO_2$ containing reference capsule shells used for machine set-up, but also to each other. With respect to capsule shell composition, there was no specific trend based on the opacifier used. For example, no issues were observed in Trial 1 (CAP-003) but both damaged capsule shells and unopened capsule shells were found in Trial 4 (CAP-004), although both of these capsule shells contain $CaCO_3$ as the opacifier.

Given the overall success of the 8 manufacturability trials, it is likely that with further experience equipment set-up could be further optimised for each capsule shell batch to reduce the capsule feeding issues noted.

## Conclusions from Manufacturability Studies on Selected $TiO_2$-free Shells

The performance of all of the $TiO_2$-free capsule shells was acceptable in the 8 manufacturability trials and could be likely improved with experience and optimized equipment set-up for the specific $TiO_2$-free capsule shell being used. However, it should be noted that manufacturability trials on empty capsule shells is less challenging than trials involving powder encapsulation.

# Overall Discussion and Conclusion

This report describes the findings of the work carried out to evaluate the properties of 13 $TiO_2$-free hard capsule shells versus 4 $TiO_2$ containing reference capsule shells against a set of key performance factors important in their use in medicinal products and in over-encapsulation to blind products for use in clinical trials. The number and type of $TiO_2$-free capsule shells selected for evaluation was principally based on what was either commercialized or close to commercialization at the start of the work. The selection included a balance of gelatin and HPMC-based $TiO_2$-free capsule shells and included different types of opacifier system and $TiO_2$-free capsule systems from different vendors. A red version of CAP-002 was also requested from the supplier but was not available in time for inclusion into the studies.

A summary of the experimental findings against these key performance parameters is included in Table 162 for the gelatin-based capsule shells and in Table 163 for the HPMC capsule shells.

## White $TiO_2$-free Capsule Shells versus White $TiO_2$ Capsule Shells

The results show that for white capsule shells, all of the $TiO_2$-free capsule shells have inferior properties to $TiO_2$ containing reference shells in terms of opacity and ability to camouflage the capsule shell contents. In some cases, they were also considerably more brittle than the $TiO_2$-containing counterparts. The reasons why a specific white $TiO_2$-free capsule shell was considered inferior to the $TiO_2$ reference are given below:

CAP-002 – Gelatin-based, Opacifier – NaPOx

This capsule shell, under certain humidity conditions, is the most opaque of the white $TiO_2$-free capsule shells and it performed well in the brittleness tests. Its major drawback is that its opacity changes with %relative humidity with the capsule shell becoming partially translucent at certain humidities. This change occurs within the normal relative humidity range experienced globally and takes time to reverse when the relative humidity alters again. This means that this capsule shell is likely to fail appearance tests on ICH stability and makes it unsuitable for use for over-encapsulation to blind clinical supplies due to the risk of unblinding. In addition, the water content of CAP-002 at 53%RH was outside the current proposed draft USP pharmacopoeial limits [15].

CAP-003, CAP-004 and CAP-005 – Opacifier - $CaCO_3$

These capsule shells could not be considered opaque and the capsule contents would be visible to the patients. There would be little possibility of a color match between a $TiO_2$-containing capsule and these capsules. CAP-003, which is an HPMC-based capsule shell, performed poorly in the brittleness tests, while CAP-004 had variable performance. Overall, CAP-005 the gelatin-based capsule shell performed better in the photostability and brittleness tests than its HPMC-based counter-parts, CAP-003 and CAP-004. The latter perhaps being due to the higher water content in gelatin-based capsules acting as a plasticizer.

CAP-006, CAP-007 and CAP-009 – Opacifiers $CaCO_3$ + A

The presence of opacifier A increased the opacity of these capsule shells compared with those containing $CaCO_3$ as the sole opacifier. However, they were still semi-transparent. It also made them more brittle and this was especially the case for CAP-007 and CAP-009 which were HPMC-based.

Table 162: Summary of the study findings for $TiO_2$-free versus $TiO_2$ containing reference gelatin capsule shells

| | | | 013[a] | 002 | 005 | 006 | 012 | 017[b] | 010 |
|---|---|---|---|---|---|---|---|---|---|
| **Empty Capsule Shells** | | | **Acceptance Criteria Met (Yes/No)** | | | | | | |
| Appearance & Opacity | Visual appearance | Opaqueness similar to $TiO_2$ reference | NA | No | No | No | No | NA | Yes |
| | Colorimetry | $\Delta E^*_{00}$ values ≤ 1 (white capsules)<br>$\Delta E^*_{00}$ values < 2 (colored capsules) | NA | No | No | No | No | NA | NA |
| Color match to gelatin- based $TiO_2$ reference | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | NA |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | NA | No | No | No | No | NA | NA |
| Capsule shell stability-to light 2 x ICH Q1B | Visual appearance | No visible difference exposed vs control | No | No | Yes | No | No | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | No | No | Yes | Yes | No | Yes | Yes |
| Capsule shell stability to %relative humidity | Visual appearance | No visible difference conditioned vs control | Yes | No | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Mechanical Robustness | % Brittle shells | ≤ 4 % brittle capsule shells at ≥33%RH | Yes | Yes | No | Yes | No | Yes | Yes |
| Manufacturability | In-process data | In-process controls within specification<br>No manufacturing issues | NA | Yes | Yes | Yes | Yes | NA | NA |
| **Blend Filled Capsules – Benserazide HCl/Levodopa, Fluvastatin or Loperamide** | | | | | | | | | |
| Appearance & Opacity & Color Match with $TiO_2$ reference batches | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | Ni | No | NA | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | NA | No | No | No | No | NA | NA |
| Mechanical Robustness ($T_0$) Lab Storage | % Brittle shells | ≤ 5% brittle capsule shells | Yes | Yes | Yes | No | Yes | No | Yes |
| In vitro performance ($T_0$) | Disintegration/Dissolution | No difference to $TiO_2$ reference batches | NA | Yes | Yes | Yes | Yes | NA | Yes |
| Capsule shell stability-to light -2 x ICH Q1B | Visual Appearance | No visible difference exposed vs control | Yes | Yes | No | Yes | No | Yes | Yes |
| Ability to protect actives against photodegradation (2 x ICH Q1B) | Assay & Related Impurities | No difference exposed versus control | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection | | | | | | |
| In vitro performance (2 x ICH Q1B) | Disintegration/Dissolution | No difference exposed versus control | Slower profiles obtained for fluvastatin capsule batches | | | | | | |
| Capsule shell stability-to accelerated stability conditions 5°C; 50°C/50% RH versus $T_0$ | Visual Appearance | No difference to $T_0$ | Yes | No | No | No | No | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | Yes | No | No | No | No | No | No |
| | % Brittle shells | ≤ 5% brittle capsule shells | Results variable for $TiO_2$ references but no trend. $TiO_2$-free capsules met criterion except for CAP-012 at 14 & 21 days. | | | | | | |
| Ability to protect actives on stability: 5°C; 50°C/50% RH versus $T_0$ | Assay & Related Impurities, | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection. | | | | | | |
| In vitro performance on stability: 5°C; 50°C/50% RH versus $T_0$ | Disintegration/Dissolution | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to disintegration and dissolution. | | | | | | |

[a]White $TiO_2$ reference gelatin capsule shell [b]Red $TiO_2$ reference gelatin capsule shell NA=Not applicable

Color Code: Green = Meets acceptance criteria; Yellow = Slight change or $\Delta E^*_{00}$ = 1-2; Red = Does not meet acceptance criteria

Table 163: Summary of the study findings for $TiO_2$-free versus $TiO_2$ containing HPMC capsule shells

| Key Performance Indicator | Parameter(s) Assessed | Acceptance Criteria | Consortium Capsule Shell Reference | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 016[a] | 003 | 004 | 007 | 009 | 014[b] | 001 | 008 | 015 | 011 |
| Appearance & Opacity | Visual appearance | Opaqueness similar to $TiO_2$ reference | NA | No | No | No | No | NA | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ values ≤ 1 (white capsules)<br>$\Delta E^*_{00}$ values < 2 (colored capsules) | NA | No | No | No | No | NA | No | NA | NA | NA |
| Color match to gelatin- based $TiO_2$ reference | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | No | NA | NA | NA |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | NA | No | No | No | No | NA | No | NA | NA | NA |
| Capsule shell stability-to light 2 x ICH Q1B | Visual appearance | No visible difference exposed vs control | No | No | No | No | No | Yes | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | No | No | No | No | No | Yes | Yes | Yes | Yes | No |
| Capsule shell stability to %relative humidity | Visual appearance | No visible difference exposed vs control | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as above | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Mechanical Robustness | % Brittle shells | ≤ 4 % brittle capsule shells at ≥33%RH | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| Manufacturability | In-process data | In-process controls within specification<br>No manufacturing issues | NA | Yes | Yes | Yes | Yes | NA | NA | NA | NA | NA |
| Appearance & Opacity & Color Match with $TiO_2$ reference batches | Visual appearance | Appearance matches $TiO_2$ reference | NA | No | No | No | No | NA | Yes | Yes | Yes | No |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | NA | No | No | No | No | NA | No | NA | NA | NA |
| Mechanical Robustness ($T_0$) | % Brittle shells | ≤ 5% brittle capsule shells | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | No |
| In vitro performance ($T_0$) | Disintegration Dissolution | No difference to $TiO_2$ reference batches | NA | Yes | Yes | Yes | Yes | NA | Yes | Yes | Yes | Yes |
| Capsule shell stability-to light 2 x ICH Q1B | Visual Appearance | No visible difference exposed vs control | Yes /No | Yes | No | No | Yes | Yes | Yes | Yes | Yes | No |
| Ability to protect actives against photodegradation (2 x ICH Q1B) | Assay & Related Impurities | No difference exposed vs control | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to light protection | | | | | | | | | |
| In vitro performance (2 x ICH Q1B) | Disintegration Dissolution | No difference exposed vs control | Slower profiles obtained for fluvastatin capsule batches except for CAP-014. Slowdown may be related to significant API degradation. | | | | | | | | | |
| Capsule shell stability- 5°C; 60°C/30% RH versus $T_0$ | Visual Appearance | No difference to $T_0$ | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes |
| | Colorimetry | $\Delta E^*_{00}$ criteria as for empty capsule shells | No | No | No | No | No | No | No | No | No | No |
| | % Brittle shells | ≤ 5% brittle capsule shells | Variable results but CAP-003, CAP-007, CAP-009, CAP-011 very brittle. | | | | | | | | | |
| Ability to protect actives on stability 5°C; 60°C/30% RH versus $T_0$ | Assay & Related Impurities | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to assay and degradation. | | | | | | | | | |
| In vitro performance following stability: 5°C; 60°C/30% RH versus $T_0$ | Disintegration Dissolution | No change versus $T_0$ | Results depended on API. No major difference between $TiO_2$-free & $TiO_2$ reference capsules with respect to disintegration and dissolution. | | | | | | | | | |

[a]White $TiO_2$ reference gelatin capsule shell [b]Red $TiO_2$ reference gelatin capsule shell NA=Not applicable

Color Code: Green = Meets acceptance criteria; Yellow = Slight change or $\Delta E^*_{00}$ = 1-2; Red = Does not meet acceptance criteria, Yes/No= Variable results

CAP-012 – Opacifiers - $CaCO_3$ + B + D

This gelatin-based capsule shell was more opaque than those containing $CaCO_3$ as the sole opacifier. However, it is not as opaque as the $TiO_2$ reference capsule shell. It had variable results in the brittleness test perhaps due to the number of opacifiers impacting on its overall solids content.

## Colored $TiO_2$-free Capsule Shells versus $TiO_2$ Colored Capsule Shells

The color of the colored capsule shells evaluated in the course of the Consortium's work was restricted to mainly red and white capsule shells due to the aforementioned availability and because these are two common capsule shell colors, which can be typically bought "off-the-shelf".

Red/Orange $TiO_2$-free Capsule Shells Containing $Fe_2O_3$

Overall, the $TiO_2$-free capsules containing the colorant, $Fe_2O_3$ performed well in the battery of tests and in many cases performed equally as well as the $TiO_2$ reference capsules. The capsule shells are opaque and therefore capable of camouflaging any color differences in the capsule contents.

$Fe_2O_3$ is not an opacifier per se but imparts opacification through its intense red color. The intensity of color makes it difficult for the human eye to detect color changes in the capsule shell e.g., following storage under accelerated stability conditions, even though colorimetry data showed that changes had occurred. However, exact color matching for the purposes of reformulating an existing product as $TiO_2$-free may be difficult as CAP-014, the HPMC-based $TiO_2$ reference and the HPMC-based $TiO_2$-free CAP-001 from the same supplier, product line and tradename had color difference values of above 2.

CAP-011 – Opacifiers – $CaCO_3$ + B+C+D

This pink semi-translucent capsule shell was the only non-red/orange colored capsule shell evaluated and was the worst performing $TiO_2$-free capsule shell of the 13 tested. It does not contain $Fe_2O_3$. Its pink color was bleached to white in the photostability studies and it was found to be brittle both as empty and filled capsule shells. In addition, its semi-transparency would not hide the color and appearance of its contents. For the above reasons it is not considered a replacement for $TiO_2$ containing pink capsule shells.

As discussed in Section 0, none of the $TiO_2$-free white capsule shells would be a suitable replacement for white $TiO_2$ containing capsule shells. However, the $TiO_2$-free capsule shells containing $Fe_2O_3$ could be a potential replacement for $TiO_2$ containing red capsules provided color-matching with existing $TiO_2$ capsule shells was possible,

The understanding of how the removal of $TiO_2$ impacts on the performance of capsule shells of other colors could not be studied. At present it is not known how $TiO_2$ free capsule shells of e,g, blue or green or yellow would behave or compare with their $TiO_2$ containing counterparts or if a suitable level of opaqueness could be achieved. Use of $TiO_2$-free red/orange capsule containing $Fe_2O_3$ only would be a major restriction on the color palette available to formulation scientists developing new medicines or reformulating commercially available ones to be $TiO_2$-free. It would also have a downstream impact on the ability to identify medicines and prevent counterfeiting. Based on the results, only $TiO_2$-free red/orange capsule containing $Fe_2O_3$ could be suitable replacements for $TiO_2$ containing capsules. If $TiO_2$ was banned in medicines, this would severely restrict the color palette available for new medicines or reformulating commercially available ones to be $TiO_2$-free, with a down-stream impact on the ability to identify medicines and prevent counterfeiting. In addition to a reduced color palette caused by the darker colors imparted by iron oxides to the capsule shell, finding an imprinting ink with sufficient contrast to the capsule shell color will be difficult because the lighter ink colors, e.g. white ink, contains $TiO_2$. The daily intake of iron oxide (E172) is also restricted by authorities such as the World Health Organization, the FDA and the Japanese authorities for safety reasons. These limits translate approximately to the equivalent of 3 x Size 0 capsules per day. Based on these limitations, $Fe_2O_3$ would not be a suitable replacement for $TiO_2$ as it would not have global regulatory acceptability and could not be used in medicines developed for global markets especially those involving multiple dosing or chronic use.

## Comments on the Methodologies Used

Visual Appearance and Colorimetry

The perception of differences in appearance and color by the human eye is subjective and depends on the lighting conditions. For this reason, lighting, background and camera settings were standardized for comparative visual appearance studies and also colorimetry was used. Color differences were calculated using the $\Delta E^*_{00}$ equation which is the most accurate color difference equation currently in use. In some studies, there were differences between the visual appearance and colorimetry results e.g. in the accelerated stability studies involving red/orange capsule shells. This was attributed to the intensity of the capsule shells'color which made the color changes difficult to detect by the human eye.

Capsule Brittleness

Capsule brittleness was tested by two different methods, one was used for empty capsules and was based on methodology used by hard capsule manufacturers. It involved dropping a weight onto a capsule shell under controlled conditions and repeating this 50 times for each capsule type. The other was used for filled capsules. It involved an analyst rolling and pinching individually 20 capsules between the fingers to check for cracking or splitting. This latter method was introduced to minimize potential analyst exposure to the API blends on capsule fracture. However, it is more operator-dependent than the former method and involves testing fewer capsules. These factors may account for some of the variation in brittleness seen with this test. Despite this, certain $TiO_2$ free capsule shells displayed unacceptable levels of brittleness using both methods.

Photostability and Accelerated Stability Studies

Extreme light conditions (2 x ICH Q1B) and accelerated stability conditions were used to assess rapidly changes in the stability of filled $TiO_2$-free capsule shells and their contents compared with the same blends encapsulated in $TiO_2$ containing capsule shells. Overall, the assay and impurity results depended on the API blend used and whether it was sensitive to light exposure, moisture, heat etc or not. No major differences were observed between the $TiO_2$ and $TiO_2$-free capsule shells and their ability to protect against degradation for any of the blends, showing that a combination of encapsulation and protective packaging would be important to protect these blends regardless of whether the capsule shell contained $TiO_2$ or not. None of the alternative opacifiers used appeared to promote API degradation.

In order to keep the number of encapsulation runs and photostability and accelerated stability studies to a manageable size, each API blend was not encapsulated into every capsule shell under evaluation. This meant it was not possible, for example, to compare fully the ability of each capsule shell to protect a moisture-sensitive compound like benserazide. The photostability and accelerated stability conditions used were typical for development studies. However, the results may not be representative of long-term stability studies.

Manufacturability

The manufacturability trials were conducted on empty capsule shells in the interests of time and efficiency. However, these studies present less of a challenge to the $TiO_2$-free capsule shells trialled than a powder-filling operation due to the interaction of the powder and the capsule shell wall which may lead to more capsule brittleness.

## Studies Outside the Scope of the Consortium's Work Plan

The following factors/aspects were outside the scope of the consortium's worlk plan and have not been evaluated.

Capsule sizes other than Size 0

Studies were conducted on Size 0 capsules based on their availability, the fact that they are commonly used especially for clinical trials and their larger size makes them more sensitive to stress than smaller capsule sizes. Therefore, they represent a worst-case scenario for smaller capsules. However, Size 00

and Size 000 capsule shells have not been assessed and, although less commonly used, they will be more sensitive to stress than the Size 0 capsule shells evaluated.

Long-term stability of the filled capsules

Although typical development studies, the photostability and accelerated stability results may not be representative of those from long-term stability studies. Long-term stability was not generated on the $TiO_2$-free capsules due to the relatively short time-frame of the project.

Impact on gelatin crosslinking

No work was carried out to evaluate whether gelatin-based capsules with the $TiO_2$ alternatives had a greater propensity to cross-link and, thus, result in a prolongation of capsule disintegration and API dissolution. Literature suggests there is an increased risk to cross linking when using calcium carbonate [16] [17]. However, in the photostability and accelerated stability tests, no prolongation of disintegration was observed.

Extensive color matching studies involving commercial products

The Consortium's work program included a limited amount of color comparison between the $TiO_2$-free capsule shells and the $TiO_2$ reference shells with disappointing results. If $TiO_2$ is banned in medicines, it would require color matching studies to determine whether it was possible to color match reformulated $TiO_2$-free products with existing commercial products.

Soft-gel capsules and hard gelatin capsules containing semi-solids

The Consortium used three API blends, two were used in immediate release products and one used in a sustained release product. The impact of $TiO_2$ alternatives on the performance of soft-gel capsules or hard gelatin capsules containing semi-solid mixtures was not assessed.

Impact on inhalation capsule puncturing performance

The Consortium's work focused on hard gelatin capsules for oral use. Capsules are not only used for oral dosage forms but also inhalation products. Any change in the properties of the capsule shell may have an impact on performance.

# Literature References

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# Appendix A

## $TiO_2$ Alternatives Consortium Members

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# Annex 4

## Safety assessment of alternatives and comparison with titanium dioxide as an opacifier and colorant for oral administration

| | |
|---|---|
| Author: | Andreas Czich, David Cragin, Julia Kenny on behalf of the Consortium Safety Team |
| Document type: | Safety Summaries |
| Document status: | Final |
| Release Date: | February 13, 2024 |

## Table of contents

# 1. Executive Summary

The safety team of the consortium evaluated the potential colourants/opacifiers included in the $TiO_2$ alternative film coating and capsule systems assessed. All selected alternative colourants, which also serve as opacifiers, are already in use in medicinal product formulations and food supplements. The safety team considered all alternatives as safe, with comprehensive safety data sets in some cases and health authority assessments available. The individual assessments are attached to this report (Appendix 1).

As with $TiO_2$, these opacifiers and colourants have been safely used in products for decades. However, some of the colourants/opacifiers have data gaps with regard to toxicity data (including genotoxicity, chronic toxicity, carcinogenicity, reproductive and developmental toxicity) compared to $TiO_2$ (Table 1) but given their history of safe human use, these non-clinical data gaps are not considered relevant. Therefore, as available safety data for those colourants/opacifiers are considered sufficient, no further toxicology studies are needed.

In addition, for a few colourants/opacifiers the presence of nanoparticles is unclear. Guidance from EMA/EFSA is needed to understand how to account for the unintended nanoparticle portions of the colourants/opacifiers and if further safety testing is required to characterise those fractions (as was the case for $TiO_2$). Applying the criteria of the EFSA nanoguidance (3), materials are considered as nanoparticles and have to be tested if their size is below 100 nm and they are not soluble in gastric fluid. Preliminary investigations demonstrated that the alternatives Zinc Oxide (ZnO), Calcium sulphate ($CaSO_4$), Calcium carbonate ($CaCO_3$), Magnesium carbonate ($MgCO_3$) and Magnesium oxide (MgO) may contain nanoparticles, but all are soluble at pH 1.2. In addition, Isomalt and Maltodextrin are freely soluble in water and do not pose a nanoparticle concern as well as Microcrystalline Cellulose and Rice Starch. For Trisodium phosphate and Tetrasodium-pyrophosphate data to confirm the absence of nanoparticles and solubility in gastric fluid are not available.

Sanofi, on behalf of the $TiO_2$ Alternatives consortium approached EMA for Scientific Advice on $TiO_2$ E171. There is an extensive data set for $TiO_2$ available, assessed by different authorities and expert groups ensuring its safety. Most notably, the carcinogenicity study (1) on $TiO_2$ using comparable material to the material used in medicines provided a robust conservative No Observed Adverse Effect Level (NOAEL) of 2250 mg/kg/day. Additionally, the JECFA concluded that there is no identifiable hazard for INS171 (similar to E171) and consequently no requirement for an ADI. However, the $TiO_2$ Alternatives consortium have proposed establishing an oral permitted daily exposure (PDE) of 2250 mg/day which will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels.

Also, the oral PDE can be applied to compare the safety of $TiO_2$ with the safety of alternative colourants/opacifiers.

Furthermore, safety evaluations by Agencies are ongoing for some of the alternative colourants/opacifiers present in some formulations, and as such, were not assessed as part of this safety review e.g.,

- Talc (E553b): ECHA is evaluating talc as a potential Category 2 carcinogen. The safety experts of the $TiO_2$ Alternatives consortium concluded that talc (pharmacopoeia grade) can be considered as safe by the oral route. Furthermore, an EFSA opinion was published in June 2018 on talc as a food additive.
- $Fe_2O_3$ (E172): an EFSA re-evaluation is currently ongoing.

Overall, the consortium considers there is no relevant difference between the safety profile of $TiO_2$ E171 and the investigated alternatives based on available data.

# 2. General information

## *2.1. Procedure*

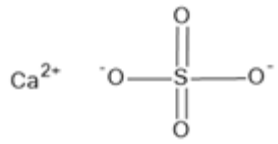
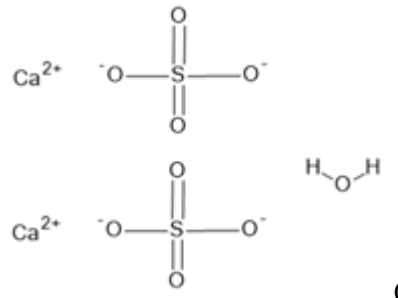
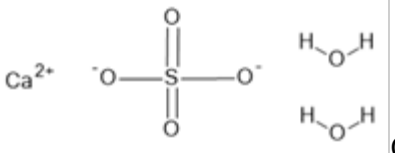
The non-clinical safety assessments considered data from peer-reviewed publications and included regulatory assessments and limits, e.g., available ADI/PDE/RDI/ULs, in cases when the compound is listed in U.S./European pharmacopoeia, is already an approved excipient in medicinal products, and/or if the compound is available in GRAS/IID/EFSA lists (references provided in the safety assessments of each material).

## *2.2. Alternative colourants/opacifiers*

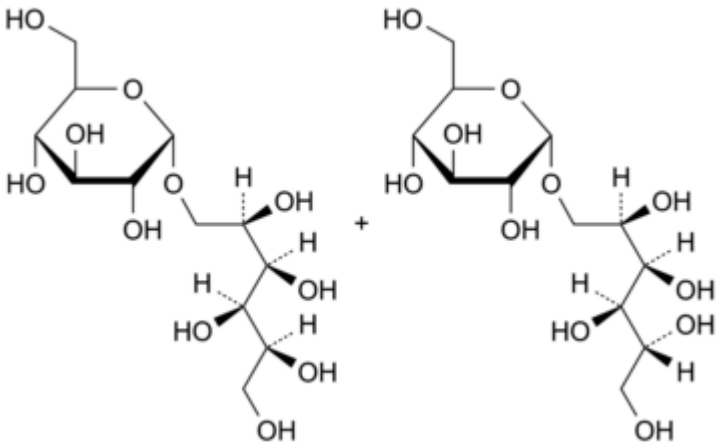
### 2.2.1. Calcium carbonate

| | |
|---|---|
| Name (IUPAC) | Calcium carbonate |
| Chemical name, synonyms | Aragonite, calcite, chalk, CI Pigment White 18, lime, limestone, marble, oyster, pearl |
| CAS No. | 471-34-1 |
| Molecular formula | $CaCO_3$ |
| Molecular weight | 100.0869 g/mol |
| Chemical structure | Amorphous or micro-crystalline |
| Physicochemical properties | Odourless and tasteless white powder, poorly soluble in pure water (47 mg/L) and alcohol, soluble in dilute acids (depending on acid strength and pH) (EFSA, 2023) |

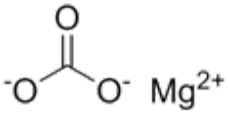
### 2.2.2. Calcium sulphate

| | |
|---|---|
| Name | Calcium sulphate |
| Chemical name | Calcium sulphate anhydrite<br>Calcium sulphate hemihydrate<br>Calcium sulphate dihydrate |
| Synonyms | Calcium sulphate, sulphuric acid calcium salt, gypsum, thiolate, drierite |
| CAS No. | 7778-18-9 (anhydrite)<br>10034-76-1 (hemihydrate)<br>10101-41-4 (dihydrate) |
| Molecular formula | $CaSO_4$ (anhydrite)<br>2 $CaSO_4$ x $H_2O$ (hemihydrate)<br>$CaSO_4$ x 2 $H_2O$ (dihydrate) |
| Molecular weight | 136.1 g/mol (anhydrite)<br>145.1 g/mol (hemihydrate)<br>172.1 g/mol (dihydrate) |
| Chemical structure |  <br>Calcium sulphate |
| |  <br>Calcium sulphate hemihydrate |
| |  <br>Calcium sulphate dihydrate |
| Physicochemical properties | Appearance: yellow-white, crystalline, odourless<br>Physical state (20 °C, 1013 hPa): solid, fine powder<br>Soluble in water up to approx. 2 g/L (at 20 °C) |

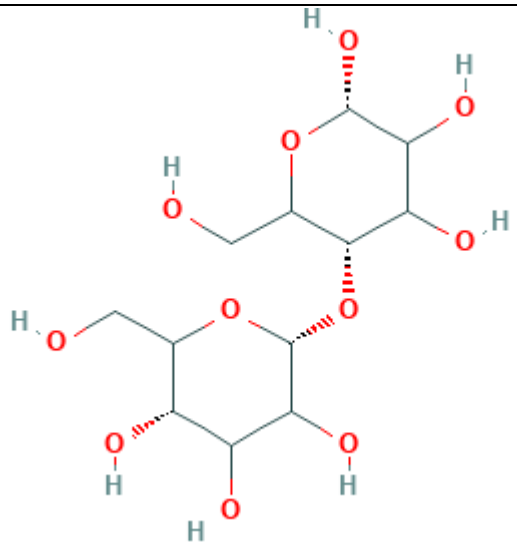
### 2.2.3. Isomalt

| | |
|---|---|
| Name | Isomalt |
| Chemical name | 1-O-α-D-glucopyranosido-D-mannitol (1,1-GPM) and 6-O-α-D-glucopyranosido-D-sorbitol (1,6-GPS) |
| Synonyms | E 953, isomaltitol, palatinit, palatinitol, hydrogenated isomaltulose; 6-O-α-D-glucopyranosyl-D-glucitol (for 1,6-GPS) |
| CAS No. | 64519-82-0 |
| Molecular formula | $C_{12}H_{24}O_{11}$ (or $C_{12}H_{24}O_{11}\cdot 2H_2O$ as dihydrate) |
| Molecular weight | 344.3 g/mol (or 380.3 g/mol for the dihydrate) |
| Chemical structure |  |
| Physicochemical properties | White or almost white powder or granules; freely soluble in water, practically insoluble in anhydrous ethanol [Ph.Eur. 2023]. |

### 2.2.4. Magnesium carbonate

| | |
|---|---|
| Name | Magnesium carbonate |
| Chemical name, synonyms | magnesium (II) carbonate; carbonate magnesium; carbonic acid, magnesium salt (1:1); magnesite |
| CAS No. | 546-93-0 |
| Molecular formula | $MgCO_3$ |
| Molecular weight | 84.31 g/mol (anhydrous) |
| Chemical structure |  |
| Physicochemical properties | Boiling point: 900°C (liberating $CO_2$)<br>Melting point: 350°C (decomposes)<br>Solubility in water: insoluble (at 20°C: 0.01 g/100 mL)<br>Appearance: odourless, white hexagonal crystals |

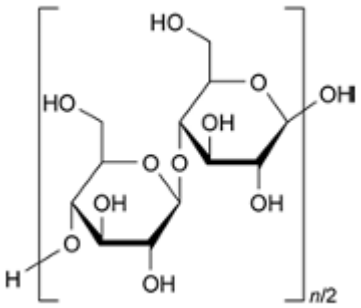
### 2.2.5. Maltodextrin

| | |
|---|---|
| Name | Maltodextrin |
| Chemical name, synonyms | Cargill Dry; C*Dry MD; C*PharmDry; Glucidex; Glucodry; Lycatab DSH; Maldex; Maldex G; Malta*Gran; maltodextrinum; Maltosweet; Maltrin; Maltrin QD; Paselli MD10 PH; Rice*Trin; Star-Dri; Tapi. |
| CAS No. | 9050-36-6 |
| Molecular formula | $C_{12}H_{22}O_{11}$ |
| Molecular weight | 342.30 g/mol |
| Chemical structure |  |
| Physicochemical properties | Freely soluble in water |

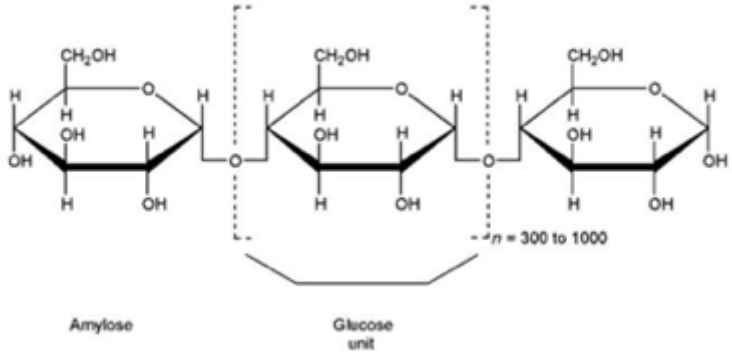
### 2.2.6. Magnesium oxide

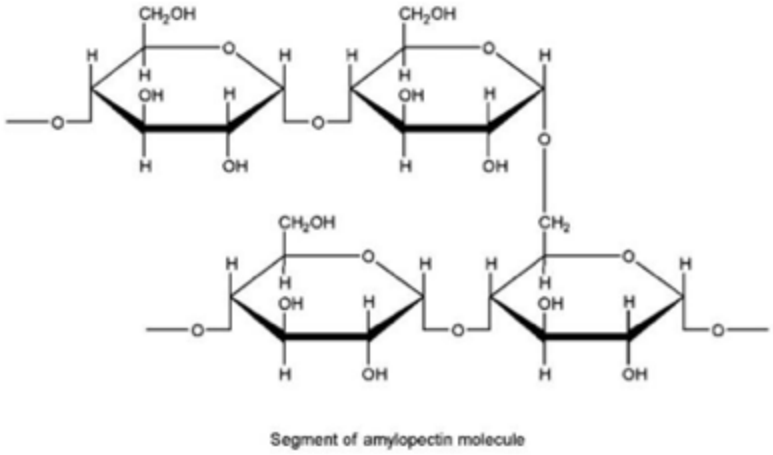
| | |
|---|---|
| Name | Magnesium oxide |
| Chemical name, synonyms | Magnesia<br>Periclase |
| CAS No. | 1309-48-4 |
| Molecular formula | MgO |
| Molecular weight | 40.3 g/mol |
| Chemical structure | Mg=O |
| Physicochemical properties | Practically insoluble in water. Insoluble in ethanol.<br>Soluble in acid, ammonia. |

### 2.2.7. Microcrystalline cellulose

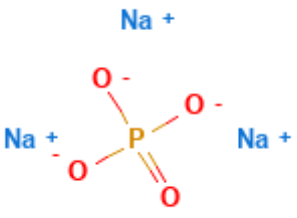
| | |
|---|---|
| Name | Microcrystalline cellulose (MCC) |
| Chemical name, synonyms | (6S)-2-(hydroxymethyl)-6-[(3S)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxane-3,4,5-triol, Diethylaminoethyl cellulose |
| CAS No. | 9004-34-6 |
| Molecular formula | $(C_6H_{10}O_5)_n$ (or $C_{6n}H_{10n+2}O_{5n+1}$) |
| Molecular weight | 342.30 g/mol (monomer)<br>As polymer about 36,000 g/mol |
| Chemical structure |  |
| Physicochemical properties | Insoluble in water, dissolves in strong acidic or alkaline conditions. Substance on the EEA market in nanomaterial form (ECHA) |

### 2.2.8. Starch

| | |
|---|---|
| Name | Potato Starch, Maize Starch, Rice Starch, Pregelatanized Starch |
| Chemical name | (5-[5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-3,4-dihydroxyoxan-2-yl]oxy-6-(hydroxymethyl)-2-methyloxane-3,4-diol |
| Synonyms | Solanum tuberosum starch, Fecule, Corn starch, Amylum pregelificatum, Compressible starch |
| CAS No. | CAS 9005-25-8 |
| Molecular formula | $(C_6H_{10}O_5)_n$ where n= 300-1000 |
| Molecular weight | 69.5x106g/mol (average) |
| Chemical structure |  |

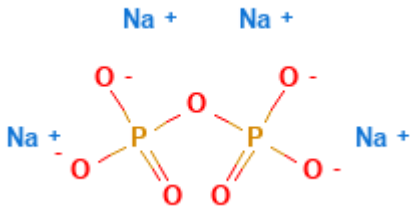
| | |
|---|---|
| Physicochemical properties | Insoluble in water at room temperature |
| Name | Waxy Maize Starch, Pegelatanized Starch |
| Chemical name | (5-[5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-3,4-dihydroxyoxan-2-yl]oxy-6-(hydroxymethyl)-2-methyloxane-3,4-diol |
| Synonyms | Amylopectin, Amylopectine, Amoica |
| CAS No. | CAS 9037-22-3 |
| Molecular formula | $C_{30}H_{52}O_{26}$ (as amylopectin) |
| Molecular weight | 828 g/mol (as amylopectin) |
| Chemical structure |  Segment of amylopectin molecule |
| Physicochemical properties | Insoluble in water at room temperature |

### 2.2.9. Trisodium phosphate (sodium phosphate, tribasic, anhydrous)

| | |
|---|---|
| Name | Trisodium phosphate |
| Chemical name, synonyms | Sodium phosphate, tribasic, anhydrous, TSP, E339 |
| CAS No. | 7601-54-9 |
| Molecular formula | $Na_3PO_4$ |
| Molecular weight | 163.941 g/mol |
| Chemical structure |  |

| | |
|---|---|
| Physicochemical properties | Freely soluble in water (120 g/L, 20°C). Insoluble in ethanol. (NIH, 2023) |

### 2.2.10. Tetrasodium pyrophosphate

| | |
|---|---|
| Name | Tetrasodium pyrophosphate (E450) |
| Chemical name, synonyms | Tetrasodium diphosphate, TSPP, E450 |
| CAS No. | 7722-88-5 |
| Molecular formula | $Na_4P_2O_7$ |
| Molecular weight | 265.90 g/mol |
| Chemical structure |  |
| Physicochemical properties | Solubility in water: 3.16 g/100 mL (cold water); 40.26 g/100 mL boiling water. (NIH, 2023) |

### 2.2.11. Zinc oxide

| | |
|---|---|
| Name (IUPAC) | Zinc oxide |
| Chemical name, synonyms | Pigment white 4, zincite, zinc white, calamine, philosopher's wool, Chinese white, flowers of zinc |
| CAS No. | 1314-13-2 |
| Molecular formula | $Zn^{2+}$ $O^{2-}$ |
| Molecular weight | 81.38 g/mol |
| Chemical structure | Zn=O |
| Physicochemical properties | White solid (2 crystalline forms: hexagonal wurtzite and cubic zincblende), odourless, insoluble in water (0.42 mg/100 g water at 18°C), rapidly soluble in dilute acids such as 3% acetic acid, 0.07M HCl, and ammonia and alkali hydroxide solutions (EFSA, 2016; Hapgood and Antic, 2023) |

# 3. Characterisation of selected materials with respect to nanoparticle solubility

## 3.1. Method

The method applied was Dynamic Light Scattering (DLS) using a Zetasizer instrument. It provides calculated results in number of particles.

