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Evaluation of different *in vitro* dissolution tests based on level A *in vitro–in vivo* correlations for fenofibrate self-emulsifying lipid-based formulations

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1. Introduction

Many problems arise due to the poor solubility of Biopharmaceutics Classification System (BCS) Class II compounds. The limited dissolution rate arising from low solubility frequently results in the low bioavailability of orally administered drugs, and generally these compounds present dissolution-limited absorption. Several strategies for dealing with the formulation problems of poorly water soluble compounds have been developed and described in the literature [1]. Lipid-based drug delivery systems and solid dispersions (SD) constitute some of the possible approaches to improving drug bioavailability. Usually, solid dispersions are prepared via melting or solvent evaporation methods. However, an innovative green process for producing solid dispersions with lipid excipients, namely the Particles from Gas Saturated Solutions (PGSS) process, seems to be a promising approach to improving the bioavailability of BCS Class II compounds [2]. This technique was used during the present study for the production of lipid-based SD and was compared with the classical melting technique.

If the development of an oral delivery formulation of BCS Class II Active Pharmaceutical Ingredients (APIs) is a frequent and great challenge to formulation scientists in the pharmaceutical industry, the development of an *in vitro* tool to estimate *in vitro* release and to optimize the formulation is not as obvious as for Class I/III drugs. Indeed, for compounds with poor aqueous solubility, maintaining sink conditions can be problematic, rendering the characterization of complete dissolution a challenging task. In order to establish sink conditions, several solubility modifiers, such as surfactants, inorganic salts and organic co-solvents are routinely added to aqueous dissolution media [3]. However, these experimental conditions are not always discriminant, nor do they mimic *in vivo* conditions. It is well known that as a tool for the quality control of pharmaceutical products, a dissolution method should be capable of discriminating products made with different materials and/or processes [4] that could lead to different outcomes *in vivo*. Indeed, a biorelevant *in vitro* dissolution test should be an indicator of how the formulation will perform *in vivo*. In this case, the adequacy of the *in vitro* dissolution method has to be shown through an *in vitro–in vivo* correlation (IVIVC) for which predictability has been established [5]. IVIVC is a tool recommended on both sides of the Atlantic (by the FDA and EMA). ICH guideline Q8 [6] specifies that a successful correlation can assist in the selection of appropriate dissolution acceptance criteria and can potentially reduce the need for further bioequivalence studies following changes to the product or its manufacturing process.

Two of the essential conditions for developing an IVIVC are firstly, that the apparent *in vivo* absorption must be dissolution rate limited and secondly, that the *in vitro* dissolution rate must be the critical dosage form attribute [7]. Bearing in mind these considerations, IVIVC establishment might be suitable for immediate release products with BCS Class II drugs such as fenofibrate, for which the dissolution is the rate limiting step for absorption. This dissolution is driven by API characteristics (such as its polymorphic form or amorphous form), the type of formulation and its excipients or even the production process used. However, although many studies regarding the performance of fenofibrate formulations can be found in the literature, few of these investigations have been successful. For example, the *in vitro* lipolysis test (frequently used for lipid-based formulations) has been shown not to adequately

Abbreviations: Af, absorbed API fraction without EHC; API, Active Pharmaceutical Ingredient; AUC, Area Under the Curve; BCS, Biopharmaceutics Classification System; C_{max}, Maximal Concentration; C_S, Saturation Concentration; DSC, Differential Scanning Calorimetry; EHC, EnteroHepatic Circulation; EMA, European Medicines Agency; FaSSGF, Fasted State Simulated Gastric Fluid; FaSSIF, Fasted State Simulated Intestinal Fluid; FDA, Food and Drug Administration; FeSSIF, Fed State Simulated Intestinal Fluid; GIT, Gastro Intestinal Tract; GLP, Good Laboratory Practice; HPLC, High Performance Liquid Chromatography; ICH, International Council for Harmonization; IVIVC, *In Vitro–In Vivo* Correlation; LC, Liquid Chromatography; MS, Mass Spectroscopy; PE, Prediction Error; PGSS, Particles from Gas Saturated Solutions; PVDF, PolyVinyliDene Fluoride; RPM, Rotation Per Minute; SD, Solid Dispersion; SEM, Standard Error of the Mean; t_{max}, maximal time; USP, United State Pharmacopeia.

