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Preparation and In Vivo Evaluation of SMEDDS (Self-Microemulsifying Drug Delivery System) Containing Fenofibrate

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ABSTRACT

The present work was aimed at formulating a SMEDDS (self-microemulsifying drug delivery system) of fenofibrate and evaluating its in vitro and in vivo potential. The solubility of fenofibrate was determined in various vehicles. Pseudoternary phase diagrams were used to evaluate the microemulsification existence area, and the release rate of fenofibrate was investigated using an in vitro dissolution test. SMEDDS formulations were tested for microemulsifying properties, and the resultant microemulsions were evaluated for clarity, precipitation, and particle size distribution. Formulation development and screening was done based on results obtained from phase diagrams and characteristics of resultant microemulsions. The optimized formulation for in vitro dissolution and pharmacodynamic studies was composed of Labrafac CM10 (31.5%), Tween 80 (47.3%), and polyethylene glycol 400 (12.7%). The SMEDDS formulation showed complete release in 15 minutes as compared with the plain drug, which showed a limited dissolution rate. Comparative pharmacodynamic evaluation was investigated in terms of lipid-lowering efficacy, using a Triton-induced hypercholesterolemia model in rats. The SMEDDS formulation significantly reduced serum lipid levels in phases I and II of the Triton test, as compared with plain fenofibrate. The optimized formulation was then subjected to stability studies as per International Conference on Harmonization (ICH) guidelines and was found to be stable over 12 months. Thus, the study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulations of fenofibrate to improve its bioavailability.

KEYWORDS: Fenofibrate, SMEDDS, pseudoternary phase diagrams, Triton-induced hyperlipidemia

INTRODUCTION

Lipid-based formulation approaches, particularly the self-microemulsifying drug delivery system (SMEDDS), are well known for their potential as alternative strategies for delivery of hydrophobic drugs,[1] which are associated with poor water solubility and low oral bioavailability.[2-4] SMEDDS formulations are isotropic mixtures of an oil, a surfactant, a cosurfactant (or solubilizer), and a drug. The basic principle of this system is its ability to form fine oil-in-water (o/w) microemulsions under gentle agitation following dilution by aqueous phases (ie, the digestive motility of the stomach and intestine provide the agitation required for self-emulsification in vivo in the lumen of the gut).[5] This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption.[6] Apart from solubilization, the presence of lipid in the formulation further helps improve bioavailability by affecting the drug absorption.[1] Selection of a suitable self-emulsifying formulation depends upon the assessment of (1) the solubility of the drug in various components, (2) the area of the self-emulsifying region as obtained in the phase diagram, and (3) the droplet size distribution of the resultant emulsion following self-emulsification.[7]

Fenofibrate is a lipid-regulating agent that has chemical, pharmacological, and clinical similarities to the other fibrate drugs, such as clofibrate and gemfibrozil.[8] Fenofibrate is a Biopharmaceutical Classification System (BCS) Class II drug with a high dose number.[9] Thus, it can be assumed that the low oral bioavailability of fenofibrate is due to its solubility and dissolution limitations.[9] Researchers have tried various methods (eg, cyclodextrin complexation, comicronization, solid dispersion) to overcome these limitations.[10-12]

Furthermore, it is reported that absorption of fenofibrate is increased by ~35% when it is administered with food rather than in a fasting state.[8,13] Thus, formulating a lipid-based system of fenofibrate can be viewed as an option for improving its oral bioavailability. Fenofibrate is available in various doses (54 mg, 67 mg, 100 mg, 160 mg, and 200 mg); for our study we selected 54 mg as the working dose to limit the total formulation volume.

The main objectives of the study were to develop and evaluate an optimal SMEDDS formulation containing fenofibrate and to assess its pharmacodynamic effect in terms of lipid-lowering potential.

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MATERIALS AND METHODS

Materials

Fenofibrate was obtained as a gift sample from Cipla Ltd (Mumbai, India). The following materials were donated by Gattefosse (Mumbai, India) and were used as received: Labrafac CM10 (C_8-C_{10} polyglycolized glycerides), Maisine 35-1 (glyceryl monolinoleate), Lauroglycol FCC (propylene glycol laurate), Labrafil 1944 CS (apricot kernel oil polyethylene glycol [PEG] 6 esters), and Labrafac PG (propylene glycol caprylate/caprate). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), Cremophor EL (polyethoxylated castor oil), and Solutol HS 15 (polyoxyethylene esters of 12-hydroxystearic acid) were obtained from BASF (Mumbai). Gelucire 44/14 (PEG-32 glyceryl laurate) and 50/13 (PEG-32 glyceryl palmistearate) were received from Colorcon Asia (Mumbai). Span 20 (sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monooleate), and PEG 400 were bought from Merck (Mumbai, India). Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France). Acetonitrile and methanol used in the present study were of high performance liquid chromatography (HPLC) grade. All other chemicals were reagent grade. Empty hard gelatin capsule shells were generously donated by ACG Capsules (Mumbai).

