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RESEARCH ARTICLE

Microemulsions as vehicles for topical administration of voriconazole: formulation and *in vitro* evaluation

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Abstract

This work was undertaken to investigate microemulsion (ME) as a topical delivery system for the poorly water-soluble voriconazole. Different ME components were selected for the preparation of plain ME systems with suitable rheological properties for topical use. Two permeation enhancers were incorporated, namely sodium deoxycholate or oleic acid. Drug-loaded MEs were evaluated for their physical appearance, pH, rheological properties and *in vitro* permeation studies using guinea pig skin. MEs based on polyoxyethylene(10)oleyl ether (Brij 97) as the surfactant showed pseudoplastic flow with thixotropic behavior and were loaded with voriconazole. Jojoba oil-based MEs successfully prolonged voriconazole release up to 4 h. No significant changes in physical or rheological properties were recorded on storage for 12 months at ambient conditions. The presence of permeation enhancers favored transdermal rather than dermal delivery. Sodium deoxycholate was more effective than oleic acid for enhancing the voriconazole permeation. Voriconazole-loaded MEs, with and without enhancers, showed significantly better antifungal activity against Candida albicans than voriconazole supersaturated solution. In conclusion, the studied ME formulae could be promising vehicles for topical delivery of voriconazole.

Keywords: Microemulsion, antifungal, permeation enhancers, *in vitro* skin permeation study, antifungal activity

Introduction

Azole antifungal agents are the most commonly used antifungals in clinical treatment of both superficial and systemic fungal infections[1]. Voriconazole is a new wide-spectrum triazole with activity against *Candida* spp., *Aspergillus* spp. and *Cryptococcus neoformans* including those resistant to the other commonly used antifungal agents. It is more potent than amphotericin B, fluconazole, itraconazole and flucytosine[2]. In general, the MICs (minimum inhibitory concentration) of voriconazole for a broad spectrum of yeast and molds are 1–2 log lower than the MICs of fluconazole[3].

Voriconazole is available as intravenous and oral formulations. Recent clinical studies proposed topical voriconazole as a new, promising therapy for fungal keratitis in combination with systemic voriconazole[4,5]. Voriconazole (1% solution) was used topically as an adjunctive therapy with systemic antifungal agents for the treatment of cutaneous aspergillosis[6]. Few studies investigated the effect of complexation with cyclodextrins on the solubility of voriconazole in aqueous medium[7,8], while no literature was available about solubilizing or formulating voriconazole for topical route. This study aimed to incorporate insoluble voriconazole in microemulsion (ME) system for topical administration. The prepared systems were optimized and evaluated *in vitro* by studying the effect of the type and ratio of different ME components on the physical and rheological characters. *In vitro* release was also evaluated. An *in vitro* permeability study was conducted to assess the ability of using this system to improve either dermal or transdermal delivery of voriconazole and to study the effect of two

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permeation enhancers. Moreover, the microbiological activity of the selected ME was tested against Candida albicans strain.

Materials and methods

Materials

Voriconazole was purchased from NIVON Specialties, India (batch no. RICM 0031106); oleic acid, sodium deoxycholate and polyoxyethylene (10) oleyl ether (Brij 97, HLB (hydrophilic-lipophilic balance) = 12.4, pH of 10% aqueous solution = 4) were obtained from Sigma Chemical Company, USA; jojoba oil was bought from Agriculture Research Center, Sinai, Egypt; polyoxyethylene-20-sorbitan mono-oleate, polysorbate 80 (Tween 80, HLB = 15, pH of 10% aqueous solution = 7), glycerol, sorbitol and liquid paraffin were bought from EL-Nasr Pharmaceutical Chemicals Company, Abu-Zaabal, Egypt; HPLC-grade acetonitrile was obtained from Aldrich, USA.

