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Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery

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Abstract

Background and Aim: Telmisartan (TEL) is an angiotensin II receptor blocker (ARB) antihypertensive agent. The aim of the present investigation was to develop a self-nanoemulsifying drug delivery system (SNEDDS) to enhance the oral bioavailability of poorly water soluble TEL. **Materials and Methods:** The solubility of TEL in various oils was determined to identify the oil phase of a SNEDDS. Various surfactants and co-surfactants were screened for their ability to emulsify the selected oil. Pseudoternary phase diagrams were constructed to identify the efficient self-emulsifying region. A SNEDDS was further evaluated for its percentage transmittance, emulsification time, drug content, phase separation, dilution, droplet size, zeta potential, pH, refractive index, and viscosity. **Results:** The developed SNEDDS formulation contained TEL (20 mg), Tween® 20 (43.33% w/w), Carbitol® (21.67% w/w), and Acrysol® EL 135 (32% w/w). The optimized formulation of the TEL-loaded SNEDDS exhibited a complete *in vitro* drug release in 15 min as compared with the plain drug, which had a limited dissolution rate. It was also compared with the pure drug suspension by oral administration in male Wister rats. The *in vivo* study exhibited a 7.5-fold increase in the oral bioavailability of TEL from the SNEDDS compared with the pure drug suspension. **Conclusions:** These results suggest the potential use of the SNEDDS to improve the dissolution and oral bioavailability of poorly water soluble TEL.

Key words: Bioavailability, poor water solubility, self-nanoemulsifying drug delivery system, telmisartan

INTRODUCTION

Oral route is the easiest and most convenient way of noninvasive administration. However, the oral drug delivery may hamper drug molecules that exhibit poor aqueous solubility. Approximately, 40% of new chemical entities exhibit a poor aqueous solubility and present a major challenge to modern drug delivery systems which leads to a poor oral bioavailability, high intra- and intersubject variability, and lack of dose proportionality. These drugs are classified as class II drug by the Biopharmaceutical Classification System (BCS), drugs with a poor aqueous solubility and high permeability.[1] Different formulation approaches like micronization, solid dispersion, and complexation with cyclodextrins have been utilized to resolve such problems. Indeed, in some selected cases, these approaches have been successful but they offer many other disadvantages. The main problem with micronization is chemical/thermal stability; many drugs may degrade and lose bioactivity when they are micronized by a conventional method. For solid dispersion, the amount of carriers used is often large, and thus if the dose of the active ingredient is high, the tablets or capsules formed will be large in volume and difficult to swallow. Moreover, the carriers used are usually expensive and the freeze-drying or spray-drying method requires particular facilities and processes, leading to a high production cost. Though a traditional solvent method can be adopted instead, it is difficult to deal with co-precipitates with a high viscosity. Complexation with cyclodextrins techniques is not applicable for drug substances which are not soluble in both aqueous and organic solvents. The realization that the oral bioavailability of poor water-soluble drugs may be enhanced when co-administered with a meal rich in fat has led to increasing recent interest in the formulation of poorly water soluble drugs in lipids. Lipid suspension, solutions, and emulsions have all been used to enhance the oral bioavailability, but more recently there have been much focus on the utility of a self-nanoemulsifying drug delivery system (SNEDDS). Being hydrophobic, i.e., more lipophilic, a lipid-based drug delivery system would ideally work for a poorly water soluble drug. Lipid-based drug delivery systems have gained considerable interest after the commercial success

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of Sandimmune Neoral (cyclosporine A), Fortovase (saquinavir) and Norvir (ritonavir).[2-5]

SNEDDS are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and cosolvents/surfactants that have the ability of forming fine oil-in-water (o/w) micro emulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids. SNEDDS spread readily in the GI tract, and the digestive motility of the stomach and the intestine provides the agitation necessary for self-emulsification.

TEL displaces angiotensin II from the angiotensin I receptor and produces the blood pressure-lowering effects by antagonizing angiotensin II-induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake, and hypertrophic response. TEL is practically insoluble in water (0.0035 mg/mL) and has high hydrophobicity (log *P* 6.66) with only 42% oral bioavailability. Hence, TEL was selected as a model drug for this study. TEL is available in various doses (20 mg, 40 mg, 80 mg and 120 mg); for our study a 20-mg dose was selected as a working dose to limit the total formulation volume. The aim of this study was to develop a SNEDDS containing a poor water soluble drug (telmisartan).

