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Dynamic dissolution-/permeation-testing of nano- and microparticle formulations of fenofibrate

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ABSTRACT

The aim of the current study was to evaluate a dynamic dissolution-/permeation-system for prediction of gastrointestinal and absorption-behavior of two commercial fenofibrate formulations. To this end, both dissolution and barrier-flux were followed simultaneously for fenofibrate powder, a microparticle formulation (Lipidil® 200 mg) and a nanoparticle formulation (LIPIDIL 145 ONE®) using a pair of side-by side diffusion cells separated by a cellulose hydrate membrane. Under such dynamic conditions, transient supersaturation arising from the nanoparticle formulation could be demonstrated for the first time.

Furthermore, the dissolution-/permeation-system introduced here allowed for in-depth mechanistic insights: Biomimetic media, despite enhancing the apparent solubility of fenofibrate *via* micellar solubilization, did not increase permeation rate, irrespective whether the micro-/ or nanoparticle-formulation was tested. Nondissolved nano-/microparticles served as a reservoir helping to maintain high levels of molecularly dissolved drug, which in turn caused high and constant permeation rates. The micelle-bound drug may also serve as a drug-reservoir, yet of subordinate importance as long as there are nano-/microparticles present.

Despite the limitations of the current experimental set-up, combined dissolution-/permeation-testing appears a valuable new tool to promote mechanistic understanding during formulation development. Last but not least, the *in vitro* dissolution and permeation behavior revealed here was in good qualitative agreement with human duodenal and plasma values reported in literature for the same formulations.

1. Introduction

Dissolution and permeation are among the most relevant drug properties for formulation development and drug substances are classified accordingly (Biopharmaceutics Classification System, BCS), where class II drugs exhibit high permeability but low solubility (Amidon et al. 1995). Based on the principles of BCS, the Developability Classification System (DCS) was proposed for identifying critical quality attributes in early drug development (Butler and Dressman 2010). In the DCS, class boundaries are modified and class II is divided into two subclasses: compounds belonging to class IIa exhibit poor dissolution rate, whereas the limiting factor for class IIB is the thermodynamic solubility. In order to overcome poor solubility as the limiting factor for drug absorption, formulation scientists seek to increase the solubility by the introduction of enabling formulations.

Formulation-induced increase of solubility and dissolution rate is commonly determined *in vitro* as a matter of routine. While extrapolating from *in vitro* dissolution curves to the performance *in vivo* is relatively likely to be successful for extended release formulations of BCS class I drugs (good solubility and permeability), the necessity to employ enabling formulations and the potential precipitation of the drug renders establishing a meaningful *in vitro-in vivo* correlation (IVIVC) more challenging for poorly soluble drugs. There are different levels of IVIVC, and ideally a linear correlation between the fraction released *in vitro* and the fraction absorbed *in vivo* can be established (Zhou and Qiu 2011).

Compendial dissolution testing aims for sink conditions. For poorly soluble drugs sink conditions can be maintained by using high volumes of dissolution medium and/or adding surfactants, both of which are unlikely to reflect *in vivo* conditions. Dissolution testing in biomimetic media is commonly expected to yield more meaningful results (Kostewicz et al. 2014). Biomimetic media (also referred to as "biorelevant media") simulate, among others, drug solubilization in mixed phospholipid/taurocholate micelles similar to those in intestinal fluids (Buckley et al. 2012). Poorly soluble drugs may be present in the gastrointestinal tract in the following states: undissolved, molecularly dissolved, and solubilized by components of intestinal fluids (Buckley et al. 2013). However, there is evidence that dissolution testing using biomimetic media does not necessarily yield a relevant IVIVC either (Do et al. 2011). At the same time recent studies indicate that there is an important difference (Frank et al. 2014) between an increase in concentration of molecularly dissolved drug, induced by an enabling

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formulation (Frank et al. 2012a, 2012c) and of solubilized drug, induced by the formulation (Fischer et al. 2011; Kanzer et al. 2010) or the dissolution medium (Fong et al. 2016; Frank et al. 2012b).

One approach to better reflect the interplay between drug dissolution and drug permeation is the simultaneous testing of both parameters. The first combined dissolution/permeation experiments were based on Caco-2 cell monolayers as a permeation barrier (Ginski and Polli 1999). Cell-based models were subsequently implemented by several groups (Kataoka et al. 2003; Kobayashi et al. 2001; Motz et al. 2007). Later, *ex vivo* models based on rat intestine (Li et al. 2011; Zhou et al. 2014) and *in vitro* models based on artificial barriers (Gantzsch et al. 2014; Kataoka et al. 2014) were developed. With the combined dissolution/permeation testing it as, for instance, possible to establish a Level A IVIVC (*i.e.* a point-to-point correlation between the *in vitro* dissolution curve and the *in vivo* plasma curve) for three different generic products of poorly soluble indapamide (Yaro et al. 2014). However, neither the influence of enabling formulations nor that of biomimetic media on the interplay between drug dissolution and permeation of poorly soluble drugs has been studied systematically.

