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Research Article

In Vitro Lipolysis Data Does Not Adequately Predict the *In Vivo* Performance of Lipid-Based Drug Delivery Systems Containing Fenofibrate

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Abstract. The present study investigated the utility of *in vitro* lipolysis performance indicators drug solubilization and maximum supersaturation ratio (SR^M) for their predictive use for the *in vivo* performance in a minipig model. The commercial Lipanthyl formulation and a series of LbDDS based on identical self-nanoemulsifying drug delivery systems (SNEDDS) containing 200 mg of fenofibrate, either dissolved or suspended, were subjected to combined gastric (pH 2) and intestinal (pH 6.5) *in vitro* lipolysis. Based on the solubilization profiles and SRM the rank-order SNEDDS (75% drug load)>super-SNEDDS (150% drug load, dissolved)=SNEDDS suspension (150% drug load, partially suspended)> Lipanthyl was established, with an increased likelihood of drug precipitation above SR^M>3. The *in vitro* performance, however, was not reproduced *in vivo* in a minipig model as the mean plasma concentration over time curves of all LbDDS were comparable, independent of the initial physical state of the drug. There was no correlation between the area under the solubilization-time curves ($AUC_{in\ vitro}$) of the intestinal step and the $AUC_{in\ vivo}$. The study suggests careful interpretation of *in vitro* performance criteria and revision of LbDDS optimization towards increased solubilization.

KEY WORDS: *in vitro* ipolysis; *in vitro*/*in vivo* correlation; lipids; SNEDDS suspensions; super-SNEDDS; supersaturation.

INTRODUCTION

The oral route, as the preferred way of drug administration, has become increasingly challenging due to the vast number of drugs candidates with poor water solubility and/or slow dissolution rate (1). Drug solubilization is a prerequisite prior to drug absorption for an intended systemic effect of an orally administered drug (2). As the gastrointestinal permeability of many poorly water-soluble drugs is often sufficiently high to enable rapid absorption in the intestinal tract, formulation scientists have developed a number of drug delivery strategies that can facilitate problems associated with limited drug solubility and dissolution rate (3,4). Among these enabling formulation approaches lipid-based drug delivery systems (LbDDS), especially self-emulsifying drug delivery systems, have become a popular formulation option for poorly water-soluble, lipophilic drugs (5). Despite the success of some marketed LbDDS (e.g., Neoral®), the number of commercially available LbDDS is still limited. This reluctance might be attributed to the fact that despite intense research in the area, the factors governing the *in vivo* performance of LbDDS are still poorly understood. Moreover, the majority of *in vivo* studies have compared the performance of LbDDS with conventional solid dosage forms rather than evaluating differences between diverse LbDDS, which could help elucidate the critical factors governing the performance of LbDDS.

In LbDDS the drug is commonly dissolved in a single or, more frequently, a blend of excipients as in the case of self-nanoemulsifying drug delivery systems (SNEDDS) (6). SNEDDS are an isotropic mixture of lipids, surfactants, cosurfactants, and cosolvents that generate ultrafine, kinetically stable emulsions in aqueous media under conditions of gentle agitation (7). The delivery of the drug in the dissolved state is considered the major advantage of SNEDDS as the dissolution step of the crystalline material thereby is circumvented (8). However, there have been concerns on the potential negative impact on drug absorption due to drug precipitation in the small intestine resulting from the partitioning of hydrophilic formulation components (e.g., cosolvents and hydrophilic surfactants) during SNEDDS dispersion in the intestinal fluids and the subsequent

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enzymatic breakdown of digestible SNEDDS excipients (9,10). The development of SNEDDS has, therefore, been focused on the avoidance of drug precipitation during *in vitro* testing, e.g., by reducing drug loads in the formulations (11). Recent studies however, have shown that the precipitation of the poorly water-soluble drugs cinnarizine, simvastatin, and halofantrine observed during *in vitro* lipolysis was not reflected in a reduced area under the plasma concentration-time curve (AUC) in the corresponding *in vivo* studies carried out in a dog model (12–14). It is interesting to note that these drugs were also shown to precipitate in an amorphous form during *in vitro* lipolysis, with a faster dissolution rate compared with the crystalline form of the drugs (13–15). Although it is not known whether or not (and in which form) the drugs also precipitate *in vivo*, these studies point at the potential importance of the nature of the solid state of drug precipitates and could explain the poor predictive value of the *in vitro* model on the *in vivo* performance.

It has recently been suggested that the propensity of drug precipitation during *in vitro* dispersion and digestion of LbDDS can serve as a potential indicator for the *in vivo* performance (16). As a measure for the likelihood of drug precipitation, the maximum supersaturation ratio (SR^M) has been suggested as a representation of the ratio of the theoretical drug concentration (in the absence of precipitation) and drug solubility in the aqueous phase (16,17). A value of SR^M>2.5 has been identified as the threshold above which drug precipitation is likely to occur. The majority of the studies evaluated the SR^M precipitation parameter *in vitro* with only very limited *in vivo* data available (11). While the proposed precipitation parameter SR^M reflected the *in vivo* performance of formulations containing moderate drug load (40% of the drug solubility in the formulation), the *in vitro* results failed to predict the *in vivo* performance at higher drug load (80% drug solubility) in a dog model.

From an industry perspective and due to patient compliance SNEDDS with drug loads high enough to reduce the pill burden are desirable. However, in conventional SNEDDS, the drug load is frequently limited by the drug solubility in the formulation. In an attempt to overcome this limitation, supersaturated SNEDDS ("super-SNEDDS") containing drug loads in the formulation above equilibrium solubility have been described recently (13,14). Despite pronounced generation of (amorphous) drug precipitates during *in vitro* lipolysis, the super-SNEDDS were equivalent or superior to conventional SNEDDS *in vivo* (13,14).

So far, the number of studies evaluating the *in vivo* performance of SNEDDS based on precipitation parameters, such as the SR^M, is very limited. The aims of the current study were, therefore, twofold: (1) to evaluate the *in vitro* and *in vivo* performance of commercial Lipanthyl and a series of SNEDDS, including super-SNEDDS, of the same relative composition containing a high drug load of fenofibrate, a compound previously shown to precipitate in a crystalline form during *in vitro* lipolysis (16) and (2) to evaluate the proposed *in vitro* precipitation parameter for its predictability on the *in vivo* performance. To facilitate the above study aims, an identical dose of 200 mg fenofibrate was used across all formulations by adjusting the overall mass of the respective formulation. A recently established *in vitro* lipolysis model was employed, combining consecutive gastric and intestinal *in vitro* lipolysis (18–20). Moreover, the *in vitro* lipolysis conditions (such as porcine bile salts and pancreatic lipase extract of porcine origin) were chosen to match the minipig model used for the subsequent *in vivo* study.

