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Title

In vitro dissolution models for the prediction of *in vivo* performance of an oral mesoporous silica formulation

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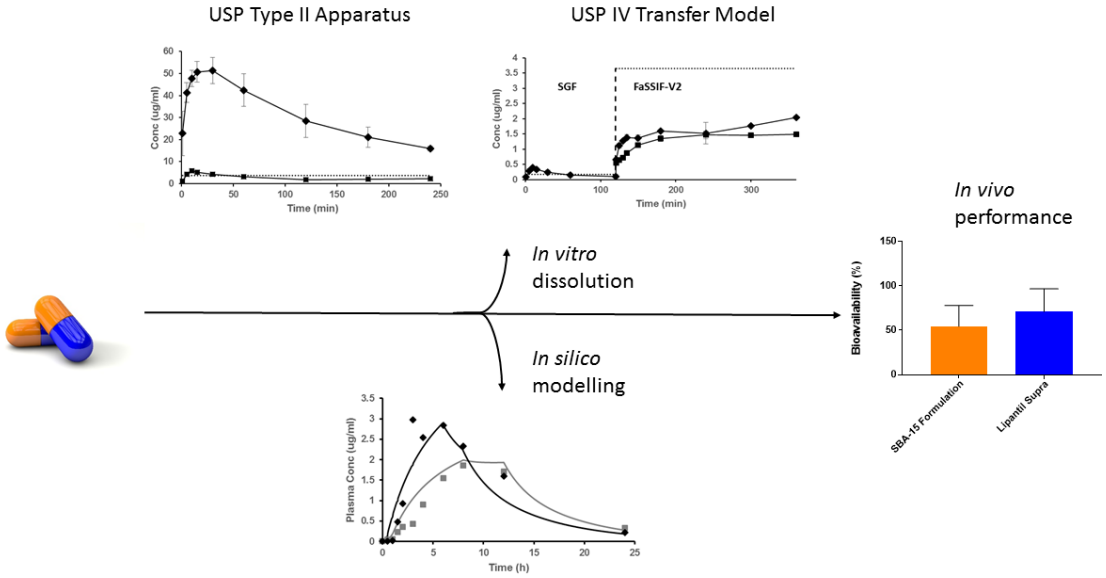
Keywords

Mesoporous silica, dissolution, transfer model, hydrodynamics, supersaturation, *in vitro in vivo* relationship (IVIVR), fenofibrate

Graphical Abstract



Abstract

Drug release from mesoporous silica systems has been widely investigated *in vitro* using USP Type II (paddle) dissolution apparatus. However, it is not clear if the observed enhanced *in vitro* dissolution can forecast drug bioavailability *in vivo*. In this study, the ability of different *in vitro* dissolution models to predict *in vivo* oral bioavailability in a pig model was examined. The fenofibrate-loaded mesoporous silica formulation was compared directly to a commercial reference product, Lipantil Supra®. Three *in vitro* dissolution methods were considered; USP Type II (paddle) apparatus, USP Type IV (flow-through cell) apparatus and a USP IV Transfer model (incorporating a SGF to FaSSIF-V2 media transfer). *In silico* modelling, using a physiologically based pharmacokinetic modelling and simulation software package (Gastroplus™), to generate *in vitro/in vivo* relationships was also investigated. The study demonstrates that the *in vitro* dissolution performance of a mesoporous silica formulation varies depending on the dissolution apparatus utilised and experimental design. The findings show that the USP IV transfer model was the best predictor of *in vivo* bioavailability. The USP Type II (paddle) apparatus was not effective at forecasting *in vivo* behaviour. This observation is likely due to hydrodynamic differences between the two apparatus and the ability of the transfer model to better simulate gastrointestinal transit. The transfer model is advantageous in forecasting *in vivo* behaviour for formulations which promote drug supersaturation and as a result are prone to precipitation to a more energetically favourable, less soluble form. The USP IV transfer model could prove useful in future mesoporous silica formulation development. *In silico* modelling has the potential to assist in this process. However, further investigation is required to overcome the limitations of the model for solubility enhancing formulations.

1. Introduction

Recent developments in drug discovery have increased the number of BCS Class II drug candidates. These drug molecules pose significant challenges in terms of oral drug delivery and biopharmaceutics [1, 2]. Drug loading onto a mesoporous silica substrate using various novel loading approaches (including solvent methods, supercritical fluid and microwave irradiation), has been considered as a possible formulation strategy to improve drug aqueous solubility [3-6]. Drug molecules are stabilized on the silica surface and within silica pores in an amorphous state, which enhances the drug's dissolution rate [7, 8]. However, further research is required to fully understand the behaviour of these mesoporous silica formulations *in vitro* and *in vivo*.

Van Speybroeck *et al* published two *in vivo* studies which investigated the impact of supersaturation and precipitation on release from these formulations [9, 10]. However, the total number of mesoporous silica *in vivo* studies published in the literature is limited [11-13]. The majority of *in vitro* dissolution experiments conducted involving mesoporous silica formulations have utilised traditional methods: USP Type II apparatus and sink conditions with simple buffer solutions as the dissolution medium [14-18]. There are limitations associated with this traditional approach to dissolution which are of particular relevance to poorly water-soluble drug candidates [19]. Augustijns *et al* recommended that non-sink *in vitro* dissolution conditions are required for silica-based formulations to provide meaningful data that can be correlated with *in vivo* studies [20]. Furthermore, as the simple buffer solutions used in most *in vitro* experiments do not represent all aspects of the fluid composition of the gastrointestinal (GI) tract, it is preferable to use biorelevant media that better simulate physiological fluids [21]. The Type IV dissolution apparatus offers the ability

to change the dissolution medium during an experiment, which results in conditions that more closely reflect the pH gradient associated with transit through the GI tract [22]. It has been reported that the Type IV dissolution apparatus is a better simulator of *in vivo* hydrodynamics than the paddle apparatus [23]. However, the number of studies which utilise this model are limited and published data with regards to the superiority of the Type IV flow-through cell over the Type II apparatus is not in agreement [24, 25].

In this study, we compared the ability of *in vitro* dissolution methods to predict *in vivo* performance of an oral mesoporous silica drug delivery system. Type II and Type IV dissolution apparatus were employed to investigate the release of a poorly water-soluble drug, fenofibrate, from this formulation. This study is the first to our knowledge to use the Type IV apparatus to assess dissolution of a BCS Class II drug-loaded mesoporous silica system. Fenofibrate was chosen as the model compound in this study. Fenofibrate is a neutral, lipophilic drug (log *p* = 5.2), which is practically insoluble in water [26]. The *in vivo* performance of the mesoporous silica formulation was assessed following oral administration in a fasted pig model and compared to the commercial fenofibrate formulation, Lipantil Supra® (which utilises NanoCrystal® technology) [27].

In vitro/in vivo correlations (IVIVC) and *in vitro/in vivo* relationships (IVIVR) are being increasing used as part of the formulation 'toolbox' to build on knowledge from *in vitro* data and forecast formulation effects. The best candidates for *in vitro/in vivo* correlations are drugs where dissolution is the rate-limiting step to drug absorption and biorelevant dissolution models are used in the experimental design [28]. In this study, data from the *in vitro* and *in vivo* experiments was analysed using a physiologically-based pharmacokinetic

modelling and simulation software package (Gastroplus™) to generate IVIVR. The potential benefits and limitations of this *in silico* modelling approach are discussed.

2. *Material and Methods*

2.1. Materials

Fenofibrate was purchased from Kemprotec Ltd. (United Kingdom). SBA-15 was obtained from Glantreo Ltd. (Ireland). Liquid carbon dioxide was supplied by Irish Oxygen Ltd. (Ireland). Fenofibric acid, sodium taurocholate (>95%) and pepsin (from porcine gastric mucosa, 800-2500 units/mg protein) were obtained from Sigma Aldrich (Ireland). Lecithin (Lipoid E PC S, >98% pure) was kindly donated by Lipoid GmbH, Germany. All other chemicals and solvents were of analytical grade or HPLC grade, and purchased from Sigma-Aldrich (Ireland). Lipantil® Supra 145mg film-coated tablets were sourced through a local community pharmacy.