Fenofibrate was chosen for the current study as a neutral, lipophilic model compound (BCS class II / DCS class IIb drug) and because there were recently published both human duodenal and plasma concentration time curves for its marketed nano- and microparticle formulations (Hens et al. 2015). Fenofibrate was first introduced to the market as a capsule containing 100 mg fenofibrate powder with a mean particle size of approximately 150 μm (Sauron et al. 2006). The recommended administration of three capsules together with food resulted in a bioavailability of approximately 60% (Shepherd 1994). Later, a capsule preparation containing micronized fenofibrate (Lipidil® 200 mg) was marketed. Increased bioavailability was achieved by reducing particles to sizes between 5 μm and 15 μm (Sauron et al. 2006): administration of 200 mg micronized fenofibrate once daily yielded the same area under the curve as 300 mg of the conventional formulation, along with less inter-individual variance of plasma levels (Munoz et al. 1994; Shepherd 1994). Using wet-milling, a nanoparticle formulation was developed (LIPIDIL 145 ONE®) with a mean particle size of <400 nm (Sauron et al. 2006) by which the bioequivalent dose was further reduced from 200 mg to 145 mg. Moreover, plasma levels varied even less and the nanoparticulate formulation (presented in the form of a coated tablet) can be administered independent from food intake (Sauron et al. 2006). Since the particle size of the nanoparticle formulation is smaller than 2 μm, (true) supersaturation is likely to occur (Mosharraf and Nyström 1995) which to some extent may account for the higher bioavailability as compared to the microparticle formulation. To the best of our knowledge, however, occurrence of supersaturation has not been experimentally proven for fenofibrate nanoparticles.

The objective of the present study was to investigate the contribution of both solubilization and (true) supersaturation on fenofibrate permeability in a dynamic setting.

2. Material and methods

2.1. Chemicals

Fenofibrate (≥99%, powder) was purchased from Sigma-Aldrich ApS (Brøndby, Denmark). Lipidil® 200 mg capsules (Mylan Healthcare GmbH, Hannover, Germany; lot 23102) and LIPIDIL 145 ONE® coated tablets (Abbott, Hannover, Germany; lot 22672) were purchased in a German pharmacy. Caprylocaproyl macrogol-8 glycerides (Labrasol®) were kindly donated by Gattefossé (Saint-Priest Cedex, France) and will be referred to by its tradename for reasons of readability. SIF powder for preparation of simulated intestinal fluids was purchased from biorelevant.com (London, UK). Highly purified water was prepared in-house using a Milli-Q® water purification system (Merck Millipore, Darmstadt, Germany). Acetonitrile, formic acid and buffer salts were purchased from Sigma-Aldrich ApS (Brøndby, Denmark).

2.2. Media

Phosphate-buffered saline (PBS) contained 1.73 g l^{-1} of sodium dihydrogen phosphate dihydrate and 4.92 g l^{-1} disodium hydrogen phosphate dodecahydrate in highly purified water. The pH was adjusted with sodium hydroxide to a value between 7.35 and 7.45; the osmolality was adjusted with sodium chloride to a value between 280 and 290 mOsmol kg^{-1}.

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared according to the protocol of the supplier.

2.3. Dissolution-/permeation-set-up

A pair of jacketed side-by-side diffusion cells of 5 ml volume each (PermeGear Inc., Hellertown, PA, USA) were separated by a hydrophilic cellulose hydrate membrane with an effective permeation area of 1.77 cm^2 (Pütz GmbH, Taunusstein, Germany), unless stated otherwise. High permeability and low non-specific adsorption of fenofibrate to the membrane were observed during preliminary studies. An appropriate amount of powdered sample was placed in the rear part of the donor cell; subsequently, the cells were assembled and filled with medium. Both, the donor and acceptor chamber were stirred with the provided cross-shaped stirbars at a fixed speed of 500 rpm (H-3 stirrer, PermeGear Inc., Hellertown, PA, USA) and the temperature was set to 37 °C.

Based on the assumption that a volume of 250 ml is the volume available in the intestinal lumen for dissolution of a single oral dose, an amount of formulation equivalent to 1/50 of the single oral dose was used in the dissolution- and in the combined dissolution-/permeation-experiments. For this purpose, LIPIDIL ONE 145® tablets (immediate-release coating) were thoroughly ground in a mortar; for Lipidil® 200 mg, conventional gelatin capsules were opened and an aliquot of the capsule content was used.

2.4. Sampling and sample handling

In order to minimize non-specific adsorptive loss from aqueous fenofibrate solutions, 1 ml and respectively 5 ml glass syringes (FORTUNA® Optima, Poulten & Graf GmbH, Wertheim, Germany) were used for sampling. All samples were immediately diluted with a twofold volume of acetonitrile (ACN) using Gastight® Syringes (Hamilton Company, Reno, Nevada, USA).