MATERIALS AND METHODS

Materials

Fenofibrate, fenofibric acid, clofibric acid, soybean oil (long-chain triglycerides), sodium hydroxide, porcine pancreatic lipase (≥3× USP activity), porcine bile extract (68% purity), sodium taurodeoxycholate, and calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA), and maleic acid and 4-bromobenzeneboronic acid (BBBA) from Fluka (Buchs, Switzerland). Kolliphor RH 40 (polyoxyl 40 hydrogenated castor oil) and Maisine 35-1 (long-chain mono-, di-, and triglycerides) were donated by BASF (Ludwigshafen, Germany) and Gattefossé (St. Priest, France), respectively. Soybean phospholipids (SPC; 99% purity) were supplied by Lipoid (Ludwigshafen, Germany). *Candida antarctica* lipase A (10 mg/mL) was a gift from Novozyme (Bagsvaerd, Denmark). HPLC-grade acetonitrile, methanol, and absolute ethanol were obtained from VWR (Herlev, Denmark). Purified water was obtained from a Siemens Ultra Clear water purification system (Guenzburg, Germany). All other chemicals were of analytical grade and were used as received unless specified otherwise.

Methods

Preparation of the Formulations

A previously characterized SNEDDS composed of 24% soybean oil, 32.2% Maisine 35-1, 30% Kolliphor RH 40, and 13.8% ethanol was used for the current study (21). The molten Maisine 35-1 and Kolliphor RH 40 were blended with soybean oil and, after cooling to room temperature, the required amount of ethanol was added before the mixture was stirred on a magnetic stirrer. Drug-loaded formulations were produced by weighing the required amount of fenofibrate directly into dust-free screw-top glass vials followed by the addition of appropriate amounts of drug-free SNEDDS. The following formulations were prepared using the same drug-free SNEDDS (Table I): (1) SNEDDS containing fenofibrate corresponding to 75% of the drug's solubility in the SNEDDS; (2) supersaturated SNEDDS (super-SNEDDS) corresponding to 150% of the drug's solubility in the SNEDDS. Supersaturation in the formulation was induced by consecutive heating and cooling cycles as described previously (13,14); (3) SNEDDS suspension containing a saturated solution of fenofibrate in SNEDDS (corresponding to 100% fenofibrate solubility in SNEDDS) plus suspended fenofibrate (corresponding to 50% of the drug's solubility in the SNEDDS; "100+50%" drug load). The SNEDDS suspension was prepared analogous to the super-SNEDDS but without the heating/cooling cycle. Preliminary X-ray diffraction analyses had confirmed that fenofibrate precipitated in the same crystalline form as the

Table I. Drug Loads, Amounts of Formulation, and Number of Capsules used for *In Vitro* and *In Vivo* Studies

	Formulation (% drug load)			
	SNEDDS (75%)	Super-SNEDDS (150%)	SNEDDS suspension (100+50%)	Lipanthyl
Total dose (mg)	200	200	200	200
Total amount of lipid formulation (g)	2.451	1.226	1.226	n/a
Number of capsules	3	2	2	1

SNEDDS self-nanoemulsifying drug delivery systems

starting material after inducing drug precipitation from a super-SNEDDS (data not shown). The SNEDDS suspension, therefore, resembled the likely composition of a super-SNEDDS after precipitation of excess fenofibrate within the formulation. The freshly prepared formulations were filled manually into hard gelatin capsules (AAEL DB, Capsugel, Strasbourg, France) in amounts corresponding to a dose of 200 mg fenofibrate before immediate use in the *in vitro* and *in vivo* studies. To allow reproducible filling of the hard gelatin capsules with SNEDDS suspension, the preconcentrate was continuously stirred to prevent sedimentation of undissolved drug. Commercially available Lipanthyl 200 M capsules containing micronized fenofibrate (Abbott AG, Baar, Switzerland), representing a conventional solid dosage form, were used as received.

Determination of Fenofibrate Saturation Solubility in Formulations and in Digestion Media

The equilibrium solubility of fenofibrate in anhydrous SNEDDS was determined after incubation of excess fenofibrate (0.2 g) in 1 g of drug-free SNEDDS at 37°C for up to 72 h using a tube shaker/rotator. The excess drug was removed in 24 h intervals by centrifugation at 14,000 rpm for 20 min at 37°C (Eppendorf 5804R, Eppendorf, Hamburg, Germany). Following appropriate dilution in methanol/chloroform aliquots of the clear supernatant were subsequently quantified for fenofibrate by HPLC as described below. Equilibrium solubility was assumed when values of two consecutive days varied less than 5%. The equilibrium solubilities of fenofibrate in 200 mL of lipase-free, gastric lipolysis medium, in 300 mL and in 350 mL of lipase-free, intestinal lipolysis media (both in the absence and presence of corresponding amounts of drug-free formulations) were determined analogous to the method described above after appropriate dilution in methanol. All the determinations were done in triplicates.

Combined Gastric and Intestinal In Vitro Lipolysis

For the current study a combined gastric/intestinal *in vitro* lipolysis protocol was employed with minor adaptations of the method recently described by Christophersen *et al.* (20). The freshly prepared capsules filled with formulations corresponding to a dose of 200 mg fenofibrate and commercial Lipanthyl capsules were immersed in a temperature-controlled reaction vessel containing 200 mL of gastric *in vitro* lipolysis medium (37°C, pH 2) using stainless steel sinkers to prevent floating of the capsules. For the preparation of gastric lipolysis medium sodium taurodeoxycholate was used due to the poor solubility of the porcine bile salts at pH 2. The pH was controlled by a Titrando 842 pH-stat and Tiamo software 1.3 (Metrohm, Zofingen, Switzerland). Gastric lipolysis was carried out for 30 min using the microbial lipase *C. antarctica* lipase A previously shown to have an activity-profile similar to human gastric lipase (Christophersen *et al.* (20,22)). Thereafter, 110.5 mL of intestinal lipolysis medium (pH 6.5) were added to the gastric medium and the pH was automatically adjusted to 6.5 by the pH-stat apparatus. Following a 3-min equilibration time, the intestinal *in vitro* lipolysis was initiated by the addition of 50 mL of freshly prepared pancreatic lipase solution resulting in a total volume of 350 mL at the beginning of intestinal lipolysis. Throughout the subsequent 60-min intestinal lipolysis, the pH was maintained at 6.5 by the titration of liberated fatty acids with 1 M NaOH, and the lipolysis rate was controlled by the constant addition of 0.5 M calcium chloride (0.09 mL/min) (23). Background titration experiments were carried out to determine the sodium hydroxide consumption during the unspecific digestion of phospholipids and impurities present in the formulation-free lipolysis media (24). The initial compositions of the gastric and intestinal lipolysis media are compiled in Table II.

