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Article

Lactoferrin Reduces Surfactant Content in the Self-Emulsifying Drug Delivery System

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Cite This: *ACS Omega* 2024, 9, 13612−13620

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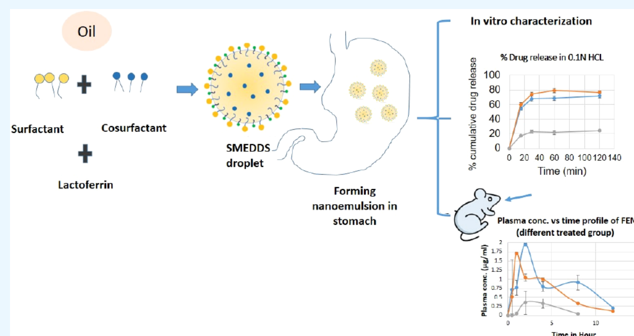
ABSTRACT: Self-emulsifying drug delivery systems (SEDDSs) can effectively be employed to formulate drugs with poor oral bioavailability due to low aqueous solubility and high first-pass metabolism. High surfactant content is an existing challenge toward the successful application of SEDDS. A SEDDS is developed with lactoferrin, a natural emulsifier to reduce the Tween content of a fenofibrate (FEN) formulation. FEN SEDDS (SEDDS without lactoferrin) and FEN Lf-SEDDS (SEDDS with lactoferrin) were developed with 30% and 21% Tween content, respectively. Both formulations containing Crodamol GTCC as a lipid component were thermodynamically stable. No significant difference was observed in zeta potential (−9.25 to −12.63 mV), drug content (>85%), and percentage transmittance (>99%) between the two formulations. FEN Lf-SEDDS resulted in higher viscosity and larger particle size than FEN SEDDS. Solidified SEDDS with Aerosil 200 significantly improved *in vitro* drug release from both formulations than pure FEN. However, FEN SEDDS and FEN Lf-SEDDS did not show a significant difference in cumulative percent release or dissolution efficiency at 120 min. It can be concluded that lactoferrin containing SEDDS with 27% lesser synthetic surfactants (Tween 80 and Span 80) can result in similar physicochemical characteristics. Oral pharmacokinetic study of FEN Lf-SEDDS in a rat model resulted in 1.3 and 5.5 times higher relative bioavailability than marketed product and pure drug, respectively. The addition of lactoferrin could substitute synthetic surfactants in self-emulsifying drug delivery systems significantly.

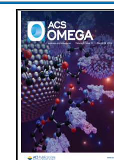


1. INTRODUCTION

Self-emulsifying drug delivery systems (SEDDSs) are liquid isotropic mixtures of natural or synthetic oils, surfactant, and optionally one or more cosurfactant.[1] Visually it appears as a thick, viscous liquid formulation generally loaded into capsules or converted to tablets after solidification. Upon contact with the aqueous medium, SEDDS spontaneously forms emulsion by mild agitation. In the last two decades, SEDDS has gained a lot of popularity to deliver highly lipophilic drugs orally. Some of the advatanges of SEDDS include higher stability, lesser chances of phase separation than conventional emulsion, significant improvement of oral bioavailability of lipophilic drug by improving solubility, and possibility of absorption by lymphatic routes.[2−4] Some oils and surfactants used in SEDDS are also claimed to be pGP transporter inhibitors, which potentially increases oral bioavailability of pGP substrate drugs.[5] Inside the gastrointestinal tract, mild agitation occurs due to peristalsis, helping SEDDS to form microemulsion or nanoemulsion. Depending on the type of emulsion formed inside the body, SEDDS is termed as self-microemulsifying drug delivery systems (SMEDDS) or self-nanoemulsifying drug delivery systems (SNEDDS). As the formulation itself remains as a concentrated mixture before adding into the aqueous medium, it is often called as emulsion preconconcentrate or micro/nano emulsion preconcentrate.[6] The formation of stable and smaller droplets without phase separation is the primary criterion of an efficient SEDDS for improved drug absorption. Surfactant plays the most important role to fulfill the key criteria. Surfactants, often mixed with cosurfactant, reduce the interfacial tension between two distinct phases, resulting in smaller size droplets. Determining the proper combination and ratio of oil vs surfactant-cosurfactant is the key to a successful oral SEDDS.[7] Tween (polysorbate) and Span (sorbitan ester) are the two primary small molecule nonionic surfactants used as emulsifier cum stabilizers in SEDDS.

The major limitation of SEDDS is the large amount of surfactant−cosurfactant required to stabilize the colloid. If

Received: September 4, 2023
Revised: February 20, 2024
Accepted: February 26, 2024
Published: March 11, 2024



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given orally, a high surfactant concentration may cause gastric irritation and intestinal mucosal damage. Intestinal permeability enhancement and damage to the local tissue by surfactant are two correlated phenomena.[8] A recent study has shown that the composition of a SEDDS containing lipid and surfactant has a significant adverse effect on the rat intestinal system.[9] The lipid component of SEDDS disrupts the intestinal microbiota, while the surfactant triggers intestinal barrier injury in a rat model.[9] Chassaing et al. (2015) have studied the effect of dietary emulsifier in a rat model and observed that a low quantity of carboxymethyl cellulose and polysorbate 80 resulted in mild intestinal inflammation and abnormal metabolic syndrome.[10] Polysorbate or Tween 80 triggers intestinal microbial dysbiosis by reducing microbial diversity in gut, resulting in intestinal inflammation.[11] A high quantity of surfactant−cosurfactant mixture also restricts the usage of a high dose drug in orally given SEDDS. Therefore, reduced drug loading and increased toxicity possibility can be considered as two major limitations of synthetic surfactant usage in SEDDS. The use of naturally occurring surfactants have lesser unwanted effects than synthetic surfactants. Formulation scientists are trying to reduce the surfactant load with more biologically compatible agents in SEDDS without compromising the stability or permeability enhancement property.

