Original Research Article

A New Lipid Based Drug Delivery System (LBDDS) for Oral Delivery of Tioconazole.

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A b s t r a c t
Purpose: Peroral delivery of some drugs with limited aqueous solubility has remained a challenge to drug formulation experts. This work seeks to address the issue of unavailability of oral delivery system of tioconazole, a potent antifungal agent.
Method: Novel lipid based drug delivery systems (LBDDS) of tioconazole, composed of oil, surfactants and co-surfactants were formulated and their physicochemical as well as pharmacokinetic parameters determined. The oil, surfactant and co-surfactant used were soya bean oil, Cremophor® S9 and Brij® 35, respectively. The droplet sizes of the tioconazole micro-emulsion obtained after self emulsification were assessed after 72 h incubation. The in vitro permeation studies of the tioconazole-loaded LBDDS were evaluated using a modified Franz cell.
Result: Permeation coefficients of the formulation were between 1.204 x 10⁻³ cm/sec and 2.178 x 10⁻³ cm/sec. In vitro microbiological test of the tioconazole LBDDS revealed increased in vitro antifungal activity (~ 1.4 times) against clinically isolated Candida albicans as compared to tioconazole solution. Preliminary in vivo pharmacokinetic studies also showed an AUC₀₋₁2 of 2930 μg/hr/ml for the optimized LBDDS formulation while that of oral suspension was 1171 μg/hr/ml and the C_max of the optimized LBDDS formulation was 797 μg/ml as against 355 μg/ml obtained for the pure drug. This showed a 2.5-fold increase in the systemic bioavailability of tioconazole from the optimized LBDDS formulation.
Conclusion: The result of this study gave insight that the issue of unavailability of tioconazole in oral delivery system could be surmounted by tactical engineering of LBDDS such as self-microemulsifying drug delivery systems (SMEDDS).
Keywords: tioconazole, bioavailability, LBDDS, Cremophor® S9, Brij® 35, permeation coefficient.

Introduction
The azole antifungals have remained the main stay in antifungal therapy and are divided into imidazoles and triazoles. The triazoles are mostly delivered orally while the imidazoles are mostly available in topical dosage forms. The imidazoles are usually not favoured for oral delivery because of extensive metabolism after oral delivery (Drugbank). Tioconazole shares the same characteristics as other members of the imidazole class. It is a Biopharmaceutics Classification Systems (BCS) Class 2 drug and suffers poor dissolution in the gastrointestinal tract (GIT). The drug is also associated with gastrointestinal side effects, which are suspected to be due to prolonged contact time between the undissolved drug and the walls of the gastrointestinal tract. Tioconazole is the most active of the imidazole antifungals [1, 2] and has wide spectrum of activity against Candida albicans, Aspergillus spp., Chlamydia and some Gram positive bacteria [3], with both fungicidal and fungistatic activity against migratory and dormant yeasts and fungi. Its fungistatic activity is due to inhibition of sterol biosynthesis in the fungi. Fungicidal activity has also been shown to be concentration and pH dependent [4], making it imperative for the need for higher drug concentration in vivo for the desired effect, which can be achieved by higher dissolution at the absorption interface. When given orally, about 60% of the drug is excreted in faeces (possibly due to low solubility), while 30% is metabolized to glucoronide metabolite. Lipid based drug delivery system (LBDDS) is a mixture of oil, surfactant and co-surfactant, which is capable of fast self-emulsification in the gastrointestinal fluid under mild agitation provided by gastrointestinal motility to form emulsion. The lipid emulsions have advantages in terms of high drug loading capacity, reduction in irritation or toxicity of the incorporated drug, the possibility of sustained release and industrial productivity, improved dissolution of the drug in the GIT [5 - 10], reduction of
pre-systemic as well as systemic clearance of drug [11, 12] and the possibility of dose reduction. They are thus considered appropriate drug carriers for highly lipophilic drugs. It is on these grounds that tioconazole LBDDS was formulated to help reduce some of the problems encountered when tioconazole is given orally, since antifungal activity relies on improved bioavailability and quick attainment of minimum inhibitory concentration (MIC) [13–15]. With some disturbing side effects of topical administration of tioconazole like local irritation, and difficulties encountered by patient in ensuring proper topical application, a convenient oral dosage form with improved oral bioavailability, reduced doses and improved antifungal efficacy was needed to replace or compliment topical therapy in sensitive patients, especially with the increase in invasive fungal infections [16, 17] and recurrent infections caused by Candida spp. and Aspergillus spp. [18–21]. The objective of this study was to prepare and evaluate stable LBDDS formulations of tioconazole, with enhanced iv vitro antifungal activity and increased oral bioavailability. Lipid nanoparticle formulation of tioconazole for parenteral administration has been studied [22].