2.5. Ultra-high performance liquid chromatography (UHPLC)

Fenofibrate was quantified using a Dionex Ultimate 3000 Binary Rapid Separation LC system (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) in combination with a Hypersil GOLD column (C18, 1.9 μm, 2.1 mm × 100 mm) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and a pre-column. Measurements were performed in isocratic flow mode with a mobile phase consisting of ACN and 0.1% formic acid in water in the ratio of 80:20 (v/v). The flow rate was 0.3 ml min^{-1} and the injection volume was 10 μl. The column temperature was 38 °C and the total run time was 3 min. Fenofibrate typically eluted after 2.1 min and was detected at a wavelength of 286 nm.

Standard series were prepared in ACN/water 2:1 (v/v). Calibration was carried out over the range of 10 ng ml^{-1} to 100 μg ml^{-1}.

For samples containing simulated intestinal fluids, the total run time was increased to 12 min. Under the chosen chromatographic conditions, fenofibrate appeared as rider peak. It was therefore verified that the peak area was determined correctly (data not shown).

2.6. Determination of apparent solubility

An excess of raw fenofibrate was dispersed in the respective medium and shaken for four days in a shaking water bath (SW23, JULABO GmbH, Seelbach, Germany) at 37 °C and 100 rpm, unless stated otherwise. The water bath was covered with aluminum foil to avoid photodegradation. After 24 h, 48 h, 72 h, and 96 h, an aliquot of 3.5 ml each was filtered through a 0.1 μm pore-size Anotop® 25 syringe filter (GE Healthcare, Little Chalfont, UK) discarding the initial 3 ml of filtrate.

2.7. Combined dissolution-/permeation-studies

For simultaneous evaluation of dissolution and permeation processes, the set-up described in 2.3 was used. Cells and barriers were assembled after placing an appropriate amount of ground nanoparticle formulation, microparticle formulation, or raw fenofibrate, respectively, in the rear part of the donor cell before the two parts were clamped together and filled with media. An isotonic dispersion of 0.6% (w/v) Labrasol® in highly purified water with the appropriate amount of sodium chloride was used as acceptor medium in all experiments. Labrasol® was used as a solubilizer and because it had been observed during preliminary studies that it prevents non-specific adsorption of fenofibrate to glass apparatus. As the donor medium, either PBS, FaSSIF, or FeSSIF respectively was employed.

At pre-determined time points, aliquots of 1 ml were withdrawn from the acceptor compartment with a syringe. Subsequently, an aliquot of 1 ml was withdrawn from the donor compartment and filtered, discarding the initial 0.8 ml of filtrate. In order to improve accuracy and precision, the syringes used for sampling from the acceptor compartment were weighed before and after withdrawal of the sample and thus the exact sample mass determined. The volumes removed from donor and acceptor compartment were replaced by 1 ml of the respective medium.

All experiments were run in triplicate over a period of 5 h while the cells were covered with aluminum foil.

2.8. Dissolution study using the donor chamber of the side-by-side diffusion cell

A simple dissolution study of the nanoparticle formulation was carried out in the set-up used for the combined dissolution-/permeation-experiments by replacing the permeation barrier with a sheet of aluminum foil. Any other conditions remained unchanged in order to study the influence of the permeation process and the respective sampling scheme from the donor on the dissolution profile. The nanoparticle formulation was chosen because it was expected to be the most affected formulation.

2.9. Preparation of saturated solutions of fenofibrate in FaSSIF for permeation experiments

In order to identify the role of the micelle-bound fraction in the interplay of drug dissolution and permeation in solubilizing media, saturated solutions of the different formulations in FaSSIF were prepared. For this, an excess of the respective formulation was added to 25 ml of FaSSIF and shaken in a shaking water bath under the same conditions as for the solubility studies. After 1 h (microparticles) or respectively 24 h (nanoparticles), the suspensions were centrifuged at 15,000 × *g* and 37 °C for 30 min (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany). These particular time intervals were chosen to assure that the equilibrium was reached and that the solution was no longer supersaturated (data not shown). The supernatant was filtered through a 0.1 μm Anotop® syringe filter and the first 3.5 ml were discarded. These solutions were used for permeation experiments.

2.10. Evaluation of permeation experiments and statistical analysis

When combining dissolution and permeation processes, the donor concentrations are not constant and initial concentrations to calculate apparent permeability coefficients (P_{app}) cannot reasonably be defined. Therefore, flux values (J) are reported here instead. Steady-state flux values were calculated from the respective slope of the regression line obtained by plotting the cumulative amount of permeated drug (dQ) divided by area (A) against time (dt):

$$J = \frac{dQ}{A \cdot dt.}$$

For comparison of data sets, an unpaired, two-tailed Student's *t*-test was applied. A value of $p \leq 0.05$ was considered as significantly different.

