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Research Article

Design of Lipid-Based Formulations for Oral Administration of Poorly Water-Soluble Drug Fenofibrate: Effects of Digestion

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Abstract. Lipid-based drug carriers are likely to have influence on bioavailability through enhanced solubilization of the drug in the gastrointestinal tract. The study was designed to investigate the lipid formulation digestibility in the simulated gastro intestinal media. Fenofibrate was formulated in representative Type II, IIIA, IIIB and IV self-emulsifying/microemulsifying lipid delivery systems (SEDDS and SMEDDS designed for oral administration) using various medium-chain glyceride components, non-ionic surfactants and cosolvents as excipients. Soybean oil was used only as an example of long-chain triglycerides to compare the effects of formulation with their counterparts. The formulations were subjected to *in vitro* digestion specifically to predict the fate of the drug in the gastro intestinal tract after exposure of the formulation to pancreatic enzymes and bile. *In vitro* digestion experiments were carried out using a pH-stat maintained at pH 7.5 for 30 min using intestinal fluids simulating the fed and fasted states. The digestion rate was faster and almost completed in Type II and IIIA systems. Most of the surfactants used in the studies are digestible. However, the high concentration of surfactant and/or cosolvent used in Type IIIB or IV systems lowered the rate of digestion. The digestion of medium-chain triglycerides was faster than long-chain triglycerides, but kept comparatively less drug in the post digestion products. Medium-chain mixed glycerides are good solvents for fenofibrate as rapidly digested but to improve fenofibrate concentration in post digestion products the use of long-chain mixed glycerides are suggested for further investigations.

KEY WORDS: fenofibrate; *in vitro* lipolysis; lipid formulation classification system; self-emulsifying/microemulsifying drug delivery systems (SEDDS, SMEDDS).

INTRODUCTION

As a new and evolving discipline, lipid-based drug delivery has attracted considerable attention as well as played an emergent role from academia to industry (1,2). Lipid-based drug carriers are likely to have influence on bioavailability through enhanced solubilization of the drug in the gastrointestinal (GI) tract and to reduce the variability of systemic exposure (3–6). Among the lipid-based formulations, self-emulsifying/microemulsifying drug delivery systems (SEDDS/SMEDDS) have been characterised more systematically from a physiological point of view (7). For these formulations, digestion can release drug from the lipid phase into a solubilised phase formed from the components of bile and lipolytic products. A well-designed lipid formulation presents the drug as a molecular dispersion in which the drug may be transferred to the mixed micellar system as the formulation is diluted into aqueous phase. The surfactant components would be expected to interact with mixed bile salt micelles with the result of changing their structure and solubilization capacity (8,9). Drug absorption from emulsions of this nature is thought likely to be faster and more consistent compared to a situation where drug is concentrated within large oil droplets.

Understanding the digestion and absorption process of lipids is of great importance for interpretation of the biopharmaceutical properties of lipid-based formulations of lipophilic drugs proposed for oral administration (10). Lipid-based formulations, thus self-emulsifying drug delivery systems have been proposed for the advantage of hydrophobic drug absorption by several mechanisms (for example, gastric emptying, emulsification, digestion), most of which relate to the ability of pancreatic lipase to digest lipid in the formulation (11). Digestion is suggested to gradually release drug, previously partitioned in the lipid phase, into the GI fluid together with digestion products. These digestion products in combination with physiological amphipathic molecules (bile components) and any surfactant present, could form a solubilising phase in which drug may remain in a dissolved state until absorption. Additionally, digestion could improve dispersion of the lipid-based formulation, thus offering a higher surface area available for drug release. The use of lipid formulations from the lipid formulation classification systems (LFCS), for developing oral dosage forms for this broad spectrum lipid-modulating agent, and a poorly water-soluble model compound, fenofibrate were investigated. The LFCS was classified by Pouton based on the polarity of the excipients blends, which was introduced as a working model in 2000 (5) and further modified in 2006 (12).

Briefly, LFCS can be differentiated by the way in which they disperse in water and according to their digestibility (12). Simply, Type I formulations are 100 % oil solutions. They generally exhibit poor aqueous dispersions but have the desirable

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properties of being rapidly digested, and absorbed completely from the intestine. Type II formulations (referred as SEDDS) consist of oils and water insoluble surfactants, form fine O/W emulsions when introduced into aqueous media. They are likely to be digested. Type III formulations are well dispersible SEDDS/SMEDDS, sometimes including cosolvents such as propylene glycol (PG). Digestion is not needed for these formulations. They are subdivided into IIIA and IIIB systems. Type IIIA consists of oils and water insoluble surfactants where as IIIB consists of oils and water-soluble surfactants. Type IV formulations are lipid free and produce micelles on dispersion but having limited or no digestibility.

In the initial phase of the present study, we have investigated the digestibility of a range of excipients. For the interest of the study, the model formulations were split into two components, the oily and the surfactant components. The assumption was made from the investigations of the previous studies (13) that fenofibrate would prefer the oily component. If fenofibrate is dissolved into an oily formulation which is polar or may be even polar with PG (Type IIIA systems) then that would not precipitate on dispersion. Subsequently, the aim was to examine the impact of different lipid-based self-emulsifying formulations on the solubilization process of poorly water-soluble drug in a dynamic *in vitro* lipolysis model.

To achieve this goal, the representative Types of lipid formulations (digestible oil, SEDDS/SMEDDS) comprising various combinations of oils together with surfactants and cosolvents were assessed for their behaviour on lipid digestion in an attempt to better predict the rate of hydrolysis and also the patterns of solubilisation. However, inclusion of surfactants within a lipid-based formulation could be disadvantageous due to the ability of many non-ionic surfactants to inhibit digestion of lipid. But the inhibition of lipolysis due to those surfactants can be significantly reduced by certain lipophilic surfactants and also with the combination of other oily excipients (mixed glycerides). In this paper, formulations containing medium-chain glycerides have been focused mainly based on several reports in the literature that indicate absorption enhancement of different compounds by this lipid (14); however, soybean oil was also used as an example of long-chain triglycerides to compare the effect of digestion. Thus medium-chain (C_6–C_{12}) mono-, di- and triglycerides, particularly C_8/C_{10} mono- and diglycerides have been used in mixed micelles to enhance the absorption of fenofibrate. These formulations will be expected to be digested during their passage through the GI tract. In the present work, a kinetic model has been projected for the enzymatic hydrolysis of lipid-based formulations. This model was applied to the initial reaction rates of the enzymatic process and used to explain the kinetic behaviour of the action of lipases with representative Types of lipid formulations.

