Formulation of Self Micro-Emulsifying Drug Delivery System for Ketoconazole

Manish Bairagi[1], Kaushelendra Mishra[2], Parulben D Mehta[3]

[1,2,3]Lakshmi Narain College of Pharmacy, Bhopal

Abstract

The present investigation was undertaken with an objective to prepare the SMEDDS of ketoconazole in order to improve the bioavailability of lipophilic drugs. A ternary phase diagram was constructed in the absence of ketoconazole. The results revealed that span 60 and PEG 600 used in ratios of 2:1 (F17-18) and 3:1 (F23-24) exhibited largest micro-emulsion area and shortest emulsification time (less than 1 min). It was observed that with increase in the ratio of the PEG 600, spontaneity of the self-emulsification process got increased. A fixed ketoconazole concentration of 5% w/w was selected to be loaded in all self-emulsifying formulations. The prepared formulations were kept in closed containers and tested for thermodynamic stability. All the formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C) and centrifugation at 10,000 g indicating good stability of formulations and their emulsions. The *in vitro* dissolution studies revealed the drug release profiles for the SMEDDS. All the formulations exhibited quick drug release characteristics and almost complete drug release in 15-20 minutes. In contrast, the pure drug exhibited only a maximum of 39.63% release in 60 min duration.

Keywords: Self-microemulsifying, ketoconazole, anti-fungal, ternary phase diagram

Introduction

Oral delivery route is the most convenient route for drug administration to achieve desired therapeutic effects and the greatest degree of patient compliance, especially for chronic condition diseases [1]. Despite some clinical oral formulations have been developed, their low oral bioavailability is still a major hurdle, leading to challenges for pharmaceutical manufacturers to design delivery systems that can provide improved pharmacokinetic profiles and therapeutic responses [2-4]. At present, numerous efforts such as efflux pump inhibitors, permeation enhancers and drug nanonization, have been made to overcome the challenges of low oral bioavailability resulting from low drug solubility, poor permeation and enzymatic degradation [5].

Ketoconazole (KTZ) is chemically 1-[4-(4-{[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1- ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-ones,a broad spectrum antifungal agent active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable due to its poor water solubility leading to shorter half-life i.e. 2 h of the drug. Topically it is used in the treatment of candidal or tinea infections of the skin [6]. Ketoconazole is a poorly water-soluble drug having log P value of 4.4. Peak plasma levels occur within 2 h, after which the therapeutic drug plasma concentration falls abruptly [7]. Several approaches have been studied for improving the bioavailability of the drug [8-11].

Self-emulsifying drug conveyance frameworks (SEDDS) represent a vital tool in improving oral bioavailability of lipophilic medications. Lipophilic medications can be solubilized in SEDDS formulations, empowering them to be administered as a unit dosage form for oral administration.

The overall goal of the present postulation was to improve the dissolvability, dissolution pace, conceivably the intestinal penetrability and bioavailability of ketoconazole by using self-microemulsifying drug delivery systems (SMEDDS) for oral administration.

Material and Methods

Standard curve for Ketoconazole

Ketoconazole 100 mg was weighed and dissolved in methanol in a 100 ml volumetric flask. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1 mg/ml. From the above solution, pipette out 2 ml and placed into 10 ml volumetric flask. The volume was made up to mark with methanol to give a stock solution containing 200 μg/mL.

Preparation of sample solution

Appropriate volume of aliquots (0.2 to 1.0 mL) from Ketoconazole secondary stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 4, 8, 12, 16 and 20 μg/mL. Absorbance of each dilution against methanol as blank was measured at 302 nm.

Drug solubility

The solubility of ketoconazole in different oils, surfactants and co-surfactants was determined according to the method of Date and Nagarsenker [12]. In this method, an excess amount of the drug was mixed with fixed amounts of the oil (castor oil, sesame oil, coconut oil, peanut oil, sunflower oil, eucalyptus oil, lemon oil, oleic acid, sunflower oil, Soyabean oil), surfactants (Tween 80, Tween 20, Span 20, Span 60) and cosurfactants (PEG 400, Propylene glycol, ethanol, butanol) and the mixtures were shaken for 48 hours at 25°C to attain equilibrium. The samples were then centrifuged to remove the undissolved drug, filtered through a 0.45 μm membrane filter, and the supernatant was suitably diluted before spectrophotometric analysis at 302 nm using UV-visible spectrophotometer to determine the amount of the drug dissolved in each excipient.

