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Research paper

Biorelevant *in vitro* dissolution testing of products containing micronized or nanosized fenofibrate with a view to predicting plasma profiles

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ABSTRACT

The ability of *in vitro* biorelevant dissolution tests to predict the *in vivo* performance of nanosized fenofibrate (Lipidil 145 ONE®) and microsized fenofibrate (Lipidil – Ter®) was evaluated in this study. *In vitro* dissolution was carried out using USP apparatus 2 (paddle method) with updated biorelevant media to simulate the pre- and postprandial states. Membrane filters with different pore sizes were evaluated for their ability to hold back undissolved, nanosized drug particles. It was shown that filters with pore sizes of 0.1 μm and 0.02 μm were able to separate molecularly dissolved drug from colloidal and undissolved particles. *In vitro* results obtained with a suitable filter were used to generate simulated plasma profiles in combination with two different models using STELLA® software: (a) under the assumption of no permeability restrictions to absorption and (b) under the assumption of a permeability restriction. The simulated plasma profiles were compared to *in vivo* data for the nanosized and the microsized formulation in the fasted and fed states. The first model approach resulted in good correlation for the microsized fenofibrate formulation, but the plasma profile of the formulation containing nanosized fenofibrate was overpredicted in the fasted state. The second model successfully correlated with *in vivo* data for both formulations, regardless of prandial state. Comparison of simulations with the two models indicates that in the fasted state, absorption of fenofibrate from the nanosized formulation is at least partly permeability-limited, while for the microsized formulation the dissolution of fenofibrate appears to be rate-determining.

1. Introduction

Many new chemical entities possess physicochemical characteristics unfavorable for oral absorption. A major hurdle is their low aqueous solubility and subsequent slow dissolution, leading to insufficient bioavailability. One of the classical approaches to increase the rate of dissolution is through decreasing particle size. The underlying basis for the correlation between particle size and dissolution rate can be explained by the following modification of the Noyes–Whitney equation:

$$DR = \frac{A_{Drug} \cdot D_{Drug}}{\delta} \cdot (C_S - C_t) \quad (1)$$

where DR is the dissolution rate, A_{Drug} is the drug surface area, D_{Drug} is the diffusion coefficient of the drug, δ is the diffusion layer thickness, C_S is the saturation solubility of the drug and C_t is the concentration of the dissolved drug at time t.

Reducing the particle size, e.g. by micronization leads to an increase in the dissolution rate and frequently to a higher and more reliable bioavailability. The success of applying this approach has been well documented in many studies [1–4]. More recently, nanosizing technology has been introduced to further decrease the particle size of the active pharmaceutical ingredient (API) and hence improve its bioavailability. Potential advantages of nanonization over micronization include the very substantial additional increase in particle surface area and, potentially, a modest increase in saturation solubility.

The scientific backgrounds of these effects are well described in the literature [5–8]. In addition, the possibility of mucoadhesion and/or direct uptake of colloidal particles across the gut membrane has been raised [6,7,9,10].

A second advantage of reducing API particle size to the nanometer range is that the dependency of absorption on food intake can in some cases be attenuated [11,12].

Fenofibrate, a lipid lowering agent, is virtually insoluble in water and exhibits a positive food effect [13]. Its oral formulation has been improved over time. An early formulation consisted of a capsule containing coarse fenofibrate in a dose of 200 mg

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(Lipanthyl®). This formulation exhibited high interindividual variability in the plasma profiles, as well as a pronounced food effect and was recommended to be administered with meals. In 2000, "suprabioavailable" tablets (Lipidil – Ter®) were introduced. This formulation combines micronization of fenofibrate with a spray-coating process resulting in a particle size of 5–15 μm [13]. Micronization leads to a higher dissolution rate and this "suprabioavailable" tablet containing 160 mg fenofibrate was demonstrated to be bioequivalent to the 200 mg coarse powder capsule, with less variability and a reduced food effect. More recently, it has become possible to reduce the particle size of fenofibrate to a D_{50} less than 500 nm with a novel wet-milling technique (Tricor®, Lipidil 145 ONE®). These formulations exhibit even better bioavailability, so that the dose could be lowered to 145 mg fenofibrate and still maintain bioequivalence to the 200 mg conventional capsule. With the nanosized formulation, it is possible to administer fenofibrate independently of meal intake, since no food effect is observed [14]. Additionally, formulations with reduced particle size have been demonstrated to provide a more efficient and better tolerated treatment of hypercholesterolemia and hypertriglyceridemia.

The goal of this work was to investigate the dissolution of fenofibrate from microsized and nanosized formulations. In doing so, the study was designed to differentiate between colloidally suspended drug particles and molecularly dissolved drug, which has been an issue for years [15,16]. First, filters of various pore sizes were identified. Second, dissolution behavior in biorelevant media (FaSSIF, FeSSIF, FaSSGF, FaSSIF-V2 and FeSSIF-V2 [17–19]) was studied to determine whether food effects can be explained by dissolution behavior as a function of prandial state. Biorelevant media have been successfully applied over the past decade to obtain *in vitro–in vivo* correlations (IVIVC) [17,20–23]. Third, *in vitro–in silico–in vivo* correlations were established by combining dissolution tests with the STELLA® 9.1.1 software (isee systems, NH, USA).