MATERIALS AND METHODS

Materials

Telmisartan was obtained as a gift sample from Torrent Research Center, Bhat, Gandhinagar, Gujarat, India. The following materials were donated by Abitec Corp., USA, and were used as received: Acconon® CC 400 (polyoxyethylene 6 capric esters), Acconon® Sorb 20 (polyoxyethylene 20 sorbitol), Acconon® E (polyoxypropylene 15 stearyl ether), Capmul® MCM (glycerol mono-dicaprylate), Capmul® GMO (glycerol mono/di-oleate), Capmul® MCM C8, Captex® 355 (caprylic/capric acid triglycerides) and Caprol® ET (polyglycerol esters). Cremophor® RH 40 (polyoxyl 40 hydrogenated castor oil) and Solutol® HS 15 (macrogol 15 hydroxystearate) were also donated from BASF, Mumbai, Maharashtra, India. Miglylol® 812 (caprylic/capric acid triglycerides) and Imwitor® 742 (glycerol monocaprylocaprate) were generously gifted from Sasol, Germany. Sefsol® 218 (propylene glycerol monocaprylate) was gifted from Nikko Chemicals, Tokyo, Japan. Labrafil® M 2125 CS (linoleoyl macrogolglycerides), Plurol Oleique® (polyglycerol oleate), and Capryol® 90 (polypropylene glycol monocaprylate) were received as a gift sample from Gettefosse, Mumbai, Maharashtra, India. Acrysol® K 140 (polyoxyl 40 hydrogenated castor oil) and Acrysol® EL 135 (polyoxyl 35 castor oil) were also gifted from Corel Pharma, Gujarat, India. Cremophor® EL (polyethoxylated castor oil) was purchased from Sigma Aldrich, India. Other chemicals like Span® 20 (sorbitan monolaurate), Span® 80 (sorbitan monooleate), Tween® 20 (polyoxyethylene sorbitan monolaurate), Tween® 80 (polyoxyethylene sorbitan monooleate), polyethylene glycol 400 (PEG 400), polyethylene glycol 200 (PEG 200), propylene glycol (PG), Carbitol® (monoethyl ether of diethylene glycol), glycerol, castor oil, olive oil, cotton seed oil, poloxamer 188, and poloxamer 407 were bought from Merck India, Mumbai, and S. D. Fine Chem, Mumbai, Maharashtra, India. Double distilled water was used throughout the study. Acetonitrile and methanol used in the present study were of high-performance liquid chromatography (HPLC) grade. All other chemicals were reagent grade. Empty, hard gelatin capsule shells were generously donated by Torrent Research Center, Gujarat, India.

Animals

Male Wister rats (weighing approximately 250 ± 30 g) were used for the bioavailability studies. The animals were maintained at temperature (24–25°C), and humidity (60%), and were supplied with food and water ad libitum. The animal requirement was approved by the Institute Animal Ethics Committee (IAEC) and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

Selection of self nanoemulsified drug delivery systems components

Oil (solubility studies)

The solubility of TEL in various buffers, oils, surfactants, and co-surfactants was measured using the shake flask method as suggested by Date and Nagarsenker. An excess amount of TEL was introduced into each excipient (2 mL) followed by sealing in vials. A vortex mixer (REMI, Mumbai, India) was used to facilitate the solubilization. Sealed vials were stirred in a water bath at 40°C for 24 h and allowed for reaching equilibrium at 30°C for 72 h. Each vial was centrifuged at 15,000 rpm for 10 min using a centrifuge (REMI, Mumbai, India) followed by the removal of undissolved TEL by filtering with a membrane filter (0.45 μm). Samples were suitably diluted with methanol and a drug concentration was obtained via a validated UV method at 297 nm using methanol as a blank (R^2 = 0.99057, %error = 1.5, CV = 2%, linearity = 1–20 μg/mL) using a double-beam UV visible spectrophotometer (Shimadzu 1700, Shimadzu Corporation, Japan). The experiment was repeated in triplicates. Results were represented as mean values (mg/mL ± SD).

