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Development and Pharmacokinetic Evaluation of Industrially Viable Self-microemulsifying Drug Delivery Systems (SMEDDS) for Terbinafine

Ankit Baheti[1], Saurabh Srivastava[1*], Deepak Sahoo[2], Rohit Lowalekar[1], Bibhu Prasad Panda[2], Bijay Kumar Padhi[1] and Rajeev Raghuvanshi[1]

[1]*Dr. Reddy's Laboratories Ltd., Hyderabad, Telangana, India;* [2]*Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India*

Saurabh Srivastava

Abstract: *Objective*: The aim of this study was to develop a formulation for lymphatic uptake with enhanced solubility of antifungal drug, terbinafine by use of self-microemulsifying drug delivery system (SMEDDS); suitable enough to be an industrially feasible and acceptable dosage form. *Methods*: Pseudo ternary phase diagrams were plotted using suitable oils, surfactants and co-surfactants. The optimized formulation was fabricated and characterized by various *in-vitro* parameters like droplet size, polydispersity index, zeta potential, cross-polarized light microscopy, thermodynamic stability, viscosity and compatibility with capsule shell. The optimized formulation was also tested in animal model for per oral conceptualization of lymphatic uptake in absence and presence of chylomicron blocker (cycloheximide) followed by the pharmacokinetic evaluation of the same. *Results*: The self-emulsification time, droplet size, polydispersity index of the optimized formulation remained unaffected in different media (water, 0.1N HCl and phosphate buffer pH 6.8) over the test time period. Crossed-polarized light microscopy examination of diluted SMEDDS formulation indicated that the dispersion was an isotropically stable system. The rate of dissolution for SMEDDS formulation was almost two folds than the marketed formulation (Lamisil®). Current investigation indicates a potential for uptake of the lipid based SMEDDS formulation through lymphatic route with enhanced solubility of the candidate drug terbinafine. The terbinafine SMEDDS when orally administered to rat with and without chylomicron flow blocking agent (cycloheximide) exhibited the area under the curve ($AUC_{0\text{-}48}$ hr) as 7425.44 ng h/ml and 10168.17 ng h/ml respectively hence indicating absorption through the lymphatic route. Thus, the study reaffirms the use of SMEDDS formulation for the drug delivery by lymphatic uptake.

Keywords: Bioavailability, lymphatic uptake, SMEDDS, terbinafine, zeta potential.

INTRODUCTION

Oral lipid based formulations have been extensively explored for enhancing solubility and bioavailability of poorly water soluble lipophilic drugs. Lipids are known to promote lymphatic uptake of the drugs and facilitates their entry into systemic circulation. Different lipid-based drug delivery systems used to enhance bioavailability of poorly water soluble drugs are; simple oil solution of the drug [1]; emulsions [2]; solid dispersions [3]; self-microemulsifying drug delivery systems (SMEDDS) [4-6]; liposomes [7]; and solid lipid nanoparticles [8]. Lipid nanoparticles have also been specifically explored for improving lymphatic transport of drugs prone to first pass metabolism such as Lopinavir, Nimodipine and Tamoxifen [9].

Fate of lipids after oral administration and mechanistic aspects of lymphatic targeting are well described in the literature [10, 11]. Most important factors influencing lymphatic uptake of drugs are; lipophilicity of the drugs, solubility in triglycerides and the nature of lipid used in the formulation. Generally, drugs with log P >5 and solubility in triglycerides > 50 mg/mL are more likely to have access to the systemic circulation via lymphatic transport mechanism. Free fatty acids of chain length lower than 12 carbon atoms are primarily transported by means of portal blood circulation whereas, free fatty acids with chain length greater than 12 carbon are re-esterified and transported via intestinal lymphatic transport.

Among different lipid based formulations, SMEDDS containing liquid lipids are capable of forming fine emulsion upon dispersion in the aqueous media. They offer a unique advantage as they can hold the drug in the dissolved state with a large interfacial surface area available for drug absorption thus resulting in a reduced variability across the bioavailability. SMEDDS are isotropic mixtures of an oil, surfactant, co-surfactant and the drug. They form fine oil-in-water emulsions when introduced into the aqueous media under gentle agitation. The digestive motility of the stomach and intestine provide the required agitation necessary for self-emulsification under *in-vivo* conditions. The small lipid droplet size produced by SMEDDS facilitates lipid digestion, resulting in more rapid incorporation of the drug into the bile salt to form mixed micelles. Additionally they can also facilitate direct drug absorption independent of the bile salt

*Address correspondence to this author at the Proprietary Products, Formulation R&D, Block 'C', IPDO Innovation Plaza, Survey Nos. 42, 45, 46 & 54, Bachupally, Qutubullapur, Hyderabad, R.R. Dist. -500072, Telangana, India; Tel: +91-9652815707; E-mail: ssrivastava@drreddys.com

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mixed micelle transport system [10]. The SMEDDS formulated with LCFA may hold potential as lymphotropic drug delivery system by facilitating the optimal production of drug-carrying chylomicrons.

Terbinafine is an allylamine antifungal agent indicated for the treatment of oncomycosis of toenail and fingernails caused by dermatophytes. Terbinafine belongs to Biopharmaceutics classification system (BCS) class IV category that is well absorbed (>70%) from GIT upon oral administration. However, bioavailability of terbinafine is approximately 40% in humans as a result of first-pass metabolism, thus making it an ideal candidate for lymphatic targeting [12].

The present research work discusses the development, *in-vitro* and *in-vivo* evaluation of SMEDDS of terbinafine for lymphatic uptake. This investigation reports successful formulation of SMEDDS containing a high dose of terbinafine (250 mg) that can release the drug rapidly in the *in-vivo* conditions, which in itself is a significant challenge. Additionally, the influence of SMEDDS on enhancement of lymphatic uptake of terbinafine was also evaluated through pharmacokinetic parameters. Cycloheximide was used to block chylomicron lymphatic flow for indirect estimation of oral bioavailability through lymphatic uptake in rats. The optimized formulation was tested *in-vivo* for enhancing the lymphatic uptake of terbinafine along with an increase in the overall bioavailability of terbinafine in rat model.

