Quality by design approach to the development of self-microemulsifying systems for oral delivery of teriflunomide: design, optimization, and *in vitro* and *in vivo* evaluation

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Objective
The present study was aimed at the development of a self-microemulsifying drug delivery system (SMEDDS) for the low water-soluble drug using quality by design (QbD) to enhance the bioavailability of drugs.
Experimental work
The components of the SMEDDS were preliminarily screened using the pseudoternary phase diagram as a solubility study. The patient-centric, quality target product profile, and critical quality attributes were earmarked. Preformulation studies were performed along with an initial risk assessment that facilitated the selection of lipids (i.e. Sefsol 218), surfactants (i.e. Acrysol EL-135), and cosurfactants (i.e. PEG 400) as Critical Material Attributes for the formulation of SMEDDS. Extreme vertices mixture design, given its utility and the pertinence to the design issue in hand, was chosen for the study. The various responses selected for this design were drug release at 20 min (%), transmittance (%), emulsification time (s), and globule size (nm). Eleven distinct formulations were prepared and measured to check the model fit. The optimization and model validation were finished by directing experimental runs.
Results and discussion
Sefsol 218 (oil), Acrysol EL-135 (surfactant), and PEG 400 (cosurfactant) showed the highest solubility. The fourier transform infrared spectroscopy (FTIR) study suggested that there may be no significant difference in the characteristic's peak at a wavenumber of the drug in the presence of excipients. The studies have shown that the application of extreme vertices mixture design and the development of formulation in QbD resulted in a powerful and viable technique for improving the bioavailability of the drug. This was confirmed by the characteristics' studies of the optimized batch like *in vitro* drug release in 20 min (73.44%), drug content (99.3%), emulsification time (25 s), transmittance (99.5%), droplet size (16.64 nm), polydispersibility index 0.170, and zeta potential –9.74 mV. A great agreement was observed among the predicted and experimental values for the average globule size and percentage of the drug released in 20 min. Furthermore, the optimal SMEDDS formulation exhibited fundamentally higher, extreme-plasma concentration, and area under the curve values a twofold higher value ($P<0.05$) than the teriflunomide suspension.
Conclusion
In summary, the present studies report successful QbD-oriented development of a novel oral teriflunomide-loaded SMEDDS formulation to noticeably improve the bioavailability of low water-soluble drugs.

Keywords:
extreme vertices mixture design, *in vivo* study, quality by design, risk estimation matrix, self-microemulsifying drug delivery system, teriflunomide, ternary phase diagrams

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Introduction

Teriflunomide, or TEF, *is* an analog of leflunomide known for treating rheumatoid arthritis. TEF is an oral pyrimidine synthesis inhibitor used for relapsing forms of multiple sclerosis. It has broad immunosuppressive effects, including a cytostatic development on proliferating T and B lymphocytes. TEF is teratogenic in rats and rabbits. Therefore, it is contraindicated in pregnant women and women of childbearing potential. TEF becomes maximally absorbed in mice, rats, rabbits, and dogs within 1 h, 6 h, 4–8 h, and 1–4 h, respectively, culminating in levels of 36 μg/ml, respectively [1,2]. The lymphocyte proliferation manifests the immunomodulatory properties of TEF. TEF is approved as a first-line

treatment of relapsing–remitting multiple sclerosis by the Food and Drug Administration, European Medicines Agency, and Swiss medic (the Swiss Agency for Therapeutic Products).

SMEEDS is one of the most helpful systems to water-insoluble drugs countering the drug's inadequate absorption of water [3,4]. Self-microemulsifying drug delivery system (SMEDDS) formulations contain anhydrous isotropic mixes of oil, surfactant, and cosurfactant that form a fine droplet (1–100 nm) of oil-in-water emulsions after dilution in an aqueous medium and with gentle agitation in the gastrointestinal tract [5]. Spontaneous development of a microemulsion conveys the drug in a solubilized form. In addition, SMEDDS' small globule size permits the quick dissolution of the drug and enlarges the surface area for absorption. This reduces the irritation produced by the contact of the drug in the gastrointestinal tract and improves its permeation across the intestinal tissue. Moreover, the drug, solubilized in oil drops, is carried by lymphatic transport through the digestive tract keeping it away from the first-pass metabolism in the liver. In addition, SMEDDS are steady formulations that are not difficult to make. The self-emulsification measure is expressed to the particular pair of oil and surfactant, surfactant concentration, oil/surfactant proportion, and the temperature at which self-emulsification occurs [6–10].

The extreme vertices mixture design (EVMD) is the most famous response surface approach for optimizing the SMEDDS design because it limits the difference related to the assessment of coefficients in a model, and delivers the ideal subset by considering the standards for augmenting data matrix determinants [6,8,11–13]. Quality can be planned, and most of the quality deficiency occurs when the process is planned and developed. The quality expert Joseph Moses Juran introduced quality by design (QbD) and its application in product development. The principles of QbD have been used in every industry to improve the quality of products and processes. In addition, well-controlled and reproducible outcomes produce the necessary therapeutic objectives of the formulation by the exact strategy called QbD [8,14–16].

In this study, TEF-loaded SMEDDS formulations were prepared and optimized by a Design of Experiments approach called EVMD. It is a mathematical technique used to select the framework's components (experimental inputs) that will produce the most insignificant impact on the product inconstancy. The Mixture Design was created using JMP Software 16 version (SAS Institute, Cary, North Carolina, USA). The Critical Material Attributes (CMAs) chosen for the examination were oil (Sefsol 218), surfactant (Acrysol EL-135), and cosurfactant (polyethylene glycol 400 or PEG 400). The critical process parameters considered conditions like the mixing speed, type of stirrer, and the mixing temperature. The critical quality attributes (CQAs) selected included globule size, emulsification time, drug loading, polydispersibility index (PDI), transmittance, and an *in vitro* drug release study. The concept of building quality into a product through the QbD application was used to develop TEF-loaded SMEDDS.

Materials and methods

Materials

The TEF was procured from Zydus Cadila Healthcare Limited, Vadodara. Labrasol, Transcutol CG, Transcutol P, MCT, Labrafac P, Capryol 90, Capmul PG 8 NF, and Labrafil M 2125 were acquired from Gattefosse, France. Capex 200 P, Capmul MCM, and Captex 355 were received from Abitech Corporation, United States. Acryl EL-135, Acrysol K 160, Acrysol K 140, and K150 were purchased from Corel Chemical Pvt Ltd, Ahmedabad, Gujarat, India. The sunflower oil, sesame oil, canola oil, and olive oil were obtained from Kiran Oil Ltd, Ahmedabad, Gujarat, India. The flaxseed oil/linseed oil, soybean oil, and coconut oil were acquired from Kush Proteins Ltd, Anand. The Sefsol 218 (mono caprylic ester) was obtained from Nikko Chemical Co. Ltd, Japan. IPM Kollicream was supplied by BASF, Germany. Tween 80, Span 80, Tween 20, and PEG were obtained from Finer Chemical Limited, Ahmedabad, India. All materials and chemicals used in this research project are of analytical quality. Deionized water was used throughout the study.

Animals

The animals used for the tests were healthy, precise, pathogen-free adult male Sprague–Dawley rats weighing from 220 to 250 g. The rats were purchased from Zydus Cadila Healthcare Ltd, Ahmedabad, India. The rodents' house was kept at a constant room temperature of 25±2°C with an air humidity of 50±10% and a light/dark cycle of 12 h. The rats were permitted constant access to a quality diet of mouse crackers and water throughout the study. In addition, the rats were allowed to adjust for 14 days before treatment. All procedures for animal care and use were conducted with the National Institute of Health instructions for the Care and Use of Laboratory Animals publication #85–23, revised

1996 [5,17]. The Institutional Animal Ethics Committee approved the animal experiment (CPCSEA No approval No./08/05-11-2020/ CPCSEA).

Experimental methods

Defining quality target product profile and critical quality attributes

The quality target product profile (QTPP) tactic establishes a planning system for product development, and it begins with 'plan as a primary concern' to ensure product efficacy and safety. QTPP precisely outlines the characteristics expected during the product's development to respond to the therapeutic objective of the drug. The aim of quality product profile structures is the origin of the pharmaceutical product development [18]. Ultimately, the QTTP forms the root for the development of the product. Through the QbD approach, the product development advocates or requires defining the QTPP, and it is one of the prerequisites to deliver therapeutic benefits as per the label claim. The QTPP for liquid SMEDDS was determined based on the patient-centric (emulsification time, drop size, drug release) and product-centric (zeta potential) quality attributes of the drug product. The CQAs identified from QTPP were interlinked to give the desired quality, safety, and efficacy to the product, showing noticeable changes when QTPP is altered [8,13,19,20]. Patel *et al.* [8] reported QTPP and CQAs.

Risk assessment

Formulation development according to QbD involves the evaluation of material and process properties that have a more significant impact on product quality. A risk assessment is a combined effort to identify and evaluate factors that can affect a product's CQA. Risk assessment tools help you identify and mitigate risks and prioritize them according to their level of impact, high, medium, or low. Table 1 provides a risk assessment matrix for formulating SMEDDS with TEF loading. Two qualitative tools were used in this study: the Fishbone chart and the risk assessment matrix. The Fishbone chart (Fig. 1) was created using JMP Software, version 16 (SAS, SAS Institute) and shows causes and sub-causes affecting CQA. Risk assessment matrices help classify risks [8,15,19].

Drug excipient compatibility study

Samples were analyzed to study the compatibility of drug excipients using an FTIR 8400 s spectrophotometer (Shimadzu, Kyoto, Japan). FTIR TEF and loaded TEF SMEDDS formulations (Sefsol 218, Acrysol EL-135 and PEG 400) were recorded using the KBr mixing method..References to drug excipients play an indispensable role in the release of drugs from formulations. TEF and excipients were preground and mixed with KBr and translucent infrared matrix in a ratio of 1:10 (sample: KBr). KBr disks were installed with a powder packing. Samples were scanned in the range of 400–4000 cm^{-1} [13]. The FTIR spectrum is shown in Fig. 2a.