2. Materials and methods

2.1. Chemicals and materials

Lipidil 145 ONE® (lot 85900, Solvay Arzneimittel, Germany) and Lipidil – Ter® (lot 87247, Solvay Arzneimittel, Germany) were purchased from pharmacies in Germany. Egg phosphatidylcholine (Lipoid E PC®, 97.9% pure, lot 108015-1-/042) was kindly donated from Lipoid GmbH, Ludwigshafen, Germany. Glycerylmonooleate (GMO, Rylo MG19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was a gift from Danisco Specialities, Brabrand, Denmark. Hydrochloric acid (31–33%) was obtained from Hedinger, Stuttgart, Germany. Ortho-phosphoric acid (85%) and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were purchased from Fluka Chemie AG, Buchs, Switzerland. Fenofibrate drug substance (lot 016K1644) and maleic acid (99% pure, lot 056K5473) were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, >97% pure, lot 2007100274) was purchased from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. All other chemicals were of analytical grade and obtained from Merck KGaA, Darmstadt, Germany. Long-life milk (3.5% fat) was obtained from Milfina, Germany.

The syringe filters used in this study are listed in Table 1.

2.2. Solubility measurements

The apparent solubilities of fenofibrate in FaSSIF and FeSSIF were taken from the literature [24]. Additional solubility measurements of fenofibrate were performed in FaSSGF, FaSSIF-V2 and FeSSIF-V2 using the shake-flask method. An excess of coarse fenofibrate in the medium was shaken on an orbital shaker at 37 °C ($n = 6$). After 24 h, the samples were filtered through a filter with a pore size of 0.45 μm (Rezist® 30, PTFE). The filtrate was analyzed by HPLC. Further, the measured solubility (24 h) of drug substance in FeSSGF was determined by HPLC using the shake-flask method for 24 h, filtering the medium through a filter of 2.7 μm and precipitating the proteins with acetonitrile [25].

2.3. Dissolution studies

The dissolution testing conditions for Lipidil 145 ONE® and Lipidil – Ter® consisted of USP apparatus 2 Erweka DT6 (Erweka, Heusenstamm, Germany), 500 ml medium volume, stirring at 75 rpm and a temperature of 37 °C. FaSSGF, FaSSIF and FaSSIF-V2 were used to simulate the preprandial state. FeSSIF and FeSSIF-V2 were used to represent the fed state in the small intestine.

The dissolution profiles were constructed from samples of 5 ml withdrawn after 5, 10, 15, 30 and 60 min, which were filtered immediately through filters with different pore sizes and diluted appropriately with methanol. The samples were replaced with fresh medium.

It was not possible to perform the dissolution test in FeSSGF, since this medium contains milk, which cannot be filtered using filters with a pore size in the range of 20–500 nm. As an approximation and despite the different colloidal structures in the two media, the dissolution rate in FeSSGF was assumed to be similar as in FeSSIF-V2, since the solubility in these media was not significantly different. To confirm that this assumption was not important to the outcome of the simulation, a sensitivity analysis with respect to dissolution rate was conducted (see Sections 2.7 and 3.4.2).

The initial dissolution rate of Lipidil – Ter® was calculated from the complete dissolution profiles. The initial dissolution rate of Lipidil 145 ONE® in FaSSGF, FaSSIF-V2 and FeSSIF-V2 was calculated after performing additional tests during which samples were withdrawn at 1, 2, 3, 4 and 5 min, filtered and immediately diluted appropriately with methanol. All experiments were carried out in triplicate.

2.4. Quantitative analysis of fenofibrate

Samples from the dissolution tests were analyzed by HPLC. The HPLC system consisted of a LaChrom® L-7110 pump, a LaChrom® L-7400 UV–Vis-Detector, a LaChrom® L-7200 autosampler (Merck Hitachi, Darmstadt, Germany) and the EZ-Chrom Elite Data System Software® (Biochrom Ltd., Cambridge, UK). The analysis was performed on a LiChroCART® RP-18 5 μm, 125 × 4 mm column (Merck, Darmstadt, Germany). The mobile phase consisted of 80% acetonitrile and 20% MilliQ-water. The pH value was adjusted with ortho-phosphoric acid to 2.5. The flow rate was set at 0.85 ml/min resulting in elution of fenofibrate at approximately 4.5 min. The concentration of drug was determined using a UV detector set at 254 nm.

2.5. Analysis of in vitro dissolution data

Differences in the *in vitro* dissolution profiles obtained using the different pore size filters were assessed using the model-independent similarity factor (f_2). The f_2-value can range from 0 to 100, values above 50 indicate a difference of less than 10% and therefore similarity between two dissolution profiles [26,27].

2.6. Analysis of in vivo pharmacokinetic data

An oral administration, two-compartment analysis (WinNonlin® model 12) was applied for evaluation of pharmacokinetic parameters using WinNonlin® Professional Edition version 4.1 software

Table 1
Syringe filters used in this study.