MATERIALS AND METHODS

Materials

Terbinafine was available with Dr. Reddy's Laboratories (Hyderabad, India). Labrafil M1994CS, Labrafil M2125CS, Maisine™ 35-1, Plurol® Oleique CC 497, Labrasol, Peceol were obtained as gift samples from Gattefosse, France. Oleic oil, olive oil, castor oil, corn oil, soybean oil were purchased from Croda Inc., PEG 400 and hard gelatin capsule 00 el were purchased from Merck India Ltd. and ACG Capsugels respectively.

Solubility Studies

Solubility estimation of terbinafine in various oils, surfactants and co-surfactants was determined using the shake flask method. A known excess amount of terbinafine was introduced into 3 ml of each excipient in a stoppered vial. The sealed vials were then heated to 40°C in a water bath to facilitate solubilization using vortex mixer and then shaken on a rotary shaker for 72 hr at 25°C. After standing for 24 hr at room temperature to reach equilibrium, each vial was centrifuged (Heraeus, Megafuse 40) at 3200xg for 15 min. Excess insoluble terbinafine was removed by filtration using whatman filter (0.45 µm). The concentration of dissolved terbinafine in various excipients was quantified through UV spectroscopy (Shimadzu, UV-1800) at 223 nm by using a validated method.

Pseudo Ternary Phase Diagram Studies

Pseudo ternary phase diagrams of oil, surfactant/co-surfactant, and water were developed using the water titration method. Based on the solubility data, lipids that could support higher solubility concentrations of terbinafine in oil were shortlisted. Surfactants and co-surfactants were selected on the basis of their emulsification efficiency. The mixtures of oil and surfactant/co-surfactant at certain weight ratio were diluted with water in drop-wise manner. For each phase diagram, a 1:1 ratio of surfactant/co-surfactant was taken. Each of the mixtures were titrated with water and visually observed for phase clarity. After identification of microemulsion region in the phase diagrams, the oil-surfactant/co-surfactant combinations were selected. Further, the selected oil-surfactant/co-surfactant combinations were investigated for the effect of terbinafine on the microemulsion region.

Preparation of Terbinafine SMEDDS

A series of SMEDDS were prepared by dissolving terbinafine into oil with varying ratios of surfactant and co-surfactant. The mixtures were then heated to 40°C and vortex-mixed to form a homogeneous mixture. Final formulation was stored at 25°C till use.

Self-emulsification Ability of Terbinafine SMEDDS in different Media

The self-emulsification efficacy of selected SMEDDS formulation was evaluated through aqueous dilution method in different media with gentle agitation at 37°C±0.5°C. Purified water, 0.1 N HCl and pH 6.8 phosphate buffer were used as dilution media (250 ml each). A visual assessment was carried out after dilution of 1g of SMEDDS formulation in 250 ml media that was magnetically stirred.

Droplet Size and Polydispersity Index Determination

The SMEDDS formulations were diluted 10 times using distilled water, 0.1N HCl or pH 6.8 phosphate buffer. The droplet size and polydispersity index of resultant microemulsion was determined immediately and after holding it for 4hr by using laser scattering particle size analyzer (Malvern zetasizer Nano ZS).

Zeta-Potential Determination

The SMEDDS formulations were diluted 10 times with distilled water. The zeta potential of resultant microemulsion was determined immediately and after holding it for 4hr by laser scattering particle size analyzer (Malvern zetasizer Nano ZS).

Cross-polarized Light Microscopy

The SMEDDS formulations were diluted 10 times with distilled water and were examined under cross-polarized light microscopy (Nikon Eclipse 50i POL) to evaluate the presence of liquid crystal structures.

In-vitro Dissolution Test

In-vitro dissolution tests were performed using USP apparatus II (Electrolab TDT 08L USP-II). The revolution speed of paddle and the bath temperature were set to 50 rpm and 37°C±0.5°C respectively. Dissolution studies were performed for commercially available tablets (Lamisil ®) and optimized terbinafine-loaded SMEDDS filled in 00el hard gelatin capsules in triplicates. Drug release from both test

and reference formulations were evaluated in 900 ml 0.1N HCl and pH 6.8 phosphate buffer. 5 ml aliquots of samples were withdrawn at 5, 15, 30, 45, 60, 90 and 120 min. with appropriate replenishment of blank buffers. The samples were analyzed spectrophotometrically at 223 nm after suitable dilution.

Thermodynamic Stability Studies

The thermodynamic stability of terbinafine loaded SMEDDS formulations were evaluated by subjecting them to three freeze-thaw cycles. Approximately 5 g of each formulation was placed in stoppered vials. Each cycle consisted of freezing at -20°C for 48 hr and then followed by thawing at 40°C for 48 hr. The samples were then centrifuged at 5000xg for 30 min. and visually observed for phase separation.

SMEDDS Viscosity and Compatibility with Hard Gelatin Capsules

Viscosity of drug loaded SMEDDS was measured using Brookfield viscometer (DVII + Pro viscometer) at shear rate of 1500 sec^{-1}. About 1g of formulation was encapsulated in hard gelatin capsules size 00 el and evaluated for any leakage after three months of storage at 25 °C ±2°C.

Stability Studies

Formulations were subjected to stability studies on real time conditions (25°C±2°C/60±5% RH) and accelerated conditions (40°C±2°C/75±5% RH) in stability chamber (Newtronics India Ltd.) for a period of 3 months. The samples were analyzed for assay, droplet size and zeta potential at pre-determined time intervals.

Determination of Terbinafine in Rat Plasma by UFLC

Instrumentations

The formulation was analysed using LC-20 AD UFLC (Ultra-Fast Liquid Chromatography) from Shimadzu. The detector used was SPD-M20A Photo-Diode Array UV Detector (Shimadzu Prominence). The wavelength was set at 223 nm. The software used was LC solution (Shimadzu). The column used was reverse phase C-18 (0.5 µm, 4.6 X 250 mm).

