AAPS PharmSciTech, Vol. 16, No. 4, August 2015 (© 2015)
DOI: 10.1208/s12249-014-0275-8

Research Article

Development and Evaluation of New Microemulsion-Based Hydrogel Formulations for Topical Delivery of Fluconazole

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Received 25 July 2014; accepted 16 December 2014; published online 16 January 2015

Abstract. The aim of the present investigation was to develop and evaluate microemulsion-loaded hydrogels (MEHs) for the topical delivery of fluconazole (FZ). The solubility of FZ in oils, surfactants and cosurfactants was evaluated to identify the components of the microemulsion. The pseudo-ternary phase diagrams were constructed using the novel phase diagram by micro-plate dilution method. Carbopol EDT 2020 was used to convert FZ-loaded microemulsions into gel form without affecting their structure. The selected microemulsions were assessed for globule size, zeta potential and polidispersity index. Besides this, the microemulsion-loaded hydrogel (MEH) formulations were evaluated for drug content, pH, rheological properties and *in vitro* drug release through synthetic membrane and excised pig ear skin in comparison with a conventional hydrogel. The optimised MEH FZ formulations consisting of FZ 2%, Transcutol P 11.5% and 11%, respectively, as oil phase, Lansurf SML 20-propyleneglycol 52% and 50%, respectively, as surfactant–cosurfactant (2:1), Carbopol EDT 2020 1.5% as gelling agent and water 34.5% and 37%, respectively, showed highest flux values and high release rate values, and furthermore, they had low surfactant content. The *in vitro* FZ permeation through synthetic membrane and excised pig ear skin from the studied MEHs was best described by the zero-order and first-order models. Finally, the optimised MEH FZ formulations showed similar or slightly higher antifungal activity as compared to that of conventional hydrogel and Nizoral® cream, respectively. The results suggest the potential use of developed MEHs as vehicles for topical delivery of FZ, encouraging further *in vitro* and *in vivo* evaluation.

KEY WORDS: fluconazole; *in vitro* skin permeation; microemulsion; microemulsion-loaded hydrogel; topical.

INTRODUCTION

Microemulsions (MEs) are defined as thermodynamically stable, fluid, transparent (or translucent) homogenous dispersions, which have a quaternary composition including oil phase, aqueous phase, surfactant and cosurfactant at appropriate ratios, which constitute a single optically isotropic dispersion with a droplet diameter usually within the range of 10–100 nm (1–4). As new pharmaceutical dosage forms, microemulsions are useful for topical delivery of drugs due to their several advantages, such as high capacity to solubilise both hydrophilic and lipophilic compounds, excellent thermodynamic stability, facile and low cost preparation, optical clarity and increased penetration of drugs through the skin (4–8). Because of their high fluidity, microemulsions are difficult to apply to the skin, but this inconvenience can be overcome through the enhancement of their viscosity by adding gelling agents, which will not affect the diffusion of drug in the resulting microemulsion gel.

In the last decade, numerous studies have highlighted the pharmaceutical importance of microemulsions as vehicles for dermal and transdermal delivery of a wide variety of drugs (9–32). In order to explain the increase in drug penetration through the skin by microemulsions, several potential mechanisms have been proposed, including (i) increased thermodynamic activity towards the skin due to their high solubility potential, (ii) the ingredients of microemulsions can act as permeation enhancers by reducing the diffusional barrier of the stratum corneum and increasing the permeation of drugs through the skin and (iii) increasing the permeation rate of the drug from microemulsions, since the physico-chemical

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properties of the drug molecules such as hydro- and liposolubility can be modified to gain some liposolubility and hydrosolubility, respectively, favouring its partitioning into the external phase of the microemulsion.

Fluconazole (FZ), a synthetic fluorinated bis-triazole derivative, is one of the most valuable antimycotic agents with a broad spectrum and advantageous physical–chemical properties, which ensure a good bioavailability and allow both oral and parenteral (i.v.) administration. It is widely used not only in severe systemic mycoses of peritoneum, lungs, urinary tract and in cryptoccocal meningitis but also in superficial, cutaneous and mucous (buccal, oropharyngeal, esophageal and vaginal) candidiasis (33,34). At present, FZ is available commercially as capsules for oral administration and as solution for i.v. infusion. Compared with other azole derivatives (*e.g.* ketoconazole, itraconazole, miconazole), fluconazole is less lipophilic (log P=0.5) and has increased antifungal activity, aqueous solubility (8 mg/mL at 37°C) and higher bioavailability, due to the presence of a halogenated phenyl ring and two triazol rings (35). The FZ efficacy in the treatment of cutaneous mycosis by oral administration has been attributed to its rapid and extensive accumulation in the stratum corneum, thus the achieved concentration of FZ in the skin being higher than its concentration in the serum and also as the minimum inhibition concentration for most dermatophytes (36–38). However, it is well known that oral administration of fluconazole is often associated with adverse effects, especially gastric disorders including nausea, gastric irritation, vomiting and abdominal discomfort, which reduces the patient compliance with long-term therapy. In order to overcome this inconvenience, topical treatment of dermatomycosis using a drug delivery system, which localises the fluconazole at the level of the skin has been recommended. Over the last years, different dosage forms including lipogels, amphiphilogels, hydrogels, emulsions, microemulsions, emulgels, microemulsion gels and liposomal gels have been investigated as vehicles for topical delivery of fluconazole (39–47).

The aim of this study was to develop new microemulsion-loaded hydrogel (MEH) formulations to be used as vehicles for topical delivery of FZ and to evaluate their potential *vs.* a traditional topical dosage form such as hydrogel. Thus, several MEH formulations and a hydrogel containing 2% FZ were prepared using Carbopol EDT 2020 as gelling agent, and their quality control, regarding physicochemical properties and stability, was performed. Furthermore, the *in vitro* drug release and skin permeation through synthetic membrane and hairless pig ear skin were investigated in order to assess the formulations performance and consequently to identify the formulation with highest delivery capacity of fluconazole.