Filter	Company	Lot	Material	Pore size [μm]
Anotop 25 Plus	Whatman, Maidstone, England	07006F	Aluminum oxide	0.02
Anotop 25 Plus	Whatman, Maidstone, England	07005C	Aluminum oxide	0.1
Minisart RC 25	Sartorius, Göttingen, Germany	17764	Regenerated cellulose	0.2
Rezist 30	Whatman, Dassel, Germany	7029474	Polytetraflouroethylene	0.45
GD/X	Whatman, Florham Park, USA	V378	Glass microfiber GF/D	2.7

Table 2
Solubility of fenofibrate in μg/ml in various media measured at 37 °C after 24 h (mean ± SD, all experiments $n = 4$, except FeSSGF $n = 6$).

Medium	Solubility, μg/ml (mean ± Sd)
FaSSGF	0.22 ± 0.01
FaSSIF	13.7 ± 0.5
FaSSIF-V2	4.67 ± 0.25
FeSSGF	147.49 ± 93.67
FeSSIF	35.6 ± 1.0
FeSSIF-V2	78.84 ± 1.0

(Pharsight Corporation, Mountain View, CA, USA). The plasma drug concentration–time profiles used to obtain pharmacokinetic parameters were taken from the literature [14]. These profiles of orally given fenofibrate were used to calculate the pharmacokinetic parameters k_{10}, k_{12}, k_{21} and V/F of microsized and nanosized fenofibrate, in the fasted and fed states. No intravenous data were available for fenofibrate.

These parameters were utilized in STELLA® 9.1.1 to simulate plasma profiles from *in vitro* dissolution data.

2.7. Computer simulation of fenofibric acid plasma profiles

The fenofibric acid plasma profiles were generated using STELLA® 9.1.1 in combination with experimental dissolution results and afterwards compared to the plasma profiles from the literature [14]. Two different model approaches were used:

(A) A model based on the Noyes–Whitney theory for dissolution, given by the following equation [28]:

$$\frac{dW_t}{dt} = \frac{D\Gamma N^{1/3}}{V\delta\rho^{2/3}} W^{2/3}(X_s - W_t) = zW^{2/3}(C_s - C_t) \quad (2)$$

where D is the diffusion coefficient of the drug, Γ is the shape factor, N is the number of particles, V is the volume of dissolution medium, δ is the diffusion layer thickness and ρ is the density of drug. The term $\frac{D\Gamma N^{1/3}}{\delta\rho^{2/3}}$ is a constant equal to z. The z-value can be derived from *in vitro* dissolution experiments. W_t is the amount of drug dissolved at time t, W is the amount of drug remaining to be dissolved and X_s is the mass of drug which saturates the dissolution medium. C_s is the saturation solubility in the dissolution medium, while C_t is the concentration of drug at time t. Assumptions in this model include the following: (i) absorption from the stomach is negligible, (ii) solid and liquid emptying from the stomach occur simultaneously and (iii) any fenofibrate dissolved is immediately transported into the mucosa and appears in the blood as fenofibric acid. The suitability of this model for lipophilic drugs (troglitazone, atovaquone, sanfetrinem, GV150013X and celecoxib) has been demonstrated previously [25,28].

(B) In the second model, an additional compartment was implemented. This compartment represents the GI mucosa and enables simulation of permeability restrictions.