Surfactant and oil miscibility

The oil and surfactant in the ratio of 1:1 were shaken at 40°C in 3 ml transparent glass vials. The miscibility was monitored optically and considered to be good when the mixture was transparent.

Screening of surfactants for emulsifying ability

The emulsification ability of different surfactants was evaluated by mixing the surfactant with the selected oily phase in a 1:1 weight ratio. The mixtures were vortex mixed and diluted up to 200 fold dilution. The ease of formation of an emulsion was assessed by observing the number of inversion of the volumetric flask required to obtain a uniform emulsion. The resulting emulsion was also examined visually for relative turbidity according to different grading systems (Grades A – E) described by Khoo et al [13] that depict the spontaneity and appearance of the nanoemulsion formed upon dilution.

Screening of co-surfactants for emulsifying ability

The ability of co-surfactants (or co-solvents) to improve the emulsification ability of surfactants was also evaluated according to the method of Date and Nagarsenker [12]. Mixtures of the selected oily phase, surfactants and co-surfactants (or co-solvents) were mixed at a ratio of 3:2:1, respectively, and then diluted with distilled water for 200 fold dilution. The appearance and the ease of formation of nanoemulsion were assessed as described above for screening of surfactants.

Construction of ternary phase diagrams

Based on the solubility of ketoconazole, lemon oil was chosen as the oil phase. Span 60 was used as the surfactant and PEG 400 was employed as the cosurfactant. Distilled water was used as the aqueous phase for development of these phase diagrams. The surfactant and co-surfactant (Smix) in were mixed in different weight ratios (1:1, 2:1, 3:1) so that the concentration of surfactant increases with respect to co-surfactant (Table 1).

The oil phase and each Smix were blended thoroughly in 9 different weight ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9). From these each ratio, 0.1 ml of mixtures was transferred to separate glass beakers. To these contents, 100 ml distilled water was added gently agitated using a magnetic bar at 37°C. The resulted emulsions were examined for clarity, phase separation, and coalescence of oil droplets on standing for 2 h. When the oil droplets easily spread out in water and formed a clear, transparent emulsion, the emulsion was judged as ''good'' emulsion, and when there was poor or no emulsion formation with immediate coalescence of oil droplets, especially when stirring was stopped, the emulsion was judged as ''bad'' emulsion.

Table 1 Composition for construction of ternary phase diagram (%w/w)

Formulation	Oil	Smix ratio 1:1	Smix ratio 2:1	Smix ratio 3:1
F1	9	1	-	-
F2	8	2	-	-
F3	7	3	-	-
F4	6	4	-	-
F5	5	5	-	-
F6	4	6	-	-
F7	3	7	-	-
F8	2	8	-	-
F9	9	-	1	-
F10	8	-	2	-
F11	7	-	3	-
F12	6	-	4	-
F13	5	-	5	-
F14	4	-	6	-
F15	3	-	7	-
F16	2	-	8	-
F17	9	-	-	1

F18	8	-	-	2
F19	7	-	-	3
F20	6	-	-	4
F21	5	-	-	5
F22	4	-	-	6
F23	3	-	-	7
F24	2	-	-	8

Preparation of ketoconazole -loaded self-microemulsifying formulations (L-SMEDDs)

Ketoconazole was added to the optimized blank ternary systems at a drug loading concentration of 5% w/w (Table 2). Final mixtures were mixed and shaken for 24 hours at 25°C in a shaking water bath to ensure complete solubilization.

