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The conflict between *in vitro* release studies in human biorelevant media and the *in vivo* exposure in rats of the lipophilic compound fenofibrate

Thao Thi Do[a], Michiel Van Speybroeck[a], Raf Mols[a], Pieter Annaert[a], Johan Martens[b], Jan Van Humbeeck[c], Jan Vermant[d], Patrick Augustijns[a], Guy Van den Mooter[a,*]

[a] *Laboratory of Pharmacotechnology and Biopharmacy, KU Leuven, Belgium*
[b] *Center for Surface Chemistry and Catalysis, KU Leuven, Belgium*
[c] *Department of Metallurgy and Materials Engineering, KU Leuven, Belgium*
[d] *Department of Chemical Engineering, KU Leuven, Belgium*

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ABSTRACT

The performance of four different lipid-based (Tween 80–Captex 200P, Tween 80–Capmul MCM, Tween 80–Caprol 3GO and Tween 80–soybean oil) and one commercially available micronized formulation (Lipanthyl Micronized®) of the lipophilic compound fenofibrate was compared *in vitro* in various biorelevant media and *in vivo* in rats. In simulated gastric fluid without pepsin (SGF_{sp}) and fasted state simulated intestinal fluid (FaSSIF), only Tween 80–Captex 200P system resulted in a stable fenofibrate concentration, but no supersaturation was obtained. The other three lipid based systems created fenofibrate supersaturation; however they did not maintain it. In fed state simulated intestinal fluid (FeSSIF), all lipid-based formulations resulted in complete dissolution of fenofibrate during the experiment, which represented a supersaturated state for Tween 80–Capmul MCM and Tween 80–Caprol 3GO systems. In both FaSSIF and FeSSIF, all lipid-based formulations yielded a higher fenofibrate concentration than the micronized formulation. Contrary to the *in vitro* results, no significant difference in the *in vivo* performance was observed among the four tested lipid-based formulations both in the fasted and the fed states. The *in vivo* performance of all lipid-based formulations was better than that of Lipanthyl Micronized®, in the fasted as well as in the fed state. The fact that for the lipid based systems the *in vitro* differences in pharmaceutical performance were not translated into *in vivo* differences can be attributed to the continuous excretion of bile in the gastrointestinal tract of rats, causing enhanced solubilizing capacity for lipophilic drugs. This study clearly points to the conflicting situation that might arise during the preclinical phase of the development of lipid based formulations of lipophilic drugs as the performance of such systems is very often evaluated by both *in vitro* release studies in human biorelevant media as well as *in vivo* studies in rats. Care must be taken to select a relevant animal model.

1. Introduction

Oral administration has attractive advantages for drug delivery including the ease of application and high patient compliance, and is the preferred route for chronic drug therapy (Shen and Mitragotri, 2002; Barakat, 2010). However, drug solubility and its dissolution are among many factors determining drug bioavailability after oral administration (Rolan and Molnar, 2006). Low solubility is the reason why BCS (Biopharmaceutics Classification System) class II drugs often show poor and variable oral bioavailability (Grove et al., 2007). Several strategies dealing with the formulation problems of poorly water soluble compounds have been developed and described in literature. Lipid-based drug delivery systems, including oil solutions, oil suspensions, emulsions and self-(micro)emulsifying drug delivery systems (SMEDDS), constitute one of the possible approaches to improve drug bioavailability (Pouton, 2000). There are different advantages using lipid-based drug delivery systems such as the drug already being in solution, allowing elimination of the dissolution step (Porter et al., 2004), and the benefits observed in the presence of food as even small doses of lipid have been shown to stimulate a post-prandial response (Khoo et al., 2003). Significant interest has been paid to lipid-based drug delivery systems after the commercial success of Sandimmune Neoral® (cyclosporine A), Fortovase® (saquinavir) and Norvir® (ritonavir) (Grove et al., 2006), as well as the proven increase in bioavailability of different compounds such as ontazolast (Hauss et al., 1998), halofantrine (Khoo et al., 1998), danazol (Porter et al., 2004) and seocalcitol (Grove et al., 2006, 2007) when

* Corresponding author at: Laboratory of Pharmacotechnology and Biopharmacy, Catholic University of Leuven, O&N II (9/921), Herestraat 49, B-3000 Leuven, Belgium. Tel.: +32 16 330300; fax: +32 16 330305.
E-mail address: Guy.Vandenmooter@pharm.kuleuven.be (G. Van den Mooter).