In this research, lactoferrin is explored as component of SEDDS. Lactoferrin, a member of the transferrin family, is an iron-binding glycoprotein with attached carbohydrate.[12] It is widely used in drug delivery as a targeting ligand for nanocarriers. Surface-treated nanoparticles with lactoferrin have good targeting efficiency and can cross the blood−brain barrier to treat brain tumor or glioma.[13,14] It also has several favorable pharmacological properties, including antibacterial, antiviral, and antifungal.[15] It contains more than 600 amino acids with a strong positive surface charge. Its isoelectric point is 8−8.5.[16] Previous studies have shown that it adsorbs at oil−water interface, producing a catioin nanoemulsion.[17] Other researchers also have reported lactoferrin's excellent ability as an emulsifying agent.[18,19]

The hypothesis behind this research work was that the reduction/replacement of Tween 80 in a SEDDS with lactoferrin could offer equal stability as a fully synthetic surfactant-based system and enhanced oral bioavailability compared to conventional oral preparation. Reduced tween or span concentration in the SEDDS may reduce the chances of undesired effects occurring in synthetic surfactants. The model API used in the study was fenofibrate (FEN), a poorly bioavailable BCS class II drug indicated as a hypolipidemic agent given orally. First, a stable SEDDS of FEN was developed with Tween 80 and then the possible reduction of Tween 80 by lactoferrin was approached. Finally, an oral pharmacokinetic study in a rat model was done to check the bioavailability enhancement by a lactoferrin-containing formulation.

2. RESULTS AND DISCUSSION

2.1. Selection of Oils. The oil with the highest FEN solubility was selected for the formulation. As presented in Table 1, SR Crodamol GTCC resulted in the highest FEN solubility, followed by soyabean oil. The solubility in the other two natural oils, like sunflower and castor oil, is significantly lower than that of soyabean oil or SR Crodamol GTCC. SR Crodamol GTCC is a synthetic oil containing medium-chain triglyceride (MCT). Long-chain triglycerides may dissolve a higher amount of drug and can help in lymphatic absorption[1] but could be very viscous. SR Crodamol GTCC was colorless, odorless, less viscous oil with satisfactory FEN solubility. Hence, it was selected as the lipid component for SEDDS.

Table 1. Fenofibrate Solubility in Different Oils

oil	drug solubility (mg/mL) (mean ± SD)
soyabean oil	52.86 ± 0.5885
sunflower oil	6.4033 ± 0.1823
castor oil	4.9433 ± 0.5635
Crodamol GTCC	80.96 ± 1.3692

2.2. Selection of Surfactant−Cosurfactant (S_{mix}) Combination. The selection of S_{mix} was made by a pseudoternary phase diagram prepared by an aqueous titration method. For spontaneous emulsification of a self-nano-emulsifying system, an optimum composition of S_{mix} for a specific oil is essentially required. In the phase diagram, the transparent region was determined, and the combination giving the highest transparent region was selected for further development. Tween 80 was combined with Transcutol P and Span 80 in different ratios, taking Crodamol GTCC as the oil component. As presented in Figure 1, the Tween 80−Transcutol P combinations displayed a lower transparent region in the phase diagram than the Tween 80−Span 80 combination. A higher transparent region provides better self-emulsification efficiency by S_{mix}. The transparency of the emulsion depends on the adsorption of surfactant on the oil−water interface, reducing surface tension and droplet size.[20] Therefore, this research selected Tween 80: Span 80 (1:2 ratio) as the optimum S_{mix} for developing FEN SEDDS. In the later stage, the concentration of S_{mix} would be reduced by lactoferrin inclusion.

2.3. Formulation of FEN-Loaded SEDDS. The optimum composition of FEN-loaded SEDDS with and without lactoferrin (FEN SEDDS and FEN-Lf-SEDDS, respectively) is given in Table 2. It can be observed that the amount of S_{mix} was reduced by adding lactoferrin. FEN SEDDS contains 90% S_{mix} in the formulation, whereas FEN Lf-SEDDS contains only 63%. The rest of the amount (27%) is made up of lactoferrin. Lactoferrin is a natural emulsifier, and hence, it can help spontaneous emulsification if added with other surfactants. The reduction of surfactant in the formulation reduces gastric irritation and the chances of toxicity. Different lactoferrin concentrations were tried to incorporate in the SEDDS by gradually reducing S_{mix}. However, above 27% of lactoferrin, the formulation became very thick and showed phase separation within 72 h of normal storage. It should be noted that FEN SEDDS was less viscous than FEN Lf-SEDDS due to the presence of lactoferrin in the latter.

2.4. Thermodynamic Stability and Self-Emulsification. Thermodynamic stability of FEN SEDDS and FEN Lf-SEDDS was performed by a heat stress and centrifugation study. There was no phase separation or turbidity observed in both the formulations. The results indicate the physical stability of both SEDDS.