Materials and methods

The following materials were used: tioconazole (99% potency, Niemeth Pharm. International, Lagos, Nigeria), Cremophor® S9 and Soluto® HS15 (BASF AG, Ludwigshafen Germany), Brij® 35 (Gattefossé, Gennevilliers Cedex France) melon seed oil, soya bean oil (Pharmacopoeial grade), Miglyol® 812 (Hüls, Witten Germany), n-hexane (Aldrich Chem. Co., Germany), dimethyl sulfoxide (DMSO) (Merck, Darmstadt Germany) and distilled water. All other reagents and solvents were of analytical grade.

Extraction of soya bean and melon seed oils

Soya bean seeds and melon seeds were dried and separately ground into coarse powders. The oils were thereafter extracted from the coarse powders by cold maceration in n-hexane for 5 days. The extracts were recovered by filtration using muslin cloth and Whatman filter papers (No. 3) consecutively. The oils were later separated from the n-hexane using rotary evaporator at 80–90°C under reduced pressure and further filtered and left under air current for 24 h to ensure total evaporation of the solvent from the oils. The oils were stored at 28 ± 2°C.

Solubility of tioconazole in the oils

Combination of titrimetric and gravimetric methods was used to determine the solubility of tioconazole in soya bean oil, melon seed oil and Miglyol® 812. In each case, fifty (50)-mg quantities of the drug were weighed out into three test tubes and increasing quantities of the various oil added in aliquots with intermittent agitation. The experiment was carried out at 30–40°C for 48 h. The amounts of the oils that dissolved the given quantity of the drug were noted. The oils containing dissolved drug and those containing no drug were weighed separately and the difference in their weight noted. The mean values of the both methods were then used as the solubility of the drug in the oils.

Selection of surfactants

The following non-ionic surfactants were assessed - Soluto® HS15, Brij® 35, Cremophor® S9. The surfactants were selected on the basis of rate of solubilization of 100 mg of the drug in 1ml of oil. The keys: A, B, C were used, with A representing fast and complete solubilization, B representing slow and complete solubilization and C representing very slow and complete solubilization.

Construction of pseudo ternary phase diagram

The surfactant, co-surfactant and oil selected were used in the construction of ternary phase diagram. The following mixtures of the surfactant and co-surfactant (S mix) were prepared: 1:1, 1:2, 1:3 and 2:1 w/w, and blended with the oil to give varying complementary ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 w/w. Water was folded in drop-wise into the various complementary ratios of S mix and oil at 28 ± 2°C under the agitation of magnetic stirrer set at 50 rpm. The experiment was visually assessed to determine the time of complete emulsification [23]. The amount of water folded in and time taken for complete emulsification were noted. The ternary phase diagrams were constructed using Sigma Plot® 11 Exact Graph and Data Analysis software (StatSoft Software Inc., USA). The globule sizes of the emulsions formed were determined by light microscopy to further assess their quality.

Preformulation isotropicity test

The various combinations of the S mix and oil were formulated at 30–40°C in a test tube under manual shaking. The formulations were kept in an undisturbed position at 28 ± 2°C and visually assessed for phase separation. The formulations that remained stable were then selected for drug loading.

Preparation of SMEDDS

A 100-mg quantity of tioconazole was dissolved in each S mix according to the composition stated in Table 1 at 30–40°C under continuous manual agitation for 30 min. The required amount of oil were then added and thoroughly mixed and left to equilibrate at 28°C. Three batches of LBDDS were produced.