3. Results and discussion

3.1. Equilibrium solubility studies

Equilibrium solubility of fenofibrate powder at 37 °C was determined in any of the media which we planned to employ in our dissolution-/permeation-studies, *i.e.* PBS, FaSSIF and FeSSIF, and a 0.6% (m/v) aqueous dispersion of Labrasol®. Under the chosen conditions, equilibrium was reached after 24 h in PBS and after 72 h in FaSSIF and FeSSIF (data not shown). Equilibrium solubility of fenofibrate in PBS was low (Table 1). Its apparent solubility in the media containing solubilizing agents was up to three orders of magnitude higher, which is attributed to micellar solubilization. The use of 0.6% (m/v) Labrasol® dispersion as acceptor medium obviously provides an apparent solubility of fenofibrate sufficient to ensure sink conditions in the acceptor compartment during our permeation studies.

As the next step, equilibrium solubility of fenofibrate in aqueous suspensions of the marketed formulations was determined and compared to that of fenofibrate powder (Table 2). While the nanoparticle formulation showed the same equilibrium solubility as fenofibrate powder, surprisingly, the solubility of fenofibrate from Lipidil® 200 mg (microparticle formulation) was found to be slightly yet significantly lower than the solubility of raw fenofibrate. In the German expert information for both Lipidil® 200 mg and LIPIDIL 145 ONE® marketed products, sodium dodecyl sulfate is listed as a formulation additive (Abbott Arzneimittel GmbH, 2015; Mylan Healthcare GmbH, 2015). This excipient may act as a solubilizing agent. But, since the measured equilibrium solubilities were equal to or even lower than that of fenofibrate powder, it was concluded that no relevant amounts of the surfactant in terms of solubilization of the active ingredient were present in the formulations. A plausible explanation for the lower equilibrium solubility of the microparticle formulation could not be identified.

3.2. Dissolution- vs. dissolution-/permeation-analysis of the nanoparticle formulation

The dissolution rate of the nanoparticle formulation was determined using our mini-scale set-up (5 ml chambers, magnetic stirring), both as a simple dissolution experiment or combined with the simultaneous absorption-mimicking set-up, respectively.

Table 1
Equilibrium solubility of raw fenofibrate in different media at 37 °C, determined after 96 h in a shaking water bath (mean ± S.D., $n = 3$).

Medium	Solubility [μg ml^{-1}]
PBS	0.3 ± 0.0
FaSSIF	10.1 ± 0.2
FeSSIF	47.0 ± 0.6
0.6% Labrasol®	289.2 ± 46.0

Table 2
Equilibrium solubility of different formulations in PBS at 37 °C, determined after 24 h in a shaking water bath (mean ± S.D., $n = 4$).

Formulation	Solubility [ng ml^{-1}]
Raw fenofibrate	308 ± 34
Microparticles	241 ± 19
Nanoparticles	299 ± 29

In the combined dissolution-/permeation-experiment, the nanoparticle formulation showed a significant extent of supersaturation in PBS (Fig. 1). At the first sampling time point (after 15 min), the concentration of dissolved fenofibrate exceeded its equilibrium solubility by a factor of two to four. Supersaturation was found to decay to equilibrium solubility levels within 90 min. It has previously been shown that filters with a pore size of 0.1 μm are capable of retaining nanoparticles present in the dispersion of the formulation (Juenemann et al. 2011). It can therefore be ruled out that the high fenofibrate concentrations measured here are an artifact arising from nanoparticles slipping through the filter pores.

In contrast, in the simple dissolution set-up, where the permeation barrier between donor- and acceptor-chamber was replaced by impermeable aluminum foil, the extent of supersaturation was rather low and not significant, as compared to equilibrium solubility. Since the duration of the supersaturated state is inversely proportional to the degree of supersaturation (Brouwers et al. 2009), we assume that in absence of a permeation barrier the initial degree of supersaturation was higher and, thus, resulted in faster precipitation. Therefore, substantially more drug had precipitated before the first sampling time point after 15 min.

It is important to note, that due to the absence of any solubilizing agents, "true" supersaturation is reported here; for definition and discussion of its impact on bioavailability see Buckley et al. (Buckley et al. 2013). As compared to the simple dissolution experiment, both a higher initial degree and a longer duration of supersaturation were observed during the combined dissolution−/permeation-experiment. This means in other words that a simple dissolution experiment may disguise the presence of supersaturated states. Such effect is obviously more readily captured by a combined dynamic system of dissolution and permeation.