Self-emulsification ability is an important parameter that indicates the efficiency of the formulation for spontaneous emulsification. Both the formulations displayed good dispersibility with transparent dispersion. The time taken for spontaneously forming a clear microemulsion was less than 105 s, which is considered satisfactory. The average time taken

https://doi.org/10.1021/acsomega.3c06645
ACS Omega 2024, 9, 13612−13620

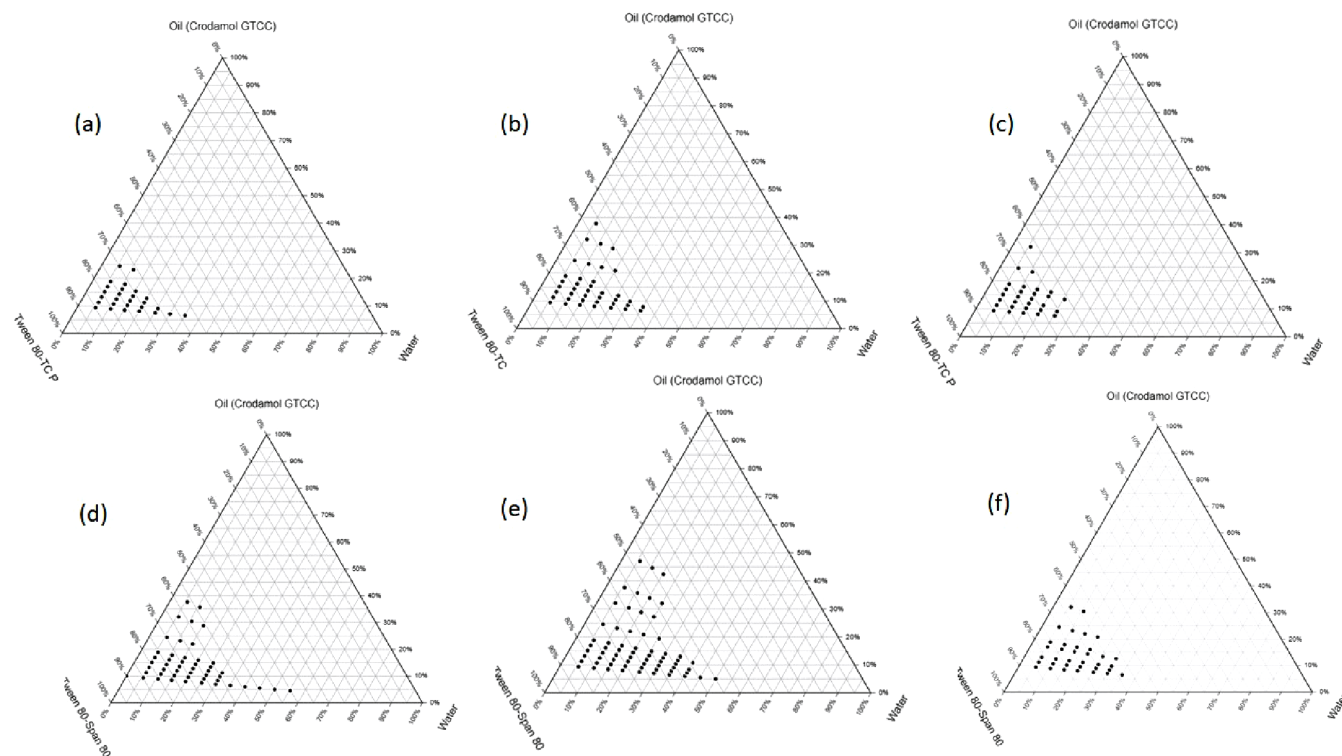

Figure 1. Pseudoternary phase diagram of crodamol GTCC and different S_{mix} combinations: (a) Tween 80: Transcutol (1:1), (b) Tween 80: Transcutol (1:2), (c) Tween 80: Transcutol (2:1), (d) Tween 80: Span 80 (1:1), (e) Tween 80: Span 80 (1:2), and (f) Tween 80: Span 80 (2:1).

Table 2. Composition of Fenofibrate SEDDS[a]

sample name	SR Crodamol GTCC (wt %)	Tween 80 (wt %)	Span 80 (wt %)	lactoferrin (wt %)
FEN SEDDS	10	30	60	-
FEN Lf-SEDDS	10	21	42	27

[a]FEN SEDDS: SEDDS of fenofibrate without lactoferrin; FEN Lf-SEDDS: SEDDS of fenofibrate with lactoferrin, Drug loading: 80 mg/mL.

for self-emulsification of FEN SEDDS and FEN Lf-SEDDS were 100.33 ± 5.87 and 90.22 ± 8.66 s, respectively. The difference was not significant (p-value >0.05).

2.5. Robustness to Dilution, %Transmittance. Robustness to dilution helps us to understand the fate of the formed emulsion when exposed to different pH mediums. After diluting in different aqueous media, no cloudiness was observed in any SEDDS. %Transmittance of both SEDDS was above 99%, indicating transparent colloidal dispersion after dilution.

2.6. Cloud Points. Cloud point is a measurement of the phase behavior of a surfactant system at elevated temperatures. It is the temperature at which phase separation and turbidity occur in a transparent microemulsion. At a specific high temperature, polyoxyethylene molecules of nonionic surfactants start dehydration, resulting in phase separation and turbidity.[21] An ideal self-emulsifying system should have a cloud point above the physiological temperature, so that it will not create phase separation in the gastric environment. Both FEN SEDDS and FEN Lf-SEDDS displayed cloud points >80 °C and indicated stability in physiological temperature.

2.7. Viscosity, Self-Emulsification Time, Zeta Potential, and Droplet Size. Viscosity, zeta potential, and droplet size of both the SEDDS are given in Table 3. Lactoferrin-loaded ME (FEN Lf-SEDDS) has a slightly higher viscosity than synthetic S_{mix} containing FEN SEDDS. Higher viscosity could be responsible for a longer self-emulsification time. In another research, pioglitazone was formulated as a self-microemulsifying delivery system with >56 cps viscosity and 42 s self-emulsification time.[22] Although there are no standard acceptance criteria, self-emulsification time should not exceed 120 s. Dispersibility analysis provided the self-emulsification time of FEN Lf-SEDDS and FEN SEDDS. In our research, the formulations' viscosity was relatively higher; hence, the self-emulsification time was relatively longer (80−100 s).