The reference particle size distribution (PSD) method of analysis was applied to monitor the dynamic particle sizes across various pH ranges: pH 1.2 (0.1N hydrochloric acid solution), pH 4.5 (acetate buffer), neutral pH 7 (water) and pH 6.8 (phosphate buffer) media. When relevant, the PSD evolution across time for a given pH media was evaluated as well.

## 3.2. Summary of Results

Physicochemical stability was initially assessed by suspending a defined quantity (g) of powder materials in a specific volume (mL) of different pH media. The impact of each media's (pH) on the materials was noted and compiled in the table given below:

| Material Name | Water | pH 1.2 | pH 4.5 | pH 6.8 |
|---|---|---|---|---|
| Titanium dioxide ($TiO_2$) | **Insoluble** | **Insoluble** | **Insoluble** | **Insoluble** |
| Zinc Oxide(ZnO) | **Insoluble** | **Soluble** | **Soluble** | **Precipitation** |
| Calcium sulfate($CaSO_4$) | **Slightly soluble** | **Soluble** | **Slightly soluble** | **Slightly soluble** |
| Calcium carbonate($CaCO_3$) | **Slightly soluble** | **Soluble** | **Soluble** | **Slightly soluble** |
| Magnesium carbonate($MgCO_3$) | **Soluble** | **Soluble** | **Soluble** | **Soluble** |
| Magnesium oxide (MgO) | **Precipitation** | **Soluble** | **Soluble** | **Precipitation** |

Based on the above compatibility data, it was evident that:

- Alternative materials namely $CaSO_4$, $CaCO_3$, $MgCO_3$ and MgO, were either completely or partially soluble in acidic and neutral pH and precipitated in pH 6.8 media. Being soluble across the pH range, it was not practically feasible to measure particle size.
- ZnO was soluble at a lower acidic pH range (1.2 to 4.5) and precipitated at pH 6.8 media. Notably, ZnO showed insolubility only at neutral pH i.e., in water and particle size was stable across time ($PS_0$= 278.9 & $PS_{96h}$= 276.7 nm), with the established PSD up to 96 hours showing a highly limited amount of particles below 100nm.

The pH-dependent particle size measurement in the nano-range could only be successfully performed for the $TiO_2$ material because the alternative materials which potentially contained a nanoparticulate fraction, such as ZnO, $CaSO_4$, $CaCO_3$, $MgCO_3$ and MgO, were all soluble in the acidic pH range. Since this pH range is the most physiologically relevant for oral ingestion of the excipients, these materials would not fall under the scope of the EFSA's guidance for nanomaterial testing (3).

# 4. Summary and Discussion on the Safety of Alternative Colourants and Opacifiers

The $TiO_2$ Alternatives consortium selected 11 potential alternatives based on the information provided by the Consortium Excipients Material Management Team. For each of the alternatives a full assessment was prepared (Appendix 1) and a summary of the data are described in section 0. Most of the colourants/opacifiers have already been assessed by different agencies and all are considered safe

for use in food, and by extension, in medicines. However, safety evaluations by agencies are ongoing for some of the alternative excipients present in some formulations investigated by the Excipients Material Management Team, and as such, were not assessed as part of this safety review e.g.,

- Talc (E553b): ECHA is evaluating talc as a potential Category 2 carcinogen. The safety experts of the consortium concluded that talc (pharmacopoeia grade) can be considered as safe by the oral route. Furthermore, an EFSA opinion was published in June 2018 on talc as a food additive.
- $Fe_2O_3$ (E172): an EFSA re-evaluation is currently ongoing.

## *4.1. Summary of Data*

The expert review of the alternative excipients by members of the $TiO_2$ Alternatives Consortium Safety Team are summarised below:

**Table 1: Summary of Safety assessments of $TiO_2$ alternatives**

| **Chem Name CAS** | **Used in Food** | **Used in Drug Formulations** | **Other Assessments** | **Unintended Nanoparticles Present** | **Summary and potential safety Data gaps** |
|---|---|---|---|---|---|
| Calcium Carbonate $CaCO_3$ 471-34-1 | E170 | FDA IID | JECFA 1965), EU SCF (1990), EFSA (2011, 2023) | Yes, but fast dissolution in the acidic environment of the stomach demonstrated (EFSA, 2011, 2023). Considered as no concern. | Comprehensive toxicology data package available, except chronic toxicity and carcinogenicity. However, for use in food, the EFSA Panel concluded that there is no need for a numerical acceptable daily intake (ADI) for calcium carbonate and that, in principle, there are no safety concerns with respect to the exposure to calcium carbonate per se at the currently reported uses and use levels in all age groups of the population, including infants below 16 weeks of age. No ADI specified |
| Calcium Sulphate $CaSO_4$ anhydrous: 7778-18-9 hemihydrate: 10034-76-1 dihydrate: 10101-41-4 | E516 | FDA IID, US and EU Pharmacopoeia | GRAS, SIDS (2003), JECFA, (1973), EFSA (2003, 2012) | Yes, but soluble at pH1.2 | Basic toxicological data are available for calcium sulphate but long-term and carcinogenicity data in animals are lacking. In the available studies, the test item has often not been well characterised and i.e., information on particle size (i.e., nanoforms) is missing. Calcium sulphate has a long history of safe use, an ADI was not specified, the tolerable upper intake limit is 2500 mg/d based on calcium intake. High doses of sulphate result in transient gastrointestinal effects. |

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| Isomalt<br>64519-82-0 | E953 | FDA IID, US and EU Pharmacopoeia | GRAS, BfR (2014), EU SCF (1984, 1989), JECFA (1985) | No (freely soluble in water) | Extensive toxicological data, including repeat-dose (up to chronic) toxicity studies, multigeneration and teratogenicity studies, genotoxicity and carcinogenicity studies are available for isomalt. Even though many of the published studies are from 1970's to 1980's and may not fully comply to current standards, and no formal fertility and peri- and postnatal development studies are available (the multigeneration study covered many of the relevant endpoints). Overall, no relevant data gaps regarding toxicity data are seen. In humans, isomalt is well tolerated at doses <20 g/day. Gastrointestinal effects, in particular flatulence and diarrhoea, were observed at ≥20 g/day. |
| Magnesium Carbonate<br>$MgCO_3$<br>546-*93*-0 | E504 | FDA IID | Magnesium: JECFA (1986), EFSA (2015)<br>EU SCF (2006),<br>BfR (2017) | Yes, but soluble at at pH 1.2 | Taking into account all available data, both the existing toxicological studies with magnesium carbonate and other Mg salts and that Mg is an essential trace element, it can be concluded that the use of magnesium carbonate as an excipient in pharmaceutical products is safe. The in vitro genotoxicity battery is missing, although there is no indication of a genotoxic potential for $MgCO_3$. |
| Magnesium Oxide<br>MgO<br>1309-48-4 | E530 | FDA IID, EU Pharmacopoeia | Magnesium: JECFA (1986), EFSA (2015),<br>EU SCF (2006),<br>BfR (2017)<br>MgO (GRAS) | MgO readily dissociates after a reaction with gastric HCl under formation of magnesium chloride ($MgCl_2$). | Considering the high NOAEL and relatively mild toxic effects associated with Mg intake, the available upper limit (UL) of 250 mg/day derived by regulatory authorities seems sufficient and it can be concluded that MgO is of low toxicity and concern. Whilst several routes of synthesis for MgO NP have been described, data on the particle size distribution of MgO for the use as a pharmaceutical excipient is lacking. Safety data of those MgO NP is rare and current studies do not fulfil the requirements by EFSA Guidance on risk assessment of |

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| | | | | | nanomaterials to be applied in the food and feed chain [EFSA, 2021]. However, based on the dissociation of MgO in gastric fluid MgO is not considered a NP |
| Maltodextrin 9050-36-6 | E1400 | FDA IID | GRAS FDA (2023) EFSA (2013) | No (freely soluble in water) | Maltodextrin is widely used across the food, cosmetic and pharmaceutical industry. Based on its metabolic profile, it has been considered non-hazardous by health authorities and is either an approved food additive or is considered safe but not classified as a food additive. No carcinogenicity studies or reproductive and developmental toxicity studies could be found for maltodextrin. |
| Microcrystalline Cellulose 9004-34-6 | E460-E469 indirect food additive (US FDA 2018) | FDA IID | JECFA (1998, 2000), EFSA (2018) | No | The available data set and toxicity information with cellulose and derivative forms is extensive. Physical properties or particle size (including the nanoparticulate fraction) and distribution are not always available and represent a data gap. In alignment with US authorities, EFSA determined no numerical ADI for microcrystalline cellulose and based on the available toxicological dataset, considered no safety concern at the reported use levels (estimated exposure 660-900 mg/kg bw day) with unmodified and modified celluloses (EFSA, 2018). |
| Rice Starch 9005-25-8 | Nutrient | FDA IID | GRAS (1979) | No | Starch is GRAS listed and considered to be safe. It is already in use as an excipient for pharmaceuticals in different regions and REACH and EFSA reports are coming to the same conclusion. No genotoxicity and chronic toxicity data are available. |
| Tetrasodium pyrophosphate 7722-88-5 | E450 | FDA IID | GRAS (2023), EFSA (2019), EU SCF (1997), JEFCA (2006) | TBD, No data on solubility in gastric fluid | The available toxicological information for each phosphate salt is limited and the overall phosphate assessment as a pharmaceutical excipient is based on read-across approaches and a group-specific toxicity assessment for several phosphate salts. While not |
| Trisodium phosphate 7601-54-9 | E339 | FDA IID | GRAS (2023), EFSA (2019), EU SCF (1997), JEFCA (2006) | TBD, No data on solubility in gastric fluid | |

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| | | | | | assuming that there would be significant differences in toxicity, different salts could express different oral bioavailability or solubility in water.<br>The EFSA derived a group ADI for phosphates and its salt of 40 mg/kg bw per day (expressed as P). Both phosphates, E339 and E450, are considered to be of low toxicity concern for human exposure as pharmaceutical excipient. |
| Zinc Oxide<br>ZnO | FDA Substances added to food list | FDA IID<br>UK, EU and US Pharmacopoeia | GRAS (2023), EU SCF 2003 , EFSA (2016) | Yes, but fast dissolution expected in the acidic environment of the stomach (EFSA, 2016), and soluble at pH 1.2 | For zinc oxide, no specific safety information was found in the open domain. However, as a food additive, zinc oxide is generally recognized as a safe substance. For zinc, detailed toxicological information can be found in the public space. In general, no adequate experimental studies are available to evaluate the carcinogenic potential of zinc or zinc compounds. In addition, the safety of zinc (oxide) nanoparticles is less well understood. |

List of references can be found in the respective reports attached to this document (Appendix 1).

# 5. Overall conclusions

Overall, the alternative excipients investigated are considered safe for use. As available safety data for those colourants/opacifiers are considered sufficient, no further toxicology studies are needed.

While no single alternative was found by the consortiums Materials Team (see reports on Alternatives for Coatings and Capsules) that can provide the functional attributes to replace TiO2 equally based on their material characteristics in the different formulations, the safety assessments of this report provide a basis for the safety evaluation of the different complex formulations containing one or more of the colourants/opacifiers assessed.

Some uncertainties around nanoparticle content need further investigation on a case-by-case basis in the context of the formulation used. For some of the colourants/opacifiers the presence of nanoparticles is not fully understood, in particular, there is no clear definition on the acceptable content of unintended nanoparticles in excipients. Guidance from EMA/EFSA is needed to understand how to account for the nanoparticle portions of the colourants/opacifiers containing unintended portions of nanoparticles and if further safety testing is required to characterise those fractions.

Of note, the risk assessments performed to date by the Safety Team of the $TiO_2$ Alternatives consortium have not taken into account that in most cases the daily exposure of the selected colourants/opacifiers in the formulations will be higher than the $TiO_2$ levels used to provide the equivalent functional attributes (e.g., iron oxide ($Fe_2O_3$) would generally be 2-3 times higher than $TiO_2$), as discussed in the report of the materials Team. It has to be highlighted that e.g., $Fe_2O_3$ exposure is limited: WHO-ADI E172 0.5 mg/kg bw, JPN Fe(OH)3 5.67 mg/day, FDA 5 mg Fe/day. This typically limits the daily dose to 3 standard size #0 capsules per day from a safety perspective.

This is in contrast to the proposed oral PDE for $TiO_2$ E171 of 2250 mg/day derived by the Safety Team of the $TiO_2$ Alternatives consortium, which is based on the NCI oral carcinogenicity study in rats and mice (1979) (1) that was identified as the most relevant study to define a point of departure that covers both systemic toxicity after lifetime exposure as well as the most severe endpoint (carcinogenicity) as a possible consequence of any genotoxic potential. The safety assessment on TiO2 which was used as basis for the scientific advice is also attached to this report (**Appendix 2**).

To clarify the discussion on nanomaterials, the Safety Team of the consortium would like to note that according to the new Guidance on the implementation of the Commission Recommendation 2022/C 229/01 (4) on the definition of nanomaterial, E171 would not fall under the definition of a nanomaterial, as the content of nanoparticles (<100 nm) is specified to be below 50 %.

# 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AI | Adequate Intake |
| BfR | German Federal Institute for Risk Assessment |
| EFSA | European Food Safety Authority |
| EU SCF | European Scientific Committee for Food |
| FDA | U.S. Food and Drug Administration |
| FDA IID | FDA Inactive Ingredients Database |
| GRAS | Generally Recognized As Safe substance database, FDA |
| IUPAC | International Union of Pure and Applied Chemistry |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| NOAEL | No Observed Adverse Effect Level |
| NP | Nanoparticle |
| PSD | Particle Size Distribution |
| PDE | Permitted Daily Exposure |
| RDI | Reference Daily Intake |
| UL | Upper Limit |

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# 8. Appendix 1: Safety Assessments of the Alternative Excipients

ithin the $TiO_2$ Alternatives consortium, safety assessments were conducted on 11 compounds that were being investigated by the Excipients Material Management Team as alternatives for $TiO_2$ used as an opacifier/colourant. The goal was to ensure the safety of the selected potential alternatives, to identify data gaps and to compare the safety data with the data available for $TiO_2$ E171.

## *8.1 Summary calcium carbonate*

# Calcium carbonate ($CaCO_3$)

(CAS No. 471-34-1; E170)

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | C Trendelenburg (Novartis) |
| Peer review: | L Wiesner, T. Broschard |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 25-Sep-2023 |

# Table of contents



# Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as coloring agent in orally administered medicinal products, the present safety assessment for calcium carbonate (CaCO3) was performed to support the justification for its potential usage as alternative coloring agent.

Few effects seen in animals and humans are associated with high calcium carbonate intakes and the (maximum) Upper Limit (UL) of 2500 mg/day for calcium established by the SCF (15), and confirmed by the NIH (12), is taking these effects into account.

In humans, trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium in observational studies, that did not show increased cardiovascular risks with higher dietary calcium intake.

Overall, the EFSA (8) concluded that the toxicological database on calcium carbonate is limited, but it does not give rise to concern, and that further toxicological studies on calcium carbonate are not necessary. For use in food, the EFSA (2023) (5) recently concluded, that there is no need for a numerical acceptable daily intake (ADI) for calcium carbonate and that, in principle, there is no safety concern with respect to the exposure to calcium carbonate per se at the currently reported uses and use levels in all age groups of the population, including infants below 16 weeks of age ('No safety concern at the reported uses and uses levels', EFSA, 2023 [5]).

# General information

| | |
|---|---|
| Name (IUPAC) | Calcium carbonate |
| Chemical name, synonyms | Aragonite, calcite, chalk, CI Pigment White 18, lime, limestone, marble, oyster, pearl |
| CAS No. | 471-34-1 |
| Molecular formula | $CaCO_3$ |
| Molecular weight | 100.0869 g/mol |
| Chemical structure | Amorphous or micro-crystalline |
| Physico-chemical properties | Odorless and tasteless white powder, poorly soluble in pure water (47 mg/L) and alcohol, soluble in dilute acids (depending on acid strength and pH) (5) |

Calcium carbonate ($CaCO_3$) occurs naturally as an odorless and tasteless white powder or as crystals. It occurs as amorphous or micro-crystalline structures with particle size varying between 40 – 120 nm diameter spherules (amorphous) and 1 - 10 µm diameter crystals (crystalline forms) (8). Typical average particle size (d50) of food grade calcium carbonate is about 5 µm, with an upper range (d98%) of 65 µm and less than 1% of particles having a diameter below 100 nm (8).

Nano form calcium carbonate (as used in some of the below summarized toxicological studies) has a particle size of 60 - 100 nm (examined by Scanning Electron Microscopy, SEM) (8). Regarding nanoparticles, the EFSA Panel (5) considered that, based on the data provided, the presence of small particles including nanoparticles in the pristine ground calcium carbonate (GCC) and precipitated calcium carbonate (PCC) used as a food additive (E 170) cannot be excluded, however they are expected to dissolve in the acidic environment of the stomach.

In pharmaceuticals calcium carbonate is used as an excipient and as an active ingredient of antacids. As a pharmaceutical excipient, it is mainly used in solid-dosage forms as a diluent as well as a bulking agent in tablet sugar-coating processes and as an opacifier in tablet film-coating (4).

# Regulatory information and published limits

Calcium carbonate is authorized in food (E170) as food additive and as food coloring substance (CI Pigment White 18). Previously an Acceptable Daily Intake (ADI) of “not limited” was established by the JECFA (10) and a group ADI of “not specified” was assigned by the EU SCF (14).

In a recent reevaluation, the EFSA (2023) (5) concluded, that there is no need for a numerical acceptable daily intake (ADI) for calcium carbonate and that, in principle, there are no safety concern with respect to the exposure to calcium carbonate per se at the currently reported uses and use levels in all age groups of the population, including infants below 16 weeks of age (‘No safety concern at the reported uses and uses levels’, [5]).

The SCF previously allocated a (maximum) Tolerable Upper Intake Level (UL) for calcium of 2500 mg/person/day (confirmed by the NIH, 2022 [12]) as a nutrient and established a Population Reference Intake (PRI) of 700 mg calcium/day (range 400-1200 mg/day depending on age and physiological status) (7, 15).

# Safety assessment

Toxicological information for calcium carbonate used in food was previously summarized and assessed by the EFSA (8). In a recent EFSA evaluation, calcium carbonate (E170) was assessed as a food

additive in foods for infants below 16 weeks of age and re-evaluated as food additive for uses in foods for all population groups (5).

Key safety information for use of calcium carbonate as excipient in pharmaceuticals for oral administration is summarized in the below sections.

## Absorption, Distribution, Metabolism and Excretion (ADME)

In the acidic milieu of the stomach, i.e., at pH 3-6, calcium carbonate dissociates into its constituent ions (8, 5). Some of the calcium is absorbed, via active transport or passive diffusion, but a large proportion (about 89-90%) is complexed to bile acids, free fatty acids and excreted with the feces (8, 5).

Regarding absorption of nano-form calcium carbonate, dissolution rate test results for GCC and PCC E170, performed at pH 3 and pH 6 mimicking the stomach conditions of adults and infants, respectively, were recently evaluated by the EFSA (5). Based on that data the Panel considered that there is no concern regarding the exposure to small particles, including nanoparticles, present in E170 when used as a food additive up to the concentrations tested and that the previous risk assessment completed by the ANS Panel in 2011 (8) does not need to be complemented with nano-specific considerations.

The average absorption of calcium from calcium carbonate has been shown to be in the range of 20-40%, however there is evidence that calcium from nano particulate calcium carbonate (NPP, pearl powder nanosized; particle diameter 0.04 to 0.4 µM) is more readily absorbed than the micro particulate form (MPP, pearl powder micronized; particle diameter 4 to 300 µM). In human subjects, a slight increase in calcium bioavailability (by +38%) was observed from NPP calcium carbonate compared to MPP calcium carbonate. In general, the absorption of calcium from calcium carbonate in nanoparticles is considered not to be markedly different than that from calcium carbonate micro particles (8).

Most of the absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and via feces and sweat) and excess carbonate/bicarbonate is excreted as carbon dioxide via respiration (15).

## Repeat-dose toxicity

From short term toxicity studies (generally less than 90 days) in mice, rats and cats, there was no evidence of relevant toxicity attributable to calcium carbonate. In general, no details on the form of calcium carbonate used in these studies were indicated by the authors (8). In a 28-day oral toxicity study with nanoparticulate (151 ±41 nm as described by the authors) at dose levels of 0, 13, 130 or 1300 mg/kg bw/day did not result in any treatment-related changes (9).

In a 91-day feeding toxicity study in rat, nephrocalcinosis was observed at 250 and 500 mg/kg/day (no details provided on the form of calcium carbonate used); but was considered not relevant for humans because the rat is a species known to be particularly sensitive to mineralization of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis (8).

In a 91-day feeding toxicity study in dog, administered dose levels were similar to rats (~ 250 and 500 mg/kg/day; no details provided on the form of calcium carbonate used); but no signs of nephrocalcinosis were observed (8).

In a more recent 90-day oral toxicity study with nano calcium carbonate in rats (16), a no-observed-adverse-effect level (NOAEL) of 1000 mg/kg/day (highest dose tested) was established based on the absence of toxicologically relevant effects.

In a combined repeat-dose oral toxicity/reproduction/developmental toxicity screening study in rat, calcium carbonate (particle size 60 - 100 nm by SEM and 0.58±0.6 µM by Sedigraph due to aggregation) was administered at dose levels up to 1000 mg/kg/day for 48 days. Non-adverse hematological and biochemical effects were noted in males at 1000 mg/kg/day and reduced plasma phosphate levels in all male treated groups. The NOAEL was established at 1000 mg/kg/day (highest dose tested) (8).

## Genetic toxicity

Nanoform calcium carbonate (particle size 60 - 100 nm by SEM and 0.58±0.6 µM by Sedigraph due to aggregation) revealed negative results in 3 in vitro genotoxicity assays (incl. Ames test, mouse lymphoma, and HuLy chromosome aberration) (8).

In a Comet assay in NIH 3T3 and MCF7 cells, calcium carbonate nanoparticles (calcite crystalline phase; size approx. 400 nm by X-ray; no agglomeration) at concentrations of 1, 5, 10, 25 and 50 µg/mL for 6 and 24 hours did not induce DNA damage at any concentration and time point in both cell lines (1).

## Carcinogenicity potential

No data are available for calcium carbonate on carcinogenicity (or chronic toxicity); however, the carcinogenicity potential was considered low/unlikely since both calcium and carbonate are natural constituents of the body (8).

## Reproductive and developmental toxicity

In pregnant rats, no developmental toxicity was observed at dietary doses up to 1500 mg/kg/day.

In a recent combined repeat dose oral toxicity/reproduction study, no effects on reproduction, including developmental toxicity, were observed with nano particulate calcium carbonate at doses up to 1000 mg/kg/day (see also repeat-dose toxicity) (8).

In other studies, in rats and mice, there was some evidence of fetotoxicity, including e.g., smaller litter size, lower total litter weights, hypertrophy of the heart, atrophy of the thymus, missing calcification centers in the developing skeletons and dentitions, of calcium when administered during pregnancy at levels in the diet ≥ 1500 mg/kg bw/day calcium carbonate. In general, no details on the form of calcium carbonate used in these studies were indicated by the authors (8).

Overall, it was concluded that calcium carbonate (≥ 1500 mg/kg/day) may cause hypercalcemia during gestation and can result in adverse effects on reproduction, fetotoxicity and elemental imbalances in the offspring. However, there is no concern for reproductive effects of calcium carbonate at intake levels below 1500 mg/kg/day (8).

## Additional safety data

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals (8).

## Human data

In humans, hypercalcemia and alkalosis often associated with renal dysfunction, metastatic calcification, and other symptoms, was observed following intake of large amounts of calcium carbonate (between 2.0 and 16.5 g/day), e.g., with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome) or large amounts of calcium-containing antacids or food supplements (8).

Meta-analysis (2, 3) indicated an increased risk of myocardial infarction and cardiovascular events in individuals given regular calcium supplementation in the management of osteoporosis, however trends reported were very modest, and are not supported by the findings in a similar 5-year study with calcium supplementation of 1200 mg/day (11).

## Data gap assessment

Based on the available data as summarized above, the following potential safety data gaps are considered relevant for further evaluation and discussion for oral administration of calcium carbonate as excipient in pharmaceutical formulations:

- Only some of the above summarized studies has been performed with calcium carbonate described as “nano form” (having a particle size of 60 - 100 nm) provided comprehensive

data on characterization of the nano material fraction fully in line with the guidance provided by EFSA (6).

## Overall conclusions

The EFSA Panel (8) concluded (for use in food) that the toxicological database on calcium carbonate is limited, but it does not give rise to concern and further toxicological studies on calcium carbonate were considered not necessary.

Few effects seen in animals and humans are associated with high calcium carbonate intakes and the (maximum) UL of 2500 mg/day for calcium established by the SCF (15), and confirmed by the NIH (12), is taking these effects into account.

In humans, trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium in observational studies, that did not show increased cardiovascular risks with higher dietary calcium intake (2). A plausible mode of action to explain these differences could be that calcium supplements acutely increase serum calcium levels which have been positively associated with an increased incidence of myocardial infarction. Ingestion of equivalent doses of calcium from dairy products is considered to have a much smaller effect on serum levels (13).

A group ADI of “not specified” established by the SCF in 1991 (14) for a group of carbonates including calcium carbonate, considers that the JECFA definition of “ADI not specified” is applicable to calcium carbonate when used as a food additive. In a recent reevaluation, EFSA (5) concluded, that there is no need for a numerical acceptable daily intake (ADI) for calcium carbonate and that, in principle, there are no safety concern with respect to the exposure to calcium carbonate per se at the currently reported uses and use levels in all age groups of the population, including infants below 16 weeks of age.

Intakes of calcium resulting from the use of calcium carbonate as a food additive, taken together with intakes of calcium from all other sources, should be below the above UL of 2500 mg/day for calcium (15).

## Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| GCC | Ground calcium carbonate |
| HuLy | Human lymphocytes |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| nm/µm/mm | nano-/micro-/millimeter |
| NOAEL | No observed adverse effect level |
| PCC | Precipitated calcium carbonate |
| SCF | Scientific Committee on Food |
| SEM | Scanning Electron Microscopy |
| UL | (Tolerable) Upper (Intake) Limit |

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## *8.2 Summary calcium sulphate*

# Calcium Sulphate ($CaSO_4$), E516

## CAS 7778-18-9
## CAS 10034-76-1
## CAS 10101-41-4

Safety assessment as excipient for oral administration

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# 1. Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as colouring agent in orally administered medicinal products, a safety assessment for calcium sulphate was performed to support the justification for its potential usage as alternative colouring agent.

Calcium sulphate is a yellow-white, crystalline solid soluble in water up to 2 g/L. Calcium sulphate dissociates in biological fluids and both calcium and sulphate ions are absorbed by the human body. Calcium sulphate is not acute toxic and was negative in skin/eye irritation as well as skin sensitization tests. Both in vitro and in vivo genotoxicity studies are negative. The most sensitive NOAEL has been determined as 100 mg/kg bw/d calcium sulphate dihydrate (corresponding to 79 mg/kg bw/d calcium sulphate anhydrite) based on changes in clinical chemistry parameters in male rats following administration for 35 days.

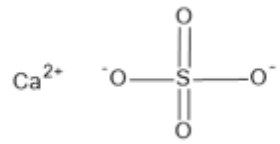
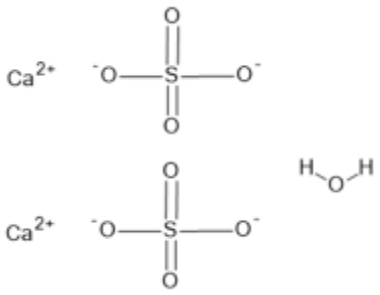
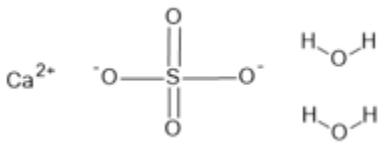
Calcium sulphate is approved as a food additive (E516), has a long history of safe use and has been evaluated by several Safety Panels (no ADI specified).

Overall, it can be concluded that a safety concern is not to be expected when calcium sulphate is used as colorant/excipient in orally administered medicinal products.

## 2. General information

Calcium sulphate, the calcium salt of sulfuric acid, is an inorganic salt occurring in different forms (anhydrite, hemihydrate and dihydrate). It occurs naturally as the mineral gypsum and arises as by-product in several industrial processes.

It is used in a variety of products including consumer and personal care products, food products as well as pharmaceuticals. Calcium sulphate's functions include the use as building material, anticaking agent, desiccant among others. In pharmaceuticals it is e.g., used as excipient and colorant.

| | |
|---|---|
| Name | Calcium sulphate |
| Chemical name | Calcium sulphate anhydrite<br>Calcium sulphate hemihydrate<br>Calcium sulphate dihydrate |
| Synonyms | Calcium sulfate, sulfuric acid calcium salt, gypsum, thiolate, drierite |
| CAS No. | 7778-18-9 (anhydrite)<br>10034-76-1 (hemihydrate)<br>10101-41-4 (dihydrate) |
| Molecular formula | $CaSO_4$ (anhydrite)<br>2 $CaSO_4$ x $H_2O$ (hemihydrate)<br>$CaSO_4$ x 2 $H_2O$ (dihydrate) |
| Molecular weight | 136.1 g/mol (anhydrite)<br>145.1 g/mol (hemihydrate)<br>172.1 g/mol (dihydrate) |
| Chemical structure | Calcium sulphate<br>  |
| | Calcium sulphate hemihydrate<br>  |
| | Calcium sulphate dihydrate<br>  |
| Physico-chemical properties | Appearance: yellow-white, crystalline, odourless<br>Physical state (20 °C, 1013 hPa): solid, fine powder<br>Soluble in water up to approx. 2 g/L (at 20 °C) |

# 3. Regulatory information and published limits

Calcium sulphate is an authorized food additive other than colours and sweeteners according to Directive No 95/2/EC (3). It is listed as E516 in Annex II of Regulation (EC) No 1333/2008 (2).

The SCF established a group ADI (=not specified) for cations including calcium (18) and a tolerable upper intake level (UL) for calcium of 2500 mg/person/d for adults (6) which was confirmed in 2012 (10).

In addition, calcium sulphate has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake was not limited (13).

Calcium sulphate is an approved additive on the Food and Drug Administration GRAS (Generally Recognized As Safe) list of food additives (11) and is, moreover, listed in FDA's Inactive Ingredient Database (IID) with several oral administration forms including tablets and capsules (12). Calcium sulphate (dihydrate) is listed in the European Pharmocopoeia (17) and the US Pharmocopoeia (16)

# 4. Safety assessment

## *4.1. Absorption, Distribution, Metabolism, and Excretion*

In biological fluids calcium sulphate completely dissociates into sulphate and the corresponding calcium cation. Both calcium ions and sulphate are absorbed by the body. Calcium ions can be absorbed via active or passive transport mechanisms across and against electrochemical gradients. Sulphate is absorbed from the intestine via an active transport mechanism. Mammalian cells contain influx/efflux transporters for sulphate. Sulphate levels are regulated by the kidney via elimination and reabsorption (14).

A feeding study in rats indicated no difference in calcium bioavailability from a diet with added calcium sulphate or other calcium sources such as carbonate or oxide. Human studies indicate that the bioavailability of calcium from calcium sulphate in mineral water is comparable to that from milk and that the sulphate anion does not affect the urinary excretion of calcium. EFSA's AFC Panel concluded that the bioavailability of calcium from calcium sulphate in other foods is not expected to differ from that of other already permitted calcium sources in foods for particular nutritional uses (6, 9).

## *4.2. Repeat-dose toxicity*

In a combined repeated dose toxicity study with reproductive/developmental toxicity screening according to OECD 422 male and female rats were administered 0, 100, 300 and 1000 mg/kg bw/d calcium sulphate dihydrate by gavage for 35 days (males) or 41-45 days (females). The NOAEL for general toxicity was determined as 1000 mg/kg bw/d (females) while the NOAEL was 100 mg/kg bw/d for males based on findings in clinical chemistry parameters. The latter corresponds to a NOAEL of 79 mg/kg bw/d calcium sulphate (14).

## *4.3. Genetic toxicity*

Calcium sulphate was tested in an OECD 471-compliant bacterial mutation assay indicating no mutagenic potential (15, 5). In an in vitro chromosome aberration assay in CHO cells in compliance with OECD 473 and GLP no genotoxic effects have been observed both with and without metabolic activation (15). Calcium sulphate dihydrate did not increase the mutant frequency in an in vitro mouse lymphoma assay with L5178Y cells (5). Negative results were also obtained in an in vivo micronucleus assay in erythrocytes of mice following single dose oral administration of calcium sulphate dihydrate up to 5000 mg/kg bw/d (5).