MATERIALS AND METHODS

Materials

Fenofibrate (2-[4-(4-chlorobezoyl) phenoxy]-2-methylpropionic acid 1-methylethyl ester) was supplied by Sigma Aldrich Co, St. Louis, MO, USA. Miglyol 812, Imwitor 988, Imwitor 308 and propylene glycol (PG, 98 % pure) were supplied by Sasol Germany GmbH, Werk Witten, Germany. The non-ionic surfactants used were Tween 80 (HLB-15) and Tween 85 (HLB-11), supplied by Sigma Aldrich Pty. Ltd., Castle Hill, NSW, Australia. All excipients were used without further purification. Soybean oil (C_{18} triglycerides), Cremophors (CrEL and CrRH40, polyoxyl castor oil), sodium taurodeoxycholate (NaTDC, 99 % pure) and porcine pancreatin (8_USP specifications activity) were from Sigma Chemical Co. St Louis, MO, USA. Lecithin (60 % pure phosphatidylcholine by HPTLC (15) from egg yolk) was a gift from Pharmacia LKB, Uppsala, Sweden, 4-bromophenylboronic acid (4-BPB) was obtained from Aldrich Chemicals Co. St Louis, MO, USA and 1 M sodium hydroxide (Titrisol), which was diluted to obtain 0.6 and 0.2 M NaOH titration solution, was purchased from Merck, Darmstadt, Germany. Water used in this study was obtained from a Milli-Q water purification system, Sartorius, Geottingen, Germany. All other chemicals and solvents were of analytical purity or high performance liquid chromatography (HPLC) grade, respectively.

Dynamic *In vitro* Lipolysis Test for Lipid Digestion

In recent years, *in vitro* lipolysis model for lipid digestion have been increasingly used as tools to assist in the design of self-emulsifying lipid-based formulations to enhance the oral bioavailability of poorly water-soluble drugs (16–18). During *in vitro* lipolysis studies, the data generated from the pH-stat can be used to quantify the rate and extent of lipolysis, and more importantly, the products of lipolysis can be examined after completion of the reaction, to determine the fate of the drug; whether it is solubilized or precipitated (12).

The procedure for the dynamic *in vitro* lipolysis experiment in this study was based on using the similar methods to those described previously (19–21), with some modifications. Briefly, for each digestion experiment, 250 mg of lipid was dispersed to 9 mL of a phospholipid/bile salt mixed micellar solution in digestion buffer (50 mM Tris maleate, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 7.5) under fed and fasted conditions. Fed and fasted intestinal contents were simulated utilising 20 and 5 mM bile salt, respectively. Phospholipids were included in the digestion mixture at a bile salt/phospholipid molar ratio of 4:1, which is the ratio secreted in bile (22). The lipid formulations were emulsified in the mixed micellar solutions prior to enzyme addition by stirring continuously for 10 min in the thermostatic jacketed glass reaction vessel. In some cases dispersion took some considerable time (>10 min). Experiments were performed at 37 °C and 1 mm of Pancreatin extract containing 1,000 tributyrin units of pancreatic lipase was then added to initiate lipolysis. Lipolysis was allowed to continue for 30 min using a pH-stat titration unit (Radiometer, Copenhagen, Denmark), which maintained the pH at 7.5. The fatty acids produced on lipolysis were titrated with 0.6 M NaOH for Miglyol812 and 0.2 M for all the other excipients, surfactants and formulations. The progress of *in vitro* lipid digestion was monitored indirectly by pH-stat and directly by HPLC analysis.

Estimation of Digestible Product in the Formulation

The moles of NaOH used can be equated with fatty acid (FA) liberated to determine the percentage of triglycerides (TG) digested throughout the course of the reaction. The moles of available fatty acid were calculated in the mass according to molar mass of the lipids in the composition and based on the assumption that 1 mol of TG equates to 3 mol of

FA equivalents, diglycerides (DG) to 2 mol of FA equivalents and monoglycerides (MG), and FA to 1 mole of FA equivalents. The total lipid mass was conserved throughout the experiment; thus the total FA was equivalent to the FA that produced after completion of the digestion. Since additional FA was produced by the digestion of lecithin, the FA produced during blank digestion experiments were subtracted from the total available FA produced during experimental lipid digests, to give true mass balance data.

To estimate the fraction of total available of FA (F_{TAF}) for Miglyol812 (M812) it was assumed that the FA content was as detailed in the manufacturers literature. For soybean oil it was assumed that the FA content was oleate. For Imwitor988 (I988, containing 50 % MG and 50 % DG) the value was estimated according to the percent of glycerides present (Table I). The number of available moles of FA per mole of surfactant has been estimated assuming that Tween85 (T85) may liberate three FA per molecule (T85 is the oleate triester of sorbitan), and Tween80 (T80) was assumed to be a pure mono-oleate of polyethoxylated sorbitan.

Preparation of Fenofibrate Formulations for Digestion Experiments

Formulations comprising either surfactant alone or combinations of lipids, surfactants and cosolvents, which represent the Types of lipid formulations were prepared in order to investigate their *in vitro* digestion properties. In all cases, formulations were made up containing fenofibrate at 80 % of its experimented equilibrium solubility in the composition. The compositions of the lipid formulations and the solubility of fenofibrate have been shown in Table II.

Briefly, for the lipolysis experiments, the formulations were exactly weighted into a 20-mL beaker and then fenofibrate was added to the formulations. The components were stirred using a magnetic stirrer until drug completely dissolved and to ensure homogeneity. Formulations were transferred into screw-capped glass vials and placed in an incubator at 37 °C to equilibrate at least overnight prior to evaluation.

In vitro lipolysis experiments of drug-loaded formulations were performed exactly as lipid digestion experiments stated before. The lipolysis reaction was monitored by continuous titration of fatty acids liberated during the reaction. To verify the solubility of the fenofibrate during lipolysis experiments, 100 μL samples were collected at times 0 (before enzyme addition), 1, 5, 10 and 30 min and dissolved in methanol for HPLC analysis. Table III shows the compositions of the biorelevant media similar to intestinal fluid with enzyme used in the *in vitro* digestion experiments, which were necessary to include in the profiling.

