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The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole

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ABSTRACT

The influence of the vehicle on the release and permeation of fluconazole, a topical antifungal drug dissolved in Jojoba oil was evaluated. Series of Cutina lipogels (Cutina CPA [cetyl palmitate], CBS [mixture of glyceryl stearate, cetearyl alcohol, cetyl palmitate, and cocoglycerides], MD [glyceryl stearate], and GMS [glyceryl monostearate]) in different concentrations as well as gel microemulsion were prepared. In-vitro drug release in Sorensen's citrate buffer (pH 5.5) and permeation through the excised skin of hairless mice, using a modified Franz diffusion cell, were performed. The rheological behavior and the apparent viscosity values for different gel bases were measured before and after storage under freezing conditions at -4 °C and were taken as measures for stability of network structure. *Candida albicans* was used as a model fungus to evaluate the antifungal activity of the best formula achieved. The results of in vitro drug release and its percutaneous absorption showed that the highest values from gel microemulsion were assured. The rheological behavior of the prepared systems showed pseudoplastic (shear-thinning) flow indicating structural breakdown of the existing intermolecular interactions between polymeric chains. Moreover, the stability study revealed no significant difference between viscosity before and after storage for different formulae except for CPA Cutina lipogel (using analysis of variance [ANOVA] test at level of significance .05). The antifungal activity of fluconazole showed the widest zone of inhibition with gel microemulsion. The gel microemulsion is an excellent vehicle for fluconazole topical drug delivery.

KEYWORDS: microemulsion, lipogel, percutaneous absorption.

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INTRODUCTION

For skin care and the topical treatment of dermatological disease, a wide choice of vehicles ranging from solids to semisolids and liquid preparations is available to clinicians and patients. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceuticals.[1,2] The effect of formulation additives on drug permeation through skin has been investigated,[3,4] in which the penetration rate of a topical agent may be influenced by drug—vehicle, drug—skin, and vehicle–skin interactions . In the clinical assessment of a topical agent, the vehicle may significantly affect drug release and skin penetration, thereby altering biological activity.[5-8] To study the effect of vehicles on the barrier function of the stratum corneum, permeation experiments using skin as a membrane are more meaningful.[9] The use of in vitro diffusion models to screen topical vehicles to optimize drug penetration is convenient. Generally, the absorption rate of a drug increases as its thermodynamic activity in the vehicle increases.[10,11] Thermodynamic activity can be expressed approximately in terms of relative solubility (the ratio of the current concentration in vehicle to the concentration of saturated vehicle).[6] Hence, absorption rate should be very high from supersaturated vehicles. However, because of the physical instability of supersaturated systems, there are no commercial dosage forms available. This problem might be overcome by application of systems that become supersaturated in situ. Such systems are represented by microemulsion systems.

Microemulsions are clear, isotropic, thermodynamically stable dispersions of 2 immiscible liquids created by the presence of a suitable surfactant, usually in conjunction with a cosurfactant. Along with the usual advantages of improved drug stability and availability afforded by surfactant solubilization, the microemulsion system has a significant impact on transdermal delivery.

Recently, lipogels—semisolid ointment-like preparations—have been investigated as vehicles for topical drug delivery.[12-14] Lipogels are based on fatty components and are obtained by gelling an oleaginous phase with a lipophilic substance. The type and concentration of the gelling agent can affect the structure on which the

rheological characteristics of the preparation depend and consequently on the requirements of physical stability and consistency.

Jojoba oil, a liquid wax, was chosen as an oleaginous phase. Jojoba oil is a standard oil-phase base for the cosmetics industry because of its promising physical properties such as high viscosity index, high dielectric constant, and high stability toward rancidity due to the presence of the natural antioxidants alpha, gamma, and delta tocopherols. In fact, its monosaturated configuration makes it more stable than other oils and helps stabilize other unstable ingredients in cosmetic formulations. The oil is made up of straight chain esters of monounsaturated long-chain fatty acids connected directly to fatty alcohols with an average total carbon chain length of 42 carbons with no side branching. It is remarkable for its molecular uniformity (97% linear wax ester) and has an amazing internal homogeneity (>87% of the esters present are of 20 or 22 carbon atoms). This unique chemical configuration accords Jojoba special characteristics unparalleled in the plant kingdom. Moreover, Jojoba oil has an exceptional shelf life, good keeping qualities, and a pleasant feel on the skin. The skin absorbs the oil within a few minutes, and transdermal penetration is suspected.[15]