Table 2 Composition of optimized ternary systems for L-SMEDDs

Formulation	Oil %w/w	Surfactant %w/w	Cosurfactant %w/w	Smix ratio
F17	70	20	10	2:1
F18	60	26.6	13.3	2:1
F23	40	45	15	3:1
F24	30	52.5	17.5	3:1

Evaluation of optimized SMEDDS formulation

Thermodynamic stability studies and cloud point

Stability of the optimized SMEDDS formulation was evaluated at different stress conditions such as heating cooling cycles (4°C and 40°C) and freeze thaw cycles (-21°C and +25°C) along with storage at specified temperature for 48 h. In order to carry out centrifugation stress study, 1 mL of the formulation was diluted to 100 mL with distilled water and centrifuged at 10000 g for 20 min and visually observed for any phase separation [14]. In order to determine cloud point temperature, 10 mL of diluted SMEDDS formulation were gradually heated on a water bath and observed for cloudiness using thermometer. The temperature at which cloudiness appeared was denoted as cloud point.

Measurement of particle size

The particle size and polydispersity index of the SMEDDS was obtained using calibrated ocular micrometer using a microscope. The particle size and polydispersity index of the best formulation was also determined using a dynamic light scattering particle size analyzer.

Measurement of zeta potential

The zeta potential of selected formulation was determined using Zetasizer. Samples were properly diluted with deionized water (1:200) and filtered through a 0.45 μm membrane filter before measurement.

Determination of drug content of ketoconazole -loaded solid SMEDDS

An accurately weighed amount of the resulting drug-loaded SMEDDS formulation was dispersed in a suitable quantity of methanol and shaken thoroughly to ensure release and dissolution of the drug in methanol. The samples were centrifuged at 3000 rpm for 15 minutes and the supernatant was filtered through a 0.45 µm membrane filter and the filtrate was assayed spectrophotometrically for the drug at a wavelength of 302 nm. The drug content in each sample was calculated as milligrams of the drug per gram of the product using the following equation:

$$drug\ content = \frac{drug\ content\ in\ the\ weight\ taken\ from\ solid\ SNEDDS}{weight\ of\ the\ solid\ SNEDDS\ taken}$$

In vitro **dissolution study**

The *in vitro* dissolution studies of different ketoconazole SMEDDS formulations were carried out in dissolution apparatus II (Paddle method) according to the requirements specified for ketoconazole capsules. The dissolution medium composed of 900 ml phosphate buffer pH 7.2 maintained at $37 \pm 0.5°C$ and the rotational speed was adjusted at 50 rpm. Phosphate buffer pH 7.2 was prepared by mixing 50 ml of 0.2M potassium dihydrogen orthophosphate with 35 ml of 0.2M sodium hydroxide and diluting to 200 ml with water. Volumes of these solutions were corrected accordingly to prepare the total volumes required for dissolution studies. An amount of SMEDDS formulation equivalent to 25 mg of ketoconazole was filled in dialysis membrane and used for dissolution studies. Samples were withdrawn at predetermined time intervals. An equal volume of fresh dissolution medium maintained at the same temperature was added to keep constant volume during dissolution study. The collected samples were filtered through 0.45 µm syringe filter, suitably diluted using methanol and then assayed for the content of ketoconazole by UV spectrophotometry at 302 nm.

Results and Discussion

Standard calibration curve of ketoconazole

The standard calibration curve of ketoconazole was constructed in methanol to obtain different concentrations ranging from 4 to 20 µg/ml, for which the absorbance readings were determined spectrophotometrically at λmax 302 nm (Figure 1). The standard calibration curve was linear over the concentration range studied and obeys Beer-Lambert's law with a correlation coefficient (R^2) 0.9911. The corresponding regression equation was found to be Y = 0.0138X – 0.0513.
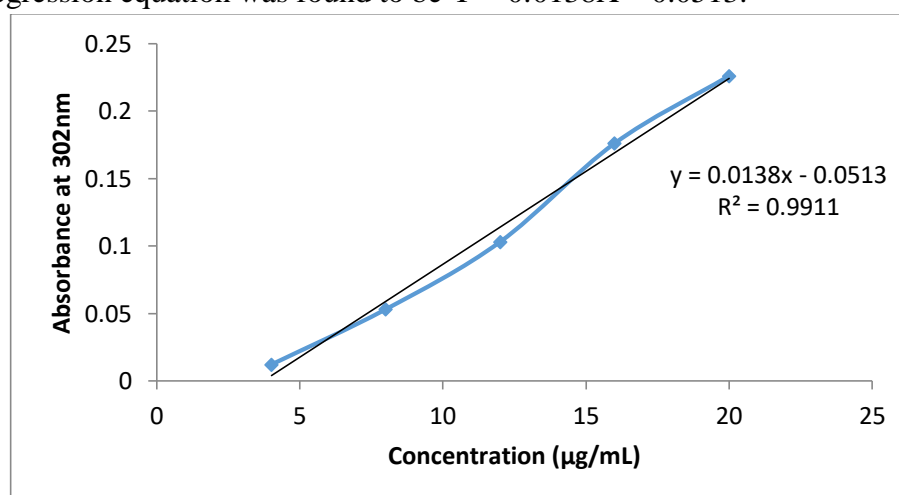