Construction of pseudo-ternary phase diagrams

Eight ME systems were prepared using two different surfactants (Tween 80 and Brij 97), two co-surfactants (glycerol and sorbitol), two oil phases (paraffin oil and jojoba oil) and water. For each system, the surfactant/co-surfactant/oil (S: CoS: O) mixtures were prepared at the 36 possible ratios and were titrated with aliquots of distilled water ranging from 10–50% w/w (in 10% increments)[9]. After being equilibrated, the mixtures were assessed visually and determined as being turbid systems, ME solutions or ME gels. Phase diagrams were plotted for each water increment.

Characterization of plain MEs

According to the ME regions in the phase diagrams, eight ME formulae were selected for further evaluations as described in Table 1. The pH values of those formulae were measured using a digital pH-Meter (CG 820 Schott-Germany S-3C). The viscosity was measured at 25°C using cone and plate rheometer (Brookfield Engineering Labs., Stoughton, MA, spindles number 40 or 52). Samples of 1 g were used and the rpm was increased gradually in a suitable range of 0.1–1 to give torque values between 10–100% with 30 s interval between each two successive speeds. The rheological behavior of each system was evaluated by plotting shear stress versus shear rate values. Flow type of each ME formula was determined based on the exponent *N* values[10]. The hystersis loop area between upward and downward curves was calculated by the trapezoidal rule and used as a measure for degree of thixotropy.

The physical stability was assessed by subjecting the chosen ME formulae to the centrifuge test at 4000 rpm for 15 min[11] and freeze-thaw cycles (a total of three complete cycles, each cycle consisting of 24 h at 25°C followed by 24 h at –5°C)[12], where the bases were examined for liquefaction and phase separation.

Preparation of voriconazole-loaded ME

Voriconazole was dissolved in the selected ME bases in an amount of 1% w/w. Two different permeation enhancers, namely oleic acid (added as 5% of the oily phase) and sodium deoxycholate (added as 1% of the water phase), were added to the prepared bases[13,14]. The prepared MEs were left to equilibrate for 24 h. They were reevaluated for clarity, homogeneity, phase separation, pH and rheological properties in addition to *in vitro* release, *in vitro* permeation and microbiological activity.

In vitro release study

An accurately weighed 1.5 g of each voriconazole-loaded ME formula was placed in a plastic dish tightly covered with a stainless steel wire screen of 350 μm mesh size[15]. The dish was dipped in 600 mL Sorensen's citrate buffer of pH 5.5 contained in the vessel of USP dissolution test apparatus II at 37°C ± 0.5°C and 75 rpm. Aliquots of 5 mL were withdrawn at time intervals up to 4 h and replaced with equal volumes of fresh warmed buffer solution. The amount of drug released was measured by UV spectroscopy at λ_{max} of 255 nm.

The release profiles were assessed by calculating the release efficiency after 4 h(RE_{4h})[16] and the median dissolution time (MDT)[17].

Table 1. Composition and rheological properties of the selected plain microemulsion formulae.

Microemulsion formula	Surfactant ratio (w/w)		Co-surfactant ratio (w/w)		Oil phase ratio (w/w)		Aqueous phase	Rheological properties	
	Tween 80	Brij 97	Glycerol	Sorbitol	Jojoba oil	Liquid paraffin	% Distilled water		
								Viscosity (c.p.)	
F1	8	—	1	—	1	—	40	8184	
F2	8	—	—	1	1	—	30	2406	
F3	8	—	1	—	—	1	40	2265	
F4	8	—	—	1	—	1	40	2053	
								Exponent *N*	Hystersis area (cm^2)
F5	—	8	1	—	2	—	50	1.2875	994.6
F6	—	8	—	1	1	—	50	5.6	3450.6
F7	—	7	1	—	—	1	50	1.3281	8108.1
F8	—	8	—	1	—	1	50	2.7233	19403.0

Effect of storage

The chosen voriconazole-loaded ME formulae were stored in closed containers on shelf at ambient conditions of temperature and humidity for 12 months to represent the changes in weather around the whole year. The withdrawn samples were examined for any changes in their physical appearance, pH, voriconzole content and viscosity values.

