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Investigating the effect of vehicle on in vitro skin permeation of ketoconazole applied in O/W microemulsions

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Abstract

The purpose of this study was to investigate the effect of vehicle on in vitro skin permeation of ketoconazole applied in o/w microemulsions. The microemulsion formulation containing 2% ketoconazole was optimized with 14% isopropyl myristate, 42% tween 80/propylene glycol (5:1) and 42% double distilled water (w/w), which showed a highest permeation rate of 63.28 ± 4.93 μg cm^{-2} h^{-1}. Candida Albicans was used as a model fungus to evaluate the antifungal activity of the best formula achieved. These results indicate that the studied microemulsion formulation might be a promising vehicle for future topical delivery of KTZ.

Key words: Microemulsion, ketoconazole, topical delivery, *in vitro* skin permeation study antifungal activity.

Introduction

Optimal drug delivery vehicles have to exert a high capacity for incorporating both lipophilic and hydrophilic drugs as well as high skin permeability. Microemulsions (MEs) as colloidal carriers are one of the promising systems who have nowadays attracted the main interest in penetration enhancement because of their localized effect. MEs are liquid dispersions of water and oil that are made homogenous, transparent (or translucent) and thermodynamically stable by the addition of relatively large amounts of a surfactant and a co-surfactant and having diameter of the droplets in the range of 10-100 nm (Danielsson and Lindman 1981). Due to their special features, MEs offer several advantages for the pharmaceutical use, such as ease of preparation, long term stability, high solublization capacity for the hydrophilic and lipophilic drugs, and improved drug delivery (Lawrence and Rees 2000).

Penetration enhancement from MEs is mainly due to an increase in drug concentration and thermodynamic activity which provides a large concentration gradient from the vehicle to the skin (Schmalfuss et al. 1997). High dose of drug can be incorporated into this system as a consequence of the super solvent properties of MEs and the dispersed phase can also act as a reservoir, making it possible to maintain an almost constant concentration gradient over the skin for a long time (Elena, Paola and Maria 2001).

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Also it has been suggested that the surfactant and the oil from the ME interact with the rigid lipid bilayer structure and acts as a permeation enhancer (Schmalfuss et al. 1997). Many studies have reported that microemulsion (ME) formulations possess improved dermal and transdermal delivery properties, mostly *in vitro* (Osborne et al. 1991, Delgado-Charro et al. 1997, Dreher et al. 1997, Schmalfuss et al. 1997, Kreilgaard et al. 2000, Rhee et al. 2001 and Lee et al. 2003) and several in vivo (Kemken et al. 1992, Kreilgaard 2002, Kreilgaard et al. 2001).

Ketoconazole (KTZ) cis-1-acetyl-4-[4-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxalan 4-yl]methoxy]phenyl] piperazine is an imidazole anti-fungal agent used in the treatment of superficial and systemic fungal infections. It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane. In the treatment of superficial and localized infection topical application of antifungal seems to be an appropriate strategy to restrict the therapeutic effect to the affected area and to reduce systemic incrimination (Souto and Müller 2005).

Topical drug delivery offers many important advantages. For instance, it is easy and painless, it protects the active compound from gastric enzymes, and it avoids the hepatic first-pass effect. Also, it is simple to terminate the therapy if any adverse or undesired effect occurs. But skin is a natural barrier, and only a few drugs can penetrate the skin easily and in sufficient quantities to be effective. Clinical efficacy of topical antifungal therapy depends on the drug ability to penetrate into the stratum corneum (SC) and the duration of treatment (Piérard et al. 1996).