Sample Preparation During In Vitro Lipolysis

Samples (3.5 mL) were withdrawn from the digestion media at predetermined time points (15 and 30 min after initiation of gastric lipolysis; 1 min after pH adjustment to 6.5 (pre-lipase addition); after 5, 15, 30, 45, and 60 min of intestinal lipolysis). Lipolysis in the samples was inhibited by immediate addition of 20 μL of the lipase inhibitor BBBA (1 M in methanol); 0.1 mL of the inhibited sample was diluted with 0.9 mL methanol and was quantified for the total fenofibrate content at the respective time points by HPLC using the method described below. Of the remaining sample, 3.0 mL was subjected to ultracentrifugation (30 min at 100,000 rpm, 37°C) in an Optima MAX-XP ultracentrifuge (Beckman Coulter, Brea, CA, USA) generating an aqueous phase and a pellet, which were both analyzed for fenofibrate by HPLC after appropriate dilution with methanol.

Solid State Characterization of Pellets

Additional samples were withdrawn after 60 min of intestinal *in vitro* lipolysis in order to characterize the solid state of the pellets generated after ultracentrifugation by X-ray powder diffraction (XRPD) using a X′Pert Pro X-ray diffractometer (MPD PW3040/60 XRD, PANalytical, Almelo, The Netherlands). The isolated pellets were placed on aluminum holders and were irradiated by a CuKα radiation

Table II. Composition of the Fasted State Lipolysis Media as Used for Gastric and Intestinal *In Vitro* Lipolysis

	pH	Pepsin (mg/mL)	Bile salts[a] (mM)	SPC (mM)	Maleate (mM)	NaCl (mM)	Lipase activity (U/mL)[b]
Gastric	2	0.15	0.080	0.020	2	34.2	10
Intestinal	6.5	–	3	0.6	10	70	179

SPC soybean phospholipids
[a] Sodium taurodeoxycholate was used for the preparation of gastric medium; porcine bile extract for intestinal medium
[b] Lipase activity in the gastric step was determined as tributyrin units and the activity in the duodenal step according to USP method (Christophersen *et al.* (20))

source (λ=1.542 Å) at 40 kV and 30 mA. The employed scan speed was 0.1285°(2)/min with a step size of 0.0884° (2) between 5° and 35° (2) start and end angle, respectively.

Quantification of Fenofibrate from In Vitro Studies

The samples obtained from solubility and *in vitro* lipolysis studies were analyzed on a Summit HPLC system (Dionex, Germering, Germany) operated by Chromeleon Software 7 (Thermo Scientific, Germering, Germany). Injected samples (20 μL) were separated on a Waters X-Bridge C18 3.5 μm, 150 mm×4.6 mm, column (Waters, Milford, MA, USA) using a previously reported method (25). In brief, a methanol: MilliQ–water mixture (85:15%, *v*/*v*) was isocratically pumped at a flow of 0.9 mL/min resulting in fenofibrate retention times of approximately 4.3 min detected at a wavelength of 288 nm. The peak areas of the samples were compared with a linear standard curve (R^2> 0.999) established between 0.5 and 100 μg/mL.

In Vitro Data Analysis

The obtained equilibrium solubilities of fenofibrate in the aqueous phase after 30 min gastric lipolysis and after 5 min of the intestinal lipolysis step were used to calculate the maximum SR^M according to Eq. 1 (11,17):

$$SR^M = \frac{\text{maximum drug concentration in the aqueous phase (gastric or intestinal)}}{\text{equilibrium solubility of the drug in the aqueous phase (gastric or intestinal)}} \quad (1)$$

The SR^M is directly related to the drug load since it reflects the maximum attainable degree of supersaturation in the absence of precipitation at a given time during digestion. The area under the fenofibrate solubilization-time curve during the course of intestinal lipolysis ($AUC_{in\ vitro}$) was determined by the linear trapezoidal method for the correlation with the *in vivo* data ($AUC_{in\ vivo}$).

In Vivo Study

The protocol for the *in vivo* study was approved by the Animal Welfare Committee, appointed by the Danish Ministry of Justice. All animal procedures were carried out in compliance with EC Directive 86/609/EEC and with the Danish law regulating experiments with animals and the NIH guidelines on animal welfare. Six male Göttingen minipigs (11.5–13.4 kg at study start) were obtained from Ellegaard Göttingen Minipigs A/S (Dalmose, Denmark) and acclimatized for 14 days before initiation of the study in an air-conditioned building with controlled environmental parameters (relative humidity, 50±10%; temperature, 20±1°C; light 06.00–18.00 h). Between the studies the animals had *ad libitum* access to standard pig food (Altromin 9023, Altromin Spezialfutter, Lage, Germany) and fresh tap water. The pigs were examined weekly by a veterinarian and observed closely after each experimental day. Before entering the experiment, the minipigs were fasted for 18–20 h with free access to water and had access to food 8 h post-dosing. In weekly intervals (wash out period seven days), each animal received 200 mg fenofibrate in freshly prepared capsules containing SNEDDS, super-SNEDDS, SNEDDS suspension, and Lipanthyl in a cross-over design. Before administration, the fenofibrate content in the formulations was confirmed by HPLC analyses. Administration of the capsules was facilitated by a biting block and subsequent administration of approximately 10 mL of tap water according to an internal standard protocol. Blood samples (2 mL) were collected at predetermined time points (predosing, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 30, 48, and 72 h post-dosing) in heparinized tubes. Following centrifugation at 3,200 rpm (15 min, 4°C), the plasma was harvested and the samples were stored at −80°C until analyses.