Surfactant (emulsification study)

Different surfactants (Cremophor® EL, Cremophor® RH 40, Solutol® HS 15, Span® 80, Tween® 20, and Tween® 80) were screened for the emulsification ability of the selected oil phase.[6] Surfactant selection was done on the basis of percentage of transparency (%transparency) and ease of emulsification. Briefly, 300 mg of the surfactants was added to 300 mg of the selected oily phase. The mixtures were gently heated at 50°C for the homogenization of the components. Each mixture, 50 mg, was then diluted with distilled water to 50 mL in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversions required to yield a homogenous emulsion. Emulsions were allowed to stand for 2 h and their %transparency was evaluated at 650 nm by a double-beam UV spectrophotometer using distilled water as a blank. Emulsions

were furthermore observed visually for any turbidity or phase separation.

Co-surfactant (emulsification study)

Six co-surfactants were screened for SNEDDS formulation, which included Carbitol®, PEG 400, PG, Capmul® MCM C8, Plurol Oleique®, and glycerol.[6] The screening of the co-surfactant was conducted on the basis of %transparency and ease of emulsification. Mixtures of 100 mg of the co-surfactant, 200 mg of the selected surfactant, and 300 mg of the selected oil were prepared and evaluated in a similar fashion as described in the above section on surfactants.

Construction of the ternary phase diagram

On the basis of solubility and emulsification study Acrysol® EL 135, Tween® 20 and Carbitol® were selected as oil, surfactant and co-surfactant, respectively. To determine the concentration of components for the existing range of the SNEDDS, a pseudoternary phase diagram was constructed using a water titration method at ambient temperature (25°C).[7] The surfactant and co-surfactant were mixed in different volume ratios (1:1, 1:2, 1:3, 1:4, 4:1, 3:1, and 2:1). Oil and surfactant/co-surfactant (S/Co-S) were mixed thoroughly in different volume ratios (1:9, 1:8.5, 1:8, 1:7.5, 1:7, 1:6.5, 1:6, 1:5.5, 1:5, 1:4.5, 1:4, 1:3.5, 1:3, 1:2.5, 1:2, 1:1.5, 1:1, 1.5:1, and 2:1) and titrated with water by dropwise addition under gentle agitation. The proper ratio of one excipient to another in the SNEDDS formulation was analyzed and the pseudoternary plot was constructed using TRIPLOT V14 software. All studies were repeated three times, with similar observations being made between repeats. Moreover, to investigate the effects of TEL on the self-emulsifying performance of the SNEDDS, the formulation amount of TEL was added to the boundary formulations of the self-emulsifying domain of the ternary phase diagrams. The self-emulsifying performance was visually assessed after infinite dilution using purified water.

Preparation of the self-nanoemulsified formulations

TEL (20 mg) was added in accurately weighed amount of oil into a screw-capped glass vial and melted in a water bath at 37°C. The surfactant and co-surfactant were added to the oily mix using a positive displacement pipette and stirred with a magnetic bar. The formulations were further sonicated (Frontline FS-4, Mumbai, India) for 15 min and stored at room temperature until their use in subsequent studies.[8]

Evaluation parameters of TEL-loaded SNEDDS

Emulsification time

The emulsification time (the time for a preconcentrate to form a homogeneous mixture upon dilution) was monitored by visually observing the disappearance of SNEDDS and the final appearance of the nanoemulsion in triplicate. A dissolution apparatus (Elactrolab Dissolution Tester USP, TDT-06 P) was employed with 500 mL water, and with a paddle speed of 50 rpm at 37°C. The SNEDDS (1 mL) was added dropwise to the medium by a dropping pipette and the time required for the disappearance of the SNEDDS was recorded.[9]

Droplet size and zeta potential determination

A total of 50 mg of the optimized SNEDDS formulation was diluted with water to 100 mL in a flask, and gently mixed by hand. The droplet size distribution and zeta potential of the resultant emulsion was determined by laser diffraction analysis using a particle size analyzer (Malvern Zetasizer, UK). The sizing of the emulsion was determined in a small volume module. The sample was directly placed into the module and the data were collected for 60 s. The particle size was calculated from the volume size distribution. All studies were repeated in triplicates, for a good agreement being found between measurements ($P < 0.05$).[6]