Mobile Phase Preparation

The mobile phase comprised of acetonitrile and aqueous phase (55:45 v/v). The aqueous phase consisted of 20 mM Potassium dihydrogen phosphate and 0.25% w/v triethylamine in distilled water. The pH of the aqueous solution was adjusted to 3.8 by addition of 1 M Phosphoric acid. The mobile phase was run isocratically. The flow rate of the mobile phase was maintained at 1.0 ml/min and the injection volumes were 25 µl. The mobile phase was degassed by using sonicator prior to its use. The UFLC column was maintained at ambient temperature.

Plasma Sample Preparation for the Determination of Terbinafine

Hundred microliter of human plasma was transferred into a 1.5 ml eppendorf tube. Three hundred microliter each of acetonitrile was added to the plasma and vortex mixed for 5 minutes. The mixture was then centrifuged at 2000xg for 15 min. The supernatant layer was transferred into another tube and filtered through a 0.45 µm filter. A 25 µl of the filtrate was then injected into the UFLC column. Terbinafine was detected at a wavelength of 223 nm with a retention time of 12.8 min.

In-vivo Pharmacokinetic Study

Two sets of *in-vivo* studies were performed using wistar rats. The first study was aimed to investigate the potential of the SMEDDS formulation in enhancing the oral bioavailability of drug whereas, the second experiment was planned to evaluate the effect of SMEDDS on the lymphatic transport of terbinafine after absorption. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee, India.

Study 1: Bioavailability Assessment (Without Chylomicron Blocking Agent)

The study for bioavailability assessment was conducted in two groups consisting of four male Wistar rats weighing 180-220 g, in each group. The animals were fasted for 12 h before drug administration and also during the sampling period but were allowed free access to water throughout the experiment. Animal groups received 22.5 mg/kg of native drug suspension (lipid-free formulation) or SMEDDS (lipid-based formulation) by oral gavage. First group was orally administered plain drug suspension to ascertain the drug bioavailability when given without lipid, second group was administered SMEDDS formulation for determination of bioavailability enhancement. Blood samples (approximately 0.3 ml) were collected sublingually at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h post dose. The blood samples were transferred into heparinized tubes. Plasma was collected by centrifuging blood at 2000xg for a period of 15 min at 4°C. About 0.1-0.15 ml aliquot of clear plasma obtained from each blood sample was pipetted into a fresh eppendorf tube. All plasma samples were stored at -20°C until completion of their analysis.

Study 2: Bioavailability Assessment (with Chylomicron Blocking Agent)

The study for bioavailability through lymphatic route by chylomicron flow blocking method was conducted in two groups consisting four male wistar rats weighing 240-280 g in each group. The animals were fasted for 12 h before drug administration and also during the sampling period but were allowed free access to water throughout the experiment. Rats were administered intraperitoneal (i.p.), 3 mg/kg of cycloheximide in saline (0.3 mg/ml) to block the lymphatic flow of chylomicron. After one hour of i.p. injection, the respective groups received 22.5 mg/kg native drug suspension or SMEDDS through oral gavage. Blood samples (approximately 0.3 ml) were collected sublingually at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h post dose and transferred into heparinized tubes. Plasma was collected by centrifuging blood at 2000xg for 15 min. About 0.1-0.15 ml aliquot of clear plasma obtained from each blood sample was pipetted into a fresh eppendorf tube. All plasma samples were stored at -20°C until completion of their analysis.

Pharmacokinetic Study

Plasma concentrations vs. time data for Terbinafine in individual rats were analyzed using Win-Nonlin® Professional software, by means of selecting the noncompartmental analysis model.

RESULTS AND DISCUSSION

Solubility Study

The solubility of terbinafine in various oils, surfactants and co-surfactants are presented in (Figs. **1** and **2**). Corn oil, soybean oil and Maisine™ 35-1 demonstrated highest solubilization potential for terbinafine. Solubility of drug in self-emulsifying excipients like oils, surfactants and co-surfactants and their combination thereof plays a critical role in development of self-microemulsifying system. This is attributed to the ability of SMEDDS to avoid precipitation of the drug on dilution with the gastrointestinal fluid in the gut lumen. Hence, the components used in formulation should have high solubilization capacity for the drug, ensuring the solubilization of the terbinafine in the resultant dispersion. Cremophor EL and Tween 80 as surfactant; PEG 400 and Labrasol as co-surfactant were selected based on their self-emulsification ability.

Pseudo Ternary Phase Diagram Studies

Based on the solubility study results, the phase diagrams were constructed containing corn oil, soybean oil and Maisine™ 35-1 as oil phase, Cremophor EL and Tween 80 were used as surfactants, Labrasol and PEG 400 were forming the co-surfactant phase. A series of SMEDDS were prepared and their self-emulsifying properties were determined by physical observation. Pseudo-ternary phase diagrams were prepared without using the drug to identify the self-emulsifying

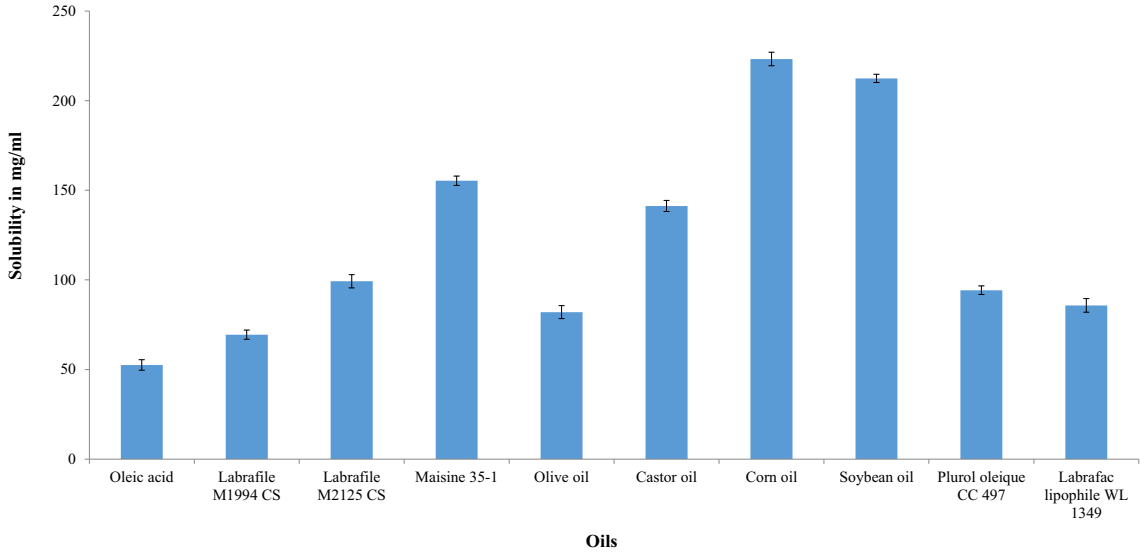

Fig. (1). Solubility of terbinafine in various oils.