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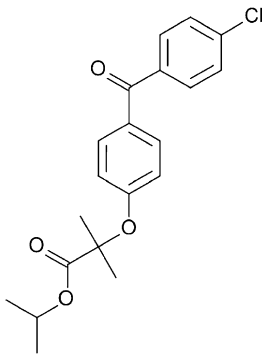

Fig. 1. Structural formula of fenofibrate.

administered in lipid-based delivery systems compared to a solid dosage form.

Various studies have been carried out in order to compare the ability of medium chain lipids versus long chain triglycerides with respect to increasing drug bioavailability. Medium chain lipids have higher fluidity, better solubilizing properties and provide a better chemical stability for the drug substance owing to the purity of the lipid and the lack of double bonds compared with long chain triglycerides (Shah et al., 1994; Grove et al., 2006). However, the latter is likely to enhance the lymphatic transport of a lipophilic drug substance, which prevents first-pass metabolism (Caliph et al., 2000; Porter et al., 2007). In some cases, such as vitamin D3 (Holmberg et al., 1990), halofantrine (Caliph et al., 2000) and danazol (Porter et al., 2004), drug bioavailability has been shown to be significantly higher after co-administration with long chain triglycerides compared with medium chain triglycerides. In contrast, the intestinal absorption of progesterone and griseofulvine was higher when administered with medium chain lipids when compared to long chain lipids (Porter et al., 2008). On the other hand, no significant differences in bioavailability were found for seocalcitol (Grove et al., 2006) and dexamethasone (MacGregor et al., 1997), irrespective of the chain length of the lipid employed. One of the remaining difficulties in formulation development during the preclinical stage is to select the optimal type of tests to evaluate the performance with respect to *in vivo* exposure. A lot of excellent research has been done to develop biorelevant (to humans) media to test *in vitro* drug dissolution or drug release (Jantratid et al., 2008; Vertzoni et al., 2004). Most often, *in vitro* dissolution tests are performed in biorelevant media and consecutively (or concurrently), based on the outcome of the biorelevant dissolution studies animal tests are executed.

The goal of this work was to investigate the relevance of combining *in vitro* release studies in human biorelevant media with *in vivo* studies in rats for the lipophilic model compound fenofibrate formulated in four different lipid based drug delivery systems (containing medium or long chain lipids) and one commercially available micronized formulation (Lipanthyl Micronized®). This combination of *in vitro* testing with animal experiments is often done in pharmaceutical industry during the early formulation development phase and is based on the philosophy "to test as much as possible with minimal efforts and costs". Often, the physiological suitability of the animal model selected is not clear and rats are frequently used in an early stage of development as they are cheap and easy to handle. Moreover, rats have been used as animal model in various published studies of lipid-based formulations (Grove et al., 2006; Yin et al., 2009). The ability of the lipid-based delivery systems and the micronized formulation to maintain fenofibrate supersaturation was studied in various biorelevant media including those simulating the fasted as well as the fed state.

Fenofibrate (Fig. 1) is a neutral, lipophilic drug ($\log P = 5.2$) (Munoz et al., 1994), which is poorly soluble in water (aqueous solubility < 0.5 mg/l) (Vogt et al., 2008); it has a high permeability and hence is considered as a Class II drug according to the BCS (Granero et al., 2005). Fenofibrate is a lipid-lowering agent, which is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease (Wysocki et al., 2004).

2. Materials and methods

2.1. Chemicals

Fenofibrate was purchased from Indis (Aartselaar, Belgium) and fenofibric acid from ABCR (Karlsruhe, Germany). Carbamazepine was bought from PharmInnova (Waregem, Belgium). Captex 200P (propylene glycol mono- and dicaprylate and mono- and dicaprate), Capmul MCM (glyceryl mono- and dicaprate) and Caprol 3GO (polyglycerol-3 oleate) were kindly provided by Abitec Corp. (Janesville, WI, USA). Soybean oil was purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Tween 80 was purchased from Alfa Aesar (Karlsruhe, Germany).

Sodium taurocholate was bought from ICN Biomedicals (Eschwege, Germany), lecithin from Nattermann Phospholipid (Köln, Germany), and chloroform from Chemlab (Zedelgem, Belgium). $NaH_2PO_4 \cdot H_2O$, NaCl and 0.1 M HCl were purchased from Fisher Scientific (Tournai, Belgium).