2.2. Preparation of Fenofibrate-Loaded Silica Formulation

Fenofibrate loaded SBA-15 was prepared according to the method previously described by Ahern *et al* [14]. The drug and mesoporous silica (2 g) at a drug:silica mass ratio of 2:3 were combined in a BC 316 high-pressure reactor (High Pressure Equipment Company, USA) and stirred using a magnetic stirring. The reactor was heated to 40 °C using heating tape and maintained at this temperature for the duration of the experiment. Temperature was monitored using a temperature monitor (Horst GmbH, Germany). The reactor cell was filled with liquid CO_2 and a high pressure pump (D Series Syringe Pump 260D, Teledyne ISCO, USA) was used to pump additional CO_2 to a final processing pressure (27.58 MPa). After 12 h, the

cell was depressurised rapidly by venting the CO_2. The processed material was collected from the cell and stored in a desiccator prior to analysis.

2.3. Drug Content Quantification

The fenofibrate content of the silica formulation was determined by thermogravimetric analysis (TGA), using a TGA 500 instrument (TA Instruments Ltd., United Kingdom). Samples in the weight range 2–10 mg were loaded onto tared platinum pans and heated from ambient temperature to 900 °C, at a heating rate of 10 °C/min under an inert N_2 atmosphere. Samples were analysed in triplicate. The drug quantity was calculated based on the weight loss between 100 and 900 °C, corrected for the weight loss over the same temperature range for a silica (SBA-15) reference sample [4]. TGA thermograms were analysed using Universal Analysis 2000 software (TA Instruments Ltd., United Kingdom). Drug-loading efficiency was calculated using Equation 1:

$$\text{Drug loading efficiency } (\%) = \frac{\text{Actual drug loading (mg)}}{\text{Theoretical drug loading (mg)}}\ x\ 100 \quad \text{(Equation 1)}$$

The theoretical drug-loading was based on mass fraction of drug and silica used to prepare samples.

2.4. Solubility Measurements

Solubility studies were carried out by the addition of excess fenofibrate to biorelevant media using a standardised shake-flask method with a shake time of 24 h at 37 °C [29]. Simulated gastric fluid (SGF) was prepared as outlined in the USP NF 26 [30]. FaSSIF-V2 was prepared as outlined in the literature [21]. Samples (2 ml volume) were removed at 24 h and centrifuged at 16,500 *g* for 13 min using a Hermle z233M-2 fixed angle rotor centrifuge,

(HERMLE Labortechnik GmbH, Germany). The supernatant was removed and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC following dilution with acetonitrile.

2.5. *In Vitro* Dissolution Experiments

USP Type II (Paddle) Apparatus: Dissolution studies were carried out in triplicate with an Erweka® DT600 dissolution test system (Erweka GmbH, Germany). Tests were performed in 500 ml of SGF or FaSSIF-V2 at 37 ± 0.5 ^{o}C at a paddle rotation of 75 rpm. Drug-loaded silica samples equivalent to 50 mg fenofibrate were placed in the dissolution medium. The dose of 50 mg fenofibrate in the release media corresponded to a theoretical concentration of 100 µg/ml following complete dissolution which represents a supersaturated state in SGF and FaSSIF-V2 (see fenofibrate solubility values in section 3.2). Samples of 4 ml volume were withdrawn at 1, 5, 10, 15, 30, 60 and 120 min intervals (with additional samples taken from the FaSSIF-V2 media at 180 and 240min). Samples were immediately replaced with an equal volume of fresh, pre-warmed medium. The withdrawn samples were filtered through a 0.20 µm PES membrane filter (Filtropur S0.2, Sarstedt AG & Co., Germany). Samples were diluted with acetonitrile prior to analysis by HPLC.

USP Type IV (Flow-Through Cell) Apparatus: Dissolution studies were carried out in triplicate with an Erweka® flow-through apparatus (DFZ 720 with HKP 720 piston pump) equipped with 22.6 mm diameter cells. The temperature of the water bath was maintained at 37 ^{o}C. The dissolution medium of either 100 ml SGF or FaSSIF-V2, recirculated in closed loop model at a flow rate of 4 ml/min. A glass ball (5mm) and 1 g of glass beads (1mm) were placed in the bottom of the cone to ensure laminar flow of the jet of fluid entering the cell. Formulation samples equivalent to 10 mg fenofibrate were placed on top of the glass beads.

Undissolved fenofibrate particles were retained in the sample holder using a glass fibre filter located in the top of the cone. The dose of fenofibrate in the release media thus corresponded to a theoretical concentration of 100 µg/ml which represents a supersaturated state in SGF and FaSSIF-V2 (see fenofibrate solubility values in section 3.2). Samples of 1 ml volume were withdrawn at 1, 5, 10, 15, 30, 60 and 120 min intervals (with additional samples taken from the FaSSIF-V2 media at 180 and 240 min). Samples were immediately replaced with an equal volume of fresh, pre-warmed medium. The withdrawn samples were filtered through a 0.20 µm PES membrane filter (Filtropur S0.2, Sarstedt AG & Co., Germany). Samples were diluted with acetonitrile and analysed by HPLC. In addition to conducting individual dissolution experiments using the Type IV apparatus employing either SGF or FaSSIF-V2, a dissolution experiment involving a SGF to FaSSIF-V2 transfer method was conducted. Samples were removed as described above for the initial SGF stage of the experiment up to the 120 min time point. The SGF dissolution medium supply beaker was then removed and replaced with a beaker containing 100 ml of FaSSIF-V2 medium. Subsequent sampling time points were taken at 1, 5, 10, 15, 30, 60 and 120, 180 and 240 min) following transfer from SGF to FaSSIF-V2 medium.

2.6. *In vivo* oral bioavailability study

The study was carried out under licences issued by the department of Health, Ireland as directed by the Cruelty to Animals Act Ireland and EU Statutory Instructions. Local university ethical committee approval was also obtained. The data from this intravenous study has been previously used for the calculation of fenofibrate clearance in pigs to allow absolute bioavailability to be determined in separate studies [31, 32]. Female landrace pigs (17–19 kg) housed at the University College Cork's Biological Services Unit were used for these

experiments. Animals were fasted for 16 h before experimentation. On day 1, an indwelling intravenous catheter was inserted in the jugular vein, under general anaesthesia, as previously described [33]. Following recovery, pigs were returned to their pens and allowed access to food and water.

On day 2 (following an overnight fast), the oral formulations containing a dose of 67 mg fenofibrate were administered in gelatin capsules with the aid of a dosing gun. After dosing the pigs received 50 ml of water via an oral syringe. Blood samples (5 ml) were withdrawn from the jugular line at time zero (pre-dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h intervals post dosing. Water was available *ad libitum* throughout the study period and the animals were fed 8 h post-dose. For the intravenous treatment (i.v.), animals were administered 25 mg fenofibrate by slow infusion, over 2 min, via 3 ml of a solution containing 8.33 mg/ml fenofibrate in 80 %w/w ethanol and 20 %w/v physiological saline into an ear vein. Blood sampling was performed as outlined above. All blood samples were collected in heparinised tubes (Sarstedt, Germany) and centrifuged immediately after withdrawal at 3220 *g* for 5 minutes at 4 °C. Plasma samples were stored at −80 °C prior to HPLC assay.

2.7. Quantitative Analysis of Fenofibrate

HPLC analysis of the *in vitro* dissolution samples was performed using an Agilent 1200 series HPLC system with an UV/Vis detector (Agilent Technologies, USA). A reversed-phase column (Kinetex C-18, 150 × 4 mm x 2.6 µm, Phenomenex Ltd. UK), mobile phase of acetronitrile and water (80:20) at a flow rate of 1 ml/min and injection volume of 20 µl were employed. The wavelength for fenofibrate detection was set at 286 nm and retention time was 4.5 min.