## 4.4. Carcinogenicity potential

No reliable data from oral carcinogenicity studies are available for calcium sulphate or its forms. A carcinogenic potential, however, is not to be expected: calcium sulphate is not genotoxic and both calcium and sulphate are essential nutritional components.

## 4.5. Reproductive and developmental toxicity

In a combined repeated dose toxicity study with reproductive/developmental toxicity screening according to OECD 422 male and female rats were administered 0, 100, 300 and 1000 mg/kg bw/d calcium sulphate dihydrate by gavage for 35 days (males) or 41-45 days (females). The NOAEL for reproductive effects was determined as 1000 mg/kg bw/d (females) corresponding to a NOAEL of 790 mg/kg bw/d calcium sulphate (15). In an embryofetal developmental toxicity study (OECD 414) in mice, rats and rabbits with calcium sulphate the NOAEL was 1600 mg/kg bw/d, the highest tested dose (5)

## 4.6. Additional safety data

Calcium sulphate is of low acute oral toxicity as confirmed in rats (LD50 > 5000 mg/kg) and mice (LD50=4052-4226 mg/kg (15). Calcium sulphate dihydrate was tested in an acute oral toxicity study according to OECD 420 resulting in an LD50 of >2000 mg/kg corresponding to a an LD50 of >1581 mg/kg for calcium sulphate anhydrite (5).

Calcium sulphate dihydrate revealed no skin sensitization potential in a Buehler Assay in guinea pigs according to OECD 406 (5). The lack of any irritating/corrosive effects has been confirmed in both a skin and an eye irritation study in rabbits according to OECD Guideline 404 and 405, respectively (5).

## 4.7. Human data

Reliable data on human toxicity and exposure of calcium sulphate is sparse. At repeated exposures of 600 mg/L (20 mg/kg bw/day) sulphate taken orally had a temporary laxative effect (14). This is in line with a study where sodium sulphate decahydrate (corresponding to 5400 mg sulphate ion) was orally administered to human volunteers. While the single dose bolus produced diarrhoea, four divided hourly doses caused only mild or no diarrhoea (1). In clinical trials using a low number of single doses of up to 4500 mg sodium sulphate decahydrate (corresponding to 2700-5400 mg sulphate ion) per person only occasional loose stools were noted (7).

There are several reports describing adverse effects of excessive calcium intake including hypercalcaemia, cardiovascular disease, nephrocalcinosis, nephrolithiasis, renal failure and prostate cancer which have been reviewed by the EFSA Panel (10). The Panel concluded that there is e.g. no link between increased calcium intake levels and chronic hypercalciuria or impaired kidney function (up to 2400 mg/d for adults) or nephrolithiasis (up to 3000 mg/d). In addition, it has been summarized that calcium intakes up to 2000 mg/d have not been linked to an increased risk of cardiovascular disease or prostate cancer.

Therefore, the Panel proposes an UL of 2500 mg/d (10) which concurs with the Recommended Daily Allowance (800 mg calcium/d corresponding to 2700 mg/d calcium sulphate) defined in Annex I of the Directive 90/496/EEC on nutritional labelling (4). This is in line with actual exposure to calcium of approx. 700-1000 mg/d from food and supplements as summarized by EFSA, 2006 (8).

## 4.8. Data gap assessment

Basic toxicological data are available for calcium sulphate while long-term and carcinogenicity data in animals are lacking. In the available studies, the test item has often not been well characterized and i.e., information on particle size (i.e., nanoforms) is missing.

# 5. Overall conclusions

Based on the available data on calcium sulphate and the long history of safe use, no safety issues are expected for the use of the three forms of calcium sulphate as colorant/excipient in orally administered medicinal products.

# 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AFC | Scientific Panel on food additives, flavourings, processing aids and materials in contact with food |
| bw | body weight |
| CHO | Chinese hamster ovary |
| d | day |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| GLP | Good Laboratory Practice |
| GRAS | Generally recognized as safe |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| kg | Kilogram |
| LD | Lethal dose |
| mg | Milligram |
| NOAEL | No observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| Ph.Eur | European Pharmacopoeia |
| SCF | E.U. Scientific Committee for Food |
| SIDS | Screening Information Dataset |
| UL | Tolerable Upper Intake Level |
| USP | United States Pharmacopeia |

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## *8.3. Summary isomalt*

# Isomalt, E 953

# CAS No. 64519-82-0

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Dr. Claudia Sehner (Boehringer Ingelheim) |
| Peer review: | Carley Corado, Andreas Czich |
| Document type: | Expert statement |
| Document status: | Final |
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# 1. Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as colouring agent in orally administered medicinal products, a safety assessment for isomalt (E953) was performed to support the justification for its potential usage as alternative colouring agent.

Isomalt is an equimolar mixture of two diasteromeric disachharides, α-D-glucopyranosido-1,6-sorbitol (1,6-GPS) and α-D-glucopyranosido-1,6-mannitol (1,1-GPM). It is a sweetener widely used in sugar-free candy and chewing gum and as a sweetening agent in confectionery for diabetics. Isomalt is used as excipient in a variety of pharmaceutical preparations including tablets or capsules, coatings, sachets, suspensions, in effervescent and chewable tablets and in lozenges.

Isomalt has been investigated in an extensive set of toxicity studies. Toxicological information for isomalt is vastly described in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation of isomalt and is mainly cited in this safety summary document (11).

In repeat-dose dietary toxicity studies (including 13-week and 1-year studies in rats and dogs and lifetime studies in mice and rats), no adverse effects were observed at dietary levels up to 3.3-10% in rats and 5-10% in dogs. Main findings (mainly) at higher dietary concentrations included diarrhoea in rats and dogs, caecal enlargement in mice and rats, and decreased body weight gain, increased blood bilirubin and decreased urea concentrations and renal pelvic nephrocalcinosis in rats.

Isomalt was not mutagenic or genotoxic in different in vitro and in vivo assays, was not carcinogenic in mice and rats, and showed no adverse developmental and reproductive effects in a multigeneration study in rats and in teratogenicity studies in rats and rabbits.

In humans, isomalt is well tolerated at doses <20 g/day. Gastrointestinal effects, in particular flatulence and diarrhoea, were observed at ≥20 g/day. The laxative effect of isomalt is a common feature of polyols. No significant or only low increases in the blood glucose and insulin levels were observed after oral intake of isomalt, and the compound is non-cariogenic.

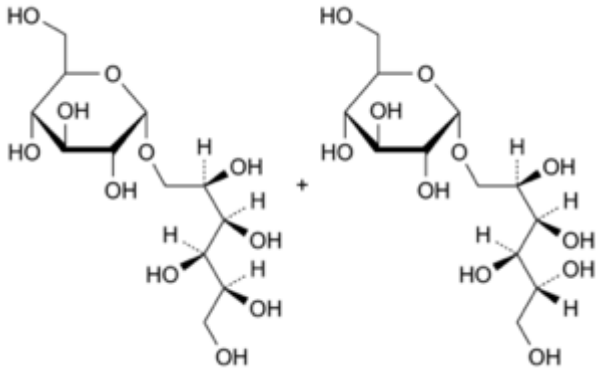
Higher sensitivity to isomalt may occur in patients with hereditary fructose intolerance (HFI) or irritable bowel syndrome (IBS). However, small daily doses of isomalt (1-3 g/day) are expected to be tolerated even in these patients.

Thus, no safety issues are expected for the use of isomalt as part of as colouring agent in orally administered medicinal products (expected doses <1 g).

## 2. General information

Isomalt is an equimolar mixture of two diasteromeric disachharides, α-D-glucopyranosido-1,6-sorbitol (1,6-GPS) and α-D-glucopyranosido-1,6-mannitol (1,1-GPM). It is a non-cariogenic sweetener widely used in sugar-free candy and chewing gum and as a sweetening agent in confectionery for diabetics. Isomalt is used as excipient in a variety of pharmaceutical preparations including tablets or capsules, coatings, sachets, suspensions, in effervescent and chewable tablets and in lozenges (11, 13, 14).

The sweeting power of isomalt is 0.45 relative to sucrose in about a 10% solution (14). Its energy value is 2 kcal/g, half that of sucrose or fructose (1).

| | |
|---|---|
| Name | Isomalt |
| Chemical name | 1-O-α-D-glucopyranosido-D-mannitol (1,1-GPM) and 6-O-α-D-glucopyranosido-D-sorbitol (1,6-GPS) |
| Synonyms | E 953, isomaltitol, palatinit, palatinitol, hydrogenated isomaltulose; 6-O-α-D-glucopyranosyl-D-glucitol (for 1,6-GPS) |
| CAS No. | 64519-82-0 |
| Molecular formula | $C_{12}H_{24}O_{11}$ (or $C_{12}H_{24}O_{11}\cdot 2H_2O$ as dihydrate) |
| Molecular weight | 344.3 g/mol (or 380.3 g/mol for the dihydrate) |
| Chemical structure |  |
| Physico-chemical properties | White or almost white powder or granules; freely soluble in water, practically insoluble in anhydrous ethanol (14). |

## 3. Regulatory information and published limits

Isomalt is included in the European Pharmacopoeia (13) and in the United States Pharmacopeia (17). In the USP-NF it is listed as excipient, being used as coating agent, diluent, suspending and/or viscosity-increasing agent and sweetening agent (17). According to the FDA's Inactive Ingredient Database (IID), the Maximum Daily Exposures (MDE) for isomalt approved in oral and sublingual products range from 73 to 630 mg/day. The oral maximum potency per unit dose for oral troches was listed as 1718.7 mg (8).

Since ingestion of isomalt may lead to fructose exposure (via sorbitol, one of the polyols in isomalt, which can be metabolized to fructose), isomalt is listed in Annex to the European Commission (EC)

guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' with the proposed Summary of Product Characteristic (SmPC) wording that "patients with rare hereditary problems of fructose intolerance should not take this medicine" (5). Although this statement has to be included for products containing isomalt without threshold, for oral products containing fructose and sorbitol it has to be included only up from a threshold of 5 mg/kg/day.

Isomalt (E 953) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 in all nutrients at quantum satis except those intended to be used in foodstuffs for infants and young children listed in point 13.1 of Part E of Annex II (2). Isomalt is listed by the FDA under 21 CFR 101.80 as eligible non-cariogenic carbohydrate sweeter (7) and is listed as food additive in the FAO/WHO Codex alimentarius (6).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated isomalt with an Acceptable Daily Intake (ADI) "not specified". The fact that high doses of isomalt exert a laxative effect in man, which is a common feature of polyols, should be taken into account when considering appropriate levels of use of polyols, alone and in combination (11).

The Scientific Committee for Food (SCF) did not consider it appropriate to establish an ADI for isomalt but considered the use of isomalt as sweetener to be acceptable provided the limitations due to the laxative action were kept in mind. Consumption of the order of 20 g/person/day of polyols is unlikely to cause undesirable laxative symptoms (15, 16).

# 4. Safety assessment

## *4.1. Absorption, Distribution, Metabolism, and Excretion*

Hydrolysis of isomalt yields glucose (50%), sorbitol (25%), and mannitol (25%). Hydrolysis by intestinal disaccharidases in the small intestine is incomplete. Further metabolism by the microbial flora of the large intestine results in complete disappearance of the sweetener from the faeces (11).

After administration of $^{14}$C-isomalt to rats (250, 1000, 2500 mg/kg), absorption ranged from 62-33%, excretion of radioactivity in expired air ranged from 33-62%, and in faeces from 18-54% over a period of 48 h, depending on the dose; approximately 5% of the administered radioactivity appeared in the urine (11).

Metabolisable energy values of isomalt was shown to be lower than that of sucrose in rats and pigs (11).

## *4.2. Repeat-dose toxicity*

In a 3-month study in rats, animals received isomalt at dietary levels of 0, 3.3, 10 and 30%. Appearance, behaviour, growth, and mortality were unaffected in the 3.3% group. Rats receiving 10% isomalt showed mild diarrhoea in the first 2 weeks, which ceased as the study continued. Rats given 30% isomalt had severe diarrhoea in the first 2 weeks, which then diminished in intensity. Body-weight gains were impaired in this high-dose group, most markedly in males. Main clinical pathology finding was increased plasma bilirubin in males at 30% isomalt and in females in all dose groups. Urea concentrations were decreased at 30% isomalt. Kidney weights were lowered in the 30% isomalt group, which may have resulted from reduced nitrogen metabolism. No histopathological findings were seen. Thus, dietary concentrations of up to 10% isomalt were tolerated without obvious organ damage. Taken the transient diarrhoea into account, 3.3% dietary isomalt was well tolerated. However, due to the elevated plasma bilirubin concentrations seen in females at all treatment levels, it was considered difficult to establish a no-effect level (11). A NOAEL of 10% isomalt (7290 and 9160 mg/kg/day in males and females, respectively) were defined in the ECHA registration dossier (3).

In a 1-year study in rats, which was part of a long-term toxicity/carcinogenicity study, animals were fed isomalt at 0, 2.5, 5 or 10% in the diet. The rats were derived from parents that had been fed the same diets prior to mating and during the gestation and lactation periods (*in utero* exposure). The only

treatment-related change consisted of an increase in the relative weights of the filled and empty caeca in males fed 10% isomalt. It was concluded that isomalt, fed at levels up to 10% in the diet to rats that had been exposed to the test substance *in utero* and then continuously during a 1-year period, did not induce any effects of toxicological importance (11).

In a 13-week study in Beagle dogs fed isomalt at dietary concentrations of 0, 5, 10, and 20% isomalt, diarrhoea was observed in animals receiving 20% isomalt and, occasionally, in the 10% dose group. No relevant effects on clinical pathology parameters and no effects on organ weights or in histopathological examination were observed. It was concluded that concentrations of up to 20% isomalt did not produce any toxic injury. Allowing for the occasional ill-formed faeces in the 10% dose group, the no-effect level was placed at 5% of the diet (~1670 mg/kg/day) (11).

In a 1-year study in Beagle dogs, in which isomalt was administered orally at concentrations of 0, 2.5, 5 or 10%, the only finding was increased occurrence of pappy to liquid faeces during treatment with isomalt (all doses, most pronounced at 10%), which was not considered of toxicological relevance. Thus, concentrations of isomalt up to 10% administered orally (~2970 mg/kg/day) over a period of 12 months were tolerated by dogs without harm (11, 3).

For life-time studies in mice and rats, see chapter 4.4.

## *4.3. Genetic toxicity*

Isomalt was non-mutagenic in the Ames test in *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 uvr A when tested with and without metabolic activation (S9 mix) at concentrations up to 5000 µg/plate (3) and in another Ames test using the *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 at up to (at least) 5000 µg/plate in the absence or presence of metabolic activation (3, 11).

Isomalt was non-mutagenic in an *in vitro* mammalian cell gene mutation test (HPRT test) in CHO cells treated for 4 h at concentrations up to 5000 µg/mL in the presence or absence of metabolic activation (S9 mix) (3).

Isomalt was also negative for the induction of micronuclei in an *in vivo* erythrocyte micronucleus test which was part of a 90-day dietary 13-week repeat-dose toxicity study in rats fed isomalt at a level of 10% (approximately 7000 and 8400 mg/kg/day in males and females, respectively) (3).

## *4.4. Carcinogenicity potential*

No evidence of carcinogenic properties of isomalt were observed in mice fed isomalt at dietary levels of 0, 2.5, 5 or 10% throughout the major part of their lifetimes (94 weeks in males due to mortality >80% in controls, 104 weeks in females). Mean body weights of females in the mid- and high- dose groups were relatively low from day 112 onwards. The absolute and relative weights of the caecum (filled and empty) were increased in both sexes fed 10% isomalt (11). It was concluded that the NOAEL for carcinogenicity in this study was 10% (~ 26950 and 18690 mg/kg/day in male and female mice, respectively). In the absence of any adverse effects in males the NOAEL for general toxicity was considered to be 10% (26950 mg/kg/day), while for females, based on reduced body weights the NOAEL was considered to be 2.5% (4050 mg/kg/day) (3).

In a study in rats exposed to isomalt at dietary levels of 0, 2.5, 5 or 10% *in utero* and then continuously during their lifetimes (128 weeks for males, 130 weeks for females), no indication of carcinogenicity or any other effect of obvious toxicological importance was found (11). It was concluded that the NOAEL for carcinogenicity in this study was 10% (corresponding to ~3650 and 4980 mg/kg/day in males and females, respectively). Based on the kidney findings (increased number of treated male and female rats showed hyperplasia of the urothelium in the renal pelvis accompanied by mineralization, whereas the number of females showing corticomedullary mineralization was decreased in the treated groups) the NOAEL for general toxicity was considered to be 5% (~1630 mg/kg/day) in males and 10% (1220 mg/kg/day) in females (3).

In conclusion, in chronic (lifetime) feeding studies in rats and mice, isomalt was not carcinogenic, but resulted in caecal enlargement in mice and rats and renal pelvic nephrocalcinosis in rats, effects common to other polyols (11, 15).

## 4.5. Reproductive and developmental toxicity

In a multigeneration reproduction study conducted in rats, isomalt at levels up to 10% in the diet (~4600 and 5900 mg/kg/day in parental males and females) did not affect fertility or reproduction, nor did it affect the health or survival of the progeny (11, 3).

In a teratogenicity study in Wistar rats, isomalt fed to pregnant female rats at dietary levels up to 10% from gestational day (GD) 0 to 21 did not induce any embryotoxic or teratogenic effects in the foetuses (11).

In a teratogenicity study in FB30 rats fed 0, 2.5, 5 or 10% isomalt in the diet from GD 0-20, food consumption in rats fed 5% or 10% isomalt was significantly reduced and the number of foetuses with retarded development was increased in the groups that received 5% and 10% isomalt. Furthermore, in a group fed 10% isomalt and allowed to deliver, there was a reduction in food consumption during gestation and a reduction in weight gain of the mothers during this period and during the phase of nurturing the young. Prenatal losses and perinatal and postnatal mortality were elevated among the pups. The surviving pups exhibited normal development, however, and no signs of delayed damage were evident in the F1 mating. When animals were adapted to isomalt over a period of 14 days before gestation, postnatal mortality in the isomalt group was not elevated. Weight gains and physiological development of the pups were comparable to those of both the concurrent controls and historical controls of this strain of rats. Therefore, it was considered unlikely that embryotoxic effects observed in strain FB30 rats were due to isomalt; these effects were probably the result of maternal impairment caused by the elevated acute doses of isomalt at the beginning of gestation. These effects were avoided by adaptation of the animal to isomalt when it was mixed with the feed before gestation (11).

In a teratogenicity study in rabbits, isomalt fed at concentrations of 2.5, 5.0, or 10% in the diet from GD 0-29 did not induce any teratogenic or embryo-foetotoxic effects in rabbits (11).

The feeding of isomalt at levels up to 10% in the diet of rats exposed *in utero* and then continuously during 1 year did not induce any toxic effects (11).

## 4.6. Local tolerance and sensitization

Isomalt was classified as not irritating to eyes in a battery of *in vitro* tests (9). It is also considered non-irritating to skin and not sensitising to skin (9, 3).

## 4.7. Human data

Isomalt is used as sweetener in a wide range of food and drinks. No exposure estimate is available, but no ADI has been set and the use is not restricted.

As reported in the FDA IID, the MDE for isomalt approved in oral and sublingual products range from 73 to 630 mg/day. The oral maximum potency per unit dose for oral troches was listed as 1718.71 mg (8).

In volunteers given 15 g $^{14}$C-isomalt orally, mostly ~10% of the administered radioactivity was excreted in the faeces and ~5% of the radioactivity was excreted in the urine, principally in the first 24 h. Serum levels of radioactivity reached a maximum within 1 h. In different studies, only small amounts of unhydrolysed isomalt were found in the urine (<0.2%), indicating that a minor proportion of the dose was absorbed unchanged (11).

Healthy volunteers and diabetics given oral doses of isomalt up to 100 g or 50 g, respectively, showed no significant or only minor increases in blood glucose levels and insulin levels (11).

Isomalt was well tolerated, and no laxative effects were noted in humans (including children, tested up from an age of 4 years) at doses up to 250 mg/kg or 10-20 g/day, but gastrointestinal effects, in particular flatulence and diarrhoea, were observed at doses ≥350 mg/kg or 20-30 g/day (15, 11). Symptoms diminished with daily dosing, indicating adaptation. Also, no effects on cardiovascular, haematology or other clinical chemistry parameters (e.g., lactate, cholesterol, triglycerides, lipids) were observed (10, 11).

The general nature of the laxative effects indicates that the condition results from osmosis across the intestinal wall owing to the presence in the lumen of unabsorbed isomalt and its metabolites (15).

Isomalt does not promote dental caries because it does not lower plaque pH to the levels associated with enamel demineralization (4). Isomalt was shown to be less cariogenic than sucrose in both animal and human experiments (15).

In patients with hereditary fructose intolerance (HFI), oral intake of isomalt, which is partly metabolized to sorbitol and then further on to fructose may have a potential risk of fructose-related adverse effects (e.g., gastrointestinal symptoms such as nausea, bloating, diarrhoea, vomiting). Considering that only maximally 25% of isomalt can be metabolized to sorbitol and part of this is further metabolized to fructose, and taking the dose of 5 mg/kg/day up from which a statement has to be made for fructose (see also chapter 3) (5) and considering this dose as a kind of a threshold for relevant effects, this corresponds to a dose of minimally 20 mg/kg/day for isomalt or minimally 1 g for a 50 kg patient, which is not expected to lead to relevant effects.

In addition, FODMAPs (fermentable oligo-, di-, and monosaccharides and polyols) including isomalt may be symptom-triggering factors in irritable bowel syndrome (IBS), and low FODMAP diets (<3 g /day) may improve symptoms in patients with IBS (12).

### *4.8. Data gap assessment*

Extensive toxicological data, including repeat-dose (up to chronic) toxicity studies, multigeneration and teratogenicity studies, genotoxicity and carcinogenicity studies are available for isomalt. Even though many of the published studies are from 1970's to 1980's and may not fully comply to current standards, and no formal fertility and peri- and postnatal development studies are available (however, the multigeneration study covering many of the relevant endpoints), overall, no relevant data gaps with regard to toxicity data are seen.

Also, human data up to high doses are available, including long-standing use as sweetener and excipient. A potential data gap is seen with respect to doses of isomalt that are well tolerated even in patients with HFI or IBS, although doses <1 g are not expected to cause safety issues.

## 5. Overall conclusions

Isomalt is a widely used sweeteners and is used as excipient in a variety of pharmaceutical products.

In repeat-dose dietary toxicity studies (including 13-week and 1-year studies in rats and dogs and lifetime studies in mice and rats), no adverse effects were observed at dietary levels up to 3.3-10% in rats and 5-10% in dogs. Main findings (mainly) at higher dietary concentrations included diarrhoea in rats and dogs, caecal enlargement in mice and rats, and decreased body weight gain, increased blood bilirubin and decreased urea concentrations and renal pelvic nephrocalcinosis in rats.

Isomalt was not mutagenic or genotoxic in different *in vitro* and *in vivo* assays, was not carcinogenic in mice and rats, and showed no adverse developmental and reproductive effects in a multigeneration study in rats and in teratogenicity studies in rats and rabbits. Embryotoxic effects and postnatal mortality observed in a study in FB30 rats were considered no direct effect of isomalt, but due to maternal impairment (reduced food consumption and weight gain) after acute high doses of isomalt; these effects were avoided by adaptation of the animals to isomalt before gestation.

In humans, isomalt is well tolerated at doses <20 g/day. Gastrointestinal effects, in particular flatulence and diarrhoea, were observed at ≥20 g/day. The laxative effect of isomalt is a common feature of

polyols. No significant or only low increases in the blood glucose and insulin levels were observed after oral intake of isomalt, and the compound is non-cariogenic.

Higher sensitivity to isomalt may occur in patients with HFI or IBS. However, small daily doses of isomalt (1-3 g/day) are expected to be tolerated even in these patients.

Thus, no safety issues are seen for the use of isomalt as part of as colouring agent in orally administered medicinal products (expected doses <1 g).

## 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AI | Adequate Intake |
| ECHA | European Chemical Agency |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| FAO | Food and Agriculture Organization of the United Nations |
| FODMAPs | Fermentable oligo-, di-, and monosaccharides and polyols |
| GD | Gestational day |
| HFI | Hereditary fructose intolerance |
| IBS | Irritable Bowel Syndrome |
| IID | FDA's Inactive Ingredient Database |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| MDE | Maximum Daily Exposure |
| NOAEL | No observed adverse effect level |
| PDE | Permitted Daily Exposure |
| RDI | Reference Daily Intake |
| SCF | EU Scientific Committee for Food |
| WHO | World Health Organization |

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polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation (ID 463, 464, 563, 618, 647, 1182, 1591, 2907, 2921, 4300), and reduction of post-prandial glycaemic responses (ID 617, 619, 669, 1590, 1762, 2903, 2908, 2920) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 9(4):2076.

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## *8.4. Summary maltodextrin*

# Maltodextrin, E1400

# CAS No. 9050-36-6

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Carley Corado, PhD (Pfizer) |
| Peer review: | Julia Kenny, Kristin Deubner |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 07 February 2024 |

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# 1. Summary

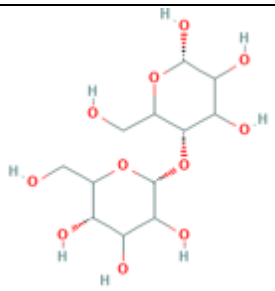
With the aim to identify alternatives to titanium dioxide ($TiO_2$) as colouring agent in orally administered medicinal products, a safety assessment for maltodextrin was performed to support the justification for its potential usage as alternative colouring agent. Maltodextrin is a non-sweet nutritive saccharide polymer, produced by hydrolysis of starch.

In a 2013 peer review on the pesticide risk assessment, EFSA indicates that maltodextrin is of low toxicological concern and no risk to human health, based on its rapid metabolism to a standard energy source (e.g., glucose) and its widespread use in food, cosmetic and medicinal products (5). Maltodextrin is used included on the FDA's Generally Recognized as Safe (GRAS) list and is used in food with no limitations other than current good manufacturing practice (1). A search of the FDA's inactive ingredient database yielded 15 results, with reported maximal daily exposures (MDE) ranging from 5 mg to 14,404 mg for orally dosed maltodextrin (6), refer to Appendix 1.

Available toxicology data indicates negligible safety concerns related to the ingestion of maltodextrin.

## 2. General information

Maltodextrin is a nonsweet nutritive saccharide polymer of D-glucose units linked primarily by a-1-4 bonds and has a dextrose equivalent of less than 20 (1). It is produced by hydrolysis from starch and is found commercially as a white hygroscopic powder. The manufacturing process mimics the digestion of starch within a human digestive tract (5). The commercially available powders are used in a wide range of food and beverage products including baked goods, infant formula, and sports drinks (9). Maltodextrin is also used in pharmaceutical formulations of glucose indicated for caloric supply and carbohydrate supplementation in case of nutrient deprivation or for metabolic disorders such as hypoglycemia. Both digestible and resistant-to-digestion types of maltodextrin are commercially utilized as food ingredients under the same denominator (9). The digestive end product of maltodextrin, glucose, is not considered to be an essential nutrient, but participates in many basic metabolic processes in the body (9). Maltodextrin has also been utilized as a contact insecticide (5).

| Name | Maltodextrin |
|---|---|
| Chemical name, synonyms | Cargill Dry; C*Dry MD; C*PharmDry; Glucidex; Glucodry; Lycatab DSH; Maldex; Maldex G; Malta*Gran; maltodextrinum; Maltosweet; Maltrin; Maltrin QD; Paselli MD10 PH; Rice*Trin; Star-Dri; Tapi. |
| CAS No.[a] | 9050-36-6, |
| Molecular formula[a] | $C_{12}H_{22}O_{11}$ |
| Molecular weight[a] | 342.30 g/mol |
| Chemical structure[a] |  |
| Physico-chemical properties | Water soluble |

[a] Pubchem, Maltodextrin-dextrose equivalent 10-15 | C12H22O11 | CID 68229136 - PubChem (nih.gov). Accessed June, 2023.

## 3. Regulatory information and published limits

Maltodextrin is included on the FDA's GRAS list and is used in food with no limitations other than current good manufacturing practice (1). A search of the FDA's inactive ingredient database yielded 15 results, with reported maximal daily exposures (MDE) ranging from 5 mg to 14,404 mg for orally dosed maltodextrin (6). Maltodextrin is also classified as a Natural Health Product by Health Canada, with both medicinal and non-medicinal uses approved (8).

Maltodextrin is not included on the approved food additive list in Europe, as according to Regulation No 1333/2008 of the European Parliament and of the Council on Food Additives, monosaccharides, disaccharides, oligosaccharides are not considered food additives (2). However, EFSA has released several statements regarding maltodextrin, including a Scientific Opinion regarding a number of health claims related to maltodextrin (4), an opinion regarding the potential for allergic reactions to wheat-based maltodextrins (3), and a peer review on the pesticide risk assessment of maltodextrin (5). In the 2013 peer review on the pesticide risk assessment, EFSA indicates that maltodextrin is of low toxicological

concern and no risk to human health, based on its rapid metabolism to a standard energy source (e.g. glucose) and its widespread use in food, cosmetic and medicinal products (5).

# 4. Safety assessment

Absorption, Distribution, Metabolism, and Excretion

Maltodextrin is rapidly broken down to a standard energy source, glucose, following ingestion (5). Sodium dependent glucose transporter SGLT1 and GLUT2 are predominantly responsible for the transport of glucose into circulation, and glucose is known to be excreted renally.

## *4.1. Repeat-dose toxicity*

Digestion resistant maltodextrin was administered to 60 rats (30 male, 30 female) via oral gavage at doses of 0, 2.5 or 5.0 g/kg for 90 days. Dose-dependent increases in weight of the cecum, cecal contents and cecum with cecal content were observed, as well as hypertrophy of the cecal mucosal epithelium. This finding is considered adaptive and not unexpected for indigestible polysaccharides, which have been found to be fermented by enterobacteria in the cecum. The NOAEL was estimated to be > 5.0 g/kg (14).

Isomaltodextrin was administered to 80 rats (40 male, 40 female) for 90 days via oral gavage at doses of 0, 100, 300, or 1000 mg/kg/day. Significant differences between dosage groups were noted for hematologic, blood chemistry, urinalysis and histologica evaluations, but none of the findings were deemed to be associated with a toxicologic response. The NOAEL for the study was determined to be 1000 mg/kg/day, equivalent to 60g/day consumed by a 60 kg human (12).

## *4.2. Genetic toxicity*

Digestion resistant maltodextrin was negative in the Ames test (Salmonella typhimurium strains TA98, TA100, TA1537, TA1535) with and without the addition of metabolic activation (14).

Isomaltodextrin was also negative in an OECD compliant Ames test (Salmonella typyimurium strains TA98, TA100, TA1535 and TA 1537) with and without metabolic activation. Isomaltodextrin was also confirmed to be negative in an OECD compliant in vivo micronucleus test in rats and mammalian chromosome aberration test (12).

## *4.3. Carcinogenicity potential*

No carcinogenicity studies were identified. Maltodextrin is not expected to pose a carcinogenicity risk based on its rapid metabolism to glucose, an endogenous energy source (10).

## *4.4. Reproductive and developmental toxicity*

No reproductive or developmental studies have been identified.

## *4.5. Additional safety data*

An OECD 404 compliant skin irritation study was conducted in NZW rabbits (n=3). A single 4-hour semi-occluded dermal application of maltodextrin was applied to intact skin. Maltodextrin was determined to be non-irritating (10).

## *4.6. Human data*

- Excessive intake (33 g/day for 4 weeks) of digestion resistant maltodextrin (FS-2H) resulted in no clinically significant adverse events (7)

- Digestion resistant maltodextrin was administered to 50 healthy volunteers (25 men, 25 women) at doses of 0.4, 0.5, 0.6, 0.8 and 1.0 g/kg (n=10/group). Condition of the first stool after intake of the test solution was the primary endpoint in the determination of an acute NOEL for diarrhoea. The NOEL for diarrhoea was determined to be 0.8 g/kg for men and >1.0 g/kg for women. No other clinical symptoms were observed that led to discontinuation for any subject (14)

- The effects of resistant maltodextrin on colon transit time (CTT) were evaluated in comparison with a placebo (non digestion-resistant maltodextrin) in 29 subjects. Resistant maltodextrin was well tolerated and improved CTT, stool volume, stool consistency and some intestinal function was observed after a 21-day intervention period (11).

- Maltodextrin has also be utilized as a placebo control (8 g/day for one week) in a dietary comparison with gamma-cyclodextrin (13)

- Isomaltodextrin was administered in a 4-week high dose ingestion study (30 g/day) and a 12-week low dose ingestion study (10 g/day) and all laboratory values were found to be within normal variation for the duration of the studies (12).

## *4.7. Data gap assessment*

Ames study that was performed was conducted with digestion resistant maltodextrin and not reported to be OECD compliant or GLP. No carcinogenicity studies or reproductive and developmental toxicity studies could be found for maltodextrin.

# 5. Overall conclusions

Maltodextrin is widely used across the food, cosmetic and pharmaceutical industry. Based on its metabolic profile, it has been considered non-hazardous by health authorities and is either an approved food additive or is considered safe but not classified as a food additive. Maltodextrin is not mutagenic and not irritating to rabbit skin. Available studies in nonclinical species and in humans indicate that maltodextrin is well tolerated at levels (up to 5 g/kg/day for 90 days in rats and up to 33 g/kg/day for 30 days in humans) that far exceed those that would be expected as an excipient (14). While no carcinogenicity or reproductive toxicity studies could be identified with maltodextrin, it is not anticipated to pose a carcinogenicity hazard or reproductive hazard based on its rapid metabolism to glucose (10).