2.2. Methods

2.2.1. Preparation of lipid-based formulations

Four formulations (Tween 80–Captex 200P, 3–1; Tween 80–Capmul MCM, 5–1; Tween 80–Caprol 3GO, 5–1; and Tween 80–soybean oil, 7–1; w/w) were prepared by mixing Tween 80 and each oil at 50–60 °C. Fenofibrate was then dissolved into the mixture of surfactant and oil by constant stirring and kept at 50–60 °C until a clear solution was obtained. The final concentration of fenofibrate in the lipid-based system was 5%. All mixtures remained clear at room temperature. The commercially available micronized formulation, Lipanthyl Micronized® (dose strength 67 mg) was obtained from Solvay Pharma (Brussels, Belgium).

2.2.2. Droplet size measurement

The droplet size of the formulations was determined at 0.5% (w/v) concentration of the formulation in water by photon correlation spectroscopy using a CGS-3 spectrometer (Malvern Instruments, Worcestershire, UK) equipped with a goniometry, auniphase 22 mV He–Ne laser operating at 632.8 nm, an avalanche photodiode detector and an ALV-5000/EPP multi-angle tau correlator. Light scattering was monitored at 90°.

2.2.3. Preparation of release media

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared according to the formula described in Vertzoni et al. (2004), and simulated gastric fluid without pepsin (SGF_{sp}; USP). The following chemicals were used for the preparation of the biorelevant media: sodium acetate (VWR, Brussels, Belgium), acetic acid (Chemlab, Zedelgem, Belgium), sodium taurocholate (practical grade) (ICN Biomedicals, Eschwege, Germany), lecithin (Phospholipon 90G,Nattermann Phospholipid, Köln, Germany), $NaH_2PO_4 \cdot H_2O$, NaCl and 1 M HCl (Fisher Scientific, Tournai, Belgium) and chloroform (Chemlab, Zedelgem, Belgium).

2.2.4. Solubility measurements

The solubility of fenofibrate was assessed in various aqueous media by the shake-flask method: an excess amount (approximately 2 mg) of fenofibrate was dispersed in 1.5 ml of medium containing placebo lipid-based formulations and shaken for 24 h at

37 °C. Samples were filtered through a 0.45 μm pore size membrane filter and analyzed with HPLC (see Section 2.2.5).

2.2.5. In vitro dissolution experiments

The dissolution behavior of the lipid-based formulations containing 5% (w/w) fenofibrate and Lipanthyl Micronized® was assessed in various media: simulated gastric fluid without pepsin (SGF_{sp}) with pH change, fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF). Experiments were performed in triplicate. To change the pH of SGF_{sp} to 6.8, after 2 h, trisodium phosphate was added to the medium. 20 ml of each dissolution medium was added in test tubes with a diameter of 22 mm, which were placed in a water bath (37 °C), and the medium was constantly stirred. The stirring intensity of magnetic stirrers (length: 10 mm; diameter: 2.5 mm) was set at position 5 of the nominal scale of the IKA machine (ca. 485 rotations per min). Formulations containing 2 mg of fenofibrate were added to the test tubes. The nominal dose of fenofibrate in the release media thus amounted to 100 μg/ml. At 5, 10, 30, 60, 120, 180 and 240 min, 1-ml samples were withdrawn and replaced by an equal volume of the fresh medium to maintain a constant total volume. Samples were filtered through a 0.45 μm pore size membrane filter and analyzed with HPLC. The HPLC analysis was performed using a Merck Hitachi pump L-7100, an autosampler L-7200 and a UV–VIS detector L-7420. The column was a Lichrospher 100 RP8 5 μm 125 mm × 4.6 mm; the mobile phase consisted of methanol: 25 mM ammonium acetate buffer pH 3.5 (72:28, v/v) and the detection wavelength was set at 287 nm. The mobile phase was used at a flow rate of 1.0 ml/min. 20 μl sample volume was injected into the system. Cumulative percentages of the drug dissolved from the preparation were calculated.

2.2.6. In vivo study

All *in vivo* experiments were carried out in accordance with the EC directive 86/609/EEC for animal experiments (license number LA1210261). Approval for this project was granted by the KU Leuven Institutional Ethical Committee for Animal Experimentation.