Zeta potential (Table 3) indicates the surface charge of the microemulsion droplet formed after dispersion, which depends

Table 3. Viscosity, Self-Emulsification Time, Zeta Potential, Droplet Size, and Polydispersity Index (PDI) of FEN-loaded SEDDS[a]

sample name	viscosity (cps) (±SD)	self-emulsification time (s) (±SD)	zeta potential (mV) (±SD)	droplet size (nm) (±SD)	polydispersity index (PDI) (±SD)
FEN SEDDS	252.4 ± 5.67	100 ± 1.93	16.63 ± 0.98	20.92 ± 1.31	0.355 ± 0.67
FEN-Lf ME	266.7 ± 3.94	80 ± 1.74	9.25 ± 0.84	29.52 ± 1.02	0.132 ± 0.19

[a]FEN SEDDS: SEDDS of fenofibrate without lactoferrin, FEN Lf-SEDDS: SEDDS of fenofibrate with lactoferrin.

https://doi.org/10.1021/acsomega.3c06645
ACS Omega 2024, 9, 13612−13620

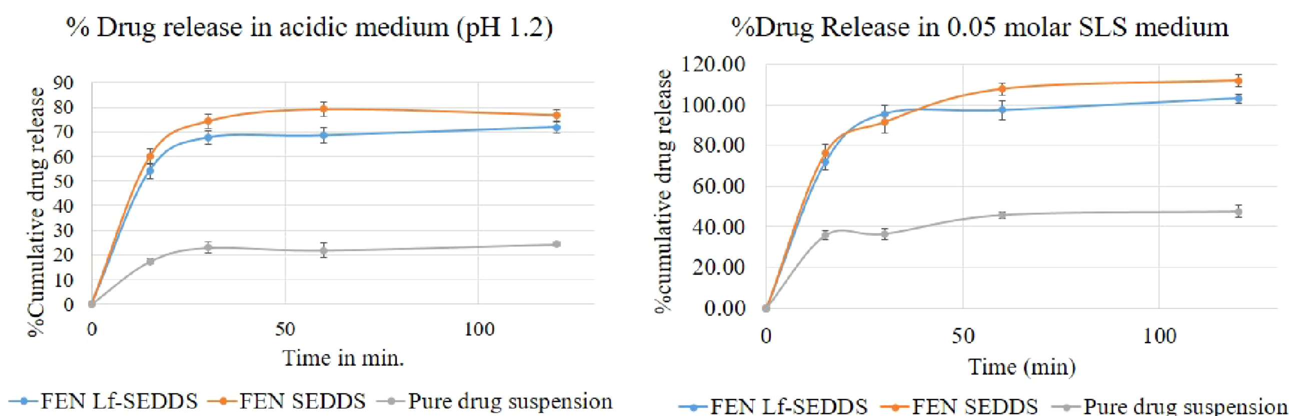

Figure 2. Percent drug release of fenofibrate in different mediums from different samples (vertical bars show standard deviation, n = 6).

Table 4. Similarity Factor (f_2) and Dissolution Efficiency (%DE) of Different Fenofibrate Formulation[a]

sample name	in drug release medium (0.1 N HCl)			in drug release medium (0.05 Molar SLS)		
	f_2 value between FEN SEDDS and FEN Lf-SEDDS	%DE (60 min)	%DE (120 min)	f_2 value between FEN SEDDS and FEN Lf-SEDDS	%DE (60 min)	%DE (120 min)
FEN SEDDS	56.70	57.02	67.51	55.93	58.26	79.94
FEN-Lf ME		50.99	60.60		56.60	74.25
pure FEN suspension		16.55	19.88		24.16	33.61

[a]FEN SEDDS: SEDDS of fenofibrate without lactoferrin, FEN Lf-SEDDS: SEDDS of fenofibrate with lactoferrin.

on the composition. The high zeta potential of the droplets creates electrostatic repulsion and prevents aggregation. In colloids with low zeta potential, attraction between droplets may increase, resulting in aggregation and a size increment. A zeta potential value of + or −30 mV indicates a stable colloidal dispersion. In this research, FEN SEDDS and FEN Lf-SEDDS had a negative zeta potential of 16.63 and 9.25 mV, which are lower than the standard acceptable range. However, unlike micro- or nanoemulsion, SEDDS is free of water and hence, the possibility of droplet aggregation during storage is not there.

The droplet size of the microemulsion significantly affects the dissolution and absorption of a drug. Lower the droplet size, higher the surface area, and better the dissolution. Hence, it is essential to measure the droplet size of SEDDS after emulsification. As presented in Table 3, FEN Lf-SEDDS displayed significantly larger average droplet size than FEN SEDDS, although both are within the conventional range of microemulsion droplets (10−300 nm).[23] The presence of lactoferrin in the SEDDS could be a reason for larger droplet sizes. Both SEDDS showed an acceptable polydispersity index (PDI) (0.1−0.355). Low PDI indicates narrow size distribution, which is beneficial for storage stability, dissolution, and absorption.

2.8. Drug Content. Drug content present in the FEN SEDDS was determined by a UV spectroscopy method. FEN SEDDS and FEN Lf-SEDDS resulted in drug contents of 85.8 ± 2.9 and 91.6 ± 1.7%, respectively. There was no significant difference in the drug content between the two SEDDS.