Preparation of solid self-microemulsifying drug delivery system formulation for FTIR study

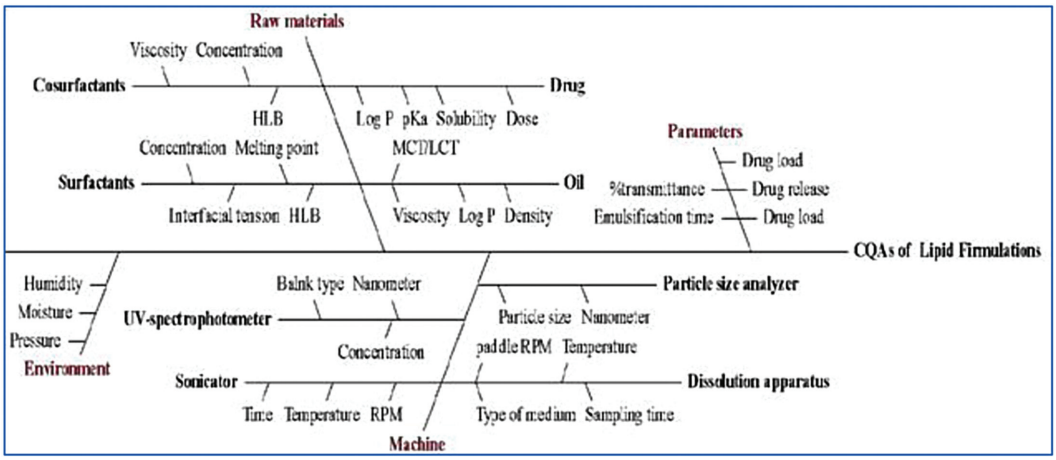
The selected liquid SMEDDS (TEF 40 mg) formulation was mixed with reliable carrier Aerosil 200. The SMEDDS was added dropwise over the solid adsorbent contained in a porcelain dish. With every addition, the mixture was homogenized using a glass rod to ensure uniform distribution of the formulation. Then, the resultant damp mass was passed through sieve no. 120 and dried at 40°C and stored at room temperature until needed for further study. As a result, the resolution of the formulation's spectra is in the frequency range of 4000–400 cm^{-1}, as shown in Fig. 2a and b. Next, small quantities of samples were placed in KBr pellets and positioned

Table 1 Risk-estimated matrix

CMA/CPP CQAs	API particle size	Oil	Surfactant	Cosurfactant	Stirring speed	Stirring time	Stirring temperature
Drug content	Low	High	High	High	Low	Low	Low
Globule size	Low	High	High	High	Medium	Medium	Medium
Zeta potential	Low	High	High	High	Low	Low	Low
Emulsification time	Low	High	High	High	Medium	Medium	Medium
PDI	Low	High	High	Medium	Medium	Medium	Medium
% Transmittance	Low	High	High	High	Medium	Medium	Medium
Drug release in 15 min	Low	High	High	High	Low	Low	Low
	High-risk factor			Medium Risk Factor		Low-risk factor	

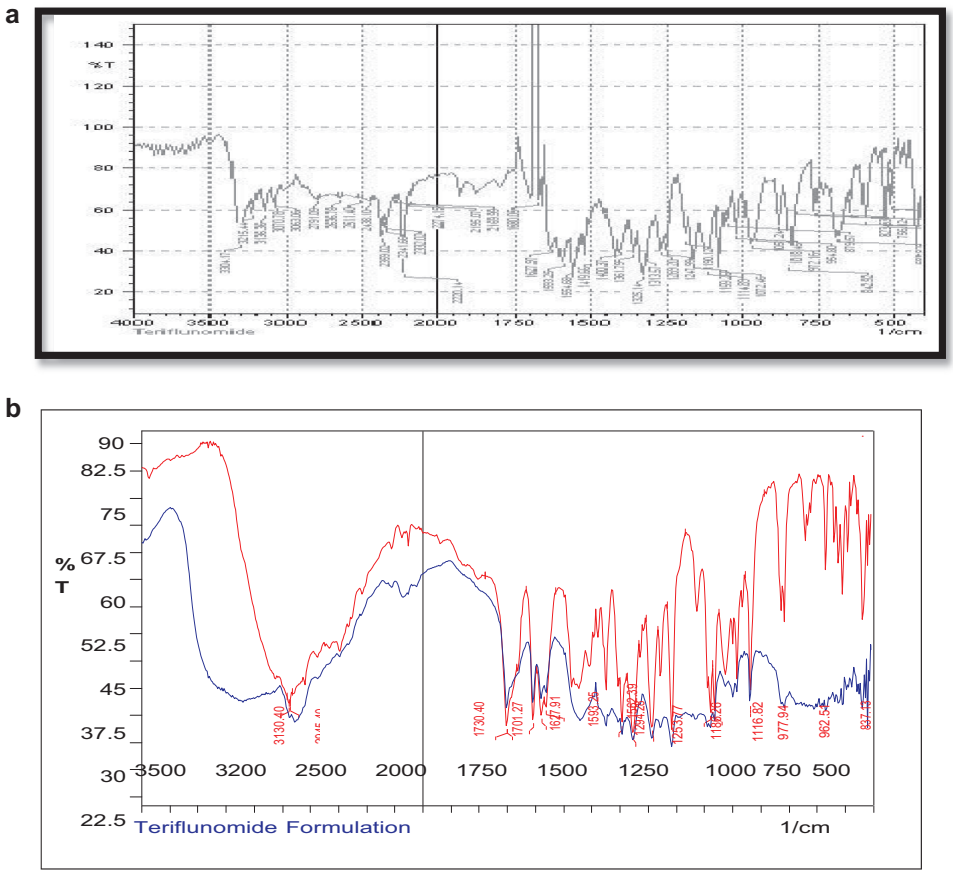
CMA, Critical Material Attributes; CPP, critical process parameter; CQA, critical quality attribute; PDI, polydispersibility index.

Figure 1



Fishbone diagram depicting causes and sub-causes affecting teriflunomide-SMEDDS quality attributes. SMEDDS, self-microemulsifying drug delivery system.

Figure 2



(a) FTIR spectra of teriflunomide and (b) drug-excipient compatibility study.

into the sample holder. The infrared spectrum was acquired by uniform scattering of the sample.

Solubility studies

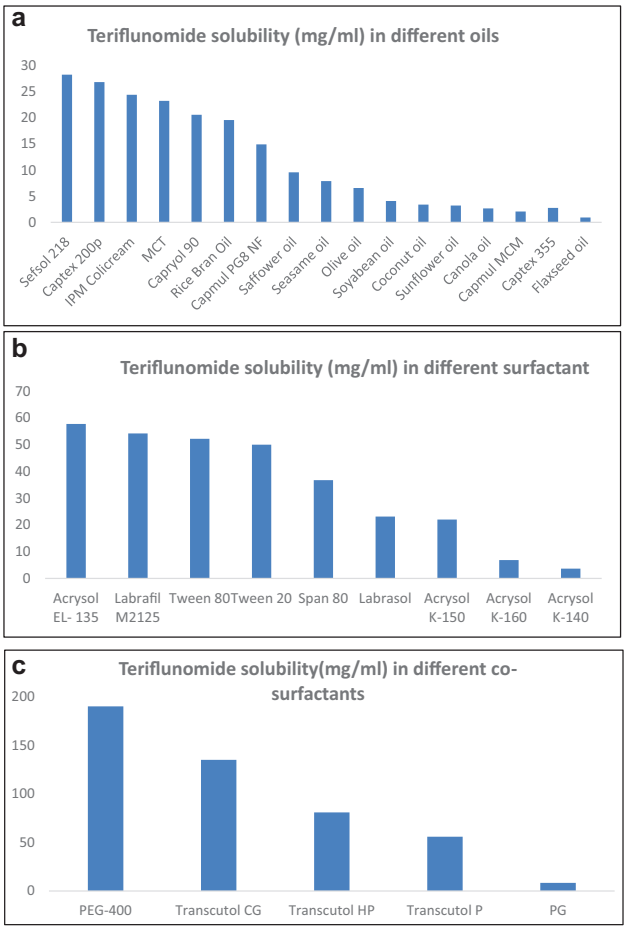
The solubility of TEF in different adjusted oils, surfactants, and cosurfactants was determined. First, an excess amount of TEF was added to 1 g of various vehicles and the mixtures were rotated using a vortex shaker (GL-88B Vortex Mixer, China) for 20 min. Then, the mixtures were shaken at 40±2°C for 48 h in a water-bath shaker (Rotary Shaker, Remi, India) to reach equilibrium. After that, the mixtures were

centrifuged (RM-12C Micro Centrifuge, Remi, India) at 3000 rpm for 20 min. Finally, the supernatant was diluted with methanol and filtered through a 0.45-µm membrane filter. The concentration of TEF in supernatants was measured at 249 nm using the UV double-beam spectrophotometer method [5,8,13].

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams are a tool for screening suitable constituents and identifying the well-suited ratios of components in SMEDDS. Based on the self-emulsifying test grading test results, pseudoternary phase diagrams were developed using the aqueous titration method. Surfactant (Acrysol EL-135) and cosurfactant (PEG 400) were determined. Initially, the weight of the surfactants and cosurfactants were mixed (Smix) in different weight ratios (1 : 1, 1.5 : 1, 1 : 3, 2 : 1, 3 : 1). The oil (Sefsol 218) and specific surfactant/cosurfactant (Smix) ratio were mixed thoroughly in various weight ratios from 9 : 1 to 1 : 9 (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9) in glass vials. One gram of the mixture was titrated with distilled water. The double-distilled water was added in 5% increments in a range of 5–95% of the total volume. The mixture was stirred in a constant temperature water bath at 37°C until it started to form a clear or light blue or light opalescent liquid. Then the mass fraction of each of the component's mixture was recorded and visual observations were noted. Ternary plot.com was used to construct the pseudoternary phase diagrams [21–23]. The optimum cosurfactant was screened by comparing the microemulsion region of the mixture in pseudoternary phase diagrams (Fig. 3).

Figure 3



Pseudoternary phase diagram: (a) surfactant (Acrysol EL-135)/co-surfactant (PEG 400) ratio (1 : 1) and oil (Sefsol 218), (b) surfactant (Acrysol EL-135)/cosurfactant (PEG 400) ratio (1.5 : 1) and oil (Sefsol 218), (c) surfactant (Acrysol EL-135)/cosurfactant (PEG 400) ratio (2 : 1) and oil (Sefsol 218), (d) surfactant (Acrysol EL-135)/cosurfactant (PEG 400) ratio (1 : 1) and oil (Sefsol 218), (E) surfactant (Acrysol EL-135)/cosurfactant (PEG 400) ratio (1.5 : 1) and oil (Sefsol 218), (F) surfactant (Acrysol EL-135)/cosurfactant (PEG 400) ratio (2 : 1) and oil (Sefsol 218).