In vitro skin permeation experiments

Permeation studies were performed using a modified Franz glass diffusion cell with an effective diffusion area of 0.7225 cm^2. The experiments were carried out using the whole skin of new-born guinea pigs. The obtained skin was frozen at −18°C and was pre-equilibrated in saline solution for 2 h at ambient temperature before the experiments. The donor cell was filled with 1 g of voriconazole ME and the receptor compartment was filled with 7 mL of physiological saline solution and continuously stirred and thermostated at 37 ± 1°C throughout the experiments. The receiving solution was completely substituted at each time interval. The solutions were analyzed by HPLC as described previously by Srinubabu et al[18].

The cumulative amount permeated through the guinea skins per unit area was plotted as a function of time. The permeation rate of the drug at a steady state (J_{ss}, mg cm^{-2} h^{-1}) through skin was calculated from the slope of linear portion of the plotted curve.

To discuss the effect of permeation enhancers, enhancement ratio (ER) was determined as the ratio of flux from ME with enhancer to flux from ME without enhancer.

The amount of voriconazole retained in the skin was determined at the end of the experiment. The skin was removed, where the effective permeation area of skin was cut, and washed three times with saline solution and wiped off. Each sample of skin was weighed and homogenized in 1 mL of methanol. The resulting solution was centrifuged for 10 min at 7000 rpm. The supernatant was analyzed for the drug.

The locally accumulation efficiency (LAC) values were obtained as the ratio of the drug accumulated into the skin to that delivered through the skin at the end of the experiment[19].

Microbiological activity

The selected MEs were tested against Candida albicans strain ATTC 90028 (obtained from the sabouraud dextrose agar at concentration of 5×10^5 cells/mL) using agar cup diffusion method[20]. The inhibition zone for each cup was measured in triplicate and compared with a reference voriconazole supersaturated solution.

Results

Construction of pseudo-ternary phase diagrams

Figures 1 and 2 show the phase diagrams for Tween 80- and Brij 97-based ME systems, respectively.

Tween 80-based systems

ME system I prepared using jojoba oil and glycerol produced clear ME of solution consistency at S:CoS:O w/w ratios of 8:1:1, 7:2:1, 6:3:1, 5:4:1, 7:1:2 and 6:2:2 at 10% water content. ME points were reduced to 8:1:1, 7:2:1 and 6:3:1 at 20% water and to 8:1:1and 7:2:1 at 30% and 40% water. No ME was formed at 50% water (Figure 1). The decrease in the ME area on increasing water content could be attributed to the large molecular size of liquid paraffin and jojoba oil and the short hydrophobic chain length of Tween 80 that would result in a decreased critical packing parameter with subsequent ME destabilization[21].

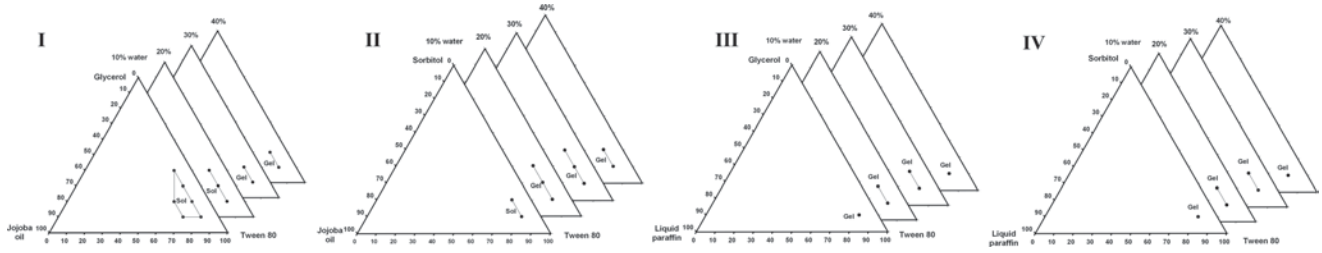

Figure 1. Pseudo-ternary phase diagrams of Tween 80-based microemulsion systems (I–IV).

Figure 2. Pseudo-ternary phase diagrams of Brij 97-based microemulsion systems (V–VIII).

Figure 1 shows ME system II, containing jojoba oil and sorbitol, at different water contents. MEs were formed at S:CoS:O ratios of 8:1:1and 7:2:1 for 10% and 40% water contents and at ratios of 8:1:1, 7:2:1 and 6:3:1 for 20% and 30% water.