2.2.6.1. Animals. Male Wistar rats (300–380 g, ca. 9 weeks of age) (Elevage Janvier, Le Genet Saint Isle, France) were used in this study. Water and food (58% carbohydrates, 33% proteins, 9% lipids) (sniff R/M-H, sniff Spezialdiäten, Soest, Germany) were available *ad libitum*. All formulations were evaluated in the fasted and fed state. Fed animals had access to food prior to the experiment, whereas the fasted rats were deprived from food 12 h prior to dosing. Fasted animals were allowed access to food again 4 h post-dose, whereas fed animals had access to food *ad libitum* during the whole time course of the experiment. Water was available *ad libitum* to all animals.

2.2.6.2. Dosing. All rats were dosed orally with 1 ± 0.05 mg of fenofibrate, as either a lipid-based formulation or Lipanthyl Micronized® ($n = 4$). All formulations were filled into hard gelatin capsules (PCcaps kit, Capsugel). All capsules were administered intragastrically using a dosing syringe plunger (PCcaps kit). After dosing the lipid-based formulations, 1 ml of water was administered.

2.2.6.3. Blood sampling. Prior to each blood withdrawal; the rats were placed in an incubator (37 °C) (Mini-thermacageMK3, Datesand, Manchester, UK) for 15 min to promote bleeding. Subsequently, the animals were placed in a cylindrical restrainer with adjustable headgate and removable tailgate (Harvard Apparatus, Holliston, MA). Blood samples (500 μl) were taken by individual venipunctures of the later tail vein at 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h post-dose. Blood was collected into heparin-coated tubes (LH, 68 I.U.) (Vacutainer, Becton Dickinson, Plymouth, UK) and plasma was harvested by centrifugation (2600 × *g*, 4 °C, 10 min). All plasma samples were stored at −20 °C pending bioanalysis. After every blood withdrawal, the rats were rereleased into their cages.

2.2.6.4. Bioanalysis.

2.2.6.4.1. Sample preparation. All plasma samples were quantified for fenofibric acid, the major active metabolite of fenofibrate. Presystemic metabolism of fenofibrate into fenofibric acid is quantitative in rats (Weil et al., 1988) as well as in humans (Weil et al., 1990). Carbamazepine was used as internal standard during sample preparation. Plasma samples (200 μl) were spiked with internal standard (100 μl of 20 μM carbamazepine in 1 M HCl). Subsequently, 1 M HCl (1.3 ml) was added in order to precipitate plasma proteins. Afterwards, all samples were extracted using 4 ml of diethylether (Lab-Scan, Dublin, Ireland). After centrifugation (2600 × *g*, 4 °C, 5 min), the organic phase was evaporated to dryness. Lastly, the extraction residue was re-suspended in 150 μl of a 50/50 (v/v, %) methanol/water mixture, transferred into autosampler vials and analyzed by HPLC–UV as described below.

2.2.6.4.2. HPLC–UV. The chromatographic system and column used for bioanalysis were the same as for the *in vitro* samples, but some modifications were made with respect to the chromatographic conditions. The mobile phase consisted of methanol: 5 mM ammonium acetate buffer pH 3.3 (66:34, v/v). The flow rate was 1 ml/min and UV detection was performed at 300 nm. Under these conditions, the retention times of the internal standard and fenofibric acid was 5 and 9.5 min, respectively. Calibration curves were linear over a concentration range of 0.048–100 μM. The intraday variability for 6.25 μM standards amounted to 1.3% ($n = 6$). Accuracy of the same standards was within the range of 95–101%. The interday variability of a 6.25 μM standard was below 4%.

2.2.6.4.3. Data analysis. The observed maximum plasma concentration (C_{max}) and the time of its occurrence (T_{max}) were determined directly from the individual plasma concentration–time profiles. The area under the plasma concentration–time curve (AUC) was calculated by linear trapezoidal method from time zero to the last sampling point. Statistical analysis of the C_{max} and AUC values of the various formulations was performed in Excel using a one-way analysis of variance (ANOVA) and the Student's *t*-test (90% confidence interval).

3. Results and discussion

3.1. Droplet size analysis

Based on a previous study (Do et al., 2009), four lipid based systems were selected for the current study: Tween 80–Captex 200P (3–1; w/w); Tween 80–Capmul MCM (5–1; w/w); Tween 80–Caprol 3GO (5–1; w/w) and Tween 80–soybean oil (7–1; w/w). They all resulted in a stable SMEDDS with relatively small droplet size upon dilution in aqueous environment. However, drug incorporated into S(M)EDDS might result in a change in droplet size distribution (Gursoy and Benita, 2004). Thus, the droplet size of the four systems loaded with fenofibrate was determined. The experiments were performed in demineralized water to exclude the influence of other agents on the droplet size. Moreover, the stability of the droplet size was also measured as a function of time, i.e. after 0, 1 and 6 h. The results are shown in Table 1.