Formulation of self-microemulsifying drug delivery system

The formulations were prepared by mixing 7 mg of TEF in oil, surfactants, and cosurfactants at 37°C temperature. The mixture was vortexed for 24 h to result in a clear solution. The resulting mixture was stored at room temperature for further use. The formulations were examined for signs of turbidity or phase separation before self-emulsification and particle size analysis studies.

Formulation optimization of self-microemulsifying drug delivery system using extreme vertices mixture design

Mixture design is a kind of statistical experimental design used for the development and optimizations of the formulations. Mixture design is used in drug-product development when the elements are in proportions of the mixture. EVMD is a constrained mixture design where the mixture ingredients are exposed to imperatives, maximum or minimum for each constituent. The components of the mixture are connected as parts which add up to 1 (100%). The measured response in the mixture tests relies on the available amount of components [8,13,14,24]. The main principle behind the mixture design is numerical to demonstrate the proportions of the mix to predict the response(s) for any mixture in the design and measures the impact of each factor alone or in a mix with different components on the response(s). The CQAs distinguished were considered as responses or area factors chosen for the study. The CQAs are the globule size (nm), emulsification time (seconds), % transmittance, and *in vitro* drug release in 20 min (%). The CMAs (variables) or independent factors chosen for the examination are oil (Sefsol 218), surfactant (Acrysol EL-135), and cosurfactant (PEG 400) [15,25].

EVMD was utilized to optimize the components of the SMEDDS formulation. The experiment was designed to use the three compositions as independent factors. In light of the solubility study and pseudoternary phase outline, the concentration of Sefsol 218 (oil; X1), Acrysol EL-135 (surfactant; X2), and cosurfactant (cosurfactant; X3) were set within ranges of 200–300, 375–500, and 234–500 mg, correspondingly. For any experiment, the amounts of X1, X2, and X3 added up to 100%. We evaluated average globule size (Y1), the percentage of drug released in 20 min (Y2), % transmittance (Y3), and emulsification time (Y4) to determine the optimal 11 SMEDDS formulations with excellent physiochemical characteristics. We used JMP 16 Software versions (SAS Institute) for developing and evaluating the experimental design. The base design allowed 11 experiments to fit a cubic model, checked for lack of fit, and estimated the practical error in the responses (Y1, Y2, Y3, and Y4) listed in Tables 2 and 3. The critical process parameter was not variable in the design due to its insignificant impact on the reactions as shown by the risk estimation matrix. The different formulations (Table 3) acquired according to the design are exposed to the portrayal. Coherent validation of different risk(s) for each of the material attributes and process factors resultant to the individual CQAs are listed in Table 4. The outline of the QbD measures in creating the mixture designs is shown in Figs 4a–c and 5a,b.

Evaluation of self-microemulsifying drug delivery system formulations

Determination of the self-emulsification time

The self-emulsification time of SMEDDS was determined using the standard USP dissolution apparatus II. First, 1 ml of each formulation was added to 200 ml of distilled water at 37±0.5°C. Then, gentle agitation was provided by a standard stainsteel dissolution paddle rotating at 50 rpm. Emulsification time was assessed visually and was completed within 1 min [13,26]. The results of the self-emulsification time are exhibited in Table 5.

Visual assessment

A measure of 1 ml of SMEDDS was diluted with 500 ml of purified water at 37±0.5°C. Gentle agitation was achieved using a standard stainlesssteel dissolution paddle rotating at 50 rpm. According to the self-emulsifying grading system the time is taken after 72 h by each formulation to form a clear homogeneous system noted in triplicates. Based on the product's final appearance, the emulsified formulations were graded as per the following grading system. Grade A: a clear bluish emulsion was obtained within 1 min. Grade B: slightly clear, bluish-white emulsion was formed within 1 min. Grade C: milky emulsion was obtained within 2 min. Grade D: a dull grayish emulsion with an oily appearance and emulsification process took more than 2 min. Grade E: poorly emulsified formulation with large oil globules floating on the surface. [8,13,27] The results are depicted in Table 5.

Transmittance

The transmittance of the optimized SMEDDS preparation was measured using a UV spectrophotometer (UV-1800, Shimadzu). Sample permeability was measured at 650 nm and distilled water was used as a blank to perform three assays. The SMEDDS product was diluted with 100 ml of distilled water to calculate the permeability at 100 µl [28]. The results of the permeation test are shown in Table 5.

Drug content

TEF from SMEDDS formulation was extracted in methanol for 2 h using the sonication technique. The solution was filtered using a Whatman filter paper. The

Table 2 Mixture design components and response

Composition and limits of experimental domain			
		Values	
Critical material attributes	Role	Low	High
Sefsol 218	Mixture	200	300
Acrysol EL-135	Mixture	375	500
PEG 400	Mixture	234	500
Responses in mixture design			
Responses	Goal	Lower limit	Upper limit
Drug release in 10 min (%)	Maximize	80	100
% Transmittance	Maximize	95	100
Emulsification time (s)	Minimize	20	60
Droplet size (nm)	Minimize	50	100

Table 3 Composition of self-microemulsifying drug delivery system as per the extreme vertices mixture design

Formulation	Sefsol 218	Acrysol EL-135	PEG 400
F1	0.2	0.375	0.425
F2	0.2	0.4375	0.3625
F3	0.3	0.375	0.325
F4	0.25	0.375	0.375
F5	0.2532	0.4432	0.3036
F6	0.3	0.466	0.234
F7	0.2	0.5	0.3
F8	0.3	0.4205	0.2795
F9	0.283	0.483	0.234
F10	0.233	0.5	0.267
F11	0.266	0.5	0.234

Table 4 Justification of risk allotment and identification of critical material attributes and critical process parameters

CMAs/CPPs	API particle size	Is it critical?	Justification
API particle size	Drug content	No	Since SMEDDS is a homogeneous system containing the drug molecularly dispersed in a self-emulsifying form, the particle size of the drug may not affect the CQA of the drug. Therefore, the material attribute, particle size, possesses a low risk
	Globule size	No	
	Zeta potential	No	
	Emulsification time	No	
	PDI	No	
	% transmittance	No	
	Drug release	No	
Type of lipid	Drug content	No	The type of lipid used to formulate the SMEDDS has little effect on the designated CQA. Most lipids used in SMEDDS dosage forms are a mixture of mono/triglycerides whose chain length varies between C8 and C10. The type of lipid used does not significantly change the length of change, lipophilicity, or HLB. Therefore, there is a lower threat associated with these types of lipids
	Globule size	No	
	Zeta potential	No	
	Emulsification time	No	
	PDI	No	
	% transmittance	No	
	Drug release	No	
Type of surfactant and cosurfactant	Drug content	No	In SMEDDS formulations, primarily the water-soluble surfactants and cosurfactants are used. Although the surfactant/cosurfactant type has a gentle to direct effect on the chosen quality attributes of the medication product, these were held as low-risk parameters in the formulation
	Globule size	No	
	Zeta potential	No	
	Emulsification time	No	
	PDI	No	
	% transmittance	No	
	Drug release	No	
Amount of lipid, surfactant, and cosurfactant	Drug content	No	It measures lipids, surfactants, and cosurfactants, and is responsible for drug dissolution, droplet size, and emulsification time, as well as implementation of the dissolution rate defined by SMEDDS. Measurement changes in lipids, surfactants, and cosurfactants affect the CQA of most pharmaceuticals. Therefore, these schemes are high-threat frontiers
	Globule size	No	
	Zeta potential	No	
	Emulsification time	No	
	PDI	No	
	% transmittance	No	
	Drug release	No	
Type of stirrer, stirring speed, and stirring time T	Drug content	No	SMEDDS formulations are isotropic mixtures in which the drug present is dissolved in the excipients and lipid emulsifiers. The solubility of the drug is largely due to the amount of lipids, surfactants, and cosurfactants. Process parameters such as the type of stirrer used, the stirring speed, and the stirring time used to mix the drug with the excipients have little effect on the listed CQA. Therefore, the risk involved is considered to be relatively low
	Globule size	No	
	Zeta potential	No	
	Emulsification time	No	
	PDI	No	
	% transmittance	No	
	Drug release	No	

CMA, Critical Material Attributes; CPP, critical process parameter; CQA, critical quality attribute; PDI, polydispersibility index; SMEDDS, self-microemulsifying drug delivery system.

Table 5 Evaluation of self-microemulsifying drug delivery system F1–F11 formulation of mixture design

Formulation code	Visual assessment grade	% entrapment efficiency	Transmittance (%) *n*=3	% drug content	Emulsification time (s) *n*=3	Droplet size (nm)	PDI	Zeta potential
F1	A	96.4	97.09±0.90	98.4	18±1	72.38	0.320	–8.80 mV
F2	A	95.7	98.03±0.70	98.7	22±1	102.23	0.230	–7.46 mV
F3	A	94.69	98.53±0.83	99.3	23±1	35.5	0.310	–6.86 mV
F4	A	97.3	99.5±0.72	99.69	25±1	16.64	0.170	–9.74 mV
F5	A	95.6	98.72±0.71	98.6	27±2	136.65	0.280	–1.29 mV
F6	A	94.03	97.93±0.55	99.03	26±1	135	0.173	–0.144 mV
F7	A	95.4	97.19±0.90	97.4	30±1	802	0.300	–8.70 mV
F8	A	95.5	98.03±0.70	98.5	26±1	94.25	0.210	–7.86 mV
F9	B	92.69	97.23±0.83	94.69	30±1	110.25	0.290	–6.26 mV
F10	A	95.3	97.8±0.72	98.3	29±1	78.25	0.180	–7.74 mV
F11	B	93.1	97.72±0.71	98.1	28±2	95.5	0.290	–2.29 mV

PDI, polydispersibility index.

methanolic extract was analyzed for the TEF content spectrophotometrically (UV-1800, Shimadzu) at 249 nm using a standard curve [8,13]. Results of the % drug content are listed in Table 5.

Robustness to dilution

SMEDDS was diluted to 1000 times with water and pH 1.2 HCL buffer solutions. The diluted microemulsion was stored for 12 h and observed for any indications of phase separation or medication precipitation [8]. Results of robustness to dilution are in acceptable range [8].