3.3. Dissolution-/permeation-analysis of fenofibrate powder, microparticles and nanoparticles

Fig. 2 shows the dissolution profiles of raw fenofibrate, micro- and nanoparticular formulations in the combined dissolution-/permeation-set-up. As opposed to the nanoparticles, neither fenofibrate powder nor fenofibrate microparticles showed any supersaturation during dissolution in PBS under the absorption-mimicking conditions (Fig. 2). The microparticles exhibited a very high dissolution rate providing high concentrations of dissolved fenofibrate close to equilibrium solubility already after 15 min and during the entire course of the experiment, despite repeated sampling and replacing the extracted volume by fresh buffer. In contrast, fenofibrate powder dissolved more slowly with considerable variability between parallel experiments. Under the conditions employed, the concentration of dissolved fenofibrate did not reach equilibrium solubility for the drug powder. In essence, while the microparticle formulation yielded faster and more reproducible dissolution profiles than raw fenofibrate, the nanoparticle formulation induced transient supersaturation.

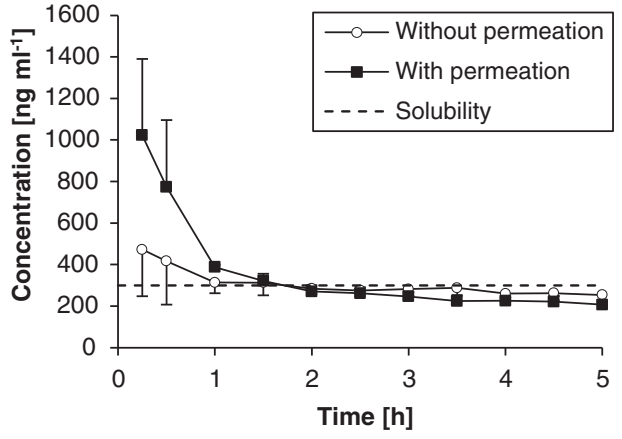

Fig. 1. Dissolution profile of ground nanoparticle formulation in PBS in a conventional dissolution experiment (open circles) and in a combined dissolution-/permeation-experiment (closed squares) (mean ± S.D., n = 3). The dashed line represents the equilibrium solubility of the formulation in PBS.

As the next step, the cumulated amounts of fenofibrate which permeated across the barrier over time were compared with the dissolution curves (Fig. 3). In all cases, the cumulated amount of permeated drug increased over time (closed symbols in the graph) in a linear manner indicating steady-state flux. Apparently, the supersaturation peak seen for the nanoparticles in the donor during the first hour did not translate into a significant permeation peak under the conditions employed. The flux rates of the different formulations were found to increase in the rank order fenofibrate powder < microparticles < nanoparticles as expected. Interestingly, for the nano- and microparticles, the cumulated amount of permeated fenofibrate exceeded the amount that is dissolved in the donor from the four- and three-hour time points onwards. This fact proves that the dissolution process in the course of the permeation experiment is of importance and the two processes interact dynamically.

3.4. Drug dissolution in biomimetic media during dissolution-/permeation-experiments

Fig. 4 shows dissolution profiles for the three fenofibrate materials in biomimetic media in the combined dissolution-/permeation-set-up. In contrast to the experiments in PBS described above, no supersaturation was observed for the nanoparticles with FaSSIF or FeSSIF as a dissolution medium (Fig. 4). Already at the first time point (15 min), both the nanoparticle formulation and the microparticle formulation showed concentrations of apparently dissolved drug which corresponded to their respective equilibrium solubilities of fenofibrate. A slower dissolution process was observed for raw fenofibrate and a plateau value was reached after 3 to 4 h. However, the plateau-concentration was significantly lower than expected from the solubility of raw fenofibrate in these respective media. This fact is due to the dynamic equilibrium where the amount of fenofibrate dissolving within a sampling time interval of 30 min is equal to the amount removed from the donor by removing a certain volume as well as the amount permeated. Due to the innate low dissolution rate of raw fenofibrate in all the media, the concentrations reached were below equilibrium solubility in the respective medium. This effect is less obvious in PBS, where the dissolution rates of all materials are low.

In both FaSSIF and FeSSIF, the concentration of fenofibrate reached with the nanoparticle formulation was slightly, yet significantly lower than that in the experiments with microparticles.

It should be kept in mind that the apparent solubility of fenofibrate in FaSSIF and FeSSIF is much higher (by factors of 30 and 110, respectively) as compared to the solubility in PBS (Table 1). In contrast, true supersaturation in PBS enhanced solubility only two- to fourfold. This large difference suggests that true supersaturation may also have occurred in the solubilizing media, but was not perceived because the huge enhancement of apparent solubility due to solubilization disguised the little effect of supersaturation.