# 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AI | Adequate Intake |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| NOAEL | No observed adverse effect level |
| NZW | New Zealand White |
| PDE | Permitted Daily Exposure |
| RDI | Reference Daily Intake |
| UL | Upper Limit |

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# 8. Appendix 1

Summary of Inactive Ingredient Database – accessed 23-Aug-2023, showing approved levels of maltodextrin:

**Search Results for: maltodextrin**

Show 30 rows | CSV | Excel

Filter:

| Inactive Ingredient | Route | Dosage Form | CAS Number | UNII | Maximum Potency per unit dose | Maximum Daily Exposure (MDE) | Record Updated |
|---|---|---|---|---|---|---|---|
| MALTODEXTRIN | ORAL | CAPSULE | 9050366 | 7CVR7L4A2D | | 200mg | |
| MALTODEXTRIN | ORAL | FILM, SOLUBLE | 9050366 | 7CVR7L4A2D | 3.2mg | | |
| MALTODEXTRIN | ORAL | GRANULE, FOR SUSPENSION | 9050366 | 7CVR7L4A2D | 285.7mg/5ml | | |
| MALTODEXTRIN | ORAL | LOZENGE | 9050366 | 7CVR7L4A2D | | 175mg | |
| MALTODEXTRIN | ORAL | PASTE | 9050366 | 7CVR7L4A2D | | 1,050mg | |
| MALTODEXTRIN | ORAL | POWDER, FOR SUSPENSION | 9050366 | 7CVR7L4A2D | | 8,000mg | |
| MALTODEXTRIN | ORAL | SOLUTION | 9050366 | 7CVR7L4A2D | | 1,451mg | |
| MALTODEXTRIN | ORAL | SUSPENSION | 9050366 | 7CVR7L4A2D | | 5mg | |
| MALTODEXTRIN | ORAL | TABLET | 9050366 | 7CVR7L4A2D | | 151mg | |
| MALTODEXTRIN | ORAL | TABLET, CHEWABLE | 9050366 | 7CVR7L4A2D | 292mg | | |
| MALTODEXTRIN | ORAL | TABLET, COATED | 9050366 | 7CVR7L4A2D | 5.6mg | | |
| MALTODEXTRIN | ORAL | TABLET, EFFERVESCENT | 9050366 | 7CVR7L4A2D | | 14,484mg | |
| MALTODEXTRIN | ORAL | TABLET, EXTENDED RELEASE | 9050366 | 7CVR7L4A2D | | 3,239mg | |
| MALTODEXTRIN | ORAL | TABLET, FILM COATED, EXTENDED RELEASE | 9050366 | 7CVR7L4A2D | NA | | |
| MALTODEXTRIN | ORAL | TABLET, ORALLY DISINTEGRATING | 9050366 | 7CVR7L4A2D | | 12mg | |

Showing 1 to 15 of 15 entries

Previous | 1 | Next

## *8.5. Summary magnesium carbonate*

# Magnesium Carbonate, E504

# CAS 546-93-0

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Thomas Broschard (Merck KGaA) |
| Peer review: | Kristin Deubner, Yi Yang |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 05-Feb-2024 |

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# 1. Summary

The present safety assessment for magnesium carbonate ($MgCO_3$) was performed to support the justification for its potential usage as alternative coloring agent for titanium dioxide.

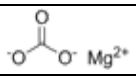
Magnesium carbonate (CAS 546-93-0) is already used as an excipient in U.S. FDA-approved drug products. Moreover, it is an accepted food additive both in the U.S. (GRAS status) and the EU (E504).

No adverse effects have been observed in experimental in vitro and in vivo studies on acute toxicity, genotoxicity, carcinogenicity and developmental and reproductive toxicity with magnesium carbonate or related magnesium salts. In repeat dose toxicity studies slight effects (transient soft stool, slightly reduced body weight) were observed in rats orally treated with very high dose levels >1,000 mg/kg bw/day which are not relevant for human exposure scenarios.

Magnesium is an essential element in human metabolism with several important physiological functions. With an amount of approx. 25g it is the fourth most common mineral constituent in the human body. Magnesium carbonate is soluble under acidic conditions such as those in the stomach. Based on all available data, the European Food Safety Authority (EFSA) established Adequate Intake (AI) values for magnesium of 350 and 300 mg/day for men and female, respectively, which is >1g when calculated as $MgCO_3$ (1,214 and 1,040 mg/day for men and females, respectively).

Taking into account all available data, both the existing toxicological studies with magnesium carbonate and other Mg salts and the physiological relevance of magnesium in the body, it can be concluded that the use of magnesium carbonate as an excipient in pharmaceutical products is safe.

## 2. General information

| | |
|---|---|
| Name | Magnesium carbonate |
| Chemical name, synonyms | magnesium (II) carbonate; carbonate magnesium; carbonic acid, magnesium salt (1:1); magnesite |
| CAS No. | 546-93-0 |
| Molecular formula | $MgCO_3$ |
| Molecular weight | 84.31 g/mol (anhydrous) |
| Chemical structure |  |
| Physico-chemical properties | Boiling point: 900°C (liberating $CO_2$)<br>Melting point: 350°C (decomposes)<br>Solubility in water: insoluble (at 20°C: 0.01 g/100 mL)<br>Appearance: odourless, white hexagonal crystals |

## 3. Regulatory information and published limits

Magnesium carbonate ($MgCO_3$) is used as an inactive ingredient/excipient in oral drug products that have been approved by the U.S. Food and Drug Administration (FDA) with maximum single dose levels of 10 mg to 250 mg (1). Moreover, the U.S. FDA affirmed in their final rule that certain magnesium salts, among which magnesium carbonate, are generally recognized as safe (GRAS) for use as direct human food ingredients (2). Also in the EU, magnesium carbonate is approved as a food additive (E504) e.g., to prevent caking and as a whitener in food processing applications (3). The European Food Safety Authority (EFSA) established Adequate Intake (AI) values for magnesium of 350 and 300 mg/day for men and female, respectively (4), which is >1g when calculated as $MgCO_3$ (1,214 and 1,040 mg/day for men and females, respectively).

## 4. Safety assessment

### *4.1. Absorption, Distribution, Metabolism, and Excretion*

Magnesium is an essential element in human metabolism and is required for over 300 enzyme reactions, including all reactions requiring adenosine triphosphate. It is essential to regulate cell permeability, and inadequate levels of magnesium will severely affect cardiovascular, neuromuscular, and renal functions.

The body contains about 25 g of magnesium, making it the fourth most common mineral constituent in the body (5). More than half the magnesium is in bone (67%); the remainder is found intracellularly in soft tissues (31%) and, to a lesser degree, in body fluids (approximately 1%) (6).

In general, magnesium levels in the body are regulated by homeostatic processes. These homeostatic processes are able to deal with moderate increases in magnesium intake: either by storage in bone or by excretion via urine, faeces or sweat (5). Magnesium balance is highly regulated by both intestinal absorption and excretion, predominantly renal. Little is excreted through sweat unless intense exercise is performed (7).

Magnesium carbonate reacts with hydrochloric acid to produce magnesium chloride, carbon dioxide and water, i.e, after oral uptake it is dissolved at the acidic conditions in the stomach.

Magnesium absorption takes place in the distal intestine, mainly as the ionized form. Percentage absorption is generally considered to be 40–50 %, but figures from 10 to 70 % have also been reported (4). The oral bioavailability of different Mg salts was investigated in newly weaned rats at two different

doses. The percentage of apparent absorption, retention from plasma levels, and urinary excretion were measured, as well as the concentration of Mg in the femur. The authors concluded that absorption of carbonate was higher than other salts including chloride phosphate, sulfate or silicate (8).

The majority of the body magnesium content is stored in bone (about 60 %) and muscle (about 25 %). A small amount is present in the serum, mainly as the free cation (4).

Magnesium levels in the body are primarily controlled by the kidney, with as little as 2% of endogenous magnesium excreted in the faeces. Normal renal regulation of magnesium usually consists of glomerular filtration and tubular reabsorption, which are hormonally controlled. Because the renal threshold for magnesium (between 1.3 and 1.7 meq/L) is near normal serum values, a portion of dietary magnesium will appear in the urine, regardless of magnesium status. The maximum renal capacity is over 2.0 g/day (5).

## *4.2. Acute toxicity*

In an acute oral toxicity study in rats with magnesium carbonate, no mortalities were observed in the observation periods and there were no clinical signs of systemic toxicity (with the exception of hunched posture noted in the initial treated animal during the day of dosing; however, no signs of systemic toxicity were noted in the additional four treated animals) or macroscopic effects noted at necropsy. The $LD_{50}$ value was therefore >2000 mg/kg bw for oral exposure and demonstrates that magnesium carbonate is not acutely toxic via the oral route (5).

## *4.3. Repeat-dose toxicity*

No repeat dose toxicity assays have been identified for magnesium carbonate. However, magnesium carbonate is soluble in diluted acids including hydrochloric acid from which magnesium chloride is formed. For oral administration, it is therefore justified to use repeat dose toxicity studies with magnesium chloride as a surrogate.

A 28 day repeat dose oral toxicity study combined with a reproduction/ developmental toxicity screening test was performed in the rat in accordance with OECD TG 422. Magnesium chloride hexahydrate was administered daily by gavage to three groups of Wistar rats for 14 days pre-mating and 14 days mating in both male and females, during gestation period and up to post natal day 3 in females. Males were dosed for 28-29 days. Dose levels of 0, 250, 500 and 1000 mg/kg bw/day were used. No adverse systemic effects were and a systemic NOAEL of 1000 mg/kg bw/day was established for magnesium chloride hexahydrate. This study is directly applicable to magnesium carbonate and hence the equivalent NOAEL for magnesium carbonate can be calculated as 414 mg/kg bw/day (5).

In a 90-day repeated dose oral toxicity study in rats, magnesium chloride hexahydrate was administered to Fischer 344 rats in the diet at concentrations of 0.1, 0.5, and 2.5% (resulting in dose levels of 0, 62, 308 and 1600 mg MgCl2/kg bw/day in males and 0, 59, 299 and 1531 mg MgCl2/kg bw/day in females). No treatment related deaths were observed during the study. Transient soft stool and a sustained increase in water consumption were observed both in males and females of the high dose group and a slight reduction in body weight gain was noted in the high dose males. There were no toxic changes in food consumption, organ weights, hematology and biochemistry and histopathological examinations in any treated group. The mean NOAEL for males and females was calculated as 303.5 mg/kg bw/day. Considering the molecular weights, a NOAEL of 125.8 mg/kg bw/day can be calculated for magnesium carbonate from the results of this study.

## *4.4. Genetic toxicity*

No genotoxicity tests have been identified for magnesium carbonate. However, a reduction of the genotoxic effect of nickel subsulfide was observed in an in vitro micronucleus and other genotoxic assays when magnesium carbonate was added (9). A read-across approach from the magnesium chloride and magnesium chloride hexahydrate to the target magnesium carbonate is scientifically

justified, as the presence of acid in the stomach converts magnesium carbonate into magnesium chloride when ingested orally. Magnesium chloride was negative in several in vitro genotoxicity studies including the Ames test, the mouse lymphoma assay and in vitro chromosome aberration studies in mammalian cells. Based on all this data it is concluded that magnesium carbonate has no genotoxic potential.

## *4.5. Carcinogenicity*

Groups of male Fischer 344 rats were injected intrarenally with either vehicle (n=20), 2 doses of 5 mg of Ni3S2 (nickel subsulphide) (n=40), 2 doses of 6.2 mg of 4 $MgCO_3$ x $Mg(OH)_2$ x $nH_2O$ (magnesium basic carbonate, $MgCO_3$) (n=20), or 2 doses of $Ni_3S_2$ plus $MgCO_3$ (n=20). After 109 weeks, no kidney tumors were found in the $MgCO_3$ group. $Ni_3S_2$ alone induced local renal tumors in 62.5% of the rats, with the first tumor appearing at week 30 after the injections. Ni3S2 carcinogenesis was strongly inhibited by $MgCO_3$. The addition delayed the onset of renal tumors by 44 weeks and lowered the final yield of tumors to 20%. The authors did not have an explanation for the mode of action of magnesium (10).

A similar experiment was performed by intramuscularly injecting male Fischer 344 rats with either 2.5 mg $Ni_3S_2$, or 6.1 mg $MgCO_3$, or both doses combined, or with vehicle (in all cases: n=20). After 79 weeks, no sarcomas in the kidneys or metastases in lungs, kidneys, or other organs were found in the $MgCO_3$ group. In the $Ni_3S_2$ group, 100% of the animals had tumors, predominantly rhabdomyosarcomas. $MgCO_3$ inhibited the carcinogenicity of $Ni_3S_2$ in a dose-related manner. The final incidence of sarcomas decreased from 100% to 55%, and the appearance of first tumors was delayed from 25 to 39 weeks.

Given the essential nature of Mg, oral administration of soluble Mg compounds would not be expected to pose a cancer risk. Supporting this statement is a 2-year study in B6C3F1mice. Magnesium chloride administered at 0, 0.5 or 2% in the diet for 96 weeks followed by 8 weeks of observation was reported to be negative (11).

Based on these non-guideline studies, indicating a more anti-carcinogenic effect, the data on genotoxicity and the physiological relevance of magnesium, a carcinogenic potential for magnesium carbonate is not to be expected.

## *4.6. Reproductive and developmental toxicity*

No reproductive or developmental toxicity study was found in the open literature for pure magnesium carbonate. However, the mineral dolomit consisting of calcium and magnesium carbonate, was neither teratogenic nor embryotoxic when given once a day orally to rats at doses up to 1,500 mg/kg bw/day from day 6–15 of pregnancy (12). In addition, experimental developmental and reproductive toxicity data are available for magnesium chloride, which can be used as a surrogate as described above: In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test no adverse effects on reproduction or development were observed in rats treated orally with magnesium chloride hexahydrate up to highest dose tested (NOAELrepr/dev = 1,000 mg/kg bw/day) (5). In a teratogenicity study according to OECD TG 414, magnesium chloride hexahydrate was not teratogenic when given orally (by gavage) to pregnant rats once a day from day 6 through 15 of pregnancy at doses of 0, 200, 400 and 800 mg/kg bw/day (5, 13). Taking into account all these studies performed with surrogate substances, magnesium carbonate is not considered to be a reproductive or developmental toxicant.

## *4.7. Human data*

Magnesium is essential to both plants and animals. The body of an average adult contains about 25 g of magnesium (14). Magnesium is present in many foods, such as meats, cereals, vegetables, and milk. The average adult ingests about 300 mg of magnesium per day. Magnesium deficiency results in

weakness, dizziness, and convulsions. Magnesium is a normal constituent of human blood, being present at 1.6-2.2 meq/L. When serum magnesium reaches 3-4 meq/L, signs of central nervous system depression, loss of reflexes, muscular tone, and power, hypotension, and bradycardia may appear. Death in cardiac arrest and/or respiratory paralysis can occur when serum magnesium reaches 10-15 meq/L (14).

In the literature, only few cases of toxic hypermagnesaemia (>2.5 mmol/L) have been published, mostly due to the (ab-)use of Mg as laxatives or antacids in single doses of >100 mmol Mg (ca. 2,500 mg). Symptoms were hypotension, nausea and vomiting (EFSA, 2006).

### *4.8. Data gap assessment*

Based on a weight of evidence approach, the safety of magnesium carbonate can be adequately proven with the available data. No relevant data gap has been identified for magnesium carbonate.

## 5. Overall conclusion

Taking into account all available data including (i) the existing toxicological studies with magnesium carbonate and other Mg salts, (ii) the physiological relevance of magnesium in the body and (iii) the solubility of magnesium carbonate particles under acidic conditions, it can be concluded that the use of magnesium carbonate as an excipient in oral pharmaceutical products is safe.

# 6. Abbreviations

| | |
|---|---|
| AI | Adequate Intake |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| GRAS | Generally recognized as safe |
| HPRT | Hypoxanthine-guanine-phosphoribosyltransferase |
| $MgCl_2$ | Magnesium chloride |
| $MgCO_3$ | Magnesium carbonate |
| MNT | Micronucleus Test |
| NOAEL | No observed adverse effect level |
| OECD | Organization for Economic Co-operation and Development |

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## *8.6. Summary magnesium oxide*

# Magnesium oxide, E-No. 530

# CAS No. 1309-48-4

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Kristin Deubner (STADA) |
| Peer review: | Verena Ziegler |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 8 February 2024 |

# Table of contents



# 1. Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as colouring agent in orally administered medicinal products, a safety assessment for magnesium oxide (MgO) was performed to support the justification for its potential usage as alternative colouring agent.

MgO readily dissociates after reaction with gastric hydrochloric acid. A scientific opinion on Mg dietary reference levels was published in 2015 by the EFSA NDA Panel (1). Here it was concluded that based on a NOAEL of 250 mg Mg per day and an uncertainty factor of 1.0 an upper limit (UL) of 250 mg Mg per day can be established for readily dissociable magnesium salts like MgO. The EFSA NOAEL is based on a mild, transient laxative effect, without pathological sequelae, which is readily reversible and for which considerable adaptation can develop within days. The NOAEL holds for adults, including pregnant and lactating women, and children from 4 years on. Few cases of toxic hypermagnesaemia have been published, mostly due to the (ab-)use of Mg as laxatives or antacids in single doses. However, mild diarrhoea can be taken as the most sensitive non-desirable effect.

MgO is used as active ingredient in medications for the treatment of Magnesium (Mg) deficiency, in antacids and laxatives as well as pharmaceutical excipient (e.g. filler), food supplement and food additive. Due to the widespread experience and application, oral toxicity after repeated MgO intake is considered to be minor.

Considering the high NOAEL and relatively mild toxic effects associated with Mg intake, the available UL of 250 mg/day derived by regulatory authorities seems sufficient and it can be concluded that MgO is of low toxicity and concern.

## 2. General information

MgO is a white hygroscopic solid mineral that occurs naturally as periclase. MgO is used as food additive and is listed as E530 in the Commission Regulation EU No 231/2012. The specifications in EU No 231/2012 as well as in European pharmacopeia (Ph Eur) differ between very bulky, white powder known as light MgO and a relative dense, white powder known as heavy MgO (2, 3).

MgO is produced by calcination of magnesium carbonate or magnesium hydroxide at different temperatures. The density of the oxide is influenced by the calcining temperature; high temperature yielding more compact forms. Light form more readily than heavy; combines with water to form magnesium hydroxide; imparts a slight alkaline reaction to water. Light-burned MgO is characterized by small crystallite size (<0.5 µm) and moderate to high chemical reactivity. Hard-burned MgO is characterized by moderate crystallite size (1-20 µm) and moderately low chemical reactivity (4, 5).

Besides its technical use, MgO is used as active ingredient in medications for the treatment of magnesium deficiency, in antacids and laxatives. MgO is also used as pharmaceutical excipient (e.g. filler), as food supplement and food additive (6, 7, 8, 9).

Advancements in nanotechnology have led to the development of nanomedicine. Among the known metal oxides MgO nanoparticles (NPs) have attracted wide scientific interest due to ease of synthesis, chemical stability, unique properties, and extensive applications in various fields. However, safety data of those particles is rare (10, 11).

| | |
|---|---|
| Name | Magnesium oxide |
| Chemical name, synonyms | Magnesia<br>Periclase |
| CAS No. | 1309-48-4 |
| Molecular formula | MgO |
| Molecular weight | 40.3 g/mol |
| Chemical structure | Mg=O |
| Physico-chemical properties | Practically insoluble in water. Insoluble in ethanol.<br>Soluble in acid, ammonia. |

## 3. Regulatory information and published limits

MgO readily dissociates after a reaction with gastric hydrochloric acid under formation of magnesium chloride ($MgCl_2$) salt. A scientific opinion on Mg dietary reference levels was published in 2015 by the EFSA NDA Panel (1). Here it was concluded that based on a NOAEL of 250 mg Mg per day and an uncertainty factor of 1.0 an upper limit (UL) of 250 mg Mg per day can be established for readily dissociable magnesium salts (e.g., chloride, sulphate, aspartate, lactate) and compounds like MgO in nutritional supplements, water, or added to food and beverages.

The Scientific Committee on Food (SCF) of the European Union (EU) had concluded that osmotic diarrhoea was the crucial effect for establishing an UL for Mg in 2001. The UL only applies to adults, including pregnant and lactating women, and children aged 4 and older. It excludes magnesium from meals and beverages. Children between the ages of 1-3 could not have a UL set for lack of data (1, 12).

Further, the German Federal Institute for Risk Assessment (BfR) recommends a maximum amount of 250 mg per daily dose of an individual food supplement. It is recommended that this amount be divided into two or more servings per day (13). Additionally, MgO is listed in European pharmacopeia as light and as heavy MgO. In Europe it is used as active ingredient as well as excipient (3).

MgO is also listed in the Code of Federal Regulations Title 21 of the US FDA in part 184 Direct food substances affirmed as generally recognized as safe. The ingredient is used in food with no limitation

other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient (14). MgO is listed as Inactive Ingredient for Approved Drug Products in chewing gum, capsules and tablets with a maximum daily exposure of up to 368 mg (15). According to a JECFA report in 1965 the overall intake of oxides of magnesium were not limited. However, the evaluation referred to bases used to adjust pH in food technology (16).

# 4. Safety assessment

## *4.1. Absorption, Distribution, Metabolism, and Excretion*

MgO readily dissociates after reaction with gastric hydrochloric acid. It is converted into $MgCl_2$ under acidic condition in the stomach and then to $Mg(HCO_3)2$ in the intestinal tract (17, 18).

Mg plays an important role in many physiological functions. Habitually low intakes of Mg and in general the deficiency induce changes in biochemical pathways (19). The total Mg body amount varies between 20 and 28 g. More than 99% of the total body Mg is located in the intracellular space, mainly stored in bone (50–65%), where, together with calcium and phosphorus, it participates in the constitution of the skeleton, but also muscle, soft tissues, and organs (34–39%), whereas less than 1–2% is present in blood and extracellular fluids. Body Mg content is physiologically regulated through three main mechanisms: intestinal absorption, renal re-absorption/excretion, and exchange from the body pool of magnesium (i.e., bones). The elimination of Mg by the kidneys increases when there is a Mg surplus and can decreases in the urine during deficits (19).

Orally supplied Mg is used depending on the initial situation of the Mg level of the body. In case of Mg deficiency of the organism, more Mg is absorbed, and when there is an abundance of Mg, less is absorbed. The resorption ratios correspond to those that are typical of homeostatically actively regulated physiological substances (degressive resorption kinetics). Thus, in the case of a physiological Mg level and intact renal function, no increased Mg storage above the upper normal range can be achieved even with higher supplementation. After absorption in the intestine, Mg is excreted mainly via the kidney. Unabsorbed Mg is excreted via the stool. Elimination of Mg usually ranges around 120 mg per day and is subject to homeostatic regulation, deviations of the Mg level from the physiological state in the organism are therefore rare (20).

## *4.2. Repeat-dose toxicity*

Oral chronic toxicity after repeated MgO intake is considered to be minor due to the widespread application as therapeutic agent, food additive or food supplement (4, 1). 60-day administration of a daily oral dose of 476 mg MgO/day only caused diarrhoea in 18 of 50 healthy probands (further details were not provided) (21).

However, in exceptional cases, toxicities with lethal outcome were described after repeated oral administration of very high doses of bioavailable magnesium compounds. In one case, the serum level of a 2.5-year old child was in the range of 8 mmol Mg/l (normal range 0.75 to 0.95 mmol/l) after several-day administration of about 2400 mg MgO per day. The initial systemic (neuromuscular) symptoms were similar to those observed after acute toxicity; death occurred after cardiac arrest (22).

## *4.3. Genetic toxicity*

MgO was not mutagenic in Salmonella typhimurium strains TA102 with or without S-9, and it reduced, but did not eliminate the mutagenicity of methylglyoxal in the same strain. MgO was also not mutagenic in the TA97 and TA100 strains with or without S-9 (4). Negative studies in the Ames assay for Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 with and without metabolic activation and in the Escherichia coli wp2 UVRA assay with and without S-9 for magnesium sulfate are consistent with the negative results reported above for MgO (4).

MgO nanoparticles (NPs) were investigated in comparison to their micron counterparts in female Wistar rats in a comet assay, a micronucleus test and a chromosomal aberration assay. High doses (1000 mg/kg bw) of MgO NPs produced significant DNA damage probably induced by oxidative stress (10).

## *4.4. Carcinogenicity potential*

A carcinogenicity bioassay of MgO conducted by subcutaneous administration to rats was described as negative by the authors (4). However, no experimental details were provided.

Given the essential nature of Mg, oral administration of soluble Mg compounds would not be expected to pose a cancer risk. Supporting this statement is a 2-year study in B6C3F1mice. Magnesium chloride administered at 0, 0.5 or 2% in the diet for 96 weeks followed by 8 weeks of observation was reported to be negative (4).

No carcinogenicity data could be found for MgO NPs.

## *4.5. Reproductive and developmental toxicity*

MgO was used as a positive control to examine the effects of an industrial magnesite dust on avian embryos. In groups injected with magnesite dust suspensions dose-related embryolethality, skeletal anomalies and delayed ossification was also observed (4).

Female Wistar rats exposed to emissions from the magnesite factory for 6 months and mated with non-exposed males had a decreased fertility index. Average litter size was also smaller and the progeny weighed significantly less than control pups on the 21st postnatal day. The F1 rats had significantly increased concentrations of Mg in the lungs and muscles (4).

However, there is no indication of reproductive or developmental toxicity after oral use of MgO, neither in the regulatory assessments nor based on literature research. Due to the widespread oral application as therapeutic agent, food additive and food supplement the risk of reproductive and developmental toxicity can be regarded as minor.

No reproductive and developmental data could be found for MgO NPs.

## *4.6. Additional safety data*

The $LD_{50}$ for oral administration of MgO in mice was reported to be 810 mg/kg. The $LD_{50}$ for oral administration in rats was reported to be 3870 mg/kg in males and 3990 mg/kg in females (4).

When fine particles of MgO are dispersed in air, whether directly or when generated by the burning or cutting of magnesium metal, the resulting MgO fume is an inhalation hazard. Occupational exposure to MgO appears to occur primarily through inhalation of dusts and fumes generated from high-temperature processes, e.g, calcining of magnesite ores. Syrian golden hamsters were administered MgO by intratracheal instillation at 3 mg per week for life. Particle size was 90%<25 µm, 46%<10 µm, 18% <5 µm, and 1% <1 µm. Necropsy of each lobe of the lung, larynx, trachea, and stem bronchi showed slight metaplasia in the tracheobronchial zone and moderate hyperplasia of the alveolar zone (4).

The oral administration of high doses of MgO NPs in rats produced biochemical alterations and accumulation in the liver and kidney tissues apart from urine and faeces. Antioxidant assays revealed prominent oxidative stress at high dose level. In vivo studies with high doses of MgO NPs showed hematological changes as well as changes in the activity of liver enzymes (10, 23).

## 4.7. Human data

In the literature, only few cases of toxic hypermagnesaemia (>2.5 mmol/L) have been published, mostly due to the (ab-)use of Mg as laxatives or antacids in single doses of >100 mmol Mg (ca. 2,500 mg). Symptoms were hypotension, nausea and vomiting (EFSA, 2006).

Easily dissociable Mg salts, especially the sulphate ("Epsom salt", "Bittersalz"), are used as "osmotic" and "saline" laxatives, respectively. However, mild diarrhoea can be taken as the most sensitive non-desirable effect if Mg supplements are taken for nutritional purposes. Mild diarrhoea occurs in a small percentage of adult subjects at oral doses of about 360/365 mg Mg per day, thus presenting the LOAEL. No laxative effects have been observed in adult men and women - also during pregnancy and lactation at doses up to 250 mg Mg per day. This dose is considered by EFSA as being the no-observed-adverse-effect level (NOAEL). The NOAEL was derived from studies in which pharmaceutical type of dosage formulation was taken in addition to Mg present in normal foods and beverages. The EFSA NOAEL is based on a mild, transient laxative effect, without pathological sequelae, which is readily reversible and for which considerable adaptation can develop within days. The NOAEL holds for adults, including pregnant and lactating women, and children from 4 years on. No data was available for children from 1 to 3 years, and since extrapolation of the UL for older children and adults on the basis of body weight was inappropriate, no UL was established for this age group. Diarrhoea induced by easily dissociable Mg-salts or compounds like MgO is completely reversible within 1 to 2 days and does not represent a significant health risk in subjects with intact renal function. Toxic hypermagnesaemia, presenting e.g. with hypotension or muscular weakness, is only seen at oral Mg doses greater than 2,500 mg, i.e. doses exceeding the UL by a factor of more than 10 (EFSA, 2006).

Elimination occurs almost exclusively via the kidneys with the urine. Individuals with impaired kidney functions run an increased risk of Mg toxicity (20).

## 4.8. Data gap assessment

Based on the available data there are some minor data gaps for the evaluation of oral administration of MgO.

Whilst several routes of synthesis for MgO NP have been described, data on the particle size distribution of MgO for the use as a pharmaceutical excipient is lacking. It appears reasonable however to assume that only minimal amounts, if any, would be present in MgO for the use in pharmaceutical formulations, and that these would pose no risk considering that MgO has been extensively used and studied.

Safety data of those MgO NP is rare and current studies do not fulfill the requirements by EFSA Guidance on risk assessment of nanomaterials to be applied in the food and feed chain (24).

# 5. Overall conclusions

MgO readily dissociates after reaction with gastric hydrochloric acid. A scientific opinion on Mg dietary reference levels was published in 2015 by the EFSA NDA Panel (1). Here it was concluded that based on a NOAEL of 250 mg Mg per day and an uncertainty factor of 1.0 an upper limit (UL) of 250 mg Mg per day can be established for readily dissociable magnesium salts like MgO. The EFSA NOAEL is based on a mild, transient laxative effect, without pathological sequelae, which is readily reversible and for which considerable adaptation can develop within days. The NOAEL holds for adults, including pregnant and lactating women, and children from 4 years on. Few cases of toxic hypermagnesaemia have been published, mostly due to the (ab-)use of Mg as laxatives or antacids in single doses. However, mild diarrhoea can be taken as the most sensitive non-desirable effect.

Considering the high NOAEL and relatively mild toxic effects associated with Mg intake, the available UL of 250 mg/day derived by regulatory authorities seems sufficient and it can be concluded that MgO is of low toxicity and concern.

# 6. Abbreviations

| | |
|---|---|
| bw | Body weight |
| BfR | German Federal Institute for Risk Assessment |
| EFSA | European Food Safety Authority |
| EFSA NDA | EFSA Panel on Dietetic Products, Nutrition and Allergies |
| EU | European Union |
| FDA | U.S. Food and Drug Administration |
| GRAS | generally recognized as safe |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LD50 | lethal dose, 50% |
| LOAEL | Lowest observed adverse effect level |
| Mg | Magnesium |
| $MgCl_2$ | Magnesium chloride |
| MgO | Magnesium oxide |
| NOAEL | No observed adverse effect level |
| NPs | Nanoparticles |
| Ph Eur | European pharmacopeia |
| SCF | Scientific Committee on Food |
| $TiO_2$ | Titanium dioxide |
| UL | Upper Limit |

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## *8.7 Summary microcrystalline cellulose*

# Microcrystalline cellulose, E460

# CAS 9004-34-6

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Lisa Wiesner (Takeda) |
| Peer review: | John Risvanis, Julia Kenny |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 20-Sep-2023 |

# Table of contents



# 1. Summary

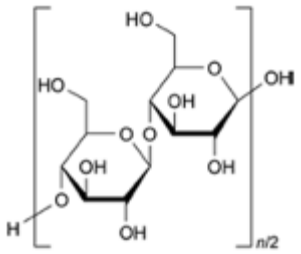
Microcrystalline cellulose with the CAS 9004-34-6 is a purified and partially depolymerized form of cellulose and used as inactive ingredient in many pharmaceutical formulations and as food additive. Cellulose is a natural polysaccharide derived from plant fibers (linear glucose homopolymer consisting of glucopyranose units linked by b-1,4-glycosidic bonds). Cellulose and its derivatives are essentially unabsorbed by the gastrointestinal tract following oral administration (2).

Microcrystalline cellulose is listed in the FDA inactive ingredient database (IID) with multiple oral administration forms (tablet, capsules, suspension, granules, powders) and potency levels. Further, it is approved as an indirect food additive (1).

Several regulatory assessment reports are available for cellulose and microcrystalline cellulose, and often a read-across approach between several cellulose derivates was performed This is justified by physico-chemical, structural, and biological similarities between cellulose forms (8). No numerical ADI limit is established by authorities, but it is generally concluded that microcrystalline cellulose (as well as celluloses as a group) is associated with no safety concern for human exposure.

## 2. General information

Microcrystalline cellulose (MCC) is an inactive ingredient in pharmaceutical formulations and is an approved food additive.

| | |
|---|---|
| Name | Microcrystalline cellulose (MCC) |
| Chemical name, synonyms | (6S)-2-(hydroxymethyl)-6-[(3S)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxane-3,4,5-triol, Diethylaminoethyl cellulose |
| CAS No. | 9004-34-6 |
| Molecular formula | $(C_6H_{10}O_5)n$ |
| Molecular weight | 342.30 g/mol (monomer)<br>As polymer: 36'000 g/mol (according to EFSA) |
| Chemical structure |  |
| Physico-chemical properties | Insoluble in water, dissolves in strong acidic or alkaline conditions. Substance on the EEA market in nanomaterial form (ECHA) |

## 3. Regulatory information and published limits

Microcrystalline cellulose is listed in the FDA inactive ingredient database (IID) with multiple oral administration forms (tablet, capsules, suspension, granules, powders) and potency levels (see Appendix 1 for details). Further, it is approved as an indirect food additive (1). In the Australia Food Standard Code (Schedule 15), Microcrystalline Cellulose is listed as substance that may be used as food additive with a limit up to 5000ppm (7).

The ADI assessment available by JECFA (4) determined no numerical ADI limit ("ADI not specified") and concluded based on the available toxicological data from humans and animals, that there is no evidence that the ingestion of microcrystalline cellulose (with particle sizes >5 µm) can cause toxic effects in humans when used in foods according to good manufacturing practice.

In a re-evaluation report by EFSA, the panel did not conclude a numerical ADI and anticipated no safety concern at the reported uses and use levels (estimated exposure of around 660-900 mg/kg bw per day) for the unmodified and modified celluloses (E 460(i); E 460(ii); E 461–466; E 468 and E 469) (5).

## 4. Safety assessment

### *4.1. Absorption, Distribution, Metabolism, and Excretion*

Cellulose and its derivatives are essentially unabsorbed by the gastrointestinal tract following oral administration or by the skin following topical application (2). There was no evidence of degradation or digestion and no radioactivity appeared in the urine of rats after oral exposure with microcrystalline cellulose. In human, radiolabeled microcrystalline cellulose was recovered in the faeces (>98%)

confirming the lack of absorption (4). Microcrystalline, powdered and modified celluloses are less fermented than other polysaccharides (such as gums, starches or pectins) (5).

## 4.2. Repeat-dose toxicity

Although no data were identified for cellulose, its derivatives have very low acute oral toxicities, are at most minimally irritating, and are not skin sensitizers (2).

In the majority of studies performed with microcrystalline cellulose and cellulose derivates, animals were dosed via diet at levels up to 10%. Effects on body weight at the highest dose tested (10%) were reported in some, but not all studies, which may reflect nutritional constraints rather than toxicity. No adverse effects were reported with most of the tested cellulose derivates, except for local effects on caecal weight and size due to the presence of undigested fiber.

In a reliable, GLP-relevant 90-day oral study performed in Sprague–Dawley rats (20/sex/group) doses in the diet containing 0 (control), 25,000 or 50,000 mg Avicel®CL-611/kg diet (microcrystalline cellulose and carboxy methyl cellulose) (equivalent to 0, 2,250 or 4,500 mg/kg bw per day). No treatment related clinical signs and no mortality occurred. No toxicologically relevant findings were present in male and females. There were slight effects on food consumption and body weight attributed to the decreased caloric intake and difference in test diet concentration compared to basal diet (e.g., some studies with cellulose derivates included high sodium concentration in the diet formulation). The authors of the study conclude a NOAEL of 4,500 mg/kg/day as the highest dose tested (5) for microcrystalline cellulose. Similar results and NOAEL ranges were concluded in further sub-chronic studies in rats with either pure microcrystalline cellulose or a mixture of cellulose derivates with mean particles sizes as low as 6 µm. The main effects seen in all repeated-dose studies were decreases in body weight gain at the highest dose, which are likely to be due to the amount/bulk of cellulose in the diet. NOAEL values reported range from 2,000 to 9,000 mg/kg bw per day (5).