Evaluation of the Initial Digestion Rate

One of the most useful parameters for comparing digestion of different formulations is to calculate initial rate of hydrolysis, i.e. the rate of FA liberated per unit time at the few starting points of the reaction. For the calculation of the initial reaction rate the slopes of the hydrolysis curve at the beginning of the reaction were calculated by linear regression analysis. A number of experimental points (Fig. 1) were incorporated, the slope determined by the least-squares linear regression method until the value of the slope of these straight-line began to decrease (23). The most stable estimate of the slope was typically observed 0–3 min after the lipase solution had been added to the reaction vessel. Sometimes a short lag phase was seen (up to 1 min), and after this the hydrolysis rate was constant.

Analysis of Post Lipolysis Products

At the end of each digestion experiment, two samples of 4.2 mL of digestion mixtures were transferred into polyallomer centrifuge tubes and 40 μL of 4-bromophenylboronic acid was added to each sample to prevent further lipolysis. Samples were then ultracentrifuged (model Optima XL-100K; Beckman, Palo Alta, CA, USA) at 334,000×*g* for 30 min at 37 °C utilising a SW-60 swinging bucket rotor to separate the different digestion phases. After ultracentrifugation, the formulation digests were separated into an aqueous phase and a precipitated pellet phase. For soybean oil, there was an oily layer appeared on top of the aqueous phase. In this study, the medium-chain lipids were mostly used except only soybean oil, which was explored as an example of the long-chain lipid to compare their performance under the same experimental conditions. Sample obtained from each of the separated phases was analysed for drug content by HPLC as described previously (24).

In addition, to compensate for the additional FA produced by the digestion processes (i.e. other than by digestion of added lipid such as lecithin, lyso-PC, etc.), a series of blank or control experiments were performed. Blank digestion experiments were performed in the same manner as the experimental run, except that lipid and drugs were not added to

Table I. Available Fatty Acid Present in Lipids and Surfactants for Titration During Lipolysis Experiments and the Extent of Digestion is Expressed as Percent (%) After the Reaction is Completed

Excipients	Average MW	Average mol FA per mol excipient	mmol of each excipient in 250 mg	mmol FA equivalents in 250 mg excipient	% Digested after 30 min
Soybean oil	871	3	0.287	0.8611	38
Miglyol812	500	3	0.5	1.5	86
Imwitor988	287	N/A	0.8711	1.31	93
Imwitor308	218	1	1.1468	1.1468	96
Tween85	1839	3	0.1359	0.408	15
Tween80	1310	1	0.191	0.191	40
Cremophor EL	2560	3	0.0977	0.293	29
Cremophor RH40	2699	3	0.0926	0.2778	7.3

Table II. The Compositions of the Formulations and Fenofibrate Solubility in Anhydrous Formulations at Equilibrium and 80 % Saturation (Adapted from Ref. (13))

Type	Formulation (% *w/w*)	Mass of Fenofibrate (at 80 % equilibrium solubility (mg/g))	Equilibrium Solubility (mg/g)
I	Soybean oil	62	78.1±3.9
	M812	82	102.4±5.3
II	[M812:I988(7:3)]/T85(1/1)	80	99.9±0.8
IIIA	[M812:I988(7:3)]/T80(1/1)	80	100.7±0.0
IIIB	I308/T80(1/1)	68	84.4±2.6
IV	T80	66	82.5±2.7
	PG/T80(1/1)	24	29.8±2.5

Mass ratios of mixtures are shown in parentheses
M812 Miglyol 812, *I988* Imwitor 988, *I308* Imwitor 308, *T80* Tween 80, *PG* propylene glycol

the BS/PL mixed micellar solutions. Digestion of the lipids under this study was corrected for background fatty acid production by subtracting the data from the blank digestion runs. By following this protocol, the current method was found to be robust and measured values were reproducible.

Data Analysis

All experiments in the current studies were carried out a minimum three times on freshly prepared samples. Prism pad® software was used to analyse the data. The results were then reported as means and standard deviations of these measurements.

RESULTS

The progress of lipid digestion in the lipolysis experiments were generally monitored *in vitro* in this study. It quantified the rate and extent of digestion indirectly via titration of the fatty acid produced (25). The location of the drug in the fractions after ultracentrifugation of the products of digestion, allowed to investigate the likely fate of the drug after lipolysis (5). Hence, drug molecules were solubilised in the aqueous phase of the lipolysis medium was expected to be available for absorption, in contrast to drug in the sediment which might not be available for absorption in *in vivo* conditions (26)

Self-emulsifying lipid-based formulations of various compositions were assessed upon lipid digestion in order to understand the formulation parameter responsible for maintaining drug in solution during the trafficking of lipid-based drug delivery systems in the GI tract.

Table III. Composition of Mixture used in *In Vitro* Digestion Experiments

Substance of the mixture		
Lipid 250 mg		
Pancreatic lipase 1 mL (1,000 TBU/mL)		
Lipolysis buffer 9 mL		
Composition of the lipolysis buffer	Conc. (fed state)	Conc. (fasted state)
Bile salt (BS, mM)	20	5
Phospholipid (PL, mM)	5	1.25
Tris maleate (mM)	0.5	0.5
Ca^{+} (mM)	0.05	0.05
Na^{+} (mM)	1.5	1.5

Initial Lipolysis Rate of LFCS Formulations

The initial rates of digestion between the formulation Types were compared in Fig. 1. The initial (0–3 min) rate of reaction was rapid and accounted for over 50 % of the final extent of hydrolysis. Rate dropped off steeply between 5 and 10 min and thereafter became slow and approximately linear over the next 20 min (Fig. 1). In this work, Type II formulations, such as M812:I988 (7:3)/T85 (1/1), produced coarse emulsions usually had high glyceride content showed a fairly high initial digestion rate. Type IIIA and Type IIIB formulations were equally rapidly digested in the initial stages. This is perhaps not surprising for Type IIIA which contains a mass of oil equal to the Type II formulation.