MATERIALS AND METHODS

Materials

Fluconazole was kindly donated by S.C. Vim Spectrum S.R.L (Romania). Solutol HS 15 (macrogol 15 hydroxystearate) (BASF Chem Trade GMBH, Germany), Monomuls 90-O 18 (glyceryl oleate) and Eumulgin B 1 PH (macrogol cetostearyl ether 12) (Cognis, Germany), Lauroglycol 90 (propyleneglycol monolaurate) and Transcutol P (diethyleneglycol monoethyl ether) (Gattefossé, France), Lansurf SML 20 (polyoxyethylene (20) sorbitan monolaurate), Lansurf SMO 81 [polyoxyethylene (5) sorbitan monooleate], Lansurf OA 10 (macrogol 400 monooleate), Lansurf OA 14 (macrogol 600 monooleate) and Lansurf CO 12 (castor oil 12 ethoxylate) (Lankem L.t.d., UK), Captex 355 (caprylic/capric triglyceride) and Captex 500 (triacetin) (Abitec Corporation, USA), hydroxypropylmethylcellulose (Methocel K4M, Colorcon Ltd, UK), Carbopol ETD 2020 (Lubrizol Advanced Materials, USA) and Gantrez AN 119 (ISP, Germany) were received as gift samples. Castor oil was supplied by S&D Chemicals (India), propyleneglycol (PG) was obtained from BASF Chem Trade GMBH (Germany), ethanol (96%) and isopropyl alcohol (IPA) were purchased from Chimopar S.A. (Romania), triethanolamine (TEA) was obtained from Fluka (Germany) and methyl- and propylparaben were purchased from Stera Chemicals (Romania). Tuffryn HT synthetic hydrophilic membranes of polysulfone (0.45 μm, 25 mm) were supplied by Pall Corporation (USA). Double-distilled water was used throughout the study. All chemicals and reagents were of pharmaceutical or analytical grade and were used without further purification.

Methods

Solubility Studies

The solubility of FZ in water, various oils (Lauroglycol 90, Transcutol P, castor oil, Captex 355 and Captex 500), surfactants (Solutol HS 15, Monomuls 90-O 18, Eumulgin B1PHA, Lansurf SML 20, Lansurf SMO 81, Lansurf OA 10, Lansurf OA 14 and Lansurf CO 12) and cosurfactants (ethanol, isopropyl alcohol and propyleneglycol) was determined using the shake flask method. Briefly, an excess amount of FZ was dispersed in 3 mL of each of the solvents in 10-mL-capacity stoppered vials separately and mixed for 10 min using a vortex mixer in order to facilitate proper mixing of FZ with the vehicles. The mixture vials were then kept and shaken at 37±1°C in an isothermal shaker bath (Memmert, Germany) for 98 h to get to equilibrium. The resulting mixtures were then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a membrane filter (0.45 μm, 25 mm, Teknokroma, Germany). The concentration of the FZ in the filtrate was determined by UV spectrophotometer (T70+, PG Instruments, UK) at the wavelength 253 nm. Each experiment was performed in triplicate.

Screening of Formulations Components

Screening of Oil. The oil phase for developing MEs of FZ was selected based on the maximum solubilising capacity for drug.

Screening and Selection of Surfactants. The surfactant for developing o/w MEs of FZ was selected based on its solubilisation capacity for FZ and Transcutol P. After performing the solubility studies, five different surfactants, including Lansurf SMO 81, Lansurf SMO 20, Lansurf OA 10, Lansurf OA 14 and Lansurf CO 12, were screened. The solubilisation capacity of surfactants for Transcutol P was determined using the technique described in some previous studies (25,48,49). Briefly, to 2.5 mL of 15% (*w*/*w*) aqueous solution of surfactant

aliquots of 5 μL of oil (Transcutol P) was added with vigorous vortexing; if a one-phase clear solution was obtained, an additional quantity of oil was added until/till the solution became cloudy. The total amount of oil added before the appearance of the turbidity of the solution was considered the solubility. The solubility was calculated using Eq. (1):

$$\text{solubility of oil}\left(\%\, w/w\right) = \frac{a \cdot 0.988}{0.375} \cdot 100 \quad (1)$$

where *a* represents the volume (mL) of Transcutol P added till the appearance of the turbidity, 0.988 is the density of Transcutol P (g/mL) and 0.375 is the quantity of surfactant contained in 2.5 mL of 15% (*w*/*w*) aqueous solution of surfactant.

Screening and Selection of Cosurfactants. The selection criterion of cosurfactant for developing o/w MEs was the area of ME region. Lansurf SMO 20 was mixed with three types of solubilisers selected as cosurfactants, namely ethanol, isopropyl alcohol and propyleneglycol. At a fixed ratio S_{mix} of 1:1 the pseudo-ternary phase diagrams were constructed. The oil and S_{mix} were used in nine different weight ratios (from 9:1 to 1:9) so that maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams.