KTZ, however, has very poor solubility characteristics in commom solvents such as water and alcohol. For topical use it is only available commercially in suspension in a semisolid aqueous cream and in shampoo. The cream provides poor bioavailability of KTZ as the discrete particles thereof do not permeate very efficiently into the skin. The shampoo is designed to apply for a very short periods which therefore does not provide the extended contact time necessary to maintain a therapeutic adequate concentration into the skin. Furthermore shampoo tends to contain anionic surfactants which may hinder the drug permeating through the skin and may also irritate the skin. There is therefore a need for a vehicle containing suitable concentration of KTZ for direct application to the skin in order to treat susceptible infective conditions. The present paper describes the attempts made to design ME formulations as vehicle from which dissolved KTZ can continue to permeate into the skin over an extended contact period for maximum thermodynamic activity. ME formulations containing a lipophilic (isopropyl myristate (IPM)) and two hydrophilic (polysorbate–80 (T-80) and propyleneglycol (PG)) skin penetration enhancers for topical delivery of poorly water-soluble KTZ were developed. In vitro permeation study was performed using Franz diffusion cell through intact rat skin to demonstrate the potential of a developed ME system for topical drug delivery of KTZ. The antifungal activity of KTZ using *Candida albicans* as a model fungus has been also evaluated.

Materials and Methods

Materials

KTZ (purity 99%) was procured as gratis sample from Alembic Ltd. (Vadodara-India). IPM, Ethyl Laurate (EL), Ethyl Oleate (EO), Wheat Germ Oil (WO), Oleic Acid (OA), Arachis Oil (AO),

Polysorbate 20 (T-20) and Polysorbate 20 (T-40) were purchased form National Chemicals (Vadodara, India). T-80 was purchased form Ranbaxy Ltd. (Delhi, India). PG was purchased from Chiti Chem Corporation (Vadodara, India). Span 20 (S-20) and Span 80 (S-80) were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India). Candida Albicans (ATCC 10231) gratis sample was procured from Food and Drug Laboratory (Vadodara, India). Double distilled water was used for the preparation of ME. All other chemicals and solvents were of analytical reagent grade.

Methods

Screening of oils and surfactants for microemulsions: The solubility of KTZ was determined in various oils and surfactants. The oils employed were IPM, EL, WO, EO, AO and OA. The surfactants used were T-80, T-40, T- 20, S-80 and S-20 having HLB values 15.0, 15.6, 16.7, 4.3 and 8.6 respectively.

Drug powder of KTZ was added in excess to each of the oils and surfactants and then vortexed for mixing. After vortexing the samples were kept for 72 hours at ambient temperature for attaining equilibrium. The equilibriated samples were then centrifuged at 5000 rpm for 30 minutes to remove the undissolved drug. The aliquots of supernatant were filtered through 0.45 μm membrane filters and the solubility of KTZ was determined by analyzing the filterate spectrophotometrically using double beam Perkin Elmer Lambda 19 (Perkin Elmer, Norwalk, CT) after dilution with methanol at 225nm. Appropriately diluted solutions of oils in methanol were taken as blank.

Construction of pseudoternary phase diagram: In order to find out the concentration range of components for the existing range of MEs, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature. Five phase diagrams were prepared with the 1:1, 2:1 3:1, 4:1 and 5:1 weight ratios of T- 80 to PG, respectively. For each phase diagram at a specific surfactant :co-surfactant weight ratio, the ratios of oil to the mixture of surfactant and co-surfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1 and 9.5:0.5. The mixtures of oil, surfactant and co-surfactant at certain weight ratios were diluted with water drop wise, under moderate magnetic stirring. After being equilibrated at ambient temperature for 24 hours, the mixtures were assessed visually and determined as being MEs, crude emulsions or gels. The stable MEs were also observed under polarizing light to confirm their isotropic nature. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type MEs. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90°.

Preparation of KTZ-loaded microemulsions: KTZ was added to the mixtures of oil, surfactant, and co-surfactant with varying component ratio as described in Table 1, and then an appropriate amount of distilled water was added to the mixture drop by drop and the MEs containing KTZ was obtained by stirring the mixtures at ambient temperature. All MEs were stored at ambient temperature.