The digestion rate of the Type IIIB formulation was explained in this case by the high 1-monoglyceride content. This is not necessarily typical of a Type IIIB formulation in its susceptibility to lipase. Often Type IIIB formulations contain

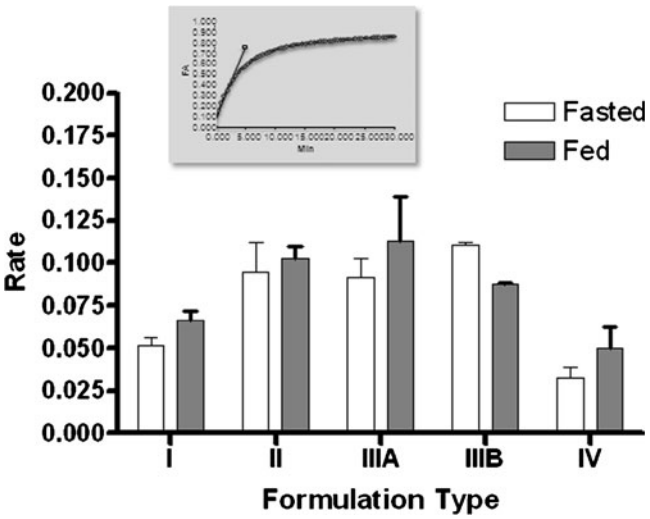

Fig. 1. Initial digestion rate of the representative Types of formulation under fasted and fed conditions. Rate of digestion was measured as rate of FA liberation (mmol FA/min, INSERT). Systems represent *Type I* M812 (MCT), *Type II* M812:I988 (7:3)/T85 (1/1), *Type IIIA* M812:I988 (7:3)/T80 (1/1), *Type IIIB* I308/T80 (1/1) and *Type IV* Pg/T80 (1/1), respectively

a lower proportion of glycerides. The Type IV systems were expectedly digested at a slower rate since T80 the only component which contains esters was hydrolysed. With the exception of the latter system, the rate and extent of digestion for both Type II and IIIA formulations were higher than that observed when the same mass (250 mg) of M812 was digested alone (Fig. 1). This is likely to be explained by the crude emulsion formed on dispersion of M812.

Initial Rate and Digestion of Various Surfactants

Within the scope of the present study, it was necessary to investigate the susceptibility of some commonly used non-ionic surfactants to digestion and particularly, to get the information of which non-ionic surfactant types are likely to inhibit pancreatin activity so their use in lipid-based formulations could be avoided.

A summary of the initial digestion rates for all surfactants at a fixed mass of surfactant per unit volume is shown in Fig. 2. The digestion rate is appeared to be higher in case of T85 than all other surfactants. The subtraction of control from Brij 30 yielded negative values across the whole profile. This observation strongly suggests that Brij 30 is not digestible whatsoever. The rate of digestion when assessed as a percentage was ranked as T85>T80>CrEL>CrRH40>Brij-30.

The digestion profiles in Fig. 3 illustrate the fraction of available FA liberated under fasted and fed conditions. The initial digestion rate (0–5 min) was rapid for the polysorbates (i.e. T85 and T80). These surfactants continued to be digested over the 30 min period at a slower rate after which they had liberated 20–30 % of the available FA content. The extent of digestion of CrRH40 both under fed and fasted conditions was limited between 7 % and 5 %. Despite its chemical similarity, CrEL behaved more like the polysorbates with about 2–30 % hydrolysis after 30 min (Fig. 3a and b). This is in agreement with previous studies that have shown CrEL and T80 to be substrates for pancreatic lipase and release esters of ricinoleate, oleic acid and polyethoxylated sorbitol, respectively (20).

Although the initial digestion rate was higher for T85, the amount of CrEL hydrolysed after 30 min was higher than T85 under fed conditions (Fig. 3b). It is interesting that the digestion

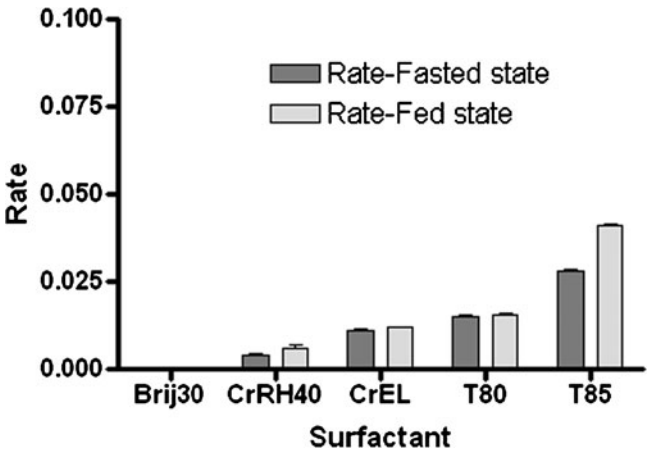

Fig. 2. The initial digestion rate of various non-ionic surfactants under fed and fasted conditions (in millimoles FA per minute). All surfactants were digested using a mass of 250 mg which, equated to the following concentrations: *Brij30* 0.693 mM, *CrRH40* 0.093 mM, *CrEL* 0.098 mM, *T80* 0.191 mM, *T85* 0.136 mM for digestion

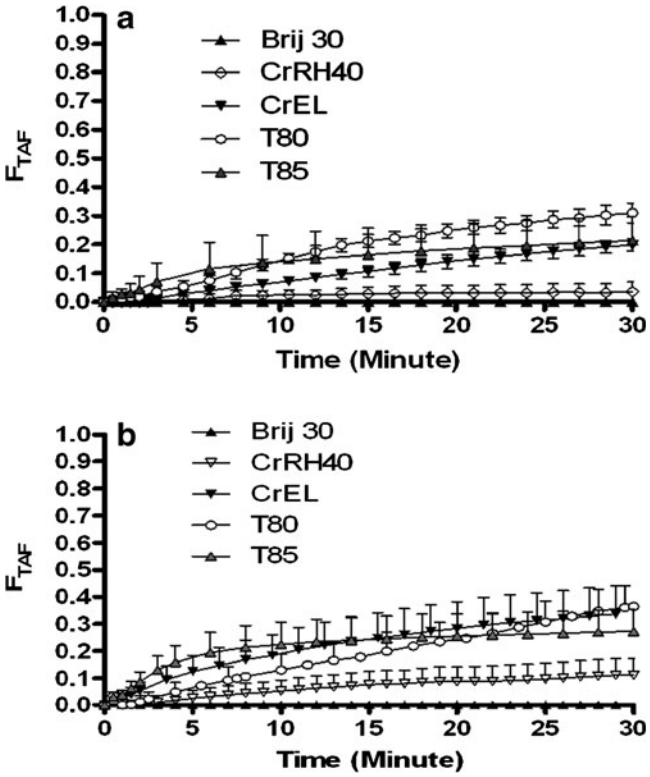

Fig. 3. Fraction of total available FA (F_{TAF}) released during 30 min digestion of various surfactants under **a** fasted and **b** fed conditions. Blank digestion experiments were subtracted to account for the FA release by the digestion of the PC present in the media. Data are mean ±SD, (n=3)

of CrRH40 and CrEL differed so markedly. The extent of digestion when assessed as a percentage was greatest for T80= CrEL=T85>Brij-30.