Figure 1 Standard calibration curve of ketoconazole

Solubility Studies

The solubility of ketoconazole was determined in oils, surfactants, co-surfactants, mixture of oils and mixture of surfactants (Figure 2).
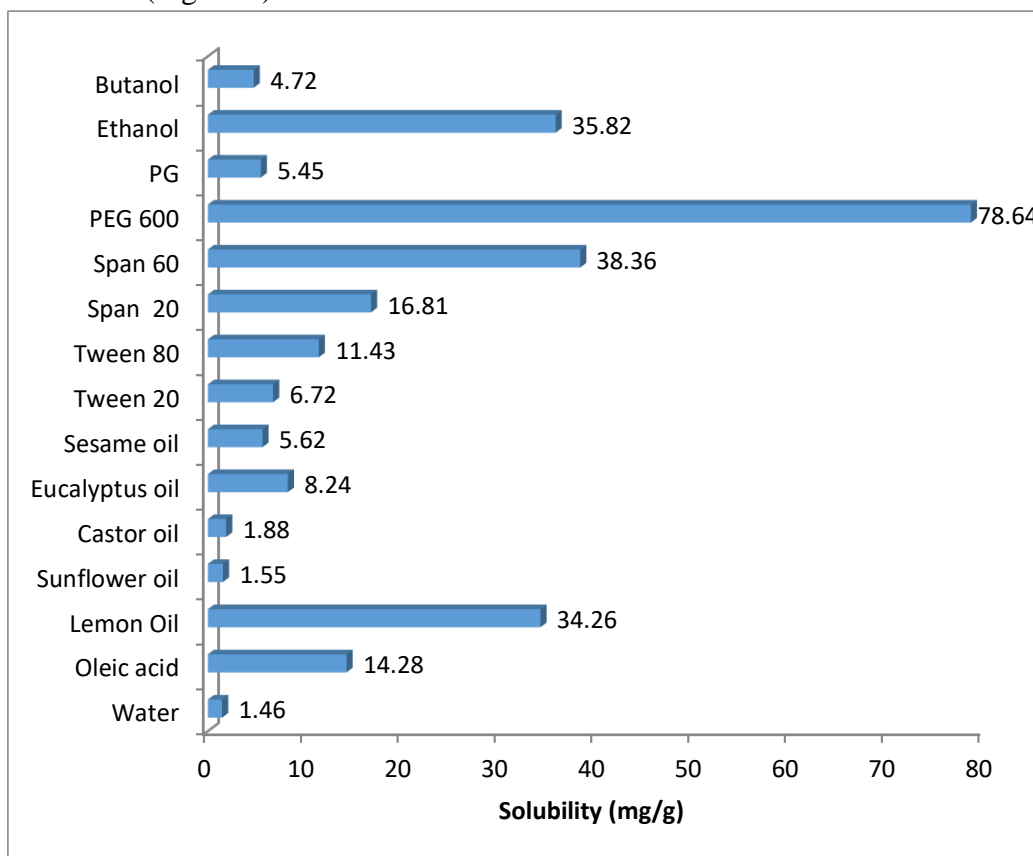

Figure 2 Comparative chart of solubility of ketoconazole

Among the tested oils, ketoconazole exhibited significantly higher solubility in lemon oil compared to all other oils. In order to form clear microemulsion judicious selection of oil, surfactant, co-surfactant and oil to surfactant/co-surfactant ratio is very important. In order to achieve this, it is recommended that a surfactant should have hydrophilic-lipophilic balance (HLB) value more than 10 to form an o/w emulsion. Lemon oil was considered as the oil phase form formulation of the microemulsion. The highest solubility was exhibited by Span 60 and it has an HLB value of 4.7 while PEG 600 has HLB value of 13.1.