Table 1. Composition of selected microemulsions (%w/w)

Microemulsion	Fluconazole	IPM	S_{mix}*	Water
ME1	2	14	42	42
ME2	2	14	49	35
ME3	2	14	54	30
ME4	2	7	65	26
ME5	2	7	71	20
ME6	2	7	77	14

* Smix = Surfactant : co-surfactant mixture (5:1)

Characterization of microemulsions: The formulated MEs were then recognized and characterized on the basis of their physical properties, which cannot only explain the performance of the system but also help in modifying their performance attributes

To measure the solubility of KTZ in the MEs excess of KTZ was added to each ME and then allowed to equilibrate for 72 hours at ambient temperature (Lianli et al. 2002). The equilibrated samples were then centrifuged at 5000 rpm for 30 minutes to remove the undissolved drug. The aliquots of supernatant were filtered through 0.45 μm membrane filters and the solubility of KTZ was determined by dilution of filtrate with methanol and measurement of absorbance at 225 nm. Appropriately diluted solutions of plain ME in methanol were taken as blank.

The droplets of the ME being smaller than $1/4^{th}$ the wavelength of visible light, permit white light to pass through the dispersed system making it transparent or translucent. The ME systems were inspected for optical transparency and homogeneity by visual observation against strong light. The systems were also checked for the presence of undissolved drug or other solid ingredient. This was also confirmed by measuring % Transmittance using colorimeter (Digital Colorimeter, D-801, Photocon) at 570 – 590 nm.

In order to verify the isotropic nature of MEs, samples were examined using cross-polarized light microscopy (Polarizing Microscope, Carlzeless, Jena, Germany). A drop of sample was placed between a cover slip and a glass slide and then observed under cross-polarized light. Isotropic material, such as MEs, in contrast to anisotropic liquid crystals, not interfere with the polarized light (Friberg 1990) and the field of view remain dark because the analyzer absorbs light passing through the polarizer.

The pH values of MEs were determined using digital pH meter (Orion pH meter 420A, Allometric Ltd., Baton Rouge, LA), standardized using pH 4 and 7 buffers before use.

The viscosity of MEs was measured using Brookfield Viscometer (Brookfield Engineering LABS, Stoughton, MA) with spindle LV – III at 100 rpm using interval of 30 seconds. All aspects of testing were controlled using optional Rheocalc Software.

The electric conductivity of MEs was measured with a conductivity meter (Equip-Tronics, EQ – 664) equipped with inbuilt magnetic stirrer. This was done by using conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as a conductor.

For determination of drug content about one gram of each ME was weighed in a 10 ml volumetric flask and dissolved in methanol. It was diluted appropriately and analyzed spectrophotometrically at 225nm. Appropriately diluted solutions of respective plain ME in methanol were taken as blank.

The average droplet size and polydispersity index of ME were measured by photon correlation spectroscopy with in-built Zetasizer (Nano ZS, Malvern Instruments, UK) at 633 nm. Helium – neon gas laser having intensity of 4mW was the light source. The droplet size was calculated using Stokes – Einstein relationship by Zetasizer Software.

In vitro skin permeation study: The *in vitro* skin permeation study was carried out under the guideline compiled by CPCSEA (Committee for the purpose of control and supervision of Experiments on animal, Ministry of Culture, Government of India) and all the study protocols were approved by the Local Institutional Animal Ethics Committee. The abdominal skins obtained from male Wistar rats weighing 230±20 g were used for in vitro permeation experiments. After hair was shaved carefully with an electric clipper, the skin was excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues were removed without damaging the epidermal surface. The excised rat skins were washed and examined for integrity, and then stored at 4 °C for 24 hours in phosphate buffer saline pH 6.8 (PBS), and then used for the permeation experiments. The permeation experiments were