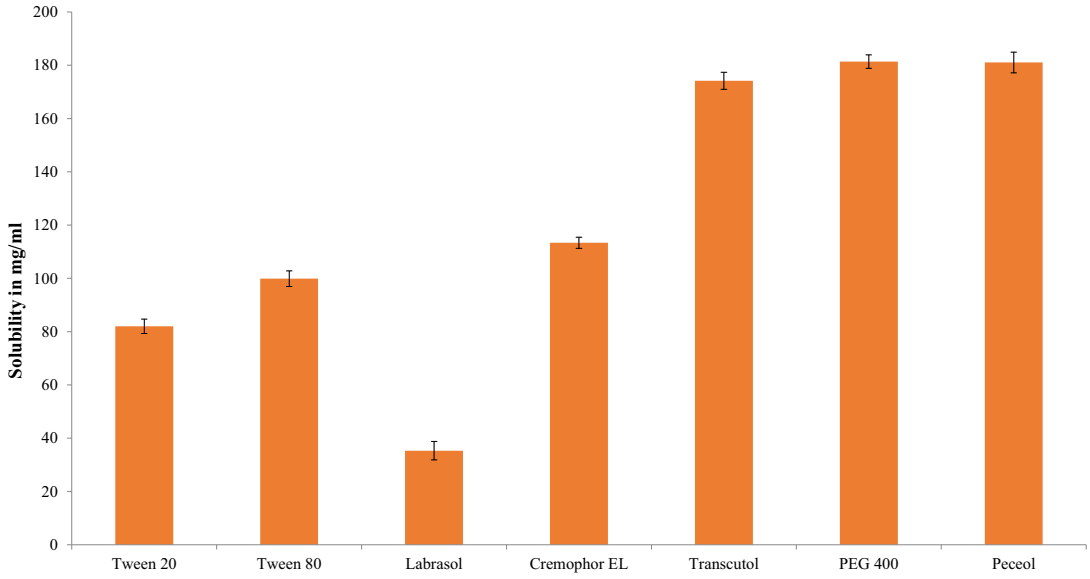

Fig. (2). Solubility of terbinafine in various surfactants and co surfactants.

regions and to optimize the concentrations of oil, surfactant and co-surfactant ratio in the SMEDDS formulations. It was observed that the efficiency of emulsification was good when the surfactant/co surfactant concentration was more than 50% w/w of SMEDDS formulation. Further, it was observed that on increasing the concentration of the surfactants, Cremophor EL and Tween 80 within the self-emulsifying region, there was an increase in the spontaneity of the self-emulsification process. It was observed that the emulsification was not efficient with less than 40% w/w of surfactant ratio; indicating the importance of surfactant/cosurfactant for the stability of SMEDDS formulation. The region of self-emulsification varies significantly with the type of surfactant and co-surfactant and also with their respective ratios.

In view of current investigation, Maisine™ 35-1 with Cremophore EL and Labrasol were shortlisted for further studies based on larger self-emulsifying regions and a greater capacity for incorporation of terbinafine (Fig. **3**). Three shortlisted formulations with surfactant to co-surfactant ratio of 3:2; 1:1 and 2:3 were selected for further studies. The composition of the shortlisted formulations is enlisted in Table **2**.

Self-Emulsification Ability of Terbinafine SMEDDS in Different Media

The SMEDDS formulations were prepared by taking 250 mg of Maisine™ 35-1 containing 250 mg of terbinafine with different ratios of surfactants and co-surfactants. The self-emulsification ability of the formulation was visually assessed in different media. The observation of the effect of water and 0.1 N HCl on the self-emulsification ability and stability of the formulations is depicted in Table **1**. Formulation Ter-1, Ter-7 and Ter-13 were found to self-emulsify within 2 minutes and no phase separation was observed up to 6 hr in the tested media.

Droplet Size, Polydispersity Index and Zeta-Potential Determination

The average droplet size in different media (water, 0.1N HCl or pH6.8 phosphate buffer) for all three formulation is shown in Table **3**. The droplet size of Ter-1 and Ter-13 was found to be significantly different in each of the media, however, in case of Ter-7 it was found to be minor [13]. The droplet size and polydispersity index of the emulsion are crucial factors because they determine the rate and extent of drug release from the formulation and in turn dictate the drug absorption. Smaller the droplet size of the emulsion, more rapid is the absorption and hence better the bioavailability. In our optimized formulation, the droplet size and polydispersity index were similar over a period of 4 hours indicating a stable formulation.

Zeta potential is defined as the potential difference between surface of the tightly bound layer and the electro- neutral region of an emulsion. It has a significant effect on the stability of the microemulsion since zeta-potential governs the degree of repulsion between adjacent, similarly charged, dispersed droplets If the zeta-potential is reduced below a certain value, the attractive forces exceed the repulsive forces and the system becomes unstable due to flocculation of the particles [14]. As depicted in Table **3**, the zeta potential of formulation Ter-7 was found to be 3.71±1.92 (mean± SD, n=3) at initial and 3.84±2.71 (mean±SD, n=3) at 4 hr. This range complies with the required zeta potential prerequisite for a stable microemulsion, *i.e.*, ±30 millivolt (mV).

Cross-polarized Light Microscopy

Examination of 10x diluted SMEDDS formulation under cross-polarized light microscopy exhibited a dark field indicating an excellent dispersion of spherical micelles or microemulsion and an isotropically stable system. Liquid crystalline

Table 1. Dispersibility test and self-emulsification time (SEF).