Construction of Pseudo-Ternary Phase Diagram

The pseudo-ternary phase diagrams were also used to obtain the concentration range of the components for the existing region of microemulsions. Surfactant (Lansurf SMO 20) and cosurfactant (propyleneglycol) were blended in the weight ratios of 3:1, 2:1, 1:1 and 1:2. These S_{mix} ratios were chosen in decreasing concentration of surfactant with respect to cosurfactant and *vice versa* for detailed study of the phase diagrams. Different mixtures of oil and surfactant/cosurfactant mixtures were prepared at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The phase diagram by micro-plate dilution (PDMPD) method, a novel technique based on the water titration method, was used for the construction of the pseudo-ternary phase diagrams (50). In brief, the individual oil–emulsifier mixtures (oil, surfactant and cosurfactant) were gradually diluted with water in a microtitre plate (96 wells, 350 μL volumes each). The microtitre plates were filled by microsyringe according to the filling scheme: The oil–emulsifier phase was added starting at A1 with 200 μL up to D4 with 5 μL, decreasing 5 μL in each well, and the water phase was then added from A2 with 5 μL up to D5 with 200 μL, increasing 5 μL in each well. The wells E1 up to H5 were filled with the next batch using the same procedure. The plates filled in this way were then sealed with adhesive storage films and shaken on the temperature controlled thermomixer at 25°C in order to ensure adequate mixing and temperature adjustment of the system. Subsequently, each plate was evaluated visually regarding the isotropy and the boundary between the homogeneous or the heterogeneous system. The microemulsion phase was identified as the region in the phase diagram where clear, easily flowable and transparent formulations were obtained.

Preparation of Fluconazole Microemulsion Formulations

According to microemulsion regions in the phase diagrams, ten microemulsion formulations were selected at different component ratios. The composition of fluconazole-loaded microemulsion formulations is given in Table I. FZ was dissolved under stirring in mixture of Transcutol P, Lansurf SMO 20 and propyleneglycol. Then, the appropriate amount of water was added to the mixture drop by drop with continuous stirring. All microemulsions were stored at 25±2°C. The final concentration of FZ in microemulsion systems was 2% (*w*/*w*).

Preparation of Microemulsion-Loaded Hydrogel of Fluconazole

Carbopol EDT 2020 was selected as suitable gelling agent to prepare the microemulsion-loaded hydrogel formulations. Carbopol EDT 2020 was dispersed slowly in the microemulsion under stirring. The concentration of carbomer in microemulsion-loaded hydrogel was 1.5% (*w*/*w*).

Preparation of Fluconazole Hydrogel

FZ was dissolved under stirring in hot propyleneglycol. The preservative mixture of methyl- and propylparaben (3:1) was dissolved in hot water under stirring. The carbomer (Carbopol EDT 2020) was dispersed in warm aqueous solution of parabens with constant stirring using a laboratory stirrer (Eurostar Digital IKA Werke, Germany) at 2000 rpm. Then, the FZ solution was added. The pH of carbopol hydrogel was adjusted using TEA.

The composition of the final fluconazole hydrogel was 2% (*w*/*w*) FZ, 20% (*w*/*w*) propyleneglycol, 0.5% (*w*/*w*) Carbopol EDT 2020, 0.1% (*w*/*w*) mixture of methyl- and propylparaben (3:1), TEA and water.

Characterisation of Fluconazole Microemulsions

The obtained microemulsions were evaluated regarding various physicochemical characteristics.

The average droplet size, polydispersity index and zeta potential of the FZ microemulsions were measured in triplicate by photon correlation spectroscopy using a Zetasizer Nano-ZS (Malvern Instruments, UK) instrument. Measurements were carried out at a fixed angle of 173° at 25°C. Microemulsions were diluted in ratio of 1:3 with ultrapure water delivered by a Simplicity UV Water Purification System (Millipore SAS, France). The refractive indexes and the viscosities of formulations were determined at 25±2°C, using a refractometer (Digital ABBE Mark II-Reichert, Depew, USA) and a rotational viscosimeter equipped with a SC4-25 spindle (Brookfield DV-I+, UK), respectively. The pH of the microemulsions was measured at 25±2°C using a pH-meter (Sension™1, Hach Company, USA). Experiments were performed in triplicate for each sample.

Table I. Composition of Fluconazole-Loaded Microemulsions

Microemulsion components	Weight (%) and formulation codes									
	ME FZ 1	ME FZ 2	ME FZ 3	ME FZ 4	ME FZ 5	ME FZ 6	ME FZ 7	ME FZ 8	ME FZ 9	ME FZ 10
Fluconazole	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Transcutol P	16.0	15.0	14.0	13.0	12.5	12.0	11.5	11.0	10.5	10.0
Lansurf SML 20—propylene glycol (2:1)	72.0	68.0	64.0	58.0	56.0	54.0	52.0	50.0	48.0	46.0
Methylparaben	0.003	0.006	0.009	0.012	0.015	0.018	0.021	0.024	0.027	0.030
Propylparaben	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.100
Distilled water	9.996	14.492	19.988	26.984	29.48	31.976	34.472	36.968	39.464	41.87

Characterisation of Fluconazole Microemulsion-Loaded Hydrogels and Hydrogel

Determination of Drug Content and pH. To determine the drug content, about 0.4 g of formulation (MEH or hydrogel) was weighted in a 25-mL volumetric flask and dissolved in ethanol 96%; FZ content of filtered solution was analysed spectrophotometrically, at 253 nm. The pH values of aqueous solutions containing 5% (*w*/*w*) MEH FZ or H FZ were determined at 25°C using the Sension™1 digital pH-meter (Hach Company, USA). Each experiment was performed in triplicate.