Selection of surfactant and cosurfactant

Selection of surfactants should be based on its emulsification efficiency for the selected oil more than its solubilizing potential for the drug [15]. Therefore, the miscibility of the above surfactants with the selected oil (lemon oil) at a 1:1 weight ratio was investigated according to the method reported by Balakrishnan [16] and Date and Nagarsenker [12]. Emulsification studies showed that Span 60 was able to produce clear microemulsion with lemon oil upon dilution, and hence, it was employed as the surfactant in further studies.

The use of a single surfactant may not be enough to achieve a transient negative interfacial energy or a fluid interfacial film. Hence, addition of a co-surfactant may provide sufficient flexibility to the interfacial film so that various curvatures can be available to form microemulsions over a wide range of composition. Results of the study of the ability of co-surfactants (or co-solvents) to improve the emulsification ability of surfactants are presented in Table 3. The co-surfactant and co-solvents used were equivalent in

improving emulsification ability of surfactants as demonstrated by grades A and B produced upon dilution with distilled water.

Table 3 Surfactant and cosurfactant/cosolvent emulsifaction study

Surfactant	Cosurfactant/cosolvent	Visual Grade
Span 60	PEG 600	A
	PG	C
	Ethanol	B
	Butanol	C

Hence blends of span 60 and PEG 600 were used for the formulation of the microemulsions. The appropriate amounts of the selected oil, surfactants and co-surfactant were determined by constructing phase diagrams.

Construction of ternary phase diagram

In order to identify the self-emulsifying regions and to optimize the percentages of different liquid SMEDDS components, a ternary phase diagram was constructed in the absence of ketoconazole (Figure 3).
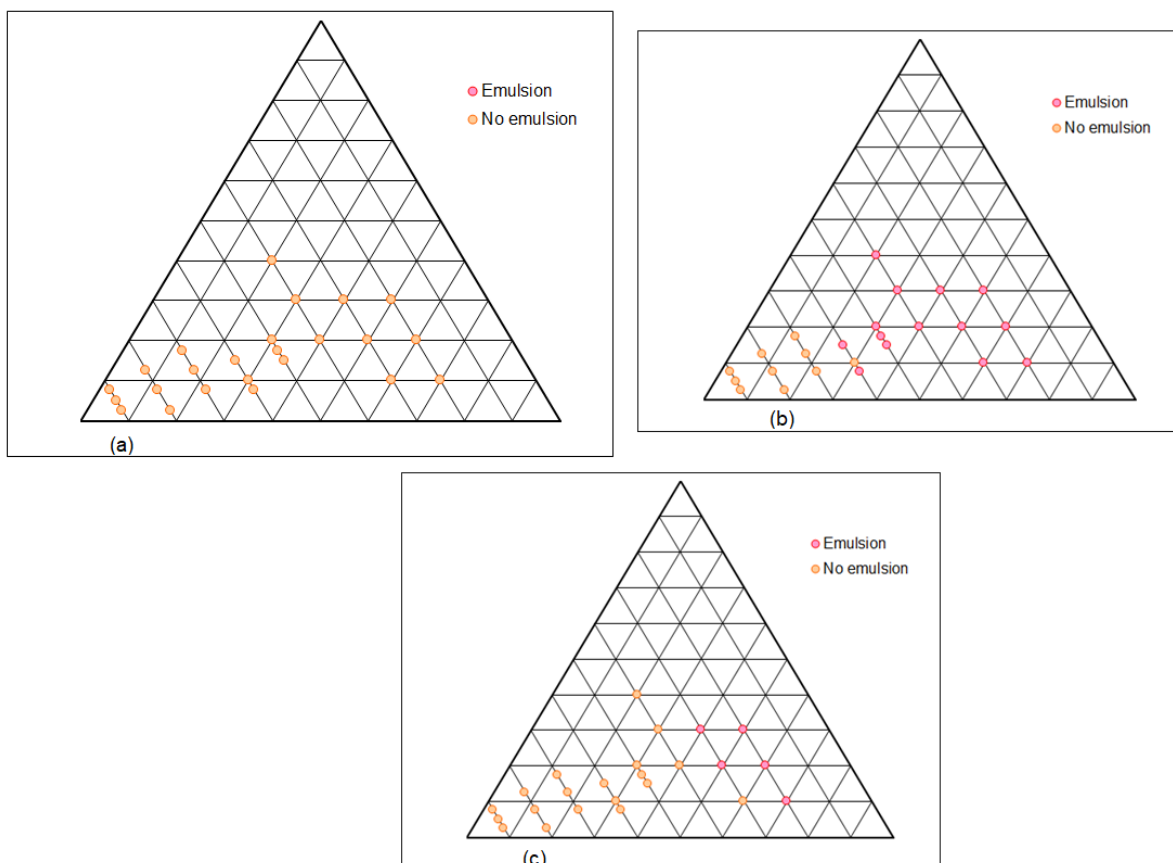