Fluconazole, a recent synthetic triazole antifungal drug for the treatment of superficial and systemic fungal infections, was chosen as the test drug. The drug has a slight solubility in water (8 mg/mL at 37°C) and a melting point of 138°C to 140°C.[16]

The present study aims at formulating fluconazole in different topical preparations; namely, a series of Cutina lipogels as well as a gel microemulsion using Jojoba oil as an oleaginous phase. An important objective of this study was to test the influence of both vehicles and their compositions as drug carriers on the rheological behavior, in vitro drug release, and percutaneous absorption using formatted skin of hairless mice, where both systems studied here contained similar basic components. The antifungal activity of fluconazole using *Candida albicans* as a model fungus has been also evaluated.

MATERIALS AND METHODS

Materials

Fluconazole (Pfizer Pharm Co, Cairo, Egypt), Jojoba oil (Agricultural Research Center, Sinai, Cairo, Egypt), Cutina CPA (cetyl palmitate, mp 43°C -47°C), MD (glyceryl stearate, mp 52°C -58°C), GMS (glyceryl monostearate, mp 58°C -60°C) and CBS (mixture of glyceryl stearate, cetearyl alcohol, cetyl palmitate, and cocoglycerides, mp 52°C -58°C) (Henkel, Düsseldorf, Germany), Brij 96 (ICI, Atlas, Essen, Germany), Capmul (Karlshamns Lipid Specialties, Columbus, Ohio), Mueller-Hinton agar (Merck Co, Darmstadt, Germany), Sabouraud dextrose agar and broth (Oxoid Co, Hampshire, England), *Candida albicans* ATCC10231 American Type Culture Collection (ATCC, Rockville, MD), all other chemicals used were of analytical grade.

Preparation of Cutina lipogels

The calculated amount of Cutina (10, 30% wt/wt) was heated to 70°C with Jojoba oil over a water bath and the appropriate amount of fluconazole (2% wt/wt) was dissolved into the melted mass. The mass was then gelled by cooling to a controlled temperature of 20 ±2°C under continuous stirring (60 rpm).

Preparation of gel microemulsion

The appropriate amount of Brij 96, Capmul, and Jojoba oil were weighed into screw-capped vials as surfactant, cosurfactant, and oil, respectively. Surfactant/cosurfactant ratio (km ratio) was kept constant at 4:1. Fluconazole was dissolved in a concentration of 2% wt/wt. The mixture was shaken by using a magnetic stirrer to ensure thorough mixing. Gel microemulsion was formed by the addition of 40% water, where mixing was enhanced by using a vortex for 2 minutes, and subsequent storage at 37 ±1°C for 24 hours for equilibration.

In vitro drug release

This study was carried out using the modified United States Pharmacopeia (USP) dissolution apparatus. A plastic dish containing 3 g of the drug-gel formula was tightly covered with a stainless steel wire screen (350-µm mesh size). The dish was then dipped in 500 mL Sorensen's citrate buffer pH 5.5, contained in a 900-mL vessel of the USP dissolution test apparatus. The release study was carried out at 32°C, and the stirring shaft was rotated at a speed of 25 rpm. Five-mL samples were withdrawn from the vessel at 0, 30, 60, 90, 120, 200, 240, and 360 minutes and filtered through a 0.45-µm millipore filter. The drug was assayed spectrophotometrically at 264 nm.

In vitro percutaneous absorption of fluconazole

The in vitro measurements of drug permeation were performed according to the general method described by Skelly et al in the Food and Drug Administration report.[17] Newly born mice, 2 to 3 weeks of age, were killed by drowning in water. Full thickness abdominal skin, free of bites and or scratches, was excised surgically. The dermal surface was carefully cleaned to remove subcutaneous tissues without damaging the epidermal surface. The skin was washed and soaked for 24 hours in 0.9% sodium chloride solution, then mounted in the Franz diffusion cell with the epidermal surface outward. The surface area available for diffusion was calculated and was found to be 3.14 cm^2. The drug formulation (3 g) was

placed in the donor compartment, while the receptor compartment contained 12 mL 0.9% sodium chloride of pH 7.4. Sodium lauryl sulphate (1%) was added to the receptor compartment to ensure sink condition. Aliquots of 0.5-mL samples were withdrawn at 0, 1, 3, 6, 12, and 24 hours from the receptor compartment, and fluconazole was assayed spectrophotometrically at 264 nm.