performed using Franz diffusion cells fitted with excised rat skins having epidermal surface outward. The effective diffusion area was 3.14 cm^2 (20mm diameter orifice), and the receptor compartment was filled with 12 ml of PBS. The diffusion cell was maintained at 37±1°C using a recirculating water bath and the solution in receptor chamber was stirred continuously at 600 rpm throughout the experiment. The formulation (1 g) was gently placed in a donor chamber. At 1, 2, 4, 6 and 8 hours aliquot of 2 mL sample were withdrawn from the receptor compartment for spectrophotometric determination and replaced immediately with an equal volume of fresh PBS. Average values of three readings of in vitro permeation data were calculated and the average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The in vitro permeation data was subjected to model fitting using the PCP Disso 3.0 software, Pune, India.

The permeation parameters of the Fick's law equation were calculated from the plot of penetrated amounts vs time. The slope of the linear portion of the plot was calculated as flux Jss ($\mu g/cm^2/h$) (Lianli et al. 2002) and the permeability coefficient was calculated using following formula (Panigrahi et al. 2005).

Kp = Jss/Cv where Kp is permeability coefficient and Cv is total amount of drug.

In vitro antifungal activity: The antifungal activity of KTZ from the optimized formulation and reference standard (KTZ dissolved in PBS) was determined using Candida Albicans ATCC 10231 as a representative fungus, adopting cup plate method (El laithy and El-Shaboury 2002). One gram each of ME and KTZ reference solution containing 2% KTZ were placed in each well with a control (vehicle-free drug). The mean inhibition zone of KTZ released from 5 plates for each formula was calculated and this value was taken as an indicator for the antifungal activity. Statistical analysis using ANOVA test at level of significance of .05 was carried out to determine the degree of significance between the test and the reference standard.

Stability studies, Visual Inspection: Shelf life as a function of time and storage temperature was evaluated by visual inspection of the ME system at different time period. Stability was monitored at 0-8°C (refrigerator), 25 ±2°C and 50±2°C temperature.

Centrifugation: In order to estimate metastable systems, the selected ME vehicles were centrifuged (Remi Laboratories, Mumbai, India) at 5000 rpm for 30 minute at 0°C.

Results and Discussion

Solubility of KTZ in oils and surfactants: To develop ME system for topical delivery of KTZ, the suitable oil and surfactant has to be chosen. So the solubility of KTZ was determined in various oils and surfactants (Table 2). Among the nonionic surfactants studied T-80 led to the highest solubility of KTZ (13.88±2.38 mg/ml). Also T-80 is known to be less affected by pH and ionic strength changes and acts as a solublizing agent (Kim et al. 2001). On the other hand, there was no significant difference in the solubility of KTZ among the various oils tested except AO and OA which exhibited low solubility as compared to other oils. However, IPM, a fatty acid ester slightly increases the solubility of KTZ (11.23 ± 1.86 mg/ml) compared with other oils, although it was not statistically significant. IPM is used as a permeation enhancer in topical formulations but the mechanism of its action is poorly understood (Goldberg-Cettina et al. 1995). Also in respect of convenience of formation and use, the oil IPM is better choice in comparison with other physiologically tolerable oils (Acharya et al. 2001). From these solubility results, T-80 and IPM were chosen as a surfactant and oil respectively, for the preparation of ME formulations of KTZ for further studies.

Table 2. Solubility of Ketoconazole in various oils and surfactants (mean±S.D., n=3)

Vehicle	Solubility (mg/ml)
Oils	
Isopropyl Myristate	11.23±1.86
Ethyl Laurate	9.48±1.45
Wheat Germ Oil	8.94±1.30
Ethyl Oleate	10.84±0.18
Arachis Oil	6.53±0.25
Oleic Acid	5.22±0.74
Surfactants	
Tween-80	13.88±2.38
Tween-40	7.14±1.45
Tween-20	6.55±1.37
Span-80	2.31±1.43
Span-20	2.47±1.78

Phase behaviour: Pseudoternary phase diagrams were constructed to obtain appropriate components and their concentration ranges for the MEs (Chen et al. 2004). The pseudo-ternary phase diagrams with various weight ratios of T-80 to PG are presented in Figure 1. The transparent ME region is presented in phase diagrams. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) ME was observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. No liquid crystalline structure was observed using cross polarizer. The area of ME isotropic region changed slightly in size with the increasing ratio of surfactant to co-surfactant.

