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Research Article

Effect of Formulation Components on the *In Vitro* Permeation of Microemulsion Drug Delivery System of Fluconazole

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Abstract. The purpose of this study was to evaluate the effect of formulation components on the *in vitro* skin permeation of microemulsion drug delivery system containing fluconazole (FLZ). Lauryl alcohol (LA) was screened as the oil phase of microemulsions. The pseudo-ternary phase diagrams for microemulsion regions were constructed using LA as the oil, Labrasol (Lab) as the surfactant and ethanol (EtOH) as the cosurfactant. The formulation which showed a highest permeation rate of 47.15± 1.12 μg cm^{-2} h^{-1} and appropriate physicochemical properties was optimized as containing 2% FLZ, 10% LA, 20% Lab/EtOH (1:1), and 68% double-distilled water (*w*/*w*). The efficiency of microemulsion formulation in the topical delivery of FLZ was dependent upon the contents of water and LA as well as Lab/EtOH mixing ratio. It was concluded that the percutaneous absorption of FLZ from microemulsions was enhanced with increasing the LA and water contents, and with decreasing the Lab/EtOH ratio in the formulation. *Candida albicans* was used as a model fungus to evaluate the antifungal activity of the best formula achieved, which showed the widest zone of inhibition as compared to FLZ reference. The studied microemulsion formulation showed a good stability for a period of 3 months. These results indicate that the studied microemulsion formulation might be a promising vehicle for topical delivery of FLZ.

KEY WORDS: antifungal activity; fluconazole; *in vitro* skin permeation study; microemulsion; topical delivery.

INTRODUCTION

Human skin is an important target site for the application of drugs. Especially in the treatment of local diseases, a topical drug delivery is an appropriate strategy to restrict the therapeutic effect on the affected area and to reduce systemic incrimination. In order to reach therapeutic drug concentrations in certain skin layers, the uppermost barrier, the stratum corneum (SC), has to be overcome. This process is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for application (1,2).

Penetration enhancement with special formulation approaches is mainly based on the usage of colloidal carriers. Colloidal carriers have attracted the main interest because they are promising systems having localized effect. The carriers, accumulate in SC or other upper skin layers are not expected to penetrate into viable skin. The common characteristic of all colloidal carriers is the submicron-sized particles which are intended to transport entrapped active molecules into the skin. Microemulsions (MEs) as colloidal carriers are one of the promising systems which have nowadays attracted the main interest in penetration enhancement because of their localized effect. 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 (3).

Penetration enhancement from ME is mainly due to an increase in drug concentration and thermodynamic activity, which provides a large concentration gradient from the vehicle to the skin (4). High dose of drug can be incorporated into this system as a consequence of the supersolvent 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. 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 (5). Many studies have reported that ME formulations possess improved dermal and transdermal delivery properties, mostly *in vitro* (6–11) and *in vivo* (12–14).

Fluconazole (FLZ) is chemically 2-(2, 4-difluorophenyl)-1, 3-*bis*-(1H-1, 2, 4-triazole-1-yl)-2-propanol, a synthetic triazole which is one of the most commonly prescribed antifungal drug. FLZ has emerged as the primary treatment option for virtually all forms of susceptible *Candida* infections in both immunocompetent and immunocompromised hosts. It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane (15). Clinical efficacy of topical antifungal therapy depends on the drug ability to

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penetrate into the SC and the duration of treatment (16). Hence, ME formulations appeared to be a viable approach for future topical delivery of FLZ. The solubilization of FLZ in MEs would improve its topical availability.

In this study, oil-in-water (O/W) MEs containing 2% FLZ have been developed to provide maximal skin permeation rates of FLZ. Also an attempt was made to study the effect of oil, surfactant/cosurfactant mixing ratios and water on the *in vitro* permeation of FLZ using rat skin. The antifungal activity of optimized ME formulation using *Candida albicans* as a model fungus has been also evaluated.