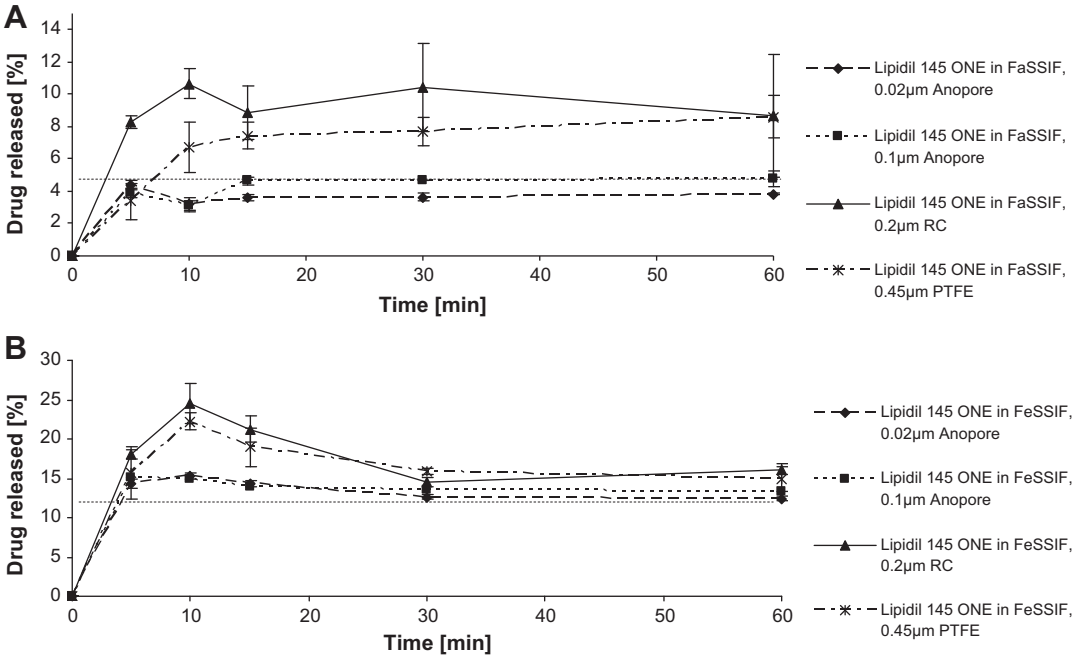

Fig. 1. Dissolution profiles of nanosized fenofibrate (Lipidil 145 ONE®) in FaSSIF (A) and FeSSIF (B) using filters with different pore sizes (0.02 μm, 0.1 μm, 0.2 μm, 0.45 μm). The dotted horizontal line represents the apparent solubility (24 h) of fenofibrate in the medium.

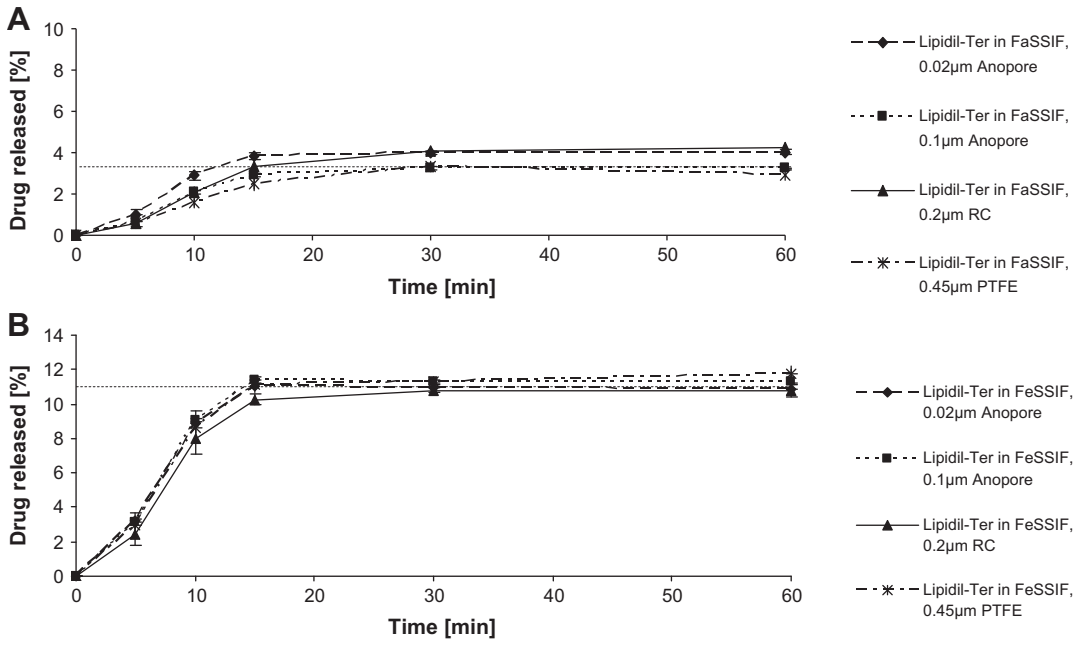

Fig. 2. (A) Dissolution profiles of microsized fenofibrate (Lipidil – Ter®) in FaSSIF and (B) in FeSSIF, using filters with different pore sizes (0.02 µm, 0.1 µm, 0.2 µm, 0.45 µm). The dotted horizontal line represents the apparent solubility (24 h) of fenofibrate in the medium.

Table 3
Comparison of dissolution profiles using the f_2-value (0.1 µm pore size as reference).

Filter pore size (µm)	Lipidil 145 ONE®		Lipidil – Ter®	
	FaSSIF	FeSSIF	FaSSIF	FeSSIF
0.02	91.53	94.86	86.09	97.09
0.2	46.84	48.54	74.71	84.53
0.45	61.81	55.86	66.58	99.54

For both models, the rate of gastric emptying was set to 2.8 h^{-1} in the fasted state and 4 kcal/min in the fed state [19,28]. Additionally, conversion of fenofibrate to fenofibric acid was assumed to be complete (e.g. [29]) in both models.

Sensitivity studies were also performed by varying the respective parameters from one-fifth to five times the physiological or measured value of the parameter used in the initial simulations.