For Tween 80–Captex 200P system, an increase in droplet size was observed during the experimental time; however no precipitation was seen. For Tween 80–Caprol 3GO and Tween 80–soybean oil systems, drug loading resulted in similar droplet sizes during the first hour; however, after that, both systems displayed fenofibrate precipitation. For Tween 80–Capmul MCM system, the precipitation already occurred after 1 h. Among these four systems, only the

Table 1
The mean droplet size of lipid-based formulations in water ($n = 5$).

System	Time 0 h		Time 1 h		Time 6 h	
	MDS[a] (nm)	PDI	MDS[a] (nm)	PDI	MDS[a] (nm)	PDI
Tween 80–Captex 200P (3–1, w/w)	16.0 ± 4.0	0.54 ± 0.03	26.6 ± 5.1	0.59 ± 0.01	44.3 ± 6.7	0.36 ± 0.02
Tween 80–Capmul MCM (5–1, w/w)	76.9 ± 8.7	1.02 ± 0.04	Precipitation		Precipitation	
Tween 80–Caprol 3GO (5–1, w/w)	90.5 ± 9.6	0.69 ± 0.02	93.4 ± 9.7	0.71 ± 0.02	Precipitation	
Tween 80–soybean oil (7–1, w/w)	5.0 ± 2.2	0.05 ± 0.01	5.2 ± 2.3	0.12 ± 0.03	Precipitation	

[a] Mean droplet size.

Tween 80–Captex 200P system with loaded fenofibrate possibly presents a SMEDDS.

3.2. *In vitro* dissolution experiments

Although the results of droplet size measurement showed that only the Tween 80–Captex 200P system could maintain a small droplet size without precipitation when loaded with fenofibrate, the *in vitro* dissolution studies examining the ability of the lipid-based formulations to maintain fenofibrate in the dissolved state were still conducted for all four fenofibrate formulations in different media, namely SGF_{sp} with pH change, FaSSIF and FeSSIF. The volume ratio of formulation towards that of the release medium in these experiments is comparable to that of the *in vivo* situation in rats (McConnell et al., 2008). As standard media for simulating the human GI tract physiology, FaSSIF and FeSSIF were selected to test the *in vitro* performance as they better reflect the complex composition of the human GI fluid. For Lipanthyl Micronized® formulation, the experiments were not conducted in SGF_{sp} because the solubility of fenofibrate in this medium is too low (0.21 μg/ml). The results are shown in Fig. 2.

In SGF_{sp} and FaSSIF media, similar dissolution profiles were observed for all four lipid-based formulations. There seems to be an agreement between the ability in retaining small droplet size upon drug loading and the ability to maintain the drug concentration at a plateau. Indeed, the Tween 80–Captex 200P system, which was the only formulation where the small droplets remained and did not result in precipitation for the duration of the experiment, is the only system achieving a stable fenofibrate concentration for 4 h in SGF_{sp} and FaSSIF. However, no supersaturation was created in this system as the fenofibrate concentration in the dissolution experiments was not higher than its solubility in these media containing the Tween 80–Captex 200P system (Table 2). For the other three non-stable systems, displaying precipitation when loaded with fenofibrate, the drug concentration in solution gradually decreased in time. The Tween 80–Capmul MCM system, showing the earliest precipitation, failed to keep fenofibrate dissolved in SGF_{sp} medium, as indicated by the instantaneous decrease in drug concentration. In FaSSIF, however, this system has a slightly different dissolution profile, with the fenofibrate concentration only starting to decrease after about half an hour. For Tween 80–Caprol 3GO and Tween 80–Soybean oil systems, no precipitation was observed in the first hour, which is expressed in the *in vitro* dissolution profile as a slight delay of the decrease in fenofibrate concentration. In these three systems, supersaturation was created, but was not maintained in both SGF_{sp} and FaSSIF media. In general, the concentration of fenofibrate in solution is higher in FaSSIF than in SGF_{sp} for all formulations, which can be explained by the presence of micelles in the FaSSIF, which can help in solubilizing poorly soluble drugs. In FaSSIF, the Lipanthyl Micronized® formulation created a very low degree of supersaturation as the fenofibrate concentration released and maintained in solution (about 16 μg/ml) was just only slightly higher than the fenofibrate solubility in FaSSIF (13.6 μg/ml). The fenofibrate concentration released from the Lipanthyl Micronized® formulation is lower than that from all four lipid-based formulations, despite the fact that there is a decrease in fenofibrate concentration in three lipid-based formulations. However, it should be pointed out that for Lipanthyl Micronized® formulation, the dissolution experiment illustrated how much drug is dissolved and maintained in solution, while as mentioned above, for the lipid-based formulation, it examines the ability to maintain the drug in the dissolved state.