MATERIALS AND METHODS

Materials

FLZ (purity 99%) was procured as gratis sample from Alembic Ltd. (Vadodara-India). Lauryl alcohol (LA), ethyl laurate (EL), ethyl oleate (EO), wheat germ oil (WO), oleic acid (OA), and arachis ail (AO) were purchased from National Chemicals (Vadodara, India). Labrasol (caprylocaproyl macrogol-8-glyceride) (Gattefosse Saint-Priest, France) was procured as gratis sample from Colorcon Asia Ltd. (Mumbai, India). Polyoxyethylene-20-sorbitan monooleate (Tween-80) was purchased from Ranbaxy Ltd. (Delhi, India). Ethanol was purchased from Baroda Chemical Ind. Ltd (Dabhoi, India). *C. albicans* (ATCC 10231) gratis sample was procured from Food and Drug Laboratory (Vadodara, India). Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

Screening of Oils for Microemulsions

Drug powder of FLZ was added in excess to each of the oils and then vortexed for mixing. After vortexing the samples were kept for 72 h at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 5,000×*g* for 30 min to remove the undissolved drug (10). The aliquots of supernatant were filtered through 0.45 μm membrane filters and the solubility of FLZ was determined by analyzing the filtrate spectrophotometrically using double beam Perkin Elmer Lambda 19 (Perkin Elmer, Norwalk, CT) after dilution with methanol at 258 nm. Appropriately diluted solutions of oils in methanol were taken as blank.

Construction of Pseudo-Ternary 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 the water titration method (17) at ambient temperature. Three phase diagrams were prepared with the 1:1, 2:1, and 3:1 weight ratios of Labrasol (Lab) to Ethanol (EtOH), respectively. For each phase diagram at a specific surfactant (S)/cosurfactant (CoS) mixing ratio (Km), the ratios of oil to the mixture of S/CoS 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 and S/CoS at certain weight ratios were diluted with water drop wise, under moderate magnetic stirring. After being equilibrated at ambient temperature for 24 h, the mixtures were assessed visually and determined as being MEs, crude emulsions, or ME gels. The stable MEs were also observed under polarizing light to conform 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 being tilted to an angle of 90°.

Eighteen different formulations with various values of oil of 5% and 10%, water of 48%, 58%, and 68% and S/CoS mixing ratio of 1, 2, and 3 were prepared for the *in vitro* skin permeation study. Effects of the contents of the oil, water, and the mixture of S/CoS on the permeation of FLZ through excised rat skins were evaluated.

Preparation of FLZ-Loaded Microemulsions

Various MEs were selected from the pseudoternary phase diagram with 1:1, 2:1, and 3:1 weight ratio of Lab to EtOH (Table I). FLZ was added to the mixtures of oil and S/CoS and then an appropriate amount of distilled water was added to the mixture drop by drop and the MEs containing FLZ were obtained by stirring the mixtures at ambient temperature. All MEs were stored at ambient temperature.

In Vitro Skin Permeation Study

The *in vitro* skin permeation study was carried out under the guideline compiled by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, Ministry of Culture, Government of India) and all the study protocols were approved by the Local Institutional Animal Ethics Committee (Protocol No.:CPCSEA/IAEC/ARCP/06-07/01). The abdominal skins obtained from male Wistar rats weighing 230±20 g (age, 6–8 weeks) were used for *in vitro* permeation experiments of 18 prepared formulations. 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 h in phosphate-buffered 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 (20 mm 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 waterbath 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 h 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.