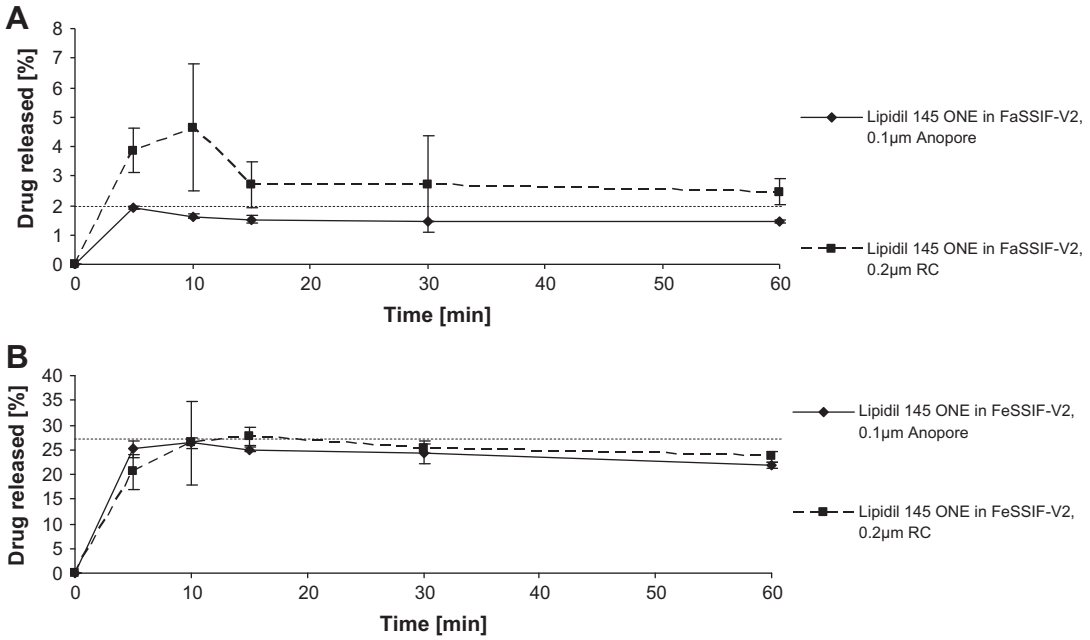

Fig. 3. (A) Dissolution profiles of nanosized fenofibrate (Lipidil 145 ONE®) in FaSSIF-V2 and (B) in FeSSIF-V2, using filters with different pore sizes (0.1 µm, 0.2 µm). The dotted horizontal line represents the apparent solubility (24 h) of fenofibrate in the medium.

2.8. Comparison of simulated plasma profiles

The simulated plasma profiles were compared to the *in vivo* data in terms of bioequivalence using the point estimate ratios of AUC and C_{max}. Bioequivalence is established when the 90% confidence intervals around the point estimates ratios for the simulated plasma profile fall in the range of 0.8–1.25 of those for the *in vivo* profile [30]. Additionally, the point estimate ratio of T_{max} is presented.

3. Results and discussion

3.1. Solubility studies

Solubility values (measured solubility at 24 h) of fenofibrate in biorelevant media are shown in Table 2. The increased solubility in FeSSIF and FeSSIF-V2 can be explained by the formation of solubilizing micelles from bile salts, lecithin, GMO and sodium oleate [21,31]. The solubility measurements in the complex milky medium FeSSGF showed very high standard deviations. This is a result of using a multi-phase system and the ensuing difficulties with reproducibility of analysis.

Table 4
Ratios of C_{max}, T_{max} and AUC from *in silico/in vivo* plasma profiles using Model A.

	Lipidil 145 ONE®		Lipidil – Ter®	
	Fasted state	Fed state	Fasted state	Fed state
C_{max}	1.41	1.16	0.91	1.32
T_{max}	0.52	0.78	1.20	0.94
AUC	0.93	0.97	0.98	0.99

3.2. Dissolution in FaSSIF and FeSSIF using various filter pore sizes

The dissolution profiles obtained using filters with different pore sizes are shown in Fig. 1 for Lipidil 145 ONE® and Fig. 2 for Lipidil – Ter®. The f_2-values for both are listed in Table 3.

Using a pore size of 0.1 μm or less, the maximum concentration of drug achieved in solution from the nanosized formulation was commensurate with the measured solubility (24 h) of fenofibrate in FaSSIF and FeSSIF. Filtration with a pore size of 0.2 μm or 0.45 μm generated concentrations exceeding the measured solubility. These results, in combination with the higher standard deviations, indicate that the apparent "supersaturation" is caused by colloidal fenofibrate, which is too fine to be held back by these filters. The f_2-value of less than 50 when comparing the profiles obtained from 0.1 μm and 0.2 μm filter pore size indicates that the choice of filter pore size is crucial to the interpretation of the dissolution profiles. To separate nanosized drug from molecularly dissolved fenofibrate in Lipidil 145 ONE®, a filter pore size of 0.1 μm or less appears to be appropriate. The use of a smaller pore size (0.02 μm) resulted in a comparable dissolution profile.

The analogous experiment with microsized fenofibrate which contains little or no colloidal fenofibrate yielded similar dissolution profiles, irrespective of filter pore size; f_2 was always higher than 65, indicating less than 5% difference between the dissolution profiles in any medium.