Emulsification time test

A 2-ml volume of the drug-loaded LBDDS was titrated with 20 ml of distilled water at 37 ± 2°C under magnetic stirrer hot plate assembly at 50 rpm agitation. The times taken for complete emulsification of each batch as well as the globule size achieved were noted.
Absolute drug content analysis

A quantity of the LBDDS formulation equivalent to 50 mg dose of the drug was emulsified by vortexing in 200 ml of SGF (pH 1.2) under magnetic stirrer hot plate assembly at 50 rpm for 60 min. One-ml volume was then withdrawn, diluted and the absorbances taken in a spectrophotometer (SP 6-450 UV-VIS, Pye Unicam, England) at 206 nm. This was repeated for different time intervals.

**In vitro permeation studies**

Modified Franz cell attached with a dialysis membrane (MWCO 5000 - 12000) was used to determine the permeation of tioconazole from the SMEDDS formulation. SGF (pH 1.2) or distilled water at 37 ± 2 °C was used as the receiving medium. The experiment was carried out using magnetic stirrer at a rotation speed of 100 rpm. A 0.5 ml volume of the medium was withdrawn and replaced with fresh medium at 10, 30, 60, 120, 240, 360 and 480 min. The withdrawn aliquots were then assayed spectrophotometrically as before and the drug concentration determined by reference to Beer’s plot. The amount of the drug that permeated per unit area was calculated using special in-house Microsoft Excel calculation software programme.

**In vitro microbiological test**

The in vitro microbiological activities of the LBDDS containing tioconazole were determined using agar diffusion technique. Volumes (0.1 ml) of the various LBDDS formulations were diluted with distilled water to obtain 10 μg/ml of tioconazole. A 10 μg/ml solution of the drug in DMSO was also prepared. Sabouraud-dextrose agar plates were prepared following manufacturer’s specifications and seeded with clinically isolated Candida albicans. The various dilutions of the drug and the formulations were then inoculated on the plate and the plate incubated for 24 h at 37 °C. The various inhibition zone diameters (IZD) generated after incubation were measured planimetrically and the average for each concentration determined after replicate determinations.

**Preliminary in vivo studies**

This study was conducted in accordance with Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of University of Nigeria, Nsukka, following the 18th WMA General Assembly Helsinki, June 1964 and updated by the 59th WMA General Assembly, Seoul, October 2008. Ten (10) Wistar albino rats weighing between 140 - 225 g were used. They were grouped into 2 groups of 5 each. Group 1 received the optimized LBDDS formulation while group 2 received oral extemporaneous dispersion of the tioconazole in distilled water (since there is no available commercial oral formulation) at a dose of 50 mg/kg. All the animals were fasted 12 h before the test and throughout the experiment. One (1 ml) volumes of blood were withdrawn from the rats’ orbital sinus using heparinised hematocrit tubes at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h. The withdrawn blood samples were centrifuged at 5000 rpm for 5 min to separate the plasma, which were stored at -4 C till analysis. For each sample, 0.2 ml of the plasma sample was diluted in SGF (pH 1.2) and assayed for drug content using a digital spectrophotometer (Jenway, Model 6405 Barloworld Scientific, England) at 251 nm. The plasma from the blood withdrawn at zero hour was similarly diluted and used as blank and for the preparation of calibration curve. Amounts of drug in the plasma were plotted against time to obtain the plasma concentration time curve, which was further evaluated to obtain the pharmacokinetic parameters.

**Data and statistical analysis**

All experiment were performed in replicates (at least, n = 3) for validation of statistical analysis. Results were presented as mean ± SD. ANOVA and student t-tests were performed on the data sets generated using SPSS. Figures were processed using Microsoft Excel and SigmaPlot® 11 Exact Graph and Data analysis. AUC of the plasma drug concentration time profile was determined using Origin® for windows.