Formulation	Water		0.1 N HCl	
	Turbidity	SEF Time	Turbidity	SEF Time
Ter-1	Clear Slightly bluish	<2 min	Clear Slightly bluish	<2 min
Ter-7	Clear Slightly bluish	<1 min	Clear Slightly bluish	<1 min
Ter-13	Slightly Bluish	<1 min	Slightly Bluish	<1 min

Table 2. Optimized formulation composition.

Component	Ter-1	Ter-7	Ter-13
Antifungal drug (mg)	250	250	250
Maisine™ 35-1 (mg)	250	250	250
Cremophor EL (mg)	300	250	200
Labrasol (mg)	200	250	300
Total weight (mg)	1000	1000	1000

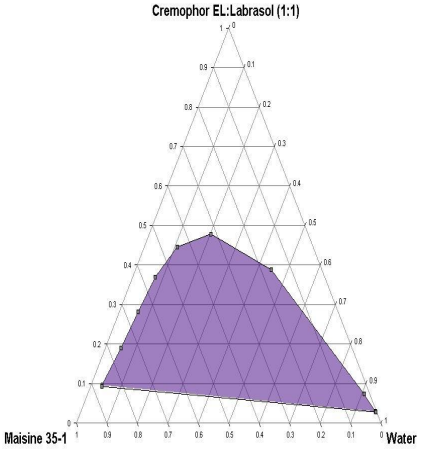

Figure A: Pseudo-ternary phase diagram of Maisine 35-1 vs Cremophor EL:Labrasol (1:1)

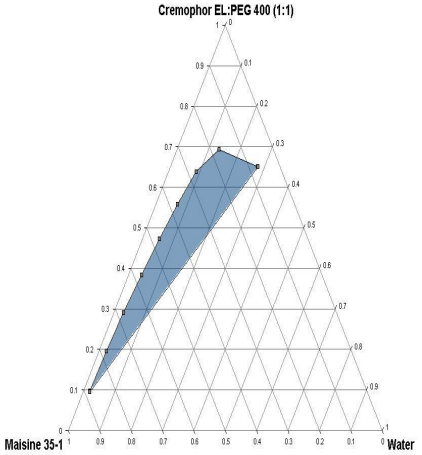

Figure B: Pseudo-ternary phase diagram of Maisine 35-1 vs Cremophor EL:PEG 400 (1:1)

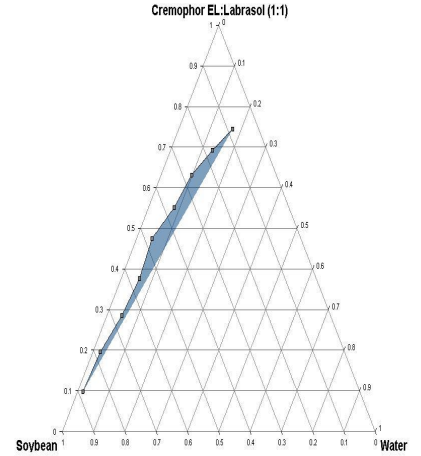

Figure C: Pseudo-ternary phase diagram of Corn oil vs Cremophor EL:Labrasol (1:1)

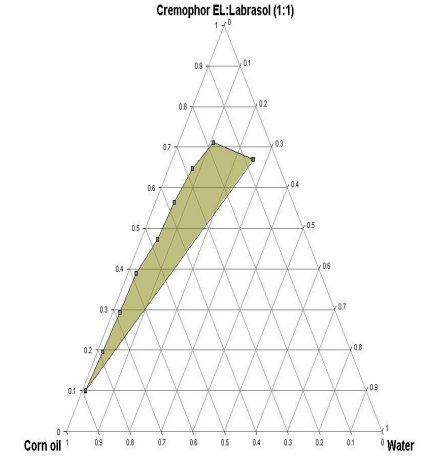

Figure D: Pseudo-ternary phase diagram of Soybean vs Cremophor EL:Labrasol (1:1)

Fig. (3). Pseudo-ternary phase diagrams of the Maisine™ 35-1, corn oil and soybean oil with different surfactants and co-surfactants.

phase depends upon the property of oil, surfactant, co-surfactant and water concentration. Different liquid crystalline phase affects the drug release from the formulation [15] and cross-polarized light microscopy is the best technique to distinguish lamellar liquid crystals from microemulsion.

In-vitro Dissolution Test

The dissolution study was carried out for some of the selected formulations, *i.e.*, Ter-1, Ter-7, Ter-13 & marketed formulation (Lamisil ®) respectively. The comparative dissolution profile for the above formulations are shown in (Figs. **3** and **4**). Dissolution study was performed in 0.1N HCl and pH 6.8 phosphate buffer and served as one of the critical screening criteria for formulation finalization. Drug dissolution from formulation Ter-7 in 0.1N HCl was found to be 100% in 45 min. and formulations Ter-1 & Ter-13 showed similar release patterns *i.e.* 97.83±3.44% & 98.55±1.35% respectively in 45 min. In contrast, drug release from marketed formulation (Lamisil®) was found to be very low *i.e.* 47.87±0.07% in 45 min. Drug dissolution from formulation Ter-7 in pH 6.8 phosphate buffer (with 0.5% sodium lauryl sulphate, SLS) was 100% in 30 min and formulations Ter-1 & Ter-13 exhibited a similar release *i.e.* 97.2±2.52% and 96.08±2.04% respectively. In comparison, the drug release from marketed formulation (Lamisil®) was found to be sig-