Quantification of Plasma Samples

The plasma samples were analyzed for fenofibric acid, the main metabolite of fenofibrate, using the validated assay previously described by Hanafy *et al.* with minor modifications (26). For sample preparation, 50 μL of plasma was spiked with internal standard (40 μL, 200 μg/mL clofibric acid in methanol) before adding 410 μL of methanol. After brief whirl mixing the samples were sonicated for 10 min in a Corpax GS20 ultrasonic water bath (Corpax, Valby, Denmark) followed by storage for 10 min at −20°C. Thereafter, the samples were centrifuged for 14 min at 15,600 rpm at 4°C (Eppendorf 5804R, Eppendorf, Hamburg, Germany). Each sample was analyzed in duplicate by injection of the clear supernatant (50 μL) and separation on the identical chromatographic system as described above using methanol/MilliQ water (68%:32%, 0.1% formic acid, *v*/*v*) as mobile phase at a flow rate of 0.6 mL/min. Samples for the standard

curve (0.05 to 3.0 μg/mL, R^2>0.997) were prepared in a similar manner by spiking blank plasma with varying concentrations of fenofibric acid in methanol, 40 μL of internal standard, and 410 μL of methanol. The accuracy of the assay was between 94 and 103% and the precision between 4.2 and 1.6% at 0.1 and 1.0 μg/mL, respectively (n=5).

Pharmacokinetics and Statistical Analyses

The pharmacokinetic parameters were determined by noncompartmental analyses using WinNonlin Professional 6.3 (Pharsight Corporation, Mountain View, USA). The AUC were calculated by the linear trapezoidal method from time zero to 8 h ($AUC_{0–8\ h}$), i.e., until the time of feeding post-dosing, and from zero to 72 h ($AUC_{0–72\ h}$). The peak plasma concentrations (C_{max}) and their time of occurrence (t_{max}) were recorded directly from the individual plasma concentration-time curves. The oral bioavailability of fenofibrate after administration of SNEDDS, super-SNEDDS, and SNEDDS suspension relative to the commercial Lipanthyl capsules were calculated based on the $AUC_{0–72\ h}$ obtained from individual animals. Analyses of variance (ANOVA) in combination with Tukey's multiple comparisons test, and Pearson correlation coefficients were computed by GraphPad Prism Version 6.03 (La Jolla, CA, USA) to determine statistical significance between group means and to determine correlations between *in vitro* and *in vivo* data (p<0.05).

RESULTS

Solubility Determination and Preparation of Formulations

The equilibrium solubility of fenofibrate in the SNEDDS preconcentrate at 37°C was attained within 48 h incubation time and totaled 108.8±4.1 mg fenofibrate/g preconcentrate. At this value the equilibrium solubility represents 100% drug load. Based on the equilibrium solubility, drug loads of 75 and 150% were calculated and the formulations were prepared accordingly to achieve the target dose of 200 mg in each formulation. In the case of SNEDDS and super-SNEDDS, the entire drug load was dissolved in the formulation, whereas in the case of the SNEDDS suspension crystalline fenofibrate was partially suspended in the formulation as described above. As a consequence of the reduced drug load in the SNEDDS compared with the other formulations it was necessary to prepare a slightly greater number of capsules for the *in vitro* and *in vivo* studies (Table I).

Combined Gastric and Intestinal In Vitro Lipolysis

All capsules containing lipid formulations (SNEDDS, super-SNEDDS, and SNEDDS suspension) ruptured and fully dispersed their content within approximately 3 min in the low pH medium of the gastric lipolysis step. Similarly, Lipanthyl capsules raptured within the same time, but instead of forming a fine emulsion the powder content (mainly micronized fenofibrate, lactose, magnesium stearate, pregelatinized maize starch, sodium lauryl sulfate, and crospovidone, according to manufacturer's information) was suspended in the medium. For all formulations, no consumption of sodium hydroxide was observed during the gastric step of *in vitro* lipolysis (data not shown). The addition of pancreatic lipase solution for the intestinal lipolysis step at pH 6.5 triggered the hydrolysis of digestible excipients and resulted in the continuous consumption of sodium hydroxide over 60 min of intestinal *in vitro* lipolysis and correlated inversely with the drug load of the formulations (R^2=0.96, p=0.02). Consistent with this observation the sodium hydroxide consumption during the digestion of super-SNEDDS (150% drug load) and SNEDDS suspension ("100+50%" drug load) were comparable. As expected for the Lipanthyl capsules, devoid of SNEDDS excipients, only small amounts of sodium hydroxide were released (<0.2 mL), comparable to those observed during the digestion of lipolysis medium in the absence of formulations.

The solubilization of fenofibrate in the aqueous phase during consecutive gastric and intestinal *in vitro* lipolysis showed pronounced differences across the different formulations (Fig. 1). For the SNEDDS (75% drug load) almost the entire dose of fenofibrate was maintained solubilized in the aqueous phase, throughout 30 min of the gastric step. However, after initiation of the intestinal step the amount of fenofibrate solubilized in the aqueous phase continuously decreased to approximately 60% at 60 min.

For the super-SNEDDS (150% drug load), considerably less fenofibrate was recovered from the aqueous phase (approximately 50–60%) reflecting the greater degree of drug precipitation prior to the first sampling time point during the gastric lipolysis step. In line with the SNEDDS data, the intestinal digestion step resulted in further reduction of fenofibrate in the aqueous phase to approximately 15% of the fenofibrate dose after 45 min of intestinal *in vitro* lipolysis. Fenofibrate solubilization was reduced during gastric lipolysis for the SNEDDS suspension ("100+50%" drug load) in which approximately one third of the entire fenofibrate dose was present in the formulation as suspended, crystalline drug. During the initial gastric step, approximately 25% of the fenofibrate dose was solubilized in the aqueous phase. The addition of intestinal medium and adjustment of the digestion medium to pH 6.5 (pre-lipase) facilitated an increased fenofibrate solubilization to levels comparable with the super-SNEDDS. The subsequent digestion of