3.3. Dissolution profiles in updated biorelevant media

The experiments were repeated in updated biorelevant media to obtain appropriate data for the simulation of plasma profiles using STELLA® 9.1.1. The dissolution profiles of Lipidil 145 ONE® are shown in Fig. 3. As in the first series of dissolution tests, only

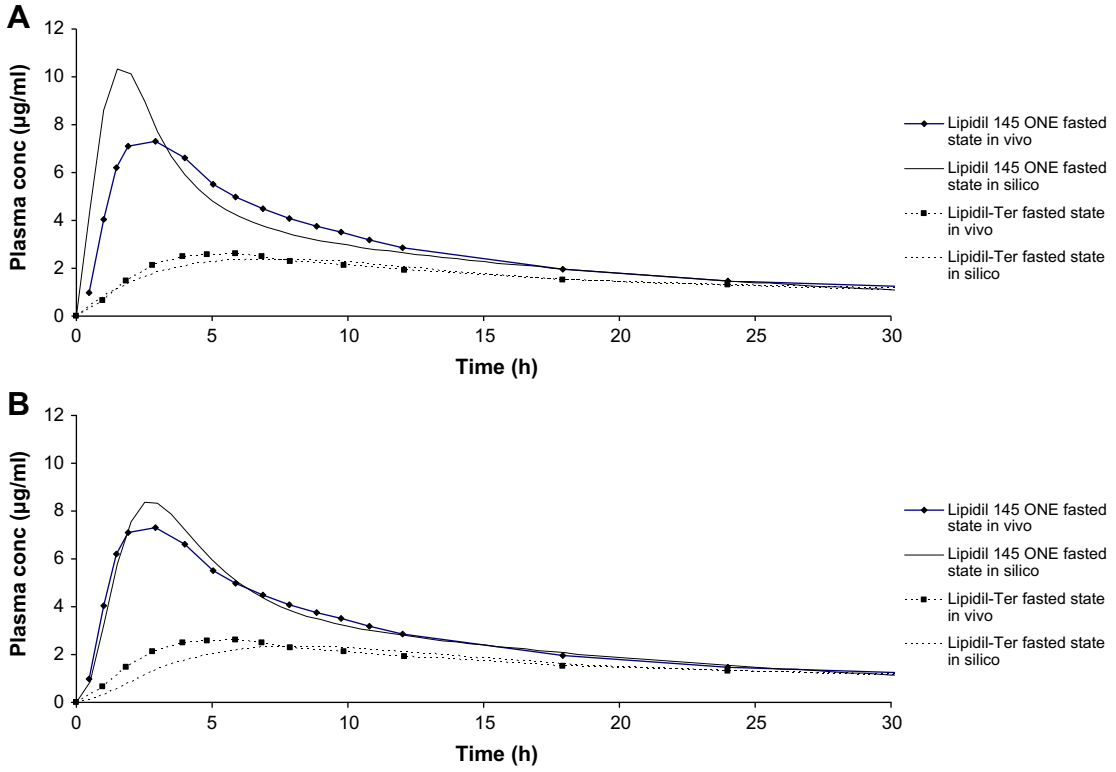

Fig. 4. Simulated plasma profiles of Lipidil 145 ONE® and Lipidil – Ter® in the fasted state generated (A) with Model A and (B) with Model B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

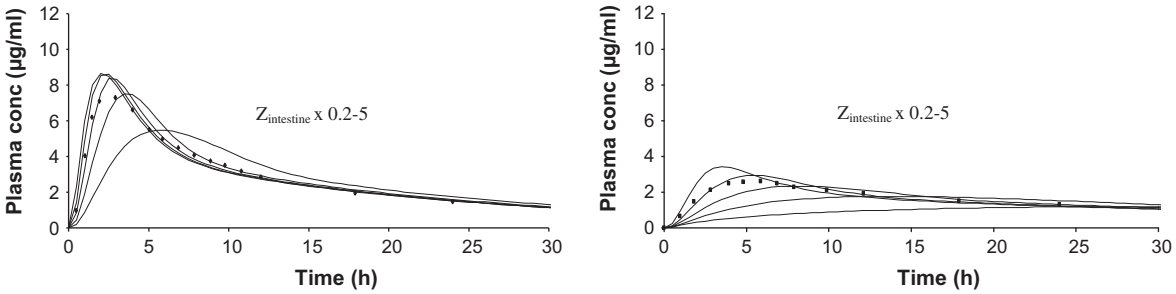

Fig. 5. Sensitivity to model parameters: influence of intestinal dissolution rate (IDR) in the fasted state for Lipidil 145 ONE® (left) and Lipidil – Ter® (right). IDR was varied from one-fifth to five times the experimental value.

Table 5
Ratios of C_{max}, T_{max} and AUC from *in silico/in vivo* plasma profiles using Model B.

	Lipidil 145 ONE®		Lipidil – Ter®	
	Fasted state	Fed state	Fasted state	Fed state
C_{max}	1.14	1.06	0.90	1.20
T_{max}	0.86	0.97	1.37	1.04
AUC	0.93	0.97	0.98	0.99

filters with a pore size of 0.1 µm or less held back nanosized API, while some colloidal fenofibrate appears to pass through filters with a pore size of 0.2 µm. Therefore, the use of a filter pore size of 0.1 µm is recommended for dissolution testing of nanosized APIs.

The oral absorption of BCS Class II drugs is mainly limited by the dissolution rate of the formulation [16,29]. However, the fate of colloidal fenofibrate is unclear. It has been hypothesized that colloidal particles in the nanometer range might adhere to the gut membrane or even be taken up directly [5,6,10,32,33]. Different models constructed with STELLA® 9.1.1 software were used to generate simulated plasma profiles in an effort to help clarify these points.