Table I. Compositions of Selected Microemulsions (% *w/w*) and Particle Size (mean±SD, $n=3$)

Formulation	Fluconazole	Km[a]	Oil	Water	S/CoS	Particle size (nm)	Polydispersity index
ME 1	2	1	5	68	25	32±0.5	0.456±0.027
ME 2	2	1	5	58	35	53±1.0	0.239±0.031
ME 3	2	1	5	48	45	58±0.7	0.432±0.019
ME 4	2	1	10	68	20	28±0.3	0.121±0.011
ME 5	2	1	10	58	30	43±0.2	0.126±0.021
ME 6	2	1	10	48	40	55±0.3	0.142±0.016
ME 7	2	2	5	68	25	67±0.4	0.132±0.027
ME 8	2	2	5	58	35	75±0.1	0.122±0.010
ME 9	2	2	5	48	45	82±0.1	0.136±0.014
ME 10	2	2	10	68	20	62±0.7	0.127±0.012
ME 11	2	2	10	58	30	72±0.5	0.116±0.023
ME 12	2	2	10	48	40	75±0.3	0.129±0.038
ME 13	2	3	5	68	25	85±0.2	0.139±0.019
ME 14	2	3	5	58	35	93±0.4	0.147±0.027
ME 15	2	3	5	48	45	96±0.2	0.137±0.010
ME 16	2	3	10	68	20	74±0.8	0.196±0.053
ME 17	2	3	10	58	30	86±1.1	0.317±0.028
ME 18	2	3	10	48	40	96±0.7	0.523±0.056

[a] Surfactant (S)/cosurfactant (CoS) mixing ratio

The permeation rate of FLZ at steady-state (Jss, micrograms per centimeter per hour) through rat skin was calculated from the slope of linear portion of the cumulative amount permeated through the rat skins per unit area *versus* time plot (17).

In order to obtain the permeability coefficient Kp (centimeters per hour), the following equation was used:

$$Kp = Jss/C_{donor}$$

where Kp is the permeability coefficient, Jss is the flux calculated at steady-state, and C_{donor} represents the applied drug concentration in the donor compartment.

Statistical Analysis

All the skin permeation studies were repeated three times and data were expressed as the mean value±SD. Statistical data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations and $P<0.05$ was considered to be significant.

Physicochemical Characterization of Optimized Microemulsion

Particle Size Measurements

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

Refractive Index and Percent Transmittance

The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing one drop of solution on the slide. The percent transmittance of the system was measured using a colorimeter (Digital Colorimeter, D-801, Photocon) at 570–590 nm.

Polarizing Microscopy

In order to verify the isotropic nature of ME, 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.

pH

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

Viscosity

The viscosity of ME was measured using a Brookfield Viscometer (Brookfield Engineering LABS, Stoughton, MA) with spindle LV-III at 100 g using interval of 30 s. All aspects of testing were controlled using optional Rheocalc Software.

Conductivity

The electric conductivity of ME was measured with a conductivity meter (Equip-Tronics, EQ-664, Mumbai, India) equipped with in-built 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.

In Vitro Antifungal Activity

The antifungal activity of FLZ from the optimized formulation and reference standard (FLZ dissolved in PBS) was determined using *C. albicans* ATCC 10231 as representative fungi, adopting a cup plate method (18). One gram each of ME and FLZ reference solution containing 2% FLZ were placed in each well with a control (vehicle-free drug). The mean inhibition zone of FLZ released from five 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 0.05 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 temperatures.

Centrifugation

In order to estimate metastable systems, the selected ME vehicles were centrifuged (Remi Laboratories, Mumbai, India) at 5,000×*g* for 30 min at 30°C.

RESULTS AND DISCUSSION

Solubility of Fluconazole in Oils

To develop ME system for topical delivery of FLZ, suitable oil has to be chosen. So the solubility of FLZ was determined in various oils (Table II). There was no significant difference in the solubility of FLZ among the various oils tested except AO and OA which exhibited low solubility as compared to other oils. However, LA, slightly increases the solubility of FLZ (14.25±1.23 mg/ml) compared with other oils, although it was not statistically significant. But in respect of convenience of formation and use, the oil LA is better choice in comparison with other oils. From these solubility results, LA was chosen as oil, for the preparation of ME formulations of FLZ for further studies.