3.5. Drug permeation during dissolution-/permeation-experiments in biomimetic media

The effect of the different media on permeation was also studied. For all three formulations, a linear increase of the cumulated amount of

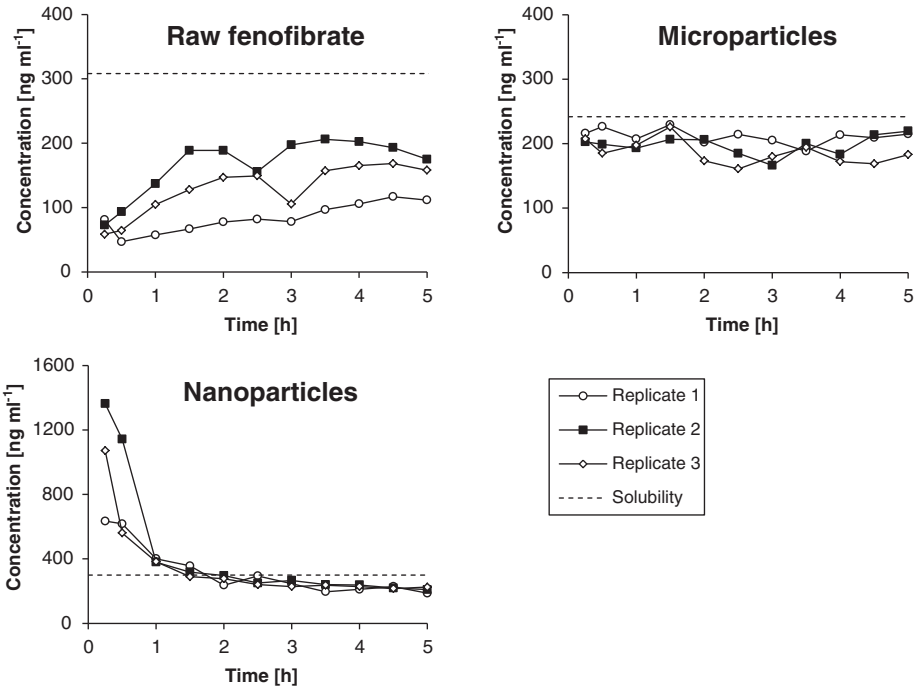

Fig. 2. Dissolution profile of raw fenofibrate, microparticle formulation and nanoparticle formulation in PBS in a combined dissolution-/permeation-experiment. The concentrations in each of the three cells are reported and the solubility of the respective formulation in PBS is indicated by a dashed line.

permeated drug over time was observed after an initial lag-phase, irrespective which donor medium was used (data not shown). The steady state flux values obtained are summarized in Fig. 5. The flux values were found to increase in the rank order fenofibrate powder < microparticles < nanoparticles, while the flux values, for a given particle type, did not change significantly with the donor medium employed. In other words, micellar solubilization by FaSSIF- or FeSSIF-micelles did not have a significant impact on permeation rate under the conditions employed.

3.6. Permeation studies of saturated solutions in biomimetic media

Even though it is widely accepted that micelle-bound drug is unlikely to permeate directly and we have shown that the micellar solubilization

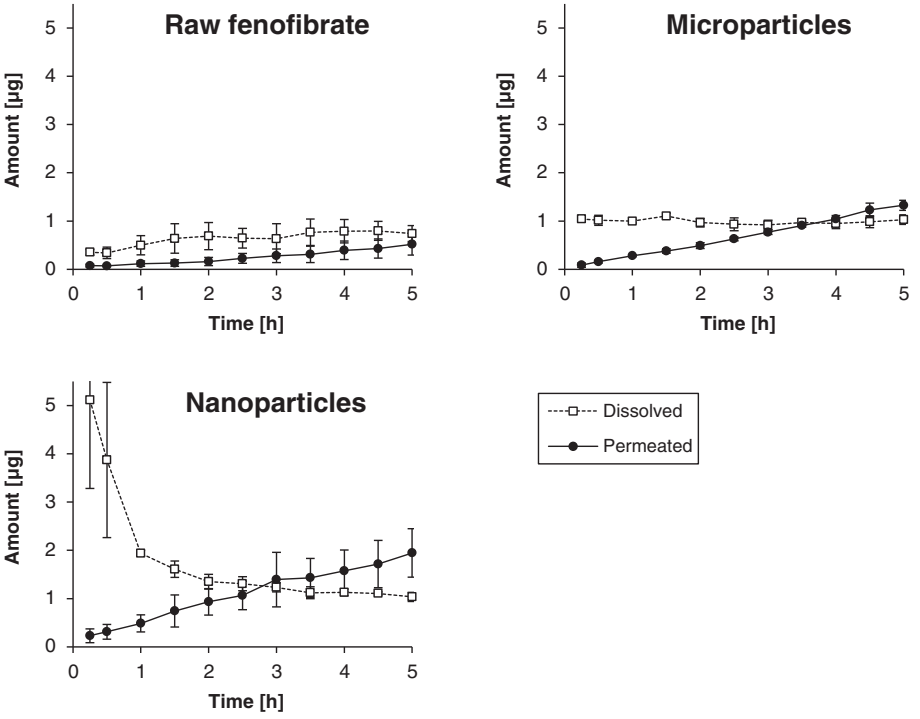

Fig. 3. Total amount of dissolved fenofibrate present in the donor cell (open squares) and cumulative amount of permeated drug (closed circles) in combined dissolution-/permeation-experiments in PBS with raw fenofibrate, microparticle formulation and nanoparticle formulation (mean ± S.D., n = 3).