In vitro drug dissolution study

The *in vitro* drug release of TEL from the optimized SNEDDS was performed by a conventional method. Hard gelatin capsules, size "0," filled with the preconcentrate (equivalent to 20 mg Tel) and pure drug (20 mg) separately were put into each of 900 mL water and a phosphate buffer, pH 1.2 and pH 7.5, at 37 ± 0.5°C with a 50 rpm rotating speed. Samples (5 mL) were withdrawn at regular time intervals (5, 10, 20, 30, 45, 60, 90, and 120 min) and filtered using a 0.45 μm filter. An equal volume of the respective dissolution medium was added to maintain the volume constant. The drug content of the samples was assayed using previously validated UV visible spectrophotometric methods. All measurements were done in triplicate from three independent samples.[6]

Dilution studies/robustness on dilution

The dilution study was done to access the effect of dilution on SNEDDS preconcentrate. In this study, the optimized formulation was subjected to various dilutions (i.e., 1:50, 1:100, and 1:500) with various diluents (i.e., water, 0.1 N HCL, phosphate buffer pH 7.5) and the droplet size was recorded.[6]

Determination of drug content

TEL from the optimized SNEDDS formulation was extracted in methanol using the sonication technique. The methanolic extract was analyzed for the TEL content spectrophotometrically, by a validated UV method at a 297 nm wavelength after suitable dilution.

In vivo studies

The rats were deprived of food but had free access to water 24 h before the day of the experiment. Two groups of rats were used for the experiments. Each group was either administered orally the TEL aqueous suspension (control group) or TEL-loaded SNEDDS. Sample of the TEL powder (20 mg) or TEL SNEDDS (650 mg equivalent to TEL 20 mg) was accurately weighed and separately dispersed into distilled water (3 mL) by mixing homogeneously for 30 s prior to dosing. Each formulation was administered to rats by oral gavage using an animal feeding needle. Under ether anesthesia, blood samples (0.5 mL) were collected via the retro-orbital vein at 15, 30, 60, 120, 240, 360, 480, and 720 min after oral administration into heparinized microcentrifuge tubes. The samples were centrifuged at 15,000 rpm for 10 min at 4°C temperature. The plasma samples (100 μL)

were separated, and 1 mL of acetronitrile was added to each of the plasma sample to precipitate the protein. The samples were then centrifuged again at 15,000 rpm, 4°C for 5 min, and the supernatant (20 μL) was directly injected on to the HPLC (LC-20AD, Shimadzu Corporation, Japan) with a PDA detector for the estimation of the TEL content by a validated chromatographic method (R^2 = 0.9759, %error = 3.1, CV = 3.5%). Intraday and interday variations at the above two concentrations were lower than 10%. The limit of detection of TEL in this method was 10 ng/mL. The chromatographic column utilized was Luna C8 (150 cm and 4.6 mm i.d.) with a 5 μm particle size. Acetonitrile and methanol (55:45) were utilized as a mobile phase at a flow rate of 1.0 mL/min with total run time of 10 min. Data from these samples were used to plot curves for TEL absorption with time.

Pharmacokinetic parameters

A noncompartmental model was employed to estimate the following pharmacokinetic parameters for each rat in each group: Peak plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), and the area under the plasma concentration versus time curve from zero to the last sampling time 12 h ($AUC_{0\rightarrow12h}$). Values are reported as mean ± SD (n = 3) and the data were considered as statistically significant ($P < 0.05$).

RESULTS AND DISCUSSIONS

Solubility study (screening of oil)

Solubility studies were aimed at identifying a suitable oily phase for the development of the TEL SNEDDS. Identifying the suitable oil having the maximal solubilizing potential for the drug under investigation is very important to achieve optimum drug loading.[4,5] The solubility of TEL in various buffers, oily phases, and 10% (w/w) surfactant solutions is presented in Table 1. Among the various oily phases that were screened, Acrysol EL 135 could solubilize the target amount of TEL (20 mg) at a relatively small amount of 210 μL. The selection of the surfactant or co-surfactant in the further study was governed by the emulsification efficiency rather than the ability to solubilize TEL.