Animals

Male Holtzman rats (weighing approximately 250 ± 30 g) were used for the comparative lipid-lowering studies. The animals were maintained at a constant light (14L: 10D), temperature (24°C-25°C), and humidity (60%) and were supplied with food and water ad libitum. The animal requirement was approved by the Institute Animal Ethics Committee (IAEC), and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

Methods

Solubility Studies

The solubility of fenofibrate in various components (oils, surfactants, and cosurfactants) was determined as follows: 500 mg of each of the selected vehicles was added to each cap vial containing an excess of fenofibrate (1 g). After sealing, the mixture was heated at 40ºC in a water bath to facilitate the solubilization. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25ºC for 48 hours. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 5 minutes, and excess insoluble fenofibrate was discarded by filtration using a membrane filter (0.45 μm, 13 mm, Whatman, Mumbai, India). The concentration of fenofibrate was then quantified by HPLC.

Pseudoternary Phase Diagrams

Pseudoternary phase diagrams of oil, surfactant/cosurfactant (S/CoS), and water were developed using the water titration method. The mixtures of oil and S/CoS at certain weight ratios were diluted with water in a dropwise manner. For each phase diagram at a specific ratio of S/CoS (ie, 1:1, 2:1, 3:1, and 5:1 wt/wt), a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 minutes. Then each mixture was titrated with water and visually observed for phase clarity and flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio. To determine the effect of drug addition on the microemulsion boundary, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component. Phase diagrams were then constructed using Tri plot v1-4 software (David Graham and Nicholas Midgley, Loughborough, Leicestershire, UK).

Preparation of SMEDDS Formulations

A series of SMEDDS formulations were prepared using Tween 80 and PEG 400 as the S/CoS combination and Labrafac CM10 as the oil (Table 1). In all the formulations, the level of fenofibrate was kept constant (ie, 8.5% wt/wt of the total formulation weight). Briefly, accurately weighed fenofibrate was placed in a glass vial, and oil, surfactant, and cosurfactant were added. Then the components were mixed by gentle stirring and vortex mixing and were heated at 40ºC on a magnetic stirrer, until fenofibrate was perfectly dissolved. The mixture was stored at room temperature until further use.

Freeze Thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at –4°C for 24 hours followed by thawing at 40°C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

Emulsion Droplet Size Analysis

One hundred microliters of each SMEDDS formulation was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle

Table 1. Developed Formulations With Their Compositions*

Components (% wt/wt)	Batch A	Batch B	Batch C	Batch D	Batch E	Batch F	Batch G	Batch H
Fenofibrate	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Labrafac CM10	28.5	29.5	30.5	31.5	32.5	31.5	31.5	31.5
S/CoS ratio	3:1	3:1	3:1	3:1	3:1	3.2:1	3.5:1	3.7:1
Tween 80	47.25	46.50	45.75	45.00	44.25	45.71	46.67	47.30
PEG 400	15.75	15.50	15.25	15.00	14.75	14.29	13.33	12.70

*S/CoS indicates surfactant/cosurfactant; PEG, polyethylene glycol.

size analysis (using Malvern Mastersizer (Worchestershire, UK) equipped with 2000 Hydro MU) with a particle size measurement range of 0.02 to 2000 µm. Particle size was calculated from the volume size distribution. All studies were repeated in triplicate, with good agreement being found between measurements.

Self-Emulsification and Precipitation Assessment

Evaluation of the self-emulsifying properties of SMEDDS formulations was performed by visual assessment as previously reported.[14] In brief, different compositions were categorized on speed of emulsification, clarity, and apparent stability of the resultant emulsion. Visual assessment was performed by dropwise addition of the preconcentrate (SMEDDS) into 250 mL of distilled water. This was done in a glass beaker at room temperature, and the contents were gently stirred magnetically at ~100 rpm.

Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were then categorized as clear (transparent or transparent with bluish tinge), nonclear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours).