Rheological Characterisation. In order to determine the viscosity and the consistency of samples, rheological studies were conducted. Viscosimetric measurements were performed using a stress-controlled rheometer (RheoStress 1, HAAKE, France) equipped with a cone-plate geometry (1/60), and data analysis was carried out by HAAKE RheoWin 3.1 software. Measurement of consistency was performed by penetrometry using a penetrometer (PNR 12, Petrolab, Germany) equipped with a micro-cone and suitable container, following the procedure described in the pharmacopoeias. In addition, the spreadability of the hydrogels was determined, as this characteristic is nearly related to consistency. The spreadability of the samples was carried out using the parallel-plate method. In brief, 1 g hydrogel was placed within a circle of 1 cm diameter premarked on the centre of a glass plate over which a second glass plate was placed and the diameter was measured after 1 min. Subsequently, every 1 min standardised weights (50, 100, 200, 250, 500 and 750 g) were placed on the upper glass plate, and the spread diameters were recorded each time. Then, the areas of respective circles were calculated and the obtained values, expressed as mean ± SD, were plotted *vs.* corresponding standardised weight. All rheological tests were performed in triplicate at 25°C.

In Vitro Drug Release Studies. The *in vitro* release of fluconazole from selected MEH formulations was determined to evaluate the effect of the formulation variables on preparations performance. The release experiments were performed on a system of six Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA) using synthetic hydrophilic membranes of polysulfone (HT Tuffryn membrane, Pall Corporation, USA). Franz diffusion cells presented an effective diffusional area of 1.767 cm^2 and 6.5 mL of receptor cell capacity. The receptor chambers were filled with freshly prepared phosphate-buffered saline solution at pH 7.4 containing 30% ethanol (*w*/*w*) to ensure sink conditions. The synthetic membranes were mounted between donor and receptor compartments of the Franz diffusion cells and were put in previous contact with phosphate-buffered saline solution at pH 7.4 containing 30% ethanol (*w*/*w*) 30 min prior placing the samples. The tested formulation (300 mg) was placed into each donor compartment. The receptor compartment was constantly stirred at 600 rpm, and the diffusion cells were maintained at 32±1°C throughout the experiment. A 0.5-mL sample of the receptor medium was withdrawn at predetermined times (1, 2, 3, 4, 5, 6, 7 and 8 h) and replaced with an equal volume of fresh receiver medium to maintain a constant volume. The collected samples were analysed for FZ content by UV spectrophotometric method, at 268 nm. The assay was linear in the FZ concentration range of 64.96–649.60 μg/mL ($y = 0.0053x + 0.0101$, $R^2 = 0.9989$). The determinations were conducted in triplicate.

In Vitro Skin Permeation Studies

Preparation of the skin. In vitro permeation studies were carried out using full thickness pig ear skin with a surface area of 1.767 cm^2. The skin was excised from 4-month-old, female or male domestic pig ears, obtained from a local slaughterhouse. The pig ears were cleaned up with tap water, immediately after excision. The outer region of the ears was clipped of bristles, and then, the skin was dermatomed to a thickness of around 500 μm. The dermatomed skin samples were immediately used for the permeation experiments or stored at −20°C for a maximum period of 2 months. Before use, the dermatomed pig skin was removed from the freezer and allowed to thaw at room temperature. The integrity of the skin was evaluated by visual examination for physical damage, excluding unsuitable samples. The thickness of each sheet was measured with a micrometre, and then, squares of skin 2–2.2 cm^2 were cut from the sheet.

In vitro permeation study. The evaluation of *in vitro* fluconazole permeation was performed on the same Franz diffusion cells system (Microette-Hanson system, 57-6AS9 model, Hanson, USA) described above. Sink conditions were

achieved in the receiver compartment with 6.5 mL freshly prepared phosphate buffered saline solution at pH 7.4 as receptor fluid. The skin pieces were mounted carefully on the Franz diffusion cells, between the donor and receptor compartments, with *stratum corneum* facing donor chamber. After that, the skin pieces mounted in the cells were allowed to rest in contact with phosphate-buffered saline solution at pH 7.4 1 h prior the application of the formulations. Further, the study was performed under the same experimental conditions as described in the above-mentioned paragraph that refers to the *in vitro* drug release studies. The sampling was performed at the following time intervals: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22 and 24 h. The collected samples were analysed for FZ content by UV spectrophotometric method, at 260 nm. The assay was linear in the FZ concentration range of 64.96–649.60 μg/mL ($y=0.0053x+0.0101$, $R^2=0.9989$). The determinations were conducted in triplicate.

Data Analysis of In Vitro Drug Release Studies. Cumulative amount of fluconazole permeated through the membrane (μg/cm^2) was plotted as a function of time (t, h). The permeation rate of drug at steady-state (flux, J_s, μg cm^{-2} h^{-1}) and the lag time (t_L, h) were calculated from the slope and the x intercept of the linear portion of the plots of cumulative amount of drug permeated *vs.* time in steady state conditions, respectively. Permeability coefficient (K_p, cm/h) was calculated by dividing the flux with initial concentration of drug in the donor compartment. The release rate (k) values were calculated using the pseudo-steady-state slopes from plots of cumulative amount of FZ permeated through membrane (μg/cm^2) *vs.* square root of time (51). Diffusion coefficient (D) values were calculated from the release rate values.

In order to investigate the release kinetics of the FZ from MEH formulations and hydrogel, the data obtained from *in vitro* drug release studies were fitted into various mathematical models, as follows:

– Zero order model:

$$M_t = M_0 + K_0 t \tag{2}$$

where M_t is the amount of drug delivered in time t, M_0 is the initial amount of drug in the solution (it is usually zero), K_0 is the zero order release constant expressed in units of concentration/time and t is the time.

– First order model:

$$\log C = \log C_0 - K_1 t/2.303 \tag{3}$$

where C_0 is the initial concentration of drug, K_1 is the first order rate constant and t is the time.

– Higuchi model:

$$M = K_H t^{1/2} \tag{4}$$

where M is the amount of drug released in time t and K_H is the Higuchi release constant.