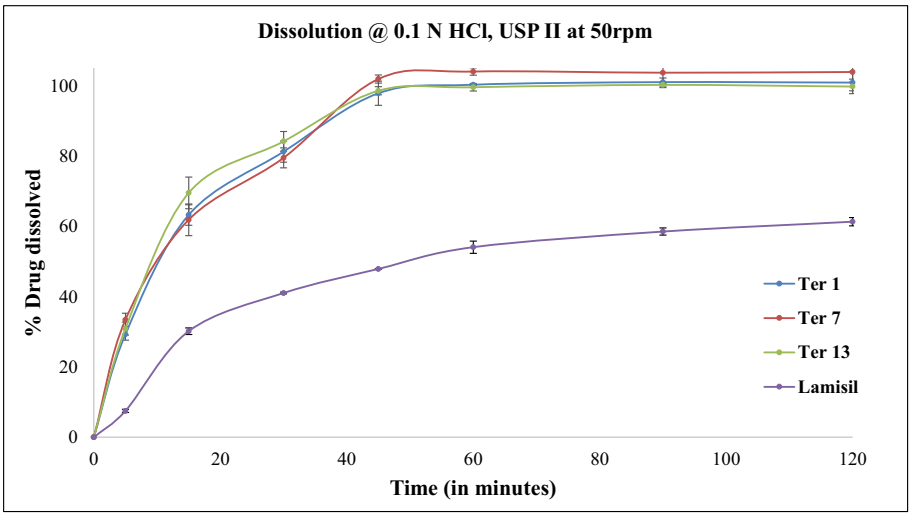

Fig. (4). Cumulative percent drug released from different SMEDDS formulations and marketed preparation (Lamisil®) in 0.1N HCl at 50 rpm.

nificantly low *i.e.* 63.06±0.02% in 30 min. The mentioned data signifies that terbinafine dissolves completely when incorporated within SMEDDS system followed by complete release because of smaller droplet size and an enhanced solubilization provided by Labrasol & Cremophore EL, which in turn allows a faster rate of drug dissolution in different media as compared to marketed product (Lamisil®) which may enhance the bioavailability as well.

Thermodynamic Stability Studies

Thermodynamic stability is a key parameter for the long term stability of drug product. Thermodynamically stable SMEDDS formulations are formed at a particular concentration of oil, surfactant and co-surfactant with no evident phase separation [16]. Ter-1, Ter-7 and Ter-13 were subjected to three freeze-thaw cycles and then subjected to centrifugation in order to screen out any meta-stable forms that may be present in dosage form. In all the cases no phase separation was observed, indicating thermodynamic stability of the formulation and the absence of any metastable forms.

SMEDDS Viscosity and Compatibility with Hard Gelatin Capsules

Formulation filled in hard gelatin capsule showed no sign of leakage when stored at room temperature for a period of three months. To minimize the risk of leaking of liquid SMEDDS from capsule it must exhibit viscosities of at least 100 centipoise (cps) [17]. All the SMEDDS formulations showed preferable viscosity as required to be filled into suitable capsule sizes (Table **4**).

Stability Studies

The drug content, droplet size and zeta potential of the three formulations were analyzed at time intervals of 1, 2 and 3 months and different temperatures. There was no significant change in their physical parameters such as clarity, homogeneity, percentage drug content, droplet size or zeta potential (Table **5**).

Table 4. Viscosity measurements for selected terbinafine formulations.

Formulation	Viscosity (cP) mean±SD	Temperature (°C)
Ter-1	251.9±1.21	22±0.5
Ter-7	244.7±1.01	22±0.5
Ter-13	245.9±1.32	22±0.5

In-vivo Study: Pharmacokinetic Evaluation

Effect of Drug Suspension vs. Lipid Based Formulation (Without Chylomicron Blocking Agent)

The *in-vivo* studies were performed to quantify terbinafine in blood after oral administration. The plasma profile of orally administered drug suspension and SMEDDS formulation were compared. The plasma concentration profile for terbinafine in which SMEDDS showed a significantly greater improvement in drug absorption as compared to drug suspension is depicted in (Fig. **6**).

The pharmacokinetic data exhibits significantly higher drug bioavailability through SMEDDS where the $AUC_{0\text{-}48}$ hr and Cmax were found to be 10168.17 ng h/ml and 469.414 ng/ml, respectively, as compared to the native drug suspension where the $AUC_{0\text{-}48}$ hr and Cmax were reported to be 4067.26 ng h/ml and 187.77 ng/ml respectively, revealing the 2.5 folds greater relative bioavailability of SMEDDS as compared to native drug suspension (Table **6**). The outcome of pharmacokinetic parameters justifies the increased drug absorption through SMEDDS in comparison to native drug suspension.

The absorption of terbinafine is likely to be improved due to one or more of the following phenomenon (i) enhanced solubilization of terbinafine, (ii) delay in gastric emptying, (iii) increase of permeability and, (iv) incorporation of drug

Table 5. Summary of stability data for Ter 7 formulation.

Parameters	Time Points	25°C±2°C/60±5% RH			40°C±2°C/75±5% RH		
		1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Assay (%)		99.323±1.7	99.021±2.7	98.793±1.7	99.125±0.52	98.205±0.52%	97.111±1.52
Droplet size (nm) mean±SD							
Water	Initial	98.3±2.42	95.313±4.23	105.2±1.33	103.2±2.3	113.42±1.22	116.5±2.21
	4hrs	105.2±1.32	108±1.36	102±2.41	121±3.87	117±2.07	111.2±1.43
0.1N HCl (pH 1.2)	Initial	111±1.42	117±0.67	111±1.22	115.42±1.22	122.7±2.2	119.3±2.12
	4hrs	102±0.41	106±2.73	103.2±2.3	121±2.21	116±1.42	123.31±4.21
Phosphate buffer (pH 6.8)	Initial	100±1.48	112±2.12	105±2.42	109±4.21	120.2±2.75	112.8±1.34
	4hrs	112±2.98	102±1.21	109±1.21	106±2.42	126±1.32	115±1.11
Polydispersity index (PDI)							
Water	Initial	0.165	0.172	0.119	0.131	0.122	0.142
	4hrs	0.157	0.182	0.126	0.221	0.212	0.124
0.1N HCl (pH 1.2)	Initial	0.129	0.144	0.192	0.112	0.145	0.122
	4hrs	0.171	0.156	0.122	0.121	0.124	0.21
Phosphate buffer (pH 6.8)	Initial	0.201	0.202	0.149	0.123	0.134	0.143
	4hrs	0.124	0.182	0.22	0.142	0.231	0.122
Zeta potential (mV) mean±SD							
Zeta potential (mV)	Initial	3.99±2.64	3.72±3.12	4.11±2.49	5.81±2.72	5.36±1.89	5.42±1.84
	4hrs	4.06±1.92	3.82±2.71	3.89±1.49	5.93±1.37	5.24±2.52	5.24±3.01