Effect of Glycerides on the Fatty Acid Liberation

The digestion profiles in Fig. 4 illustrate the fraction of hydrolysed F_{TAF} as a function of time for various glyceride excipients under fasted and fed conditions. I988 (mixer of mono- and diglycerides) and Imwitor 308 (I308, 80 % monoglycerides) hydrolysed quite readily. More than 80 % fatty acids released from I308 and I988 within 5 min of the lipolysis reaction and have gone completion over 95 % after 30 min reaction period. Both I988 and I308 dispersed well in bile salt micelles. The digestion profiles for M812 (MCT) and soybean oil (LCT) from the current study show that the medium-chain triglycerides (MCT) was hydrolysed much faster than the long-chain triglycerides (LCT), which has been described previously in the literature (27). Almost 90 % of M812 was digested after 30 min time both under fed and fasted conditions. Whether in case of soybean oil, only 25 % digestions were completed under fasted (Fig. 4a) and 38 % under fed conditions (Fig. 4b).

Digestion of Representative LFCS Formulations

The formulation of SEDDS/SMEDDS containing digestible lipids and surfactants often produce efficient emulsification and also improve the solvent capacity of the complete formulation (28).

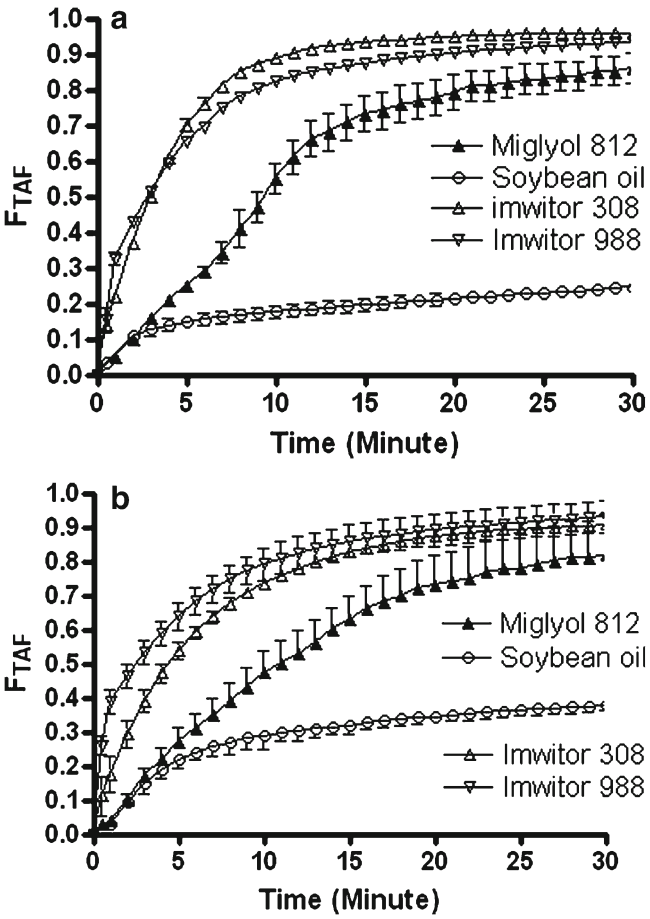

Fig. 4. F_{TAF} established by pH- stat titration techniques during 30 min digestion period for various glycerides under **a** fasted and **b** fed conditions. Data are mean±SD, (n=3, blank subtracted; fraction calculated as, Moles of NaOH titrated over time divided by the total amount of available fatty acid)

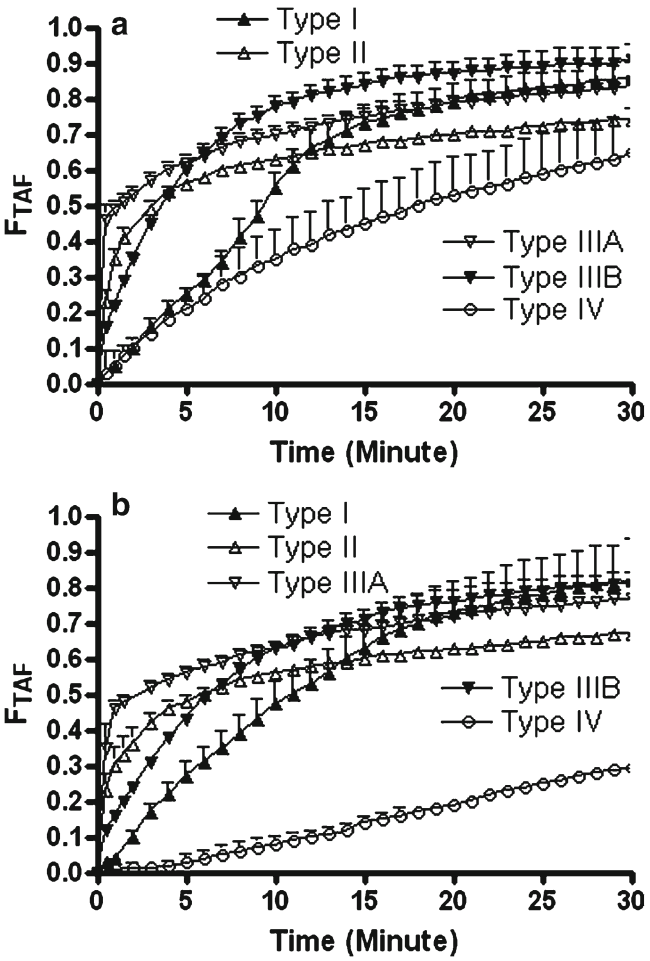

Fig. 5. F_{TAF} liberated after 30 min during *in vitro* digestion experiment for representative Types of formulations under **a** fasted and **b** fed conditions. Data are mean±SD (n=3, blank subtracted). Systems represent *Type I* Miglyol 812, *Type II* M812:I988 (7:3)/T85 (1/1), *Type IIIA* M812:I988 (7:3)/T80 (1/1), *Type IIIB* I308/T80 (1/1) and *Type IV* PG/T80 (1/1), respectively

Figure 5 displays the digestion profiles of the representative LFCS lipid formulations under fasted and fed conditions. Approximately, 70–90 % of the titratable fatty acids were released under fasted conditions from Type I, II and IIIA and IIIB formulations within 30 min lipolysis reaction time (Fig. 5a). In contrast, PG/T80 (1/1) formulation, which represents Type IV systems, was hydrolysed relatively slowly as evidenced by the fatty acid titration profiles (Fig. 5). However, the extent of digestion for Type II and IIIA formulations were improved compare to that seen when the same mass of M812 (Type I) was digested alone. As M812 is immiscible in aqueous solution, it forms crude emulsion on dispersion and consequently could have a variable digestion rate.