– Korsmeyer–Peppas model:

$$M_t/M_\infty = K_P t^n \tag{5}$$

where M_t/M_∞ represents the fraction of drug released at time t (M_∞ being the equilibrium concentration of drug in the release solution), K_P is the Korsmeyer–Peppas release rate constant, and n is the diffusion coefficient. In this case, the first 60% drug release data were incorporated.

The following plots were made: cumulative percentage drug released *vs.* time (zero-order kinetics), log cumulative percentage of drug remaining *vs.* time (first-order kinetics), cumulative percentage drug released *vs.* square root of time (Higuchi model) and log cumulative percentage drug release *vs.* log time (Korsmeyer–Peppas model).

In Vitro Antifungal Activity. The antifungal activity of selected MEH formulations, conventional hydrogel and Nizoral® cream was evaluated against *Candida albicans* strains isolated from selected patients with morphologically identified candidiasis. The agar plate diffusion method was used to investigate the efficacy of selected hydrogels and commercial cream against the above-mentioned strains. Bacterial colonies from blood agar plates were used for preparation of the inoculums. *C. albicans* was grown on Sabauroud's agar medium. Colonies were diluted in sterile 0.85% NaCl solution, and suspensions were adjusted to approximately 3×10^8 colony forming units (CFU)/mL using a turbidimeter (DEN-1 McFarland Densitometer, Biosan). The Petri dishes containing Sabouraud's dextrose agar were inoculated with tested fungal suspension strain by spreading it on the agar surface. The plates were dried at room temperature for 10 min and then 100 mg of tested preparations (MEH formulations, conventional hydrogel and Nizoral® cream) were placed on their surface. The plates were incubated at 37°C±0.1°C for 48 h. Antifungal activity was expressed as the mean zone of inhibition measured for all the samples after the incubation period. All determinations were made in triplicates for each fungal strain.

Statistical Data Analysis. Statistical analysis was performed using Statistica 7.0 software. Data were shown as mean±standard deviation (SD) and were considered statistically significant at $P<0.05$.

RESULTS

Screening of Formulations Ingredients

Screening of Oil and Water

The solubility of FZ in different oils as well as in distilled water is listed in Table II.

Screening of Surfactants

The results of the solubility study involving the surfactants and cosurfactants are also presented in Table II.

The solubility of Transcutol P in Lansurf SML 20 and Lansurf OA 14 was 823.33% and 658.67% (*w*/*w*) respectively of selected oil (Transcutol P). The solubilisation capacity of the other three surfactants (namely Lansurf SMO 81, Lansurf OA 10 and Lansurf CO 12) for Transcutol P could not be determined because their water solubility is lower than 15% (Lansurf SMO 81 and Lansurf OA 10 are dispersible in water and Lansurf CO 12 is partially soluble in water), and consequently, aqueous solutions could not be obtained.

Screening of Cosurfactants

Addition of cosurfactants provides further reduction in the interfacial tension and increases the fluidity of interfacial surfactant film, which can take up different curvatures, thus expanding the area of existence of microemulsion system (1,2). Consequently, ethanol, isopropyl alcohol and propylene glycol were selected as cosurfactants.

The microemulsion area in the pseudo-ternary phase diagrams was used to assess the emulsification potential of these cosurfactants. Figure 1 presents the pseudo-ternary phase diagrams constructed for Transcutol P (oil phase), water, Lansurf SML 20 and cosurfactant at a fixed ratio S_{mix} 1:1.

Construction of Pseudo-Ternary Phase Diagram

The construction of pseudo-ternary phase diagrams was used to determine the appropriate concentration ranges of components (aqueous phase, oil phase, surfactant and cosurfactant) in the regions of forming microemulsions. Figure 2 presents the pseudo-ternary phase diagrams of Transcutol P, Lansurf SML 20 and water systems in the presence of cosurfactant (propylene glycol) with various weight ratios of Lansurf SML 20/propylene glycol.

Formulation and Preparation of Fluconazole Microemulsions

From the microemulsion region of pseudo-ternary phase diagram constructed for the systems containingTranscutol P, Lansurf SML 20/propylene glycol in 2:1 weight ratio and water, ten mixtures (formulations) along the water dilution line of oil: S_{mix} mass ratio 2:8 have been selected (Fig. 2b). This selection will thus permit to study the effect of formulation components on the microemulsion characteristics. The composition of the studied formulations is shown in Table I.

Characterisation of Fluconazole Microemulsions

The measured physical characteristics of the developed FZ microemulsions are shown in Table III.

Characterisation of Fluconazole Hydrogel and Microemulsion-Loaded Hydrogels

The FZ content of studied formulations (microemulsion-loaded hydrogels and hydrogel) and their pH and viscosity values are indicated in Table IV. In addition, the results of penetration measurements are presented in Table IV.

The results of spreadability measurements are presented as extensiometric curves in Fig. 3.