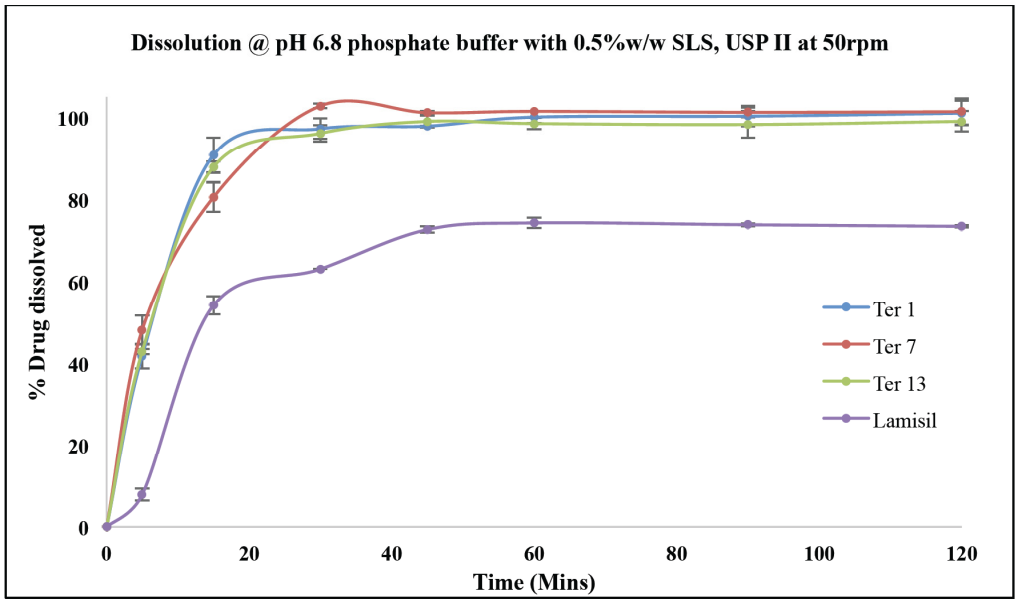

Fig. (5). Cumulative percent drug released from different SMEDDS formulations and marketed preparation (Lamisil®) in 6.8 pH phosphate buffer with 0.5% w/w SLS at 50 rpm.

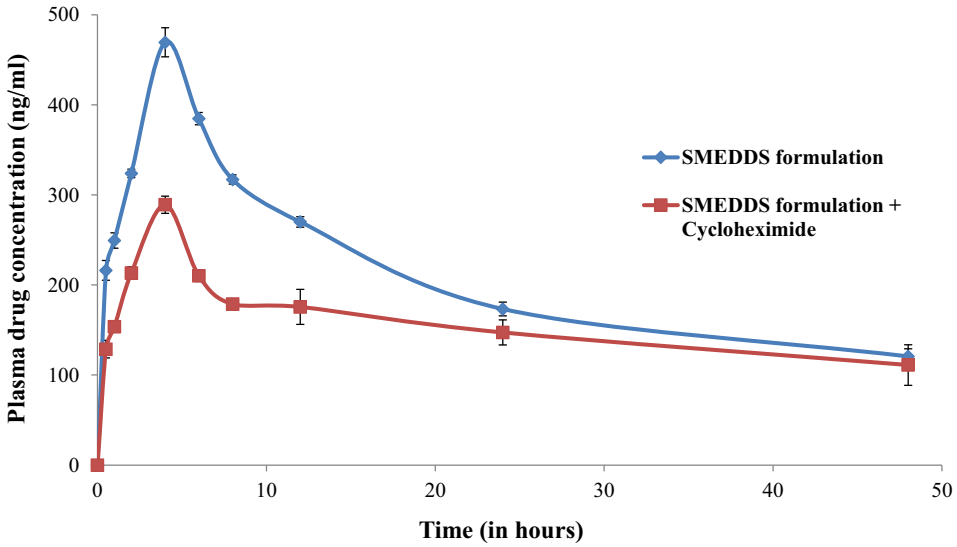

Fig. (6). Effect of chylomicron blocker on plasma drug concentration of terbinafine in SMEDDS formulation.

into lipoproteins. The effects of large amount of surfactants and cosurfactants, contribute towards a smaller lipid droplets size, with a greater surface area and mucosal permeability which in turn are crucial for enhancement of the bioavailability of terbinafine loaded-SMEDDS in comparison with terbinafine suspension. Terbinafine dissolved in the gastrointestinal media could be directly absorbed as the microemulsion droplets in the gastrointestinal tract without any dissolution step. Non-ionic surfactants not only enhance solubility but also improves dissolution of the drug, it may reduce the interfacial surface tension and enhance penetration of the drug through the epithelial cells. The pharmacokinetic data attributed towards the enhancement in dissolution and absorption of the terbinafine administered through SMEDDS formulation; however the rationale for the same *i.e.* lymphatic uptake/systemic circulation needs to be ascertained.

Table 6. Pharmacokinetic parameters of terbinafine after oral administration in rat in plain drug suspension and SMEDDS.

Pharmacokinetic parameters	Plain drug suspension	SMEDDS
K (hr^{-1})	0.021	0.021
$t_{1/2}$ (elimination) (h)	0.02	0.02
Cmax (ng/ml)	187.765	469.414
T_{max} (h)	4	4
AUC $^{0-48h}$ (h*ng/ml)	4067.256	10168.17

Effect of Drug Suspension vs. Lipid Based Formulation (With Chylomicron Blocking Agent)

The contribution of lymphatic uptake on overall absorption of terbinafine needs to be assessed. Thus, chylomicron blocking agent (Cycloheximide) was used to block the lymphatic uptake of drug and thus help in indirect assessment of the amount of drug absorbed through the lymphatic route (Fig. **7**).