The quantity of fluconazole diffused per unit area of the membrane (Q/A) was calculated for each time interval, and the diffusion coefficient through the membrane (D) was determined according to the following equation:

$$D = \frac{B.h}{C_0} \quad (1)$$

where, B is the slope of the curve relating Q/A against time, h is the thickness of the membrane (95 μmm as measured by the dial micrometer), and C_o is the initial drug concentration (60 mg).

Rheological and stability studies

The different gel bases were tested for their rheological characteristics at 25°C using Brookfield viscometer (DV-III Programmable Rheometer, Brookfield Engineering LABS, Stoughton, MA). The measurement was made over the whole range of speed settings from 0.3 to 35 rpm with 30 seconds between each 2 successive speeds, and then in a descending order. The hysteresis loop between upward and downward curve was determined, and the flow behavior of the different gel bases was studied according to the following equation[18]:

$$\text{Log } S = N \text{ Log } D - \text{Log } \eta \quad (2)$$

where D is the shear rate in sec^{-1}; S is the shear stress in $dyne/cm^2$; η is the viscosity in cp; and N is the slope of log S against log D plot, which indicates the deviation from Newtonian flow. When N is less than 1, dilatant flow is indicated, and if greater than 1, pseudoplastic flow is assured. Stability testing was applied for different gel bases, where the course viscosity measurements were repeated for different formulae after storage under freezing conditions (-4°C) for 48 hours.

Infrared study

Studies of the infrared spectra of fluconazole, plain and medicated Cutina, and microemulsion gel were conducted with an infrared spectrophotometer (IR – 435, Shimadzu, Tokyo, Japan) by spreading the gel sample as a thin layer between 2 hexagonal plates using sodium chloride cell.

Antifungal activity

Fluconazole is fungistatic and inhibits the biosynthesis of ergosterol, the major sterol found in the fungal cell membrane.[19] Primary resistance in *Candida albicans* and *Cryptococcus neoformans* has not been reported, but treatment failures or relapses should indicate the need for sensitivity testing especially where the infection has been exposed to long-term treatment with a triazole.[20,21] The efficacy of fluconazole was shown in animals challenged with lethal inocula of *Candida*, *Cryptococcus*, *Aspergillus*, or *Coccidioides*. The antifungal activity of fluconazole from the optimum formula (gel microemulsion) as well as the reference standard (fluconazole dissolved in 0.9% NaCL) was determined using *Candida albicans* as a representative fungi, adopting the cup plate method. The mean inhibition zone was calculated for each plate, and this value was taken as an indicator for the antifungal activity.

Standard calibration curve of fluconazole using cup plate method

A single well-isolated colony of *Candida albicans* of at least 1 mm in diameter was picked from the culture plate (sabouraud dextrose agar) using a disposable plastic loop (10 μL) and suspended into a tube containing 10 mLof sabouraud dextrose broth. The resulting suspension was vortexed for 15 seconds, then incubated at 35°C for 24 hours. The resulting growth turbidity was adjusted at 0.5 Mcfarland standard. One mL of the inoculum was mixed with the melted Mueller-Hinton agar, then poured into a sterile petri dish (15 cm in diameter), and allowed to solidify. Wells were done by punching a stainless steel cylinder onto the plate and removing the agar by a Pasteur pipette to form a well. Six concentrations of fluconazole were made by dissolving the desired amount of fluconazole in a sterile solution of 0.9% sodium chloride. Each concentration was placed in each well, and the plates were incubated aerobically at 37°C for 24 hours. After incubation, the inhibition zone diameter around each well was measured using a ruler, and a graph of inhibition zone versus drug concentration was plotted. The curve was found to be linear from 800 to 2000 μg /mL of fluconazole with a correlation coefficient of 0.998.

Microbiological assay of fluconazole

The microbiological assay of fluconazole from gel microemulsion and the reference standard (fluconazole dissolved in 0.9% NaCL) was done as previously mentioned in the calibration curve construction. One gram each of gel microemulsion and fluconazole reference solution containing 2% fluconazole were placed in each well with a control (vehicle-free drug). Mean inhibition zone of fluconazole released from 5 plates for each formula was calculated. 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.