Figure 3 Ternary phase diagram for (a) Smix (1:1)-water-lemon oil (b) Smix (2:1)-water-lemon oil (c) Smix (3:1)-water-lemon oil

Different batches of SEDDS were formulated and visually observed for their self-emulsifying properties. The ternary phases were judged as microemulsion and no emulsion formation on the basis of their turbidity

measurements and visual observations for transparency. The concentration of components was expressed as percent volume/volume (%v/v) in ternary phase diagram. The results revealed that span 60 and PEG 600 used in ratios of 2:1 (F16-18) and 3:1 (F22-24) exhibited largest microemulsion area and shortest emulsification time (less than 1 min). It was observed that with increase in the ratio of the PEG 600, spontaneity of the self-emulsification process got increased. It was observed that higher concentration of surfactant mixture (Smix) or lower concentration of oil resulted in formation of clear transparent emulsions with micro-sized droplets. This could be due to higher HLB value of Smix and better solubilization in PEG. The transparent emulsions (F17, F18, F23, F24) were visually evaluated for clarity and stability after 48h at room conditions. All tested emulsions remained clear transparent even at the end of 48h. Hence, these ternary phases were selected for ketoconazole loaded SMEDDs.

Ketoconazole -loaded self-microemulsifying formulations (L-SMEDDs)
The ternary phase diagrams revealed the optimum concentration of the oil and the surfactant mix that could be used for the formulation of ketoconazole loaded SMEDDs. A fixed ketoconazole concentration of 5% w/w was selected to be loaded in all self-emulsifying formulations. It was expected to provide spontaneous emulsification of SMEDDS with a low tendency of drug precipitation upon aqueous dilution. Also, using fixed concentration of ketoconazole in all formulations was proposed to exclude the effect of varying the drug concentration on the self-emulsifying efficiency of the systems.

Thermodynamic stability and cloud point determination
The prepared formulations were kept in closed containers and tested for thermodynamic stability. Thermodynamic stability studies were carried out to determine the effects of temperature variation and centrifugation on precipitation or phase separation of the formulated SMEDDS.
All the formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C) and centrifugation at 10,000 g indicating good stability of formulations and their emulsions. Determination of cloud point is an essential parameter for the selection of a stable SMEDDS particularly when composed with non-ionic surfactants. "The cloud point temperature (lower consolute temperature) indicates the temperature at which the transparent monophasic system was transformed into cloudy biphasic system as dehydrated surfactant molecules associated together as precipitate, which can affect the formulation adversely. It is recommended that the cloud point for SMEDDS should be higher than body temperature (37°C), which will avoid phase separation occurring in the gastrointestinal tract. The cloud point temperature of the tested SMEDDS was found to be in the range of 89-94°C (Table 4). Thus, it can be inferred that the developed formulation was stable and do not require a precise storage temperature and it develops a stable emulsion upon administration at physiological temperature in vivo.