On the other hand, under fed conditions (Fig. 5b), Type IIIB systems [I308/T80 (1/1)] were digested to the same extent as the Type II–IIIA formulations except PG/T80 (1/1, % *w*/*w*) formulation of Type IV systems, which lowered the rate of digestion significantly.

Drug solubilization in the Post Digestion Products of LFCS Formulations

It was required in the present investigation to assess the solubilisation capacity of the representative Types of formulations for fenofibrate upon *in vitro* digestion. The drug can be located either in the core or near to the surface of the micelle, depending on the lipophilicity and the dissociation constant, which was characterised and evaluated by investigating the composition of the aqueous phase and the concentration of fenofibrate in the aqueous phase.

Fenofibrate is a prodrug (29). It is practically insoluble in water, with an aqueous solubility of 3 μg/L. The distribution and solubilisation pattern of fenofibrate across the different phases of the digestion medium resulting from the representative lipid formulations under fed and fasted conditions are summarized in Table IV.

The data demonstrate that Type I formulations presents comparably higher amount of drug in aqueous phase (AP) both under fed and fasted conditions (Table IV). Although, M812 was almost completely hydrolysed and produced a large aqueous phase, it was only able to carry 5–7 % drug in the aqueous phase. In contrast, during the soybean oil digestion, approximately 21 % and 36 % drug under fasted and fed conditions were solubilised in the aqueous phases which were obviously higher than any other formulation systems but largely the oil remained undigested after 30 min digestion

Table IV. Percent Fenofibrate in Aqueous Phase (AP) and Pellet Phase (PP) After 30 min Digestion Under Fed and Fasted Condition, (mean ± SD, n=3)

		Fasted			Fed		
Type	Formulation (% *w*/*w*)	% Drug in AP	% Drug in PP	% Total recovery	% Drug in AP	% Drug in PP	% Total recovery
I	M812	5.21±0.0	63.9±0.0	69.11	7.93±0.0	65.1±0.0	73.03
	Soybean oil	21.2±1.4	0.8±0.1	22.00[a]	36.3±1.7	0.9±0.1	37.20[a]
II	M812:I988(7:3)/T85(1/1)	5.2±0.5	68.3±4.9	73.50	7.1±0.2	66.2±6.8	73.30
IIIA	M812:I988(7:3)/T80(1/1)	3.5±1.2	66.3±4.7	69.80	4.9±0.3	62.9±12	67.80
IIIB	I308/T80(1/1)	2.63±0.8	61.6±5	64.23	4.4±1.2	61.7±9.3	66.10
IV	PG/T80(1/1)	5.9±0.1	72.3±1.1	78.20	13±1.4	63.7±4.3	76.70
	T80	6.5±0.1	77.1±2.3	83.60	5.9±0.2	79.9±1.7	85.80

[a] In case of soybean oil, majority of the drug remained in the undigested oil phase after completion of the reaction

period (Table IV). The addition of lipophilic surfactant T85 which combines well with medium-chain mono-, di- and triglycerides representing Type II systems produce coarse emulsion on dilution with BS/PL solutions. The digestion rate was increased in case of the mixed glycerides (M812/I988) with 50 %T85, which shows slightly higher amount of drug in aqueous phase (AP, Table IV). Type IIIA formulations which include hydrophilic surfactant T80 by replacing T85 produced fine emulsion on dilution with simulated BS/PL solution. The digestions of these formulations result less amount of drug in AP comparably with Type II formulations. This drug concentration further decreased in AP after digestion of Type IIIB formulations. Type IV formulations contain all water-soluble materials produced microemulsions on dispersion. The formulations of this system have low fenofibrate solubility which digests well but retain majority of the drug in the pellet phase after digestion. Formulation which is blended with cosolvent and surfactant always has better dispersion characteristics, as the cosolvent helps dispersal of the surfactant.

Table V represents the concentration of fenofibrate in the formulations before start and after 2 and 30 min completion of the lipolysis reaction. The result shows that M812, which represents formulation Type I lost 21 % drug after 30 min digestion period. In addition, M812:I988 (7:3)/T85 (1/1) and M812:I988 (7:3)/T80 (1/1) of Type II and IIIA formulations reduce their solubility by 15 % and 11 % respectively. Furthermore, almost 10–20 % fenofibrate also found missing after digestion in case of the formulation of I308/T80 (1/1) in varying proportions and T80 itself in Type IIIB and IV systems.

Effect of Bile Salt Concentration on Digestion

The key mechanism by which lipid-based formulations improve the bioavailability of hydrophobic drugs has already been suggested to involve solubilization of drug in bile salt and phospholipid micelles (30). If these micelles significantly increase the solubility of a poorly water-soluble drug, stimulation of the lipid digestion cascade, and hence release of bile at the time of dosing could lead to improved bioavailability

Figure 6 shows the solubility of fenofibrate in bile salt solutions. The excess drug was dissolved within the range of 0 to 25 mM bile salt. The solubilization of fenofibrate in the bile salt micellar solution was investigated. It can be seen from the result that the higher concentrations of bile salt increased the solubility of fenofibrate from 5 to 50 μg/mL. The solubility increased in presence of higher bile salt concentration indicates that fenofibrate would be benefited during digestion in the gut under fed condition. As would be expected, the total solubility of the drug is proportional to bile salt concentration (25). Based on the *in vitro* digestion studies using the biorelevant media, it is anticipated that the bile salt mixed micelles in the intestine under both fed and fasted conditions are not able to solubilise fenofibrate more than 10 % of the unit dose. It is known that, in the absence of or at low concentrations of bile salts, the absorption of fatty acids occurs to a relatively lower and slower extent (31).