In Vitro Dissolution Studies

The quantitative in vitro release test was performed in 900 mL of buffer pH 1.2 using US Pharmacopeia XXIV dissolution apparatus 2. The paddles were rotated at 100 rpm. The SMEDDS formulations were put into hard gelatin capsules (0 sizes) and used for drug release studies; results were compared with those of plain fenofibrate. During the release studies, a 5-mL sample of medium was taken out and subjected to drug analysis using HPLC. The removed volume was replaced each time with 5 mL of fresh medium. For determination of the in vitro dissolution of plain fenofibrate, the medium was changed to buffer pH 1.2 containing Tween 80 (equivalent to the amount used in the formulation). Dissolution studies were also performed in other media (buffer pH 4.5 and 7.2) to examine the effect of pH on drug release.

HPLC Analysis of Fenofibrate

The concentration of fenofibrate in the samples was determined by HPLC analysis. The HPLC analysis system consisted of a Jasco PU 980 Intelligent Pump and Jasco MD-2015 Plus Multi-Wavelength Detector (Tokyo, Japan). The chromatographic column was a C-18 Lichrosphere 10RP-18e (5 µm) 4.6 mm × 250 mm (Merck). The chromatographic conditions were mobile phase acetonitrile:water (80:20% vol/vol); flow rate 1 mL/min; loop size 100 µL; detection at 289 nm; and retention time 7.5 ± 0.5 minutes.

Lipid-Lowering Studies

Male Holtzman rats (weighing 250±30 g) were used for the study. They were kept in air-conditioned rooms (24ºC-25ºC) with constant humidity. The rats were caged and allowed water and food ad libitum before they were distributed by weight into experimental groups. The rats fasted overnight and were then intraperitoneally injected with 250 mg/kg Triton WR 1339 (Tyloxapol, Sigma Chemical Co, St. Louis, MO) dissolved in 0.9 percent saline. Control groups of rats were given the vehicle (plain saline), and experimental groups were given plain fenofibrate (9 mg/kg body weight) or the SMEDDS formulation (equivalent to 9 mg/kg fenofibrate). Without anesthesia and by restraining rats by hand, the oral dosing was performed by intubation using an 18-gauge feeding needle (the volume to be fed was 1.0 mL in all cases). To study the effect of formulation components on lipid lowering, 1 more group was included; this group was fed a placebo formulation. Blood samples were drawn at 0 hours, 24 hours, and 48 hours. Serum was separated by centrifugation at 10 000 g and used for biochemical analysis. Serum cholesterol and triglycerides were estimated in normal, control, and drug-treated groups by reported methods.[15,16] Low-density lipoprotein (LDL) levels were estimated using the Friedwal formula. Statistical analysis of the collected data was performed using 1-way analysis of variance.

Stability Studies

The SMEDDS formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at

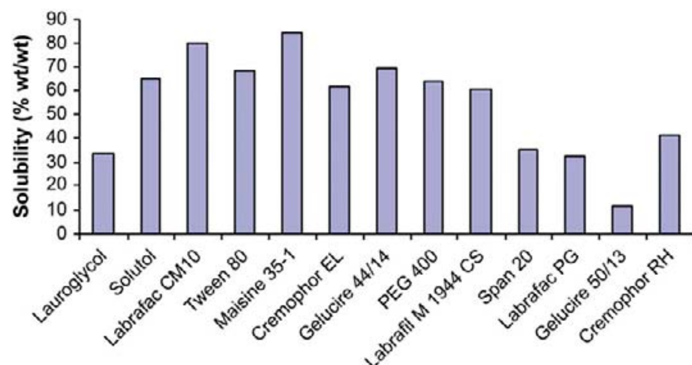

Figure 1. Solubility of fenofibrate in various components. PEG indicates polyethylene glycol.

25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers (Thermolab, Mumbai, India) with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 6 months for intermediate and accelerated conditions and 12 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating HPLC method.

RESULTS AND DISCUSSION

Solubility Studies

One important consideration when formulating a self-emulsifying formulation is avoiding precipitation of the drug on dilution in the gut lumen in vivo.[1] Therefore, the components used in the system should have high solubilization capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion. Results from solubility studies are reported in Figure 1. As seen from the figure, Maisine 35-1 and Labrafac CM10 showed the highest solubilization capacity for fenofibrate, followed by Tween 80 and PEG 400. Thus, for our study we selected Maisine 35-1 and Labrafac CM10 as oils and Tween 80 and PEG 400 as surfactant and cosurfactant, respectively.

Pseudoternary Phase Diagrams

Self-microemulsifying systems form fine oil-water emulsions with only gentle agitation, upon their introduction into aqueous media. Surfactant and cosurfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation.[17,18] Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion.