Droplet size determination and polydispersibility index

A measure of 1 ml of all formulations was diluted with 100 ml of water in a volumetric flask. The volumetric carafe was reversed twice to guarantee the total dispersion of the formulation. After ensuring wide dissemination of the formulation, the droplet size of the resultant microemulsion was determined by photon connection spectroscopy that examined the variance in light scattering due to the Brownian movement of the globules as a function of time utilizing a Zetasizer Nano sequence (Malvern Instruments, Grovewood Road, Malvern, WR14 1XZ, United Kingdom). Light dispersion was observed at 25 and at 90°C [8,11]. Results are depicted in Table 5 and in Fig. 6a,b.

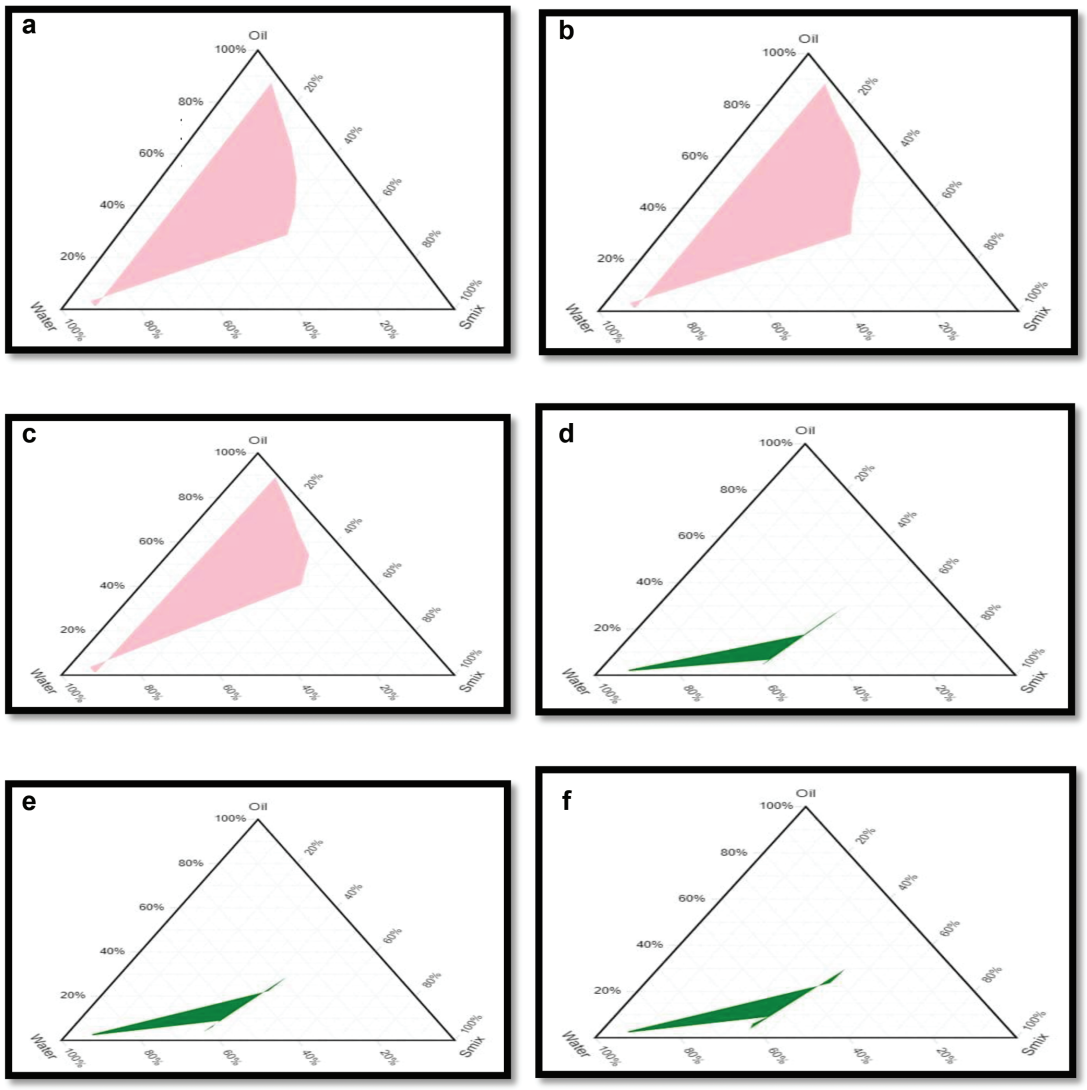
Zeta potential determination

The stability of the emulsion is directly related to the magnitude of the surface charge. One milliliter of SMEDDS was poured into a beaker, diluted 100 times with distilled water, and constantly stirred on a magnetic stirrer. Then, the zeta potential of the SMEDDS was determined using a Malvern Zetasizer [29]. Results are presented in Table 5 and Fig. 6c,d.

In vitro dissolution studies

In vitro release studies of SMEDDS were carried out using a USP dissolution apparatus type-II with a rotating paddle in 900 ml dissolution media (pH 6.8 phosphate buffer solution), at 50 rpm, and maintained at a temperature of 37±0.5°C (900 ml). These studies were to examine the drug release from SMEDDS. The SMEDDS formulation (equivalent to 7 mg TEF) and pure drug (7 mg) were filled in a soft gelation or hard gelatin capsule and introduced into the dissolution medium [28,30,31]. At predetermined time intervals of 5 min (up to 1 h), 10 ml of the samples were withdrawn and filtered through a 0.45 μm Whatman filter paper [8]. At the same time, a new dissolution medium was replaced in the mechanical assembly to keep a consistent volume. The amount of the TEF released into the dissolution medium was determined spectrophotometrically at 249 nm. The dissolution

Figure 4



(a) Actual versus predicted plots for different responses, (b) color map correlation for the screen factors, (c) ternary mixer profiler displaying the impact of formulation components on the responses. Actual versus predicted plots for different responses.

experiments were performed in triplicates [30]. The graph shown in Fig. 7 was plotted to analyze formulations and pure drugs (drug release vs. time).

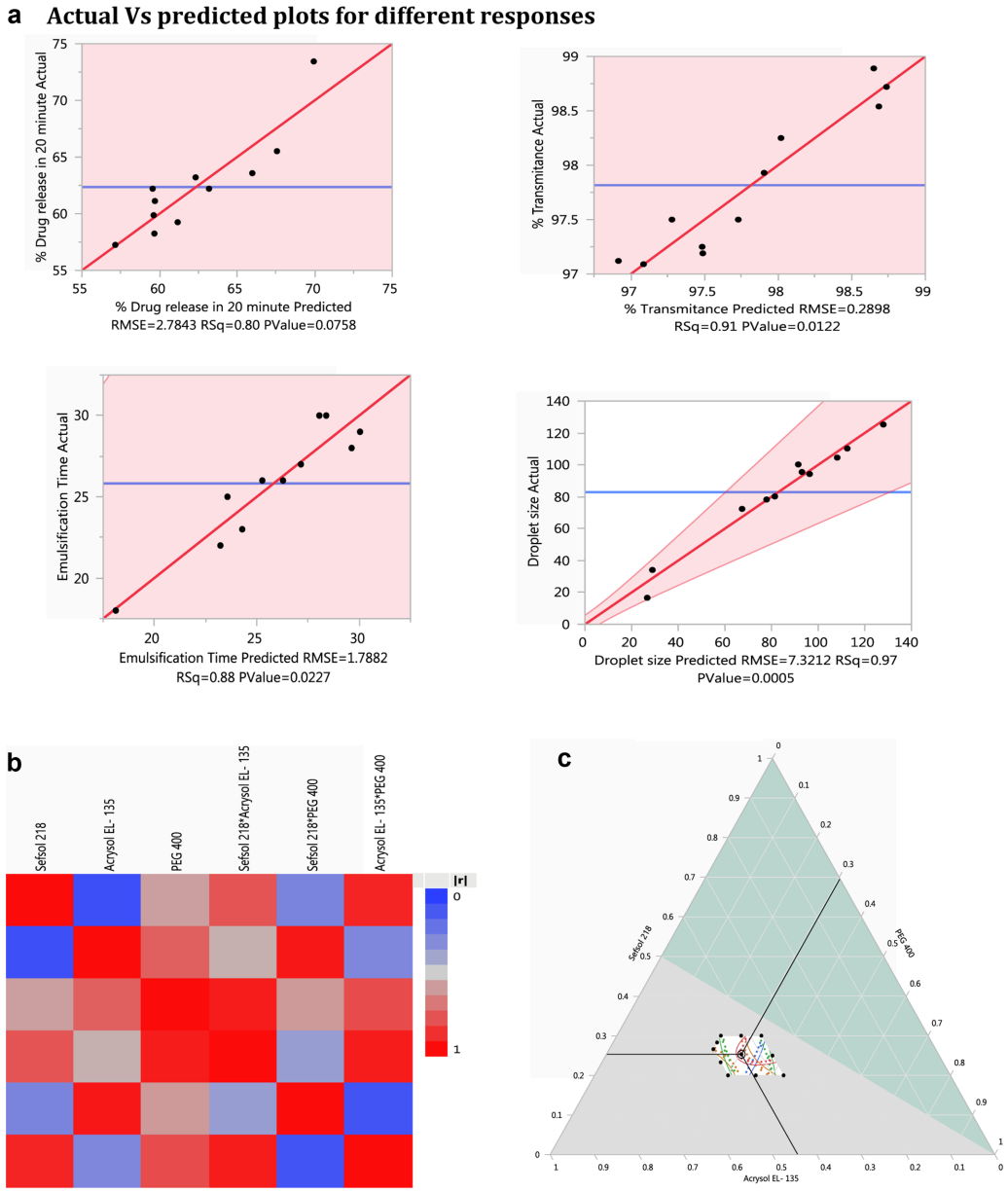
Thermodynamic stability study

The thermodynamic stability of TEF-loaded SMEDDS was observed using the heating–cooling cycle and centrifugation. In the heating–cooling cycle, SMEDDS formulations were stored at a refrigerator temperature of 2–4°C, and at room temperature for at least 48 h and then studied. All those formulations, which were kept constant at this temperature, were subjected to a centrifugation test. In the centrifugation tests, SMEDDS formulations were passed from the heating–cooling cycle and centrifuged at 3500 rpm for 30 min, The thermodynamic stability study showed that phase separations were not found for all the formulations of SMEDDS [8]. In the freeze–thaw cycle, three freeze–thaw processes were performed in temperatures between −21 and +25°C and stored at each temperature for not less than 48 h for all the formulations.