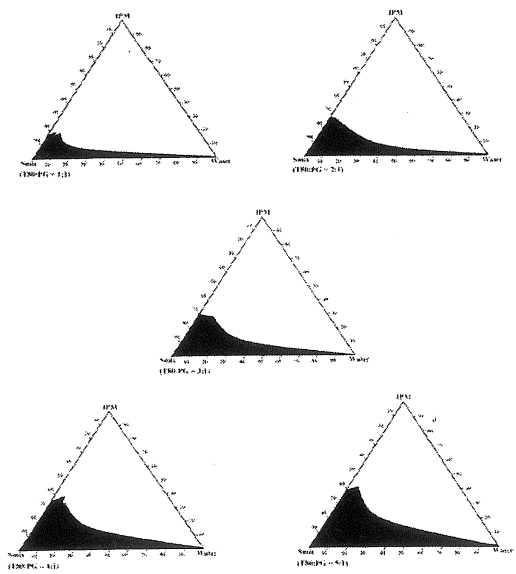

Figure 1. The pseudo-ternary phase diagrams of the oil-surfactant mixture-water system at the 1:1, 2:1, 3:1, 4:1 and 5:1 weight ratio of Tween - 80 to Propylene Glycol at ambient temperature, dark area represent microemulsions region.

Preparation and characterization of KTZ loaded microemulsions: Various MEs were selected from the pseudoternaty phase diagram with 5:1 weight ratio of T-80 to PG. Here, KTZ was

added into oily phase and the drug-loaded MEs were prepared by mixing oily phase containing KTZ with water. The physicochemical properties of MEs are reported in Table 3.

The samples were examined by ocular inspection in a cross polarizer for sample homogeneity and birefringence. The MEs appeared completely dark when observed under cross polarizer. The observations indicated that all the MEs were optically isotropic colloidal dispersions.

All the MEs formed were transparent and appeared like a homogenous single-phase liquid, when observed for visual clarity against strong light. No traces of undissolved drug or other solid ingredient were found in all samples. Percentage transmittance of all prepared ME formulations was found in the range of 96 to 99% T (data not shown).

The drug solubility in the MEs studied was found to be increasing with increasing surfactant: co-surfactant concentrations (Table 3). These could be attributed to higher solubility of KTZ in T-80. However there was no significant difference in solubility of KTZ in various MEs studied.

The ME formulations had appropriate observed pH values varying from 5.9 to 6.2 for topical application (Table 3). Incorporation of KTZ did not significantly affect the observed pH value of the ME formulations.

The ME 1 formulation had the lowest viscosity value (35.54 ± 1.19 mPas) among the ME formulations studied (Table 3). In general the viscosity of the MEs was found to be increasing with increasing surfactant: co-surfactant concentrations. There was no significant difference found between the viscosities of plain and drug loaded MEs.

As has been reported previously, in order to study electrical conduction of non-ionic MEs, a small amount of aqueous electrolyte must be added to provide the charges necessary for the charge transport (Weigert et al. 1997). However, the addition of salt can significantly affect the phase behaviors and structural properties of MEs and that even may result in phase separation. For this reason, in this study, the conductivity measurements were performed without deliberate incorporation of an electrolyte. The investigated ME formulations containing non-ionic surfactant mixture, oil and water showed electroconductive behaviour inspite of its non-ionic nature. The ME 1 formulation had relatively high conductivity (154.5 ± 4.7 μS/cm) among the ME formulations studied (Table 3). From the electroconductive study it could be concluded the tested ME formulations were of o/w type. The conductivity results obtained showed that loading KTZ and the addition of the appropriate amount of water phase into the formulation had no negative effects on system stability. When an unstable emulsion system and phase separation occurs, the conductivity values are greatly reduced (Li et al. 2003).