In the present study both Maisine 35-1 and Labrafac CM10 were tested for phase behavior studies with Tween 80 and PEG 400 as the S/CoS mixture. As seen from the ternary plot (Figures 2 and 3), Labrafac CM10 gave a wider microemulsion region than did Maisine 35-1 at all S/CoS ratios. Thus, Labrafac CM10 was selected as the preferred vehicle for the optimized formulation. The microemulsion existence

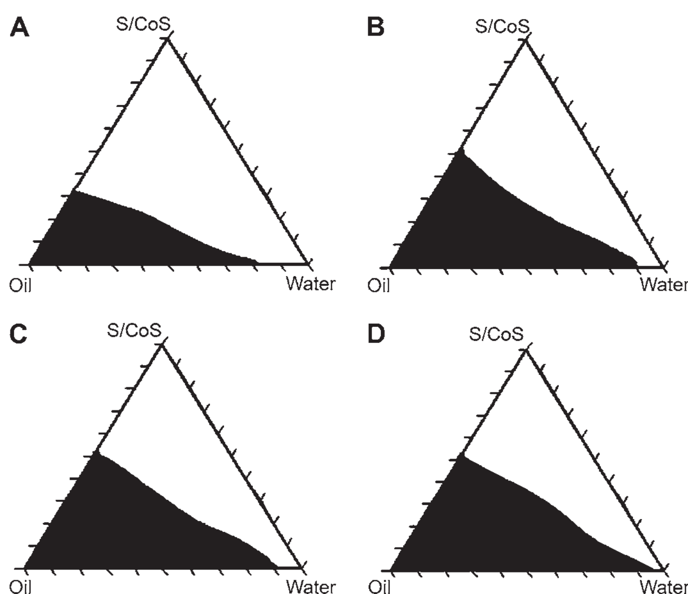

Figure 2. Pseudoternary phase diagram of system with the following components: oil = Labrafac CM10, surfactant = Tween 80, and cosurfactant = polyethylene glycol 400. S/CoS ratio of A is 1:1, B is 2:1, C is 3:1, and D is 5:1. S/CoS indicates surfactant/cosurfactant.

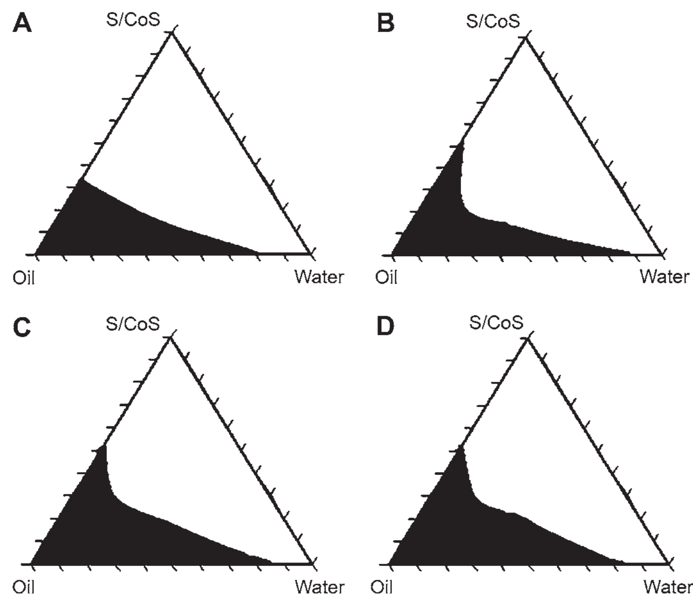

Figure 3. Pseudoternary phase diagram of system with the following components: oil = Maisine 35-1, surfactant = Tween 80, and cosurfactant = polyethylene glycol 400. S/CoS ratio of A is 1:1, B is 2:1, C is 3:1, and D is 5:1. S/CoS indicates surfactant/cosurfactant.