**Result and Discussion**

The solubility of the drug in soya bean oil was higher compared to the other oils studied. Based on this fact, soya bean oil was selected as the oil phase. The solubility rate of the drug in the surfactants also favoured the selection of Cremophor® S9 and Brij® 35 as surfactants and co-surfactants respectively. The choice of Cremophor is due to its ability to form emulsions, while the Brij®35 is a non-ionic surfactant with good toxicity profile and with the ability to form nano-sized globules [24]. The solubility of drug in the oils were; soya bean oil (56.9 mg/g), melon seed oil (52.0 mg/g) and Miglyol 812® (53.7 mg/g) while those of the surfactants were Cremophor S9® = B, Brij 35® = A and Solutol® HS 15 = C.

**Ternary phase diagram and morphological characterization**

This experiment was performed to determine the ratio of surfactant and co-surfactant that would form the best emulsion. The results obtained are presented in Figs. 1a – 1d. From the figures, the largest area of emulsification region was got at Smix ratio of 1:1 of the surfactants and co-surfactant (Fig. 1a) and was therefore selected for further studies. The result of the morphological studies on the reconstituted emulsions is presented in Fig. 2 as the photomicrographs obtained in different biorelevant media. The globule sizes of the emulsions were found to decrease as the Smix:oil ratio increased i.e. they are inversely related. The droplet size of the LBDDS as determined by light microscopy ranged from 70.8 ±2.4 μm to 100.2 ± 5.6 μm (Table 2). Fig. 2 shows the photomicrographs of the formulations in different media and at different dilutions. The figure showed no significant change (p<0.05) in globule size which can be attributed to stability conferred by the surfactant mixture.
Preformulation isotropicity test
The result of isotropicity test shows that stability of the preparation increased as the Smix:oil ratio increased. All the preparations except Smix:oil ratio of 6:4 to 9:1 lost isotropicity within 72 h and were therefore considered unstable. Loading of drug into the LBDDS also influenced isotropic stability of the formulation. At 100 mg of the drug per 1 ml loading of the formulation, the isotropic stability was maintained while at higher amounts of the drug, the stability was compromised. This was also used to confirm the excipients combination as well as drug dose that gave a stable formulation.

Absolute drug content analysis
Absolute drug content analysis performed on the LBDDS formulations is necessary to determine the dosing units of the formulations. The result showed that the amount of drug contained in units of the LBDDS formulations were: 99.2 ± 0.2 % for formulation A; 98.7 ± 2.0 % for formulation B; and 99.6 ± 0.7 % for formulation C. They were within acceptable range, which indicates good control of the formulation process and increase in the level of confidence on the products.

In vitro antifungal activity
The in vitro activity of the LBDDS formulations was compared to a solution of tioconazole in DMSO and the result recorded as inhibitory zone diameter (IZD). The in vitro activity was determined to ascertain the possibility of loss, retention or improvement of activity of tioconazole as a result of formulation into this novel dosage form. The result revealed that the LBDDS B with absolute drug content of 98.7 ± 2 % containing 26 % of soya bean oil, 40% of Cremophor® S9 and 34 % of Brij® 35 showed greater activity against Candida albicans with IZD of 22.0 ± 0.1 mm as against 16.0 ± 0.3 mm recorded for the tioconazole solution. The increase in antifungal activity of the LBDDS formulation may be attributed to the improved permeation of the drug into the fungal cell wall brought about by appropriate choice and combination of excipients used in the formulation of the LBDDS. IZDs recorded for other batches were: LBDDS A (20.5 ± 0.2 mm) and LBDDS C (19.0 ± 0.2 mm). In all, there were significant (p<0.05) increase in the in vitro inhibition of C. albicans by the LBDDS formulation compared with the drug solution.

Product stability assessment
It is always very important to assess the stability of novel formulations. Stability could be viewed from the degradation of the active ingredient or physical property of the formulation. In this case, physical stability of the formulations were studied, wherein, the time taken for the formulation to separate was noted. Since the formulations were emulsions, unstable formulations would separate into different layers. At 100 mg/ml concentration of the drug, the formulation remained stable at 28 ± 2 °C for over 3 months, while at 150 mg/ml the formulation lost stability within 2 weeks and at 300 mg/ml, the formulation lost stability within 24 h. To confirm the effect of concentration on the stability of the formulation, the formulation that lost stability, were diluted back to 100 mg/ml. When diluted back, they regained their stability and this informed the decision of limiting the dose to 100 mg/ml. Antimicrobial assessment was also carried out within this period, which revealed no significant difference (p<0.05) in the potency of the stored formulation and freshly prepared ones.