Preliminary screening of surfactants

Nonionic surfactants are generally considered less toxic than ionic surfactants. They are usually accepted for oral ingestion.[10] In this study, the six nonionic surfactants (Tween® 80, Tween® 20, Cremophor® EL, Cremophor® RH 40, Solutol® HS 15, and Span® 80) were selected out of which some are reported to have bioactive effects, such as action on the tight junction by Solutol® HS 15, lymphotropic characters by Tween® 80, Tween® 20, and Span® 80, and the inhibitory effect on P-gp and CYP enzymes by Cremophor® RH40 and Cremophor® EL.[11] These findings were confirmed by Zhang *et al.*[9] who demonstrated increased AUC and C_{max} for orally administered digoxin in rats when coadministered with Tween®. It has been reported that the well-formulated SNEDDS is dispersed within seconds under gentle stirring conditions which ultimately depends on the emulsification ability of the surfactant.[11] Results inferred that the oily phase Acrysol® EL 135 exhibited the highest emulsification efficiency with Tween® 20 [%transparency: 100, 5 flask inversions (5s)] for the homogenous emulsion formation. On the other hand, Acrysol® EL 135 showed poor emulsification properties with other surfactants employed, requiring a higher number of flask inversions [Table 2]. The aforementioned results suggested the use of Acrysol® EL 135 as an oily phase with Tween® 20 as a surfactant for further study.

Table 1: Solubility study of TEL in various excipients and buffers at 25°C

Oils	Solubility[a] (mg/mL)	Surfactants and co-surfactants	Solubility[b] (mg/mL)	Buffers/media	Solubility[a] (mg/mL)
Acconon® E	10.13 ± 1.06	Cremophor® EL	78.47 ± 3.90	pH 1.2	0.12 ± 0.01
Acconon® Sorb-20	13.82 ± 3.51	Cremophor® RH 40	5.05 ± 0.91	pH 4.5	0.07 ± 0.03
Acconon® CC 400	11.49 ± 4.34	Solutol® HS 15	73.02 ± 4.21	pH 6.8	0.08 ± 0.04
Capmul® MCM	11.8 ± 3.12	Tween® 80	107.63 ± 3.18	Water	0.08 ± 0.05
Capmul® GMO	14.52 ± 5.09	Tween® 20	104.98 ± 3.03		
Captex® 355	1.90 ± 0.23	Span® 80	65.41 ± 2.14		
Caprol® ET	1.48 ± 0.11	Span® 20	61.34 ± 1.34		
Sefsol® 218	22.92 ± 5.98	Carbitol®	114.69 ± 4.54		
Miglylol® 812	32.12 ± 6.01	PEG 400	80.72 ± 2.46		
Imwitor® 742	11.62 ± 2.57	PEG 200	69.29 ± 3.45		
Labrafil® M 2125 CS	23.76 ± 3.19	Propylene Glycol	49.09 ± 3.52		
Capryol® 90	19.98 ± 4.59	Capmul® MCM C8	3.92 ± 0.98		
Triacetin®	20.95 ± 5.67	Plurol Oleique®	53.85 ± 1.45		
Acrysol® K 140	43.02 ± 7.32	Glycerol	44.32 ± 1.23		
Acrysol® EL 135	95.76 ± 6.90	Polaxomer 188	33.09 ± 1.98		
Olive oil	5.61 ± 1.23	Polaxomer 407	8.06 ± 0.97		
Castor oil	8.17 ± 1.06				
Cotton seed oil	9.13 ± 3.02				

[a]Data are expressed as mg/mL ± SD (n = 3); [b]Values are for the 10% w/w surfactant solution

Preliminary screening of co-surfactants

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation.[11] In view of the current investigation, six co-surfactants, namely, Carbitol®, PEG 400, PG, Capmul® MCM C8, Plurol Oleique®, and glycerol were compared. As depicted in Table 3, Acrysol® EL 135 exhibited good emulsification with all co-surfactants, with Carbitol® showing the maximum transmittance (100%) followed by PG (99.4%). Herein, the solubility of the drug in different co-surfactants may judge the final selection. Results of the solubility study demonstrated in Table 1 inferred a higher solubility in Carbitol®. It is worthy to note that all dispersions exhibited an instantaneous emulsion formation with only five flask inversions [Table 3]. This could contend the importance of co-surfactant addition to the surfactant-containing dispersions.