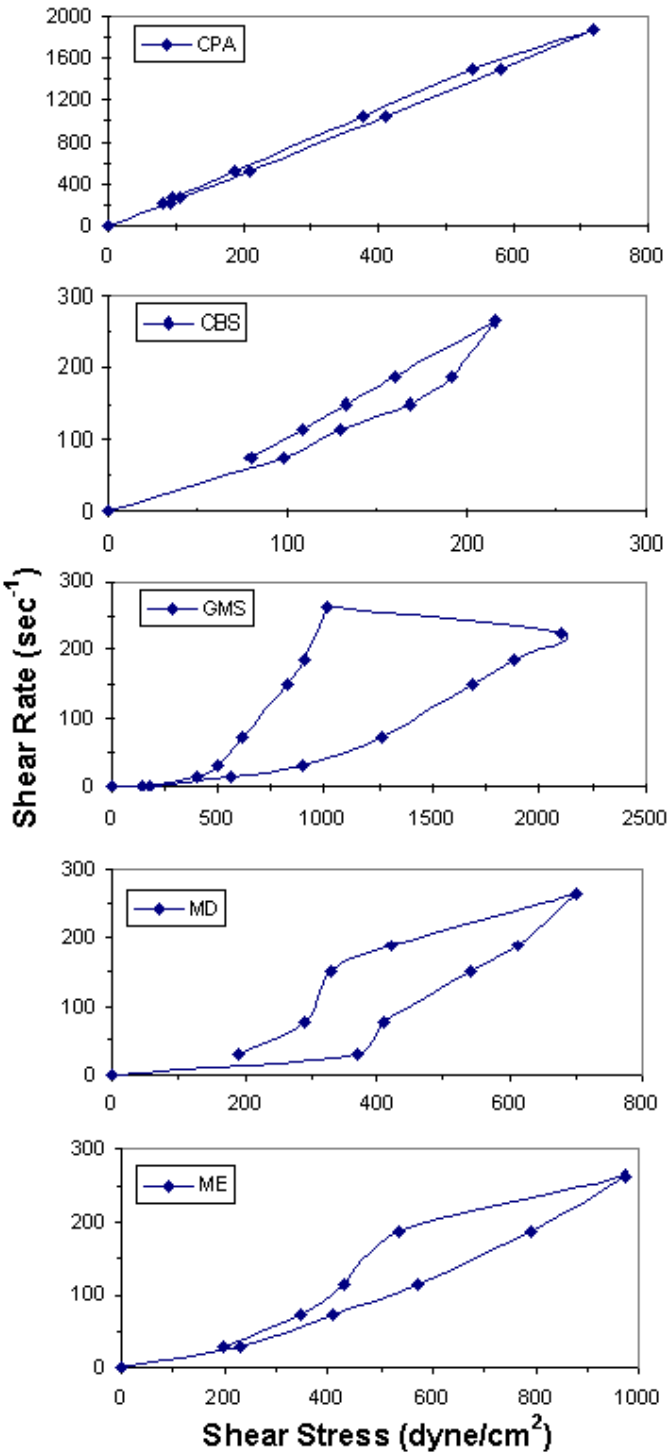

Figure 1. Rheograms of Cutina CPA, Cutina CBS, Cutina GMS, Cutina MD, and Microemulsion gel bases.

RESULTS AND DISCUSSION

Rheological and stability studies

In gel systems, consistency depends on the ratio of solid fraction, which produces the structure, to liquid fraction. Differences in concentration and kind of the gelling agents result in changes in the occurring structure consistency.[22]

The rheological behavior of thixotropic pseudoplastic systems, generally typical of the lipogels and gel microemulsion studied here, showed marked changes as a consequence of the kind of gelling agent. Needless to say, thixotropy is a desirable characteristic in pharmaceutical gels. In this flow, the molecules at rest entangled together with the association of immobilized solvent. Under the influence of shear, the molecules tend to become disentangled and align themselves in the direction of flow. The molecules thus offer less resistance to flow and this together with the release of some of the entrapped water accounts for the lower viscosity.[23]

The flow behavior of different gel bases is described in **Figure 1** and **Table 1**. The apparent viscosity values and the extent of the hysteresis loop area were used as measures of lipogel consistency. Although solid contents were equal, these values appeared markedly different revealing variability in network structure. Cutina CPA showed lower viscosity values, indicating weak structure as proved by the fast drop in shear stress with increasing shear rate. The shape of the rheograms indicates that CPA lipogel is easier to apply than other gels. The strain produced on the gelled masses caused extensive breakdown in the structure and consequent fall in viscosity. In addition, Farrow's constant, which is a measure of the degree of pseudoplasticity, was shown to be lowest in the case of CPA lipogel (N = 1.03, which is nearly Newtonian) and higher in the case of Cutina MD (N = 4.4). In contrast to Cutina CPA, Cutina GMS type produced lipogel with remarkable viscosity at minimum rate of shear, which is characterized by a well-ordered and close structure. The shape of the rheogram indicates difficult spreading with a requirement of high shear stress. Using the same chemical kind of gelling agent but changing the ratio of mono- to diglycerides (Cutina MD & CBS), masses were obtained showing similar rheological behavior but with lower consistency and compactness than Cutina GMS as deduced from the viscosity values.