ME systems III and IV were prepared using liquid paraffin as the oil phase and glycerol or sorbitol as co-surfactants, respectively. Both systems produced ME of gel consistency at S:CoS:O ratios of 8:1:1 and 7:2:1 at 20% and 30% water and at ratio of 8:1:1 for 10% and 40% water.

Brij 97-based systems

MEs were obtained at a wider range of S:CoS:O ratios (8:1:1, 7:2:1, 6:3:1, 5:4:1, 7:1:2, 6:2:2, 5:3:2 and 4:4:2) according to the CoS type and water content. Both Tween 80 and Brij 97 are POE-based non-ionic surfactants but they are known to have different compositions. Brij 97 has a single polar long POE chain linked to the oleyl group through polyhydric sorbitan in relation to four short POE chains in Tween 80. This single POE chain may provide greater anchorage of Brij 97 to the surface relative to Tween 80. In addition, the smaller cross-sectional area of the hydrophilic head group of Brij 97 occupies a relatively smaller area at the interface resulting in better oil solubilization than Tween 80[22,23].

Figure 2 shows that contrary to Tween-based MEs, increasing water content in Brij 97-based MEs increased the ME existence area. Brij 97 hydrophilic chains are strongly hydrated and connected with hydrogen bonds, allowing the interaction with more water droplets[24].

For both Tween-based and Brij-based systems, ME regions were concentrated in the co-surfactant and oil-poor part. Sorbitol and glycerol are highly polar co-surfactants, they tend to concentrate at the interface and in aqueous phase resulting in gradual removal of the surfactant from the interface[25].

Characterization of plain MEs

Characterization of the selected ME bases showed that the pH values ranged from 4.96 to 5.46 for Brij 97-based MEs and from 7.63 to 7.72 for Tween 80-based ones. The higher pH values recorded for Tween 80-based systems could be attributed to the higher pH value of its 10% aqueous solution (pH = 7) in relation to that of Brij 97 (pH = 4). In addition, hydrophilic chains of Brij 97 bind with water molecules via hydrogen bonds to a higher extent than Tween 80. This bound water differs in its thermodynamic properties and physicochemical characteristics from free water[26]. No marked effect was recorded for other ME components on the pH values.

Figure 3 shows that Tween-based MEs (F_1–F_4) recorded lower viscosity values than those of Brij 97-based ones (F_5–F_8). Tween 80 possesses a high molecular volume, which forms loose interfaces; on the other hand, the small molecular volume of Brij 97 forms tightly packed interfaces[27].

Moreover, Tween-based MEs showed Newtonian flow (the determination coefficients, R_{xy} between shear rate and shear stress were close to 1). Their viscosities were obtained from the slopes of the linear shear stress versus shear rate plots and collected in Table 1. On the other hand, Brij-based MEs exhibited pseudoplastic flow with thixotropic behavior. Degrees of pseudoplasticity (Farrow's number) ranged from 1.29 to 5.60 (Table 1).

All studied ME formulae withstood the temperature changes and centrifugation without phase separation.

Characterization of voriconazole-loaded MEs

Drug-loaded MEs remained clear and homogenous. Incorporation of voriconazole did not significantly affect the observed pH values of the MEs.

Table 2 shows that no change in the flow behavior was recorded for jojoba oil-based ME formulae (F5 and F6). They behaved as pseudoplastic. Exponent *N* values increased in relation to those of the corresponding plain MEs, indicating higher degrees of pseudoplasticity. Lower thixotropy degrees were proved by the smaller hysreresis loop areas. On the other hand, the addition of voriconazole changed the flow behavior of liquid paraffin-based MEs (F5 and F6) from pseudoplastic to dilatant ($N < 1$), which is not acceptable for topical use.