Model verification and optimization

Model verification and optimization were carried out by incorporating the different responses (CQAs) acquired for all 11 formulations were incorporated in the design to check the model fit and for the optimization of the formulation ingredients for the desired responses. The validation of the design was carried out with the help of a ternary mixture profiler. The profiler involves a ternary plot, surface plot for every response, factor (oil/surfactant/cosurfactant), response settings, and control rules [25]. The verification formulation (VF) was prepared as per the ternary mixture profiler and checked for

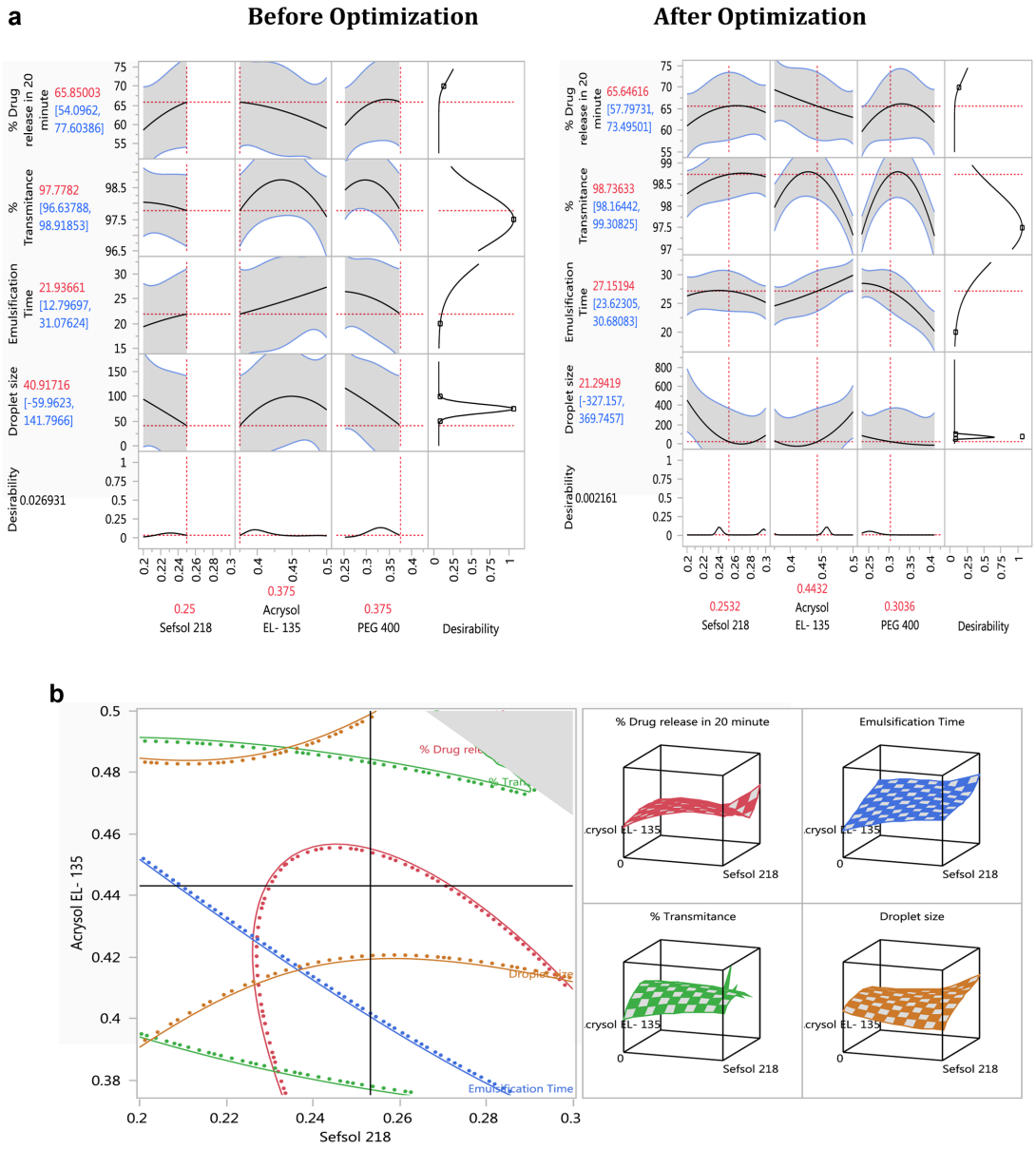
Figure 5



(a) Prediction profiler for multiple responses before and after optimization and (b) contour and surface plots exhibiting the impact of formulation components on responses.

predicted CQAs. The intercombination and intracombination conduct of parts toward the individual responses was introduced in counter-surface plots (Fig. 5b). An attractive, quality capability approach finishes the concurrent optimization of the formulation by mixture design. The general overall desirability was obtained from the attractive individual desirability reached for every response. The worldwide desirability quality capacity value ranges from 0 to 1. The prediction profiler was acquired preimprovement and postimprovement as introduced in Fig. 5a. According to the enhanced prediction profiler, the optimized formulatio was prepared and assessed for responses shown in Table 6. The experimental results acquired for the OF were then compared with the model anticipated responses. The model approval was done through the ternary mixture profiler. The ternary blend profiler gives the ideal space in the ternary graph (Fig. 4c). The individual proportion of oil, surfactant, and cosurfactant inside the perfect region did not influence the dependent factors (responses) of the SMEDDS formulation. The VF was directed according to the mixture design (Table 6). The VF was compared with experimental values and the predicted values. The lack of contrast, in the

Figure 6



(a) Result of zeta potential for the verification formulation-SMEDDS. (b) Results of droplet size and PDI for the verification formulation-SMEDDS. (c) Results of droplet size and PDI for the optimization formulation-SMEDDS, (d) results of droplet size and PDI for the optimization formulation-SMEDDS. PDI, polydispersibility index; SMEDDS, self-microemulsifying drug delivery system.

Table 6 Composition of verification formulation and optimized formulation-self-microemulsifying drug delivery system

Formulation	Sefsol 218	Acrysol EL-135	PEG 400
Verification formulation	0.2532	0.4432	0.3036
Optimized formulation	0.25	0.375	0.375

changes of observed and predicted responses, demonstrates better integrity of fit. Ternary mixture profiler was checked for the predicted CQAs [13].

Transmission electron microscopy

The morphology of the emulsion drop for the optimized TEF-loaded SMEDDS formulation was determined utilizing a transmission electron microscopy (JEM 1010; Jeol Ltd., Tokyo, Japan) with a speed increase voltage of 80 kV. The enhanced SMEDDS formulation was diluted with water (1 : 1000). One drop of the sample was stored on a copper network and dried at 25°C.

In vivo oral absorption study

The Institutional Animal Ethics Committee approved the animal experiment (CPCSEA No approval No./ 08/05-11-2020/CPCSEA). The rats fasted for about 12–18 h with free access to water, and then test samples of TEF were administered to them through oral gavage at a dose of 2.1 mg/kg. The rats were randomly

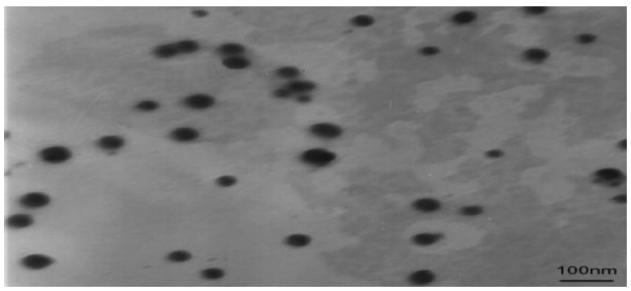
separated into two groups, each containing five animals. Group I: crude TEF powder was suspended in 1 ml of 0.5% (w/v) aqueous sodium carboxymethyl cellulose before administration. Group II: the optimized TEF-loaded SMEDDS formulation was precisely weighed and diluted with 1 ml water. After that, blood samples were collected from the retro-orbital plexus into heparinized tubes at a predetermined time point (0 , 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 min) and centrifuged at 3800 rpm for 15 min Plasma samples were stored at −80°C. The entire plasma tests (50 µl) were mixed in with 100 µl of 10 mM sodium acetic acid derivation (pH 5), 1.5 ml of methyl tert-butyl ether, and 15 µl of standard interior arrangement (500 ng/ml TEF half methanol), and vortexed for 20 min. Next, the samples were centrifuged at 3800 rpm for 10 min.

Then, 1.2 ml of the supernatant was moved to a test cylinder and dissipated to dryness under nitrogen. Last, the dried buildup was reconstituted in 400 µl of half methanol, and the blend was swirled and spun at 3800 rpm for 5 min [17,31,32].

HPLC assay of plasma samples

At the time of analysis, 200 µl plasma samples were taken in a 10 µl standard [ρ (methyl-p-hydroxybenzoate) = 100 mg/l]. The samples were vortexed for 20 s and then centrifuged at 13 000 rpm for 10 min.Subsequently, 100 µl of clear supernatant of the mixture was blown to dry at 40°C. The residue dissolved in the mobile phase of 200 µl. After centrifugation, the supernatant of 20 µl was drawn with the pipette and analyzed by high-performance liquid chromatography (HPLC) (Shimadzu Corp.). A Shimpack ODS C18 column (Shimadzu Corp.), 5 µm particle size, 250 mm×00054.6 mm, was used as a stationary phase. Methanol and 1% phosphate buffer (pH 7.4) was utilized as the mobile phase at a flow rate of 0.7 ml/min, corresponding to a column pressure of about 65 bar (6500 kPa). Peaks were detected at an absorbance wavelength of 294 nm and a column temperature of 40°C [5,17].

Figure 7



Dissolution profile of SMEDDS formulations F1–F11 and pure drug. SMEDDS, self-microemulsifying drug delivery system.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using Kinetica 5.0 PK/PD Analysis, Demo Version (Thermo Scientific, Waltham, Massachusetts, USA 02451–02454), and the standard PK parameters (mean±SD) of TEF were obtained. The experimental analysis was performed three times.

Results and discussion

Risk assessment

The development of dosage forms as part of the QbD framework included the assessment of material and process attributes that have a significant impact on product quality. We used a fishbone diagram (Fig. 1) to identify the potential variables that affect the CQA of our products. Due to the simplicity of the manufacturing process for SMEDDS formulation, material properties such as oils, surfactants, and cosurfactants contributed more to the product reaction than process properties. Therefore, in this study, the procedural features contained in the SMEDDS formulation, such as the stirring time, temperature, and stirring speed, are the least preferred as they contribute minimally to product instability. Therefore, the risk associated with process parameters is rated low (Table 1). The actual development of the SEEDS formulation depends on the correct determination of excipients at typical ratios in the designated formulation [8,13].