The KTZ content of the ME formulations were within the range of 98-100% (Table 3).

The ME 1 formulation had the lowest average particle size (18.1 ± 0.2 nm) with polydispersity index (PI) of 0.128 ± 0.027 (Table 3). Average droplet size and PI of all ME formulations ranged from 15 to 50 nm and 0.128 to 0.142 respectively. It was found that 90% of the droplets had small droplet size, less than 50 nm. PI is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PI value the more homogenous are the particles. The PI showed that all ME formulations had narrow size distribution.

Table 3. Physicochemical parameters of tested microemulsion formulations (mean±S.D., n=3)

Formulations	Solubility (mg/ml)	pH	Viscosity (mPas)	Conductivity (µS/cm)	Drug Content (%w/w)	Diameter (nm)	Polydispersity index
ME1	26.81±1.23	6.22±0.03	35.54±1.19	154.5±4.7	99.15±1.68	18.1±0.2	0.128±0.027
ME2	29.38±1.74	5.98±0.07	40.41±1.13	107.2±4.9	99.11±2.12	23.7±0.2	0.138±0.022
ME3	30.17±1.27	6.16±0.06	44.55±1.71	72.40±2.2	99.16±1.48	28.6±0.4	0.139±0.031
ME4	29.51±1.40	6.11±0.05	46.92±1.31	55.30±7.5	98.19±1.45	32.2±0.5	0.131±0.018
ME5	31.19±1.77	6.07±0.03	58.42±1.43	40.45±3.5	100.14±1.65	40.1±0.5	0.142±0.021
ME6	34.14±1.47	5.93±0.04	70.01±1.44	37.25±6.9	99.12±1.79	47.3±0.7	0.135±0.019

In vitro skin permeation study: Table 1 shows the different ME formulations composed of IPM, T-80, PG and water at different concentrations. The effects of the content of oil and surfactant mixture on the skin permeation of KTZ were evaluated. The skin permeation profiles are presented in Figure 2, the Jss and Kp values are shown in Table 4. Among the ME formulations tested, ME1, which was composed of 2 % KTZ, 14 % IPM, 42 % Tween-80/1, 2-propylene glycol (5:1, w/w) mixture and 42 % water, showed the highest permeation profile. The Jss of KTZ from ME1 was 63.28 ± 4.93 µg/cm^2/h and Kp was 2.9 ± 0.62 cm/h, 4.15 times higher than those of the KTZ saturated aqueous solution in PBS, which were 15.25 ± 1.19 mg/cm^2/h and 0.71 ± 0.21 cm/h, respectively. Various release kinetic models were applied to elucidate the mechanism of drug release from the ME formulations. Drug release from the optimized formulations ME 1 followed the Peppas models (R = 0.9869, n = 0.41), respectively, suggesting a diffusion based mechanism of drug release as the diffusion exponent values were less than 0.5 (Costa and Lobo 2001). The permeation rate of KTZ was almost linearly improved as a function of loading dose and the permeation of MEs accorded with Fick's first diffusion law.

Table 4. The permeation parameters of the ketoconazole loaded microemulsions and saturated aqueous solution (mean±S.D., n=3)

Formulation	Jss (µg/cm^2/h)	K_p (cm/h)
ME-1	63.28 ± 4.93	2.9 ± 0.62
ME-2	47.49 ± 2.53	1.9 ± 0.97
ME-3	37.25 ± 3.53	2.0 ± 0.86
ME-4	40.52 ± 1.65	2.1 ± 0.43
ME-5	49.92 ± 2.79	2.3 ± 0.38
ME-6	39.58 ± 3.08	1.6 ± 0.24
Saturated aqueous solution	15.25 ± 1.19	0.71 ± 0.21