Using oleic acid, as the permeation enhancer, decreased the ME viscosities due to its oily nature,

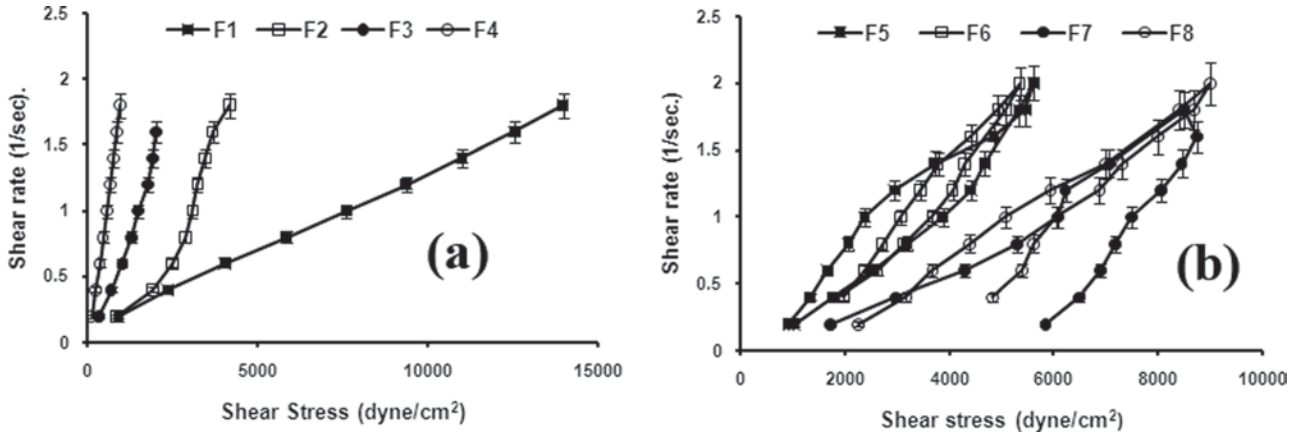

Figure 3. Rheological behavior of plain (A) Tween-based and (B) Brij 97-based microemulsions.

Table 2. Composition and evaluation parameters for voriconazole-loaded microemulsion formulae.

Microemulsion formulae		Viscosity at minimum shear rate (c.p.) × 10^3	Exponent *N*	Hystersis area (cm^2)	*MDT (min), mean ± SD	**RE_{4h} (%), mean ± SD
F5	·No enhancer	286	1.6	128.2	19.8 ± 0.9	60.4 ± 5.3
	·5% oleic acid	278	2.1	210.4	23.9 ± 1.4	49.8 ± 4.7
	·1% sodium deoxycholate	650	16	38504.4	18.9 ± 0.8	72.3 ± 3.9
F6	·No enhancer	127	6.4	2503.9	18.9 ± 1.3	69.2 ± 3.6
	·5% oleic acid	104	7.8	2904.0	20.4 ± 2.6	64.0 ± 4.7
	·1% sodium deoxycholate	447	12.6	27809.1	16.2 ± 1.6	77.6 ± 3.2
F7	·No enhancer	773	0.52		—	
	·5% oleic acid	495	0.46		—	
	·1% sodium deoxycholate	1230	0.51		—	
F8	·No enhancer	730	0.8		—	
	·5% oleic acid	345	0.46		—	
	·1% sodium deoxycholate	964	0.64		—	

Composition of the four microemulsion formulae is listed in Table 1.
*MDT is mean dissolution time calculated as follws:

$$MDT = \frac{\sum_{i=1}^{n} t_{mid} x \Delta M}{\sum_{i=1}^{n} \Delta M}$$

**RE_{4h} is release efficiency after 4 h, calculated as follows:

$$DE = \int_0^t \frac{y.dt}{y_{100} t} x\,100$$

whereas sodium deoxycholate resulted in higher viscosities due to its ability to form helical complex of macromolecular dimensions[28]. No more changes were recorded regarding homogeneity or pH.

In vitro release study

Figure 4 shows the release profiles of voriconazole from the two selected ME formulae, with and without enhancers. Voriconazole release was successfully prolonged due to the previously discussed high viscosities and compact structure of Brij-based MEs.