Table II. Solubility of Fluconazole in Various Oils (mean±SD, $n=3$)

Oils	Solubility (mg/ml)
Lauryl alcohol	14.25±1.23
Ethyl laurate	11.48±1.52
Wheat germ oil	10.84±1.30
Ethyl oleate	09.94±1.08
Arachis oil	03.43±0.06
Oleic acid	02.32±0.04

Phase Behavior

Pseudo-ternary phase diagrams were constructed to obtain appropriate components and their concentration ranges for the MEs (17). The pseudo-ternary phase diagrams with various weight ratios of Lab to EtOH are presented in Fig. 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 light microscopy. The area of ME isotropic region changed slightly in size with the increasing ratio of S/CoS.

In Vitro Skin Permeation Study

Table I shows the different ME formulations composed of LA, Lab, EtOH, and water at different concentrations. The effects of the content of oil and S/CoS mixture on the skin permeation of FLZ were evaluated. The contents of oil and water were varied from 5% to 10% and from 48% to 58% to 68%, respectively, while the content of S/CoS mixture was varied at 20%, 25%, 30%, 35%, 40%, and 45%. The Jss and Kp values are shown in Table III. Among the ME formulations tested, ME 4, which was composed of 2% FLZ, 10% LA, 20% Lab/EtOH (1:1, *w/w*) mixture and 68% water, showed the highest permeation profile. The Jss of FLZ from ME 4 was 47.15±1.12 μg cm^{-2} h^{-1} and Kp was 2.33±0.41 cm/h, 9.12 times higher than those of the FLZ saturated aqueous solution in PBS, which were 5.17±0.12 g cm^{-2} h^{-1} and 0.26±0.04 cm/h, respectively. The permeation rate of FLZ was almost linearly improved as a function of loading dose and the permeation of MEs accorded with Fick's first diffusion law (Fig. 2). Also statistical comparison of the flux throughout 8 h showed that ME 4 provided flux ($P<0.05$) higher than that of saturated aqueous solution of FLZ.

The content of S/CoS mixture in ME affected the skin permeation rate of FLZ significantly. As the content of S/CoS mixture was decreased from 45% to 20% at S/CoS=1, the skin permeation rate of FLZ increased by threefold. This may be due to an increased thermodynamic activity of the drug in the ME at the lower content of S/CoS mixture as FLZ is poorly water soluble and yet solubilized in the S/CoS mixture. On the other hand, as the content of water in MEs was decreased from 68% to 48%, the skin permeation of the drug was decreased for the same reason. It was also found that skin permeation of the drug in ME was significantly influenced by the content of EtOH (CoS) in S/CoS mixture. When the S/CoS was decreased from 3 to 1, the skin permeation rate of FLZ was increased by 6.7-fold. The highest percutaneous absorption of FLZ was obtained for MEs with Km (S/CoS ratio)=1. It has been reported that, with increasing the content of EtOH, the size of the internal phase was decreased making the surface area of the droplet increased significantly. The influence of EtOH in aqueous solutions upon the transport behavior of several permeants across the skin has been evaluated (19,20). It has been also reported that EtOH may alter or form additional polar pathways in the SC as a result of a combination of changes in protein conformation, reorganization within the lipid polar head region or lipid