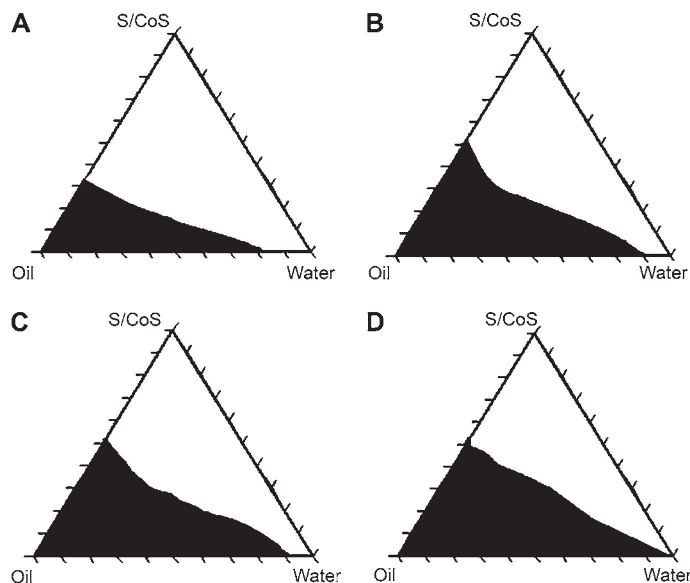

Figure 4. Pseudoternary phase diagram of system with the following components: oil = drug-enriched Labrafac CM10, surfactant = Tween 80, and cosurfactant = polyethylene glycol 400. S/CoS ratio of A is 1:1, B is 2:1, C is 3:1, and D is 5:1. S/CoS indicates surfactant/cosurfactant.

area increased as the S/CoS ratio increased. However, it was observed that increasing the surfactant ratio resulted in a loss of flowability. Thus, an S/CoS ratio between 3:1 and 4:1 was selected for the formulation study.

PEG 400 is reported to be incompatible with hard gelatin capsules when used in high concentrations.[19] Thus, while optimizing the S/CoS ratio, we tried to keep the concentration of PEG 400 as low as possible (<15% wt/wt of total formulation), as we had a final aim of putting the SMEDDS formulations into liquid-filled hard gelatin capsules. Figure 4 shows phase diagrams in the presence of the drug. As seen from the figure, the inclusion of drug narrowed the microemulsion existence area, because inclusion of the drug in the lipid phase led to expansion of the lipid phase and consequently a need for a higher S/CoS ratio for stabilization.

Droplet Size Analysis

The droplet size distribution of various formulations is given in Table 2. An increase in the ratio of the oil phase (Labrafac CM10) resulted in a proportional increase in particle size, because of the simultaneous decrease in the S/CoS proportion. Increasing the S/CoS ratio led to a decrease in mean droplet size. Batch H, with the highest proportion of surfactant (47.3% wt/wt Tween 80) at a fixed amount of oil (31.5% wt/wt), had the lowest mean particle diameter. This could be attributed to an increased surfactant proportion relative to cosurfactant. It is well known that the addition of surfactants to the microemulsion systems causes the interfacial film to stabilize and condense, while the addition of cosurfactant causes the film to expand; thus, the relative proportion of surfactant to cosurfactant has varied effects on the droplet size.[20,21]

Self-Emulsification and Precipitation Studies

The results of self-emulsification and precipitation studies are given in Table 2. It was seen that an increase in the proportion of Labrafac CM10 in the composition resulted in decreasing self-emulsification time up to a concentration of 31.5% wt/wt, beyond which it resulted in generation of nonclear dispersion. The decrease in self-emulsification time can be assumed to be due the relative decrease in surfactant concentration, leading to decreased viscosity of the formulation. The S/CoS ratio of 3:1 was kept constant for the initial formulation study. However, it was found that the resultant dispersion showed precipitation and thus was not stable, because of the presence of PEG 400. PEG 400 can be assumed to act as a cosolvent for fenofibrate (as seen from solubility studies), and thus it increases the solubilization capacity of the vehicle (Labrafac CM10). However, when the preconcentrate (SMEDDS) is dispersed in water, PEG 400, being water-soluble, is anticipated to enter the water phase and redistribute mainly between the water phase and the emulsion-water interface, resulting in a loss of solvent capacity of the vehicle. A similar observation was reported

Table 2. Evaluation Parameters of Various Formulations

	Particle Size Distribution (µm)					
Formulations	**D (0.1)**	**D (0.5)**	**D (0.9)**	**Dispersion Time (secs)**	**Clarity**	**Precipitation**
A	0.113	0.156	0.209	98 ± 5	Clear	Unstable
B	0.124	0.167	0.217	78 ± 3	Clear	Unstable
C	0.128	0.175	0.226	67 ± 2	Clear	Unstable
D	0.133	0.180	0.249	51 ± 3	Clear	Unstable
E	0.159	0.201	0.357	55 ± 4	Nonclear	—
F	0.131	0.179	0.242	52 ± 1	Clear	Unstable
G	0.129	0.171	0.239	59 ± 4	Clear	Unstable
H	0.119	0.161	0.229	58 ± 2	Clear	Stable