The drug-excipient compatibility study

The infrared spectrum of TEF was recorded on an FTIR-8400S in the range of 4000–400 cm^{-1}. The FTIR spectrum of TEF showed an absorption peak at 3136.36 cm^{-1} (O-H), 1730.40 cm^{-1} (C=O), 1627.91 cm^{-1} (C=C), and 1593.25 cm^{-1} (H-C-H). These peaks can be considered as characteristic peaks of TEF. The FTIR spectra of pure TEF and overlapping spectra of TEF-loaded SMEDDS formulations are exhibited in Fig. 2a and b. Comparison of vibration frequency of FTIR spectra of TEF and TEF-loaded SMEDDS formulation suggests that there may be no significant distinction in the characteristics peak at a wavenumber of the drug in the presence of excipients. This suggests that the formulations were well suited with the excipients.

Solubility of teriflunomide

A solubility study was performed to identify the suitable oil phase, surfactant, and cosurfactant to

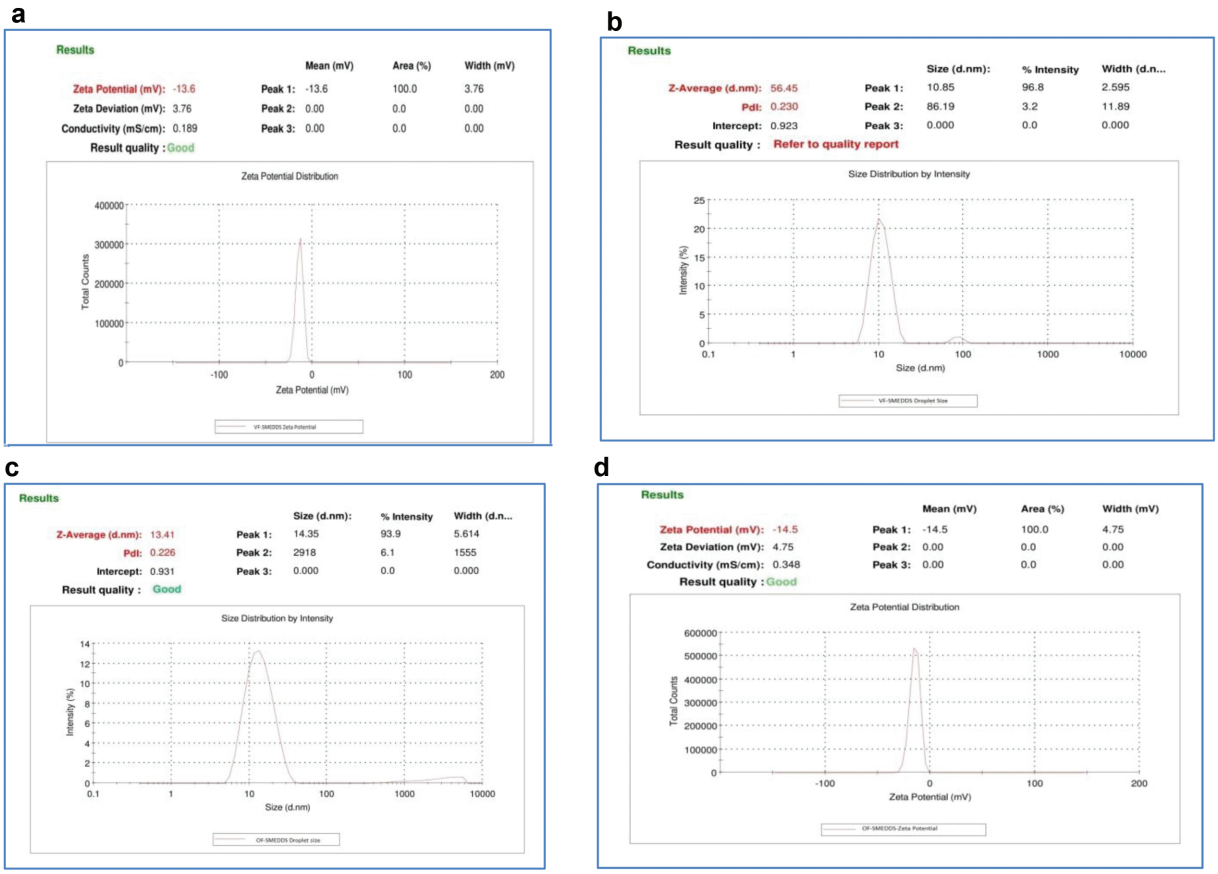
optimize TEF-loaded SMEDDS formulation. Maximum solubilizing capacities of components (oil, surfactant, and cosurfactant) are vital to achieving the optimum drug-loading content.

As per the solubility of TEF in oil, the solubility of TEF was carried out in 17 different oils, the results of which are shown in Fig. 8a. The screening of appropriate oil is the primary requirement of SMEDDS development. Therefore, solubility studies aimed at identifying a suitable oil having the maximal solubilizing potential for the development of SMEDDS. Sefsol 218 was shown to have the maximum amount of solubility of TEF (28.35 mg/ml) and therefore was preferred for further studies. The other oils exhibited different solubilizing capacities as reported in Fig. 8a.

The surfactants involved in the SMEDDS system have high hydrophilic-lipophilic balance (HLB) values (8.6–16.7 HLB value) and surfactant hydrophilicity, whose purpose is the spot formation of oil-in-water droplets and rapid distribution of formulation in aqueous media (e.g. gastrointestinal fluid). The drug dispersed within the SMEDDS formulation would remain soluble for an extended period at the absorption site.

TEF's solubility in 10 different surfactants is represented in Fig. 8b. Among the solubility data of TEF in other surfactants, maximum solubility of the drug is observed in Acrysol EL-135 (57.25 mg/ml) and Labrafil M2125 (54.20 mg/ml). The oily phase Sefsol 218 exhibited the highest emulsification efficiency with Acrysol EL-135 to form a homogeneous emulsion, whereas Labrafil M 2125 produced good transparency in the formulation. On the other hand, Sefsol 218 showed poor emulsification properties with other surfactants. Therefore, Acrysol EL-135 surfactant was selected for SMEDDS formulation. To improve the self-emulsified formulation, the proper composition of low and excessive HLB surfactants was needed to form a stable microemulsion. Therefore, Acryl EL-135 with an average HLB of 15 and PEG 400 with an HLB of 4 was used. Sefsol 218 was entrapped in the surfactant (Acrysol EL-135) with high HLB, which increased the emulsification method upon dilution with an aqueous medium. These excipients were reported to provide better stability of the emulsion.

Figure 8



Teriflunomide solubility in different oils, surfactants, and cosurfactants.

The cosurfactant reduces the oil–water interface, fluidizes the interfacial film's hydrocarbon vicinity, and permits spontaneous formation. Therefore, the choice of the cosurfactant is critical. It is no longer the most straightforward way to shape microemulsion formation and solubilization in microemulsion. Figure 8c shows the TEF solubility in five different cosurfactants. The solubility data of TEF shows it is exceptional in cosurfactants with a maximum quantity of drug solubilized in PEG 400 (190 mg/ml), and the data indicates a transparency of 98.4%. Therefore, PEG 400 was used as a cosurfactant in the SMEDDS formulation. These investigations clearly distinguish the various cosurfactants' abilities to enhance the emulsification in surfactants and increase the microemulsion formulation's spontaneity by increasing the cosurfactant.

Considering the oil, surfactant, and cosurfactant construct components of SMEEDS, the drug needed to be completely soluble in all three parts of the mixture. Therefore, the solubility of the drug was considered when choosing the best oil, surfactant, and cosurfactant. The solubility of the drug is also essential in deciding the dose of SMEDDS. Hence, SMEDDS needed to consist of an oil, surfactant, and cosurfactant that could accommodate the quantity of the drug. Another factor that may be affected through solubility is the partitioning effect. If the drug is not appropriate or stable in the mixture, it will diffuse in the direction of water with the formulation of a microemulsion. Considering each of these facts, the choice of excipients was an essential factor for successful formulation. The solubility of oil data (Fig. 8a) shows that TEF has good solubility in synthetic oil compared with vegetable oil. So, Sefsol 218 was selected as the oil phase. Acryl EL-135 acts as a surfactant because of its high HLB value and it showed good solubility of TEF in the oil phase. The third component of SMEDDS, the cosurfactant PEG 400, helped the surfactant to stabilize the microemulsion system.

Pseudoternary phase diagram

The self-micro emulsifying system produces oil-in-water emulsion with gentle agitation into the aqueous medium. A surfactant or cosurfactant prefers to be adsorbed onto the interface, lowering the interfacial energy, and giving a mechanical barrier to coalescence. Then, the reduction of energy improves the microemulsion formulation and simultaneously improves the microemulsion formulation's thermodynamic balance. Therefore, the choice of oil, surfactant, cosurfactant, and the mixing ratio of oils to Smix played an essential function in the microemulsion formulation.

The results of preliminary studies were used to construct the ternary phase diagrams to study the relationship between the phase behavior and composition of SMEDDS. The results also helped determine the concentration range of components for the formulation of a microemulsion. It used a mixture of surfactant (Smix) with high and low HLB values in the current work. Sefsol 218 has a low HLB value while Acrysol EL-135 has a higher HLB value. A combination of low and high HLB surfactants leads to more rapid dispersion and finer emulsion droplet size in the aqueous phase. Sefsol 218 and Acrysol EL-135 in a 2 : 1 ratio confirmed a wider microemulsion region and formed quicker microemulsion than in a 1.5 : 1 and 1 : 1 Smix, which is deciding to formulation development.

The optimal concentration of oil, surfactant, and cosurfactant of the microemulsion formulation is recorded in Fig. 3a–c. SMEDDS was prepared in oil to Smix (1 : 1). The data displayed in Fig. 3c shows that up to 1 : 9–4 : 6 parts of the oil to Smix ratio gave a clear solution when titrated with 100 elements of the water. This is because Smix parts are higher than oil parts. A ratio of 4 : 6 parts oil to Smix produced turbidity in the solution, indicating that SMEDDS was unstable on dilution. SMEDDS was prepared using the 1.5 : 1 oil to Smix ratio. The data in Fig. 3b shows that in a ratio range from 1 : 9 to 3.5 : 6.5, parts of oil to Smix, a clear solution was created when it was titrated up to 100 parts of water. This happened because oil parts are less than Smix parts. Surfactants decrease the interfacial tension between the oil and water phase leading to a clear solution. SMEDDS was prepared using a 2 : 1 oil to Smix ratio. The data found in Fig. 3c revealed that up to a 1 : 9 to 4 : 6 parts of oil to Smix ratio produced clear solutions when titrated up to 100 parts of water.