2.9. Solidified SEDDS. SEDDS is a thick, oily liquid, which could be delivered by soft gelatin capsules (Sandimmune Neoral, Norvir, Rocaltrol, Convulex) and hard gelatin capsules (Lipirex, Gengraf).[24] However, the liquid nature of SEDDS remains a constraint toward its commercial success. Conversion of liquid SEDDS to solids not only makes downstream processing and final dosage form preparation easier but it also provides other array of benefits. Solidified SEDDS can provide a longer duration of drug release when combined with suitable release controlling polymer such as hydroxyl propeyl methyl cellulose.[25] Solid SEDDS improves intestinal drug solubility and dissolution by stabilizing super saturated drug state in intestine and modulating lipid excipient digestion.[26] Solid carriers of SEDDS can be combined with a precipitation inhibitor to maintain the in vivo supersaturated drug state. Another important aspect of using a porous carrier to solidify SEDDS is improved drug loading and higher stability. Nucleation and crystallization of lipophilic drug are hindered when it is adsorbed into the porous structure of carrier and retained in molecularly dispersed or dissolved state. Previous study with lovasatatin SEDDS solidified with Aerosil showed a synergistic drug dissolution and improved bioavailability.[27] In another research, Meola et al. have shown significant enhancement in simvastatin oral bioavailability by formulating lipid-based hydrophilic silica containing hybrid formulation.[28] In this research, we have screened two porous silica carriers (Neusilin UFL2 and Aerosil 200) and one nonporous adsorbent (calcium carbonate) for solidification of FEN-loaded SEDDS. The aim of solidified formulation was to achieve satisfactory flow property and compressibility with the least percentage of adsorbent. The result showed that Aerosil 200 (0.67 g/mL of liquid) is required in the least quantity, followed by Neusilin UFL 2 (1.35 g/mL of liquid) and calcium carbonate (2.30 g/mL of liquid) for passable Carr Index value (21−25). Hence, based on the reported benefits in dissolution and acceptable flow property/compressibility, Aerosil 200 was selected as the adsorbent to convert liquid SEDDS to solid granules.

2.10. In Vitro Dissolution of Solidified FEN SEDDS. Comparative dissolution study between solid SEDDS containing lactoferin (FEN Lf-SEDDS) and without Lactoferrin (FEN

https://doi.org/10.1021/acsomega.3c06645
ACS Omega 2024, 9, 13612−13620

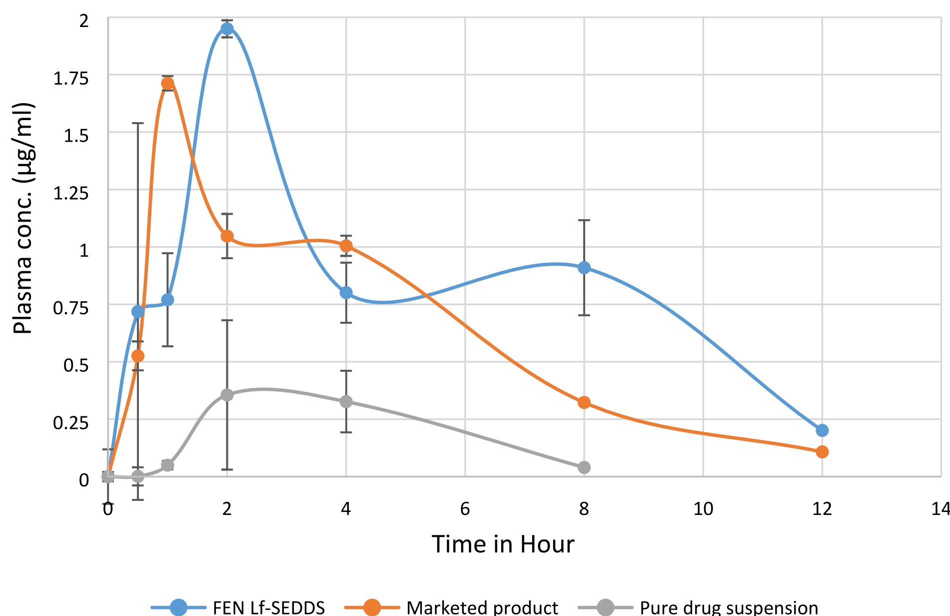

Figure 3. Plasma concentration vs time profile of fenofibrate in different formulation-treated animal models (n = 3).

SEDDS) was done. Cumulative % drug release vs time in 0.1 N HCl and 0.05 molar SLS medium is presented in Figure 2. Similarity factor (f_2), %DE at 60 min, and %DE at 120 min are presented in Table 4. Both self-emulsifying systems containing FEN displayed significantly better dissolution than the pure drug. At 1 h, FEN SEDDS showed almost 80% of drug release in 0.1 N HCl, which is slightly higher than FEN Lf-SEDDS (68.54%) but significantly higher than pure FEN (21.88%). Enhancement of dissolution from the self-emulsifying system is due to several factors, such as the formation of smaller droplets with high surface area and increased wettability due to the presence of surfactant.[29] However, at 2 h in an acidic medium, complete dissolution of the drug was not achieved from either FEN Lf-SEDDS (71%) or FEN SEDDS (76%). Very poor solubility of FEN in the acidic medium is a reason for such an incomplete release. As observed in Table 4, f_2 values in 0.1 N HCl medium between FEN SEDDS and FEN Lf-SEDDS were above 50, indicating a similar drug release profile. DE% at 120 min displayed 67.51 and 60.60 values from FEN SEDDS and FEN Lf-SEDDS, respectively. FEN SEDDS without lactoferrin resulted in slightly higher (p-value < 0.05) %DE than lactoferrin-containing system. It could be correlated with a relatively lower droplet size of FEN SEDDS than FEN Lf-SEDDS after forming microemulsion inside the medium. DE% was more than three times higher in FEN SEDDS than the pure drug suspension. Hence, it can be said that the self-emulsifying system improved the in vitro drug release profile of fenofibrate in an acidic medium.