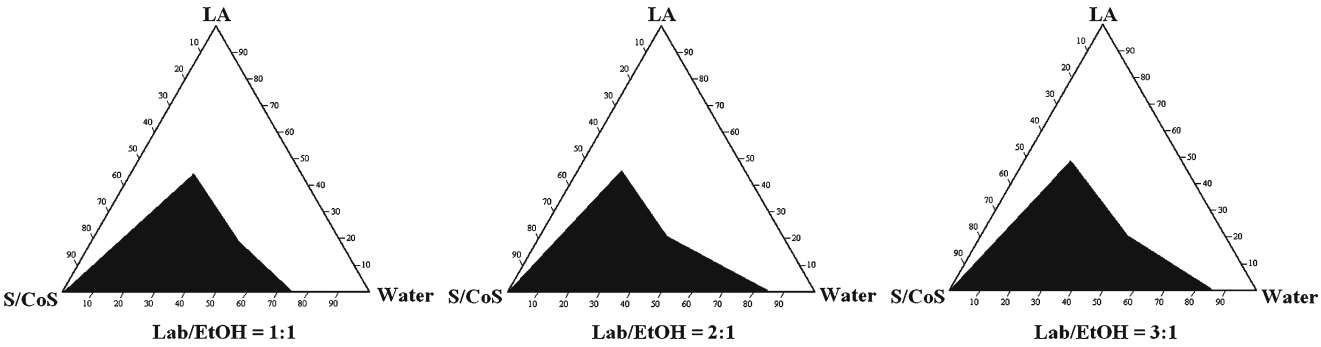

Fig. 1. The pseudo-ternary phase diagrams of the oil-surfactant/cosurfactant mixture–water system at the 1:1, 2:1, and 3:1 weight ratio of labrasol to ethanol at ambient temperature, *dark area* represent microemulsions region

extraction. The content of oil also showed similar effects on the skin permeation of FLZ but its mechanism is different from that of the S/CoS. As the content of oil was increased, the number of the internal phase was increased, which increased the skin permeation rate of the drug (21).

The topical formulations for the treatment of skin infections must provide proper concentrations of the drug at 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 (18). Interestingly, the percutaneous absorption studies were shown to be superior from the ME formulations. Maximum drug permeation and 9.12 times higher drug release were achieved in comparison to saturated aqueous solution. These results clearly indicate that FLZ, when used in ME, was more efficiently penetrated compared with saturated aqueous solution.

The higher permeability rate of FLZ from ME formulations is most probably due to the S/CoS and the oily phase, which act as penetration enhancers. The enhancer can increase the transport through skin by modifying the diffusion or partitioning coefficient of drug (22). 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.

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. 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 pathways. 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 FLZ. On the other hand, the hydrophilic

Table III. The Permeation Parameters of the Fluconazole Loaded Microemulsions and Saturated Aqueous Solution (mean±SD, n=3)

Formulations	Jss (μg/cm^2/h)	K_p (cm/h)
ME 1	31.43±0.75	1.55±0.27
ME 2	18.86±0.45	0.93±0.27
ME 3	15.72±0.37	0.78±0.14
ME 4	47.15±1.12	2.33±0.41
ME 5	20.96±0.50	1.04±0.18
ME 6	17.15±0.41	0.85±0.15
ME 7	9.82±0.23	0.49±0.09
ME 8	5.89±0.14	0.29±0.05
ME 9	4.91±0.12	0.24±0.04
ME 10	14.73±0.35	0.73±0.13
ME 11	6.55±0.16	0.32±0.06
ME 12	5.36±0.13	0.26±0.05
ME 13	4.69±0.11	0.23±0.04
ME 14	2.81±0.07	0.14±0.02
ME 15	2.35±0.06	0.12±0.02
ME 16	7.04±0.17	0.35±0.06
ME 17	3.13±0.07	0.15±0.03
ME 18	2.56±0.06	0.13±0.02
Sat. solution	5.17±0.12	0.26±0.04

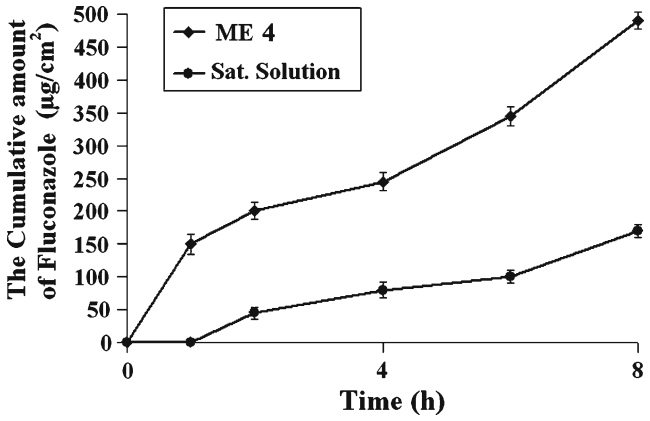

Fig. 2. Permeation profile of fluconazole through rat skin from optimized microemulsion formulation and reference (mean±SD, n=3)