In vivo plasma samples were quantified for fenofibric acid (the major active metabolite of fenofibrate). Based on a method by Griffin *et al* [32], a volume of 0.5 ml plasma was spiked with 50 µl of internal standard (sulindac) and vortexed. Proteins were precipitated through the addition of 0.5 ml of 25 %w/v NaCl solution and 1 ml of 1 %w/v H_3PO_4 in methanol with thorough mixing. Samples were centrifuged at 11,500 *g* for 9 min using a Hermle z233M-2 fixed angle rotor centrifuge (HERMLE Labortechnik GmbH, Germany). The supernatant (20 µl) was injected onto a Synergi C18 reversed phase column (250 x 4.6 x 2.6 µm, Phenomenex Ltd. UK) using the Agilent system described above. The mobile phase consisted of acetronitrile and water (80:20) adjusted to pH 2.5. The flow rate was set at 1 ml/min resulting in elution of fenofibric acid and fenofibrate at 6.5 and 10.5 min, respectively. The concentration of drug was determined at 286 nm.

2.8. *In vitro* and *in vivo* data analysis

The extent of fenofibrate release was calculated as area under the dissolution curve (AUC) using Prism (ver. 5, GraphPad Software Inc., USA.). Peak fenofibrate concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual dissolution profiles. Intravenous pharmacokinetic parameters were fitted to a two compartment model using the PKPlus™ module in Gastroplus™ (ver. 8.6, Simulations Plus Inc., USA). AUC for fenofibric acid after oral administration of both formulations was calculated for 24 h post-dosing using Prism. The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs. time profiles. Absolute bioavailability (F_{abs}) was calculated according to Equation 2:

$$F_{abs} = \left(\frac{\text{AUC oral}}{\text{AUC i.v.}}\right) x \left(\frac{\text{Dose i.v.}}{\text{Dose oral}}\right) \qquad \text{(Equation 2)}$$

Relative bioavailability was calculated as the ratio of $AUC_{0\rightarrow24}$ obtained after oral administration of the silica and Lipantil Supra® formulations. The relative extent of fenofibrate release from both formulations in the three different dissolution models was calculated as the ratio of AUC values. Results are reported as mean ± standard deviation.

In vitro dissolution data comparing the two formulations was tested for significance ($p < 0.05$) using a two-tailed, independent sample *t*-test, assuming Gaussian distribution and equal variance. Statistical analysis of the C_{max} and AUC values from the dissolution profile for both formulations were performed using a one-way analysis of variance (ANOVA) and post hoc Tukey's multiple comparisons test. *P*-values of <0.05 were considered significant. Paired *t*-tests were used to determine the statistical significance ($p < 0.05$) of calculated *in vivo* bioavailability and pharmacokinetic results, as each animal acted as its own control in this crossover study. All statistical analyses were performed using GraphPad Prism Version5, USA.

2.9. *In Silico* Predictive Modelling

In silico modelling was conducted using GastroPlus™ (ver. 9.0, Simulations Plus, USA.). The ADMET Predictor™ module was used to estimate fenofibrate physiochemical characteristics. Predictive mathematical models were generated using the IVIVCPlus™ component of the software. In this study, the Loo-Riegelman two-compartment method was implemented to deconvolute the *in vivo* oral plasma concentration profiles using intravenous data as previously published [31, 32]. An IVIVR was generated by correlating the fraction of drug absorbed *in vivo* with the fraction of drug dissolved *in vitro* (for the initial period of fenofibrate dissolution i.e. time points up to T_{max}). The data was then convoluted to generate a predicted plasma concentration-time profile, which was compared with the

observed *in vivo* data. The software displayed C_{max} and AUC for the observed and predicted profiles. It also generated the prediction error between the two profiles which can be used to evaluate the predictability of the correlation as described by the FDA [34]. The FDA require an average absolute percentage error (%PE) of 10 % or less for AUC and C_{max} for internal predictability. The %PE for each formulation should not exceed 15 %.

3. *Results*

3.1. Drug Content Quantification

Fenofibrate loading onto SBA-15 was 251.3mg drug/g silica (25.13 % ± 0.68). The low variability observed in the drug loading for this SC-CO_2 process is indicative of a homogeneous drug distribution on the silica surface [5]. The drug loading efficiency, calculated using Eq. 1, was 62.83%. The loading technique converted the fenofibrate to a non-crystalline solid phase as previously reported by Ahern *et al* [14].

3.2. Fenofibrate Solubility

Fenofibrate solubility in SGF was determined as 0.17 ± 0.05 µg/ml. Fenofibrate solubility in FaSSIF-V2 (3.64 ± 0.62 µg/ml) was significantly enhanced, which indicates that fenofibrate is solubilised in the micelles of simulated intestinal fluid [29].

3.3. *In Vitro* Dissolution

3.3.1. USP Type II (Paddle) Apparatus

Dissolution experiments using the USP Type II (paddle) method were conducted under supersaturated conditions (580 times drug saturated solubility in SGF and 27 times the saturated solubility in FaSSIF-V2). There was no detectable release of fenofibrate from the

silica formulation in SGF. This could be explained by the mechanism of drug release previously published for mesoporous silica systems *in vitro* [35, 36]. These reported dissolution profiles involve an initial burst release (where the majority of loaded drug is released) followed by a sustained secondary release [37]. As a result of the drug's low solubility in conditions of low pH and the absence of additional excipients to enhance solubility or stabilise dissolution, the release of fenofibrate was not quantifiable (the limit of quantification was 200 ng/ml). Fenofibrate dissolution from the Lipantil Supra® formulation resulted in a sustained supersaturation (Fig. 1). Drug release increased over the first 15 mins (C_{max} 2.79 ± 0.70 µg/ml), before reaching a plateau for the remainder of the experiment. The supersaturation ratio during this plateau phase (defined as C/C_s, where C_s is the saturated solubility) was 15.49. This marked solubility increase is most likely due to the composition of the Lipantil Supra® formulation which contains surfactants, sodium lauryl sulfate (SLS) and sodium docusate. SLS has been previously shown to significantly increase the solubility and dissolution rate of fenofibrate through a combination of wetting, micellar solubilisation and deflocculation [38, 39].

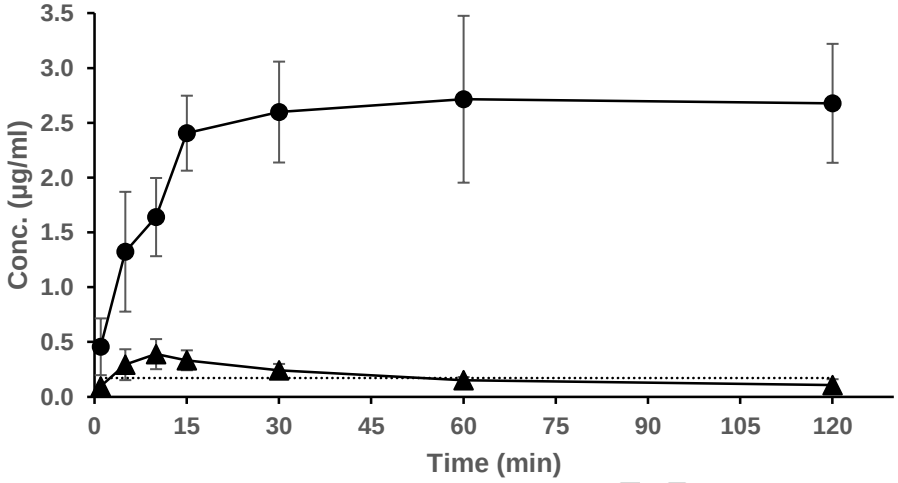


Fig 1. Fenofibrate release profiles for Lipantil Supra® in SGF at 37°C; (▲) indicates Type IV apparatus, (●) indicates Type II (paddle) apparatus. Dotted line indicates equilibrium solubility of fenofibrate in SGF. (n=3, Y error bars indicate standard deviation)

Compared to SGF, fenofibrate release from both formulations was significantly higher in FaSSIF-V2 due to the greater amount of physiologically relevant surfactants in the medium. FaSSIF-V2 allows for increased micellar solubilisation of the drug (Fig. 2). However, the extent of release from the commercial product was significantly higher than release from the silica formulation ($p<0.001$). Fenofibrate release from the silica formulation in FaSSIF-V2 media exhibited the classic 'burst release' profile characteristic of silica formulations [35, 36]. Drug dissolution maintained supersaturation levels for the first 30 min of the experiment (C_{max} = 5.76 ± 0.28 µg/ml, supersaturation ratio of 1.58). However, at 60 min, release had dropped below fenofibrate thermodynamic solubility levels. Fenofibrate release from the Lipantil Supra® formulation demonstrates high levels of supersaturation (C_{max} = 53.68 ± 2.73 µg/ml, peaking at a supersaturation ratio of 14.74). Drug dissolution decreases

after 30 min for the remainder of the experiment but never drops below supersaturation levels over the 4 h experiment.