The topical formulations for the treatment of skin infections must provide proper concentrations of the drug in target site for therapeutic activity. In the case of superficial fungal skin infections, in which the main location of the pathogen is in epidermis, the drug must penetrate into the SC in proper concentrations to inhibit the fungus growth (Alberti et al. 2001). Interestingly, the percutaneous absorption studies were shown to be superior from the ME formulations. Maximum drug permeation and 4.15-fold improvement in drug release were achieved in comparison to saturated aqueous solution. These results clearly indicate that KTZ, when used in ME, was more efficiently penetrated compared with saturated aqueous solution.

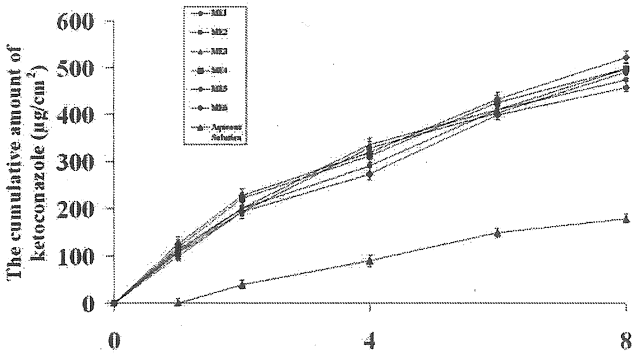

Figure 2. Permeation profile of ketoconazole through rat skins from microemulsion formulations and reference (mean ± S.D. n = 3).

The higher permeability rate of KTZ from ME formulations is most probably due to the surfactants and the oily phase, which act as penetration enhancers (Lawrence and Rees 2000). The enhancer can increase the transport through skin by modifying the diffusion or partitioning coefficient of drug (Naik et al. 2000). IPM as a permeation enhancer had a strong permeation enhancing effect and could increase the diffusion coefficient in skin, which could result in the increase of permeation coefficient (Chen et al. 2007). It was found that skin permeation of the drug in ME was significantly influenced by content of IPM. As the content of oil was increased the number of internal phase was increased, which further increased the skin permeation rate of the drug.

The content of surfactant mixture in ME was also found to affect the skin permeation flux of KTZ significantly, but its mechanism was different from that of the oil. ME containing a lower amount of T-80 and PG, provided higher flux. This may be due to an increased thermodynamic activity of the drug in ME at the lower concentration of surfactant and cosurfactant, as KTZ is poorly water soluble and yet solublized in the surfactant mixture (Rhee et al. 2001, Jang-Hoon et al. 2004). Thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of drug into skin (Walters et al. 1998).

Several mechanisms have been proposed to explain the advantages of ME for the topical delivery of drugs. First, a large amount of drugs can be incorporated in the formulation due to the high solubilizing capacity. Second, the steady-state flux of the drug from ME may be increased, since the affinity of a drug to the internal phase in ME can be easily modified to favor partitioning into SC, using a different internal phase, changing its portion in ME or adjusting its property. Furthermore, the surfactant and cosurfactant in the ME may reduce the diffusional barrier of the SC by acting as permeation enhancers (Bianca et al. 2000). For efficient percutaneous absorption of drugs, the histological and histochemical structure of the SC must be taken into consideration. Drugs can permeate the SC through two micropathways, one is the intercellular route and the other is the transcellular way. Of these routes, the intercellular route plays a major role in the percutaneous uptake of drugs. It is known that a complex mixture of essentially neutral lipids that are arranged as bilayers with their hydrophobic chains facing each other, form a hydrophobic bimolecular leaflet. Most of the lipophilic drugs pass through this region, and it is called the lipid pathway. Polar head groups

of lipids face an aqueous region forming a polar route that hydrophilic drugs generally prefer (El laithy and El-Shaboury 2002).