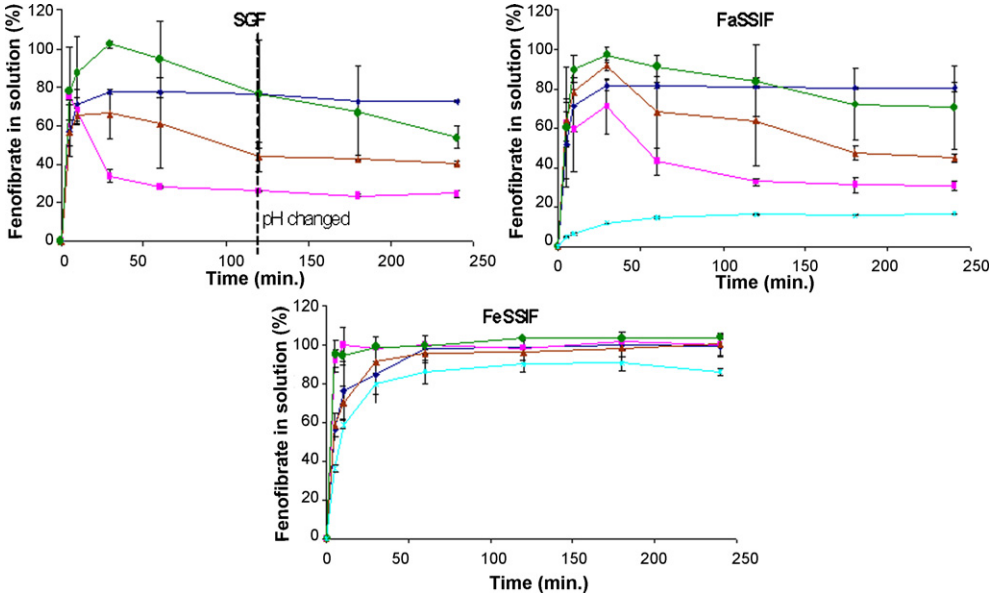

Fig. 2. *In vitro* dissolution profiles of four lipid-based formulations (Tween 80–Captex 200P (♦); Tween 80–Capmul MCM (■); Tween 80–Caprol 3GO (▲); Tween 80–soybean oil (●)) and Lipanthyl® micronized formulation (×) in SGF_{sp} (A), FaSSIFF (B) and FeSSIF (C). Average and standard deviation are depicted ($n = 3$).

Table 2
Solubility of fenofibrate in different media containing placebo lipid-based formulations ($n = 3$).

System	Solubility (μg/ml)		
	SGF	FaSSIF	FeSSIF
Pure fenofibrate (without lipid-based formulation)	0.21 ± 0.01	13.6 ± 0.1	79.1 ± 1.2
Tween 80–Captex 200P (3–1, w/w)	105.5 ± 3.3	119.5 ± 5.0	149.5 ± 1.2
Tween 80–Capmul MCM (5–1, w/w)	40.3 ± 1.4	49.2 ± 0.4	87.0 ± 1.1
Tween 80–Caprol 3GO (5–1, w/w)	45.4 ± 0.4	54.2 ± 0.9	90.9 ± 1.5
Tween 80–soybean oil (7–1, w/w)	69.6 ± 1.3	82.9 ± 1.7	118.6 ± 2.1