FEN dissolution was checked into an aqueous medium with 0.5% SLS as this is the official dissolution medium of FEN capsule as per the USP-NF monograph.[30] Complete dissolution of the drug was achieved in this medium from both self-emulsifying systems (FEN SEDDS and FEN Lf-SEDDS). As observed in Figure 2, within 30 min >80% and at 120 min ≈100% of the drug was released from both FEN SEDDS and FEN Lf-SEDDS. However, pure drug displayed a maximum dissolution of 33.61% in the SLS medium. f_2 value indicates a similarity in drug release profile between FEN SEDDS and FEN Lf-SEDDS. %DE at 120 min of FEN SEDDS and FEN Lf-SEDDS were 2.2 and 2.4 times higher than pure drug. The values indicate that SEDDS can effectively increase the drug dissolution in SLS containing an aqueous medium. Higher dissolution occurred for several reasons: (i) fine droplet size provides higher effective surface area and better contact with the medium, and (ii) the presence of surfactant increases the wettability of fenofibrate in the aqueous medium. As FEN is a BCS class II drug, it can be assumed that improvement in FEN dissolution will also increase its bioavailability.

2.11. In Vivo Pharmacokinetic Study. The plasma concentration vs time profile of FEN studied in the rat model is presented in Figure 3. No drug is detected at 24 h in the plasma samples of marketed product and pure drug suspension treated groups. Hence, all the pharmacokinetic parameters were calculated based on a 12 h profile. AUC_{0-12}, $AUC_{0-\alpha}$, C_{max}, t_{max}, and k_{el} of all the three samples (FEN Lf-SEDDS, FEN suspension, and FEN marketed product) are given in Table 5. In the in vitro drug release study and physicochemical characteristics, FEN Lf-SEDDS showed comparable results with FEN SEDDS. Therefore, in the in vivo study, we used only FEN Lf-SEDDS and compared it with a marketed product and pure fenofibrate suspension. The results showed a significant increment (p-value <0.05) in C_{max} and AUC s in FEN Lf-SEDDS than the pure drug or marketed product. The

Table 5. In Vivo Pharmacokinetic Parameters of Different Sample-Treated Animal Groups

sample	C_{max} (μg/mL)	t_{max} (h)	K_{el} (/h)	AUC_{0-12} (μg·h/mL)	$AUC_{0-\alpha}$ (μg·h/mL)
EN Lf-SEDDS	1.95	2	0.360	10.30	10.40
marketed product	1.71	1	0.303	7.64	7.99
pure drug suspension	0.356	2	0.127	1.63	1.94

https://doi.org/10.1021/acsomega.3c06645
ACS Omega 2024, 9, 13612−13620

intensity of absorption, determined by the C_{max}, was the highest in the self-emulsifying system, followed by the marketed product and pure drug. However, the rate of drug absorption was a little faster in the marketed product, as indicated by t_{max}, compared to self-emulsifying systems and the pure drug. Relative bioavailability, calculated with $AUC_{0-\alpha}$ of FEN Lf-SEDDS was 130% of the marketed product and 534% of the pure drug suspension. The acceptance limit of bioequivalence of a test product is 80−125% of that of the reference. In this case, the developed FEN Lf-SEDDS showed higher relative oral bioavailability (1.3 times) than the marketed product, which is just above the bioequivalence limit. If this formulation with enhanced bioavailability is to be translated in application to patients, then a preclinical dose response study is required to adjust the therapeutic dosage. Several reasons can be postulated for the enhanced oral bioavailability. The self-micemulsifying system, after entering the gastrointestinal medium, forms fine droplets of microemulsion spontaneously. We assume that FEN SEDDS was able to deliver the drug in a noncrystalline presolubilized state. Hence, it provides better absorption than crystalline form of marketed FEN. Additionallly, the presence of silica as nanostrauctured porous carrier increases lipid surface area and facilitates lipid−lipase interaction favoring higher in vivo drug dissolution.[28] It has been observed by other researchers that solid Luteolin self-emulsifying drug delivery system resulted in better oral bioavailability than its liquid counterpart or pure drug.[31] A supersaturated zone occurs once a drug dissolves at a higher rate and extent from a self-emulsifying system. In a solid self-emulsifying system, the adsorbent restricts drug precipitation from the supersaturated state and promotes absorption. This effect could be more pronounced if a precipitation inhibitor is used in addition to the porous carrier. Another possible reason for enhanced absorption from FEN Lf-SEDDS is due to the structural formation of medium-chain triglyceride present in the oil component of the self-microemulsifying system.[29] Tween and Lactoferrin can increase the FEN permeability through the intestinal epithelium. Surfactants also help bioavailability improvement by inhibiting pGP efflux transporter activity.[5]

3. CONCLUSION

The reduction of surfactant concentration in a SEDDS can be critical for its self-emulsification ability, particle size, and stability. The present research work establishes the use of lactoferrin, a natural emulsifier, as a partial substitute for Tween 80 and Span 80 in FEN SEDDS. A stable SEDDS (microemulsifying) was developed using lactoferrin along with reduced quantity of S_{mix}, which resulted in comparable particle size, zeta potential, thermodynamic stability, and drug content. The liquid SEDDS was converted to solids by adsorbing it on Aerosil 200 for easy downstream processing and better dissolution. In vivo pharmacokinetic study displayed almost 10 times enhanced oral bioavailability in lactoferrin containing SEDDS compared to pure fenofibrate suspension. The study presents a proof of concept for the use of lactoferrin as a part of an emulsifier cum stabilizer system for self-emulsifying drug delivery. Earlier it was observed that lactoferrin can modulate gut microbiota positively by helping the growth of gut inhabitant bacteria. Hence, the use of lactoferrin in SEDDS can not only reduce the adverse effect of synthetic surfactant but also help the functioning of gut microbiota. Researchers can further explore the dual effect of lactoferrin, as a natural stabilizer and a gut microbiota regulator in the SEDDS system.