The pharmacokinetic parameters using chylomicron blocking agent of SMEDDS exhibited AUC_{0-48} hr and C_{max} as 7425.44 ng h/ml and 289.14 ng/ml, respectively, when compared to the native drug suspension where AUC_{0-48} hr and C_{max} were reported to be 3562.57 ng h/ml and 178.98 ng/ml respectively (Table **7**). The reported data indirectly reflects the fraction of terbinafine transported directly into the systemic circulation, as the lymphatic pathway was blocked through cycloheximide.

Table 7. Pharmacokinetic parameters of terbinafine after oral administration in rat in plain drug suspension and SMEDDS with chylomicron blocker.

Pharmacokinetic parameters	Plain drug suspension	SMEDDS
K (hr^{-1})	0.023	0.011
$t_{1/2}$ (elimination) (h)	30.13	63
Cmax (ng/ml)	178.981	289.14
T_{max} (h)	4	4
AUC $^{0-48h}$ (h*ng/ml)	3562.57	7425.44

By comparing the values of AUC_{0-48} hr, it could be stated that blocking the chylomicron flow with cycloheximide has significantly decreased the plasma concentration *i.e.* from 10168.17 ng h/ml to 7425.44 ng h/ml, after oral administration of SMEDDS whereas, there was no significant difference in the values of plasma concentration (AUC_{0-48} hr) was observed *i.e.* 4067.26 ng h/ml to 3562.57 ng h/ml, in case of native drug suspension (Table **8**). These results indicate that some fraction of terbinafine loaded in SMEDDS formulation is being transported to systemic circulation via lymphatic route (through chylomicron synthesis). Lipoprotein is an

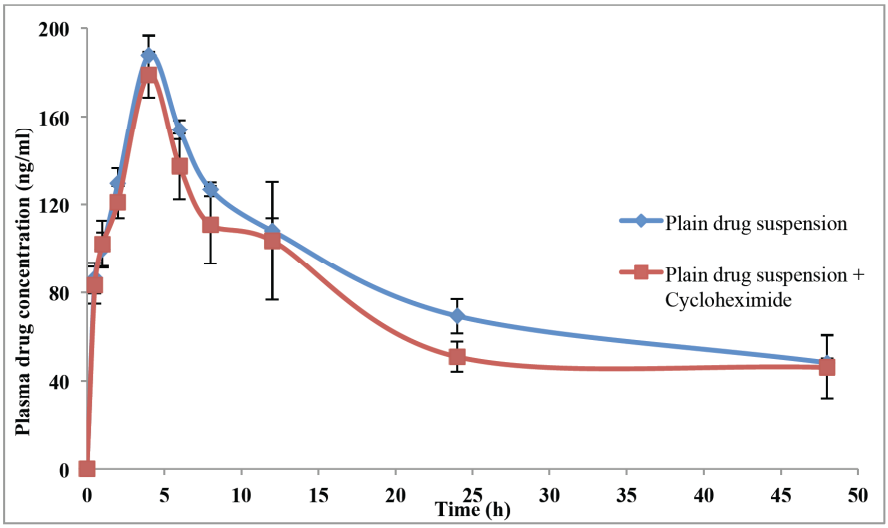

Fig. (7). Effect of chylomicron blocker on plasma drug concentration of terbinafine in plain drug suspension.

Table 8. Bioavailability due to the direct systemic transport, the intestinal lymphatic transport and the total bioavailability.

Formulation	Bioavailability due to direct systemic transport (h*ng/ml)	Bioavailability due to lymphatic transport (h*ng/ml)	Total bioavailability (h*ng/ml)
Plain drug suspension	3562.57	504.686	4067.256
SMEDDS formulation	7425.44	2742.726	10168.17

essential component to initiate formation of chylomicron and cycloheximide blocks this protein synthesis and in turn inhibits the secretion of chylomicrons from the enterocyte, apparently through anti-microtubular effects [18-20].

Comparison of Bioavailability With and Without Chylomicron Blocking Agents

Prediction of the fraction transported through lymph in the cycloheximide model is based upon an indirect estimation [21]. The fraction transported to the systemic circulation achieved in the animals pre-treated with cycloheximide was subtracted from the total bioavailability in the animals pre-treated with saline. However, the standard errors in the plasma concentration curves from both the animals dosed with saline and cycloheximide were relatively low and did not indicate any variation. This indicates that, when the lipid source is sufficiently solubilized upon administration, a lower lipid threshold is necessary to trigger the biochemical cascades responsible for the lymphatic transport.

CONCLUSION

An optimized SMEDDS formulation incorporating the complete dose of terbinafine and smaller globule size was obtained by using 25% Maisine® 35-1, 25% Cremophor EL and 25% Labrasol. Viscosity measurement (towards optimal) and passing the leak test exhibits the suitability of liquid filled hard gelatin capsules when compared to costlier soft gelatin capsules. The data set of thermodynamic stability on storage as per the ICH recommendation provides sufficient confidence for the suitability of the product under different climatic zones. Additionally, the *in-vitro* dissolution of the SMEDDS showed a significantly higher rate of dissolution as compared to marketed formulation (Lamisil®). Current investigation clearly indicates a potential for lymphatic uptake of lipid based SMEDDS formulation with enhanced solubility of the candidate drug terbinafine. The optimized formulation of terbinafine SMEDDS when orally administered to rat in presence and absence of chylomicron flow blocking agent (cycloheximide) showed the area under the curve ($AUC_{0\text{-}48}$ hr) as 7425.44 ng h/ml and 10168.17 ng h/ml respectively indicating the absorption through the lymphatic route. Hence, the study affirms an advantageous use of industrially viable SMEDDS formulation of terbinafine for the drug delivery through lymphatic uptake.

The results of present research encompasses the development of SMEDDS formulation of terbinafine incorporating the complete dose (250 mg) with potential lymphatic targeting, enhanced solubility and adequate viscosity to be filled in acceptable capsules size. Owing to the above considerations, the nascently formulated SMEDDS of terbinafine, not only appears to be a vital formulation strategy for developing an industrially viable and acceptable dosage form of poorly water soluble drug terbinafine but also promises the improvement of bioavailability through lymphatic uptake avoiding the hepatic first pass metabolism.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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PATIENT CONSENT

Declared none.

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