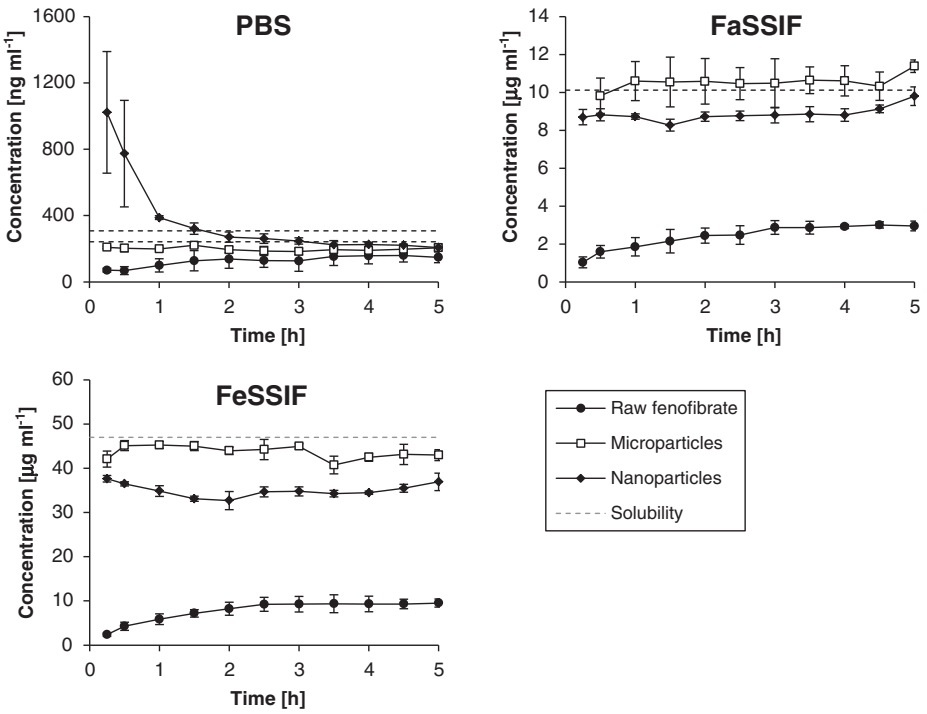

Fig. 4. Dissolution profile of raw fenofibrate (closed circles), microparticle formulation (open squares) and nanoparticle formulation (closed diamonds) in a combined dissolution-/permeation-experiment in PBS, FaSSIF and FeSSIF (mean ± S.D., n = 3). The dashed line represents the solubility in the respective medium determined with raw fenofibrate (FaSSIF and FeSSIF) or the highest and the lowest solubility determined with the different formulations (PBS).

does not increase the permeation rate in the present case either, micellar bound drug still could play a role in the dynamic interplay of drug dissolution and permeation. When testing drug permeability from a saturated solution in FaSSIF (in contrast to suspensions used in the above discussed dissolution-/permeation-studies), still a significant flux could be measured (Fig. 6). It has been previously shown for other poorly soluble drugs (such as ABT-102) that the true solubility in FaSSIF is the same as in aqueous buffer (Frank et al. 2012b). Assuming that this also applies to fenofibrate in the present case, more than half of the amount of truly dissolved fenofibrate, which had been present in the donor at the beginning of the experiment permeated into the acceptor compartment within the 5 h of experiment. Despite this substantial change in donor concentration, the permeation rate was constant and no decline was observed. This indicates that drug which had originally been bound in micelles was continuously released to the aqueous phase in the form of molecularly dissolved drug and became available for permeation. Hence, the micelle-bound fraction can be regarded as a drug reservoir and the re-distribution from the micelles is a considerably fast process.