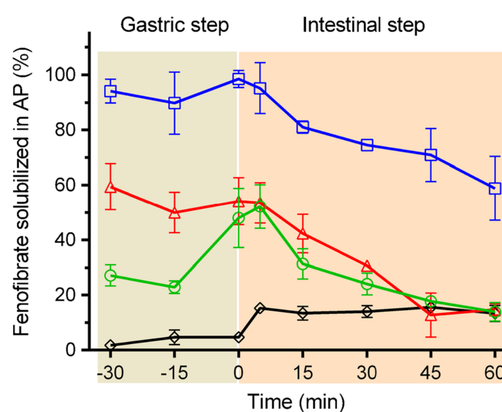

Fig. 1. Relative distribution of fenofibrate in the aqueous phase during 30 min of gastric (pH 2, *gray shaded*) and 60 min intestinal (pH 6.5, *orange shaded*) *in vitro* lipolysis of SNEDDS (75% drug load, *squares*), 150% drug-loaded super-SNEDDS (*triangles*), "100+50%" drug-loaded SNEDDS suspension (*circles*), and Lipanthyl (*diamonds*), mean±SD, n=3. The drug load in each formulation was 200 mg of fenofibrate

the SNEDDS suspension triggered a decline in the fenofibrate solubilization analogous to the super-SNEDDS. By contrast, during the gastric *in vitro* lipolysis step of the conventional Lipanthyl formulation comparably small amounts of the drug (5%) were solubilized in the aqueous phase, indicating that the presence of sodium lauryl sulfate in the commercial product contributes little to drug solubilization. The addition of the intestinal medium caused an increase of solubilized fenofibrate to approximately 15%. As expected, and in contrast to the lipid formulations, the initiation of intestinal lipolysis had no negative impact on the solubilization of fenofibrate throughout the remaining intestinal digestion step.

Solubilization of Fenofibrate in the Digestion Media

In the presence of blank SNEDDS 646.9±6.7 μg/mL of fenofibrate was solubilized in the gastric medium whereas in the intestinal medium (adjusted to pH 6.5, pre-lipase) the fenofibrate solubilization was reduced to 430.0±6.8 μg/mL and decreased only slightly to 389.1±5.7 μg/mL in the total volume (350 mL) of intestinal medium at the beginning of intestinal lipolysis. The absolute amounts of lipid formulation excipients present in the media decreased in the case of super-SNEDDS and SNEDDS suspension due to the higher drug loading, hence the solubilization of fenofibrate was determined in the presence of the corresponding, reduced amount of excipients for these formulations. As expected, the solubilization capacity for fenofibrate was lower than for the SNEDDS (291.6±7.0 μg/mL in the gastric medium, 219.7± 6.3 μg/mL and 205.2±8.6 μg/mL in 300 and 350 mL of intestinal medium, respectively). By contrast, the solubilization capacities of fenofibrate in the lipid-free media (representing the powder formulation present in Lipanthyl) were considerably lower at 4.9±0.5 and 19.2±1.6 μg/mL for the gastric and intestinal media, respectively.

Maximum Supersaturation Ratio

Based on the equilibrium solubilities and the measured concentrations in the aqueous phase, the maximum SR^M across the different formulations during the gastric and intestinal *in vitro* lipolysis steps were calculated and are compiled in Table III.

The attainable maximum SR^M was moderate for the 75% drug-loaded SNEDDS (approximate SR^M=1.5) but was increased for the super-SNEDDS (approximate SR^M=2.8) and SNEDDS suspension (approximate SR^M=3.4). The shift from gastric to intestinal lipolysis medium was not reflected in substantially different SR^M, independent from the formulation and the relative drug load. The very high SR^M calculated for Lipanthyl reflected the fact that the same amount of drug was present in this formulation as in the lipid formulations. However, as Lipanthyl is devoid of the excipients that were present in SNEDDS (e.g., Kolliphor RH40) the commercial product could not enhance the low solubilization capacity of the blank digestion medium.

Solid State Characterization of the Pellets

The solid state properties of the precipitated fenofibrate were investigated by XRPD. The diffractograms of the isolated pellets obtained after 60 min of intestinal *in vitro* lipolysis and ultracentrifugation are depicted in Fig. 2. Consistent with previous results the characteristic diffraction patterns of crystalline fenofibrate were visible in all pellets except the negative control, in which drug-free SNEDDS were subjected to *in vitro* lipolysis (27).

In Vivo Study

Following oral administration fenofibrate is extensively metabolized in the enterocytes to fenofibric acid, hence the plasma concentration *vs* time curves of fenofibric acid are shown in Fig. 3, and the corresponding pharmacokinetic parameters are tabulated in Table IV.

In general, there was considerable inter- and intra-individual variability in the plasma concentrations, complicating interpretation of the data. Where considered appropriate the data in Table IV is presented either as the mean±SD or the median and the range (in addition the mean values are reported in this section).

After administration of 200 mg fenofibrate as lipid-based formulation the course of the plasma concentration profiles was comparable, regardless of the drug being initially completely dissolved (75% drug load SNEDDS and super-SNEDDS) or (partly) suspended (SNEDDS suspension). The mean maximum plasma concentrations (C_{max}) were 1.0 μg/mL (SNEDDS) and 1.3 μg/mL both for super-SNEDDS and SNEDDS suspension. It is noteworthy that the upper range of the maximum plasma concentration for the SNEDDS formulation was 1.8 μg/mL, whereas 2.7 and 2.2 μg/mL were observed for super-SNEDDS and SNEDDS suspension, respectively (Table IV). By contrast, the maximum plasma concentration did not exceed 1.2 μg/mL after the administration of Lipanthyl. The area under the plasma concentration-time curve was calculated both for the time until the minipigs had access to food post-dosing ($AUC_{0–8\ h}$) and the last sampling time point ($AUC_{0\text{-}72h}$). The $AUC_{0\text{-}72h}$ was comparable across the tested formulations which also resulted in no significant difference in the relative bioavailability between the formulations. With regard to the initial $AUC_{0–8\ h}$ (Fig. 3, insert; Table IV), all

Table III. Maximum Supersaturation Ratios (SR^M)[a] During the Gastric and the Intestinal Lipolysis Step Across the Different Formulations

Condition	Time (min)	SNEDDS	super-SNEDDS	SNEDDS suspension	Lipanthyl
Gastric (pH 2)	30	1.5	3.4	3.4	203.4
Intestinal (pH 6.5, pre-lipase 300 mL)	0	1.6	3.0	3.0	34.8
Intestinal (pH 6.5, post-lipase 350 mL)	5	1.5	2.8	2.8	29.8

The SR^M was calculated as the maximum drug concentration in the aqueous phase (gastric or intestinal) divided by the corresponding equilibrium solubility of the drug in the aqueous phase (gastric or intestinal)