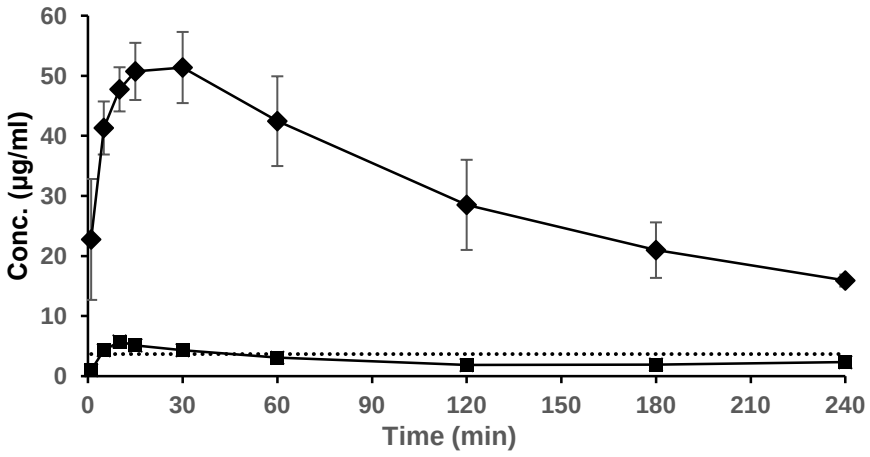


Fig. 2 Fenofibrate release profiles from mesoporous silica formulation (■) and Lipantil Supra® (◆) in FaSSIF-V2 media at 37°C for Type II (paddle) apparatus. Dotted line indicates fenofibrate solubility in FaSSIF-V2 media. (n=3, Y error bars indicate standard deviation)

A summary of the *in vitro* release parameters are detailed in Table 1. The high levels of release observed in this dissolution experiment with FaSSIF-V2 alone might not be indicative of *in vivo* performance. *In vivo*, the formulation will experience the low pH of the stomach initially (where the drug has extremely low solubility), which may cause significant precipitation. Precipitation to a lower energetically favourable, less water-soluble form can have a dramatic effect on drug release following transit to the small intestine environment mimicked by FaSSIF-V2. *In vitro* experiments simulating this transition were hence conducted and are described in the section 3.3.3.

Table 1. Summary of *in vitro* dissolution parameters. Mean values +/- standard deviation are provided (n=3)

Type II (Paddle) Apparatus			
Formulation	$C_{max\ FASSIF}$ (µg/ml)	$AUC_{240min\ FASSIF}$ (µg/ml.min)	T_{max} (min)
Mesoporous Silica	5.76±0.28	631±9	10±0
Lipantil Supra®	53.68±2.73	7493±1177	20±8.7
Type IV (Flow Through Cell) Apparatus			
Formulation	$C_{max\ FASSIF}$ (µg/ml)	$AUC_{240min\ FASSIF}$ (µg/ml.min)	T_{max} (min)
Mesoporous Silica	2.96±0.79	398±81	20±8.7
Lipantil Supra®	4.45±0.29	924±36	15±0
Transfer Model			
Formulation	$C_{max\ FASSIF}$ (µg/ml)	$AUC_{240min\ FASSIF}$ (µg/ml.min)	T_{max} (min)
Mesoporous Silica	1.49±0.07	322±18	160±69.3
Lipantil Supra®	2.04±0.06	364±30	240±0

3.3.2. USP Type IV (Flow-Through Cell) Apparatus

Non-sink conditions in the Type IV model were equivalent to those employed for USP II apparatus (580 times drug equilibrium solubility in SGF and 27 times the saturated solubility in FaSSIF-V2). Similar to results observed for the Type II model, fenofibrate release from the mesoporous silica formulation in SGF was not quantifiable. Fenofibrate release from Lipantil Supra® in SGF reached supersaturation levels for the first 30 min of the experiment (C_{max} = 0.41 ± 0.09µg/ml) but at 60 min release had fallen to the drug's equilibrium solubility (Fig. 1). As illustrated in Fig. 1, the C_{max} level and the extent of fenofibrate release was significantly higher using the Type II apparatus compared to the Type IV flow through cell ($p < 0.005$). This indicates that hydrodynamic differences between the two model apparatus

have a significant impact on the dissolution process and the final dissolution profile. This is discussed in more detail in Section 4.

Similar to the Type II apparatus, both formulations exhibit enhanced drug release in the FaSSIF-V2 media using the Type IV apparatus. The extent of release from Lipantil Supra® was significantly higher than that of the silica formulation ($p < 0.001$, Fig. 3). However, release from the SBA-15 system does not reach supersaturation levels in the Type II apparatus. Fenofibrate release from the Lipantil Supra® formulation peaks at 15 min (C_{max} = 4.45 ± 0.29µg/ml, supersaturation ratio of 1.22), then drops to remain at the thermodynamic solubility level for the duration of the four hour experiment. A summary of *in vitro* release parameters is provided in Table 1.

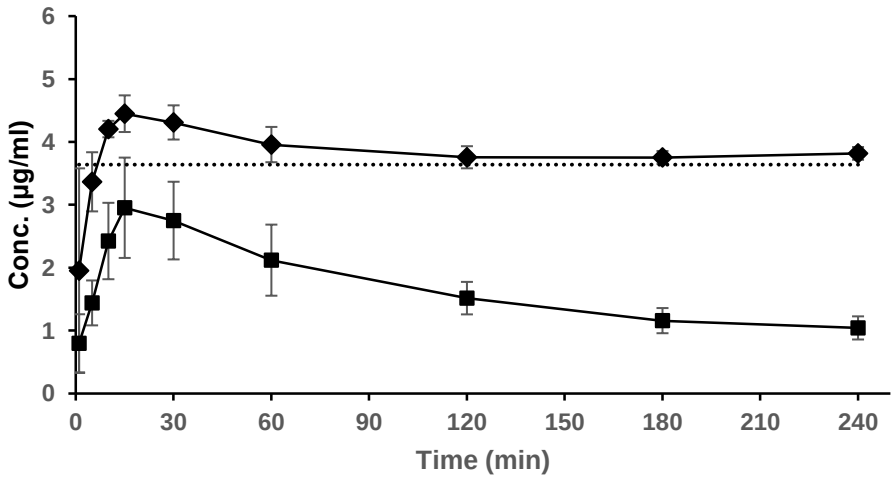


Fig. 3 Fenofibrate release profiles from mesoporous silica formulation (■) and Lipantil Supra® (◆) in FaSSIF-V2 media at 37°C for Type IV (flow through cell) apparatus. Dotted line indicates fenofibrate solubility in FaSSIF-V2 media. (n=3, Y error bars indicate standard deviation)

3.3.3. Transfer Model in USP Type IV (Flow-Through Cell) Apparatus

In the Type IV transfer model, samples were first exposed to SGF for 120 min followed by FaSSIF-V2 media for 240 min, to simulate GI transit in the dissolution model. As described in section 3.3.2, the Lipantil Supra® formulation reached supersaturation levels in the SGF, whereas release from the mesoporous silica system was unquantifiable (Fig. 4). The shape of the dissolution profile for the silica formulation in the transfer model (FaSSIF-V2 stage) is different from that of the Type II or Type IV FaSSIF-V2 profiles (Fig. 4). The classic 'burst' release in FaSSIF-V2 media was not evident using the transfer model and neither formulation reached supersaturation levels in the FaSSIF-V2 media (C_{max} = 2.04 ± 0.06 µg/ml for Lipantil Supra® and C_{max}= 1.49 ± 0.07 µg/ml for the silica formulation). It is probable that the reduction in the extent of dissolution in FaSSIF-V2 is due to fenofibrate precipitation upon exposure to SGF media for both formulations. While C_{max} for Lipantil Supra® was significantly higher than for the silica system ($p < 0.001$), there was no significant difference in the overall extent of fenofibrate release over the duration of the experiment ($p > 0.1$). This is in contrast to the differences in the extent of fenofibrate release for both formulations in FaSSIF-V2 media alone in USP Type II and USP Type IV apparatus (Table 1).