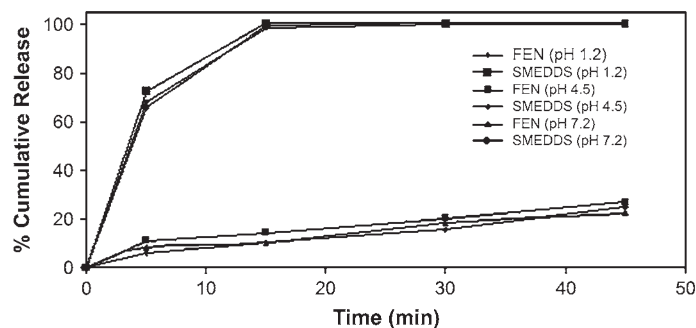

Figure 5. Comparative results of drug release from plain fenofibrate and the SMEDDS formulation in different dissolution media; n = 6 and range of variability = 1% to 3%. FEN indicates plain fenofibrate; SMEDDS, self-microemulsifying drug delivery system.

for a composition containing ethanol as the cosolvent.[22,23] Thus, the problem of precipitation was solved by increasing the surfactant proportion (S/CoS [3.7:1]) in the system.

In Vitro Dissolution Studies

Drug release from the SMEDDS formulation (Batch H) was found to be significantly higher as compared with that of plain fenofibrate (Figure 5). It could be suggested that the SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain fenofibrate. Thus, this greater availability of dissolved fenofibrate from the SMEDDS formulation could lead to higher absorption and higher oral bioavailability. It was also seen that changes in the dissolution medium (buffer pH 1.2, 4.5, and 7.2) had no effect on the drug release from either plain fenofibrate or the SMEDDS formulation (Figure 5). This observation can be explained by the fact that fenofibrate has no ionizable group and thus its solubility and dissolution is pH-independent.

Lipid-Lowering Studies

The study was performed to evaluate the pharmacodynamic potential of a developed formulation (Batch H) against plain fenofibrate using a Triton-induced hyperlipidemia model. Triton is a nonionic surfactant that induces hyperlipidemia by inhibiting peripheral lipoprotein lipase enzymes responsible for removal of lipid particles from the body.[24] The administration of Triton leads to transient elevation of lipid levels, which reach a peak at 18 to 24 hours after administration (phase I) and start to lower again the following day (phase II).[24,25] This experimental model has been previously used for screening the activity of the antilipidemic agent benzafibrate (a fibric acid derivative).[26] Thus, for our present study this method was used to evaluate the lipid-lowering activity of the developed formulation. The precise mechanism by which fenofibrate exerts its antihyperlipidemic effect has not been clearly established. However, it has

Table 3. Effect of Treatment on Serum Lipids in Phase I and Phase II of Triton Test*

Treatment Group (n = 6)	Cholesterol (mg/dL)	Cholesterol (% inh)	Triglycerides (mg/mL)	Triglycerides (% inh)	LDL (mg/mL)	LDL (% inh)
Phase I						
Basal	59.1 ± 12.4	—	55.6 ± 9.8	—	35.1 ± 10.4	—
Triton	167 ± 12.3	—	217 ± 25.5	—	173 ± 9.1	—
Placebo	163 ± 11.7	—	224 ± 22.1	—	178 ± 8.6	—
Plain drug	101 ± 4.5†	55.2	110 ± 9.1†	61.5	86.6 ± 3.2‡	57.5
SMEDDS	64.9 ± 5.3‡	92.9	71.2 ± 8.2§	92.3	40.3 ± 5.0‡	93.3
Phase II						
Basal	55.2 ± 10.1	—	56.2 ± 10.1	—	29.9 ± 9.2	—
Triton	105 ± 4.3	—	119 ± 4.1	—	93.2 ± 5.2	—
Placebo	107 ± 7.1	—	117 ± 3.0	—	96.8 ± 4.7	—
Plain drug	80.1 ± 2.8‡	40.8	71.5 ± 4.6†	62.2	58.3 ± 2.8†	57.4
SMEDDS	57.5 ± 7.2‡	98.8	62.5 ± 7.2‡	94.4	30.2 ± 6.2‡	100

*Data presented as mean ± SD. Statistically significant difference between treated group and control group. LDL indicates low-density lipoprotein; SMEDDS, self-microemulsifying drug delivery system.
†$P < .05$.
‡$P < .01$.
§$P < .001$.

been reported that fenofibric acid, the active metabolite of fenofibrate, lowers plasma lipids apparently by inhibiting synthesis and also by stimulating the catabolism of triglyceride-rich lipoproteins. It was observed that fenofibrate and its formulation were found to affect the serum lipid level in both phase I and phase II.