Statistical analysis ($p < 0.05$) proved that F5, prepared using glycerol as the co-surfactant, showed significantly higher MDT and smaller RE_{4h} values in relation to F6. This retardant effect could be related to the higher viscosity of F5 (Table 2).

In spite of their higher viscosity values, sodium deoxycholate-based MEs showed higher release rates and extents in relation to those containing oleic acid (Table 2). This could be attributed to the higher affinity of the hydrophobic voriconazole to oleic acid.

Effect of storage

No marked changes were recorded in the stored MEs except for slight decrease in viscosity values. This decrease may be due to loss of some water during storage[29].

In vitro skin permeation experiments

As can be seen in Figure 5, the permeation of voriconazole from the selected ME formula started rapidly without lag time. ME without enhancer delivered 202 μg/cm^2 after 5 h. The absorption-enhancing mechanism of ME could be explained by the large amounts of surfactant and co-surfactant that may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancers. In addition to the incorporation of large amount of the drug due to the high solubilizing capacity of ME. Furthermore, the small particle size of the ME may also affect its efficiency[30,31]. ME without enhancer showed significantly higher LAC ratio ($p < 0.05$) than those with permeation enhancers, suggesting that the main effect of this system was to accumulate the drug in the skin (Table 3). The aforementioned mechanisms caused the retention of voriconazole in the horny layer with little diffusion through the inner more hydrophilic skin layers probably because of the lipophilicity of the drug, which shows higher affinity for the subcutaneous lipid matrix. When oleic acid and sodium deoxycholate were incorporated, a more improvement in voriconazole diffusion through the skin was obtained. Oleic acid enhances membrane permeability by fluidizing the subcutaneous lipids[32]. Sodium deoxycholate had been reported to produce structural changes in the stratum corneum with consequent increased permeability. Moreover, deoxycholate, as an anionic surfactant, results in high membrane concentrations of drugs via solubilization in mixed micelles[33].

Statistical analysis showed that the two MEs with enhancer provided higher fluxes after 5 h than that of the ME alone ($p < 0.05$). The ERs of MEs with oleic acid and sodium deoxycholate were 1.39 and 1.55, respectively (Table 3). The higher permeation of voriconazole from sodium deoxycholate-based ME was consistent with the higher release rate and extent from this formula in relation to that from oleic acid-based one.

Microbiological activity

Statistical analysis ($p < 0.05$) showed that voriconazole ME with and without enhancers had significantly larger zones of inhibition than voriconazole supersaturated solution probably due to the antifungal activity of jojoba oil[34] (Table 3).

Discussion

ME was proposed as a carrier for topical delivery of voriconazole due to its high solubilizing ability and its permeation-enhancing properties. A series of non-toxic commonly used components were selected for the preparation of eight different ME systems. Tween 80 and Brij 97 are safe, very weakly irritant non-ionic surfactants with high solubilizing power[35,36]. Jojoba oil is stable, highly lipophilic, non-toxic oil commonly used to enhance the efficacy of topical drugs[37]. Liquid paraffin is one of the most common ingredients in skin care products and colored cosmetics. It is lightweight, inexpensive oil that is odorless and tasteless.

The effect of the type and ratio of the selected ME components on the formulation of ME was studied. High surfactant concentrations formed ME with gel-like behavior. This is in agreement with Kantaria et al.[38] Moreover, the increase of the surfactant portion resulted in increased probability of ME formation in the eight systems. Similar results were reported by Nandi et al.[39] The ME regions,