Droplet Size, Polydispersity and zeta potential of L-SMEDDs
The mean droplet size and polydispersity index (PDI) determined for different ketoconazole-loaded SMEDDS (F17-18, F23-24) are shown in Table 4. Incorporation of different amount of Smix into ketoconazole-loaded SMEDD formulations resulted in significantly different droplet size. Among the tested formulations, SMEDDS formulations prepared with 3:1 Smix ratio exhibited lower droplet size compared to formulations in which the amount of surfactant was low.

Table 4 Stability and characterization of L-SMEDDS

Formulation	Thermodynamic Stability				Surface characterization		
	Cloud point (°C)	Centrifugation	Cooling/Heating	Freeze/Thawing	Mean droplet size (µm)	PDI	Zeta potential
F17	89.65	No phase separation	No Phase inversion	No Phase inversion	487.15	0.427	-24.5
F18	92.17	No phase separation	No Phase inversion	No Phase inversion	329.31	0.392	-26.1
F23	90.46	No phase separation	No Phase inversion	No Phase inversion	341.03	0.211	-31.1
F24	93.59	No phase separation	No Phase inversion	No Phase inversion	317.15	0.431	-29.7

It was observed from the results that decreasing the oil content of the formulations resulted in a decrease in the size of formulation droplets.

Self-emulsifying formulations possess a negative charge on the oil droplets due to the presence of anionic groups of free fatty acids contained in their composition; the oil, surfactant and co-surfactant. The obtained high negative values of zeta potential indicate that the tested formulations are less likely to flocculate or aggregate during storage or in biological environment.

In vitro dissolution study

The *in vitro* dissolution studies revealed the drug release profiles for the SMEDDS. All the formulations exhibited quick drug release characteristics and almost complete drug release in 15-20 minutes (Figure 4). In contrast, the pure drug exhibited only a maximum of 39.63% release in 60 min duration.
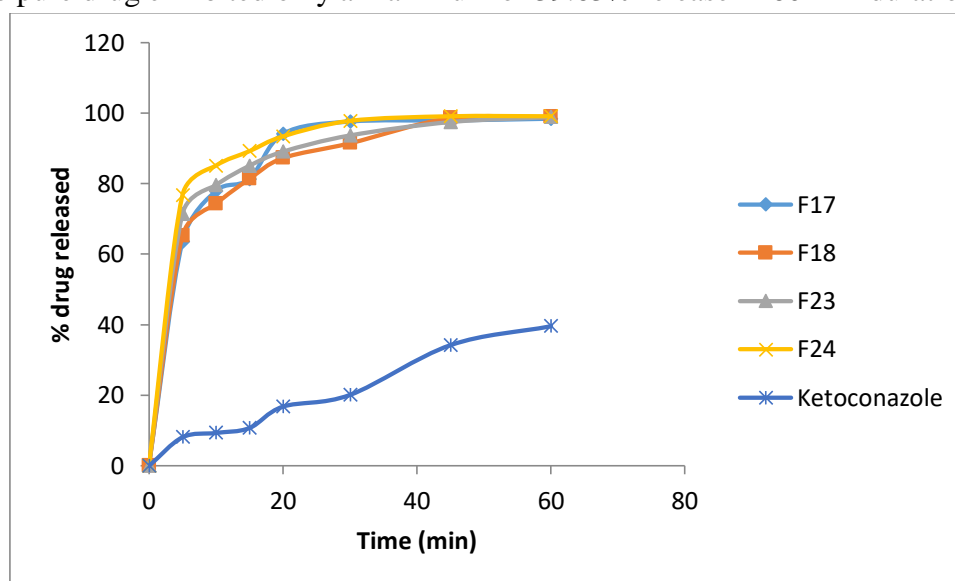

Figure 4 *In vitro* dissolution profile of SMEDDS and ketoconazole

Ketoconazole-loaded liquid SMEDDS formulations (F17, F18, F23 & F24) exhibited optimal dissolution performance. High dissolution profiles of liquid SMEDDS are due to quick formation of o/w microemulsions with small droplet size upon exposure to dissolution medium with gentle agitation. In addition, the presence of the drug in a dissolved state in liquid SMEDDS formulations avoids the dissolution rate-limiting step required for crystalline drugs.