Microscopic Analysis of the Pellet Formed After Digestion

Figure 7 shows the images taken from the pellet (sediment) phase after 30 min digestion in the presence and in the absence of fenofibrate with different formulations using the light microscope (Olympus BH2, Japan).

It was of interest to establish whether or not the drug was present in the pellet in a crystalline state. The formulations digested in the absence of drug had predominantly needle shaped hairy crystals in the pellet, which are assumed to be calcium salts of medium-chain fatty acids (Fig. 7a–d). The

Table V. Percent Fenofibrate in Mixed Bile Salt Micelles Before and After Starting Lipolysis Reaction Under Fasted Conditions

			% fenofibrate after reaction (with lipase)	
Type	Formulation	% fenofibrate before reaction (without lipase)	2 min	30 min
I	M812	100	92	79
II	M812:I988(7:3)/T85(1/1)	100	94	85
IIIA	M812:I988(7:3)/T80(1/1)	95	88	84
IIIB	I308/T80(1/1)	96	85	75
IV	T80	100	96	91

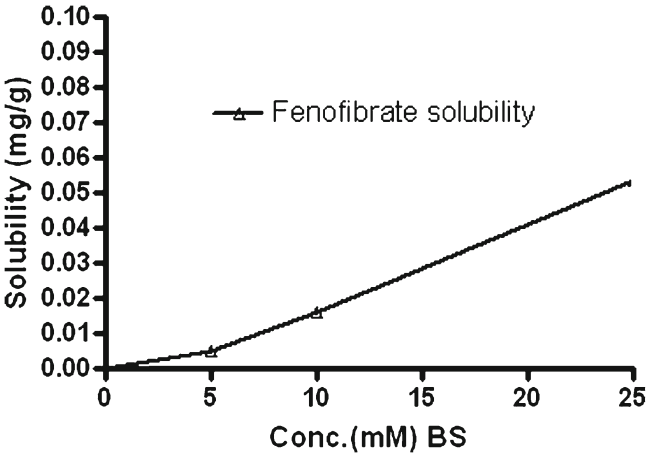

Fig. 6. Fenofibrate solubility in bile salt/phospholipid micelles solutions (the ratio of BS: PL=4:1). The values are expressed as mean±SD (n=4)

fenofibrate formulations typically had several rod and large crystals whose 2D appearance was parallelogram (Fig. 7e–h) in nature with larger width than the needles observed in the absence of drug. This is strong evidence that the fenofibrate was in a crystalline form after digestion of the formulation. Differential scanning calorimetry of pellets was used to investigate whether a clear melting endotherm for fenofibrate (at 80.4 °C for pure fenofibrate) could be detected in the pellet. Indeed, a peak for fenofibrate was observed in the pellet phase but it was not possible to use this as a quantitative assay for fenofibrate.

Overall Comparison: the Fate of Drug After Digestion

Although the equilibrium solubility and dispersion characteristics of fenofibrate in the previous studies were determined to a large extent using water as the aqueous media (13), the *in vitro* lipolysis experiments under fed and fasted conditions were supported in making *in vivo* predictions. Hence, the equilibrium solubility of fenofibrate in mixtures which represent diluted formulations was correlated with the fate of drug in real-time after dispersion and *in vitro* digestion of each formulation (Fig. 8).

The equilibrium solubility and the dynamic dispersion experiments were conducted by dissolving fenofibrate in which the formulations were diluted 1 in 100 with water. The formulations which effectively maintained drug in a solubilised state during the dispersion experiment (Type I, II and IIIA formulations) were closely assessed in the *in vitro* digestion experiments. These studies clearly illustrate that even though, Type I, II and IIIA formulations are able to support more than 90 % drug in solution during dispersion (13) but failed to prevent drug precipitation during digestion. Only 5–8 % drug of the dose remained solubilised after 30 min of digestion as evidenced by the lipolysis data in Fig. 8.

On dispersion and digestion of the representative Types of formulations, significant drug precipitation was evident from Type IIIB and IV systems and only less than 20 % of the dose remained solubilised after 30 min As a result, the drug absorption thus the oral bioavailability is predicted to be limited *in vivo* from most of the representative lipid formulation systems. This prediction was made on the basis of formulating drug with medium-chain glycerides in the studies.

DISCUSSION

The utilisation of a simple and robust method has been demonstrated in this work, which characterises lipid formulations with respect to susceptibility to enzymatic degradation by pancreatic lipase. The result from the rate of digestion shows that the initial rate of lipolysis is influenced by the inclusion of lipid in composition. Digestion profiles of all the excipients and the formulations (except soybean oil) demonstrate that during lipolysis, fatty acids were liberated rapidly, and the maximal rate of digestion was observed in the first 5–10 min and no residual oil phase was apparent after ultracentrifugation of the digestion media.

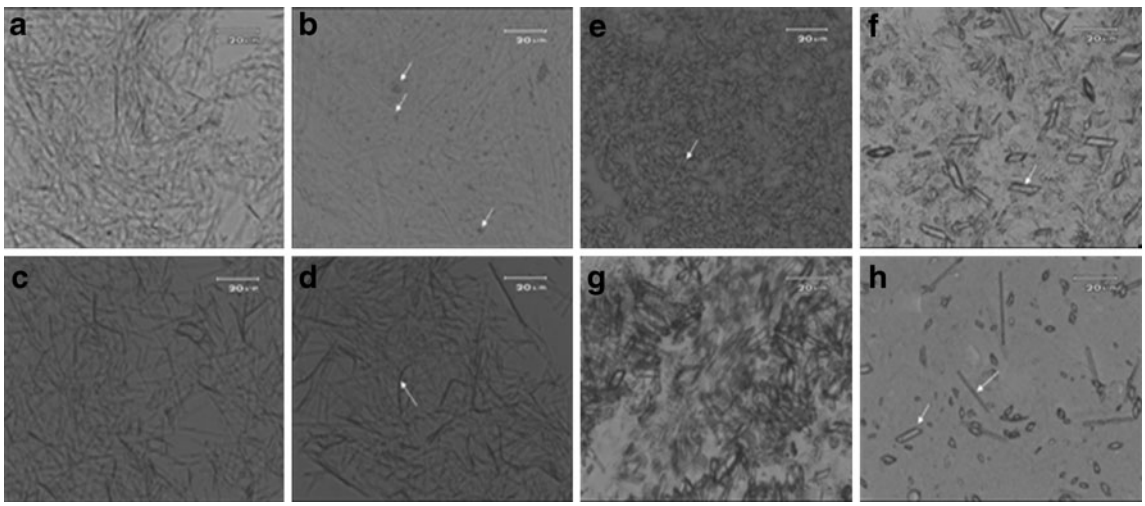

Fig. 7. Micrographs showing the images of pellet phase (PP) after 30 min digestion of drug free formulation of **a** and **b** M812, **c** and **d** M812:I988 (7:3)/T80 (1/1) under fasted and fed conditions. **e** and **f** represent I308/T80 (1/1) with drug under fasted and fed conditions, and **g** and **h** PG/T80 (1/1) with drug under fed & fasted conditions. *Scale bar* represents 20μm. The *arrow* shows the needle and rod shape crystals present in the PP