Table IV. Antimicrobial Activity of Optimized Microemulsion in Comparison to Reference Standard Using *Candida albicans*

	Zone of inhibition (mm)					
Formulation	1	2	3	4	5	Mean±SD
Microemulsion	20	22	22	20	26	22.0±2.49
Reference	11	12	15	13	10	12.2±1.92

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 FLZ can then permeate more easily through the lipid pathway of the SC (23).

Moreover, the particle size of the ME may also affect its efficiency, where its small particle size makes it an excellent carrier for promoting FLZ percutaneous uptake as the number of vesicles that can interact on a fixed area of SC will increase when the particle size decreases. This may be the reason why MEs of other formula whose particle sizes were larger than that of ME 4 showed relatively lower FLZ permeation.

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 (18).

Finally, the optimized composition of ME containing 2% FLZ was confirmed as 10% IPM, 20% Lab/EtOH (1:1, *w*/*w*) mixture and 68% water (ME 4) which showed highest permeation profile, high FLZ solubilizing capacity (37.54± 1.1 mg/ml) and appropriate physicochemical characters.

Physicochemical Characterization of Optimized Microemulsion

The ME formulations had the average particle size in the range of 28 to 96 nm. Particle size of plain ME and drug loaded ME were determined and there was no significant difference observed in average particle size after loading the drug. The ME 4 formulation had the lowest average particle size 28±0.3 nm with polydispersity index (PI) of 0.121±0.011 (Table I). 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 ME formulation had narrow size distribution. The refractive index of the developed ME was found to 1.333 and percent transmittance >99% which proved the transparency of the system. The samples were examined by ocular inspection in a cross polarizer for sample homogeneity and birefringence. The ME appeared completely dark when observed under cross polarizer which indicated that it was optically isotropic. The ME formulation had appropriate observed pH value (6.2±0.3) for topical application. Incorporation of FLZ did not significantly affect the observed pH value of the ME formulations. The developed system had the low viscosity (27.54±0.19 mPas) and high conductivity (145.5±1.90 μS/cm). There was no significant difference found between the viscosities of plain and drug loaded MEs. The investigated ME formulation containing non-ionic surfactant mixture, oil and water showed electroconductive behavior in spite of its non-ionic nature. From the viscosity and electroconductive study it could be concluded the ME 4 formulation was of o/w type.

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 IV). 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 FLZ through fungal cell walls to inhibit ergosterol synthesis (18).

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 months 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 5,000×*g* for 30 min. Average particle size and conductivity of prepared microemulsion batches were measured at different time intervals and no significant difference was observed in these parameters up to 3 months.

CONCLUSION

The effect of formulation components on the *in vitro* skin permeation of ME drug delivery system containing FLZ and the application of ME systems for topical delivery of FLZ was investigated. It was concluded that the efficiency of ME formulation in the topical delivery of FLZ was dependent upon the contents of water and LA as well as Lab/EtOH mixing ratio. The percutaneous absorption of FLZ from ME was enhanced with increasing the LA and water contents, and with decreasing the Lab/EtOH ratio in the formulation. Developed FLZ ME formulations shows better efficacy than

saturated solution of the drug for the treatment of candidiasis. Nevertheless, significant work still needs to be carried out to elucidate the mechanisms of drug delivery into the skin, which are dictated, by internal structure and the special characteristics of the ME.

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