4. EXPERIMENTAL SECTION

4.1. Materials. Spray-dried bovine lactoferrin powder (pinkish white, free-flowing, purity >95%, moisture <5%) was procured from lactoferrin.Co, Australia. Fenofibrate IP (white crystalline powder, assay: 99.6%, loss on drying: 0.19%) was generously provided by Sun Pharma Laboratories Limited, India. SR Crodamol GTCC (super-refined synthetic medium chain triglyceride, colorless, odorless liquid, specific gravity: 0.95, viscosity: 25 mPass) was received as a generous gift from Croda India Ltd., Mumbai. Tween 80 was procured from Research-lab Fine Chem Industries, Mumbai. Edible grade sunflower oil, soyabean oil, and castor oil are procured from local market. Transcutol P was procured from Sigma-Aldrich, USA. All other reagents used in this research were of chemical and analytical grade. Double distilled water was collected from the distillation system of the research lab.

4.2. Selection of Oil. Solubility study was used to select the most suitable oil with the highest drug solubility. Briefly, an excess amount of the drug (FEN) was taken in an Eppendorf tube containing 1.5 mL of different oils (Crodamol GTCC, sunflower oil, soyabean oil, and castor oil), cyclo-mixed, and kept in an orbital shaker (100 rpm, 48 h) at room temperature. After completion of shaking, the samples were centrifuged, and the supernatants were filtered through a 0.22 μm syringe filter. The filtrates were diluted accordingly in methanol and analyzed by UV spectroscopy at a 287 nm wavelength for quantitative estimation of drug content in the oil sample. A predeveloped standard curve was used for quantitation.

4.3. Selection of Surfactant−Cosurfactant by Pseudoternary Phase Diagram. Tween 80 was taken as surfactants initially due to its nonionic nature, low reported toxicity, and the highest miscibility with fenofibrate. To select the most desirable surfacatant−cosurfactant ratio, a pseudoternary phase diagram was constructed by the aqueous titration method. Oil chosen from a previous study was used for the phase diagram. Tween 80 individually was mixed with different cosurfactants (Transcutol P, Span 80) in 1:1, 1:2, and 2:1 ratios to prepare S_{mix}. Then the oil and S_{mix} were added in various volume ratios involving 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 in small transparent test tubes followed by cyclomixing. For every S_{mix} and oil ratio, aqueous titration was done with gradual increments (5% v/v) of water up to 95% of the total volume. After each portion of water addition, cyclomixing and subsequent visual observation of the tubes were performed to check transparency or turbidity. The volume of added water, until a visually translucent emulsion is formed, is recorded and entered into the Triplot 4.1 software to construct the pseudoternary phase diagram.

4.4. Formulation of Drug-Loaded SEDDS With and Without Lactoferrin. Based on the phase diagram, a desirable Smix combination and ratio were selected. Drug was dissolved in the oil chosen (SR Crodamol GTCC). S_{mix} was prepared by using the selected surfactant−cosurfactant combination. Oil-containing drug and S_{mix} were mixed vigorously in a cyclomixer for 2−3 min, followed by sonication in a bath sonicator for 6 min. Lactoferrin containing SEDDS was prepared similarly, except for the preparation of S_{mix}. Lactoferrin was mixed with Span 80 (selected from the phase diagram), followed by the addition of tween to it to make the S_{mix}. The rest of the process was the same. The final

https://doi.org/10.1021/acsomega.3c06645
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composition of drug-loaded SEDDS with and without lactoferrin is listed in Table 2.

4.5. Thermodynamic Stability. Thermodynamic stability of both SEDDS was determined by centrifugation and heat stress (heating−cooling cycle). The chosen formulation was centrifuged at 8000 rpm for 30 min and observed for visual phase separation, creaming, or cracking. The formulations with no phase separation were stored at 4 and 45 °C for 48 h and visually observed for clarity, phase separation, and drug precipitation.[32]

4.6. Percentage Transmittance. UV spectroscopy was used to measure the percentage transmittance. SEDDS samples were diluted 100 times in water, and absorbance was measured at a 550 nm wavelength. Percentage transmittance was calculated using the following eq 1:[33]

$$\text{Transmittance} = (1 - \text{Absorbance}) \times 100 \qquad (1)$$

4.7. Robustness to Dilution. The robustness of SEDDS to dilution was studied by diluting it 50, 100, and 1000 times with 0.1 (N) HCl pH 1.2, distilled water, and phosphate buffer (pH 6.8). After storing the diluted samples for 24 h, they were visually observed for phase separation or drug precipitation.

4.8. Dispersibility. The experiment aimed to assess the self-emulsification effectiveness of the developed SEDDS. 1 ml of SEDDS (1 mL) was dispersed in 500 mL of deionized water and 0.1 (N) HCl pH 1.2, separately with constant magnetic stirring (speed 50 rpm at 37 °C). The time required for the sample to form a clear transparent dispersion was noted.

4.9. Viscosity. The viscosity of the prepared formulations was measured by a rotational viscometer at 100 rpm with spindle no. 63 (Brookfield Engineering Laboratories, LVDV-III U).

4.10. Determination of Droplet Size and Zeta Potential. The mean droplet size, size distribution, and zeta potential of the formulated SEDDS were determined by a Malvern Zetasizer (Malvern, NanoZS 90). The samples were diluted 50 times with deionized water before analysis.