## 4.3. Genetic toxicity

Mixtures of microcrystalline cellulose (85%) and guar gum did not induce mutagenic effects in the presence or absence of a metabolic activation system in bacterial reverse mutation assays, in a gene mutation assay in mouse lymphoma cells, in an in vitro test for unscheduled DNA synthesis and in the mouse bone marrow micronucleus assay (4, 5). Further, negative results of other unpublished genotoxicity assays with microcrystalline cellulose preparations were mentioned by EFSA (5). Overall, microcrystalline cellulose, as well as other forms of cellulose, were concluded to not raise concern for genotoxicity.

## 4.4. Chronic Toxicity and Carcinogenicity potential

Chronic toxicity studies have been performed with microcrystalline cellulose, and main effects observed in animal studies were decreased body weight gain at the highest dose, likely due to the amount/bulk of cellulose in the diet leading to nutritional imbalance. In a chronic (72 weeks) feeding study with microcrystalline cellulose, some dystrophic calcification of renal tubules was observed in the high dose group (15,000 mg/kg bw per day) and there was no increase in tumour incidence above controls. The NOAEL values reported ranged up to 9,000 mg/kg bw per day and it was overall concluded that there was no reason to expect carcinogenic properties with microcrystalline cellulose or other cellulose derivates (5).

## 4.5. Reproductive and developmental toxicity

Cellulose derivates were tested in mice, rats, hamsters and/or rabbits with oral dosing via gavage (5). Adverse effects on reproductive performance or developmental effects were not observed with modified and unmodified celluloses at doses greater than 1,000 mg/kg bw by gavage (often the highest dose tested). Formulation particle sizes ranged from as low as 1-50 µm (5). Several reproductive and

developmental toxicity studies on microcrystalline cellulose and derivatives did not identify any adverse effects at oral doses of up to 5 g/kg/day (3, 4).

### *4.6. Human data*

Patients received up to 35 g/person repeated doses of microcrystalline cellulose or powdered cellulose and it did not adversely affect clinical chemistry and hematological parameters. Further, no effect on absorption and or metabolism of the dietary constituents was observed (5). The available data in humans indicate that daily doses of up to 6,000 mg for around 8 months were not associated with adverse effects; however, in line with many other dietary fibres, large bolus intakes of celluloses were occasionally associated with laxation, but there was a lack of dose–response (5).

### *4.7. Data gap assessment*

The available data set and toxicity information with microcrystalline cellulose is extensive. Physical properties or particle size (including the nanoparticulate fraction) and distribution are not always available and represent a data gap.

## 5. Overall conclusions

The available toxicological information microcrystalline cellulose is extensive, and multiple studies were executed with cellulose derivatives. Microcrystalline cellulose is approved as indirect ingredient, in the use as a food additive as well as pharmaceutical excipient.

Data gaps are present for some of the cellulose derivative forms as well as the nanoparticle formulation of microcrystalline cellulose. Read-across was performed for the assessment of cellulose derivates by several regulatory bodies.

The ADI assessment available by JECFA (4) determined no numerical ADI limit (“ADI not specified”) and concluded based on the available toxicological data from humans and animals, that there is no evidence that the ingestion of microcrystalline cellulose (with particle sizes >5 µm) can cause toxic effects in humans when used in foods according to good manufacturing practice.

In alignment to US authorities, EFSA determined no numerical ADI for microcrystalline cellulose and based on the available toxicological dataset, considered no safety concern at the reported use levels (estimated exposure 660-900 mg/kg bw day) with unmodified and modified celluloses (5).

## 6. Abbreviations

ADI Acceptable Daily Intake

AI Adequate Intake

EFSA European Food Safety Authority

FDA U.S. Food and Drug Administration

JECFA Joint FAO/WHO Expert Committee on Food Additives

NOAEL No observed adverse effect level

PDE Permitted Daily Exposure

RDI Reference Daily Intake

UL Upper Limit

## 7. References

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# 8. Appendix 1

Summary of Inactive Ingredient Database - accessed 23-Aug-2023, showing approved levels of microcrystalline cellulose:

**Search Results for: microcrystalline cellulose**

Show 30 rows | CSV | Excel

Filter:

| Inactive Ingredient | Route | Dosage Form | CAS Number | UNII | Maximum Potency per unit dose | Maximum Daily Exposure (MDE) |
|---|---|---|---|---|---|---|
| MICROCRYSTALLINE CELLULOSE | BUCCAL | TABLET | 9004346 | OP1R32D61U | | 18mg |
| MICROCRYSTALLINE CELLULOSE | INTRAVITREAL | IMPLANT | 9004346 | OP1R32D61U | 1.66mg | |
| MICROCRYSTALLINE CELLULOSE | ORAL | CAPSULE | 9004346 | OP1R32D61U | | 2,169mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | CAPSULE, COATED PELLETS | 9004346 | OP1R32D61U | | 456mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | CAPSULE, DELAYED RELEASE | 9004346 | OP1R32D61U | | 366mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | CAPSULE, EXTENDED RELEASE | 9004346 | OP1R32D61U | | 1,246mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | CAPSULE, GELATIN COATED | 9004346 | OP1R32D61U | | 62mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | GRANULE | 9004346 | OP1R32D61U | | 29,520mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | GRANULE, DELAYED RELEASE | 9004346 | OP1R32D61U | 789.6mg | |
| MICROCRYSTALLINE CELLULOSE | ORAL | GRANULE, FOR SUSPENSION | | OP1R32D61U | | 173mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | PELLET | 9004346 | OP1R32D61U | | 251mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | POWDER, FOR SOLUTION | 9004346 | OP1R32D61U | | 690mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | POWDER, FOR SUSPENSION | 9004346 | OP1R32D61U | | 4,441mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | SUSPENSION | 9004346 | OP1R32D61U | | 1,285mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | SUSPENSION, EXTENDED RELEASE | 9004346 | OP1R32D61U | | 1,120mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET | 9004346 | OP1R32D61U | | 5,117mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, CHEWABLE | | OP1R32D61U | | 1,725mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, CHEWABLE, EXTENDED RELEASE | 9004346 | OP1R32D61U | | 144mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, COATED | 9004346 | OP1R32D61U | | 920mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, DELAYED RELEASE | 9004346 | OP1R32D61U | | 2,210mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, DELAYED RELEASE PARTICLES | 9004346 | OP1R32D61U | | 580mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, EXTENDED RELEASE | | OP1R32D61U | | 3,679mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, FILM COATED | 9004346 | OP1R32D61U | | 992mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, FILM COATED, EXTENDED RELEASE | 9004346 | OP1R32D61U | | 615mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, FOR SUSPENSION | 9004346 | OP1R32D61U | | 20,100mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, ORALLY DISINTEGRATING | 9004346 | OP1R32D61U | | 1,800mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, ORALLY DISINTEGRATING | 9004346 | OP1R32D61U | | 1800mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TABLET, ORALLY DISINTEGRATING, DELAYED RELEASE | 9004346 | OP1R32D61U | | 1,576mg |
| MICROCRYSTALLINE CELLULOSE | ORAL | TROCHE | 9004346 | OP1R32D61U | | 250mg |
| MICROCRYSTALLINE CELLULOSE | SUBLINGUAL | TABLET | 9004346 | OP1R32D61U | 43.2mg | |

Showing 1 to 30 of 30 entries

Previous

## *8.8. Summary Rice Starch*

# Starch

## Rice, Potato or Maize Starch (CAS No. 9005-25-8)
## Waxy Maize Starch (CAS No. 9037-22-3)

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Andreas Czich (Sanofi) |
| Peer review: | Verena Ziegler |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | February 08, 2024 |

# Table of contents



# 1. Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as opacifier in orally administered medicinal products, a safety assessment for Starch was performed to support the justification for its potential usage as alternative colouring agent. This document provides a summary of available safety data and related information for the following excipients: native starches including potato starch, maize starch (both CAS No. 9005- 25-8), and waxy maize starch (CAS No. 9037-22-3) and partially pregelatinized and pregelatinized starches (both CAS No. 9005-25-8).

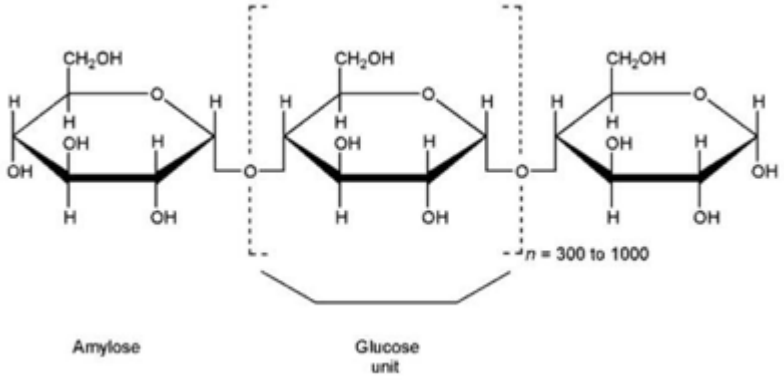
## 2. General information

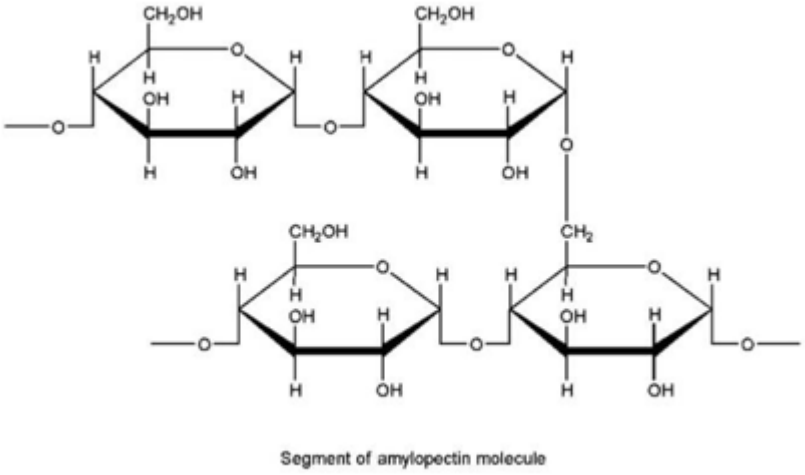
Native starches consist of linear amylose (with an average molecular mass of 105 -106g/mol) with a degree of polymerization of 1000–10,000 glucose units and branched amylopectin (with an average molecular mass of $10^6$-$10^7$ g/mol) and a degree of polymerization that may exceed one million, two polysaccharides based on α-(D)-glucose (1). Both polymers are organized in a semicrystalline structure, and in the starch granule, amylopectin forms the crystalline portion. The different configuration of these polymers results in different behaviour in cold aqueous solutions. Amylose (only linear 1,4 bonds) shows a high tendency for crystallization (retrogradation) resulting in insoluble adducts, whereas amylopectin (branched polymer) shows slow jellification, forming opaque and highly viscous preparations after some days (2). The diameter of the granules ranges from less than 1 µm to more than 100 µm, whereas shape can be angular, oval, round, spherical or irregular (3).

The molecular weight depends on the origin and the nature of the starch. Starches with high amylose content have lower molecular weight and a relatively more linear structure than those with a high content of amylopectin (4). Potato starch contains 20-23% of amylose, and its molecular weight (MW) is 69.5x106 g/mol (5), while the average particle diameter is of 46 µm. Among ten rice starch varieties, amylose content varied between 7.50 and 28.58%. The average size of the 10 investigated rice starch granules was 4–7 µm (6, 7). Maize (or corn) starch contains 24-28% of amylose, with MW of 51x106 g/mol and average particle diameter of 16 µm (1,2,4,5). A single amylopectin molecule from maize starch has molecular weight of 828.72 g/mol (6). Waxy maize or rice starch is a native starch variety with only <2% Amylose (8).

Pregelatinized starch is a starch that has been chemically and/or mechanically processed to rupture all or part of the starch granules. Starch gelatinization is a process of breaking down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water. This irreversibly dissolves the starch granule in water and depolymerization also occurs during the pregelatinization processes. Water acts as a plasticizer. Some types of pregelatinized starch may be modified to render them compressible and flowable in character (2, 9, 10).

Typically, pregelatinized starch contains 5% of free amylose, 15% of free amylopectin, and 80% unmodified starch. The USP32–NF27 does not specify the botanical origin of the original starch, but the PhEur 6.3 specifies that pregelatinized starch is obtained from maize (corn), potato, or rice starch. Normally the fully pregelatinized starch contains 20–30% amylose and the rest amylopectin, which is about the same ratio (1:3) as for the partially pregelatinized form (2).

| | |
|---|---|
| Name | Potatoe Starch, Maize Starch, Rice Starch, Pregelatanized Starch |
| Chemical name | (5-[5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-3,4-dihydroxyoxan-2-yl]oxy-6-(hydroxymethyl)-2-methyloxane-3,4-diol |
| Synonyms | Solanum tuberosum starch, Fecule, Corn starch, Amylum pregelificatum, Compressible starch |
| CAS No. | CAS 9005-25-8 |
| Molecular formula | $(C_6H_{10}O_5)_n$ where n= 300-1000 |
| Molecular weight | $69.5x10^6$g/mol (average) |
| Chemical structure |  |
| Physico-chemical properties | Insoluble in water at Room Temperature |

| | |
|---|---|
| Name | Waxy Maize Starch, Pregelatanized Starch |
| Chemical name | (5-[5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-3,4-dihydroxyoxan-2-yl]oxy-6-(hydroxymethyl)-2-methyloxane-3,4-diol |
| Synonyms | Amylopectin, Amylopectine, Amoica |
| CAS No. | CAS 9037-22-3 |
| Molecular formula | $C_{30}H_{52}O_{26}$ where n= 300-1000 amylopectin segments |
| Molecular weight | $51x10^6$ g/mol average |
| Chemical structure |  Segment of amylopectin molecule |
| Physico-chemical properties | Insoluble in water at Room Temperature |

## 3. Regulatory information and published limits

Starch is listed on Annex IV of the Regulation that includes the products that are exempted from registration under the REACH Regulation on the basis that “sufficient information is known about these substances that they are considered to cause minimum risk because of their intrinsic properties” (Art. 2.7). This entry covers all botanical origins from which starch can be produced. Reference to “corn, wheat and sorghum, and from roots and tubers such as potatoes and tapioca”, is introduced in the text of the Regulation by the expression “such as” and it is therefore made as a way of example (11). Additionally, native starch is regarded as one substance, irrespective its botanical origin, when deciding that it is eligible to Annex IV (12).

Considering the fact that native starches are metabolized with metabolites being a standard energy source (e.g. glucose), and considering also their uses as a food ingredient and additive in cosmetics and in medicinal products, native starches are of low toxicological concern and no risks to human health are expected from its use (13) and an ADI was implied to be not necessary (14, relatively to maltodextrin).

The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) delivered a scientific opinion on the essential composition of infant and follow-on formula, where it is reported that permitted carbohydrates in infant formulas are lactose, maltose, sucrose, glucose, maltodextrins, glucose syrup (or dried glucose syrup), pre-cooked starch and gelatinized starch free from gluten. Maltodextrin can be used under the following conditions: “Unrestricted within specifications for total carbohydrates” which 45-70 g carbohydrate/day assuming an energy intake from formula of 500 kcal/day (average requirement for energy of boys and girls aged three to four months) (15).

EFSA reported that, based on the available evidence and assuming that infants can tolerate starch in amounts of around 5.5 g/kg/day, and that average body weight at birth is 3.25 kg, this would translate

into a daily starch intake of 18 g/day which could theoretically be tolerated by newborns. However, lower tolerances have also been reported. Assuming an average formula consumption of 500 kcal/day, a daily starch intake of 18 g/day. The Panel also notes that there are considerable uncertainties about the amount of starch which can be tolerated by newborns and that no adverse effects from current amounts of starch in infant formula have been reported (16).

In cosmetics, maize starch is widely used, mainly as an abrasive, absorbent, skin protectant, and a viscosity increasing agent and is used at concentrations up to 99% (17).

As reported in the Food and Drug Administration's Inactive Ingredient Database (IID), native starches are used in oral formulations up to 3280 mg/day (starch, corn) and 1600 mg/day (pregelatinized) Maximum Daily Exposure (MDE) in capsule (18).

# 4. Safety assessment

Toxicological studies on native or pregelatinized starches are limited. Both amylose and amylopectin have been evaluated as safe and without limitation for daily intake (2, 19).

Starches are metabolized to metabolites such as glucose. It has a long history for use as a food supplement, excipient in cosmetics and in medicinal products. Based on the above considerations, native starches can be considered of low toxicological concern and no risks to human health are expected from its use (2, 13)

## *4.1. Absorption, Distribution, Metabolism, and Excretion*

Nutritional properties of carbohydrates depend on their rate and extent of digestion and absorption in the small intestine (20). The type of monosaccharide absorbed, and the presence of other nutritional components such as fat, dietary fiber, and protein, also influences the physiological response to carbohydrates. Only monosaccharide species like glucose, fructose and galactose can be absorbed via active membrane transport systems. Disaccharides and polysaccharides have to be split into their monosaccharide components to be absorbed (21).

From a biological point of view, starch is classified in slowly digestible starch (SDS), rapid digestible starch (RDS), and resistant starch (RS) on the basis of the rate of enzymatic digestion.

Native starches provide carbohydrates to body cells, to be metabolized up to monosaccharides, such as glucose, the only molecules to be absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and is continued in the small intestine by pancreatic amylase. Starch with high amylopectin content is more easily digested, whereas starch with a low amount of amylopectin act as a source of slowly digestible starch (SDS). The degradation products – mainly maltose and oligosaccharides – are hydrolysed further to glucose by a set of enzymes, "disaccharidases", bound to the brush border membrane of enterocytes. The same enzymes hydrolyse the dietary disaccharides. Glucose is absorbed efficiently by a secondary active carrier coupled with sodium (sodium glucose transporter 1, SGLT1). The absorption of monosaccharides is regarded as the rate-limiting step (15). Starches that are relatively high in amylose content tend to be more resistant to digestion than starches with higher amylopectin content. Considering this, starch can be divided into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (22). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine leading to a rapid elevation of blood glucose and usually a subsequent episode of hypoglycaemia. These rapid and large increases in blood glucose levels can further lead to cell, tissue and organ damage(23). RS is not digested in the upper gastrointestinal tract but is fermented by the colonic microflora, producing short chain fatty acids that provide additional energy to the body along with butyrate that is beneficial to colonic health. SDS is digested slowly throughout the small intestine to provide sustained glucose release with a low initial glycemia and subsequently a slow and prolonged release of glucose, leading to prolonged energy availability, compared to more rapidly digestible starch (20). Pregelatinized starch is a RDS starch totally digestible in the small intestine (24).

Absorbed monosaccharides are transported to the liver and then to the systemic circulation. The cellular uptake is mediated by a number of glucose transporters (GLUT1-4), variously expressed in different tissues. Insulin is a key hormone for the uptake and metabolism of glucose. The plasma insulin concentration increases immediately after ingestion of starches. Unlike glucose, fructose enters body cells without the need for insulin. The metabolism of fructose, however, favours lipogenesis more than glucose. In liver cells, fructose is phosphorylated to fructose-1-phosphate that can be converted to fatty acids, providing a route for lipogenesis in addition to that shared with glucose (via glucose/fructose-6-phosphate). Both fructose and galactose, the latter arising from hydrolysis of lactose, are also transformed to glucose mainly in the liver (15).

## *4.2. Repeat-dose toxicity*

Although repeated dose toxicity studies known in literature were not designed for toxicological evaluation per se, the general lack of adverse effects with high dosages, also tens of g/kg/day, provides support that the ingestion of native starches would not pose any safety concerns.

Male rats given starch as a 60% (w/v) paste in distilled water by gavage for 14 consecutive days at levels up to 168 g/kg bw/day showed little, if any, signs of intoxication. In these animals, water was absorbed from the paste in the stomach and upper bowel, and the starch was converted to a calculus. Probably as a result, considerable hypertrophy of the smooth muscle of the gastro- intestinal tract was seen after 14 days of exposure. A subsequent increment of the daily dose for 2-7 weeks resulted in some inhibition of growth at dose levels of 10% of body weight. At dose levels of 20% of body weight, increased susceptibility to pneumonia and bowel obstruction owing to the inability of the animal to evacuate the starch calculi were observed (25).

Male Wistar rats (n=10) fed diets containing 71% of different starches as dietary carbohydrate for 3 weeks showed no indications of short-term toxic effects. When 16% raw potato starch was added to 55% maize starch, a marked increase in caecal weights was noted relative to animals receiving only maize starch (71%, equivalent to 35.5 g/kg/day). Marked thickening of caecal mucosa and submucosa were noted at histological examination. In addition, lymphatics were prominent, and there were indications of hypertrophy of the musculature and slight oedema of the mucosa and submucosa. It is noted by the authors that raw potato starch is relatively resistant to pancreatic amylase. The caecal enlargement after starch ingestion is caused by its resistance to enzymatic hydrolysis, changes in intestinal microflora, osmotic load in the caecum, cell hyperplasia and rate of turnover of mucosal cells (26).

Slight growth retardation was seen in rats exposed for 4 weeks to raw potato starch at a dietary level of 40% (equivalent to 20 g/kg/day) (27).

Waxy maize starch was used as reference (control) substance in some repeated toxicity studies performed to assay a toxicological profile of some modified starches.

Using Sprague-Dawley rats, a control group of 25 females fed a diet containing 30% waxy maize starch (equivalent a 15 g/kg/day) both in a 1-year study in weanling rats (Experiment I) and in a separate 9-month study utilizing 9-month-old rats (Experiment II). Body weight, food consumption, urine volume, urine pH and crystal content or faecal mineral content were within normal range in both experiments (28).

Groups of 8 Pitman-Moore miniature pigs were weaned at 3 days of age (a model similar to human infants), and were fed formula diet containing 5.4% waxy maize starch (equivalent to about 1.5 g/kg bw/d) or three modified starches for 25 days. Body weight gain, chemical values for blood and serum, and relative organ weight, as well as carcass composition and liver composition were within normal ranges (29).

A transgenic rice line (TRS) with high amylose level has been developed by antisense RNA inhibition of starch branching enzymes. In a 90-day toxicology feeding experiment in Sprague-Dawley rats fed with diets containing 70% of either TRS rice flour, its near-isogenic rice flour or the control diet. The clinical performance variables (body weight, body weight gain and food consumption) were measured and

pathological responses (hematological parameters and serum chemistry at the midterm and the completion of the experiment, urinalysis profile and serum sex hormone response at the completion of the experiment) were performed. Besides, clinical signs, relative organ weights and microscopic observations were also compared between TRS group and its near-isogenic rice group. In this 90-day feeding study, no adverse effect was observed in rats consuming native rice or transgenic TRS rice (30).

## *4.3. Genetic toxicity*

No data available.

Generally, Native starches, as food ingredients, are considered free from potential genotoxicity (31).

## *4.4. Carcinogenicity potential*

Feeding of unmodified potato starch and maize starch to groups of rats at dietary levels up to 30% (equivalent up to 15 g/kg/day) in a 2-year test and 10% (food intake not indicated, but equivalent to 5 g/kg/day) in a 3-generation test did not result in distinct toxicologically significant effects (32).

Rats fed a cooked diet containing 62% unmodified maize starch (equivalent to 31 g/kg bw/d) for 2 years also did not show significant toxicological effects, including reproductive effects over 3 generations (33).

A search of the NTP website identified 51 monographs where starch capsules were tested as a negative control.

## *4.5. Reproductive and developmental toxicity*

Native starches, as natural ingredient of food, may be considered devoid of potential reproductive and developmental toxicity, based on the dietary reproductive toxicity studies available for raw potato and maize starches. No effect on reproductive performance or maternal and developmental toxicity were observed in the three-generation reproductive studies at dietary levels of up to 62% (equivalent to 31 g/kg/day) (32, 33).

The transgenic rice line (TRS) enriched with amylose and resistant starch (RS) was developed by antisense RNA inhibition of starch-branching enzymes. In the 3 generation study, clinical performance, reproductive capacity and pathological responses including body weight, food consumption, reproductive data, hematological parameters, serum chemistry components, organ relative weights and histopathology were examined. Some statistically significant differences were observed in rats consuming the high amylose rice diet when compared to rats fed the near-isogenic control rice diet or the conventional (non-rice) standard diet. These differences were generally of small magnitude, appeared to be random in nature, and were within normal limits for the strain of rat used, and were therefore not considered to be biologically meaningful or treatment related. Therefore, it was concluded that in this three generation study, no adverse reproductive or developmental effect was observed in rats consuming transgenic TRS rice diet compared with the conventional non-transgenic TQ (near-isogenic non-GM rice line) rice diet. Therefore, transgenic TRS rice should be as safe as the near isogenic TQ rice (34).

No prenatal developmental toxicity studies were available.

## *4.6. Additional safety data*

In literature, for CAS 9005-25-8, only the intraperitoneal LD50 in the mouse is available: 6600 mg/kg (35).

A repeated insult patch test (HRIPT) was done on 99 participants (26 male, 73 female, ages 18-70) using feminine powder containing 97% corn starch. A patch was applied to skin for 24 hours, after which it was removed. The same area was then repatched either 24 or 48 hours after the removal. This was

repeated until 9 induction patchings were completed. Approximately 2 weeks after induction patching, a challenge patch was placed on a new site and then removed after 24 hours. The induction and challenge sites were observed at 48, 72, and 96 hours after the removal of the challenge patch. Four cases of faint, minimal erythema were observed during the induction phase, and there was no observed irritation during the challenge phase. This same methodology was repeated with 109 participants (35 male 74 female, age 18-68) completing the study. There was no observed irritation in the participants (17).

Allergic reactions to starch are extremely rare and individuals apparently allergic to one particular starch may not experience adverse effects with a starch from a different botanical source (2).

### *4.7. Human data*

Clinical data in pediatric population

Pancreatic α-amylase, the chief enzyme which hydrolyses starch, has lower concentrations in the infant's duodenum than in adults (36). A theoretical concern in feeding glucose polymers to young infants is that pancreatic α-amylase, is secreted in a low quantity during the first six months of life. However, intestinal glucoamylase, salivary amylase and mammary amylase from breast milk may compensate for pancreatic amylase deficiency in infants. Clinical studies in young infants have shown significant hydrolysis of glucose polymers in the proximal intestine (37, 38, 39).

Fomon (40) suggested that starch is tolerated up to daily intakes of 5.5-6 g/kg body weight per day and that most infants from one to five months of age are able to digest 10-25 g of starch per day.

Based on the available evidence, considering a body weight at birth of 3.25 kg, a daily starch intake of 18 g/day could theoretically be tolerated by newborns. The authors however suggest that lower tolerances might be applicable for some individuals (15, 40).

### *4.8. Data gap assessment*

Beside the daily natural uptake of starch by food, for many different types of starch, toxicological data including carcinogenicity studies exist. In few cases like wheat starch, gluten could be an impurity, this is not reported for starch from other plant sources. The differences between the various types of Starch are mainly in the ratio of Amylose and Amylopectin and the granule size. Both characteristics do not have an impact on the safety of the different types.

Overall, no relevant data gap is identified.

## 5. Overall conclusions

Toxicological studies on native or pregelatinized starches are limited. However, both amylose and amylopectin have been evaluated as safe and without limitation for daily intake (2, 19). According to FDA IID native starches are used in oral formulations up to 3280 (starch, corn) mg/day and 1600 mg/day (pregelatinized) Maximum Daily Exposure (MDE) in capsule (17). In addition, starch is listed in the Inactive ingredient data base from FDA.

Starches are metabolized to metabolites such as glucose. It has a long history for use as a food supplement, excipient in cosmetics and in medicinal products. Therefore, starch is considered to be safe as indicated by its GRAS status, it is already in use as excipient for pharmaceuticals in different regions and REACH and EFSA reports are coming to the same conclusion.

Based on the above considerations, native starches can be considered of low toxicological concern and no risks to human health are expected from its use (2, 13).

# 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AI | Adequate Intake |
| ECHA | European Chemicals Agency |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| GRAS | Generally Recognized As Safe |
| HRIPT | Human Repeated Insult Patch Test |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| MDE | Maximum Daily Exposure |
| MW | Molecular weight |
| NOAEL | No observed adverse effect level |
| NTP | National Toxicology Program |
| PDE | Permitted Daily Exposure |
| RDI | Reference Daily Intake |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| SCF | E.U. Scientific Committee for Food |
| SDS | Slowly digestible starch |
| RDS | Rapid digestible starch |
| RS | Resistent starch |
| TRS | Transgenic rice line |
| TQ | near-isogenic non-GM rice line |
| UL | Upper Limit |

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## *8.9. Summary trisodium phosphate (sodium phosphate, tribasic, anhydrous) and tetrasodium phosphate*

# Trisodium phosphate (sodium phosphate, tribasic, anhydrous), E339

# CAS 7601-54-9

# and

# Tetrasodium pyrophosphate (TSPP), E450

# CAS 7722-88-5

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | Lisa Wiesner (Takeda) |
| Peer review: | Dr. Claudia Sehner, John Risvanis, Ph.D. |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 20-Sep-2023 |

# Table of contents



# 1. Summary

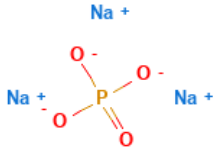
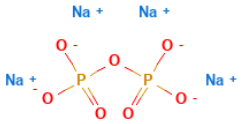
A safety assessment for trisodium phosphate and tetrasodium pyrophosphate was performed to support the justification for its potential usage as alternative colouring agent to titanium doixide ($TiO_2$). A previous safety summary was provided by the supplier Lonza for both phosphates, and the consortium agrees with their conclusion. For transparency, Appendix 1 includes the complete supplier assessment.

Trisodium phosphate (sodium phosphate, tribasic, anhydrous) with CAS 7601-54-9, also known as E339, is an approved food additive and food coloring substance in the EU and listed in the FDA Inactive Ingredients Database (IDD) with approved amounts up to 528 mg and no related safety issues. Tetrasodium pyrophosphate (E450) with CAS 7722-88-5, is an approved food additive in the EU and listed in the FDA Inactive Ingredients Database (IID) with approved amounts up to 298 mg (please refer to Appendix 2 for additional details) and no related safety issues.

Toxicological information for sodium phosphate is vastly described in the EFSA assessment report. The group acceptable ADI (Acceptable Daily Intake) for phosphates expressed as phosphorus is 40 mg/kg/day (1).

# 2. General information

Phosphates are used as pharmaceutical excipients and food additives. Phosphates are essential for living organisms (e.g., regulation of metabolic processes, supplying energy, or being a component DNA, RNA or phospholipids and present in bones) (2).

| | |
|---|---|
| Name | Trisodium phosphate |
| Chemical name, synonyms | Sodium phosphate, tribasic, anhydrous, TSP, E339 |
| CAS No. | 7601-54-9 |
| Molecular formula | $Na_3PO_4$ |
| Molecular weight | 163.941 g/mol |
| Chemical structure |  |
| Physico-chemical properties | Freely soluble in water (120 g/L, 20°C). Insoluble in ethanol (6) |
| | |
| Name | Tetrasodium pyrophosphate (E450) |
| Chemical name, synonyms | Tetrasodium diphosphate, TSPP, E450 |
| CAS No. | 7722-88-5 |
| Molecular formula | $Na_4P_2O_7$ |
| Molecular weight | 265.90 g/mol |
| Chemical structure |  |
| Physico-chemical properties | Solubility in water: 3.16 g/100 mL (cold water); 40.26 g/100 mL boiling water (6) |

## 3. Regulatory information and published limits

Currently, phosphates (E 338–341, E 343, E 450–452) are authorized food additives in the EU with maximum permitted levels (MPLs) ranging from 500 to 20,000 mg/kg in 104 authorised uses (1). Phosphates – as a group of additives – have been previously assessed by EU SCF (most recently in 1997) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (most recently in 2001), determining a “maximum tolerable daily intake (MTDI)” of 70 mg/kg bw per day (expressed as phosphorus) for the sum of phosphates and polyphosphates, both naturally present in food and ingested as food additives (7). In the GRAS FDA list (generally recognized as safe), sodium phosphates are generally recognized as safe when used in accordance with good manufacturing practice (3).

EFSA launched a re-evaluation of phosphoric acid-phosphates-di, tri- and poly-phosphates (E338-341, E343 and E450-452) as food additives and concluded a group “Acceptable Daily Intake (ADI)” of 40 mg/kg/day for phosphates expressed as phosphorus to be protective for the human population (1).

## 4. Safety assessment

### *4.1. Absorption, Distribution, Metabolism, and Excretion*

Inorganic phosphate (including trisodium phosphate and Tetrasodium pyrophosphate) as food additive dissociates in the gastrointestinal lumen to salt and phosphate. Phosphate is then well absorbed as free

orthophosphate in the small intestine with amounts ranging between 55 and 90% of the dose and absorption may be dependent on the sodium active transport, as well as the calcium content in the diet. Excretion is via the kidney through glomerular filtration (1).

Cations of phosphates are parts of human tissues that occur naturally in food. It it is assumed that their intake would not cause adverse effects in humans, considering the intake does not disturb the homeostatic mechanism controlling the electrolyte balance of the body (2).

## *4.2. Repeat-dose toxicity*

Results of multiple oral toxicity studies in rats and dogs ranging from 28 to 150 days length, demonstrated the kidney to be a target organ of phosphates at high doses. Excess phosphate intake causes increased bone demineralization and release of calcium. This mechanism is part of a physiological regulatory mechanism leading to calcification of the kidney and tubular nephropathy. EFSA summarized the acute and repeat-dose toxicity studies and noted that the highest reliable NOAEL for kidney effects is 500 mg/kg bw per day corr. to 116 mg P/kg bw per day, as identified in a 90-day rat study with tetrasodium diphosphate. In the same study, a dose of 1000 mg/kg bw per day (corr. to 233 mg P/kg bw per day) was identified as LOAEL and demonstrated to induce kidney effects (1). For chronic studies, see chapter 4.4.

## *4.3. Genetic toxicity*

Phosphoric acid, phosphates, diphosphates, triphosphates, and polyphosphates have been tested for genotoxicity in a variety of *in vitro* and *in vivo* assays. The in vitro tests included the Ames/Salmonella typhimurium mutagenicity assay (with and without metabolic activation), the Saccharomyces cerevisiae mutagenicity assay (with and without metabolic activation), the chromosome aberrations assay (Chinese hamster fibroblasts), and the in vitro cytogenetics assay (human lung cells). The in vivo tests included the dominant lethal test (rats), host-mediated assay (mice), and the mouse translocation test. In neither of the studies, did any of the tested phosphates produce a positive response (1).

## *4.4. Carcinogenicity potential*

Three 2-year dietary carcinogenicity studies in rats are available, one conducted with sodium triphosphate and two with sodium polyphosphate. No relationship between treatment with the phosphates and tumor development was observed in any of the studies. EFSA Panel concluded that phosphates do not have any carcinogenic potential. The key adverse effects in the three lifetime studies as well two additional chronic toxicity of 6-month duration were calcification in the kidneys and tubular nephropathy. The lowest tested level of phosphate causing an effect in the kidney was approx. 750 mg/kg bw (corr. to 229 mg P/kg bw per day in a 2-year study with sodium metaphosphate [metaphosphate is the general term for any polyphosphate salt with ≥4 phosphate units]). Two reliable NOAELs could be identified to be 250 mg/kg bw per day (corr. to 63 mg/kg P bw per day) and 250 mg/kg bw per day (corr. to 76 mg/kg P bw per day) with sodium triphosphate and sodium hexametaphosphate, respectively (2).