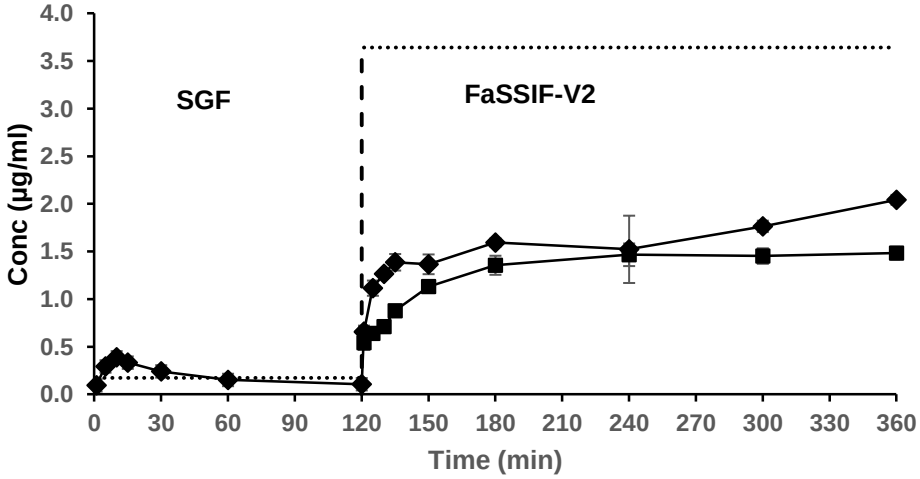


Fig. 4 Fenofibrate release profiles from mesoporous silica formulation (■) and Lipantil Supra® (◆) in USP IV Transfer Model at 37°C (incorporating SGF to FaSSIF-V2 transfer). Dotted line indicates fenofibrate solubility in SGF and FaSSIF-V2 media. (n=3, Y error bars indicate standard deviation)

3.4. *In Vivo* Oral Bioavailability

The plasma concentration profiles obtained following oral administration of 67 mg of fenofibrate, in the form of either Lipantil Supra® or the silica formulation, to fasted pigs are displayed in Fig. 5. A maximum plasma concentration of 3.96 ± 1.29 µg/ml was observed for Lipantil Supra® at 5.0 ± 2.4 h. The absorption of fenofibrate from the silica formulation was slower with a C_{max} of 2.34 ± 1.23 µg/ml at T_{max} 9.5 ± 3.0 h. A summary of the *in* vivo parameters for both formulations is provided in Table 2. The classical 'burst' release of the silica formulation, observed during both dissolution experiments conducted in FaSSIF-V2 alone was not evident in the *in vivo* pig model. No corresponding sharp onset of fenofibrate absorption was observed for the silica formulation. A slower rate of drug absorption was noted which indicates a slower release profile as noted in the transfer model FaSSIF-V2

phase. This indicates that the transfer model better simulates how the formulation will perform *in vivo*.

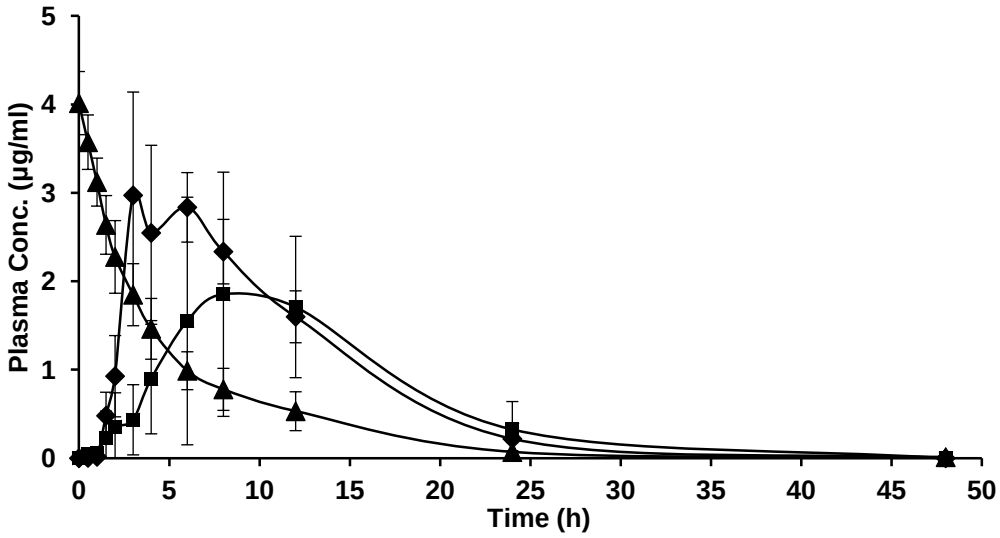


Fig. 5 Plasma concentration of fenofibric acid vs. time profiles after oral administration of 67 mg fenofibrate to fasted pigs, (■) indicates mesoporous silica formulation, (◆) indicates Lipantil Supra®, (▲) indicates intravenous preparation. (n=4, Y error bars indicate standard deviation

Table 2. Summary of *in vivo* pig model parameters. Mean values +/- standard deviation are provided (n=4). (*) denotes values which are significantly different ($p<0.05$).

Formulation	C_{max} (µg/ml)	$AUC_{0\rightarrow24h}$	T_{max}	$F_{abs0\rightarrow24h}$
Mesoporous Silica	2.34±1.23*	26502±11377	9.5±3	54.55±23.42
Lipantil Supra®	3.96±1.29*	34536±12527	5±2.4	71.08±25.78

Absolute bioavailability was determined for both formulations relative to an intravenous control. An absolute bioavailability of 54.55 ± 23.42% was observed for the silica formulation which was not significantly different to that of the commercial product, 71.08 ± 25.78% ($p > 0.1$). This confirms the potential of the silica system to enhance the bioavailability of fenofibrate. However the silica formulation has a slower onset of release when compared with the Lipantil Supra® (T_{max} of 5.0 ± 2.4h and 9.5 ± 3.0h, respectively). The relative bioavailability *in vivo* of the silica formulation versus Lipantil Supra® was 73.33 ± 17.07%.

To enable comparison of the *in vitro* dissolution and the *in vivo* bioavailability results, the ratio of extent of drug release (as a ratio of the silica formulation to Lipantil Supra® AUC) for the Type II, Type IV and USP IV transfer methods were plotted adjacent to the relative bioavailability of both formulations *in vivo* (Fig. 6). Fig. 6 highlights the differences between the extent of release from both formulations using the Type II (A) and Type IV (B) apparatus and similarity using the Type IV Transfer Model (C) and oral bioavailability in the in-vivo pig model (D). To facilitate further quantitative comparison, the ratio of extent of release of the silica vs. commercial formulation were calculated (Fig. 7). The ratios of extent of release determined for the Type II and Type IV dissolution data were 8.55±1.28% and 43.32±10.50% respectively. In contrast, the ratio of extent of fenofibrate release from the Type IV transfer method data was 89.16±12.49%. This ratio did not significantly differ from the relative *in vivo* oral bioavailability of these formulations ($p > 0.05$, Fig.7). This indicates that the USP IV transfer method was a superior predictor of *in vivo* performance compared to USP II and IV with FaSSIF-V2 alone.