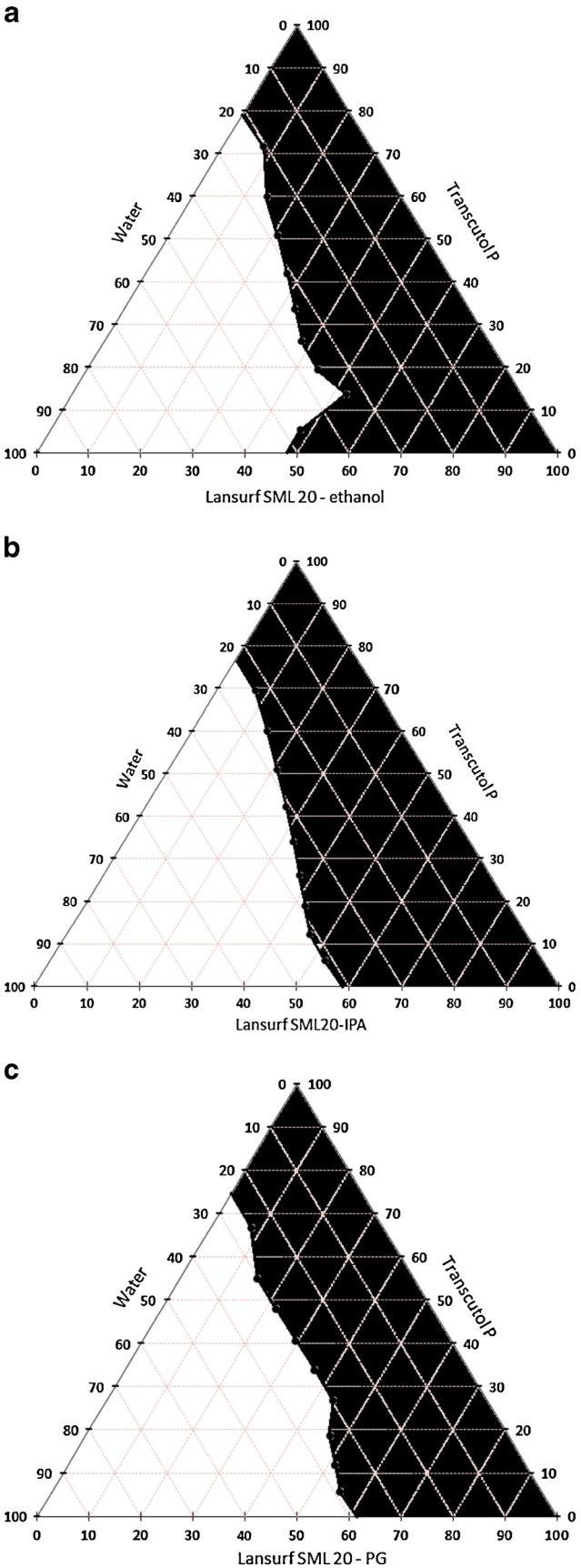

Fig. 1. Pseudo-ternary phase diagrams of systems composed of Transcutol P, Lansurf SML 20, water and different cosurfactants (**a** ethanol, **b** isopropyl alcohol and **c** propylene glycol) at S_{mix} 1:1

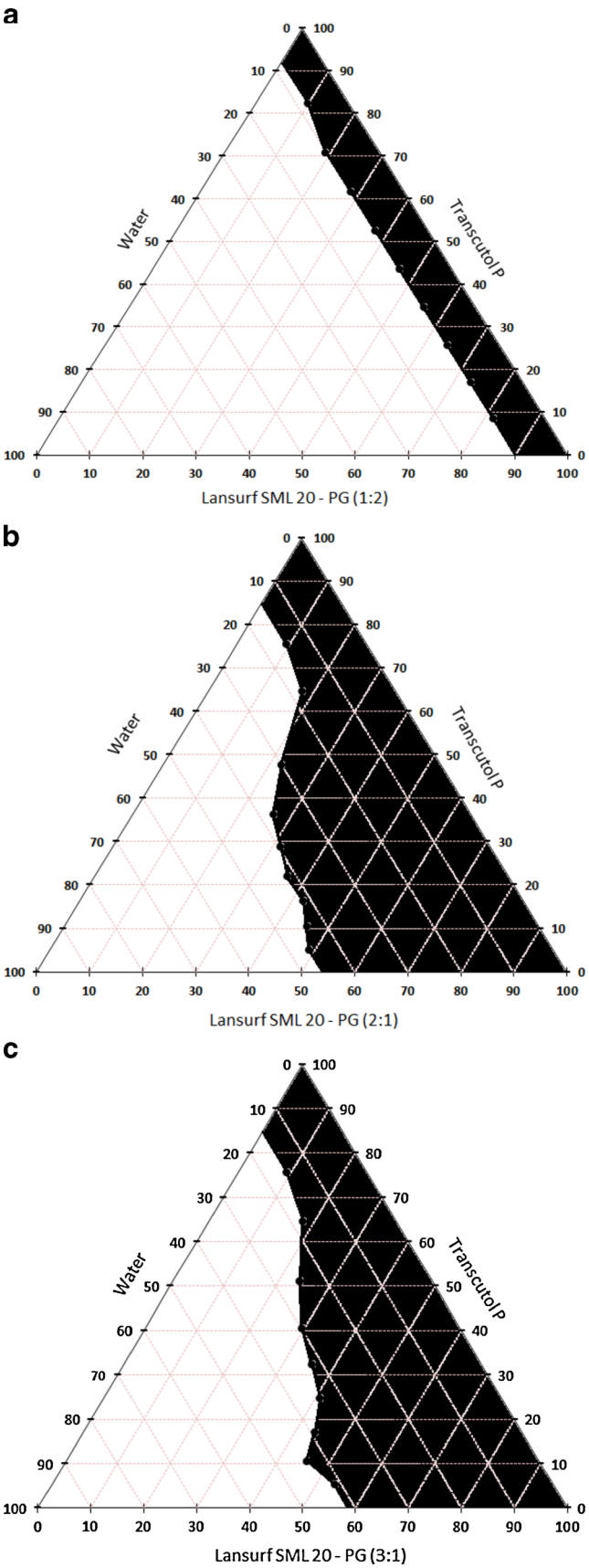

Fig. 2. Pseudo-ternary phase diagrams of systems composed of Transcutol P (oil phase), Lansurf SML 20 (surfactant), propylene glycol (cosurfactant) and water at different S_{mix} (**a** 1:2, **b** 2:1 and **c** 3:1)

In Vitro Drug Release Studies. In order to assess the formulations performance, the fluconazole loaded hydrogels were studied for *in vitro* drug permeation and release through synthetic membrane. The results are illustrated in Fig. 4 and listed in Table V.