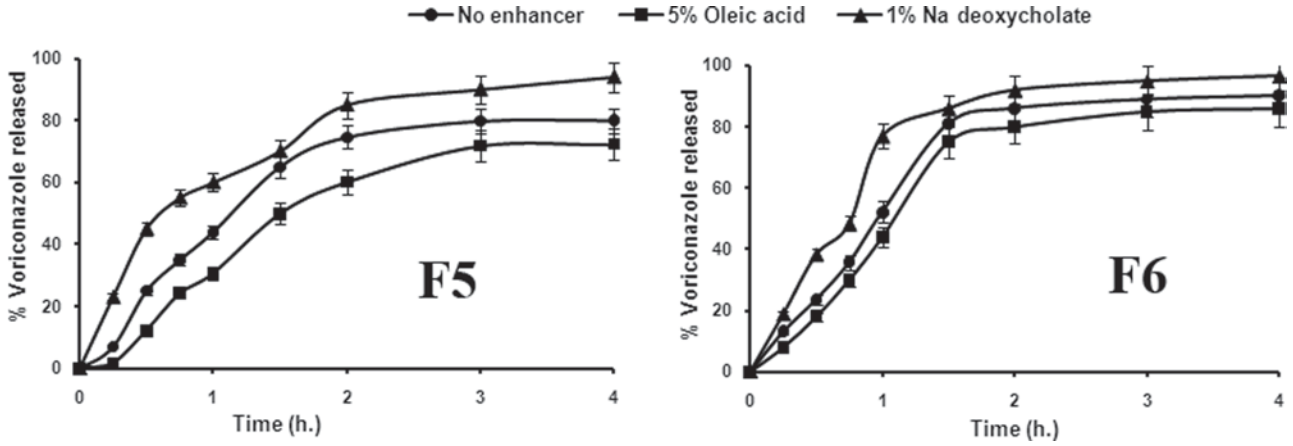

Figure 4. *In vitro* release profiles of voriconazole from the selected microemulsion formulae with and without permeation enhancers.

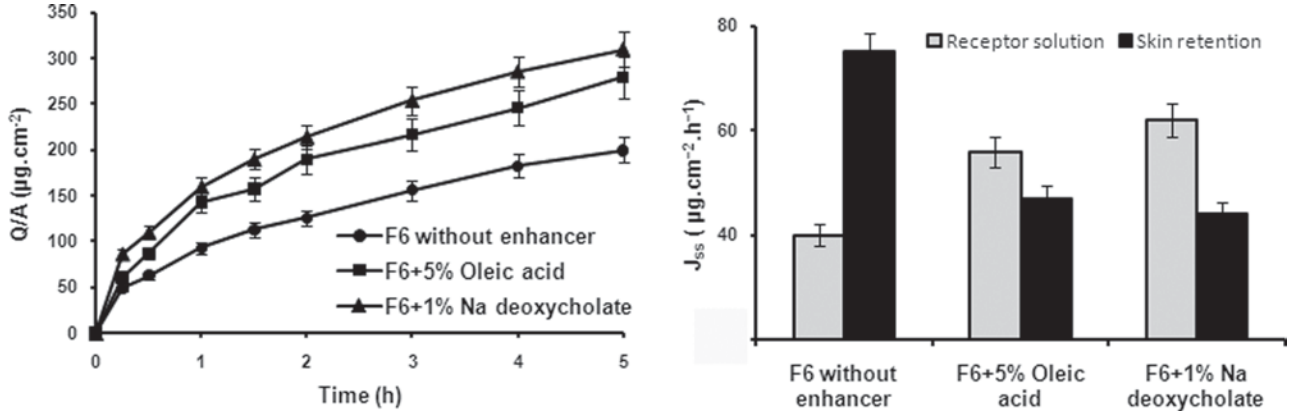

Figure 5. The results of *in vitro* skin permeation test of voriconazole from microemulsion formula F6 through guinea pig whole skin.

Table 3. Permeation parameters and microbiological activity of voriconazole microemulsions after 4 h.

Microemulsion formula	Voriconazole delivered (µg/cm² ± SD)	*LAC	ER	**Zone of inhibition (mm)
F6 without enhancer	202.00 ± 12.6	1.54	—	28 ± 1.30
F6 + 5% oleic acid	279.42 ± 13.8	0.83	1.39	31 ± 0.95
F6 + 1% sodium deoxycholate	310.00 ± 15.6	0.72	1.55	36 ± 1.70

ER, enhancement ratio. All values are mean of three readings.

*LAC = Locally accumulation efficiency $= \frac{\text{voriconazole accumulated into the skin}}{\text{voriconazole delivered through the skin}}$

**Zone of inhibition of plain microemulsion = 17 ± 2.16.

obtained upon using Brij 97 as surfactant, were larger than those with Tween 80.