SNEDDS self-nanoemulsifying drug delivery systems

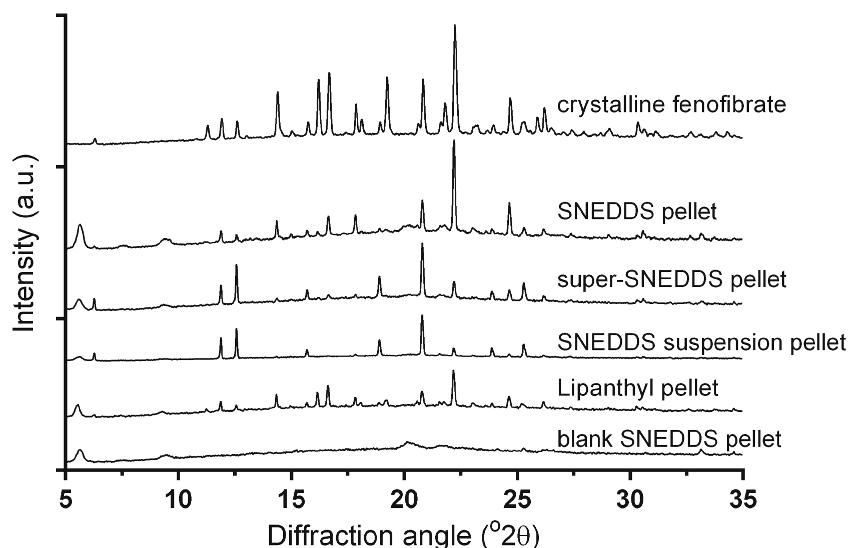

Fig. 2. Diffractograms of the isolated pellets obtained after 60 min of intestinal *in vitro* lipolysis

SNEDDS showed a trend towards increased $AUC_{0–8\ h}$, which was more pronounced for the super-SNEDDS and SNEDDS suspension compared with SNEDDS, whereas the treatment with Lipanthyl resulted in a comparably low initial $AUC_{0–8\ h}$.

Correlation of In Vitro and In Vivo Data

The areas under plasma concentration-time curves ($AUC_{in\ vivo}$), both for the initial 8 h ($AUC_{0–8\ h}$) and the last sampling time point ($AUC_{0–72\ h}$) post-dosing, were plotted against the area under the solubilization-time curves ($AUC_{in\ vitro}$) of the intestinal step (Fig. 4). As illustrated and confirmed by analysis of correlation (Pearson correlation coefficient, r=0.15 and r=0.18, for $AUC_{0–8\ h}$, and $AUC_{0–72\ h}$, respectively, p>0.8), no correlation was found between the $AUC_{in\ vitro}$ and the $AUC_{in\ vivo}$ data.

DISCUSSION

Lipid-based drug delivery systems, such as SNEDDS, have been investigated for the effects of droplet size, lipid composition, the degree of solubilization/precipitation, and the ability to maintain supersaturation during *in vitro* dispersion and *in vitro* digestion on the *in vivo* performance (9,11,12). Moreover, recent advances in the field have highlighted some critical factors during *in vitro* dispersion and *in vitro* digestion (16,17,28). It should be noted that in these previous studies the investigated formulations were

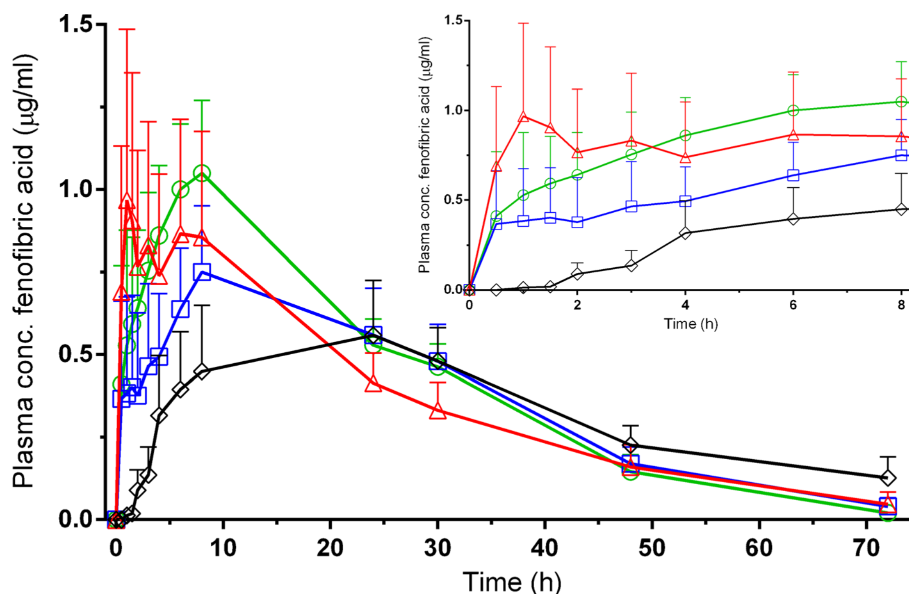

Fig. 3. Plasma concentration of fenofibric acid over 72 h and the initial 8 h (*insert*) after oral administration of SNEDDS (*squares*), super-SNEDDS (*triangles*), SNEDDS suspension (*circles*), and Lipanthyl (*diamonds*) to six minipigs (mean±SEM)

Table IV. Pharmacokinetic Parameters for Fenofibric Acid After Administration of 200 mg Fenofibrate to Six Minipigs Are Reported as Mean ±SD Unless Indicated Differently

	C_{max} (μg/mL)[a]	t_{max} (h)[a]	$AUC_{0-8\ h}$ (μg h/mL)	AUC_{0-72h} (μg h/mL)	Rel. BA (%)
SNEDDS	0.9 (0.2–1.8)	8.0 (0.5–30)	4.1±3.7	25.7±15.5	116.7±51.2
Super-SNEDDS	1.0 (0.1–2.7)	8.0 (1–30)	6.4±6.6	26.1±12.3	114.9±63.6
SNEDDS suspension	1.3 (0.4–2.2)	8.0 (1–30)	6.3±4.0	26.4±9.0	158.2±139.5
Lipanthyl	0.9 (0.4–1.2)	24 (8–72)	1.9±2.3	23.7±7.5	100

SNEDDS self-nanoemulsifying drug delivery systems, C_{max} peak plasma concentrations, t_{max} time of occurrence, *AUC* areas under the plasma concentration-time curves

[a] Median and range in brackets

rather diverse with respect to the nature of the lipid, and the relative lipid, surfactant, and cosolvent composition. By contrast, less literature is available regarding the evaluation of *in vitro* performance criteria and their ability to predict *in vivo* results for formulations of the same relative composition, which might aid in the identification of critical *in vitro* parameters (29,30). Therefore, the current study set out to compare the *in vitro* and *in vivo* performance of a series of SNEDDS of the same relative composition containing high drug loads. Fenofibrate was used as a model compound as the drug precipitates in crystalline rather than in an amorphous form facilitating the evaluation of recently proposed *in vitro* performance parameters, such as solubilization and SR^M for their *in vivo* relevance.