In vitro permeability studies
In vitro permeability assessment of the drug from the formulated LBDDS B, which was the optimized formulation showed permeation fluxes of 2.062 μg/cm².sec and 2.280 μg/cm².sec in SGF (pH 1.2) and distilled water respectively. The permeation coefficients of LBDDS B in SGF and distilled water were: 2.178 x 10⁻³ cm/s for SGF and 2.401 x 10⁻³ cm/s for distilled water. Compared with the flux and permeation coefficient of tioconazole (Flux: 0.827 and 0.735 μg/cm².sec in SGF and distilled water respectively; permeation coefficient: 8.265 x 10⁻⁴ cm/s and 7.350 x 10⁻⁴ cm/s in SGF and distilled water respectively), there was about 2.64 % increase in permeation attesting further to the improvement in the in vitro performance of this dosage form. The selection of LBDDS B was as a result of its consistent in vitro performance vis-à-vis antimicrobial property, physical stability and consistent globule size and permeation characteristics. The in vitro permeation result presented here strongly indicates improved permeation of the drug through the artificial membrane which has been shown to mimic permeation in vivo. Other formulation and their respective permeation coefficients are shown in Table 3. In all, there was higher permeation in distilled water than in SGF because of the pH differences. The permeation coefficients were obtained from the steady-state flux values got from the linear ascent of the plot of amount permeated against time (the slope), making use of the following equations [25].

\[ P = J/C_0 \]  \hspace{1cm} (1)

Where \( P \) is the permeation coefficient; \( C_0 \) is the initial drug concentration in the drug compartment. \( J \) represents the steady-state flux obtained from equation 2.

\[ J = dQ/Adt (\mu g/cm^2.s) \]  \hspace{1cm} (2)

Where \( Q \) indicates the quantity of substances crossing the artificial membrane, \( A \) is the area of the artificial membrane exposed and \( t \) is the time of exposure.

In vivo bioavailability
The systemic bioavailability of tioconazole from LBDDS formulation was 2.5 fold higher than that of oral suspension of the drug. The AUC-12 of LBDDS was 2930 μg/ml.hr while that of oral suspension was 1171 μg/hr/ml. The AUC is an important parameter for measuring bioavailability of drug from dosage forms since it represents the total integrated area under the blood
concentration time profile, which represents the amount of drug reaching the systemic circulation. The $T_{\text{max}}$ of both LBDDS and the pure drug suspension was 1 hour, while $C_{\text{max}}$ of the LBDDS was 797 $\mu$g/ml and that of the pure drug dispersion was 355 $\mu$g/ml, representing also a 2.25-fold increase in $C_{\text{max}}$. The increased AUC and $C_{\text{max}}$ for tioconazole administered as LBDDS compared with tioconazole suspension may be as a result of lowering of systemic drug concentration by first pass effect for tioconazole administered as a suspension. Lipid particle formulations have been known to be absorbed through the lymphatics, which can avoid the phenomenon of first pass effect and consequently, improve bioavailability consistent with reported works [26, 27]. The visual behavioural assessment of the rats after dosing of the bland and drug loaded formulation of LBDDS B to ascertain possible GI related side effect or other side effects did not reveal any difference between the two groups. This experiment was carried out after washout period of ten days.

Conclusion

Lipid based delivery system of tioconazole containing soya bean oil, Cremophor® S9 and Brij® 35 can be further explored for development of improved oral delivery system of triazole antifungals and as alternative to other oral imidazoles for the treatment of invasive and systemic fungal infection caused by Candida albicans. Oral delivery of tioconazole by formulation as LBDDS could be convenient and as well improve activity of triazoles, as there was increased antifungal activity $\text{in vitro}$ and increased bioavailability $\text{in vivo}$ for a model triazole used in this study.