The nature of microemulsion formed in the aqueous medium depends on the concentration of Smix (a mixture of Acrysol EL-135 and PEG 400) in the formulation. Figure 3a–c depict that as the concentration of Smix increased and the oil concentration decreased, it improved the clarity of the self-micro emulsifying system. In addition, the surfactant reduced the oil–water interface, which made rapid dispersion of SMEDDS in an aqueous medium and reduced particle size when diluted with water.

Evaluation of self-microemulsifying drug delivery system formulation

Visual assessment

The tendency to form emulsion was judged qualitatively as 'good' when droplets spread quickly in the water and created a fine transparent emulsion. It was rated 'bad' when droplets become milky or there was no emulsion formation with instant coalescence of oil droplets. F1–F11 batches produced a fast-forming microemulsion, which could be clear in appearance and is the ideal property of SMEDDS formulation. This grading system was used to identify the visual assessment. The outcomes are displayed in Table 5.

Transmission test

The transparency of the SMEDDS formulation was confirmed by measuring the transparency of the microemulsion as a transmittance (%).The % transmittance in all the eight formulations was found to be in the range of 99.03–100%. Among all the formulations, F2 shows the highest % transmittance. Table 7 shows that all formulations were clear and transparent. Therefore, the higher % transmittance ensures the formation of droplet sizes in the nanorange and the drug in the formulation has a large surface area for drug release.

Drug content and entrapment efficiency

The drug content of all SMEDDS formulations observed ranged from 98.4 to 99.69%. The F4 formulation indicates the highest drug content (99.69%), and it displayed good drug distribution in the formulation. All results are shown in Table 5. The drug entrapment efficiency of all SMEDDS formulations observed ranged from 92.69 to 97.3%. The F4 formulation indicates the highest % (97.3%), and F9 SMEDDS formulation exhibits the lowest entrapment efficiency (92.69%). It shows excellent drug distribution in SMEDDS formulations. All results are shown in Table 5.

Determination of self-emulsification time

The efficiency of self-emulsification could be estimated using determining the rate of emulsification. The SMEDDS should disperse completely and rapidly when subjected to aqueous dilution under mild agitation. The self-emulsification time description for all 11 formulations is recorded in Table 5. All the SMEDDS formulations were emulsified within 30 s. Among all the formulations, F9 and F11 appeared bluish-white and, as per the grading system, they were each graded as Kavitha and colleagues described similar results; that the bluish-white appearance of the formulation was observed due to a higher amount of the lipid in the formulation and the Smix were not enough to emulsify the lipid. The remaining nine formulations appeared blue after emulsification. They were graded as an A. After emulsification tests, all the formulations were watched for 2 h and displayed neither turbidity nor precipitation of any constituents of the system.

Robustness to dilution

First, 1 ml of SMEDDS was diluted with 1000 ml of water and 0.1 N HCl. Then, the diluted SMEDDS formulation was stored for 12 h. The formulation indicated no precipitation or phase separation after 12 h.

Droplet size

The droplet size of the microemulsion is a critical issue in self-emulsification performance. Droplet size should be less than 100 nm as it determines the rate and quantity of drug release and absorption. According to the reported literature, there may be no specific boundary between self-emulsifying drug delivery system and SMEDDS. The self-emulsification system is similar to SMEDDS, and the only difference is in the droplet size. The average droplet size in all the formulations were in the range from 16.64 to 136.65 nm, indicating that all the particles were in the nanometer range and there was a homogeneous distribution of particle size. The formulation F4 showed the tiniest particles (16.64 nm), while F5 showed larger particles. See Table 5 for the full results.

Table 7 Predicted and experimental values for verification formulation-self-microemulsifying drug delivery system and optimized formulation-self-microemulsifying drug delivery system

Responses	VF-SMEDDS			OF-SMEDDS		
	Predicted value	Experimental value	% difference	Predicted value	Experimental value	% difference
Droplet size (nm)	12.15	10.85	–10.69	21.29	14.45	–4.23
Emulsification time (s)	23.75	37.21	0.35	27.15	25	–2.88
% transmittance	97.87	96.96	2.76	98.73	99.4	1.59
% drug release in 20 min	63.75	85.94	0.849	64.54	73.44	–5.50

Differences % = [(Experimental value – Predicted value) / (Predicted value)] × 100. OF, optimized formulation; SMEDDS, self-microemulsifying drug delivery system; VF, verification formulation.

Polydispersibility index

PDI determines the size range (0.1–1); it is 0.1 to 1 of droplets in the system and is used to express the particle size distribution. Kavitha and colleagues reported that the value acquired close to zero indicates the uniform droplet in the distribution system. The more uniformity in the formulation, the better the physical stability. Ideally, SMEDDS should be widely distributed with particles smaller than 100 nm, and PDI should be less than 0.3. If particles have a size of more than 100 nm, it should be a maximum of up to 23%. The PDI value of all the formulations was found to be in the ranges from 0.170 to 0.310 and indicates the development of uniform emulsion with good stability attributes. The data in Table 7 shows that formulations F2, F4, F5, and F6 have a PDI of less than 0.3, while formulations F1 and F3 have a PDI greater than 0.3.

Zeta potential

Almost all macroscopic substances in contact with liquid media have an electronic charge on their surfaces. It is the most crucial indicator of this charge, which may predict and control the stability of an emulsion. Due to stable suspension, the charged particles repel each other, which overcomes the natural tendency to aggregate. The best value of zeta potential is less than −30 mV, which indicates that formulations have been stable for a long time [8]. The zeta potential of all SMEDDS formulations was found to be in the ranges from −0.144 to −9.74 mV. The results are found in Table 5 and Fig. 9a–d.

In vitro dissolution study

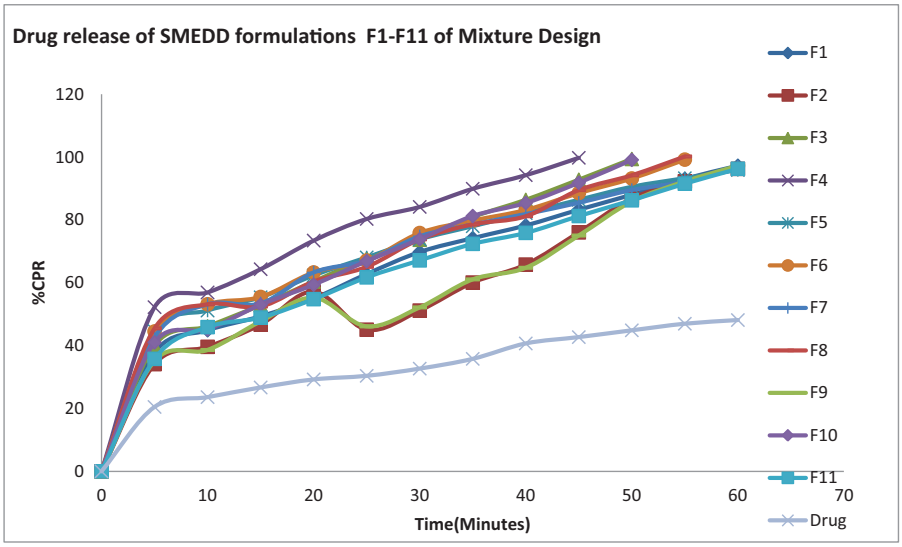
In vitro dissolution studies were performed to examine the drug release from the 11 different formulations (F1–F11) and pure drugs. Dissolution studies were performed for the SMEDDS formulation in 6.8 phosphate buffer solutions. There are no significant differences in the dissolution study of the 11 SMEDDS formulations. All SMEDDS formulations showed that 100% of the drugs were released within 45–60 min as compared with the pure drug at 48.15%.

SMEDDS formulations resulted in the spontaneous formation of a microemulsion with small droplet size, allowing TEF's faster release rate in dissolution media. The F4 SMEDDS formulation gave a uniform drug release in 45 min because it had the smallest particle size (16.64 nm) and less PDI value (0.170 mV). The *in vitro* dissolution studies have shown that formulations of TEF, in the form of SMEDDS formulation, increased the dissolution properties. Based on the *in vitro* release study, F4 formulations were optimized, with a 99.78% drug release in 45 min. All results are compiled in Fig. 7.

Thermodynamic stability study

The temperature stability study was carried out by keeping the samples at two different temperatures (2–4°C and room temperature) for 48 h and then a visual inspection was performed. All the SMEDDS formulations did not show any evidence of phase separation, flocculation, or precipitation. Therefore,

Figure 9



Transmission electron microscopy (TEM) result of the optimized SMEDDS formulation. SMEDDS, self-microemulsifying drug delivery system.

Table 8 Effect test report

% drug release in 20 min		% transmittance	
Source	Probability>*F*	Source	Probability>*F*
(Sefsol 218-0.2)/0.191	0.2426	(Sefsol 218-0.2)/0.191	<0.0001*
(Acrysol EL-135-0.375)/0.191	0.0274*	(Acrysol EL-135-0.375)/0.191	<0.0001*
(PEG 400-0.234)/0.191	0.0002*	(PEG 400-0.234)/0.191	<0.0001*
Sefsol 218* Acrysol EL-135	0.695	Sefsol 218*Acrysol EL-135	0.3116
Sefsol 218* PEG 400	0.3666	Sefsol 218*PEG 400	0.6803
Acrysol EL-135* PEG-400	0.6686	Acrysol EL-135*PEG 400	0.0354*
Emulsification Time in seconds		Droplet size	
Source	Prob > F	Source	Prob > F
(Sefsol 218-0.2)/0.191	<0.0002*	(Sefsol 218-0.2)/0.191	<0.0001*
(Acrysol EL-135-0.375)/0.191	<0.0002*	(Acrysol EL-135-0.375)/0.191	<0.0002*
(PEG 400-0.234)/0.191	0.0111*	(PEG 400-0.234)/0.191	0.1914
Sefsol 218*Acrysol EL-135	0.9657	Sefsol 218*Acrysol EL- 135	0.6034
Sefsol 218*PEG 400	0.7128	Sefsol 218*PEG 400	0.7671
Acrysol EL-135*PEG 400	0.9249	Acrysol EL-135*PEG 400	0.4231

Significance value is $p < 0.05$.

all formulations were stable at each temperature in the range 2–4°C and at room temperature [8]. In addition, SMEDDS formulations are thermodynamically stable, having a specific centralization of oil, surfactant, and water with no phase separation, creaming, or breaking.