## *4.5. Reproductive and developmental toxicity*

Extensive toxicological information with phosphates is available and mainly summarized by EFSA, 2019a (1).

Reproductive and developmental toxicity studies were conducted in mice, rats, rabbits and hamster although generally not conducted to current OECD guidelines. Nevertheless, in the performed studies, no signs of reproductive or developmental toxicity at any dose tested was observed. Therefore, the endpoint is concluded to be negative for phosphates, like sodium phosphate.

### *4.6. Human data*

Epidemiology studies conducted in humans had limitations towards confounding factors (e.g., diet and physical activity) and could not find consistent associations between dietary phosphorus intake and cardiovascular-related outcomes. Two other studies investigated the effect of serum phosphorus on BMD (bone mineral density), but the results could not provide sufficient and reliable data to assess the role of phosphates on bone health. Taking into account several clinical case reports in human at added phosphate doses threefold lower than the causing adverse renal effects in animals (4800 mg/day or 68.6 mg P/kg bw per day) elicited renal impairment in human (1). No impairment of the renal function was reported with daily doses up to 2,000 mg phosphorus (28.6 mg/kg per day).

In several of the studies using phosphorus doses up to 2,000 mg/day, the subjects had soft stools or diarrhoea which is not to be seen as adverse but is classified as discomfort. When higher doses are given, such as the doses for bowel cleansing in preparation for colonoscopy (e.g., 11,600 mg/kg or 165.7 mg/kg bw) these doses acted as a cathartic agent and this effect is seen as adverse (1).

### *4.7. Data gap assessment*

The available toxicological information for each phosphate salt is limited and the overall phosphate assessment as food additive and pharmaceutical excipient is based on read-across approaches and a group specific toxicity assessment for several phosphate salts. While not assuming that there would be grave differences in toxicity, different salts could express different oral bioavailability or solubility in water.

## 5. Overall conclusions

The EFSA derived a group ADI for phosphates and its salt of 40 mg/kg bw per day (expressed as P). This was based on the NOAEL from repeat-dose toxicity studies in rat (76 mg/kg bw per day as P), adding the background dietary phosphorus of 91 mg/kg bw per day giving a total value of 167 mg P/kg bw per day and applying an uncertainty factor of 4 (2 for interspecies toxicokinetic differences x 2 for interindividual toxicodynamic differences). Both phosphates, E339 and E450, are considered to be of low toxicity concern for human exposure as pharmaceutical excipient.

This conclusion is supported by the listing of both phosphates as food additives in the EU and in the Inactive Ingredient Database by FDA, and as approved excipients in pharmaceutical formulations (buccal tablet, capsule, powder, and oral suspension formulation).

## 6. Abbreviations

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AI | Adequate Intake |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| NOAEL | No observed adverse effect level |
| PDE | Permitted Daily Exposure |
| RDI | Reference Daily Intake |
| UL | Upper Limit |

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2. EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D, Castenmiller, J, de Henauw, S, Hirsch-Ernst, K-I, Kearney, J, Knutsen, HK, Maciuk, A, Mangelsdorf, I, McArdle, HJ, Pelaez, C, Pentieva, K, Siani, A, Thies, F, Tsabouri, S, Vinceti, M, Aggett, P, Fairweather-Tait, S, Martin, A, Przyrembel, H, Ciccolallo, L, de Sesmaisons-Lecarré, A, Martinez, SV, Martino, L and Naska, A, 2019. Scientific Opinion on the dietary reference values for sodium. EFSA Journal 2019;17(9):5778, 191 pp. https://doi.org/10.2903/j.efsa.2019.5778
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# 8. Appendix 1

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To whom it may concern

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Basel, effective date 16 June 2022

## Toxicological assessment for E339 and E450

**Summary**
In order to support innovative solutions for Capsule Health Ingredients (CHI) at Bornem site for substituting Titanium Dioxide (TiO2) as coloring agent in medicinal products, a toxicological assessment for two food additives - E339 and E450 - is needed to support the justification for their usage as coloring alternatives to TiO2.

The following table lists the two alternative compounds, and the following chapters of this memo document, will list the publicly available safety information for both phosphate compounds:

Table 1: Compound characteristics and numerical identifier of E339 and E450, already approved as food additives

| Compound | CAS | Structure | MW | Health hazards | ADI/PDE |
|---|---|---|---|---|---|
| **Trisodium phosphate Trisodium orthophosphate, E339iii** | 7601-54-9 |  | 163.941 g/mol<br><br>Na3PO4 | Causes skin irritation. | EFSA Panel derived a group acceptable daily intake |

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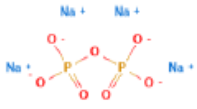
| | | | | | |
|---|---|---|---|---|---|
| Tetrasodium Pyrophosphate, E450iii, TSPP | 7722-88-5 |  | 265.90 g/mol<br><br>Na4P2O7 | Harmful if swallowed. Causes skin irritation. Causes serious eye damage. Causes serious eye irritation. May cause respiratory irritation. | (ADI) for phosphates expressed as phosphorus of **40 mg/kgbody weight (bw) per day** |
| Sodium, Natrium (Na) | 7440-23-5 | Na | 22.9 g/mol | None | EFSA states an adequate intake of 2gram sodium/day for adults. For infants, the AI is 0.2 gram/day. |

**Safety assessment**

Phosphates are authorized food additives in the EU in accordance with Annex II and III to Regulation (EC) No 1333/2008. Annex II to this regulation further defines a general maximum level of 10'000 mg/L or mg/kg as appropriate (expressed a P2O5) for phosphates and polyphosphates (E338-452), unless a different maximum level is specified in relation to individual foods or categories of foods. These specific limits vary between 800 mg/kg or mg/L for food category "04.2.4.1 fruit preparations" to *quantum satis* (not subject to quantity restrictions) in the food category "17.1 food supplements" (Regulation (EC) No 1333/2008). Phosphates – as a group of food additives – also have been previously assessed by EU SCF (most recently 1997) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (most recently 2002), determining a 'maximum tolerable daily intake'(MTDI) of 70 mg/kg bw per day (expressed as phosphorus) for the sum of phosphates and polyphosphates, both naturally present in food and ingested as food additives. FDA lists (sodium) phosphates as generally recognized as safe (GRAS) when used in accordance with good manufacturing practice (FDA, 21CFR182.1778).

Most recently, EFSA launched the re-evaluation of phosphoric acid–phosphates – di-, tri- and poly-phosphates (E 338–341, E 343, E 450–452) as food additives and concluded a group Acceptable Daily Intake (ADI) of **40 mg/kg/day** for phosphates expressed as phosphorus to be protective for the human population (EFSA, 2019a).

Inorganic phosphate as food additive dissociates in the gastrointestinal lumen to salt and phosphate. Phosphate is then well absorbed as free orthophosphate in the small intestine and absorption may be dependent on the sodium active transport, as well as the calcium content in the diet. Excretion is via the kidney through glomerular filtration.

Numerous toxicology studies are available for phosphates used as food additives, but they are generally old and not necessary performed according to current (GLP) guidelines.
Further, cations of phosphates are parts of human tissues that occur naturally in food. It is assumed that intake of them does not cause adverse effects in humans, considering the intake does not disturb the homeostatic mechanisms controlling the electrolyte balance of the body. For sodium, the EFSA panel, concludes an adequate intake (AI) for the adult EU population of 2 gram/day, and for infants aging 7-11 months of 0.2 gram/day (EFSA, 2019b). The salt is not further investigated in this memo document, and the following chapters state a summary of the available toxicology studies for phosphates.

**Toxicology data**

Results of multiple studies in rats and dogs ranging from 28 to 150 days demonstrated the kidney to be a target organ to phosphates at high doses. At high doses, excess phosphate causes increased bone demineralization and release of calcium. This mechanism is part of a physiological regulatory mechanism leading to calcification of the kidney and tubular nephropathy. EFSA summarized the acute and repeat-dose toxicity studies, and noted that the highest reliable NOAEL for kidney effects is 500 mg/kg bw per day corresponding to 116 mg/kg bw per day phosphorus, as identified in a 90-day rat study with tetrasodium diphosphates. In the same study, a dose of 1,000 mg/kg bw per day corresponding 233 mg/kg bw per day phosphorus was identified as LOAEL demonstrated to induce effects in the kidney (EFSA, 2019a).
Phosphoric acid, phosphates, diphosphates, triphosphates and polyphosphates have been tested for genotoxicity in a variety of in-vitro and in-vivo assays. In neither in-vitro nor in-vivo assays did any of the tested phosphates produce a positive response. Standard tests conducted were the Ames assay in *Salmonella,* and *E.coli,* as well as chromosomal aberration test of Comet assay (EFSA, 2019a).
Three 2-year carcinogenicity studies in rats are available, one with sodium triphosphate and two with sodium polyphosphate. In none of the studies were there any relationship between treatment with the phosphates and tumour development. EFSA Panel thus concluded that phosphates do not have any carcinogenic potential. The key adverse effects in these three life time studies as well as in two chronic toxicity studies of 6 months duration were calcification in the kidneys and tubular nephropathy. The lowest tested level of phosphate causing an effect in the kidney was approximatively 750 mg/kg bw (corresponding to 229 mg P/kg bw per day) in a 2-year study with sodium metaphosphate. Two reliable NOAELs could be identified, 250 mg/kg bw per day (corresponding to 63 mg/kg P bw per day) and 250 mg/kg bw per day (corresponding to76 mg/kg P bw per day) with sodium triphosphate and sodium hexametaphosphate, respectively (EFSA, 2019a).
For the reproductive and developmental toxicity endpoint, there are some studies conducted in mice, rats, rabbits or hamsters, but none follow the current GLP guidelines. Still, in the performed studies in named animal species, showed no signs of reproductive or developmental toxicity at any dose tested. Thus, this endpoint is concluded to be negative.

**Data gap assessment**

Epidemiology studies conducted in humans, had limitations towards confounding factors (e.g., diet and physical activity) and could not find consistent associations between dietary phosphorus intake and cardiovascular-related outcomes. Two other studies, investigated the effect of serum phosphorus on BMD (bone mineral density), but the results could not provide sufficient and reliable data to assess the role of phosphates on bone health. Taking into account several clinical case reports in humans, a chronic exposure indicate that adverse effects on the kidney have been reported in human at added phosphates doses threefold lower than that causing adverse renal effects in animals (4,800 mg/day (68.6 mg/kg per day) elicited renal impairment in humans) (EFSA, 2019a).

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The assessment for phosphates as food additives is based on read-across approaches and a group specific toxicity assessment, as there is not sufficient study information available for each phosphate salt separately. While not assuming that there would be grave differences in toxicity, different salts could still express different oral bioavailability or solubility in water.

**Exposure assessment**

Based on the below product composition for a size 0 Capsugel® white TiO2-free empty hard gelatin capsule as recently launched by Lonza CHI, the following amount of phosphates are considered per capsule:

Qualitative and Quantitative composition*

| Composition per unprinted capsule part | | Body represents about 60% of the capsule weight | | | | | |
|---|---|---|---|---|---|---|---|
| | | Body (44.407) | | Cap (44.407) | | Total Capsule | |
| Name of ingredient(s) | Function | % | mg/body | % | mg/cap | % | mg/capsule |
| Gelatin | Structure | QSP 100% | 53.8687 | QSP 100% | 35.9124 | QSP 100% | 89.7811 |
| Tetrasodium pyrophosphate | opacifier | 5.3983% | 3.1094 | 5.3983% | 2.0729 | 5.3983% | 5.1823 |
| Trisodium phosphate | opacifier | 1.0797% | 0.6219 | 1.0797% | 0.4146 | 1.0797% | 1.0365 |
| | | 100 | 57.6000 | 100 | 38.4000 | 100 | 96.0000 |

*The quantities given are based on the theoretical calculations of the average specification weight of the capsule.
Due to the nature of raw materials, their sourcing and technology improvements, the dyestuff content data indicated are target values and actual values may vary.

Figure 1: Extract of the Capsule product composition information, showing the amount of phosphates per empty size 0 hard gelatin capsule.

In total, considering both phosphate compounds – tetrasodium pyrophosphate and trisodium phosphate – the amount of 5.18 mg (1.2mg P) + 1.03mg (0.26mg P) = 6.2 mg phosphate (corresponding to ~1.5 mg Phosphor/day) per capsule is expected as estimated daily intake (EDI). Taking into account the determined ADI of 40 mg/kg/day per person expressed for phosphorus, the margin of safety for the 50kg patient can be calculated by the ratio between ADI and EDI. In the calculation it is assumed, that the realistic daily capsule intake is 12 capsules per day (12*1.5mg phosphor/day) as per reference of The Physicians' Desk Reference 70th Edition, 2016.

The calculation is the following:

$$\text{Margin of Safety } (MoS) = \frac{2'000\frac{mg}{day}(ADI)}{18\frac{mg}{day}(EDI)} = \sim 110$$

The safety margin is >1, hence, low risk to the patient is concluded.

**Conclusion**

The regulatory derived ADI for phosphate salts, are based on the dietary NOAEL in rats based on chronic repeat-dose toxicity studies. The lowest and most reliable NOAEL defined is 76 mg/kg/Phosphate per day, adding a daily dietary intake of 91 mg/kg/Phosphate per day and person to the consideration. The Point of Departure for ADI calculation is a total of 167 mg/kg/day. To this value, the chemical-specific adjustment

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factor for phosphate of 4 (based on TK/TD considerations between rats and human (interspecies differences) is to be applied resulting in an ADI value of 42 mg/kg bw per day, rounded to 40 mg/kg bw per day.
Considering all available toxicity data, the high NOAEL, and relatively mild toxic effects associated with phosphate salts, the available ADI derived by regulatory authorities seems sufficient and it can be concluded that both E339 and E450 are both of low toxicity and concern.

The margin of safety calculation further shows, that the estimated daily intake (EDI) of the phosphor amount per 12 empty capsules, is around 110-fold lower than the acceptable daily intake (ADI) published by EFSA, confirming low risk to the patient.

Given the purity criteria for E339iii and E450iii defined in Regulation (EU) 231/20122 laying down specifications for food additives are met and the ADI and GMP manufacturing criteria are followed, it can be concluded that there are no safety concerns related to the use of both phosphate salts in capsules intended as an oral dosage form at typical daily dose consumption levels associated with medicinal products.

**Signatures**

| Name | Role | Signature / Date (DD/MMM/YYYY) |
|---|---|---|
| Lisa Wiesner | Toxicologist | Lisa Wiesner, Toxicologist, MSc<br>Digitally signed by Lisa Wiesner, Toxicologist, MSc<br>Date: 2022.07.18 11:02:24 +02'00' |
| Bram Baert | Regulatory Affairs | Bram Baert<br>Digitally signed by Bram Baert<br>Date: 2022.07.18 13:54:09 +02'00' |

**Summary of Author CV**

Lisa Wiesner, MSc.
Toxicologist, Corporate EHS, Lonza AG (July 2018-now) with over 6 years' experience in Occupational Toxicology (previously Novartis) and a Master's Degree in Environmental Sciences.

- Responsible for compilation, review and approval of health hazard assessment monographs of drug substances, chemical intermediates, raw materials and others
- Contributor in establishing scientific principles in the field of occupational toxicology and patient safety
- Member of Swiss Society of Toxicology
- Regular Attendance of international toxicology meetings
- Trained by professional toxicologist in pharmaceutical industry (Ester Lovsin Barle, PhD, MSc Tox, ERT (former Novartis, now Takeda Pharmaceuticals)

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**Abbreviations**

EFSA = European Food Safety Authority
JECFA = Joint FAO/WHO Expert Committee on Food Additives
PDE = Permitted Daily Exposure
ADI = Acceptable Daily Int
EDI = Estimated Daily Intake
EU = European Union
MTDI = Maximum Tolerable Daily Intake
TSPP = Tetrasodium Pyrophosphate
TiO2 = Titanium Dioxide
AI = Adequate Intake
TK/TD = Toxicokinetics / Toxicodynamics

**References**


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- ICH. (2011). ICH Harmonised Tripartite Guideline: Impurities: Guideline for Residual Solvents (Q3C(R5)). Current Step 5 version, Nov-2021 (Parent Guideline dated 17-Jul-1997)
- Product Development Sheet, Capsugel. TiO2-free white HGC
- The Physicians' Desk Reference 70th Edition, 2016

# 9. Appendix 2

Summary of FDA Inactive Ingredient Database – accessed 23-Aug-2023, that shows the approved amount of sodium pyrophosphate:

**Search Results for: pyrophosphate**

Show 30 rows | CSV | Excel

| Inactive Ingredient | Route | Dosage Form | CAS Number | UNII | Maximum Potency per unit dose |
|---|---|---|---|---|---|
| CALCIUM PYROPHOSPHATE | DENTAL | PASTE, DENTIFRICE | 7790763 | X69NU20D19 | 39%w/w |
| CALCIUM PYROPHOSPHATE | ORAL | TABLET | 7790763 | X69NU20D19 | 298.04mg |
| SODIUM PYROPHOSPHATE | BUCCAL | SOLUTION | 7722885 | 0352864B8Z | NA |
| SODIUM PYROPHOSPHATE | DENTAL | PASTE, DENTIFRICE | 7722885 | 0352864B8Z | 1%w/w |
| SODIUM PYROPHOSPHATE | INTRAVENOUS | INJECTION | 7722885 | 0352864B8Z | 12mg |

Showing 1 to 5 of 5 entries

**Search Results for: SODIUM PHOSPHATE, TRIBASIC**

Show 30 rows | CSV | Excel | Filter:

| Inactive Ingredient | Route | Dosage Form | CAS Number | UNII | Maximum Potency per unit dose | Maximum Daily Exposure (MDE) | Record Updated |
|---|---|---|---|---|---|---|---|
| SODIUM PHOSPHATE, TRIBASIC | BUCCAL | FILM, SOLUBLE | | A752Q30A6X | 0.05mg | | |
| SODIUM PHOSPHATE, TRIBASIC | ORAL | GRANULE | | A752Q30A6X | | 1mg | |
| SODIUM PHOSPHATE, TRIBASIC | ORAL | POWDER, FOR SUSPENSION | | A752Q30A6X | | 650mg | |
| SODIUM PHOSPHATE, TRIBASIC, ANHYDROUS | ORAL | POWDER, FOR SUSPENSION | 7601549 | SX01TZ03QZ | | 89mg | |
| SODIUM PHOSPHATE, TRIBASIC, ANHYDROUS | ORAL | SUSPENSION | 7601549 | SX01TZ03QZ | | 528mg | |
| SODIUM PHOSPHATE, TRIBASIC, DODECAHYDRATE | ORAL | CAPSULE, DELAYED RELEASE PELLETS | 10101890 | B70850QPHR | | 11mg | |
| SODIUM PHOSPHATE, TRIBASIC, DODECAHYDRATE | ORAL | GRANULE | 10101890 | B70850QPHR | | 5mg | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | INTRAMUSCULAR | INJECTION | 27176085 | J9085FKF29 | 4.3mg | | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | INTRAVENOUS | INJECTION | 27176085 | J9085FKF29 | 4.3mg | | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | ORAL | POWDER, FOR SUSPENSION | 27176085 | J9085FKF29 | 88mg | | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | ORAL | TABLET | 27176085 | J9085FKF29 | 88mg | | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | ORAL | TABLET, DELAYED RELEASE | 27176085 | J9085FKF29 | 24.5mg | | |
| SODIUM PHOSPHATE, TRIBASIC, MONOHYDRATE | SUBCUTANEOUS | INJECTION | 27176085 | J9085FKF29 | 4.3mg | | |

Showing 1 to 13 of 13 entries

Previous | 1 | Next

## *8.10. Summary zinc oxide*

# Zinc oxide (ZnO)
(CAS No. 1314-13-2)

Safety assessment as excipient for oral administration

| | |
|---|---|
| Author: | C Trendelenburg (Novartis) |
| Peer review: | Z Sobol, Y Yang |
| Document type: | Expert statement |
| Document status: | Final |
| Release Date: | 25-Sep-2023 |

## Table of contents



# 1. Summary

With the aim to identify alternatives to titanium dioxide ($TiO_2$) as coloring agent in orally administered medicinal products, the present safety assessment for zinc oxide (ZnO) was performed to support the justification for its potential usage as alternative coloring agent. For zinc oxide, sparse safety information was found in the open domain.

In animals, ingestion of zinc or zinc-containing compounds at high doses has resulted in a variety of systemic effects in the gastrointestinal tract, hematological and immune systems as well as alterations in the blood lipid profile. In addition, lesions have been observed in the liver, pancreas, and kidneys of animals (1).

The weight of evidence from in vitro and in vivo genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity (1, 10, 18; 19).

No adequate experimental studies are available to evaluate the carcinogenic potential of zinc (19).

Zinc is not teratogenic and does not exhibit reproductive toxicity in rats below 1 g/kg body weight. Administration of extreme zinc doses of 1 g/kg or higher during pregnancy caused a significant reduction in fetal growth, birth weight and still births (10).

In humans, studies of chronic and sub-chronic toxicity of zinc are well documented. Like in animals, ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal (e.g., distress) and hematological systems and alterations in the blood lipid profile. Many of these changes are similar to those observed during copper deficiency.

Based on human studies, the Scientific Committee on Food (SCF) established a (human) no observed adverse effect level (NOAEL) of 50 mg/person per day for zinc (6, 10, 9), which is based on the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) (10).

For food, the EFSA derived a tolerable Upper Intake level (UL) of 25 mg zinc/day for adults, including pregnant and lactating women, by applying an additional uncertainty factor of 2 to the above NOAEL (10).

The safety of zinc oxide nanoparticles is less well understood and none of the below summarized studies provided comprehensive data on characterization of the nano material fraction or a full toxicological database in line with the nanomaterial guidance provided by EFSA (7).

## 2. General information

| | |
|---|---|
| Name (IUPAC) | Zinc oxide |
| Chemical name, synonyms | Pigment white 4, zincite, zinc white, calamine, philosopher's wool, Chinese white, flowers of zinc |
| CAS No. | 1314-13-2 |
| Molecular formula | $Zn^{2+}$ $O^{2-}$ |
| Molecular weight | 81.38 g/mol |
| Chemical structure | Zn=O |
| Physico-chemical properties | White solid (2 crystalline forms: hexagonal wurtzite and cubic zincblende), odorless, insoluble in water (0.42 mg/100 g water at 18°C), rapidly soluble in dilute acids such as 3% acetic acid, 0.07M HCl, and ammonia and alkali hydroxide solutions (8, 13) |

Although zinc oxide (ZnO) occurs naturally as the mineral zincite, most zinc oxide is produced synthetically. It is widely used as an additive in numerous materials and products including cosmetics (e.g., oral care products), food additives and packaging materials, and other chemical products.

Zinc oxide completely absorbs UV light < 366 nm and therefore in cosmetics is widely used as a sunscreen agent to block UVA and UVB. Zinc oxide nanoparticles between 200 and 400 nm reflect and scatter light but 40–100 nm particles absorb and scatter UV and absorb visible wavelengths, making the screen transparent (13).

In pharmaceuticals, zinc oxide can be used as a mild antibacterial or antifungicidal agent. Zinc oxide is also used in small quantities in subcutaneous injections, e.g., as a stabilizer in subcutaneous insulin injections as insulin hexamers form with zinc (13).

## 3. Regulatory information and published limits

As food additive, zinc oxide is listed on the U.S. FDA's list of generally recognized as safe (GRAS) substances (3) and included in the Substances Added to Food (formerly EAFUS) list compiled by the FDA.

Zinc oxide is also listed in the British, Japanese, European and US pharmacopeias (13) and included in the FDA Inactive Ingredients Database, e.g., in pharmaceutical products for SC or IV injection, suppositories, topical lotions and solutions for inhalation.

Recommended Dietary Allowances (RDAs) for zinc are 11 mg/day for men and 8 mg/day for women (1).

Based on human studies, the Scientific Committee on Food (SCF) established a (human) no observed adverse effect level (NOAEL) of 50 mg/person per day for zinc, and this was subsequently confirmed by EFSA (6, 10, 9). The NOAEL of 50 mg/person per day is based on the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) (8).

For food, the EFSA derived a tolerable Upper Intake level (UL) of 25 mg zinc/day for adults, including pregnant and lactating women, by applying an additional uncertainty factor of 2 to the above NOAEL (10).

ATSDR (1) has derived an intermediate-duration and chronic oral Minimal Risk Level (MRL) of 0.3 mg Zn/kg/day based on effects on erythrocyte superoxide dismutase, a sensitive indicator of body copper status, and changes in serum ferritin in women given supplements containing zinc gluconate for 10 weeks (20). It was highlighted that the MRL is calculated based on the assumption of healthy dietary levels of zinc (and copper) and represents the level of exposure above and beyond the normal diet that is believed to be without an appreciable risk of toxic response. The MRL is based on soluble zinc salts; it is less likely that insoluble zinc compounds, like zinc oxide, would have these effects at similar exposure levels.

Similarly, EPA has derived an oral reference dose (RfD) of 0.3 mg/kg/day for zinc.

# 4. Safety assessment

For zinc oxide, sparse safety information was found in the published literature and public databases.

For zinc, administered in a variety of forms, detailed toxicological information by any administration route can be found in the monograph of the ATSDR (1).

EFSA (8) evaluated the safety of zinc oxide nanoparticles for use in food contact materials.

Key concepts from the ATSDR monograph and the EFSA safety assessment are summarized below.

## *4.1. Absorption, Distribution, Metabolism and Excretion (ADME)*

Following oral intake, fast dissolution of zinc oxide (including zinc oxide in nanoform) into Zn2+ ions is expected in the acidic environment of the stomach (8).

Zinc absorption ranged from 8 to 81% following short-term exposures to zinc supplements in the diet. Differences in absorption are probably due to the type of diet. Persons with adequate nutritional levels of zinc absorb approximately 20–30% of all ingested zinc (1).

## *4.2. Repeat-dose toxicity*

In animals, ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal tract, hematological and immune systems as well as alterations in the blood lipid profile. In addition, lesions have been observed in the liver, pancreas, and kidneys of animals (1). Some key animal data (from ATSDR, 2005 [1]) following oral administration of zinc oxide (and other zinc salts) are summarized in the below sections.

**Gastrointestinal systems**

Intestinal hemorrhages were observed in ferrets that ingested 390 mg zinc/kg/day as zinc oxide for 2 weeks. These ferrets exhibited a 75% reduction in food intake. No intestinal hemorrhaging was observed in ferrets fed 195 mg/kg/day for up to 21 days.

**Hematological systems**

Decreased hemoglobin, hematocrit, erythrocyte, and/or leukocyte levels were observed in rats, mice, rabbits, dogs, ferrets, and preruminant calves. In rats, the lowest LOAEL for hematological effects was 4 mg/kg/day (8 mg/kg every other day) for an increased frequency of basophilic-stippled erythrocytes in rats exposed every other day for 14 days. The highest NOAEL in rats was 191 mg zinc/kg/day as zinc acetate in a 3-month drinking water study. For mice, NOAEL and LOAEL values of 104 and 1110 mg zinc/kg/day as zinc sulfate, respectively, were identified in a 13-week feeding study. In rabbits, slight decreases in hemoglobin levels were observed in rabbits fed 174 mg zinc/kg/day as zinc carbonate. Zinc oxide consumption caused anemia in dogs (76.5 mg zinc/kg/day), ferrets (195 mg zinc/kg/day), and preruminant calves (64 mg zinc/kg/day). Hematological alterations were not observed in cats

exposed to up to 83.2 mg zinc/kg/day as zinc oxide or in adult mink exposed to zinc at up to 297.4 mg zinc/kg/day as zinc oxide or to rats exposed to 53 mg zinc/kg/day as zinc sulfate.

**Blood lipids**

Increases in serum cholesterol levels were observed in two studies where rats were fed either 2.8 or 10 mg zinc/kg/day as zinc acetate for 2–7 months. Other studies have shown no effect on total cholesterol, HDL cholesterol, or serum triglyceride levels in rats ingesting 3 or 25 mg zinc/kg/day of unspecified zinc compounds.

**Renal effects**

Several intermediate-duration studies have demonstrated renal effects in animals exposed to zinc oxide, zinc sulfate, and zinc acetate.

Zinc sulfate caused an increase in the absolute and relative kidney weights and regressive kidney lesions (not specified) in female mice that consumed 1110 mg zinc/kg/day in the diet for 13 weeks, but no effects occurred in rats that consumed 565 mg zinc/kg/day or in mice that consumed 104 mg zinc/kg/day under similar conditions.

In ferrets, severe diffuse nephrosis was observed exposed to 195 mg zinc/kg/day as zinc oxide in the diet.

In rats exposed to 191 mg zinc/kg/day as zinc acetate for 3 months, epithelial cell damage in the glomerulus and proximal convoluted tubules and increased plasma creatinine and urea levels were observed. The NOAEL for the effects on creatinine and urea was 95 mg zinc/kg/day. It is unclear whether the microscopic changes were observed at lower doses. No histopathological changes in the kidneys were observed in three rats that drank water containing 98.3 mg zinc/kg/day as zinc oxide for 35–36 weeks; however, interpretation of the results of this study is severely limited by the small number of rats used.

Renal tubular dilation, with proteinaceous casts and hemosiderin deposits, was observed in the kidneys of sheep that ingested 18 mg zinc/kg/day as zinc oxide for 49-72 days. It is not known if sheep are a good model for human toxicity because they are ruminants.

Minks exposed to 195 mg zinc/kg/day as zinc oxide for 7-97 days in the food developed a diffuse nephrosis, though it did not increase with increasing dose.

**Immunological and lymphoreticular effects**

Zinc plays a role in the normal development and maintenance of the immune system, such as in the lymphocyte response to mitogens and as a cofactor for the thymic hormone thymulin (1).

Decreased lymphocyte activity (incorporation of 3H-thymidine in response to concanavalin A) was reported in mink kits from dams that had ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to conception and throughout gestation and lactation. The dose to the kits is unknown.

In contrast, no effect was observed on antibody titer (IgG and IgM) or the mitogenic response of splenic B cells isolated from mice fed 76.9 mg zinc/kg/day as zinc sulfate for 4 weeks and challenged with B cell antigens either in vivo or in vitro. The in vitro mitogenic response of T cells isolated from these mice was increased.

In mice exposed in utero to 136 mg zinc/kg/day, with exposure continuing postnatally, there were increases in direct plaque-forming activity of spleen cells and in lymphocyte proliferation in response to mitogen stimulation.

## 4.3. Genetic toxicity

Genotoxicity studies conducted in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenic effects following zinc exposure (1, 10, 19).

**In vitro**

Zinc was negative in most tests for induction of gene mutations in bacterial or mammalian cells (1, 10, 19), in particular, zinc sulfate and zinc acetate were not mutagenic in Salmonella typhimurium. However, zinc 2,4-pentanedione was mutagenic at 400 ug zinc/plate in Salmonella typhimurium (with and without S9) (16).

Zinc chloride was not mutagenic in the mouse lymphoma TK assay (WHO, 2001). Zinc acetate was found positive both in the mouse lymphoma TK assay (0-13 ug/mL and 4.2-42 ug/ml, with and without S9) and in the chromosome aberration assay in Chinese hamster ovary (CHO) cells (25-45 ug/mL and 45-80 ug/ml, with and without S9) (16).

Zinc chloride (3×10-4 – 3×10-5 mol/liter) induced chromosomal aberrations in human lymphocytes (without S9 activation) (4).

Zinc acetate and zinc 2,4-pentanedione were negative in the UDS assay in rat hepatocytes (Thompson et al, 1989). Zinc chloride at concentrations of up to 20 µg/ml did not induce cell transformation in Syrian hamster embryo (SHE) cells (10, 19).

**In vivo**

Zinc sulfate did not induce micronuclei in the mouse when administered orally at doses up to 0.3 mmol/liter per kg (11).

The induction of chromosome aberrations has been studied in bone marrow cells harvested from animals exposed to elevated levels of zinc (up to 15 g/kg). Taken as a whole, studies of this endpoint yielded in equivocal and sometimes contradictory results (19). For example, for zinc chloride conflicting results, negative or positive at high doses, were reported for the induction of chromosomal aberrations in the mouse bone marrow (5, 17; 12).

Zinc sulfate did not induce sex-linked recessive lethal mutations in Drosophila at 5 mmol/liter (11).

Zinc chloride did not induce dominant lethal mutations in mice at 15 mg/kg (17).

In vivo (oral) exposure to zinc sulfate resulted in single strand breaks in mice leukocytes dose-dependently at all doses levels (5.7-19.95 mg/kg) 24 hours post-treatment, as measured by the Comet assay (2).

**Conclusions for genetic toxicity**

The weight of evidence from the in vitro and in vivo genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity However, zinc salts can be cytotoxic at high concentrations, as noted in numerous studies (reviewed by 18; 19).

## 4.4. Carcinogenicity potential

No adequate experimental studies are available to evaluate the carcinogenic potential of zinc or zinc compounds (10, 19).

Reproductive and developmental toxicity

Zinc is not teratogenic except when high doses (20 mg/kg body weight) are injected intraperitoneally to mice during pregnancy (10). Similarly, zinc does not exhibit reproductive toxicity in rats until very high

doses of 1 g/kg body weight given during pregnancy and which caused a significant reduction in fetal growth, birth weight and still births. A total failure of reproduction occurred in rats on zinc intakes of 2 g/kg body weight (10).

## 4.5. Additional safety data

none

## 4.6. Human data

Between 2 and 4 g of zinc are distributed throughout the human body. Most zinc is found in the brain, muscle, bones, kidney, and liver. The zinc content in bone for example amounts to 100 µg/g bone dry weight (15).

Except by inhalation as fume or dust, zinc oxide is generally considered a nontoxic material. It is moderately toxic to humans by ingestion in its pure form (13).

**Acute toxicity**

Acute toxicity is infrequent in humans. Several cases of food poisoning are reported resulting from storage of food or drink in galvanized containers. Symptoms of acute zinc toxicity include nausea, vomiting, epigastric pain, abdominal cramps, and diarrhea. This change in presenting symptoms could be a result of the type of zinc ingested. An industrial hazard associated with inhalation of zinc oxide fumes is “metal fume fever”. Subjects present with malaise, fever, headache, nausea and dryness of mouth and throat (10).

**Chronic and sub-chronic toxicity**

Studies of chronic and sub-chronic toxicity of zinc are well documented. Like in animals, ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal (e.g., distress) and hematological systems (see below) and alterations in the blood lipid profile in humans (1, 10).

Prolonged intakes of zinc supplements ranging from 50 mg/day up to 300 mg/day have been associated with a range of biochemical and physiological changes. These changes include hypocupremia, leucopenia, neutropenia, sideroblastic anemia, decreased concentrations of plasma copper and decreased activity of the copper containing enzymes, superoxide dismutase and caeruloplasmin, altered lipoprotein metabolism and impaired immune function. Many of these biochemical and physiological changes are similar to those observed during copper deficiency. Nevertheless, there are problems with hazard identification in that these changes are not specific to copper deficiency and the clinical relevance of some are unknown. Sensitive sub-populations may include subjects with hemochromatosis and/or insulin dependent diabetes. Zinc excess in water may decrease iron absorption. Hepatic zinc concentration is increased in hemochromatosis and there is some evidence that zinc absorption may be increased.