Moreover, the effect of subjecting different gel formulations to freezing conditions on the rheological behavior was studied. The statistical analysis of the viscosity data before and after storage under freezing conditions using ANOVA test at level of significance of .05 revealed non-significant difference in viscosity measurements before and after storage for all gels except for Cutina CPA lipogel. This finding would confirm the stability of the gels under study and the structure breakdown of the existing

Table 1. Rheological Parameters of Different Pseudoplastic Gel Bases

Gel Base	Rheological Parameters			
	Min. vis. (cp)	Max. vis. (cp)	Farrow's N	Hysteresis Area
Microemulsion	798	370	1.99	31094
Cutina CPA	41	38.5	1.03	44597
Cutina CBS	141	81.5	1.64	4935
Cutina MD	450	158	4.4	36745
Cutina GMS	32962	247	2.99	182370

CPA indicates cetyl palmitate; CBS, misture of glyceryl stearate, cetearyl alcohol, cetyl palmitate, and cocoglycerides; MD, glyceryl stearate; GMS, glyceryl monostearate.

intermolecular interaction for Cutina CPA polymeric chains.

In vitro drug release and percutaneous absorption

Interestingly, the in vitro release data as well as the percutaneous absorption studies were shown to be superior from the microemulsion formula. Maximum drug permeation and 1.5-fold improvement in drug release were achieved in comparison to Cutina lipogels. These results clearly indicate that Jojoba oil, when used in microemulsion, was more efficiently penetrated compared with Cutina lipogel formulae. This is thought to be in concordance with Thacharodi et al[24] who found that oil/water microemulsion of lipophilic skin penetration enhancers were more efficient than their true solutions in the percutaneous absorption enhancement of nifedipine.

The release results of Cutina lipogel formulae were inversely related to gelling agent concentration and to the consequent viscosity of the gel base[25,26] as can be dictated from **Figures 2** and **3**, where a progressive decrease in release rate was observed with increasing Cutina components from 10% to 30%. Thus, gels having a compact and close structure may have a slower release rate than one of lower consistency. These differences may be attributed to the variation in shape and dimension of the crystallites of the solid fraction and their ordering in the 3-dimensional structure within the resulting network, where the liquid phase is held by adsorption, capillarity, and molecular interaction mechanisms.[27-29] Accordingly, the loose and less well-ordered CPA structure enables the solid particles to align in the flow direction produced by stirring, giving rise to a high release rate among all Cutina lipogels studied. It should be mentioned here that although Cutina GMS lipogel was characterized by a thick and more compact close structure, its meshes do allow quite free flow of the liquid phase. In any case, the extent of ordering in the solid components and the size of the network mesh may influence the release rate of a drug dissolved in the liquid fraction[30] by mechanically hindering the free diffusion of drug molecules.

The amount of fluconazole released from all gel formulations studied here showed a linear relationship with the square root of time (correlation coefficient = 0.999); therefore, the release rate of the test drug was expressed following the theoretical model by Higuchi.[31]

To explain a probable mechanism by which gel microemulsion enhances the release and percutaneous absorption of drugs efficiently, the histological and histochemical structure of the stratum corneum must be taken into consideration.

Drugs can permeate the stratum corneum through 2 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.

A dermally applied microemulsion is expected to penetrate the stratum corneum and to exist intact in the whole horny layer, altering both the lipid and the polar pathways.[24] The lipophilic domain of the microemulsion can interact with the stratum corneum in many ways. The drug dissolved in the lipid domain of the micromulsion can directly partition into the lipids of the stratum corneum, or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. In effect, these interactions will lead to increased permeability of the lipid pathway to the drugs.