Construction of pseudoternary phase diagrams

A series of the SNEDDS were prepared and their self-emulsifying properties were observed visually. Pseudo-ternary phase diagrams were constructed in the absence of TEL to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant, and co-surfactant in the SNEDDS formulations. The ratio of surfactant to co-surfactant was very effective for a stable and an efficient SNEDDS formation. The phase diagrams were constructed at surfactant/co-surfactant ratios of 4:1, 2:1, 1:1, 1:2, and 1:4 (w/w). The gel-like region was found to become large with the increasing concentration of Tween® 20, while the self-nanoemulsifying region expanded with the amount of Carbitol® increasing. The maximum self-nanoemulsifying region had to be at a ratio of 1:4. However, the drug precipitation was observed after several hours at ratios of 1:2 and 1:4. Co-surfactants are beneficial to form a nanoemulsion at a proper concentration range. However, an excessive amount of the co-surfactant will cause the system to become less stable for its intrinsic high aqueous solubility and lead to the droplet size increasing as a result of the expanding interfacial film.[9] Hence, the optimal ratio of surfactant to co-surfactant was selected to be 2:1 [Figure 1].

Based on above results, a three-component SNEDDS formulation was established containing 32% Acrysol® EL 135 as oil (on the basis of the solubility study and required target amount of TEL, 20 mg), 43.33% Tween® 20 as the surfactant, and 23.3% Carbitol® as the co-surfactant (on the basis of phase diagrams). It has been reported that the drug incorporated in the SNEDDS may have some effect on the self-emulsifying performance.[3] In our study, no significant differences were found in self-emulsifying performance when compared with the corresponding formulations with TEL.

Evaluation of the optimized SNEDDS

In the self-emulsifying systems, the free energy required to form an emulsion was very low, thereby allowing a spontaneous formation of an interface between the oil droplets and water.

Table 2: Emulsification efficiency of various surfactants and co-surfactants

Surfactants/ co-surfactants	%Transparency[a]	No. of inversions[a]
Tween® 80	98.1	20
Tween® 20	100	5
Cremophor® EL	94.2	30
Cremophor® RH 40	99.5	35
Solutol® HS 15	70.8	40
Span® 80	50.5	50
PEG 400	99.3	30
PG	99.4	10
Glycerol	89	25
Plurol Oleique®	88.6	25
Carbitol®	100	5
Capmul® MCM C8	80	40

[a]Data are expressed as means (n = 3)

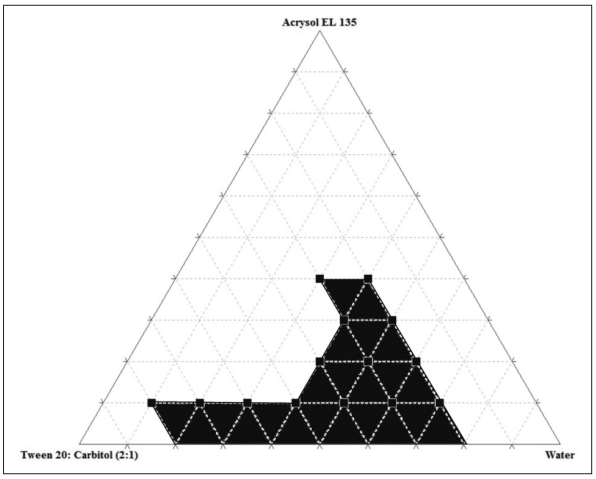

Figure 1: Pseudoternary phase diagram with surfactant/cosurfactant (km) = 2:1

Table 3: Dilution study of the optimized TEL-loaded SNEDDS formulation

Dilution media	Fraction of dilution	Phosphate buffer pH 1.2		Water		Phosphate buffer pH 7.5	
		Immediately	After 24 h	Immediately	After 24 h	Immediately	After 24 h
TEL-loaded SNEDDS	1:50	44.0[a] (0.21[b])	44.1 (0.23)	45.3 (0.33)	43.5 (0.13)	44.9 (0.56)	46.1 (0.53)
	1:100	42.0[a] (0.21[b])	42.1 (0.23)	41.3 (0.33)	42.5 (0.13)	42.9 (0.56)	42.1 (0.53)
	1:500	40.0[a] (0.21[b])	41.5 (0.23)	40.7 (0.33)	41.6 (0.13)	40.9 (0.56)	41.2 (0.53)