A significant reduction in erythrocyte superoxide dismutase activity (47% decrease), hematocrit, and serum ferritin, compared to pretreatment levels, were observed in female subjects who received supplements (as capsules) of 50 mg zinc/day as zinc gluconate for 10 weeks (20). This study/end point was selected as the basis for the derivation of an oral Minimal Risk Level (MRL) (1; see above).

### *4.7. Data gap assessment*

Based on the publicly available data as summarized above, the following potential safety data gaps are considered relevant for further evaluation and discussion for oral administration of zinc oxide as excipient in pharmaceutical formulations:

- the safety of zinc oxide nanoparticles is less well understood and none of the above summarized studies provided comprehensive data on characterization of the nano material fraction or a full toxicological database in line with the nanomaterial guidance provided by EFSA (7).

- No adequate experimental studies are available to evaluate the carcinogenic potential of zinc oxide or zinc oxide containing nano particulate fraction.

## 5. Overall conclusions

For zinc oxide, no specific safety information was found in the open domain.

In animals, ingestion of zinc or zinc-containing compounds at high doses has resulted in a variety of systemic effects in the gastrointestinal tract, hematological and immune systems as well as alterations in the blood lipid profile. In addition, lesions have been observed in the liver, pancreas, and kidneys of animals (1).

The weight of evidence from in vitro and in vivo genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity (1, 10, 18, 19).

No adequate experimental studies are available to evaluate the carcinogenic potential of zinc (19).

Zinc is not teratogenic and does not exhibit reproductive toxicity in rats until very high doses of 1 g/kg body weight given during pregnancy and which caused a significant reduction in fetal growth, birth weight and increased incidence of still births (10).

In humans, studies of chronic and sub-chronic toxicity of zinc are well documented. Like in animals, ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal (e.g., distress) and hematological systems and alterations in the blood lipid profile. Many of these changes are similar to those observed during copper deficiency, however they are not specific to copper deficiency and the clinical relevance of some are unknown.

Based on human studies the Scientific Committee on Food (SCF) established a (human) no observed adverse effect level (NOAEL) of 50 mg/person per day for zinc (6, 10, 9), which is based on the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) (8).

For food, the EFSA derived a tolerable Upper Intake level (UL) of 25 mg zinc/day for adults, including pregnant and lactating women, by applying an additional uncertainty factor of 2 to the above NOAEL (10).

## 6. Abbreviations

| | |
|---|---|
| ATSDR | Agency for Toxic Substances and Disease Registry |
| CHO | Chines hamster ovary (cells) |
| EFSA | European Food Safety Authority |
| FDA | U.S. Food and Drug Administration |
| GRAS | Generally recognized as safe |

| | |
|---|---|
| HDL | High density lipoprotein |
| IgG / IgM | Immunoglobulin G / M (antibody) |
| IV | intravenous |
| LOAEL | Lowest observed adverse effect level |
| M | molar (Mol per liter) |
| MRL | Minimum risk level |
| nm | nanometer |
| NOAEL | No observed adverse effect level |
| RDA | Recommended Dietary Allowances |
| RfD | Reference dose |
| S9 | Supernatant after centrifugation of liver homogenate at 9000 rpm |
| SC | subcutaneous |
| SCF | Scientific Committee on Food |
| SHE | Syrian hamster embryo (cells) |
| UDS | Unscheduled DNA synthesis |
| UL | (Tolerable) upper (intake) level |

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## 9. Appendix 2: Safety assessment of titanium dioxide

The TiO2 Alternatives consortium also reviewed the safety of TiO2 in the context of its use in medicines and has provided the rationale to propose an oral PDE. The establishment of the PDE will reassure patients that TiO2 use is actively monitored and controlled at safe levels.

Safety Assessment of Titanium Dioxide (E171) for oral dosage forms and derivation of a proposed oral Permitted Daily Exposure (PDE)

Release Date: 31 January 2024

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# List of Abbreviations

| | |
|---|---|
| ACF | Aberrant Crypt Foci |
| ADI | Acceptable Daily Intake |
| BALT | Bronchus-associated lymphoid tissue |
| CEFIC | European Chemical Industry Council |
| EFSA | European Food Safety Authority |
| EFSA FAF | EFSA Panel on Food Additives and Flavourings |
| EMA | European Medicines Agency |
| EOGRT | Extended One Generation Reproductive Toxicity Study |
| FSA | Foods Standards Agency of the Government of the United Kingdom |
| FSANZ | Food Standards Australia New Zealand |
| FDA | US Food and Drug Administration |
| GIT | Gastrointestinal Tract |
| HC | Health Canada |
| HPRT | Hypoxanthine-guanine-phosphoribosyl-transferase Test |
| IUPAC | International Union of Pure and Applied Chemistry |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOD | Limit of Detection |
| MHLW | Ministry of Health, Labour and Welfare of Japan |
| MNT | Micronucleus Test |
| NOAEL | No Observed Adverse Effect Level |
| NCI | National Carcinogenicity Institute |
| NIHS | National Institute of Health Sciences |
| PDE | Permitted Daily Exposure |
| PND | Post-Natal Day |
| PoD | Point of Departure |
| PQRI | Product Quality Research Institute |
| ROS | Reactive Oxygen Species |
| SCCS | Scientific Committee on Consumer Safety |
| SCHEER | Scientific Committee on Health, Environmental and Emerging Risks |
| SEM | Scanning Electron Microscopy |

| | |
|---|---|
| UK COM | UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment |
| UK COT | UK Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment |
| Ti | Titanium |
| TiO2 | Titanium Dioxide |
| TDMA | Titanium Dioxide Manufacturers Association |
| WoE | Weight-of-Evidence |

# 1. Introduction

In response to the potential ban of $TiO_2$ (E171, anatase) in medicines in the European Union (EU), the $TiO_2$ Alternatives Consortium Safety team examined the data on the potential health hazards of $TiO_2$. A review of the many decades of data on TiO2 found that:

- Any genotoxicity observed with $TiO_2$ is likely secondary to physiological stress and not due to direct DNA damage.
- One study that suggested $TiO_2$-related effects, i.e., Bettini et al., 2017(1), is flawed and not reproducible.
- Nearly all regulatory agencies have reached a different conclusion compared to the EU and state that the food additive E171 does not pose a human health concern.
- The National Cancer Institute (2) carcinogenicity study is valid and is the most appropriate study for assessing the long-term effects of $TiO_2$ and setting an oral PDE. Although a PDE is not normally necessary for low hazard substances, a PDE for $TiO_2$ was determined.

It's reasonable to restrict the food and medicinal use of $TiO_2$ to products with a defined particle size and to the calculated PDE of 2250 mg/day.

Scientific information and establishment of the PDE will serve for risk-benefit evaluation on the use of low amounts of $TiO_2$ contained in tablets and capsules in oral medicinal products and will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels.

$TiO_2$ is present in about 600 different forms. There are three naturally occurring crystallographic forms of $TiO_2$: anatase, brookite and rutile. Rutile is the most common and stable form. $TiO_2$ (E171, anatase) has been used as an excipient in medicinal products as a white colorant and opacifier in tablets and capsules for over fifty years. E171 has unique properties, such as providing light protection to many active ingredients and formulations, and to provide opacity of the core material to ensure uniform appearance when used in minimal quantities.

Therefore, the review focuses on E171, anatase, which contains a distinct fraction of nanoparticles. In a scientific opinion, the EFSA FAF panel (European Food Safety Authority Panel on Food Additives and Flavourings) published in 2019 (3) a specification for E171, based on data provided by the European Chemical Industry Council (CEFIC). The average median diameter of the constituent particles obtained by three laboratories using scanning electron microscopy (SEM) was reported, for the five brands of anatase, to range between 104 and 166 nm and the percentage of particles by number < 100 nm ranges from 11.4 to 45.6%. Therefore, the FAF panel concluded the following specification: less than 50% of constituent particles by number in E 171 have a minimum external dimension < 100 nm. In addition, constituent particles < 30 nm amounted to less than 1% by number.

Historically, $TiO_2$ has been assessed for safety by many regulatory authorities and has consistently been found safe for its intended applications. In contrast, a re-evaluation performed by the EFSA in 2021 (4)

concluded that a safety risk of $TiO_2$ in food could not be excluded, resulting in a ban of its use as food additive by the European Commission as a precautionary measure. In this case, the EFSA did not identify an immediate health concern linked to $TiO_2$ when used as a food additive, but rather their main concerns were centred around uncertainties on the safety of $TiO_2$ nanoparticles, and as such, the panel concluded that $TiO_2$ as a food additive (E171) could no longer be considered safe. The most significant uncertainty claimed by the EFSA experts was that the potential genotoxicity of $TiO_2$ particles could not be ruled out (primarily due to conflicting data in the public domain).

After the EFSA opinion, many non-EU authorities performed their own assessments, which concluded that there is no evidence for a direct genotoxic risk based on the current data available. Importantly, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (5) recently reached a different conclusion from the EFSA and published a re-evaluation of $TiO_2$, referencing a daily intake of 10 mg/kg by food as an appropriate estimate (corresponding to 700 mg/day for a 70 kg person). Based on this estimate, JECFA concluded that in the absence of any identifiable hazard associated with E171 the Acceptable Daily Intake (ADI) remains “not specified”. It is important to note that for medicinal products the daily intake is significantly lower than the exposure of $TiO_2$ in foods, i.e., between 0.075 and 45 mg per coating/capsule (across company survey). This document summarises the conclusions of the authorities and discusses the scientific data in section 2.2.1.

In addition, new data have become available since the EFSA re-assessment. Those are summarized in section 2.2.5. This newly available information is taken into consideration for the PDE calculation.

Although it is unusual from a toxicological perspective to derive a PDE for a non-hazardous compound, a PDE calculation using scientifically robust data will increase confidence of patients in the safety of medicinal products containing $TiO_2$ and will allow the pharmaceutical industry to continue to provide patient access to life-saving medicines and to develop innovative high-quality medicines in the future.

## *1.1. Importance of $TiO_2$ in Medicines*

TiO2 (E171, anatase) is primarily used in medicinal products as a white colorant and opacifier in tablets and capsules. It has unique properties, such as providing light protection to many active ingredients and formulations and to provide opacity of the core material to ensure uniform appearance when used in minimal quantities.

$TiO_2$ has played a key role in the safety, efficacy and compliance of the majority of medicines in Europe for over 50 years. $TiO_2$ is considered safe for use and requires relatively low amounts compared to the alternatives. It provides key attributes that ensures the stability of the oral formulations to ensure the active ingredients remain efficacious. The uniform appearance afforded to the tablets by $TiO_2$ provides patients with confidence in the quality of their medicines, which help drive compliance in taking these medicines. As a pure mineral, $TiO_2$ (E171, anatase) meets the most stringent requirements governing the safety of medicines, including those set by the European Pharmacopoeia, Japanese Pharmacopoeia and US Pharmacopoeia.

$TiO_2$ is ubiquitous in medicines globally. Although the exact number is difficult to establish, it is estimated that approximately 91,000 human medicinal products and 800 veterinary medicinal products in the EU contain $TiO_2$, and the number globally is likely to be significantly higher (6). Reformulating these products solely based on precautionary measures, in the absence of an identified hazard, and not taking into account risk-benefit considerations for medicines would certainly lead to the disruption of the supply of many medicinal products.

The proportion of nanoparticles in E171 is considered a key factor of concern. The International Union of Pure and Applied Chemistry (IUPAC) defined nanoparticles as particles of any shape with dimensions in the $1 \times 10^{-9}$ and $1 \times 10^{-7}$ m range. The EU legal definition in cosmetics is close to this definition. Nanoparticles are particles having, based on their number distribution, more than 50% of the particles below 100 nm. The general definition of nanoparticles by the EFSA is that they cover a size range from 1–100 nm (7).

In 2019, the EFSA FAF Panel published a scientific opinion on the proposed amendment of the EU specifications for $TiO_2$ (E171) with respect to the inclusion of additional parameters related to its particle size distribution (3). This scientific opinion was based on particle size analyses SEM provided by interested business operators, reporting median diameter of constituent particles for five brands of anatase ranging from 104 to 166 nm and a percentage of particles by number <100 nm ranging from 11.4% to 45.6% and 0.19 – 1.52 % by volume.

Based on these results, the panel proposed to insert a specification of more than 100 nm for median minimal external dimension in the current EU specifications for E171, which is equivalent to less than 50% of the number of constituent particles with a minimal external dimension below 100 nm (3). Based on this definition, E171 used in formulations would not fall under the EU cosmetics definition of nanoparticles.

## *1.2. Regulatory status*

On 14 January 2022, the European Commission adopted a ban on the use of $TiO_2$ (E171) as a food additive, amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food additive $TiO_2$ (E 171) (2022/63/EU) (8). Since 2022, $TiO_2$ has not been authorised in food categories (with a transition period of 6 months implemented 7 August 2022).

Regulation 2022/63/EU provisionally maintains the inclusion of E171 in the list of approved colours allowed for use in medicines. The recitals note that this is to avoid shortages of medicinal products containing $TiO_2$ as this could impact public health and animal health and welfare. It is also noted that the replacement of $TiO_2$ requires investigation and testing of suitable alternatives to ensure that quality, safety and efficacy of medicines are not negatively affected.

Subsequent to the EFSA opinion, several regulatory agencies performed thorough assessments on the use of $TiO_2$ in food, medicines, cosmetics and consumer articles, and their conclusions are summarised in **Table 1** below.

**Table 1: Summary of Regulatory Assessments-Conclusions**

| Agency | Conclusion |
|---|---|
| EFSA (2021) (4) European Food Safety Authority | While the EFSA Panel review lacked a definitive indication of a safety concern for E171, uncertainties associated with presented data allowed the EU Commission and Parliament to ban E171 in food products by invoking the precautionary principle. |
| UK COT (2022) – UK Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment (9) | The UK's Food Standards Agency (FSA) said after reviewing the evidence, no safety concerns have been identified, which means that $TiO_2$ (E171) will remain a permitted food additive in England and Wales. Food Standards Scotland (FSS) reached the same conclusion. The UK COT posted their interim safety assessment on $TiO_2$, which also include the initial opinions from the UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM). The final report from these committees is due in March 2024, upon which the FSA will base their final position. In essence, based on the interim report, they do not agree with the EFSA assessment, and by extension, do not see a need to replace $TiO_2$ in pharmaceuticals. |
| HC (2022) – Health Canada (10) | Based on a review of the available scientific data relevant to food uses of $TiO_2$, HC Food Directorate's position is that there is no conclusive scientific evidence that the food additive $TiO_2$ is a concern for human health. |
| FSANZ (2022) – Food Standards Australia New Zealand (11) | Based on the data currently available, FSANZ concludes there is no evidence to suggest that dietary exposures to food-grade $TiO_2$ are of concern for human health. |
| SCHEER (2023) (12) Scientific Committee on Health, Environmental and Emerging Risks | Although the SCHEER acknowledge there is uncertainty on the hazard characterization, they also state that the Margin of Safety for oral exposure for the pigmentary fine $TiO_2$ is sufficiently high to indicate safe use. When the absence of an ultrafine fraction can be demonstrated with appropriate methodology, pigmentary $TiO_2$ in toys can be considered to show safe use with no or negligible risk after oral exposure. |
| MHLW (2023) (13) Ministry of Health, Labour and Welfare of Japan | National Institute of Health Sciences (NIHS) experts stated it is difficult to support the EFSA opinion. Additionally, based on the results from Agaki et al. 2023 (14), it is thought that the absorption of $TiO_2$ from the gastrointestinal tract is extremely low. Therefore, it is difficult to rationally explain the EFSA interpretation, which assumes that orally administered $TiO_2$ reached target tissues such as the bone marrow at a concentration that would explain its induction of genotoxicity. |
| US FDA (2023) (15) US Food and Drug Administration | US FDA has reaffirmed that $TiO_2$ does not present a hazard when ingested in food at a concentration of up to 1% w/w in food (21CFR73.575) updated Oct 17, 2023. |
| JECFA (2023) (5) Joint FAO/WHO Expert Committee on Food Additives | Considering the very low oral absorption of INS 171 (very similar to E171), and in the absence of any identifiable hazard associated with INS 171 in the diet, the Committee reaffirmed the ADI "not specified" established at the Thirteenth meeting in 1969. |

| | |
|---|---|
| SCCS (2023) (16) European Commission Scientific Committee on Consumer Safety | Having considered all the information (including that evaluated by EFSA, 2021 (4), the SCCS considers that the available evidence is not sufficient to exclude the genotoxicity potential of almost all of the types of TiO2 grades used in oral cosmetic products. The only exception are two nano grades (RM09 and RM11) for which the provided genotoxicity data indicate no genotoxicity concern. In view of the concerns on the potential genotoxicity of the $TiO_2$ grades considered in their scientific assessment, the SCCS is of the opinion that the Applicants should draw up a proposal for specifications of the different TiO2 grades used in those cosmetic products that could lead to oral and inhalation exposure. The SCCS will be able to assist the Commission in reviewing the proposal. |

Several countries followed the EFSA recommendation without independent review of the data. Switzerland, Israel, Turkey and other non-EU countries have published legislation banning E171 in foods along the same path as EU. In South America, and in most parts of Africa and Asia (e.g., Korea) the evaluation is ongoing.

### *1.3. Rationale for deriving an oral PDE for $TiO_2$*

Although TiO2 is considered safe by most global agencies and expert panels (including the JECFA (5), following a meeting with the EMA Quality Working Party (Oct 23), industry was verbally advised to propose an oral PDE to help increase the confidence of patients in the continued safety of human medicines containing $TiO_2$.

An oral PDE for $TiO_2$ will support the $TiO_2$ Alternatives consortium's effort to compare $TiO_2$ to alternatives, including excipients for which specified oral PDEs or ADIs are established. Since marketing authorisations are based on a demonstrable risk-benefit analysis, an oral PDE will also allow the pharmaceutical industry to continue to provide patients access to life-saving medicines and to develop innovative high-quality medicines in the future.

An important consideration post-EFSA opinion is that the subsequent physicochemical analysis (including nanoparticulate characterisation) of the test material used in the carcinogenicity study (Unitane 0-220, also referred to as INS 171) demonstrated its comparability to E171, and therefore industry considers that the inclusion of these historical carcinogenicity data in the human risk assessment of $TiO_2$ are pivotal for derivation of the PDE and will provide a conservative estimate. The establishment of the PDE will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels.

## 2. Quality background information

In 2019, EFSA published a draft specification for E171 based on data provided by the Titanium Dioxide Manufacturers Association (TDMA). Based on this specification, comparisons can be performed with test materials used in previous toxicological studies. In April 2022, after publication of the EFSA opinion, the TDMA provided a retrospective analysis of the $TiO_2$ test article used in the National Cancer Institute (2) rodent carcinogenicity study. The report of this analysis is attached to the briefing document (**Error! Reference source not found.**).

Unitane 0-220 used in the 1979 NCI study is essentially pure anatase (>99.5% anatase) and has no surface treatments or coatings but does contain small quantities of particle growth and crystal phase control agents (<0.26% alumina, <0.05% potassium oxide and <0.2% phosphate).

Unitane 0-220 has a median diameter of 106-135nm and 20-44% of particles are <100nm (see TDMA report, **Error! Reference source not found.** – Sample 1 106-118nm/35-44% and Sample 2 124-

135nm/20-26%). Hence, Unitane 0-220 is well within the specification of E171 and within the range of current E171 grades measured in the submission to EFSA, i.e., <50% of constituent particles by number in E171 have a minimum external dimension < 100 nm, and where current grades of E171, anatase ranged between 104 and 166 nm and the percentage of particles by number < 100 nm ranged from 11.4 to 45.6%.

These data were considered and accepted by HC and other authorities. Based on these data the result of the carcinogenicity study conducted by the NCI (2) was considered as valid. The conclusion was that there was no evidence of carcinogenicity, chronic toxicity, or other non-neoplastic lesions of the gastrointestinal tract (GIT) in rats and mice. These data were considered key for the hazard identification assessment of $TiO_2$, leading to the conclusion that currently no human health hazard is identified, which is also the conclusion of several other authorities using the same data set (see Table 1).

# 3. Non-clinical background information

## *3.1. Regulatory Assessments*

As mentioned in section 1.2. , many authorities have already assessed the safety profile of $TiO_2$. Several hundreds of studies were reviewed by the authorities; this section highlights their conclusions and not the individual data for simplification. The summaries of different outcomes are presented in the next sections.

**European Food Safety Authority (EFSA)**

The EFSA Panel concluded in March 2021 (4) that E171 can no longer be considered as safe when used as a food additive based on their review of available study data and clarifying information submitted to EFSA through 09 February 2021, which showed a gap in data relating to genotoxicity of nano-grade $TiO_2$ and the absence of a cut-off value for particle size with respect to genotoxicity. Further, the EFSA Panel indicated that no clear correlation was observed between the physicochemical properties of $TiO_2$ particles, such as crystalline form, size of constituent particles, shape and agglomeration state, and the outcome of either in vitro or in vivo genotoxicity assays. Based on the available evidence, the EFSA Panel concluded that a concern for genotoxicity could not be ruled out. The Panel also reported the absence of appropriately designed carcinogenicity studies of nano-grade $TiO_2$ and maintained that reviewed studies with E171 showed indications of an induction of the preneoplastic lesion aberrant crypt foci (ACF) in the colon.

The EFSA Panel acknowledged that absorption of $TiO_2$ particles is low following oral ingestion but highlighted the potential for accumulation in the body due to its long half-life. Studies on general and organ toxicity, including a recent OECD-compliant extended one generation reproductive toxicity study (EOGRT) using E171, did not result in adverse effects up to a limit dose of 1000 mg/kg bw/day, but the EFSA Panel maintained that some findings regarding immunotoxicity and inflammation with E171 may be indicative of adverse effects. While the EFSA Panel review lacked a definitive indication of a safety concern for E171, uncertainties associated with presented data allowed the EU Commission and Parliament to ban E171 in food products by invoking the precautionary principle.

**UK Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment (COT)**

The COT and the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) are independent scientific committees that provide advice to the Food Standards Agency (FSA), the Department of Health and other government bodies on matters concerning the toxicity of chemicals in the UK.

The COT published an interim position paper (9) on the safety of $TiO_2$ in January 2022. In this position paper they raised several concerns on the 2021 EFSA evaluation.

They highlighted the inconsistencies between the outcomes of the 2020 SCCS Opinion, where it was determined that the genotoxic effects of $TiO_2$ manifest either via a threshold or secondary mechanism, and the outcomes of the 2021 EFSA evaluation, where the FAF (Food Additives and Flavourings) Panel concluded that it was unclear if a threshold mode of action could be assumed (3).

The main reason for this inconsistency was that the EFSA panel included additional data unrelated to E171 in their assessment e.g., data on materials made solely of engineered nanoparticles were included based on the assumption by EFSA that industry disperse E171 into nanoparticles by sonication. However, this was questioned by members of the COT as it was noted that pure nano $TiO_2$ would lose its technical function in the food (as it would not provide colour) and would therefore not be of use. The $TiO_2$ Alternatives consortium agrees and would add that the same is true for the technical function needed in medicinal products and as such, no sonication methods would be applied prior to the ingestion of medicines.

In addition, the COT highlighted COM's preliminary comments that the EFSA Panel used data with questionable quality in respect to the robustness of the data, the use of data from laboratories not proficient in genotoxicity studies in a regulatory context and the weight given to studies with low reliability scores e.g., the comet assays in vitro. The lack of a good dataset and a well-defined test compound (due to the poorly defined specifications) was also considered as a severe limitation and weakness of the EFSA assessment. In addition, members of the COM were concerned about the potential for publication bias in the studies evaluated by EFSA (i.e., where negative studies were less likely to be published). Additionally, the COT noted that the COM considered an indirect, threshold mode of action and that the positive effects were likely attributable to the nano-fraction.

The COT also questioned the conclusions with regards to the ability of $TiO_2$ to induce ACF and the findings of the studies on neurotoxicity were also considered inconsistent by the COT. It was noted that the EOGRT study did not report any effects and that most of the other studies on these endpoints were of nanomaterials. Members were advised that in the EFSA evaluation, the issue of the test material in the EOGRT not being dispersed by sonication was taken into consideration with regards to the conclusions. They considered that had it been dispersed and stabilised in the nano form, some effects could possibly have been observed. The COT, as previously, questioned the relevance of such dispersion to real world use.

In their assessment the COT also agreed with the comments of the COM with regards to risk communication that "As it stands the conclusion is highly risk adverse based on the weak evidence available, and it might create unnecessary concern to the public." They considered that care should be taken when expressing the conclusions and they were uncomfortable with EFSA's binary communication on a dataset with a lot of uncertainties.

The COT and COM considered that the weight of evidence (WoE) did not support the conclusions drawn by EFSA. Therefore, the FSA launched their own review of the safety of TiO2 (currently ongoing).

**Health Canada (HC)**

HC's Food Directorate did not identify any compelling health concerns for the use of $TiO_2$ as a food additive in their 2022 review. HC found that adverse effects reported following oral exposure of $TiO_2$ are largely derived from non-standard studies that administered stable, homogenized suspensions of ultrasonically dispersed particles that do not fully represent exposure to $TiO_2$ as a constituent of food. Data showing $TiO_2$ initiated or promoted the formation of ACF in the colon were limited to Bettini et al. (1), a single non-guideline study in which E171 was administered for 100 days to male rats after ultrasonication that dispersed particles in a simple drinking water matrix which prevented agglomeration. In contrast, in vivo studies conducted following the publication of Bettini et al. (1) showed no evidence that E171 induced the formation of preneoplastic lesions in the colon at doses orders of magnitude higher:

- An OECD guideline-compliant study of food-grade TiO2 dispersed in distilled water by sonication and administered to rats via oral gavage at doses up to 100 times higher than in Bettini et al. (1) for 90 days (a similar duration to Bettini et al. 2017 [1]) demonstrated no treatment-related effects,

including no histopathological changes in the gastrointestinal tract (**Error! Reference source not found.**),

- A non-guideline study conducted to replicate the findings of Bettini et al. (1) in a dietary rat model with E171 administered at doses up to ~24-30 times higher than in Bettini et al. (1)for 100 days (an identical duration to Bettini et al. 2017 [1]) showed no effect on histopathologic evaluations of small and large intestines and no effects on ACF, (**Error! Reference source not found.**), and
- An OCED guideline- compliant extended one-generation reproductive toxicity study (EOGRT) in rats at doses up to 100 times higher than in Bettini et al. (**Error! Reference source not found.**) administered as E171 formulated in the diet for ~18-19 weeks (i.e., exceeding the exposure period of the Bettini (1) study demonstrated no indications of general toxicity, no reproductive or developmental toxicity, and no evidence of ACF observed in male or female rats (Laboratory Pharmacology and Toxicology (LPT), 2020 as cited in EFSA 2021 [4]).

No evidence of carcinogenicity, chronic toxicity, or other non-neoplastic lesions of the gastrointestinal tract were observed in a well-conducted two-year cancer bioassay in male and female mice and rats using very high dietary concentrations (up to 50,000 ppm or 5% w/w) of Unitane 0-220 (2). Subsequent physicochemical analysis (including nanoparticulate characterisation) of Unitane 0-220 by the chemical industry demonstrated this to be comparable to E171 and taken together with the fact the manufacturing methods for food-grade $TiO_2$ have not changed significantly over time, supported the inclusion of the historical carcinogenicity data in the human risk assessment of $TiO_2$ by HC. Further, HC found no consistent evidence of inflammation or immunotoxicity in the GIT of rodents exposed to food-grade $TiO_2$ via the dietary route based on the WoE from multiple studies.

HC concluded that there is no immediate concern for the genotoxicity of food-grade $TiO_2$ with the three studies considered the most reliable and relevant producing negative results in vivo (19, 20, 21). While some positive genotoxicity results with food-grade $TiO_2$ and non-food-grade $TiO_2$ materials have been reported both in vitro and in vivo, HC concluded there was low confidence in the reliability and relevance of these findings due to poor study design, non-compliance with OECD test guidelines, the use of inappropriate cell lines or test articles, as well as uncertainty in the biological relevance of the positive genotoxic effects.

There was no evidence of reproductive or developmental toxicity or gross or histopathological abnormalities in male or female reproductive organs in a recent GLP- and OECD guideline-compliant EOGRT study in rats following dietary exposure to food-grade $TiO_2$ at doses up to 1000 mg/kg bw/d (LPT, 2020 as cited in EFSA 2021 [4]). Further, there was no evidence of neurotoxicity, developmental neurotoxicity, or behavioural changes in rodents exposed to food-grade $TiO_2$ in the diet. Reviewed data showed no evidence that food-grade $TiO_2$ was intrinsically immunogenic in vitro; however, it may modulate immune responses to allergenic proteins by acting as an adjuvant in vitro and more research is required to confirm any potential significance of this finding in vivo.

Studies in humans have consistently demonstrated accumulation of pigment including $TiO_2$ in macrophages in the base of Peyer's patches of the terminal ileum but not elsewhere in the GIT. Importantly, no association between the presence of particles and immune activation or pathological state has been observed.

While some uncertainties in the database were identified that would benefit from further research, the weight of available evidence suggests these data gaps are not significant enough to warrant a more precautionary approach. Thus, HC's Food Directorate did not identify any compelling health concerns for the use of $TiO_2$ as a food additive in their 2022 review.

**Foods Standards Australia New Zealand (FSANZ)**

Given concerns raised by the EFSA on $TiO_2$ leading to the removal of authorisations for use as a food additive in Europe, the FSANZ reviewed the scientific literature and issued a call for information relevant to $TiO_2$ safety in food (11). Information received included new scientific data that addressed concerns raised by the EFSA.

Newly available studies of food-grade $TiO_2$ in rats submitted to FSANZ indicate very low oral bioavailability (≤0.0013%), and evidence from human studies reviewed by FSANZ suggests that absorption of $TiO_2$ following oral exposure is very limited. FSANZ concluded that there is no evidence of DNA damage from food grade $TiO_2$ and no evidence of cancer in dietary studies with mice and rats at high concentrations of food-grade $TiO_2$ over the lifetime of the animals. Further additional studies of food-grade $TiO_2$ in rats found no evidence of general toxicity, and no harmful effects on reproduction, development, or the gastrointestinal, immune, and nervous systems.

FSANZ maintained that the different modes of exposure (e.g., drinking water with sonicated food-grade $TiO_2$, sonicated food-grade $TiO_2$ by oral gavage, and dietary exposure) in reviewed animal studies could explain varied results. For instance, an increased incidence of preneoplastic lesions in the colon of rats exposed to sonicated food-grade $TiO_2$ dispersed in drinking water were not replicated in rats administered sonicated food-grade $TiO_2$ by gavage at a 10-fold higher dose. Further, the results of the study using sonicated food-grade $TiO_2$ dispersed in drinking water are not consistent with results of a two-year carcinogenicity bioassay. No evidence of toxicity or carcinogenicity was observed in rats and mice administered a comparable food-grade $TiO_2$ at even higher dietary concentrations. FSANZ considered the results of feeding studies more relevant than studies using sonicated food-grade $TiO_2$ and concluded that evidence from such studies indicates that dietary exposure to food-grade $TiO_2$ is unlikely to induce preneoplastic or neoplastic lesions in the colon or other tissues.

The FSANZ review also included a recent OECD guideline-compliant EOGRT study in rats with food-grade $TiO_2$ administered via the diet at doses up to 1000 mg/kg bw/day that found no evidence of systemic toxicity, developmental or reproductive toxicity or developmental neurotoxicity. Further, no evidence of developmental immunotoxicity was observed with $TiO_2$ in this study.

Based on the review of an expanded data set in comparison with that available to the EFSA Panel, FSANZ concluded there is no evidence to suggest that exposure to food-grade $TiO_2$ is a concern for human health.

**Scientific Committee on Health, Environmental and Emerging Risks (SCHEER)**

The SCHEER is an independent non-food scientific committee that provides the European Commission with scientific advice for preparation of policy and proposals relating to consumer safety, public health and the environment. SCHEER relies on a WoE approach that describes how the quality and reliability of the conclusions, and their uncertainties are reached. Conclusions of the SCHEER scientific opinion (12)relevant for the oral route of exposure are as follows:

- Pigmentary fine $TiO_2$ grades can be considered to have no genotoxic potential provided the presence of a nano-fraction can be excluded, as direct binding of ultrafine $TiO_2$ particles to DNA was demonstrated in several in vitro studies but no such DNA interaction was shown for fine fraction (or micro-) pigmentary particles,
- The WoE for a genotoxic hazard of $TiO_2$ is weak,
- Available oral studies are not sufficient to draw firm conclusions on the potential carcinogenicity of pigmentary $TiO_2$ particles, but the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of $TiO_2$ on tumour development and the WoE for tumour promoting activity of $TiO_2$ particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain,
- The NCI 1979 carcinogenicity study with Unitane 0-220 (most similar to food grade $TiO_2$ E171) were negative,
- The oral bioavailability of $TiO_2$ particles from the GIT is very low and likely influenced by their size as absorption is higher for smaller particles than for larger ones,
- The overall WoE for adverse effects is judged to be weak given that toxicology data and an adverse outcome pathway (AOP) assessment of fine and ultrafine $TiO_2$ are both considered highly consistent and of medium to high quality, but uncertainties regarding immunotoxic, genotoxic and carcinogenic activity diminish the reliability and consistency of the determined 1000 mg/kg bw/day no observed adverse effect level (NOAEL).

SCHEER acknowledged that an indication for induction of ACF in the colon of animals was observed after exposure to food grade $TiO_2$ (E171) dispersed in drinking water at a human relevant dose (1), but also acknowledged that the relevance of the study for conclusions on carcinogenicity is limited, that two subsequent studies with food grade $TiO_2$ in diet did not confirm ACF effects, and that recent studies of 6nm sized anatase $TiO_2$ primary particles at high doses for 28- or 90-days did not show any indication for abnormality of colonic crypts. SCHEER maintained that different results in reviewed studies available might indicate that there is a matrix effect of the exposure vehicle on the outcome. Considering the adverse outcome pathway and the weak WoE for genotoxic potential of a possible nano-fraction after oral exposure, the SCHEER concluded that the point of departure (PoD) for oral exposure can be based on a threshold for toxicity with a NOAEL of 1000 mg/kg bw/day for general repeated-dose oral toxicity.

**U.S. Food and Drug Administration (FDA)**

FDA has reaffirmed that TiO2 does not present a hazard when ingested in food at a concentration of up to 1% w/w in food (15) updated on October 17, 2023.

**Joint FAO/WHO Expert Committee on Food Additives (JECFA)**

The JECFA discussed all available data in its Ninety-seventh meeting (Safety evaluation of certain food additives) from 31 October–9 November 2023 (5). In this meeting, the Committee considered additional toxicological studies relevant to the safety assessment of INS171 that investigated the toxicokinetics, acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity, and reproductive and developmental toxicity, as well as special studies addressing the short-term initiation/promotion potential for colon cancer. JECFA also evaluated estimates of dietary exposure to $TiO_2$, estimating the maximum 95th percentile to be 10 mg/kg bw/day, which was used for the risk evaluation of INS 171 in the diet.

INS 171 consists of uncoated $TiO_2$ anatase particles including a minor fraction of nano size particles. Food-grade $TiO_2$ is identified and labelled as E171 by the EU. INS 171 and E171 are equivalent except that INS 171 does not include the $TiO_2$ coating of pearlescent pigments (INS 176). Therefore, in line with the HC review, the JECFA also considered the historical carcinogenicity data from the NCI to be relevant for the risk assessment of INS 171 and by extension, E171.