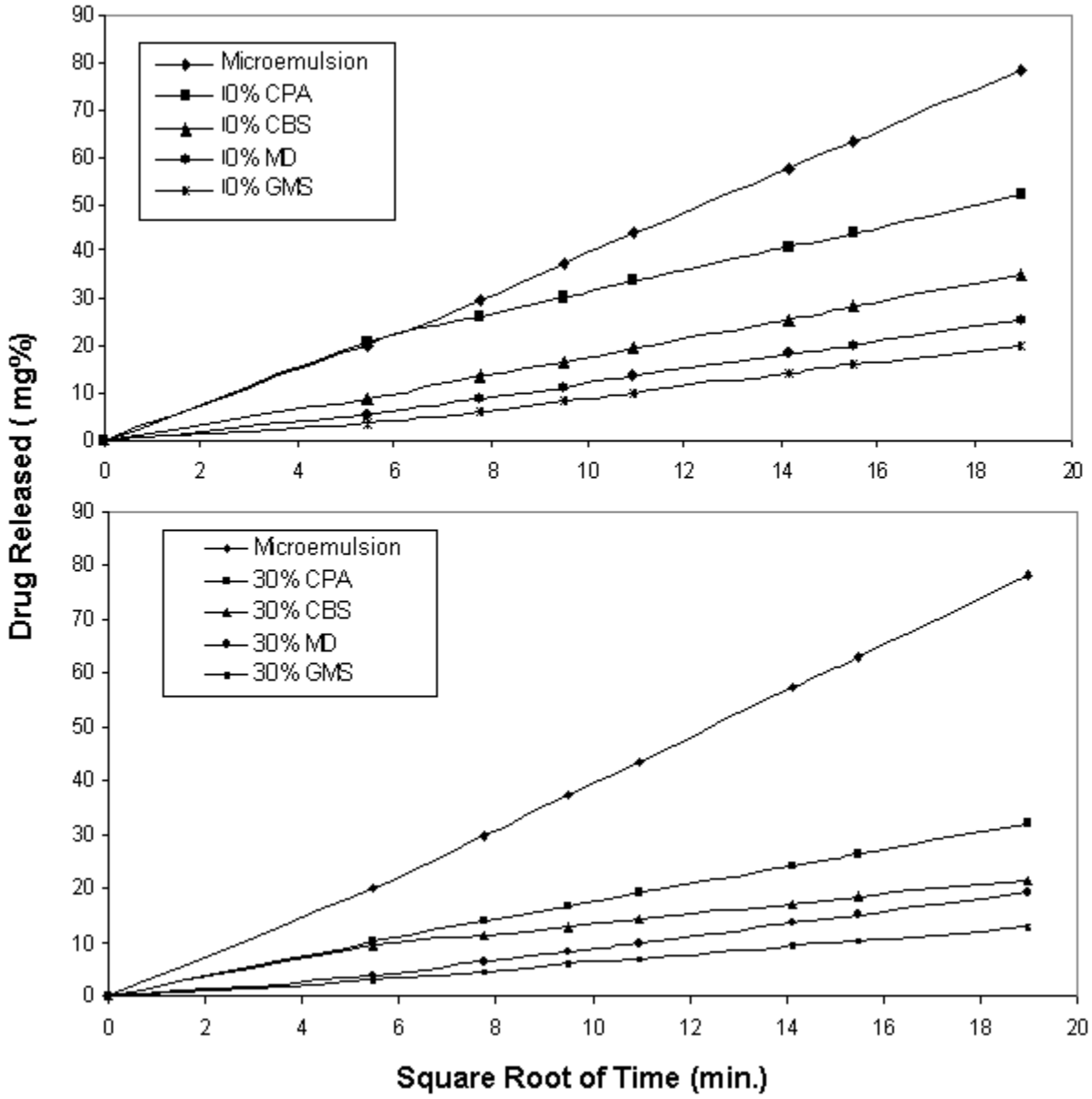

Figure 2. Release profile of fluconazole from 10% & 30% of different gel bases.

On the other hand, the hydrophilic domain of the microemulsion can hydrate the stratum corneum to a greater extent, and plays an important role in the percutaneous uptake of drugs. When the aqueous fluid of the microemulsion enters the polar pathway, it will increase the interlamellar volume of the stratum corneum 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 fluconazole can then permeate more easily through the lipid pathway of the stratum corneum.