The probability of ME formation decreased upon increasing the co-surfactant portion. This is in agreement with a previous observation[40] for ME system composed of mineral oil, Brij 96, water and propylene glycol. Moreover, glycerol, as a co-surfactant, favored ME formation for different surfactants, oils and water contents, except for system III, where quite larger monophasic areas were obtained with sorbitol-based MEs. The effect of water content varied according to the surfactant type. Moreover, increasing the water content shifted the ME toward gel-like consistency because oil/water MEs have higher viscosities than those of water/oil systems[41].

Increasing oil ratio reduced ME formation. Jojoba oil enhanced ME formation in Tween-based systems, whereas liquid paraffin was superior for Brij-based MEs except for system VII at 50% water.

Table 1 shows the composition of the selected formulae for further evaluation. ME systems of gel consistency were selected due to their suitability for topical application. Eight formulae were chosen, one to represent each system. The formulae were selected to contain the highest percentage of water and surfactant and the lowest oil content. It was reported that drug flux from ME increased by increasing its water content[42,43].

Characterization of the selected ME bases showed that they recorded pH values suitable for topical application with no risk of irritation or alteration of the cutaneous tegmentum[44].

Studying viscosity and rheological properties of MEs are necessary for characterizing them physically and controlling their stability[45]. Surfactant type showed the most pronounced effect on the rheological properties of the prepared MEs. Tween-based formulae showed Newtonian behavior, whereas Brij-based ones showed pseudoplastic flow with thixotropic behavior. Similar findings were reported[46] for ME system composed of isopropyl myristate/Tween80/soybean lecithin/water. Using glycerol as the co-surfactant and jojoba oil as the oily phase produced Tween-based MEs of higher viscosities than sorbitol and liquid paraffin, respectively. The observation for co-surfactant effect is in agreement with the findings of Gekko and Makino[47].

Due to the suitability of the pseudoplastic flow and thixotropy behavior for the topical pharmaceutical preparations, both in engineering design and consumer application, Tween-based MEs were excluded. Brij-based formulae were loaded with voriconazole alone and in combination with two permeation enhancers resulting in 12 medicated ME formulae (Table 2).

Characterization of voriconazole-loaded MEs showed that no significant changes in physical properties were recorded upon drug incorporation. As a result of viscosity measurements, it was observed that viscosity values of drug-loaded MEs were lower than the values of unloaded formulae. Similar observation was reported by Dreher et al.[48]

No changes in flow type was recorded upon incorporating voriconazole in jojoba oil-based MEs. Contrary to that, liquid paraffin MEs (F7 and F8) behaved as dilatants and were excluded from further studies.

The effect of co-surfactant type on the voriconazole release was related to its effect on ME viscosity, whereas that of the enhancer was resulting from the drug affinity to the ME vehicle. Similar findings were previously reported[49].

ME formula F6 was selected to compare voriconazole accumulation in and diffusion through the skin from ME with and without permeation enhancers. The LAC value of ME without enhancer was higher than that of MEs with enhancer, indicating that the presence of permeation enhancers favored transdermal rather than dermal delivery. Similarly, it was found that dermally applied ME penetrated the stratum corneum and remained intact in the whole horny layer[50].

Sodium deoxycholate was more effective than oleic acid for enhancing the voriconazole permeation. This was in agreement with Dhiman et al[51].

Therapeutic drug levels that cover the minimum inhibitory concentrations (MIC) of most fungi can be reached by topical application of the formulated ME both dermally and transdermally[3]. This voriconazole ME could be used as a single substitute for both topical and systemic routes of administration for the treatment of topical fungal infections.

Conclusion

A ME consisting of jojoba oil as oil phase, Brij 97 as surfactant, sorbitol as co-surfactant and distilled water could be an effective vehicle for topical delivery of voriconazole. There is significant penetration of voriconazole from this system through intact guinea pig skin. The data from our *in vitro* study has been encouraging but further evaluation is needed to elucidate the clinical efficacy of this topical dosage form.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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