Table 3 gives the effect of treatments on serum lipid levels in phase I (24 hours). Fenofibrate produced a fall in serum cholesterol (55.18% inhibition), triglyceride (61.49% inhibition), and LDL (57.54% inhibition). The SMEDDS formulation, as expected, performed better than fenofibrate, resulting in a significant reduction of serum cholesterol (92.89% inhibition), triglycerides (92.32% inhibition), and LDL levels (93.29% inhibition).

It has been reported that there is a natural tapering in cholesterol and triglyceride values in phase II of the Triton test. However, this normal clearance of serum lipid in phase II of the Triton test can also be triggered by the presence of a drug in the circulation.[26] Fenofibrate is known to have a longer stay in the blood circulation, as it has a biological half-life of 20 hours.[27] Thus, a longer duration of action is guaranteed provided that there is an optimal initial plasma drug level, which is generally determined by the bioavailability of the drug. We also evaluated the effect of fenofibrate and the SMEDDS formulation in phase II of the Triton test. As seen from Table 3, plain fenofibrate lowered cholesterol (40.84% inhibition), triglyceride (62.24% inhibition), and LDL (57.40% inhibition). The SMEDDS formulation resulted in a greater reduction of cholesterol (98.84% inhibition), triglyceride (94.36% inhibition), and LDL (100% inhibition). Thus, the higher lipid-lowering activity of the SMEDDS formulation in both phase I and phase II of the Triton test can be explained by the fact that the SMEDDS formulation resulted in complete dissolution of fenofibrate, which could have increased absorption and thereby led to a higher plasma drug concentration (higher bioavailability). The low bioavailability of fenofibrate is attributed to its poor aqueous solubility. The above difference in pharmacodynamic activity and the results from in vitro dissolution studies thus suggest that the SMEDDS formulation resulted in higher oral bioavailability owing to higher solubilization of fenofibrate from the SMEDDS formulation as compared with plain fenofibrate.

Stability Studies

Generally, SMEDDS formulations are put into hard gelatin capsules as the final dosage form. However, liquid-filled hard gelatin capsules are susceptible to leakage, and the

Table 4. Evaluation Data of SMEDDS Formulation Subjected to Stability Studies (n = 10)*

Sampling Point	Weight Variation (mg)	Disintegration Time (mins)	% Drug Content	Particle Size D (0.9) µm	t90% (mins)
(A) 25ºC/60% RH					
0 days	624 ± 4	3.9 ± 1.1	101.5 ± 0.7	0.251	<15
3 months	634 ± 4	4.2 ± 0.9	99.9 ± 2.7	0.248	<15
6 months	632 ± 5	4.7 ± 1.3	98.8 ± 3.1	0.264	<15
9 months	637 ± 4	4.2 ± 0.5	97.7 ± 3.7	0.242	<15
12 months	639 ± 2	4.2 ± 1.2	96.6 ± 0.8	0.254	<15
(B) 30ºC/65% RH					
0 days	624 ± 4	3.9 ± 1.1	101.5 ± 0.7	0.251	<15
3 months	637 ± 4	4.3 ± 2.3	96.6 ± 3.1	0.238	<15
6 months	634 ± 3	4.2 ± 0.2	96.4 ± 1.4	0.240	<15
(C) 40ºC/75% RH					
0 days	624 ± 4	3.9 ± 1.1	101.5 ± 0.7	0.251	<15
3 months	639 ± 3	4.1 ± 2.9	96.6 ± 3.0	0.241	<15
6 months	640 ± 5	4.1 ± 0.2	96.2 ± 0.4	0.233	<15

*SMEDDS indicates self-microemulsifying drug delivery system; RH, relative humidity; t90% = time taken for 90% drug release.

entire system has a very limited shelf life owing to its liquid characteristics and the possibility of precipitation of the drug from the system. Thus, the developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form.

Table 4 gives the results of the evaluation test conducted on stability samples. The formulation was found to be stable for 6 months at intermediate and accelerated conditions and 12 months at long-term conditions. There was no significant change in the drug content, drug release (t90%), or particle size of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There were also no significant changes in the appearance, disintegration time, or microemulsifying property. Furthermore, the formulation was found to show no phase separation, drug precipitation, or capsule leaks. Thus, these studies confirmed the stability of the developed formulation and its compatibility with hard gelatin capsules.