[a]Data are expressed in nanometers as means, n = 3; [b]Values in parentheses represent the polydispersibility index

Moreover, since the drug released will be in nanosize, it will increase the effective surface area for dissolution and ultimately *in vivo* absorption.

Emulsification time

In SNEDDS, the primary means of self-emulsification assessment is visual estimation. The efficiency of self-emulsification could be estimated primarily by determining the rate of emulsification which is an important index for the assessment of the efficiency of emulsification,[3] that is, the SNEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. The emulsification time study showed that the optimized formulation employed could emulsify within 25 s.

Droplet size and zeta potential determination

The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption.[12] We observed that the optimized formulation gave the smallest particle size (40 ± 4.23 nm, mean ± SD, n = 3) than other SNEDDS formulations and was chosen for further studies. The charge of the oil droplets of SNEDDS is another property that should be assessed for increased absorption.[13] The charge of oil droplets in SNEDDS was negative due to the presence of free fatty acids; the zeta potential of the optimized formulation was -23.9 ± 0.42 (mean ± SD, n = 3). In general, the zeta potential value of ±30 mV is sufficient for the stability of a nanosuspension.[14] In our formulation, it is -23.9 ± 0.42 which means it complies with the requirement of the zeta potential for stability [Figure 2].

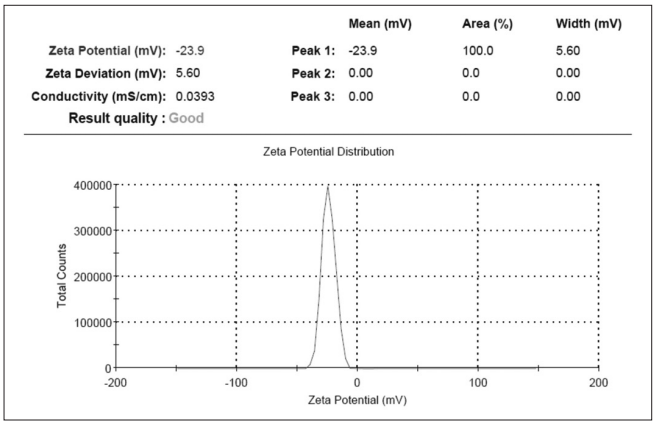

Figure 2: Zeta potential determination of the optimized TEL-loaded SNEDDS

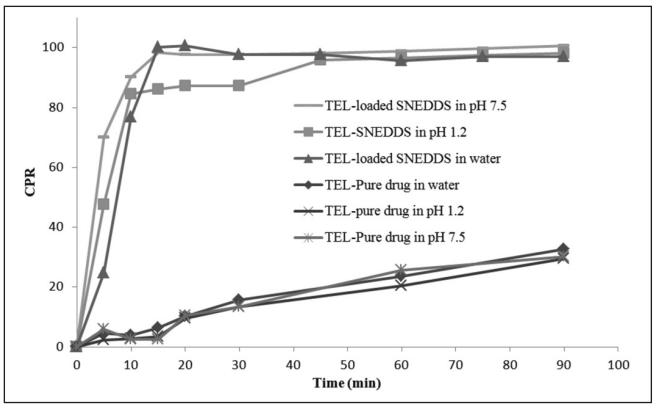

Figure 3: *In vitro* drug release of TEL from the SNEEDS in water and phosphate buffer, pH 1.2 and pH 7.5, compared with the pure drug

In vitro drug release

Dissolution studies were performed for the optimized SNEDDS formulation in water and phosphate buffer, pH 1.2 and pH 7.5, and the results were compared with the pure drug. It was also seen that changes in the dissolution medium (buffer, pH 1.2 and 7.5, and water) had no effect on the drug release from either plain TEL or the SNEDDS formulation [Figure 3]. This observation can be explained by the fact that TEL has no ionizable group and thus its solubility and dissolution is pH independent. As the emulsification time is below 25 s, the maximum percentage of the drug is released within 15 min; however, the dissolution studies were conducted for 2 h to observe the variation or occurrence of precipitation over a time.