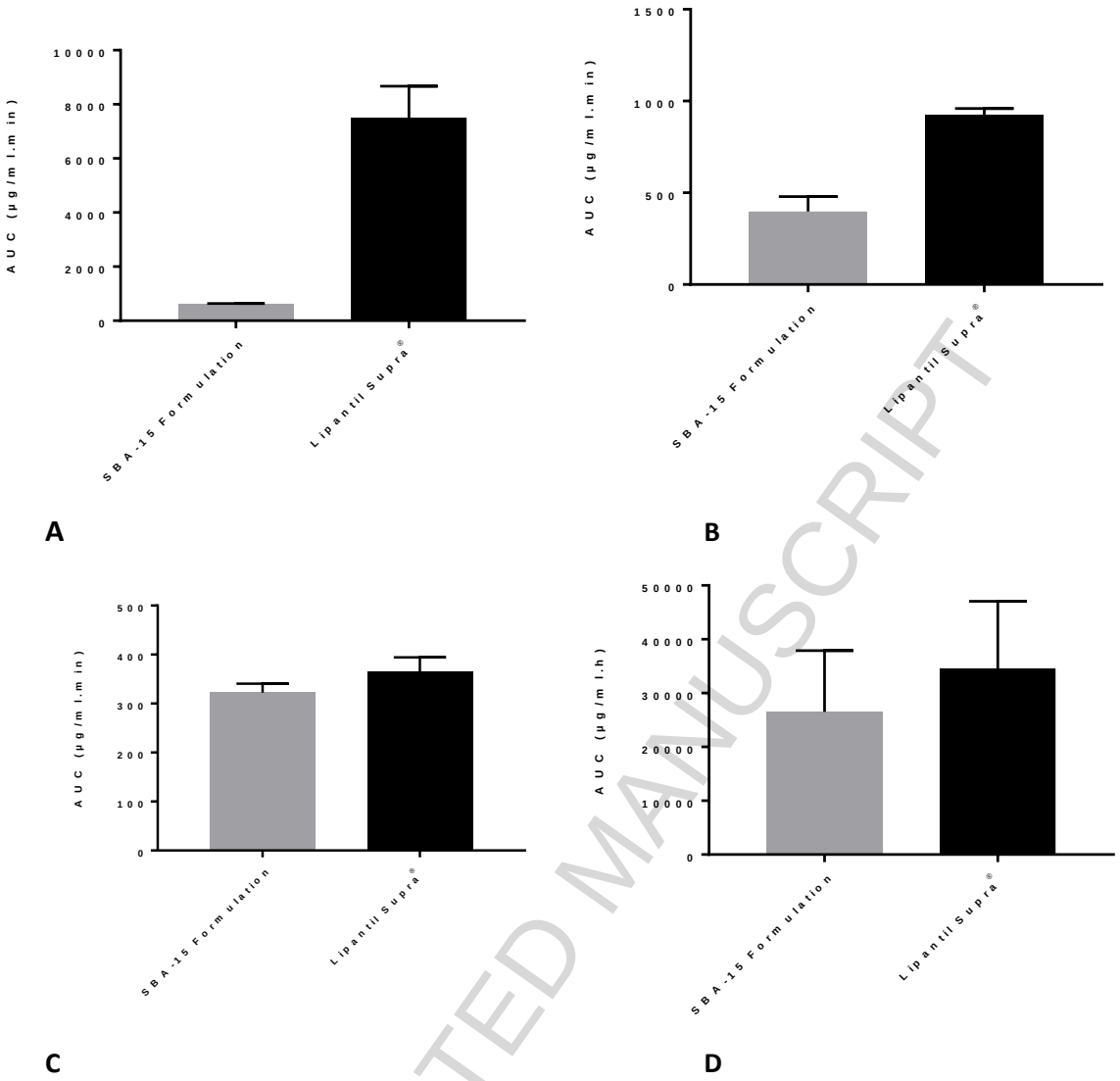


Fig. 6 Extent of release (AUC) and oral bioavailability of fenofibrate from a mesoporous silica and Lipantil Supra® formulations. A = extent of release using Type II apparatus, B = extent of release using FaSSIF-V2 media in a Type IV apparatus, C = extent of release using Type IV Transfer model, D = oral bioavailability determined using an *in vivo* pig model. Graphs show AUC over 240 min for Type II and Type IV, 360 min for Transfer and 24 hours for *in vivo* pig model). (n=3 for *in vitro* dissolution models, n=4 for *in vivo* pig bioavailability model, Y error bars indicate standard deviation)

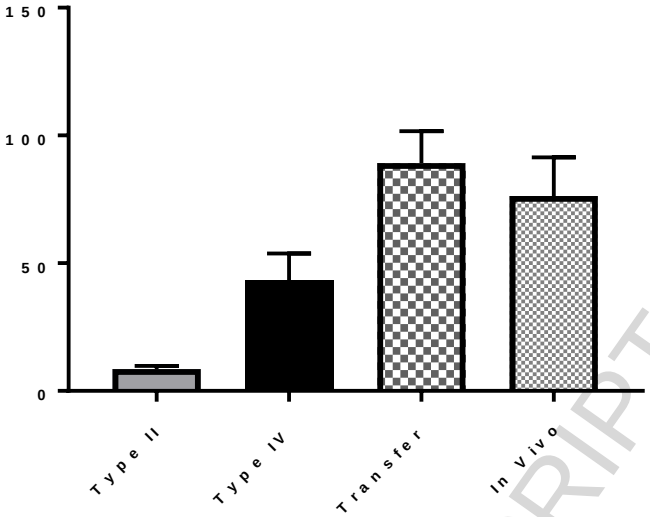


Fig 7. Ratio of extent of release of fenofibrate from the silica formulation vs. the commercial product, Lipantil Supra®, for the *in vitro* dissolution experiments and the *in vivo* pig study. Graphs show ratio of AUC release of silica formulation: Lipantil Supra® over 240 min for Type II and Type IV, 360 min for Transfer and 24 hours for *in vivo* pig model). (n=3 for *in vitro* dissolution models, n=4 for *in vivo* pig bioavailability model, Y error bars indicate standard deviation)

3.5. *In Silico* of IVIVR Modelling

The IVIVRs for the two formulations were generated using Gastroplus™ software. Previously published intravenous data was used to deconvolute the *in vivo* oral plasma concentration profiles using the Loo-Riegelman model [31, 32]. This two compartment model was chosen over a single compartment model as it has been reported that it is not possible to perform a rigorous pharmacokinetic analysis of an absorption process from oral data, unless the parameters of the model have first been derived from a separate intravenous experiment [40-42].

No quantitative IVIVR could be established with the dissolution profiles from the Type II apparatus. IVIVRs could be generated for the Type IV (FaSSIF-V2) and the Type IV transfer Model (Fig. 8 and Fig. 9 respectively). Deconvolution of the Type IV data produced a linear best-fit correlation between the fraction of *in vitro* release and the fraction of absolute bioavailability (R^2 = 0.883 for the silica formulation and R^2 = 0.802 for the Lipantil Supra®).