CONCLUSIONS

An optimized SMEDDS formulation consisting of Labrafac CM10 (31.5% wt/wt), Tween 80 (47.3% wt/wt), PEG 400 (12.7% wt/wt), and fenofibrate (8.5% wt/wt) was successfully developed with an increased dissolution rate, increased solubility, and, ultimately, increased bioavailability of a poorly water-soluble drug, fenofibrate. The developed formulation showed higher pharmacodynamic potential as compared with plain fenofibrate. Results from stability studies confirmed the stability of the developed formulation. Thus, our study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulations of fenofibrate to improve its bioavailability.

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REFERENCES

1. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur J Pharm Sci*. 2000;11:S93-S98.

2. Constantinides PP. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm Res*. 1995;12:1561-1572.

3. Kim HJ, Yoon KA, Hahn M, Park ES, Chi SC. Preparation and in vitro evaluation of self-microemulsifying drug delivery systems containing idebenone. *Drug Dev Ind Pharm*. 2000;26:523-529.

4. Kang BK, Lee JS, Chona SK, et al. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *Int J Pharm*. 2004;274:65-73.

5. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolysed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. *Int J Pharm*. 1994;106:15-23.

6. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. *Pharm Res*. 1992;9:87-93.

7. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *Int J Pharm*. 2001;212:233-246.

8. *Physician's Desk Reference 2000.* 54th ed. Montvale, NJ: Medical Economics Company; 2000:476-477.

9. Kasim NA, Whitehouse M, Ramachandran C, et al. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol Pharm*. 2004;1:85-96.

10. Patel AR, Vavia PR. Effect of hydrophilic polymer on solubilization of fenofibrate by cyclodextrin complexation. *J Incl Phenom Macrocycl Chem*. 2006;56:247-251.

11. Law D, Wang W, Schmitt E, Qiu Y, Krill SK, Fort JJ. Properties of rapidly dissolving eutectic mixtures of poly(ethylene glycol) and fenofibrate: the eutectic microstructure. *J Pharm Sci*. 2003;92:505-515.

12. Curtet B, Teillaud E, Reginault P, inventors. Fournier Innovation et Synergie, assignee. Novel dosage form of fenofibrate. US patent 4 895 726. January 23, 1980.

13. Tricor. *fenofibrate [package insert]*. North Chicago, IL: Abbott Laboratories. 2002.

14. Khoo SM, Humberstone AJ, Porter CJ, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int J Pharm*. 1998;167:155-164.

15. Zlatkis A, Zak B, Boyle AJ. New method for direct determination of serum cholesterol. *J Lab Clin Med*. 1953;41:486-492.

16. Foster IB, Dunn RT. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clin Chem*. 1973;19:338-340.

17. Groves MJ. The self-emulsifying action of mixed surfactants in oil. *Acta Pharm Suec*. 1976;13:361-372.

18. Schulman JH, Montagne JB. Formation of microemulsions by amino alkyl alcohols. *Ann N Y Acad Sci*. 1961;92:366-371.

19. Mei X, Etzler FM, Wang Z. Use of texture analysis to study hydrophilic solvent effects on the mechanical properties of hard gelatin capsules. *Int J Pharm*. 2006;324:128-135.

20. Gao ZG, Choi HG, Shin HJ, et al. Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A. *Int J Pharm*. 1998;161:75-86.

21. Constantinides PP, Scalart JP. Formulation and physical characterization of water-in-oil microemulsions containing long- versus medium-chain glycerides. *Int J Pharm*. 1997;158:57-68.

22. de Campo L, Yaghmur A, Garti N, Leser ME, Folmer B, Glatter O. Five-component food-grade microemulsions: structural characterization by SANS. *J Colloid Interface Sci*. 2004;274:251-267.

23. Szekeres E, Acosta E, Sabatini DA, Harwell JH. A two-state model for selective solubilization of benzene-limonene mixtures in sodium dihexyl sulfosuccinate microemulsions. *Langmuir*. 2004;20:6560-6569.

24. Nutting DF, Tso P. Hypolipidemic effects of intravenous pluronic L-81 in fasted rats treated with Triton WR-1339: possible inhibition of hepatic lipoprotein secretion. *Horm Metab Res*. 1989;21:113-115.

25. Schurr PE, Schultz JR, Parkinson TM. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. *Lipids*. 1972;7:68-74.

26. Krishnamurthy A, Thapar GS. Effect of benzafibrate and nicotinic acid on Triton-induced hyperlipidemias in CFY rats. *Indian J Med Res*. 1991;94:395-398.

27. Mahley RW, Bersot TP. Drug therapy for hypercholesterolemia and dyslipidemia. In: Brunton LL, Lazo JS, Parker KL, eds. *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 11th ed. New York, NY: McGraw Hill; 2006:957-958.