In order to predict and evaluate the *in vitro* fluconazole permeation behaviour from the studied microemulsion-loaded hydrogels through synthetic hydrophilic membrane, fitting into a suitable mathematical model is required. Data obtained from the *in vitro* drug permeation of the MEH FZ formulations were fitted to various mathematical models like zero-order, first-order, Higuchi and Korsmeyer–Peppas model. The results of curve fitting into the above-mentioned mathematical models were evaluated by the value of the highest correlation coefficient and are presented in Table VI.

In Vitro Skin Permeation Studies. The results of the *in vitro* permeation and release of fluconazole through pig ear skin are shown in Fig. 5 and summarised in Table VII.

Similarly to *in vitro* drug release studies, the results obtained from the *in vitro* FZ permeation of all hydrogel formulations through pig ear skin were kinetically evaluated by the same mathematical models, namely zero-order, first-order, Higuchi and Korsmeyer–Peppas model. The results of curve fitting into above-mentioned mathematical models are presented in Table VIII and were evaluated by the value of the highest correlation coefficient.

In Vitro Antifungal Activity

The antifungal activity evaluation results of fluconazole from different hydrogel formulae (microemulsion-loaded hydrogels and conventional hydrogel) compared with Nizoral® cream are shown in Table IX.

Among the three microemusion-loaded hydrogels, MEH FZ 8 with the lowest propyleneglycol content resulted to be the most effective one, being as effective as the conventional hydrogel and more effective than the commercial cream. Formulation MEH FZ 7 showed similar antifungal activity with that of MEH FZ 8. In contrast, the MEH FZ 5 showed the lowest antifungal activity as compared to all other tested preparations.

DISCUSSIONS

Screening of Ingredients for Different Formulations

Screening of Oil and Water

The solubility of fluconazole was found to be highest in Transcutol P, followed by castor oil (2.18-fold lower), Captex 500 (3.65-fold lower) and Lauroglycol 90 (6.57-fold lower), and the lowest was in Captex 355 (Table II). This may be attributed to the optimal solubilising properties of Transcutol P, a high-purity solvent from glycol ethers category, having a great ability to dissolve large amounts of lipophilic and hydrophilic drugs. Further, formulation of microemulsion using oil with high drug solubility would require incorporation of less oil to incorporate the desired drug dose, which in turn would

Table II. The Solubility of Fluconazole in Water, Oils, Surfactants and Cosurfactants at 25±2°C

Component	Solubility (mg/mL)
Water	5.551±0.041
Lauroglycol 90	20.368±0.287
Transcutol P	133.742±0.230
Castor oil	61.472±1.117
Captex 355	2.969±0.069
Captex 500	36.687±0.052
Solutol HS 15	41.841±1.428
Monomuls 90-O-18	49.203±2.375
Eumulgin B 1 PH	42.454±2.093
Lansurf SML 20	59.877±0.081
Lansurf SMO 81	736.196±0.054
Lansurf OA 10	268.712±0.025
Lansurf OA 14	271.779±0.106
Lansurf CO 12	108.589±0.132
Ethanol	126.994±0.027
Isopropyl alcohol	60.614±0.048
Propyleneglycol	103.436±0.159

require lower surfactant concentration to achieve oil solubilisation, increasing the safety and tolerability of the system. Therefore, Transcutol P was selected as the oil phase for the development of microemulsions containing FZ.

Screening of Surfactants

The surfactant selection is critical for the development of MEs, as it considers the surfactant effectiveness and also their toxicity. The surfactant effectiveness is related to the proper HLB value, leading to the spontaneous formation of a stable ME formulation. The toxicity is important because the MEs formation usually requires large amounts of surfactants, which may cause skin irritation in the topical administration. Therefore, it is crucial to select the surfactant with a proper HLB value, but with a minimum necessary concentration in the formulation. Other important criteria for surfactant selection are the drug solubility and solubilisation capacity for oil, respectively. It is not necessarily true the surfactant that has good solubilising power for drugs would have equally good affinity for the oil phase (48).

In the present study, five nonionic surfactants, namely Lansurf SMO 81, Lansurf SML 20, Lansurf OA 10, Lansurf OA 14 and Lansurf CO 12, were chosen for screening. Nonionic surfactants were selected because of their low toxicity and irritation potential, stability and low sensitivity to pH changes or the presence of electrolytes or charged macromolecules. On the other hand, selection of surfactant was primarily governed by its solubilisation efficiency for the selected oil phase, and its solubility potential for FZ was considered as an additional advantage.

The results of the solubility study involving the surfactants (Table II) showed that Lansurf SMO 81 has the highest solubilising potential for FZ, followed by Lansurf OA 14, Lansurf OA 10, Lansurf CO 12 and Lansurf SML 20. However, after selection of Transcutol P as oil phase, the surfactant was chosen based on the highest solubilisation capacity for the oil phase (Transcutol P). Because the experimental method commonly used to determine the solubilisation potential of a surfactant for a certain oil could not be applied in the case of Lansurf SMO 81, Lansurf OA 10 and Lansurf CO 12 because of their low aqueous solubility, only Lansurf SML 20 and Lansurf OA 14 were tested. The amounts of Transcutol P solubilised by Lansurf SML 20 and Lansurf OA 14 were, respectively, 8.23- and 6.58-fold higher than their own weight. Both surfactants proved to be very good solubilisers for Transcutol P, which can be attributed to the similarity of their structures (polyethylene glycol structure in both surfactants and diethylene glycol structure of Transcutol P). The difference between the two surfactants in terms of ability to solubilise and emulsify Transcutol P can be explained by the values of the hydrophilic-lipophilic balance (HLB). Lansurf SML 20 having a higher HLB value (16.7) was more effective than Lansurf OA 14 with a lower HLB value (13.6). As Lansurf SML 20 solubilised the maximum amount of Transcutol P, it was selected as the surfactant for microemulsions development.