Authors’ contributions

Attama A A conceived of the study and participated in its design and coordination, and in the statistical analysis, and helped to draft the manuscript. Ayogu I J carried out the extraction of the oil, preparation and evaluation of the SMEDDS, performed the statistical analysis and helped to draft the manuscript. Kenechukwu F C participated in the design of the study, and in the evaluation of the SMEDDS and helped to draft the manuscript. Ogbonna J D N participated in the design of the study, and in the evaluation of the SMEDDS and helped to draft the manuscript. Okore V C participated in the design and coordination of the study and helped to draft the manuscript.

Acknowledgement

The kind gift of Cremophor® S9 and Solutol® HS15 by BASF AG, Ludwigshafen Germany is acknowledged. Dr. A. A. Attama is highly grateful.

References


Table 1: Percentage composition of ingredients in the LBDDS

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Soy bean oil (%)</th>
<th>Cremophor S9 (%)</th>
<th>Brij 35 (%)</th>
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<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>26</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>44</td>
<td>39</td>
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</table>

Table 2: Emulsification time test

<table>
<thead>
<tr>
<th>Media</th>
<th>Formulation</th>
<th>Time (s)</th>
<th>Globule size (µm ± SD)</th>
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<tbody>
<tr>
<td>Distilled water</td>
<td>A</td>
<td>60</td>
<td>90.8 ± 8.1</td>
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<tr>
<td></td>
<td>B</td>
<td>57</td>
<td>70.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>54</td>
<td>90.8 ± 8.8</td>
</tr>
<tr>
<td>SGF</td>
<td>A</td>
<td>63</td>
<td>100.2 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>67</td>
<td>80.1 ± 3.1</td>
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<tr>
<td></td>
<td>C</td>
<td>51</td>
<td>90.8 ± 7.2</td>
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Table 3: *In vitro* permeation studies

<table>
<thead>
<tr>
<th>Media</th>
<th>Formulation</th>
<th>Flux (J) µg/cm².sec</th>
<th>Permeation coefficient (P) cm/sec</th>
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<tbody>
<tr>
<td>SGF A</td>
<td>1.506</td>
<td>1.549 x 10⁻³</td>
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<tr>
<td>B</td>
<td>2.062</td>
<td>2.178 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.127</td>
<td>1.204 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>1% Tioconazole suspension</td>
<td>0.827</td>
<td>8.265 x 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>Distilled water A</td>
<td>1.713</td>
<td>1.762 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.280</td>
<td>2.401 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.310</td>
<td>1.370 x 10⁻³</td>
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<tr>
<td>1% Tioconazole suspension</td>
<td>0.735</td>
<td>7.350 x 10⁻⁴</td>
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</tr>
</tbody>
</table>

![Ternary phase diagram of the S_mix (surfactant-co-surfactant mixture) at 1:1 ratio showing the region of emulsion formation.](image)

**Fig. 1a.** Ternary phase diagram of the S_mix (surfactant-co-surfactant mixture) at 1:1 ratio showing the region of emulsion formation.
Fig. 1b. Ternary phase diagram of the $S_{\text{mix}}$ (surfactant-co-surfactant mixture) at 2:1 ratio showing the region of emulsion formation.

Fig. 1c. Ternary phase diagram of the $S_{\text{mix}}$ (surfactant-co-surfactant mixture) at 3:1 ratio showing the region of emulsion formation.
Fig. 1d. Ternary phase diagram of the $S_{\text{mix}}$ (surfactant-co-surfactant mixture) at 1:2 ratio showing the region of emulsion formation.

Fig. 2a. Photomicrograph of LBDDS B before drug loading. Bar represents 200 µm.
Fig 2b. Photomicrograph of drug loaded LBDDS B in distilled water (diluted). Bar represents 200 µm.

Fig. 2c. Photomicrograph of drug loaded LBDDS B in SGF (pH 1.2) (diluted). Bar represents 200 µm.
FIG. 3. *In vivo* bioavailability profile of 1% w/v tioconazole suspension in distilled water and LBDDS B.