The incorporation of a gastric step in current lipolysis models, which are commonly limited to an intestinal step, is desirable as gastric digestion can contribute up to approximately 20% of the total lipid digestion (31). However, availability and economic considerations have hampered the use of, e.g., recombinant human gastric lipase for *in vitro* lipolysis. In lieu of recombinant human gastric lipase, *C. antarctica* lipase A has recently been used successfully in combination with intestinal *in vitro* lipolysis to predict the *in vivo* performance of SNEDDS in beagle dogs (20). In agreement with the previous study under fasted conditions, no consumption of sodium hydroxide was observed in the current study during 30 min of gastric *in vitro* lipolysis. It should be noted that the titration method is an indirect way to monitor hydrolysis of triglycerides that depends on the ionization of fatty acids. At pH 2, the fatty acids are protonated (pK_a approximately 4–5), thus they are not available for titration. The lack of sodium hydroxide consumption during the gastric step, therefore, does not imply the absence of hydrolysis but rather demonstrates that the degree of gastric lipolysis is not directly accessible using the currently established titration method.

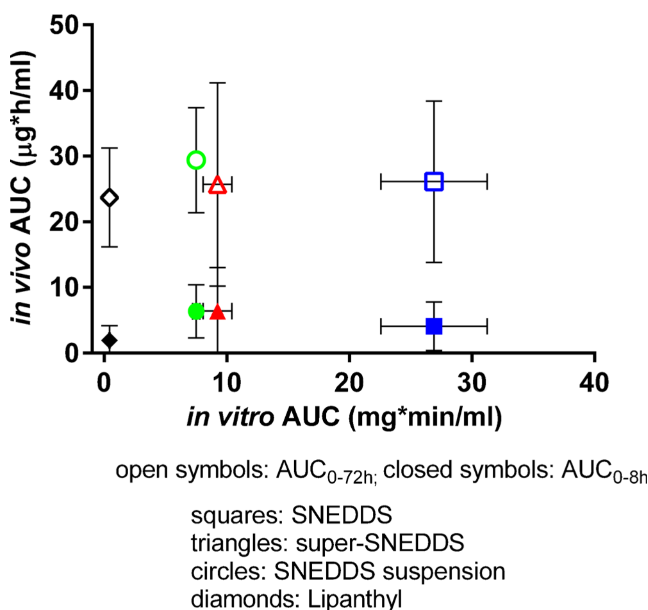

Fig. 4. Relationship between the areas under the plasma concentration-time curves ($AUC_{in\ vivo}$) and the area under the solubilization-time curves ($AUC_{in\ vitro}$) during intestinal lipolysis. *Open symbols* represent the $AUC_{0-8\ h}$, *closed symbols* $AUC_{0-72\ h}$. Formulations include SNEDDS (*squares*), super-SNEDDS (*triangles*), SNEDDS suspension (*circles*), and Lipanthy (*diamonds*)

The solubilization of fenofibrate remained largely unchanged during the gastric step for each formulation suggesting that any occurring lipolysis did not significantly affect the solubilization of the drug. Fenofibrate is a neutral drug and its solubility in biorelevant media has been shown to depend on the amount and the type of surfactants used (25). Since the concentration of bile salts in the gastric medium was low, the high solubilization of fenofibrate in the dispersed lipid formulations can mainly be attributed to the excipients present in the formulations. Consistent with this the solubilization of powdered fenofibrate increased fivefold in the intestinal medium compared to the low levels in the gastric medium in the absence of formulation- derived excipients, but increased dramatically in the presence of SNEDDS (60- to 90-fold). Starting from an initially high solubilization capacity in the gastric medium the solubilization capacity of fenofibrate decreased in the intestinal medium in spite of the increased bile salt levels present in the intestinal medium. This can be attributed to the larger media volumes in the intestinal step and the concomitant dilution of the SNEDDS excipients as the solubilization of fenofibrate is primarily based on formulation excipients and not on bile salts. As an example, the 1.5-fold dilution of the digestion media following the switch from the gastric (200 mL) to the intestinal step (300 mL) corresponded well with the reduction in fenofibrate solubility by the same factor.

During intestinal *in vitro* lipolysis at pH 6.5 the continuous digestion of the three lipid formulations could be monitored by the consumption of sodium hydroxide (data not shown). In line with an earlier study, the drug load inversely correlated with the sodium hydroxide consumption, reflecting the displacement of digestible lipid components by

the incorporated drug (32). For each lipid formulation, the proceeding digestion was reflected in a continuously decreasing fenofibrate solubilization. At the same time the SR^M changed only moderately, again due to the dilution of the medium. In contrast, for Lipanthyl the change from gastric to intestinal medium, in the absence of SNEDDS excipients, gave rise to improved fenofibrate solubilization mediated by the elevated bile salt concentration which significantly reduced the SR^M.

Based on the assumption that only drug in solution is available for absorption, the considerably enhanced fenofibrate solubilization generated by 75% drug load SNEDDS (Fig. 1) compared with the other formulations during the intestinal *in vitro* lipolysis step suggested that SNEDDS would also be superior in the *in vivo* performance, whereas the super-SNEDDS and SNEDDS suspension could be expected to yield similar plasma concentrations, and Lipanthyl would show the poorest *in vivo* performance. In agreement with previous studies drug precipitation was evident in cases where the SR^M exceeded values of around 3 (super-SNEDDS and SNEDDS suspension), indicating a high "precipitation pressure" of the systems (16,17). Conversely, the low SR^M of approximately 1.5 generated by 75% drug load SNEDDS was reflected in larger amounts of solubilized fenofibrate. Thus, based on the SR^M values the same *in vitro* rank-order of the formulations (SNEDDS> super-SNEDDS/SNEDDS suspension>Lipanthyl) could be established as obtained from the drug solubilization curves. The evaluation of the formulations in the minipig model, however, poorly reflected the *in vitro* solubilization data. The administration of the various lipid formulations resulted in very similar *in vivo* performance (C_{max}, t_{max}, $AUC_{0–72\ h}$, and relative bioavailability), whether the drug was initially present in solution (either below or above saturation solubility), suspended in the formulation, or whether the drug was maintained in solution or precipitated during *in vitro* lipolysis. Importantly, the enhanced solubilization of fenofibrate and the lower SR^M calculated for SNEDDS was not reflected in enhanced *in vivo* performance, contrasting the *in vitro* results. Compared with 75% drug load SNEDDS there was a trend to faster absorption and increased plasma concentrations during the initial 8 h following administration of super-SNEDDS and SNEDDS suspension which was not expected according to the *in vitro* solubilization data of these formulations. Some agreement between the *in vitro* and *in vivo* performance was observed for Lipanthyl, at least when comparing the initial $AUC_{0\text{-}8h}$ and the slow and incomplete solubilization during *in vitro* lipolysis.