Topically applied ME is expected to penetrate the SC and to exist intact in the whole horny layer, altering both the lipid and the polar path-ways (Thachrodi and Panduranga 1994). The lipophilic domain of the ME can interact with the SC in many ways. The drug dissolved in the lipid domain of the ME can directly partition into the lipids of the SC, or the lipid vesicles themselves can intercalate between the lipid chains of the SC, thereby destabilizing its bilayer structure. In effect, these interactions will lead to increased permeability of the lipid pathway to KTZ. On the other hand, the hydrophilic domain of the ME can hydrate the SC to a greater extent, and plays an important role in the percutaneous uptake of drugs. When the aqueous fluid of the ME enters the polar pathway, it will increase the interlamellar volume of the SC lipid bilayers, resulting in the disruption of its interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers; a lipophilic penetrant like KTZ can then permeate more easily through the lipid pathway of the SC (El laithy and El-Shaboury 2002).

Moreover, the particle size of the ME may also affect its efficiency, where its small particle size makes it an excellent carrier for promoting KTZ percutaneous uptake. as the number of vesicles that can interact on a fixed area of SC will increase when the particle size decreases.

In fact, no stout mechanism could be considered in explaining the superiority of the ME over the other vehicles, but the combined effect of both the lipophilic and hydrophilic domains as well as the particle size of the ME was responsible for its enhancing activity.

Finally, the optimized composition of ME containing 2% KTZ was confirmed as 14% IPM, 42% Tween-80/1, 2-propylene glycol (5:1, w/w) mixture and 42% water which showed highest permeation profile with appropriate physicochemical characters.

In vitro antifungal activity: The values of mean zone of inhibition (the antifungal activity) of the tested ME formulation was larger than that of reference standard (Table 5). The plain formula used in the study showed no antifungal activity. The ANOVA showed that there is a significant difference in the tested ME zone of inhibition in comparison to the reference standard at $P<0.05$, where the calculated F is larger than the tabulated F. The enhanced in vitro antifungal activity of tested ME may be attributed to enhanced penetration of oil globules containing KTZ through fungal cell walls to inhibit ergosterol synthesis.

Table 5. Antimicrobial activity of optimized microemulsion in comparison to reference standard using *Candida albicans*.

Formulation	Zone of Inhibition (mm)					
	1	2	3	4	5	Mean ± SD
Microemulsion	20.5	22.5	24.5	22.5	24.5	22.9 ± 1.67
Reference	13	12	14.5	13.5	9.5	12.5 ± 1.90

Stability studies: Stability studies of the ME formulations were carried out by subjecting them to visual inspection (without stress) and centrifugation (under stress). The visual inspection test was carried out for 3 month by drawing sample at weekly interval for the first month and

monthly interval for the subsequent months. The visual observation conducted by showing no evidence of phase separation or any flocculation or precipitation. These samples also showed no sign of phase separation under stress when subjected to centrifugation at 5000 rpm for 30 minutes.

Conclusion

The ME for KTZ can be prepared by using the pharmaceutically acceptable materials like IPM, T-80, PG, and water as suitable polar solvent. Present investigations have been demonstrated that skin permeation ability of KTZ was significantly increased by ME compared to conventional vehicles, e.g., aqueous solution, which might result from the special characteristics of ME. The favourable topical drug delivery properties of prepared MEs are indicated to be attributable to the large concentration gradients provided by the high KTZ solubility potential of the vehicles without concurrent increase in vehicle affinity for the KTZ, and/or to a potential penetration enhancer effect of the individual constituents. Moreover, the particle size of the ME may also affect its efficiency, where its small particle size makes it an excellent carrier for promoting in vitro skin permeation of KTZ. These studies indicated that KTZ ME formulations could be the viable alternative to the current topical formulations available for the treatment of superficial fungal infections. Nevertheless, significant work still needs to be carried out to confirm these interesting conclusions.

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