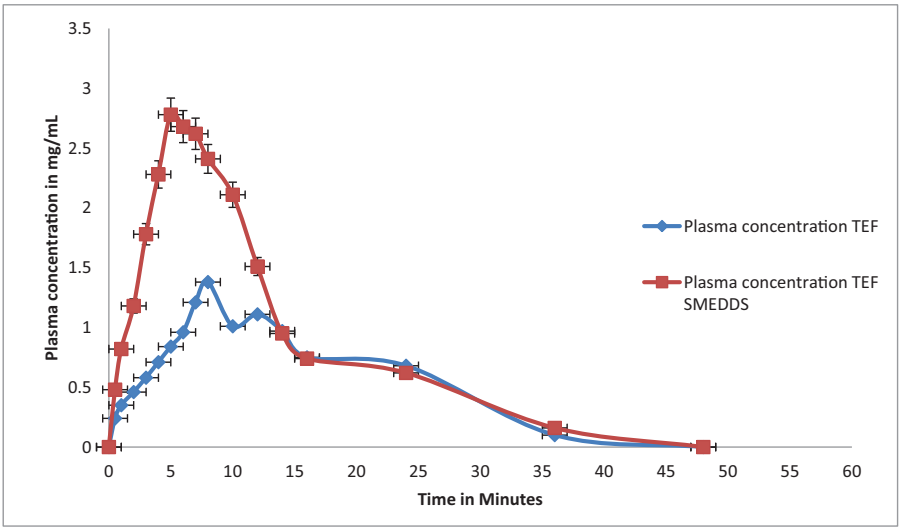
Design authentication and optimization of formulation

Mittal and Kavitha and colleagues reported the same interpretation of the color map correlation. The design assessment was first done by the color map relationship. The color map was obtained for the SMEDD components as introduced in Fig. 4b. The shading map checks each factor's effect alone or mixes various factors on fundamental responses. The brilliant red areas denote the best mixture, while the dull red, faint, and blue colors are recorded in descending order of efficacy in the necessary responses. Thus, the shading map demonstrates that the design is reasonable for screening components to acquire the SMEDDS formulations that meet all of the preset quality attributes. We examined the information from all 11 formulations of SMEDDS by fitting different regression models with the intercept to zero. The numerical significant models were determined for % drug release in 20 min, % transmittance, emulsification time (s), and globule size (nm). The changed R^2 and P value were achieved for all of the reactions and utilized to assess the model fit. The prediction plots, obtained for all four reactions, are introduced in Fig. 4a. The predictive models, % drug release in 20 min (R^2=0.80 and 0.0758), % transmittance (R^2=091 and P=0.0122), emulsification time (s) (R^2=0.88 and P=0.0227), and droplet size (nm) (R^2=0.97 and P=0.0005) were all statistically significant. The effect test reports (P value) were obtained for all the responses described in Table 8. The experimental versus predicted values and the impact test report obtained for the CQAs have a nearby mathematical instantaneousness, addressing the model's legitimacy. The contour and surface plots, obtained for every response, are introduced in Table 7 and Fig. 5b. The obscured region in the diagram's contour plot area addresses the nonsuitable region of the design, and the white area gives the optimized operational design space. The anticipated and the trial values acquired for the VF and optimization formulation SMEDDS did not shift fundamentally (Table 7 and Fig. 5a). The % differences, acquired for both VF and OF, were inside a 5% deviation. The globule size, % drug release in 20 min, % transmittance, and emulsification time for both VF and optimization formulation (Fig. 5a) confirm the formation of SMEEDS with excellent stability attributes. Also, the transmission electron microscopy investigation for the optimal formulation SMEDDS showed that the emulsion drops were spherically shaped in the nanometer range, had narrow droplet distribution, and indicated physical stability of the optimized SMEDDS formulation, as demonstrated in Fig. 9.

In vivo oral absorption and pharmacokinetic study

We investigated the pharmacokinetic study of TEF after oral administration of the optimal TEF-loaded SMEDDS formulation and the TEF suspension into rats. Plasma levels of TEF were determined and plotted against time, Fig. 10. For 0–60 min, plasma concentrations of the TEF in rats receiving the optimized SMEDDS formulation were significantly higher than those in rats receiving the TEF suspension. It may be attributed to primary high augmentation because of the quick dissolution prompted by an optimized SMEDDS formulation.

Figure 10



Plasma concentration–time profiles of TEF in rats after oral administration of TEF suspension and TEF SMEDDS Formulation. SMEDDS, self-microemulsifying drug delivery system; TEF, teriflunomide.

Table 9 Pharmacokinetic parameters of teriflunomide after oral administration of teriflunomide suspension and optimized teriflunomide-loaded self-microemulsifying drug delivery system formulation (mean±SD, *n*=6)

Parameters	Teriflunomide suspension	SMEDDS formulation
Tmax (h)	7.89±1.19	4.61±0.75
Cmax (mg/ml)	1.38±0.12	2.78±0.61
T1/2 (h)	1.28±2.42	1.41±2.11
AUC0→∞ (h×mg/l)	26.86±7.86	20.89±7.68
Relative bioavailability (%)	–	85.89

AUC, area under the curve; SMEDDS, self-microemulsifying drug delivery system.

A noncompartmental pharmacokinetic analysis method was used to investigate the pharmacokinetic behavior of curcumin. Microsoft Excel was used to calculate the pharmacokinetic parameters from the experiments. The total area under the plasma concentration time curve was determined by the trapezoidal rule using plasma TEF concentration versus time data from time zero to the last sampling time, that is 6 h plus the extrapolated area (from the last experimental time to infinity). The relative bioavailability of the representative SMEDDS formulation to the control was calculated as follows:

$$\text{Relative bioavailability \%} = [(AUC_{SMEDDS} \times Dose_{control}) / (Dose_{SMEDDS} \times AUC_{control})]$$

where AUC_{SMEDDS} means the area under the plot of plasma concentration of a drug versus time after SMEDDS gives insight into the extent of exposure to a SMEDDS and its clearance rate from the body.

The *AUC* control represents the total curcumin solution exposure across time.

The apparent elimination half-life ($t_{1/2}$) was calculated from the estimated elimination rate constant (kel) by linear regression of the log of the plasma concentrations as in 0.693/kel. The elimination rate constant (kel) can be calculated directly from those parameters using the equation kel equals clearance divided by the volume of distribution. The maximum plasma concentration (c_{max}) and time to maximum concentration (t_{max}) after oral administration were determined directly from the concentration versus the time curve.

The pharmacokinetic parameters are shown in Table 9. The result exhibits that AUC 0→∞ values of oral F was 26.86±7.86 h*mg/ml and that of TEF SMEDDS was 20.89±7.68 h×mg/ml, yielding a relative bioavailability of 85.89%. This optimization could be attributed to the improvement in TEF's solubility and dissolution rate by the optimized SMEDDS formulation, which successfully expanded film smoothness and aided in disseminating the drug through the biological layer. Results have revealed an enhanced absorption profile of embelin-loaded S-SNEDDS compared with the conventional preparation at each point of time. These may be because of the enhanced aqueous solubility and dissolution features of embelin. The peak plasma concentration (C_{max}) of TEF after oral

administration of TEF-loaded SMEDDS (2.78 ±0.61 mg/ml) was two times greater than TEF suspension (1.38±0.12 mg/ml). The time at which the uppermost concentration was observed (T_{max}) was found to be 4.61 h for TEF-loaded SMEDDS, while for TEF suspension preparation, it was observed to be 7.87 h. Student's *t* test with a *P* value of less than 0.05 confirmed a significant difference between the prepared SMEDDS formulation and TEF suspension. Therefore, the optimal SMEDDS formulation exhibited fundamentally higher, most extreme-plasma concentration, and AUC values (twofold higher qualities, separately; $P<0.05$) than the TEF suspension.In addition, the average residence time of the optimal SMEDDS formulation was significantly shorter than that of the TEF suspension ($P<0.05$). TEF dissolves rapidly from the SMEDDS formulation and peaks immediately. Hence, the relative bioavailability of TEF in the optimal TEF-loaded SMEDDS formulation is significantly higher than the relative bioavailability of TEF in similar TEF-loaded SMEDDS formulations reported in the literature [2]. The TEF-loaded SMEDDS formulation increases the oral bioavailability of TEF between 200 and 330% contrasted and the TEF suspension. Furthermore, the aggregate sum of the enhanced SMEDDS batch announced (200–1000 µl). We inferred that the optimal SMEDDS design created in this investigation, effectively enhanced the oral absorption of TEF and reduced the volume of distribution.

Conclusion

TEF is used to treat multiple sclerosis. SMEDDS was a promising approach for the formulation of poorly water-soluble drugs. We achieved the predetermined quality characteristics of TEF-loaded SMEDDS with the implementation of QbD concepts throughout the development process. We studied the detailed analysis of the three independent variables, Sefsol 218, Acrysol EL-135, PEG 400, and their effects on the quality attributes such as droplet size, emulsification time, % transmittance, and % drug release with the application of a statistical mixture design. This study showed the potential use of QbD in the development of SMEDDS. The presence of the developed TEF-loaded SMEDDS was clear, and the microemulsion droplets were spherical with a narrow particle size of 14.35 nm, PDI of 0.226, and a zeta potential of −14.5 mV. The dissolution results demonstrated that the cumulative dissolution rate of TEF-loaded SMEDDS could reach more than 90% drug release within 60 min. The optimized SMEDDS formulation showed fundamentally higher, most extreme-plasma concentration, and AUC values (two-fold and 3.3-fold higher value, separately; $P<0.05$) than the TEF suspension. A good agreement was observed between model prediction and experimental values of percentage droplet size in nm (Y1), % drug release in 20 min (Y2), and percentage transmittance (Y3), and emulsification time in seconds (Y4). Thus, the findings show that optimizing TEF-loaded SMEDDS formulation could be potentially used to improve the oral absorption and bioavailability of TEF.

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Conflicts of interest

There are no conflicts of interest.

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