The JECFA took into account that INS 171 was not carcinogenic in an adequately conducted 2-year study in mice and rats at gender-averaged doses of up to 7500 mg/kg bw/day for mice and 2500 mg/kg bw/day for rats, the highest doses tested. The JECFA confirmed the assessments of other agencies that there was no evidence of reproductive or developmental toxicity in studies in rats at INS 171 doses of up to 1000 mg/kg bw/day, the highest doses tested. However, they also stated that “JECFA reviewed all available research on genotoxicity risk and determined that the evidence is insufficient, owing mostly to the lack of suitable testing methodologies for nanoparticles.” This indirectly implies, that value of the indicator assays like the comet assay in vitro is not relevant to describe the genotoxic potential, at least in the current format. Therefore, JECFA recommended more research to address the current uncertainty about the distribution of $TiO_2$ particle sizes in food and to develop genotoxicity tests that are more appropriate for nanoparticles.

Finally, the JECFA concluded that considering the very low oral absorption of INS 171, and in the absence of any identifiable hazard associated with INS 171 in the diet, it was appropriate to reaffirm the ADI “not specified” established at the Thirteenth meeting in 1969.

**Scientific Committee on Consumer Safety (SCCS)**

The SCCS provides opinions on health and safety risks (chemical, biological, mechanical, and other physical risks) of non-food consumer products (e.g., cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products) and services. In light of the EFSA opinion, the

European Commission requested the SCCS to re-assess the safety of $TiO_2$ with a focus on genotoxicity and exposure via the inhalation and oral route (lip care, lipstick, toothpaste, loose powder, hair spray), since the currently available scientific evidence supported an overall lack of dermal absorption of $TiO_2$ particles.

The expert panel was presented with a set of five questions, of which three are considered to have relevance for oral medicines (their previous interpretations considering exposure via dermal and inhalation routes remain unchanged). SCCS categorized the reliability of the available literature and unpublished reports provided by the Cosmetic Europe Titanium Dioxide Consortium by using the ToxR Tool (22) which applies modified Klimisch criteria.

The expert panel concluded:

- There exists insufficient evidence to exclude the genotoxic potential of almost all $TiO_2$ particles, with the exception of the two nano-grades RM09 and RM11, where a negative hypoxanthine-guanine-phosphoribosyl-transferase test (HPRT) and micronucleus test (MNT) in vitro confirmed the absence of a genotoxic potential,
- In line with this interpretation, SCCS felt unable to recommend any safe levels for $TiO_2$ (including pigmentary grade) in cosmetics,
- Overall, the SCCS evaluation is in line with the EFSA statement but acknowledges that the situation for cosmetics is different from food ingredients in that oral uptake of cosmetics is usually incidental and thus quantitatively much lower, and primarily via oral buccal exposure versus through the GIT,
- In contrast to others, their assessment is based on in vitro data from the Comet Assay, whereas elsewhere this assay is given much less weight as an indicator test as it is not equivalent to stable mutations or chromosome damage,
- A valid in vitro micronucleus or chromosomal aberration test (assuring all nanotoxicology state-of-the-art principles are applied) with adequately selected E171-equivalent material(s) would be needed to overrule the current conclusion,
- A lot of weight is given to the Kirkland et al. (23) review and the SCCS conclusions are in agreement with the Kirkland et al. conclusions (“the profile of genotoxicity results from the most robust studies with titanium dioxide does not fit the response pattern which would be expected for a genotoxic carcinogen”),
- SCCS is of the opinion that the Applicants should draw up a proposal for specifications of the different $TiO_2$ grades used in cosmetics.

Thus, SCCS is the only committee that follows EFSA’s opinion that a genotoxic potential of $TiO_2$ cannot be excluded. However, in both cases this interpretation is based on data from assays that are considered by most other groups as not providing data reliable enough for such a conclusion. Of note, the SCCS suggest that well conducted OECD-compliant in vitro tests (micronucleus or chromosome aberration test) would adequately mitigate the genotoxicity concern (data that is currently lacking).

## *3.2. Toxicokinetics*

Several toxicokinetic studies were discussed in the regulatory documents. The most relevant study is briefly discussed in the following paragraphs:

HC reported in their assessment a GLP-compliant, multi-site toxicokinetics study of 5 different grades of $TiO_2$, E171, anatase, which was carried out in accordance with OECD 417 test guidelines (**Error! Reference source not found.**) and a summary report of this unpublished study was submitted to HC by industry (EBRC 2022). In this study, male and female CD (Sprague Dawley) rats (number not stated, although the test guideline stipulates a minimum of four animals per sex per dose group) received either a vehicle control or a single dose of 1000 mg/kg bw of $TiO_2$ administered by oral gavage and the total Titanium (Ti) content of whole blood was measured up to 96 h post-dose. The relative oral bioavailability of the various grades of $TiO_2$ was compared to a soluble Ti reference substance (Titanium (IV) bis(ammonium lactato) dihydroxide solution – 50% (w/v) in $H_2O$) that was administered orally (100 mg/kg

bw) or intravenously (10 mg/kg bw). The test articles included a food-grade form of $TiO_2$ identified as E171-E which had a median particle diameter (SD) of 99.9 ± 2.0 nm and contained approximately 50-51% of constituent particles in the nanoscale (LNE 2020). The other four particles are considered not relevant for this document. Details of the vehicle and the dispersion protocol were not provided.

The mean blood Ti concentrations of male and female rats were below 0.2 µg Ti/g blood following oral administration of all test articles. Administration of the soluble Ti reference resulted in blood Ti concentrations up to 90 µg/g blood and 0.9 µg/g blood following i.v. and oral dosing, respectively. The highest blood Ti concentrations following oral dosing were observed in the group that received the food-grade $TiO_2$ test item E171-E. The maximum relative oral bioavailability of E171-E was determined to be 0.0013%. The measured blood Ti levels of the other four forms of $TiO_2$ were below the limit of detection (LOD) after background correction (LOD not stated). The authors concluded that oral bioavailability of all $TiO_2$ grades tested was close to the LOD of the analytical system. They also stated that most reagents used in the process contain low but measurable background concentrations of Ti, which makes analysis of low levels challenging. In addition, the authors reported that the background level of blood Ti in controls rats was highly variable, especially in males, which is consistent with time zero levels measured during dietary studies.

## *3.3. Summary of Other Toxicological Data Relevant for the PDE Assessment*

### 3.3.1. Reproductive Toxicity Studies

All available data on reproductive studies are discussed in various regulatory assessments and will not be discussed in the following paragraphs. The $TiO_2$ Alternatives consortium selected the most recent and robust study for E171 to rule out the risk of effects on the reproductive system and in addition the risk of general toxicity. The following study was conducted according to the OECD protocol with well characterized material.

An EOGRT was submitted to EFSA as part of the re-evaluation of E171 (Laboratory Pharmacology and Toxicology 2020 as cited in EFSA 2021 [4]). The EOGRT study was conducted in male and female rats according to OECD TG 443 (25) and was GLP compliant. In the F0 generation, E171 was administered in the diet at doses of 0, 100, 300, or 1000 mg/kg/day from 10 weeks prior to mating until weaning of the F1 generation. The F1 received the same doses in the diet from weaning until post-natal day (PND) 4 or 8 of the F2 generation and the F2 generation was exposed through the milk until the termination of the study at PND 4 or 8. Total duration of dosing was dependent on the endpoints under evaluation, with maximal exposure up to 18-19 weeks.

No effects on sexual function or fertility were observed in either males or females. In addition, no treatment-related pre- or postnatal losses were observed in the F0 or F1 generations and the average litter size in all groups was comparable to the control group. No effects on pre- or postnatal development were observed and external or internal abnormalities were detected in the F1 or F2 pups at the termination of the study.

Neurofunctional endpoints were also evaluated in F1 and F2 offspring. While some changes in grip strength and hindlimb splay were observed, no dose response was observed, indicating a low likelihood that this change is test article related.

The T-cell-dependent anti-KLH response (keyhole limpet hemocyanin [KLH] assay) was also conducted as an evaluation of immunotoxicity. All tested animals in the study had a weak immunogenic response to KLH, which indicated that no conclusion could be drawn on the effect of the developing immune system.

### 3.3.2. Chronic Toxicity Studies

General toxicity endpoints were evaluated in the EOGRT study in the F0 and F1 generations. A satellite group in the F0 generation was also evaluated for occurrence of ACF. No premature deaths or changes

in general appearance or behaviour were observed in any generation for the duration of the study. No test article related changes in clinical pathology were observed in either generation, as well as no changes observed in urinalysis parameters. Additionally, no treatment related effects on T4 (thyroxine), T3 (triiodothyronine), TSH (thyroid-stimulating hormone), oestradiol, oestrone or testosterone were observed in either generation. Gross pathology and microscopic assessments were conducted, and no test article-related effects were observed. Finally, evaluation of the colon of satellite F0 animals demonstrated the absence of ACF in control or treated animals, leading to the conclusion at oral doses of E171 up to 1000 mg/kg/day did not induce ACF in the colon.

### 3.3.3. Summary - Genetic Toxicology Assessment by Authorities

The WoE supports that TiO2 does not present a genotoxic hazard in vivo, as concluded by FSANZ (11), the UK COT (9), HC (10), MHLW (13), JECFA (5) and US FDA (15). While some positive genotoxicity results with food-grade $TiO_2$ and non-food-grade $TiO_2$ materials have been reported in the literature, health authorities around the world have excluded such findings from their assessment due to low confidence in their reliability and/or biological relevance.

**Food Safety Australia New Zealand (FSANZ)**

In animal studies there is no evidence of DNA damage from food-grade titanium dioxide. There is also no evidence of cancer or other harmful effects in studies with mice and rats fed diets containing very large concentrations of food-grade titanium dioxide over their lifetime" (11).

**UK Committee on Toxicology (COT)**

The interim position of the COT (9) summarised the COM's current position relating to the potential genotoxicity of $TiO_2$. Specifically, the COM highlighted the issues of drawing conclusions using a highly heterogenous and questionable quality dataset (particularly those published in obscure and non-genotoxicity journals). Regarding the mode of action for genotoxicity, the COM agreed that the evidence indicated an indirect interaction with DNA with a threshold for genotoxicity. Although some in vitro tests reported a positive result these appeared to mainly relate to nanoparticles with the micro-sized particles mainly giving negative results. The in vivo studies tended to be of better quality and negative. The relatively low nano-fraction in E171 (i.e., often less than 3.2% by mass) and its low bioavailability could be important factors when considering risk assessment.

Members of the COM and COT considered that the evidence did not allow definitive conclusions to be drawn, and therefore, they did not agree with the overall EFSA conclusions on the genotoxicity of E171 $TiO_2$. They also agreed that the EFSA's position might cause unnecessary concern to the public. The COT suggested that the COM should independently review the database on genotoxicity and apply the COM's Guidance on determining thresholds, which is currently ongoing.

**Health Canada:**

"Overall, the Food Directorate's comprehensive review of the available science of $TiO_2$ as a food additive showed:

no evidence of cancer or other adverse effects in mice and rats exposed to high concentrations of food-grade $TiO_2$ (long-term or lifetime study) no changes to DNA in various animal studies

**Ministry of Health, Labour and Welfare of Japan**

MHLW initiated a 90-day repeated oral administration study conducted at the National Institute of Health Sciences, Japan.

The study examined accumulation of $TiO_2$ in the liver, kidney, and spleen following the oral administration of anatase $TiO_2$ NPs with a crystallite diameter of 6 nm for 28 or 90 days. This study also measured genotoxicity endpoints in the form of micronuclei in the liver after 28 days of repeat dosing and DNA double strand breaks (gamma H2AX foci) at sites of deposition of yellowish-brown materials in the nasal cavity, bronchus-associated lymphoid tissue, trachea, Peyer's patches, and cervical and mediastinal lymph nodes after 90 days of repeat dosing. Neither micronuclei nor double strand DNA breaks were induced in hepatocytes after oral administration of 1000 mg/kg/day $TiO_2$ NP.

NIHS Experts said it is difficult to support the EFSA opinion. Additionally, based on the results from Agaki et al (14), it is thought that the absorption of $TiO_2$ from the gastrointestinal tract is extremely low. Therefore, it is difficult to rationally explain the EFSA interpretation, which assumes that orally administered $TiO_2$ reached target tissues such as the bone marrow at a concentration that would explain its induction of genotoxicity.

Similarly, the **JECFA** noted that although there were limitations and equivocal findings in the available data, they concluded that "available data did not provide convincing evidence of genotoxicity for $TiO_2$ (INS 171)." (5).

**USA FDA**

FDA has reaffirmed that TiO2 does not present a hazard when ingested in food at a concentration of up to 1% w/w (15) updated on October 17th 2023.

### 3.3.4. Expert Publications:

In addition, a panel of experts (not employed by companies that manufacture and sell $TiO_2$), was convened to perform the review of the genotoxicity of $TiO_2$ (expertise in genetic toxicology, general toxicology, bioavailability, carcinogenicity, and nanoparticle characterisation).

Only studies with genetic toxicology endpoints covered by validated OECD protocols were reviewed to ensure comparable data quality. From 337 datasets with available genotoxicity data on $TiO_2$, by using a structured WoE approach, considering the relevant endpoints, study protocols and material characterizations, only 34 (10.1%) studies eventually provided relevant data. Of these, 10 were positive (i.e., reported evidence that $TiO_2$ was genotoxic), all of which were from studies of DNA strand breakage (comet assay) or chromosome damage (micronucleus or chromosome aberration assays). All the positive findings were associated with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis, or combinations of these. Considering that DNA and chromosome breakage can be secondary to physiological stress, it is highly likely that the observed genotoxic effects of $TiO_2$, including those with nanoparticles, are secondary to physiological stress.

It must be mentioned that the expert panel re-evaluated the data in each dataset included in the Kirkland et al. (23) final assessment (and sometimes did not confirm the authors findings), whereas the EFSA accepted the authors' conclusions without further review for datasets included in the EFSA (4) final assessment. The conclusion of the expert panel was that "Existing evidence does not therefore support a direct DNA damaging mechanism for titanium dioxide (nano and other forms)"

Kirkland et al. (23) concluded that carefully designed studies of apical endpoints (gene mutation, micronucleus or chromosome aberrations), following OECD recommended methods, performed with well characterised preparations of $TiO_2$, would allow firmer conclusions to be reached.

In a recent publication, Landsiedel et al. (26) summarised that it appears that most of the genotoxic damage caused by nanomaterials is due to secondary mechanisms, such as damage to cellular organelles, including lysosomes and mitochondria, leading to the release of reactive oxygen species (ROS) and other reactive species, as well as inflammation-dependent oxidative stress, ultimately resulting in oxidative DNA lesions. In their review of the genotoxicity of nanomaterials, they described the limitations of the in vitro comet assay, a key assay used by EFSA and SCCS in their assessment, which led to their precautionary conclusion. The $TiO_2$ Alternatives consortium agrees with their

statement that the comet assay suffers from rather low specificity and that the DNA damage detected in such tests is not necessarily converted into stable mutations.

### 3.3.5. Overall Conclusion - Genotoxicity

Overall, current data shows there is no evidence that $TiO_2$ has direct mutagenic potential *in vitro* or *in vivo*. The genotoxic effects observed as primary DNA damage (strand breaks) and chromosomal damage were often associated with indirect mechanisms, such as oxidative damage and/or high levels of cytotoxicity. Importantly, this damage appears to be efficiently repaired and does not result in gene mutations nor tumour induction.

The $TiO_2$ Alternatives consortium also considers the Agakj 2023 (14) study as a key high-quality study for hazard identification. The study uses anatase particles at the lower end of the particle size distribution and is considered as the worst-case scenario for low particle size $TiO_2$ and complements the data for E171. Given that this study did not find evidence of genotoxicity (as well as general toxicity) is consistent with the conclusions of an independent expert panel, which stated that all reported positive findings in genotoxicity studies were associated with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis, or combinations of these factors (23). Thus, the WoE supports the conclusion that food-grade $TiO_2$ and non-food-grade $TiO_2$ materials are not genotoxic.

In addition, the negative oral carcinogenicity data considered in previous EFSA assessments were excluded in the recent assessment (4) mainly due to a lack of nanomaterial characterisation. However, these are essential for informing the biological significance of in vitro and in vivo genotoxicity study results. Crucially since the EFSA opinion, it has been shown that the test material used in the carcinogenicity study was highly comparable to E171 (including the nanoparticulate fraction), which was key for other non-EU regulatory agencies to accept these data. E171 is not carcinogenic in rats and mice when administered at very high concentrations in the diet and the $TiO_2$ Alternatives consortium believe that these data should be used to mitigate any ambiguity in the genetic toxicity data for human risk assessment purposes (see 3.4. ).

### 3.3.6. New and/or most relevant toxicological studies considered to establish an oral PDE

In addition to the different assessments from the agencies, recent information and some new publications were considered by the $TiO_2$ Alternatives consortium to underline the safety of $TiO_2$ E171 used in oral formulations in medicinal products.

**Summary of NCI Bioassay of TiO2 for possible carcinogenicity (1979)** with new compound characterization data supporting the relevance of this study.

The National Cancer Institute (2) evaluated the carcinogenicity of $TiO_2$ in two-year bioassays in rats and mice. In the studies, B6C3F1 mice (50 animals/sex/group) and Fischer 344 rats (50 animals/sex/group) were administered $TiO_2$ in the diet at levels of 0, 2.5, and 5% for 103 weeks and were sacrificed at week 104. In mice, the dosages were equivalent 0, 3250, 6500 mg/kg/day in males, and 0, 4175, 8350 mg/kg/day in females, respectively. In rats, the dosages were equivalent to 0, 1125, 2250 mg/kg/day in males, and 0, 1450, 2900 mg/kg/day in females, respectively. Five percent of the diet is the maximum level allowed for chronic toxicity studies because above that level nutritional abnormalities occur, and is the maximum feasible dose recommended for dietary administration by today's standards (27). In these studies, there was no effect on survival, body weight gain, and no incidences of any tumours above historical controls or concurrent controls. Also, there was no evidence of increased non-neoplastic effects above background, and specifically there was no evidence of increased incidences of preneoplastic changes, such as hyperplasia, and no increases in non-neoplastic effects above background. There were also no effects on the GIT, including the colon. The NOAEL was identified as 5% in diet, corresponding to 6500 mg/kg/day in male mice, 8350 mg/kg/day in female mice, 2250 mg/kg/day in male rats, and 2900 mg/kg/day in female rats, respectively (i.e., the highest doses tested).

Of note, the form of $TiO_2$ used in the NCI studies was referred to as Unitane® 0-220, in the anatase form. A recent physicochemical characterization of this material demonstrated that Unitane® 0-220 is very similar to E171 in terms of particle size distribution (TDMA report, April 2022).

**Oral toxicological study of TiO2 nanoparticles with a crystallite diameter of 6 nm in rats**

Japan's National Institute of Health Sciences (NIHS) recently conducted 28- and 90-day studies of the oral toxicity of titanium nanoparticles with a crystallite diameter of 6 nm in rats (14). In these studies, rats received oral administration of $TiO_2$ at doses of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days and doses of 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days.

No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, hematology, serum biochemistry, or organ weight. Histopathological examination revealed $TiO_2$ particles as depositions of yellowish-brown material. The particles observed in the gastrointestinal lumen were also found in the nasal cavity, epithelium, and stromal tissue in the 28-day study. In addition, they were observed in Peyer's patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea in the 90-day study.

NIHS noted that no adverse biological responses, such as inflammation or tissue injury, were observed around the deposits. Titanium concentration analysis in the liver, kidneys, and spleen revealed that $TiO_2$ NPs were barely absorbed and accumulated in these tissues. Immunohistochemical analysis of colonic crypts showed no extension of the proliferative cell zone or preneoplastic cytoplasmic/ nuclear translocation of β-catenin either in the male or female 1000 mg/kg bw/day group. Regarding genotoxicity, no significant increase in micronucleated or γ-H2AX-positive hepatocytes was observed.

Additionally, the induction of γ-H2AX was not observed at the deposition sites of yellowish-brown materials (considered to consist of $TiO_2$). NIHS concluded no effects were observed after repeated oral administration of $TiO_2$ with a crystallite diameter of 6 nm at up to 1000 mg/kg bw/day regarding general toxicity, accumulation of titanium in the liver, kidney, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.

### 3.3.7. Other *in vivo* studies considered relevant

The only study that found oral exposure to E171 $TiO_2$ initiated or promoted the formation of preneoplastic lesions in the colon was Bettini et al. (1), a single non-guideline study in which E171 was administered for 100 days to male rats after ultrasonication that dispersed particles in a drinking water matrix to prevent agglomeration. Overall, this does not reflect the situation where patients are taking oral medicines and food, thus is considered as not physiologically relevant.

While Bettini identified a preneoplastic risk, this study had several shortcomings that make it irrelevant to human risk and has been refuted by three high-quality studies conducted following the publication of Bettini et al. (1). These studies showed no evidence that E171 induced the formation of preneoplastic lesions in the colon using doses orders of magnitude higher than Bettini or dietary routes of administration that are more physiologically relevant to pharmaceutical use:

An OECD guideline-compliant study of food-grade TiO2 dispersed in distilled water by sonication and administered to rats via oral gavage at doses up to 100 times higher than in Bettini et al. (1) for 90 days found no treatment-related effects, including no histopathological changes in the gastrointestinal tract (17)

A non-guideline-compliant study conducted to replicate the findings of Bettini et al. (1) in a dietary rat model, with E171 administered at doses up to ~24-30 times higher than in Bettini et al. (1) for 100 days, showed no effect on histopathologic evaluations of small and large intestines and no effects on aberrant cryptic foci (ACF) (18)

An OECD guideline-compliant EOGRT study in rats given E171 in the diet at doses up to 100 times higher than in Bettini et al. (1) for ~18-19 weeks demonstrated no indications of general toxicity, no

reproductive or developmental toxicity, and no evidence of ACF observed in male or female rats (Laboratory Pharmacology and Toxicology, 2020 as cited in EFSA 2021 [4])

## 3.4. Overall Conclusion on the Safety of $TiO_2$ E171

Bettini et al. (1) demonstrated the absence of genotoxicity in vivo at low oral doses as no increase in DNA damage was detected in Peyer's patches of E171 and NM-105 ($TiO_2$ nanoparticles)-treated rats. This is consistent with an independent WoE review of the genotoxicity from 34 robust datasets that does not support a direct DNA damaging mechanism for $TiO_2$ in either the nano or micro form (23). Genotoxicity data from the most recent well-conducted study with $TiO_2$ nanoparticles also confirmed this (14). Further, no evidence of carcinogenicity, chronic toxicity, or other non-neoplastic lesions of the GIT were observed in a well-conducted, two-year cancer bioassay in male and female mice and rats using very high dietary concentrations (up to 5% w/w) of TiO2 highly comparable to currently used food-grade $TiO_2$ (2).

In addition, there was no evidence of reproductive or developmental toxicity in GLP and OECD TG 443 studies (25) in rats at E171 doses up to 1000 mg/kg bw/day, the highest doses tested.

All major data were also presented at a PQRI Workshop in November 2023, and a position paper was provided to EMA (28). The information provided at the PQRI Workshop and summarised in this position paper show, that there are no significant safety concerns with the use of TiO2 (E171) in food or pharmaceutical products. This has been the conclusion of several other global regulatory agencies as well as JECFA who have all decided that no action is needed to limit the existing use of TiO2 that are allowed at this time in each of their markets. All experts involved in the PQRI Workshop and who are authors of this paper recommend that EMA strongly support the continued use of E171 in pharmaceuticals in their proposal to the European Commission in April 2024.

Based on all available data it is reasonable to conclude that E171 has no identifiable hazard. The WoE does not demonstrate genotoxicity safety concerns connected to the use of ($TiO_2$) as a colour additive and opacifier. This conclusion is consistent with conclusions reached following the scientific reviews by HC and FSANZ authorities and the JECFA.

Therefore, for E171, the NCI oral carcinogenicity study in rats and mice (1979) was identified as the most relevant study to define a PoD as it covers both systemic toxicity after lifetime exposure as well as the most severe endpoint (carcinogenicity) as a possible consequence of any genotoxic potential.

## 3.5. Derivation of an oral PDE

The term titanium dioxide ($TiO_2$) covers several hundred materials with different physicochemical properties. Its biological effects are related to some key physicochemical properties, i.e., particle size, charge, crystallinity, shape, and agglomeration state. Many of the toxicological studies conducted do not provide sufficient characterization of these parameters (23). However, the WoE suggests $TiO_2$ presents no identifiable hazard. For E171, there are some key studies that would allow a PDE to be calculated, although most regulatory authorities and the JECFA do not consider E171 a hazard.

EFSA's concern about the genotoxic potential of E171 stems from the incidental presence of nanoparticles. No risk-benefit assessment for incidental nanoparticles has been performed so far, which is an important consideration for use in medicinal products. In the EU there is no guidance on the presence of incidental nanoparticles in excipients in medicinal products, whereas in the US, the presence of nanoparticles in an existing excipient was recently discussed in an FDA Guidance (29). In their view if these excipients containing nanoparticles with a history of use in humans are used in the same way as they have been used historically with the same dose level and in drug products with the same route of administration then they are considered low risk (Drug Products, Including Biological Products, that Contain Nanomaterials. Guidance for Industry. FDA, April 2022 Pharmaceutical Quality/CMC) (29).

It is therefore important that a position on incidental nanoparticles in excipients used in medicinal products is established in the EU. $TiO_2$ can serve as a first case for such a risk-benefit assessment by establishing a PDE for $TiO_2$ containing <50% incidental nanoparticles by number that ensures patient confidence in safety.

Currently, the main challenge is the use of $TiO_2$ (or E171) in various products e.g., food, cosmetics, consumer products, medicinal products and that it is subject to different legislations in particular in Europe. The most relevant in this case is the food legislation EU 1333/2008 (positive list of colours) (30) and EU 231/2012 (E-number purity criteria) (31), as it is referred to in both the pharmaceutical (Dir 2009/35/EC (32) referring to both) and cosmetic (only referring to the purity criteria) regulations. The precautionary ban of $TiO_2$ in food in the EU, in the absence of solid data demonstrating a real risk, leaves the pharmaceutical industry and patients in an uncertain situation. Further confusion for patients is provided by the fact that other regions do not ban $TiO_2$ from food or as an excipient in medicinal products. As outlined in the document, these other non-EU agencies do not see a relevant hazard that would limit the use of E171.

Based on all available data it is reasonable to conclude that E171 has no identifiable hazard and the precautionary measure for E171 is no longer appropriate. In addition, the low bioavailability of the E171 has to be considered in a final risk assessment. This is particularly true for medicines, with E171 contents generally in the range of 0,075 – 45 mg/tablet (across company survey). Although it is unusual from a toxicological perspective to derive a PDE for a non-hazardous compound, a PDE calculation using scientifically robust data might increase confidence of patients in the safety of medicines containing $TiO_2$ and will allow pharmaceutical industry to continue to provide patient access to life-saving medicines and to develop innovative high-quality medicines in the future.

Establishing a PDE requires the selection of the most relevant repeat dose toxicity study in the most sensitive species as a Point of Departure (PoD). For E171, the NCI oral carcinogenicity study in rats and mice (1979) was identified as the most relevant study as it covers both systemic toxicity after lifetime exposure as well as the most severe endpoint (carcinogenicity) as a possible consequence of any genotoxic potential. The form of TiO2 used in this study was referred to as Unitane 0-220, in the anatase form. Recent physicochemical investigations (essentially pure anatase (>99.5% anatase), median diameter of 106-135nm, 20-44% of particles by number are <100 nm)) have demonstrated that Unitane 0-220 is very similar to E171 which justifies the use of this study as the most relevant study.

In the NCI study, the test item was administered in the diet at levels of 2.5% and 5%, in addition to a control group at 0%. It is widely accepted that a dietary level of 5% is the highest achievable dose in such chronic animal feeding studies as nutritional abnormalities occur above this level and is the maximum feasible dose recommended for dietary administration by today's standards (27). Considering the body weight and food consumption of the animals, different doses were calculated in each species and gender. On a mg/kg bw/day basis the INS 171 doses in rats were calculated to be lower than those given to mice, where the highest doses tested were considered NOAELs, and were equivalent to 2250 mg/kg/day in male rats and 2900 mg/kg/day in female rats. The study found no effect on survival or body weight gain, and no incidence of tumours above historical or concurrent controls in rats or mice. There was no evidence of increased non-neoplastic effects above background, including preneoplastic changes such as hyperplasia, nor were there any effects on the GIT, including the colon. Based on the results of this study, the lowest high dose NOAEL in rat was determined to be 2250 mg/kg bw/day (equivalent to the 5 % dose) and was selected as the PoD for the oral PDE as the most conservative estimate.

The PDE calculation with adjustment factors was performed according to the principles stated in the widely accepted ICH Q3C(R8) (33) / ICH Q3D(R2) (34) guideline. The PDE is based on the proposed specification for E171 provided by EFSA FAF Panel 2019 (3).

Lowest NOAEL = 2250 mg/kg/day (male rats)

F1 Extrapolation from rat to human: 5

F2 Interindividual variability: 10

F3 Lifetime exposure: 1

F4 Non-carcinogenic: 1

F5 NOAEL established: 1

Human body weight: 50 kg

$$\frac{2250\,\frac{mg}{kg\ x\ day}}{5\ x\ 10\ x\ 1\ x\ 1\ x\ 1}\ x\ 50\ kg = 2250\ \mathrm{mg/day}$$

**Oral PDE TiO2 (E171, anatase) = 2250 mg/day**

Using the lowest NOAEL from mice (i.e., the lowest high dose tested of 6500 mg/kg bw/day, male mice), and applying the factor of 12 for extrapolation from mice to human would lead to a similar PDE of 2708 mg/day.

Taking all data (low bioavailability, negative in vivo mutagenicity and carcinogenicity) and calculations together, the $TiO_2$ Alternatives consortium is proposing an oral PDE of 2250 mg/day to support the risk-benefit assessment of E171 as an excipient in oral pharmaceutical products, despite the fact that no hazardous properties have been identified for this material.

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# List of Appendixes

**Appendix 1: List of Consortium companies and supportive Trade Associations**

***$TiO_2$ Alternatives Consortium members:***

(1) **PFIZER Inc.**, whose administrative offices are at 235 East 42nd Street New York, NY 10017, United States of America ("**PFIZER**").

(2) **JANSSEN PHARMACEUTICA NV,** whose administrative offices are at Turnhoutseweg 30, B-2340, Beerse, Belgium ("**JANSSEN**").

(3) **ABBVIE Inc.,** whose administrative offices are at 1 North Waukegan Road, North Chicago, IL 60064 USA ("**ABBVIE**").

(4) **BAYER AKTIENGESELLSCHAFT,** whose administrative offices are at Kaiser-Wilhelm-Allee 1, 51373 Leverkusen, Germany, ("**BAYER**").

(5) **GLAXOSMITHKLINE RESEARCH & DEVELOPMENT LTD.,** whose administrative offices are at England and Wales, located at 980 Great West Road, Brentford, Middlesex, TW8 9GS, UK, ("**GSK**").

(6) **ELI LILLY AND COMPANY,** whose administrative offices are at Lilly Corporate Center, Indianapolis, Indiana 46285, U.S.A, ("**LILLY**").

(7) **MERCK KOMMANDITGESELLSCHAFT AUF AKTIEN,** whose administrative offices are at Frankfurter Straße 250, 64293 Darmstadt, Germany ("**MKDG**").

(8) **NOVARTIS AG,** whose administrative offices are at Novartis Campus Fabrikstrasse 2, CH-4056 Basel, Switzerland ("**NOVARTIS**").

(9) **SANOFI AVENTIS DEUTSCHLAND GMBH,** whose administrative offices are at Industriepark Höchst, 65926 Frankfurt am Main, Germany ("**SANOFI**").

(10) **INSTITUT DE RECHERCHES INTERNATIONALES SERVIER,** whose administrative offices are at 50 rue Carnot, 92284 Suresnes Cedex, France, ("**IRIS**").

(11) **TAKEDA PHARMACEUTICALS INTERNATIONAL AG,** with offices at Thurgauerstrasse 130, 8152 Glattpark-Opfikon, Switzerland ("**TAKEDA**").

(12) **TEVA PHARMACEUTICALS EUROPE BV,** whose administrative offices are at Piet Heinkade 107, Amsterdam, 1019GM, the Netherlands ("**TEVA**").

(13) **F. HOFFMANN LA-ROCHE LTD.,** whose administrative offices are at Grenzacherstrasse 124, CH-4070 Basel, Switzerland ("**ROCHE**").

(14) **BRISTOL-MYERS SQUIBB PHARMACEUTICALS LIMITED**, whose administrative offices are at Uxbridge Business Park, Sanderson Road, Uxbridge, Middlesex., UB8 1DH, United Kingdom ("**BMS**").

(15) **GSK CONSUMER HEALTHCARE SARL, A HALEON GROUP COMPANY,** whose administrative offices are at Route de L' Etraz 2, 1260 Nyon, Switzerland ("**HALEON**"**).**

(16) **STADA Arzneimittel AG,** whose administrative offices are at Stadastrasse 2-18, 61118 Bad Vilbel, Germany ("**STADA")**.

(17) **EUROPEAN RESEARCH AND PROJECT OFFICE GMBH**, whose administrative offices are at Heinrich-Hertz-Allee 1, 66386 St. Ingbert, Germany ("**EURICE")**.

(18) **ASTELLAS PHARMA EUROPE BV,** whose administrative offices are at Sylviusweg 62, 2333, Leiden, the Netherlands, ("**ASTELLAS**").

(19) **MYLAN PHARMA UK LTD.** whose administrative offices are at Station Close, Potters Bar, Hertfordshire, EN6 1 TL, England ("**MYLAN**").

(20) **MERCK SHARPE & DOHME** whose administrative offices are at New Jersey limited liability, having a place of business at 126 East Lincoln Avenue, Rahway, NJ 07065, USA ("**MSD**").

(21) **BOEHRINGER INGELHEIM INTERNATIONAL GMBH,** whose administrative offices are at Binger Strasse 173, 55216 Ingelheim am Rhein, Germany ("**BI**").

***Trade Associations:***

Given there is no identifiable hazard for titanium dioxide, as confirmed by the 2023 WHO/JECFA assessment, the proposal to establish a PDE for titanium dioxide is also supported by the Trade Associations listed below, where establishing the PDE will reassure patients that $TiO_2$ use is actively monitored and controlled at safe levels.

- AESGP (Association of the European Self-Care Industry)
- CEFIC (European Chemical Industry Council)
- EFPIA (European Federation of Pharmaceutical Industries and Associations)
- EUCOPE (European Confederation of Pharmaceutical Entrepreneurs)
- IPEC (International Pharmaceutical Excipient Council)
- MfE (Medicines for Europe)

**Appendix: Characterisation of INS171 (Report provided by CEFIC)**

TDMA report: Comparison of current food grade titanium dioxide (E 171) with historical samples of Unitane O-220. Apr 2022.

2022-04-12 - TDMA1175b - Comp

Conclusion

It can be seen that Unitane O-220 is very similar in all physical and chemical characteristics to the current E171 grades and lies within the draft E171 specification.