3.4. Simulated plasma profiles of fenofibric acid

3.4.1. Fasted state

3.4.1.1. Model A. The updated biorelevant media are closer to the physiological compositions and should therefore be more suitable for simulation purposes [17]. Therefore, the results from dissolution tests in these media were used to simulate plasma profiles in STELLA®. The most appropriate analytical method was used (filter pore size 0.1 µm). Model A generates plasma profiles of fenofibric acid on the assumption of instant absorption of any fenofibrate dissolved in the GI tract (i.e. no restriction of fenofibrate permeability) and subsequent conversion of fenofibrate to fenofibric acid. The ratios of C_{max}, T_{max} and AUC from simulated and *in vivo* plasma profiles are shown in Table 4. Model A correlates very well to the

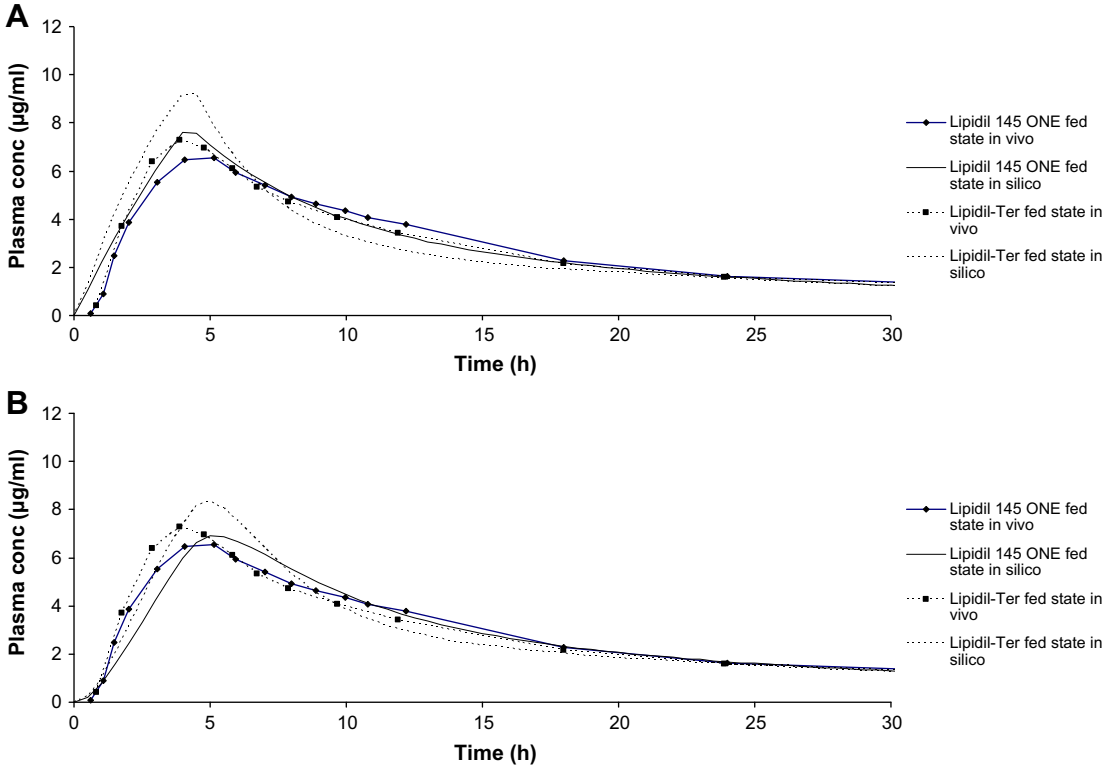

Fig. 6. Simulated plasma profiles of Lipidil 145 ONE® and Lipidil – Ter® in the fed state generated (A) with Model A and (B) with Model B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