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

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

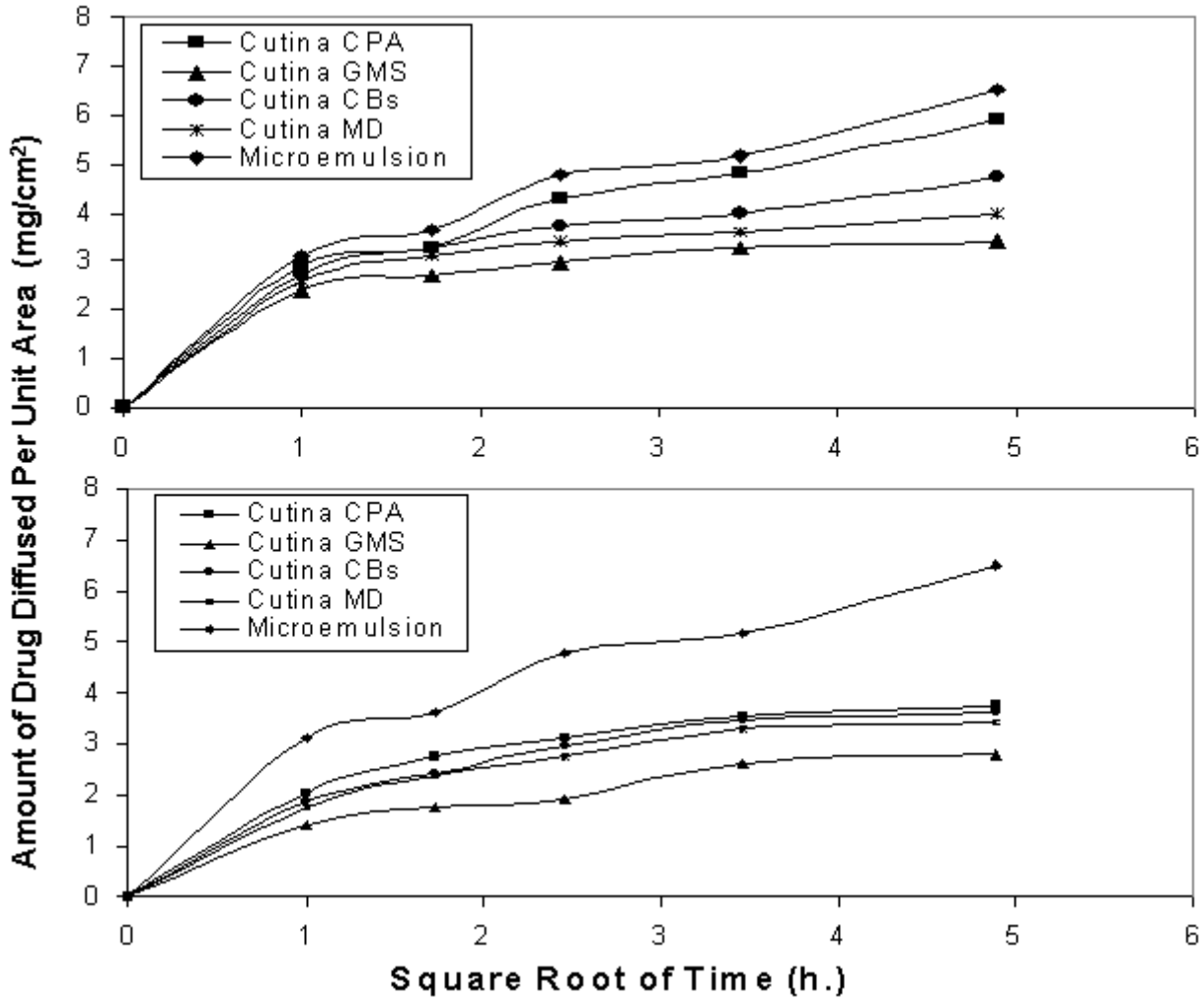

Figure 3. In vitro diffusion rate of fluconazole from 10% & 30% of different gel bases using mice skin.

Infrared study

Figure 4 shows the infrared spectra of fluconazole, plain and medicated Cutina, and microemulsion gel. The spectrum of fluconazole shows 1 absorption band at 3400 cm^{-1} due to hydroxyl stretching. This band was not affected by drug formulation in different Cutina and microemulsion gel bases, which emphasized the absence of any possible interaction between the drug and gel components.

Antifungal activity

Table 2 manifested that the mean zone of inhibition (the antifungal activity) of the tested microemulsion formula is larger than that of the reference standard. It is noted that the plain formula used in this study showed no antifungal activity. The ANOVA showed that there is a significant difference in the gel microemulsion zone of inhibition in comparison to the reference standard at $P < .05$, where the calculated F is larger than the tabulated F.