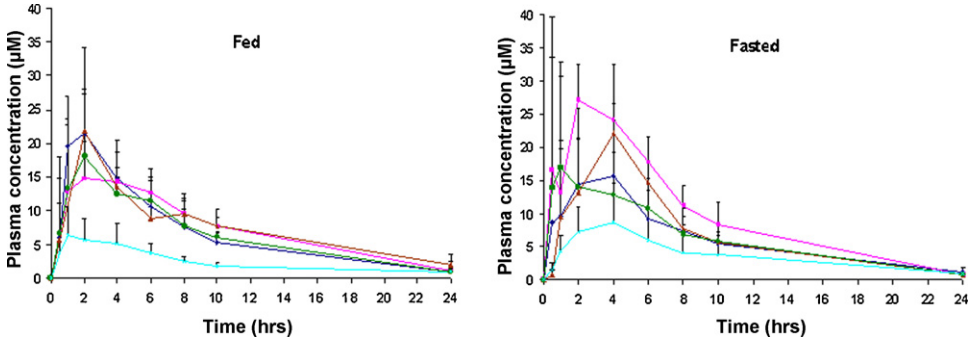

Fig. 3. Plasma concentration–time profiles of fenofibric acid in the fasted state and fed state of lipid-based formulations (Tween 80–Captex 200P (♦); Tween 80–Capmul MCM (■); Tween 80–Caprol 3GO (▲); Tween 80–soybean oil (●)) and Lipanthyl Micronized® formulation (×).

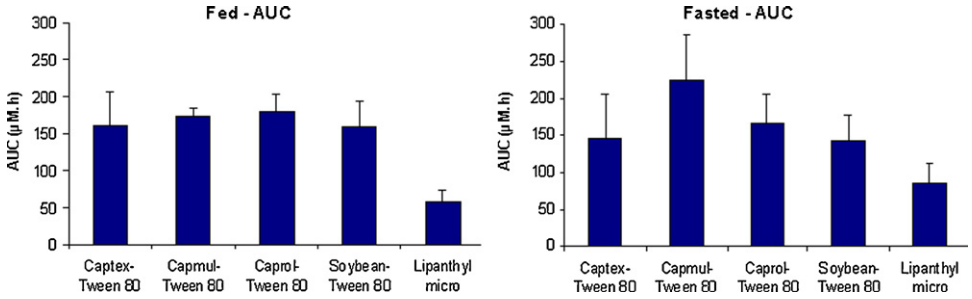

Fig. 4. Average AUC of fenofibric acid. The error bars indicate the standard deviation.

In FeSSIF, all lipid-based formulations could reach complete dissolution of fenofibrate and this fenofibrate concentration was maintained until the end of the experiment. Among the four lipid-based formulations, Tween 80–Capmul MCM and Tween 80–Caprol 3GO created and maintained supersaturation. The two other systems did not yield supersaturation as the solubility of fenofibrate in the FeSSIF containing system is higher than the concentration used in the dissolution test (Table 2). For the Lipanthyl Micronized® formulation, a much higher drug concentration was obtained and a higher degree of supersaturation was achieved in FeSSIF as compared to FaSSIF. However, this high concentration is still lower than those obtained with lipid-based formulations. These data suggest that, in the fed state, all released fenofibrate would remain in solution long enough to allow for absorption to take place.

3.3. *In vivo experiments*

The plasma concentration–time profiles of fenofibric acid after administration of different formulations in the fasted and fed state in rats are presented in Fig. 3; the corresponding $AUC_{0-24\,h}$ and C_{max} values are shown in Figs. 4 and 5. In the fed state, the $AUC_{0-24\,h}$ values of the lipid-based formulations are statistically not significantly different (90% confidence level), which shows good agreement with the *in vitro* dissolution results in FeSSIF (all lipid-based formula-

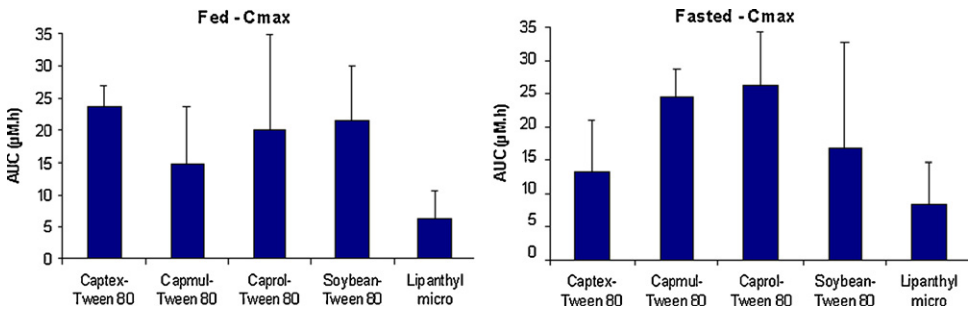

Fig. 5. Average C_{max} of fenofibric acid. The error bars indicate the standard deviation.