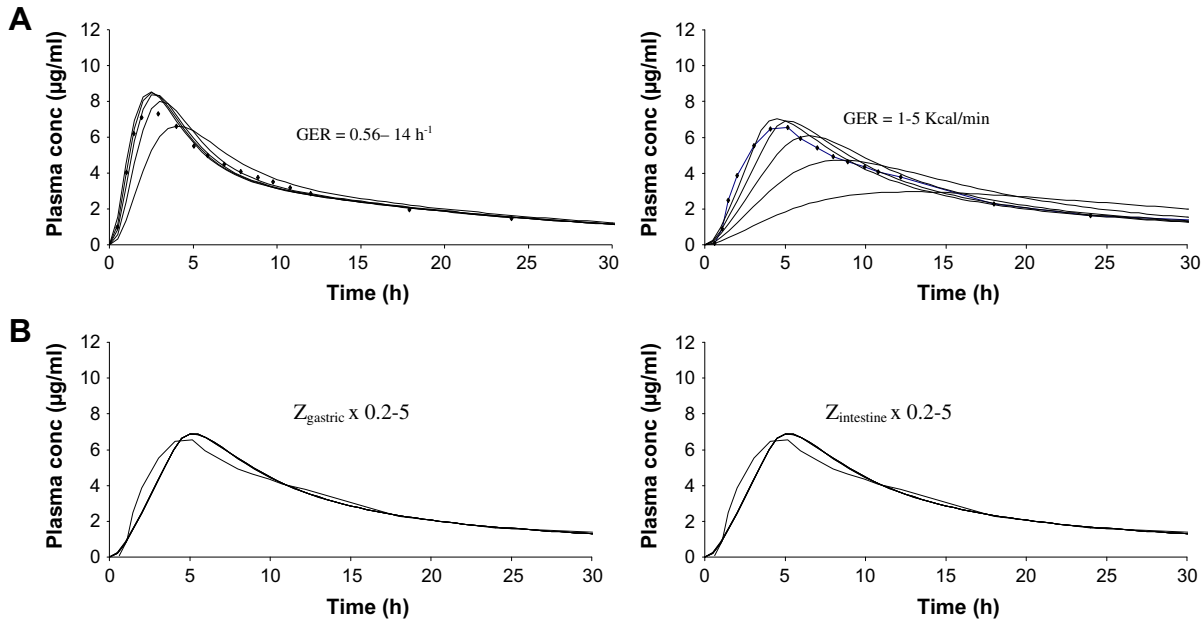

Fig. 7. Sensitivity to model parameters: (A) influence of gastric emptying rate (GER) in the fasted (left) and fed (right) state for Lipidil 145 ONE®. For the fasted state, the GER was varied from one-fifth to five times the average population value. For the fed state, GER was varied from 1 kcal/min to 5 kcal/min (average population value: 4 kcal/min). (B) influence of dissolution rate in the fed state for Lipidil 145 ONE® for gastric (left) and intestinal (right) dissolution. The results obtained in dissolution experiments were varied from one-fifth to five times the experimental value.

plasma profile of the microsized Lipidil – Ter® (Fig. 4A), indicating that for this formulation dissolution of the drug is the single rate-determining step to its appearance in the blood.

The hypothesis that nanoparticles are directly taken up through the mucosa is not supported by the results with Model A for fenofibrate nanocrystals. Model A leads to overestimation of C_{max}, even though it is assumed that only dissolved drug contributes as the driving force for absorption.

A possible reason for the difference between the simulated profiles and *in vivo* profiles for the nanosized formulation is that dissolution of fenofibrate from this formulation is faster than its uptake into the mucosa, even though fenofibrate is often assumed to be a BCS Class II drug. Buch et al. proposed that, for lipophilic drugs in general [16], results for nanosized API often infer that dissolution might not be the only issue for absorption. Therefore, a permeability limitation was built into Model B.

3.4.1.2. Model B. The implementation of an absorption step (i.e. permeability limitation) results in better simulation of the *in vivo* profile for the nanosized formulation, while the simulation for the dissolution-controlled microsized form is maintained (Fig. 4B). A sensitivity analysis, varying the intestinal dissolution rate from one-fifth to five times the rate obtained from the dissolution of the formulation in biorelevant media supports the hypothesis that some permeability limitations exist for the absorption of fenofibrate from the nanosized formulation, since the simulations at lower dissolution rates do not result in a more appropriate simulation (Fig. 5). Similar behavior has been recently reported for another poorly soluble API, aprepitant [34]. The point estimates ratios are given in Table 5.

3.4.2. Fed state

The simulated plasma profiles correlated well with the *in vivo* profiles for both Models A and B (Fig. 6A and B) in the fed state.

The lack of sensitivity to the choice of model may be explained by the zero-order gastric emptying (GE) rate in the fed state. The GE rate seems to be an important limiting factor in the fed state for the absorption of fenofibrate: solid drug particles enter the small intestine gradually over time, followed by rapid dissolution and absorption, so that the rate at which fenofibrate is transferred to the intestine from the stomach is rate limiting. Due to the more rapid GE rate in the fasted state, GE does not play a limiting role in the overall absorption process when the dosage form is taken on an empty stomach. The sensitivity analyses of gastric emptying rates in the fasted and fed state (Fig. 7A) support this hypothesis.

Nevertheless, the simulations slightly overpredict C_{max} in the fed state. Possible reasons are that (i) the fraction absorbed in the fed state is not 100%, (ii) presystemic metabolism of fenofibrate to fenofibric acid is hindered by food components such that fenofibrate is more slowly presystematically metabolized to fenofibric acid or (iii) the use of a linear gastric emptying rate for simulation purposes does not reflect the *in vivo* situation perfectly [11,35,36].

4. Conclusion

It is clearly shown that only filters with pore sizes of $\leqslant$0.1 µm result in accurate dissolution profiles for nanosized fenofibrate. The use of a larger pore size results in an overprediction of dissolution profiles and should be avoided for nanosized formulations.

The use of biorelevant media in dissolution testing in combination with the STELLA® 9.1.1 software enabled *in vitro–in silico–in vivo* correlation for both microsized and nanosized fenofibrate in both the fed and fasted state. When changing from the microsized to the nanosized formulation, the rate-determining step for absorption may change from completely dissolution-controlled to at least partly permeation-controlled in the fasted state. In the fed state, gastric emptying appears to be rate-determining for absorption of fenofibrate from both the microsized and the nanosized formulation.

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This work is dedicated to the memory of Dr. Ekarat Jantratid (1975–2010).

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