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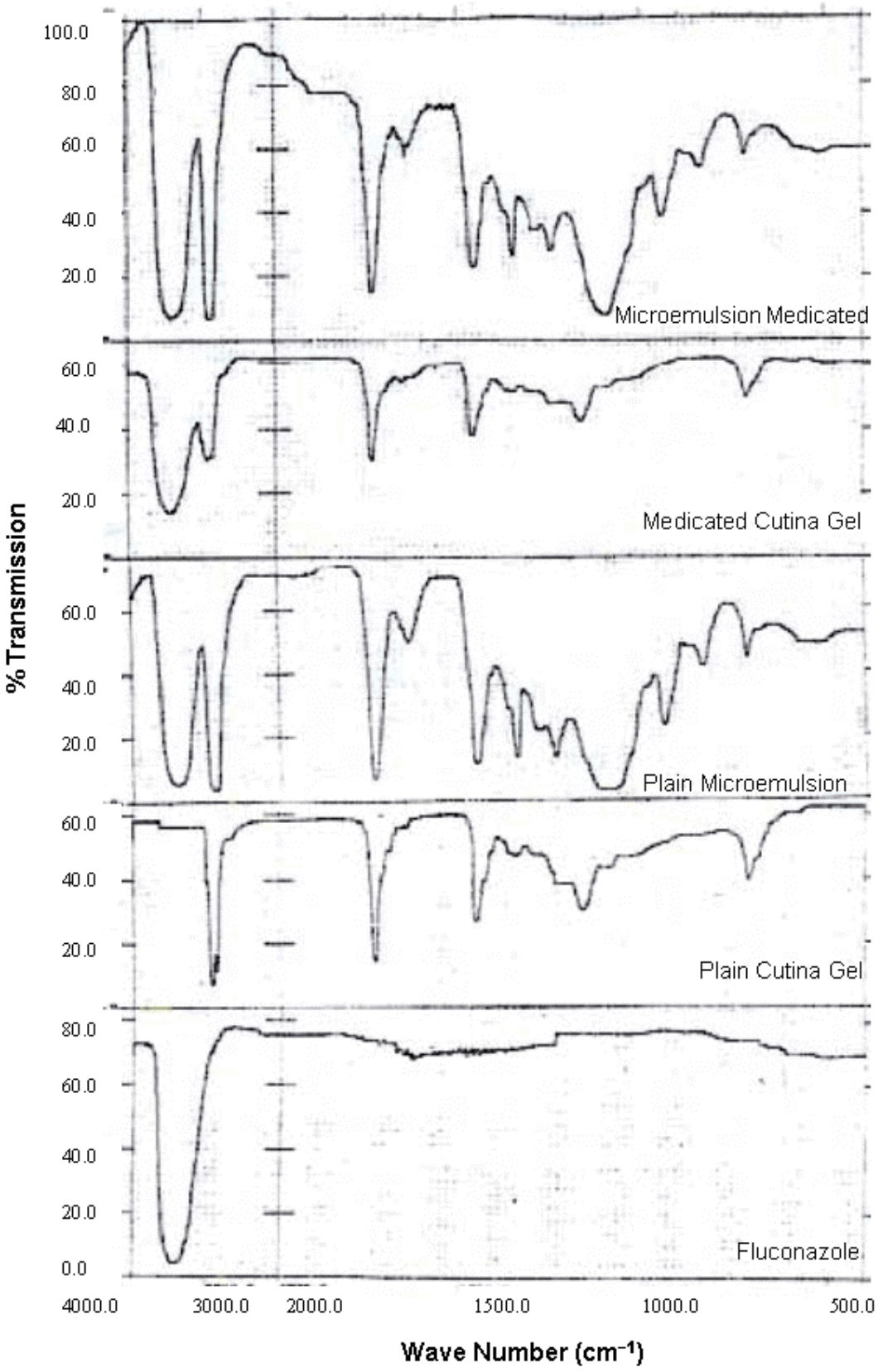

Figure 4. IR Spectra of different plain and medicated gel bases.

Table 2. Antimicrobial activity of gel microemulsion in comparison to reference standard using *Candida albicans*

Formula	Zone of Inhibition (mm)					
	1	2	3	4	5	Mean
Microemulsion	20	23	25	26	19	22.6
Reference	10	12	15	13	16	13.2

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