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predict the *in vivo* performance of this API [8,9], or else to present limitations [10]. There are also some examples where biorelevant dissolution media have been used to test the dissolution rate of fenofibrate [11,12]. However, in this case, the correlation seemed to depend greatly on the animal species selected to carry out the *in vivo* study [13]. The use of a biphasic *in vitro* dissolution test seems to be more promising [14–16]. It has been demonstrated in the literature that a biphasic dissolution test might be useful in the performance evaluation of fenofibrate formulations, especially if these formulations lead to *in vivo* supersaturation [17]. Moreover, Durdunji et al. [18] developed a biphasic dissolution system by coupling USP apparatus IV with USP apparatus II. This combined apparatus was able to achieve a level A correlation for an immediate release formulation of Deferasirox, a BCS Class II compound. Level A is the highest level of correlation and represents a point to point relationship between *in vitro* dissolution rate and the *in vivo* absorption rate or cumulative absorption. As already mentioned, this level of IVIVC is a useful tool that can accelerate the drug development process, helping to set dissolution specifications or possibly being used as a surrogate for bioequivalence studies. *In vivo* studies in human subjects provide highly relevant information for establishing IVIVCs. However, such studies face various complexities, including the need for justification by an Ethics Committee with additional limitations in terms of throughput and cost. It is for this reason that *in vivo* studies on animals are sometimes preferred in order to screen formulations and to establish IVIVC. Human studies may then be performed in order to confirm and translate predictions to the human situation. Animal studies would therefore be of great interest in testing a molecule that might exhibit a risk in humans and for which the formulation might optimize exposure to the API. Bearing this in mind, the pharmacokinetic study, which formed part of our investigation, was carried out with animals. Usually, *in vivo* pharmacokinetic studies on animals are performed on dogs, rats/mice or pigs (Landrace or Minipig). However, animal studies often yield species differences and the question arises as to which animal species is most representative for humans. In the present study, the pig was selected as the model for different reasons. In fact, pigs are considered as a translational model in biomedical research because of their anatomical, physiological and biochemical similarities to humans. Indeed, the size of the pigs'GIT regions, in relation to total body weight, is generally very similar to that of humans. Moreover, as with humans, the pig is monogastric, and acid secretion occurs in response to stimuli, such as food intake. Pig bile has a similar composition to human bile, and it is stored in the gall bladder and secreted into the duodenum as in humans.

The aim of this paper was to evaluate different *in vitro* dissolution tests capable of achieving a level A IVIVC. These dissolution tests were developed in order to analyze different self-emulsifying lipid-based formulations containing a BCS Class II drug model compound (fenofibrate). Our premise was that if one test were proven successful in this correlation, it would be of great help for our development of future formulations.

2. Material and methods

2.1. Materials

CO_2 (99.998%) was supplied by Air Liquide (Liège, Belgium). Fenofibrate (Ph.Eur.7) was provided by Moehs (Barcelona, Spain) and Gelucire® 50/13 by Gattefossé (Saint-Priest, France). Octanol (general purpose grade) was purchased from Fisher Scientific® (Loughborough, UK) and hydrochloric acid (HCl 37% for analysis) from Merck® (Darmstadt, Germany). Water was purified via a Millipore® system (18.2 MΩ/cm resistivity, Milli-Q) before filtration through a 0.22 μm Millipore Millipak® – 40 disposable filter units (Millipore Corporation, USA). Methanol, acetonitrile, ammonium acetate and glacial acetic acid were of ULC/MS grade from Biosolve® (Valkenswaard, the Netherlands). SIF Powder Original was purchased from Biorelevant.com (Surrey, United Kingdom).

2.2. Tested formulations

Four fenofibrate self-emulsifying lipid-based formulations were tested during this study. All formulations were a binary mixture of fenofibrate and Gelucire® 50/13 as the self-emulsifying excipient. The differences between these formulations were the proportions of the API and the excipient and the technique used for the formation of the solid dispersion particles.

Three formulations were produced via a PGSS process using supercritical carbon dioxide. During this process, the materials were loaded into a saturation vessel and placed in contact with carbon dioxide at the established pre-expansion pressure and temperature conditions, and were then mixed for approximately 15 min. Following this period of mixing, the valve between the saturation and expansion vessels was opened and the gas saturated solution was expanded out through the nozzle. When the expansion was completed, the expansion vessel was opened and the particles were collected and stored. Further explanation regarding this technique and its optimization can be found in a previous publication [2]. Regarding the different proportions of the API and the excipient, three formulations were selected based on the solubility of fenofibrate in Gelucire® 50/13, which had previously been evaluated by differential scanning calorimetry (DSC) [2]: one formulation was below the maximal level of solubility (150 mg/g), one was close to the maximal solubility (220 mg/g) and one was well above the maximal solubility (750 mg/g). Moreover, the three tested PGSS formulations were characterized by two different production parameters i.e. pressure and temperature.

In summary, the four tested formulations used in the present study were:

- **PGSS 220:** this was a previously-developed optimized PGSS product [2]. This formulation was dosed at 220 mg of fenofibrate per gram of Gelucire® 50/13 and was produced at a temperature of 78 °C and a pressure of 80 bars.
- **PGSS 150:** this was a PGSS product dosed at 150 mg of fenofibrate per gram of Gelucire® 50/13. The conditions of production were at an intermediate level within the PGSS processing range used, i.e. an autoclave temperature of 65 °C and an autoclave pressure of 160 bars.
- **PGSS 750:** this was a PGSS product dosed at 750 mg of fenofibrate per gram of Gelucire® 50/13. The conditions of production were also at an intermediate level within the PGSS processing range used (65 °C and 160 bars).
- The final formulation (**SD 220**) was a solid dispersion containing the same proportion of fenofibrate – Gelucire as the optimized PGSS formulation (220 mg of fenofibrate per gram of Gelucire® 50/13) but, in this case, it was produced by a melt mixing process. This solid dispersion was subsequently micronized in order to obtain a particle size close to the size obtained with the PGSS process. This process is also described in more detail in [2]. This formulation was chosen for the testing in order to highlight a possible improvement in bioavailability generated by the PGSS process compared to a classical method such as melt mixing.

2.3. In vitro dissolution tests

During the present study, five different dissolution media were tested: four single phase dissolution media and one biphasic med-