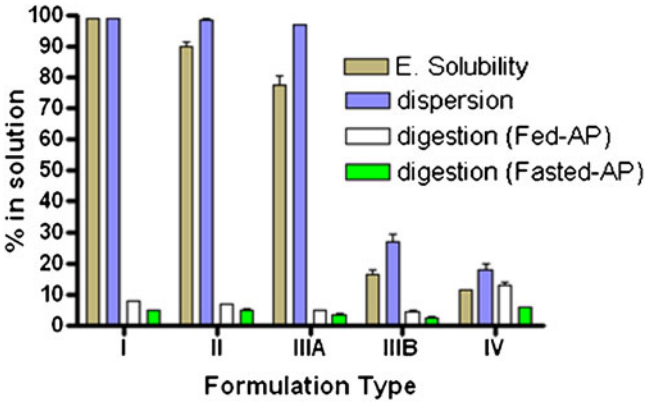

Fig. 8. Overall comparison: % fenofibrate remained in solution after 30 min dispersion and digestion in aqueous phase under fed and fasted conditions. Equilibrium solubility was performed using 99 % diluted formulations with water. Systems represent *Type I* Miglyol812 (MCT), *Type II* M812:I988 (7:3)/T85 (1/1), *Type IIIA* M812:I988 (7:3)/T80 (1/1), *Type IIIB* I308/T80 (1/1) and *Type IV* Pg/T80 (1/1), respectively

The interaction between the co-administered lipid and drug as the formulation is exposed to, and digested and dispersed by the GI environment, will depend on the Type of lipid formulation as exemplified by the lipid classification systems and the physiological properties of the particular drug (12). The ability of most of the formulations to maintain drug in solution was markedly affected by lipid digestion.

More usually Type II and IIIA formulations which include vegetable oil triglycerides, with the addition of mono-diglyceride and surfactants will be expected to be digested during their passage through the GI tract. But as these formulations contain a lot of MG and or may be T85 materials, it is very likely to have a liquid or a material which is liquid but sedimented down. This sediment will go into the bottom of all other phases and form pellet phase. So the drugs are likely to be in solubilised form in those sedimented liquid materials. It is still unknown to define what would be the product of sedimented phase.

For example I308 (>80 % MG) produces MG rich phase which is actually more dense than water and a good solvent to dissolve or solubilise the drug. Phase separation is occurred depending on the ratio of mono-, di- and triglycerides present in the formulation. Formulations containing mixed glycerides (medium-chain mono-, di- and triglycerides) in Type II and IIIA systems resulted in a lesser drug concentration in the aqueous phase in compare to the formulation of MCT on its own. A similar trend or even poorest result was obtained for Type IV formulations. The overall indication therefore, from these dynamic *in vitro* lipolysis experiments was that a large amount of drug retained in the pellet phase and the average of 70 % drug recovered as a whole from the representative Types of formulations. There was only a trivial differences of the drug concentration that shown in the aqueous phases of all the formulations. Furthermore, it was clear that no significant differences were observed between the distributions of the fenofibrate concentration following the *in vitro* digestion of the formulations.

Thus, the mechanism by which the drug is maintained in solution will depend on the solubilization of drug by mixed bile salt micelles and the products of lipolysis. The investigation with medium-chain digestion product of all different Types of formulations after completion of the digestion had already demonstrated the poor fenofibrate solubility and in the long-chain products (in case of soybean oil) it showed comparably higher solubility. This significantly suggests that fenofibrate would benefit most from formulation within a long-chain triglyceride system and strongly recommend the inclusion of long-chain lipids in the formulation blends in future.

The study also describes the assessment of a range of non-ionic surfactants for their ability to be digested itself as well as within the formulations by pancreatic lipase. This approach after digestion studies provided necessary information to aid development and selection of promising formulations by identifying surfactants capable of digestion. Results from these investigations suggest that the nonoxynol type or the alkyl ethoxylated surfactants such as T80 and T85 are digested but the ether type surfactants (for example, Brij 30) are not going to be digested. Thus Brij 30 was indicated as not to be the preferred choice of surfactants for use in the final formulations. In fact, most of the surfactants we have used in the formulations tend to be esters. In addition, the inclusion of surfactants within the formulations did not affect largely the patterns of drug solubilisation. Indeed, no significant differences were observed between the solubilisation profiles in the aqueous phase of Type I, II IIIA and IIIB and IV lipid formulations. However, the addition of hydrophilic surfactants and or cosolvent slightly reduced the fenofibrate concentration in the aqueous phase.

The beneficial effect of the inclusion of lipids in the formulations was evidenced by the poor performance of the surfactant-only solutions (Type IV systems) and with formulations having a high ratio of surfactant, cosolvent to lipid (Type IIIB systems). As it is clear in this study that the formulations containing medium-chain lipid failed to prevent fenofibrate precipitation, the use of long-chain lipid offered efficient solubilisation upon digestion.

The present investigation suggest that the valuable information can be obtained from the *in vitro* lipolysis model, leading to the optimal selection of suitable lipid components for enhanced oral bioavailability of poorly water-soluble compound, such as fenofibrate. The results obtained in the above digestion studies generally confirmed that fenofibrate may not be well absorbed with medium-chain glycerides.

CONCLUSION

The present investigations suggest that the ability of most of the formulations to maintain drug in solution was markedly affected by lipid digestion. Thus, the valuable information can be obtained from the *in vitro* lipolysis model, leading to the best possible selection of suitable lipid components for enhanced oral bioavailability of poorly water-soluble compound, such as fenofibrate. The results obtained in the above digestion studies generally confirmed that fenofibrate may not be well absorbed with medium-chain glycerides.

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