Compared with Lipanthyl, all SNEDDS showed an increased rate of drug absorption, as indicated by the higher C_{max}, and partial AUC ($AUC_{0–8\ h}$). However, the initial benefits of faster onset and higher $AUC_{0\text{-}8h}$ were not sufficient to result in an improved relative bioavailability of fenofibrate after 72 h from SNEDDS compared to Lipanthyl. The faster initial drug absorption in the present investigation is in agreement with an earlier study by Hanafy *et al.* where a nanosuspension and solid lipid nanoparticle formulation of fenofibrate showed enhanced $AUC_{0–8\ h}$ and C_{max} in a rat model compared to micronized drug while the performance of the two colloidal formulations was comparable (26). The authors concluded that particle size reduction from the micro- to the nanometer range were likely to explain the observed differences between the formulations. In the current study, fenofibrate was, at least initially, molecularly dissolved in the 75% drug load SNEDDS and super-SNEDDS formulation. The particle size of drug precipitating during *in vitro* digestion is not known, neither is it accessible to which extend the drug precipitates *in vivo*. It might be possible that *in vivo* fenofibrate precipitates in particles of similar size from all the investigated SNEDDS leading to similar drug absorption during the transit in the large gastrointestinal tract of the pig (33). The anatomic peculiarities of the pig, e.g., the reported long gastric emptying time (34), might also facilitate complete absorption of fenofibrate from the slowly dissolving Lipanthyl formulation resulting in no apparent differences in the overall extent of bioavailability between all investigated formulations. Similar to the current study a discrepancy between *in vitro* and *in vivo* performance has recently also been reported by Thi *et al.* (35). The authors found marked differences during *in vitro* dissolution studies of fenofibrate-loaded SNEDDS of various lipid and surfactant composition in biorelevant media, whereas identical *in vivo* performances across the investigated SNEDDS were observed in a rat model. In contrast to the current findings, however, the *in vivo* performance of the SNEDDS was significantly enhanced compared to commercial Lipanthyl (i.e., the same micronized drug as used in the current study). The large variations between individual animals observed in the present *in vivo* study precluded the detection of significant differences between the treatments and might reflect pharmaceutical (e.g., the degree and extent of precipitation in the intestine) and physiological differences (e.g., gastric emptying, metabolism, and enterohepatic circulation) between animals (33,34,36,37). The gastric emptying might explain, e.g., the variation in t_{max} within each treatment (Table IV) but requires further investigation with regard to the comparably late occurrence of t_{max} in the minipig model, which to the authors best knowledge, has not been reported for other species (35,38–40). It might be possible that the evaluation of the *in vivo* performance by the quantification of the fenofibrate metabolite fenofibric acid is affected by the metabolic conversion of the parent compound to fenofibric acid and the subsequent enterohepatic circulation of the metabolite, which has been shown to be species dependent (36). Nevertheless, the discrepancies between the *in vitro* and *in vivo* results in current and aforementioned studies suggest that the administration of fenofibrate as SNEDDS is more relevant for the *in vivo* performance than the initial physical state of the drug (dissolved or suspended) and the solubilization profile obtained from *in vitro* lipolysis. This contrasts previous studies correlating the amount of solubilized drug in the aqueous phase after *in vitro* lipolysis with the *in vivo* performance (11,41,42). These studies used danazol as a model drug, and further differed from the current study in terms of the employed formulations, digestion protocols, and animal models. With regard to the digestion protocols, currently two methods are being followed: either the continuous addition of calcium ions over the course of *in vitro* lipolysis to precipitate liberated fatty acids as calcium soaps; or the bolus addition of a relatively large amount of calcium ions (typically, 5 mM) at the beginning of the digestion experiment (24,43). In the past, both approaches were not

able to consistently correlate *in vitro* solubilisation data with *in vivo* performance. It is, therefore unlikely that the calcium addition in the digestion protocols could account for the limited correlation. In fact, a very recent study by Griffin *et al.* in which fenofibrate-containing LbDDS of various complexity were investigated the bolus addition of calcium was employed for the *in vitro* lipolysis protocol (44). Similar to the findings in the current study the group was not able to correlate solubilization data from *in vitro* lipolysis with *in vivo* data obtained in Landrace pigs. Given the pronounced differences in the physicochemical and biopharmaceutical properties of currently available drugs caution should be exercised when extrapolating the findings of the current study using fenofibrate A growing number of evidence, however, suggests that formulation optimization towards increased drug solubilization during *in vitro* lipolysis might be misleading for the successful development of SNEDDS (12–14,29,30). It would be desirable to extend the currently employed *in vitro* digestion models by an absorption step which could provide valuable information beyond drug solubilization. One possible way might be the utilization of recently described cell co-cultures capable to mimic the absorption barrier for drugs while being potentially more tolerant towards the presence of formulation-derived components and simulated intestinal media (45,46). Physicochemical alternatives to cell culture models include phospholipid vesicle-based barriers (47) and ultrafiltration (48), which could also help to improve the prediction of the *in vivo* performance from *in vitro* digestion data and, ultimately, lead to a more rational development of SNEDDS.

CONCLUSIONS

The evaluation of three SNEDDS with identical lipid composition and a solid dosage form during combined gastric and intestinal *in vitro* lipolysis revealed differences with regard to the amount of solubilized drug in the aqueous phase. The likelihood of drug precipitation was correctly predicted by the maximum SR^M. However, based on drug solubilization profiles and SR^M as *in vitro* performance criteria it was not possible to predict formulation performance in a minipig model suggesting to exercise caution when interpreting *in vitro* data obtained from *in vitro* lipolysis.

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