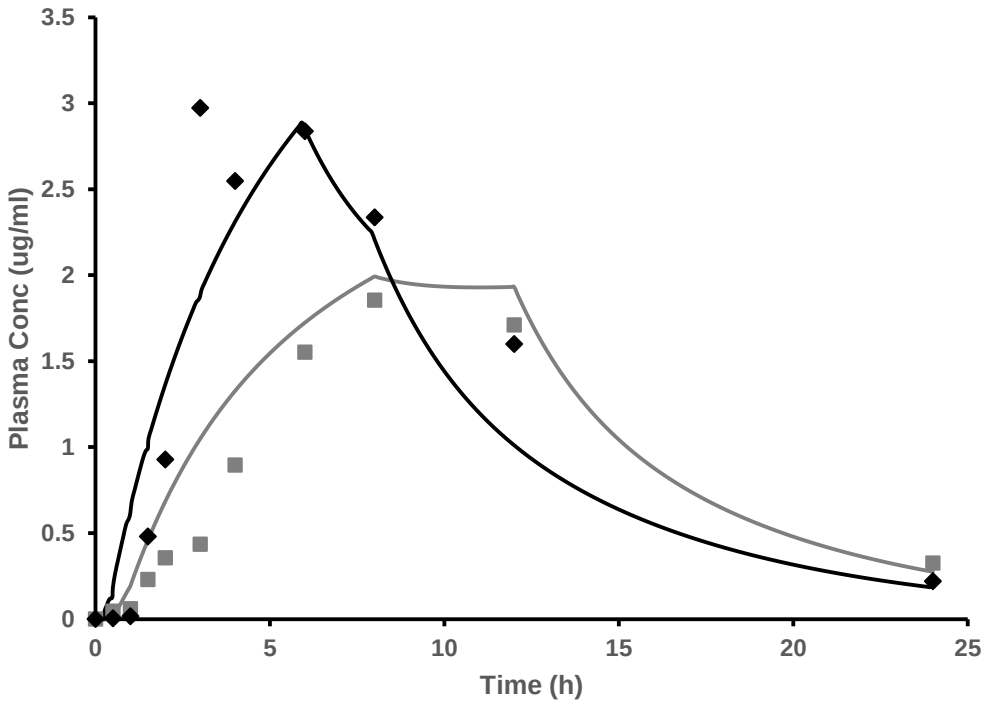


Fig. 8 Plasma concentration profiles for observed data (designated by the markers – (■) indicates mesoporous silica formulation, (◆) indicates Lipantil Supra®) and predicted plasma concentration-time profiles based on Type IV apparatus (designated by the solid lines - SBA-15 formulation (grey) and Lipantil Supra® (black))

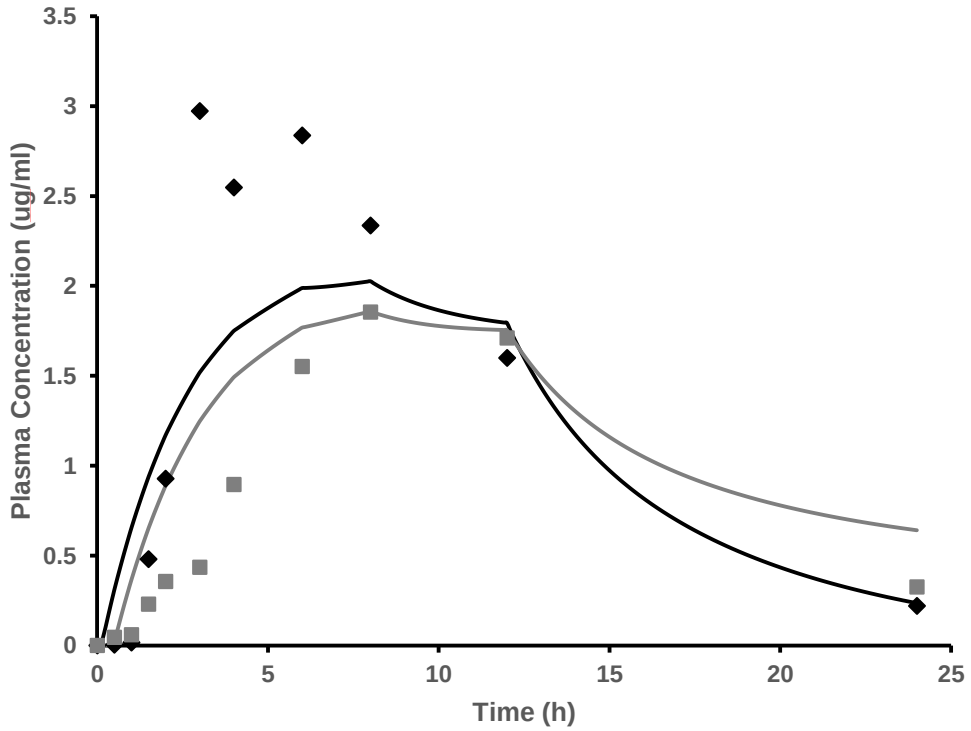


Fig. 9 Plasma concentration profiles for observed data (designated by the markers – (■) indicates mesoporous silica formulation, (◆) indicates Lipantil Supra®) and predicted plasma concentration-time profiles based on USP IV Transfer model (designated by the solid lines - SBA-15 formulation (grey) and Lipantil Supra® (black))

The mean absolute prediction error (MAE) was 5.18% for C_{max} and 12.14% for AUC (this falls outside the FDA limit of 10% error). The full list of validation statistics is shown in Table 3. Optimisation of the deconvoluted transfer model data produced a second-order polynomial best-fit correlation (R^2=0.771 for the SBA-15 formulation and R^2=0.569 for the Lipantil Supra®). The MAE was 16.02% for C_{max} and 15.55% for AUC, indicating the correlation is not as powerful as the Type IV model using FaSSIF-V2 media alone. The IVIVRs generated using Gastroplus™ software identify the Type IV apparatus as more effective at forecasting *in vivo* performance than the traditional paddle apparatus. However, it also suggests that the Type

IV dissolution, using FaSSIF-V2 alone, is the best prediction model. This is in contrast to data generated based on the extent of release discussed in section 3.4, which identified the Type IV Transfer model as the superior *in vitro* dissolution model.

Table 3. Summary of *in vitro/in vivo* relationship parameters. Observed and predicted C_{max} and AUC values and the correlation (R^2) between the observed and predicted plasma profiles generated by the Gastroplus™ IVIVCPlus® software are shown

	C_{max} (µg/ml)			AUC (µg/ml.h)			Reconstructed Plasma Concentration-Time Profile from Convolution Tab (R^2)
	Observed	Predicted	% Prediction Error	Observed	Predicted	% Prediction Error	
Type IV Apparatus							
Mesoporous Silica Formulation	1.854	1.993	-7.497	24.31	26.45	-8.803	0.883
Lipantil Supra®	2.973	2.888	2.859	31.87	26.94	15.47	0.802
USP IV Transfer Model							
Mesoporous Silica Formulation	1.854	1.858	-0.216	24.31	28.97	-19.17	0.771
Lipantil Supra®	2.973	2.027	31.82	31.87	28.07	11.92	0.569

4. Discussion

To date mesoporous silica drug formulations have been widely investigated *in vitro* using USP Type II dissolution methods. There have been limited studies to determine whether the enhanced dissolution observed during these *in vitro* dissolutions tests are capable of forecasting their oral *in vivo* bioavailability. This study demonstrates that the *in vitro* dissolution performance of a mesoporous silica fenofibrate formulation varies depending on dissolution apparatus and experiment design. The findings show that a USP IV dissolution method incorporating a SGF to FaSSIF-V2 media transfer was the best predictor of *in vivo* oral bioavailability in a pig model.

The study showed that the C_{max} and $AUC_{0\rightarrow240min}$ of both the commercial, Lipantil Supra®, and silica formulations in FaSSIF-V2 was significantly higher in the Type II compared to the Type IV apparatus. This observation is most likely due to hydrodynamic differences between the two dissolution models. The hydrodynamic properties of the Type II apparatus have been studied in detail and significant limitations have been recognised [19, 43-45]. The USP IV has the potential to operate at lower agitation rates than the paddle apparatus, resulting in lower fluid velocities considered to be more biorelevant [23, 46]. *In vivo* studies have shown that oral dosage forms can be exposed to small volumes of fluid in the gastro-intestinal tract, which can also be modelled using the Type IV apparatus [47]. This is the first study, to our knowledge, which has used the Type IV flow-through apparatus to investigate release from drug-loaded mesoporous silica systems. It is also the first to compare the Type IV and the Type II apparatus directly to study the dissolution behaviour of this formulation approach. The similarity between the relative bioavailability of the silica formulation versus Lipantil Supra®, and the relative AUC (extent of release) of the formulations determined

using the USP IV transfer method, suggests that the USP IV is a more biorelevant *in vitro* test for these formulations. These results indicate that a supersaturation/precipitation process plays a significant role in the dissolution process of these systems and is best simulated using a transfer model.