Conclusion

The bioavailability of the lipophilic drugs can be enhanced by formulating them as SMEDDS. From the release behavior witnessed through the present investigation it could be proven that the bioavailability of the lipophilic drug (ketoconazole) could be almost doubled by formulating it as SMEDDS.

References

1. Desai PP, Date AA, Patravale VB. Overcoming poor oral bioavailability using nanoparticle formulations–opportunities and limitations. Drug Discovery Today: Technologies 2012; 9: e87-e95.

2. Choonara BF, Choonara YE, Kumar P, Bijukumar D, du Toit LC, Pillay V. A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. Biotechnology Advances 2014; 32: 1269-82.

3. Pathak K, Raghuvanshi S. Oral Bioavailability: Issues and Solutions via Nanoformulations. Clinical Pharmacokinetics 2015; 54: 325-57.

4. Daugherty AL, Mrsny RJ. Regulation of the intestinal epithelial paracellular barrier. Pharmaceutical Science & Technology Today 1999; 2: 281-7.

5. Kuentz M. Lipid-based formulations for oral delivery of lipophilic drugs. Drug Discovery Today: Technologies 2012; 9: e97-e104.

6. Patel RP, Patel H, Baria AH. Formulation and evaluation of Liposomes of ketoconazole. Int J Drug Deliv Technol. 2009; 1:16-23.

7. Elder EJ, Evans JC, Scherzer BD, Hitt JE, Kupperblatt GB, Saghir SA, Markham DA. PDrugDevelopment and Industrial Pharmacy reparation, Characterization, and Scale-up of Ketoconazole with Enhanced Dissolution and Bioavailability. Drug Development and Industrial Pharmacy. 2007; 33: 755-765

8. Chauhan SP, Kanojiya AD, Patel PA, Seth AK, Shah NV, Aundhia CJ, Javia AR. Ketoconazole loaded solid self emulsifying drug delivery system: formulation and in-vitro characterization. Pharma Science Monitor. 2013; 4(4 S1): 425-441

9. Bhattacharya A, Bajpai M. Development of oral bioavailability of sel-emulsifying formulation of ketoconazole. International Journal of Pharmaceutical Sciences and Nanotechnology. 2013; 5(4). DOI: 10.37285/ijpsn.2012.5.4.5

10. Deore SK, Surawase RK, Maru A. Formulation and evaluation of o/w nanoemulsion of ketoconazole. Research Journal of Pharmaceutical Dosage Forms and Technology. 2019; 114): 269-274.

11. Mokale V, Naik S, Khatal T, Sonawane SH, Potoroko I. Formulation development and in vitro multimedia drug release study of solid self-microemulsifying drug delivery system of ketoconazole for enhanced solubility and pH-independent dissolution profile. In: Encapsulation of active molecules and their delivery system, Elsevier, 2020; pp 211-231.

12. Date AA, Nagarsenker M.. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. International Journal of Pharmaceutics 2007; 329:166-72.

13. Khoo S-M, Humberstone AJ, Porter CJH, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. International Journal of Pharmaceutics 1998; 167:155-64.

14. Inugala S, Eedara BB, Sunkavalli S. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: in vitro and in vivo evaluation. European Journal of Pharmaceutical Sciences 2015; 74: 1-10

15. Duc Hanh N, Mitrevej A, Sathirakul K, Peungvicha P, Sinchaipanid N. Development of phyllanthin-loaded self-microemulsifying drug delivery system for oral bioavailability enhancement. Drug development and industrial pharmacy 2015; 41:207-17.

16. Balakrishnan P, Lee B-J, Oh DH, Kim JO, Hong MJ, Jee J-P, Kim JA, Yoo BK, Woo JS, Yong CS, Choi H-G. Enhanced oral bioavailability of dexibuprofen by a novel solid Self-emulsifying drug delivery system (SEDDS). European journal of pharmaceutics and biopharmaceutics 2009; 72: 539-45.