Effect of dilution studies/robustness on dilution

Distilled water was used as a dispersion medium in the present study. No significant difference is observed when the nanoemulsions prepared by nonionic surfactants were dispersed in water or phosphate buffer, pH 7.5 or pH 1.2.[15,16] Dilution studies of the optimized formulation have been shown in Table 3.

Drug content

The drug content of the optimized formulation was found to be 99.34 ± 0.42 (mean ± SD, n = 3).

In vivo studies

TEL determination in the rat blood was carried out using a validated HPLC technique that has been successfully developed in-house. The plasma concentrations versus time profile graph is shown in Figure 4, and the pharmacokinetic parameters are summarized in Table 4. Dosing the aqueous suspensions of TEL resulted in

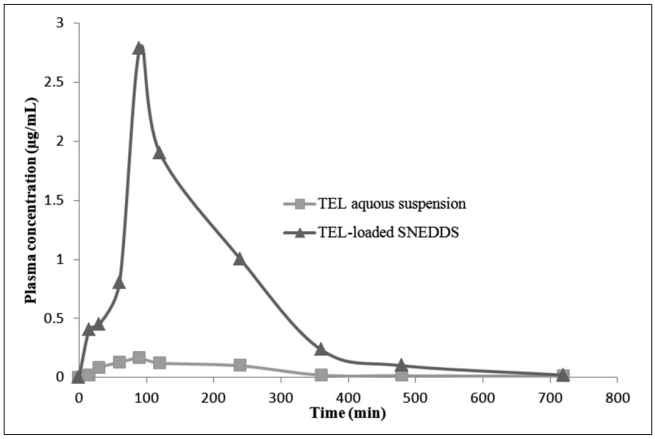

Figure 4: Plasma concentration versus time profiles after the oral administration of the TEL-loaded SNEDDS, compared with TEL pharmacokinetics after the dosing aquoues suspension

Table 4: Pharmacokinetic parameter after the oral administration of the optimized formulation of the TEL-loaded SNEDDS

Formulation	C_{max}[a] (μg/mL)	T_{max}[a] (min)	$AUC_{0\rightarrow12h}$[a] (ng h/mL)
TEL-loaded SNEDDS	9.45 ± 0.99	100	1037.90 ± 34.67
Pure drug suspension	0.56 ± 0.08	100	138.61 ± 23.11

[a]All the values are in mean ± SD (n = 3)

the lowest average TEL plasma concentrations. However, the AUC was 7.5 times greater when TEL was administered as the SNEDDS, compared with the AUC obtained for the aqueous TEL suspension. The optimized SNEDDS resulted in a C_{max} of 9.45 μg/mL, which was 16.87-fold higher than that obtained with the same dose of TEL administered as an aqueous suspension. The T_{max} (100 min) after SNEDDS dosing was the same as that obtained within aqueous suspensions (100 min) which indicates a good potential of SNEDDS to have a higher C_{max} without any change in the T_{max}. These results reveal that the formulation of TEL as SNEDDS results in a significant increase in absorption, compared with that from the aqueous suspensions.

CONCLUSION

In this study, SNEDDS of TEL were prepared and evaluated for their *in vitro* and *in vivo* behavior. The optimized formulation consisting of Acrysol® EL 135 (32%w/w), Tween® 20 (43.33%w/w), and Carbitol (21.67%w/w) exhibited faster release profiles with a rapid rate of emulsification. The optimized SNEDDS formulation of TEL showed a significant increase in the dissolution rate and oral absorption compared to the aqueous drug suspension. Thus, SNEDDS can be regarded as a novel and commercially feasible alternative to current TEL formulations. However, further studies in higher animals and human beings need to be performed before this formulation can be commercially exploited.

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