4.11. Cloud Point Measurement. In a conical flask, SEDDS was diluted 100 times in deionized water and kept on a hot plate. The temperature of the hot plate was gradually increased by 5 °C increment. Cloud point temperature is the temperature at which transparent emulsion becomes visually cloudy.[32]

4.12. Drug Content in Liquid SEDDS. SEDDS equivalent to 0.5 mg of FEN was taken in a volumetric flask and diluted to 100 mL of methanol. The flask was then sonicated in a bath sonicator at room temperature for 30 min. Aliquots were taken and filtered through a 0.45 μm syringe filter, and absorbance was measured by UV spectroscopy at 287 nm. % drug loading was calculated using the following eq 2:

$$\%\ \text{drug content} = \frac{\text{Derived drug content in sample}}{\text{Theoretical drug content}} \times 100 \qquad (2)$$

4.13. Solidification of SEDDS. Liquid SEDDS was solidified for easy administration by adsorbing it onto a suitable adsorbent. 1 mL of liquid sample was gradually added to 1 g of adsorbent (calcium carbonate/Aerosil 200/Neuselin UFL2) with continuous manual mixing by a mortar to convert them into granules. The physical properties of the granules were monitored visually for dry/wet mass and by flow property analysis.

4.14. Drug Content in Solidified SEDDS. FEN content in solidified SEDDS was measured by a method similar to that mentioned in the previous section (drug content in liquid SEDDS).

4.15. In Vitro Dissolution of Solidified SEDDS. In vitro dissolution with solidified SEDDS was done in a USP type II apparatus. Solidified SNEDDS equivalent to 120 mg of fenofibrate from each sample (FEN SEDDS, FEN Lf-SEDDS, FEN API, and marketed FEN tablet) was added separately in 900 mL of dissolution medium (0.1 N HCl and 0.05 molar sodium lauryl sulfate, SLS). The dissolution medium was maintained at 37 ± 0.5 °C with a 100 rpm stirrer speed. At regular intervals of 15, 30, 60 and 120 min, aliquots (2 mL) were withdrawn, filtered through a 0.45 μm syringe filter, and measured for quantitative estimation of FEN by UV spectrophotometer at 287 nm. The cumulative percent drug release concerning time was plotted from the quantitated data. Dissolution efficiency at 60 and 120 min (DE60% and DE120%) was calculated using the following eq 3:[34]

$$\text{DE} = \frac{\int_0^t y\, dt}{y_{100} \times t} \times 100 \qquad (3)$$

where y = area under the dissolution curve from time 0 to 't,' y_{100} is 100% drug release at time t.

The derived values were compared among all three samples. Similarity factor (f_2) of in vitro dissolution profile was determined between FEN SEDDS and FEN Lf-SEDDS using the following eq 4:[34]

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n} (R_j - T_j)^2\right]^{-0.5} \times 100\right\} \qquad (4)$$

where n is the sampling number and R and T are the percent dissolved of the reference and test products at each time point j, respectively.

4.16. Pharmacokinetic Analysis. In vivo pharmacokinetic study was done in healthy Wistar Albino rats to compare the relative bioavailability of orally delivered lactoferrin containing FEN SEDDS with the marketed tablet and FEN suspension. The study was approved by the Animal Ethics Committee, SPPSPTM, and SVKM's NMIMS (Approval No: CPCSEA IAEC p-47 | 2022). The rats (6−7 weeks of age and 220−250 g) were randomly divided into groups and kept in wire cages (four per cage) in a controlled environment (24 ± 2 °C; 55 ± 10% relative humidity) for 2 weeks of acclimatization before experimentation. Standard dry food and water were provided regularly throughout this time.

The rats were divided into three groups. Animals of group I received marketed FEN tablet, group II received FEN-loaded lactoferrin containing SEDDS (FEN Lf-SEDDS), and group III were given FEN suspension in water. Animal dose of FEN (mg/kg body weight) was calculated using the following eq 5:[35]

$$\text{AED} = \text{HED} \times \frac{\text{Km (Human correction factor)}}{\text{Km of animal of 250 gm body weight}} \qquad (5)$$

where AED is the animal equivalent dose, and HED is the human equivalent dose.

After administering the dose to the rats by oral gavage, blood samples were collected from the retro-orbital artery at an

interval of 0.5, 1, 2, 4, 8, 12, and 24 h. Plasma was separated from the collected blood samples by centrifuge (8000 rpm, 10 min). FEN was extracted from plasma samples by liquid–liquid extraction method. 90 μL of plasma was transferred to a fresh Eppendorf tube, and 10 μL propranolol (Internal Standard, IS) was added to it. The final concentration of propranolol was maintained at 20 μg/mL in all samples. After mixing the plasma and IS by cyclomixer, 900 μL methanol was added to each tube, followed by vigorous mixing for 5 min and centrifugation at 8000 rpm for 20 min. The supernatant organic layers were separated and evaporated under nitrogen. The dried residue was reconstituted with the mobile phase and subjected to HPLC-UV analysis.

A predeveloped and verified reversed-phase HPLC method coupled with UV detection (275 nm) was used to quantify FEN in the plasma sample. The method parameters were as follows; column: Intensil C18 (250 mm × 4.6 mm, 5 μm particle size), mobile phase: 20 mM ammonium acetate buffer: acetonitrile (60:40 v/v), run time: 10 min, injection volume: 40 μL. C_{max}, t_{max} of the drug was derived from FEN plasma concentration vs time profile. AUC_{0-t}, $AUC_{0-\alpha}$, and k_{el} (elimination rate constant) were determined using a non-compartmental pharmacokinetic model approach. Relative bioavailability was calculated using eq 6:

$$\text{Relative bioavailability} = \frac{AUC_{0-\alpha}}{\text{(Sample)}} \times AUC_{0-\alpha}\ \text{(reference)} \quad (6)$$

Pharmacokinetic data were statistically analyzed by two-way ANOVA.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are thankful to the SVKM's NMIMS, Mumbai for providing financial assistance for the research by the SEED grant (11.06.2021).

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ACS Omega 2024, 9, 13612−13620