tions could reach and maintain complete dissolution). However, no agreement was found between the *in vivo* results in the fasted state and *in vitro* dissolution in FaSSIF. In the fasted state, no significant differences in the $AUC_{0-24\,h}$ values were found among the lipid-based formulations, while in FaSSIF, they showed a different ability to keep fenofibrate in solution. Moreover, when comparing the *in vivo* performance of the same formulation in fasted and fed state, no significant differences (90% confidence level) were found. The explanation for the lack of agreement between the *in vitro* and *in vivo* results in the fasted state is the physiology of the rat's gastro-intestinal tract. As the rat does not have a gallbladder, it has a continuous secretion of bile into the duodenum irrespective of the nutritional state (DeSesso and Jacobson, 2001). The difference between fasted and fed state in rats is mainly attributed to the solubilization ability of food components, rather than an increase in intestinal solubility caused by bile secretion. The bile components influence the emulsification process, which helps to maintain the drug in solution. In this way, bile can enhance the *in vivo* performance of formulations that show poor *in vitro* results.

In both the fasted and fed state, the commercial product Lipanthyl Micronized® exhibits a significantly lower (90% confidence level) pharmaceutical performance than all lipid-based formulations, except for the Tween 80–Captex 200P system in fasted state. Interestingly, the results of the *in vitro* dissolution experiment in FeSSIF showed that the fenofibrate concentration reached and remained in solution from the Lipanthyl Micronized® formulation was only about 10% lower than that from lipid-based formulations. However, the lowest $AUC_{0-24\,h}$ value obtained in fed state from lipid-based formulations, which was from Tween 80–soybean oil formulation (142.7 μM h), was even 2.5-fold higher than that from the Lipanthyl Micronized® formulation (58.0 μM h). This indicates that the improved *in vivo* performance of the lipid-based formulations in this study cannot be solely related to the increase in drug concentration in solution, which is in accordance to what has been suggested by Porter, namely that lipid-based formulations can affect drug absorption, bioavailability and disposition after oral administration in different ways such as by enhancing drug solubilization, the recruitment of lymphatic drug transport processes and/or the interaction with enterocyte-based transport processes (Porter et al., 2007).

Upon oral administration to rats, the four different lipid-based formulations of fenofibrate did not show significant differences (90% confidence level) in the $AUC_{0-24\,h}$ values. Regardless of the fact that the differences between fasted and fed state were leveled, the four lipid-based formulations gave a comparable *in vivo* performance, despite the differences of the lipids used in the formulations, which result in different characteristics such as the droplet size and probably lead to differences in the digestion process. The micronized formulation performed the least *in vivo* as well as *in vitro*. In this respect, the dissolution experiments in biorelevant media were able to rank the lipid based formulations above the micronized formulation.

The results obtained in the rat experiment suggest that it is of less importance to consider whether precipitation of a co-formulated drug is prevented as the formulation interacts with the GI environment, whereas the opposite conclusion will come from the *in vitro* studies with the biorelevant media. Interactions with the GI tract include the potential ability of lipids or other excipients to stimulate secretion of biliary lipids and alter gastric transit, the potentially significant changes to formulation properties that might occur on digestion and interaction with bile salt (Porter et al., 2007). Unfortunately, this essential information cannot be obtained in rats. As the bioavailability of lipid-based formulations is so dependent on the dynamic processes in the gastro-intestinal tract, an *in vivo* study in another animal model may be needed to gain better extrapolation to humans.

4. Conclusion

Our results show the disagreement between the *in vitro* performance in human biorelevant release media and *in vivo* studies in rats for lipid-based formulations of the lipophilic drug fenofibrate and question the relevance of rats, animals that continuously excrete bile, in this type of studies. The better *in vivo* performance of the lipid-based formulations compared to the micronized form of fenofibrate suggested that also other factors than the increase of drug concentration in solution were involved, since the lowest $AUC_{0-24\,h}$ value obtained in fed state from lipid-based formulations was even 2.5-fold higher than that from the Lipanthyl Micronized® formulation. No differences in *in vivo* performance were observed among the four tested lipid-based formulations despite their different lipid composition.

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