Yet, the release kinetics from the micelles appears to be slower than the dissolution process from the particles: for both micro- and nanoparticles, the flux from the FaSSIF solution was at a level of approximately 60% of the flux value obtained with the FaSSIF suspension. Interestingly, as previously seen with the suspension, the flux from the saturated solution upon removal of the nanoparticles was significantly higher than from that of the microparticles (by 27%) even though the initial concentration of solubilized and free drug in this experiment differed only by

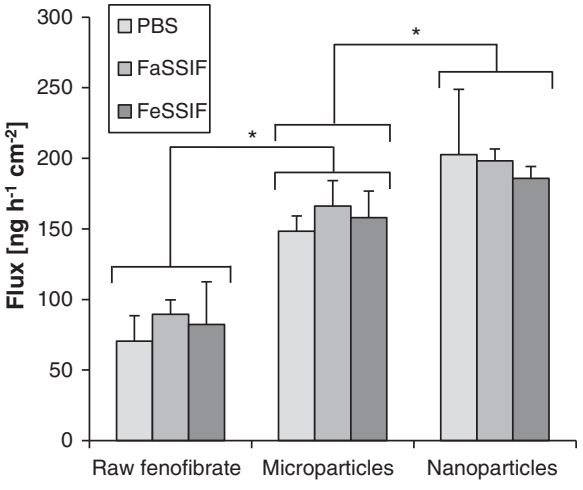

Fig. 5. Flux values of raw fenofibrate, microparticle formulation and nanoparticle formulation determined in dissolution-/permeation-experiments with PBS, FaSSIF or FeSSIF as donor medium (mean ± S.D., n = 3). * Significant difference ($p < 0.05$).

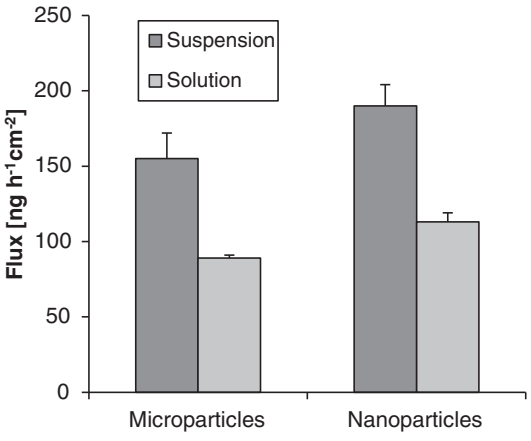

Fig. 6. Flux values of a suspension and a solution of microparticle and nanoparticle formulation in FaSSIF (mean ± S.D., n = 3).

5%. This observation may be explained by the different excipients of the formulations which might have affected the composition of the micelles so that they released fenofibrate at a different rate.

3.7. General discussion

Our observations are in line with a number of earlier studies, which investigated the impact of solubilization on drug permeability:

It has been reported previously for a dispersion of the poorly water-soluble compound ABT-102 (below and above the critical micellar concentration of the surfactants contained in the formulation) that there was an obvious discrepancy between apparent solubility and permeability obtained in a Caco-2 permeation experiment; the same was found when comparing mere buffer with simulated intestinal media (Frank et al. 2012b). Yet, only FaSSIF could be tested in comparison to Hank's balanced salt solution due to the limited robustness of the cells representing the permeation barrier. Our present study has now systematically shown that this observation is independent of the degree of solubilization and that it also applies to different types of formulations.

The relatively small permeation area of the current experimental set-up restricted the overall permeation, and a decrease in donor concentration as it occurs *in vivo* during the gastrointestinal absorption process (Hens et al. 2015) could not be simulated. All our reported flux values are steady-state values. Nevertheless, a significant difference between the three formulations was found. A relatively low flux was expected for raw fenofibrate since the donor concentration was the lowest of the three formulations, regardless of the employed medium. The flux of the nanoparticle formulation was higher than for the microparticles by an average of 22%.

Furthermore, the permeation rate of the fenofibrate from the nanoparticles was higher than that from the microparticles, even though during the combined dissolution/permeation experiments with simulated intestinal fluids the concentrations measured in the donor compartment (comprising truly dissolved and solubilized drug) were higher for the microparticles compared to the nanoparticles. Based on this observation, one might (erroneously) have expected a higher flux for the microparticles. This means in other words that using solubilizing agents in classical dissolution testing can mask underlying effects which improve permeability, and their use may lead to wrong conclusions when formulations are compared.

4. Conclusion

When dynamically combining dissolution- with permeation-testing, transient supersaturation arising from a fenofibrate nanoparticle formulation could be demonstrated for the first time. Furthermore, the dissolution-/permeation-system employed here allowed for in-depth mechanistic insights:

1) that micellar solubilization despite enhanced apparent solubility did not increase the permeation rate. This finding was independent of both the type of solubilizing biomimetic medium and of the formulation tested.

2) that undissolved nano-/microparticles served as a reservoir maintaining elevated levels of molecularly dissolved drug, which was crucial for maintaining a constant flux.

3) that there also is an interplay between truly dissolved and solubilized drug, in which micelle-bound drug can act as a drug reservoir, yet of subordinate role as long as there are nano-/microparticles present.

Despite the current limitations of the experimental set-up, combined dissolution-/permeation-testing appears thus a valuable new tool to promote mechanistic understanding during formulation development. Last but not least did the *in vitro* dissolution and permeation behavior revealed here fit qualitatively with human duodenal and plasma values of the same formulations reported in literature.

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