The advantages of using a transfer model to investigate formulation approaches which promote drug supersaturation, and are therefore prone to precipitation, have been reported by other groups [48-50]. Both formulations in this study utilise a supersaturation formulation strategy to enhance the oral bioavailability of fenofibrate [51]. The transfer model is a two compartment dissolution method to simulate GI transit from the stomach to the intestine. Fenofibrate release from the mesoporous formulation in SGF was below the HPLC assay detection limit. In contrast, fenofibrate release to supersaturation levels from Lipantil Supra® was evident in the gastric component of the transfer model. The exposure of the formulations to the acidic component in the transfer model affected their subsequent release profile in FaSSIF-V2 media. This is evident in the significant decrease in C_{max} for both formulations in the Type IV transfer model compared to the Type IV FaSSIF-V2 only model. The exposure of high energy amorphous drug forms to an aqueous environment where it has very limited solubility is reported to promote recrystallization of the drug to a lower energy less soluble form [50]. Partial recrystallization and precipitation of drug in the silica formulation would explain the reduction in C_{max} and $AUC_{0\rightarrow240min}$ upon exposure to FaSSIF-V2 media.

The importance of controlling supersaturation in mesoporous silica formulations has been investigated by Van Speybroeck *et al* [9, 10]. Although supersaturation has been explored intensively *in vitro*, there is little evidence to support what occurs in the *in vivo*

environment. A recent study investigated the impact of gastrointestinal dissolution, supersaturation and precipitation of posoconazole in humans [52]. After administration of the formulations, gastric and duodenal fluids were aspirated and blood samples were collected in parallel. Supersaturation followed by significant intestinal precipitation was reported. This is in contrast to a study which reported limited duodenal precipitation for ketoconazole and dipyridamole in a human study [53]. In this study, previously reported *in vitro* dissolution dipyridamole data over-estimated the subsequent *in vivo* observations [48]. This indicates that drug supersaturation/precipitation is a complex process which can depend on the physiochemical properties of the drug, the formulation and physiological variables [50].

The relationship between dosage form and physiological variables, such as the fed and fasted state warrant discussion. In the study, in-vitro dissolution studies were performed in FASSIF-V2 media designed to mimic the fastest state and pigs were dosed following an overnight fast, with food administered 8 hours post dose. The oral bioavailability of poorly water-soluble drugs, such as fenofibrate, is limited by their poor solubility within gastrointestinal fluid [54] and oral bioavailability can be variable depending on the food effect [55]. Formulation strategies have be shown to enhance bioavailability by reducing or eliminating the food effect [29, 31, 56]. For example, the commercial micronized fenofibrate formulation, Lipantil Micro®, displays food dependent bioavailability while the Lipantil® Supra formulation, encompassing NanoCrystal® technology, enables food independent administration and dose reduction [57]. These findings emphasise the need for studies of this nature which use *in vivo* reference data to optimize *in vitro* dissolution models and inform the development of bio-enabling formulation strategies.

The IVIVRs generated using the Gastroplus™ software support the relationships observed between the relative bioavailability of the formulations and their relative AUC (extent of release) determined from raw dissolution profiles. IVIVRs were determined for the Type IV FaSSIF-V2 model and the Type IV transfer model. No quantitative IVIVR could be determined for the Type II (paddle) apparatus. Correlations suggest that the Type IV apparatus with FaSSIF-V2 alone was better at forecasting *in vivo* performance than the transfer model. This finding can be explained by outlining the mechanism in which the model is generated and hence model assumption. The initial part of the dissolution profile (time points up to C_{max}) was used to generate the IVIVR. The assumption of the model was that the second part of the dissolution profile, which corresponded to a reduction in drug concentration due to drug precipitation, may be an artefact of the *in vitro* dissolution model, particularly in the absence of an absorption sink. The initial dissolution phase was correlated with the deconvoluted *in-vivo* plasma profile and then re-convoluted to generate a predicted plasma profile.

The IVIVR generated for the Type IV transfer model revealed significant limitations of the model; the MAE was 16.02% for C_{max} and 15.55% for AUC and a large prediction error for the Lipantil Supra® C_{max} of 31.82% was noted (more than double the FDA approved error limit of 15% for an individual formulation). This error can be attributed to the raw dissolution data for the Lipantil Supra®, specifically the concentration of fenofibrate at the fourth and fifth time point. This reduction (dip) in the release profile, while not statistically significant ($p > 0.5$) did significantly affected the *in vitro/in vivo* correlation simulated by the Gastroplus™ software. This sensitivity is a limitation of the current multi-step approach of deconvolution and correlation when comparing a small number of immediate release

formulations. Removal of fifth time point from the analysis improved the correlation but consequently reduced the power of the Type IV FaSSIF-V2 transfer model model IVIVR.

In silico modelling requires further investigation to overcome the limitations outlined above. To date, modified release formulations have proved the most successful as regards development of effective IVIVR [58]. Work to improve *in silico* modelling for formulations which employ supersaturation to improve the bioavailability of poorly water-soluble drugs will be of significant benefit in their development.

5. Conclusion

This study demonstrates that the dissolution performance of a fenofibrate mesoporous silica formulation varies depending on the dissolution apparatus and the dissolution experimental design. The findings show that a USP IV transfer dissolution model was best at forecasting *in vivo* performance. This observation is most likely due to hydrodynamic differences between the two apparatus and the ability of the transfer model to better simulate GI transit. This is advantageous in forecasting *in vivo* behaviour for formulations which promote drug supersaturation and as a result are prone to precipitation. As this drug supersaturation/precipitation process is complex and depends on both formulation and physiological variables, studies which relate *in vitro* to *in vivo* data can help optimise *in vitro* models used in formulation development. Future work should focus on investigating the effect of silica formulation additives and dissolution media composition on in-vitro dissolution and the oral bioavailability of these systems. *In silico* modelling has the potential to assist in this process. However, further development is required to overcome the limitations outlined in this study for solubility enhancing formulations.

6. Acknowledgements

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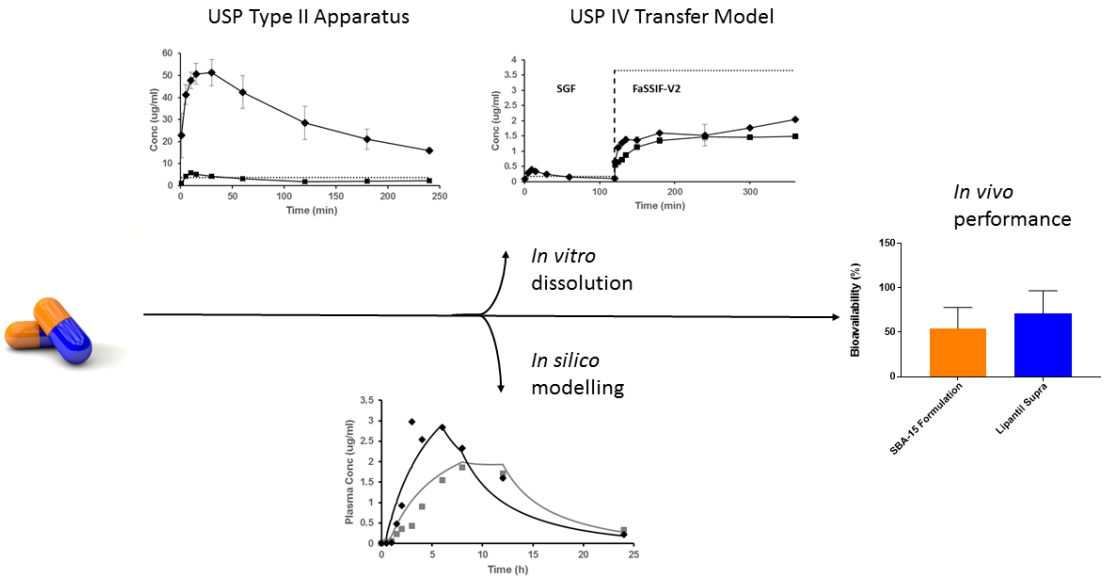

Graphical abstract