Screening of Cosurfactants

Comparing the size of the microemulsion region in the phase diagrams obtained at a fixed ratio S_{mix} (1:1), keeping the surfactant the same but replacing the cosurfactant, it was observed a very slight enhancement in the microemulsion area when the chain length was increased from ethanol (Fig. 1a) to isopropyl alcohol (Fig. 1b). However, increasing the number of hydroxyl groups from isopropyl alcohol to propylene glycol led to a slight decrease in the microemulsion region (Fig. 1c). The ME area obtained for the three tested cosurfactants showing no significant differences in size, additional selection criteria, namely solubilisation potential for FZ and the value of dermal toxicity, were taken into consideration. The solubility of fluconazole was higher in ethanol and propylene glycol (126.994±0.027 and 103.436±0.159 mg/mL, respectively) as

Table III. Mean Droplet Size, Polydispersity Index, Viscosity, Refractive Index, Zeta Potential and pH of the Fluconazole Microemulsion Formulations

Formulation code	Droplet size (nm)	Polydispersity index	Viscosity (mPa)	Refractive index	Zeta potential (mV)	pH
ME FZ 1	4.098±0.12	0.065	60.0±0.76	1.4409±0.02	−0.075±0.07	5.09±0.06
ME FZ 2	4.165±0.54	0.086	60.1±0.30	1.4365±0.01	−0.289±0.05	5.09±0.12
ME FZ 3	4.084±0.76	0.051	58.0±0.72	1.4319±0.05	−0.010±0.13	5.22±0.08
ME FZ 4	4.143±0.21	0.056	50.4±0.56	1.4241±0.07	−0.590±0.08	5.25±0.03
ME FZ 5	4.165±0.18	0.124	50.0±0.73	1.4218±0.05	−0.950±0.04	5.21±0.13
ME FZ 6	4.250±0.47	0.170	45.0±0.87	1.4188±0.03	−0.172±0.14	5.23±0.07
ME FZ 7	4.138±0.43	0.098	40.0±0.65	1.4150±0.01	−0.265±0.09	5.24±0.02
ME FZ 8	4.153±0.36	0.087	43.2±0.77	1.4125±0.02	−0.143±0.11	5.21±0.02
ME FZ 9	4.942±0.09	0.205	40.1±0.82	1.4092±0.01	−0.405±0.06	5.22±0.01
ME FZ 10	5.002±0.87	0.219	35.0±0.65	1.4069±0.05	−0.648±0.17	5.25±0.04

Table IV. Drug Content, pH, Viscosity and the Penetration Measurements of the Fluconazole Hydrogel Formulations

Formulation code	Drug content (%)	pH	Viscosity (Pa s)	Penetration value (mm)
MEH FZ 1	105.46±0.22	4.69±0.12	1.62±0.28	128.0±3.19
MEH FZ 2	107.12±0.34	4.70±0.09	1.51±0.32	127.0±2.75
MEH FZ 3	98.90±0.53	4.71±0.08	1.61±0.16	128.0±1.06
MEH FZ 4	99.74±0.25	4.72±0.10	1.55±0.37	127.5±2.25
MEH FZ 5	99.95±0.62	4.70±0.07	1.37±0.66	130.7±2.08
MEH FZ 6	106.15±0.38	4.71±0.10	1.95±0.45	102.0±1.52
MEH FZ 7	107.05±0.46	4.72±0.11	1.47±0.16	128.7±2.83
MEH FZ 8	103.42±0.76	4.71±0.05	1.81±0.42	111.0±1.67
MEH FZ 9	104.25±0.28	4.72±0.10	1.92±0.73	106.3±0.72
MEH FZ 10	101.45±0.45	4.73±0.08	1.80±0.24	117.3±1.42
H FZ	100.14±0.33	7.16±0.42	1.97±0.13	100.4±4.85

compared to isopropyl alcohol (60.614±0.048 mg/mL). In addition, the reported dermal toxicity [LD_{50}] of ethanol (20.0 mg/kg) and propylene glycol (20.8 mg/kg) were lower than that of isopropyl alcohol (12.8 mg/kg). Considering these and the fact that propylene glycol is less volatile than ethanol, the former was selected as the cosurfactant for formulating fluconazole MEs.

Construction of Pseudo-Ternary Phase Diagram

The microemulsion region in the pseudo-ternary phase diagrams increased slightly in size with the increase in surfactant concentration of S_{mix} from 1:1 (Fig. 1c) to 2:1 (Fig. 2b) and 3:1 (Fig. 2c), possibly because of progressive reduction of the interfacial tension. In contrast, when cosurfactant concentration with respect to surfactant was increased to the S_{mix} 1:2, it was observed that the microemulsion area considerably decreased as compared to S_{mix} 1:1, indicating that the optimum emulsification has been achieved. This decrement in the microemulsion region was most likely due to a decrease in surfactant concentration by the increased amount of propylene glycol. Briefly, larger microemulsion areas were observed in S_{mix} 2:1 and 3:1 as compared to the other ratios, indicating that surfactant and cosurfactant weight ratio (S_{mix}) have marked effect on phase properties. *i.e.* size and position of microemulsion region. For both cases, S_{mix} 2:1 and 3:1, the following similarities were observed: (a) the maximum concentration of oil that could be solubilised (75.5% *w*/*w* at 9.2% *w*/*w* of S_{mix}) was attained at the oil/S_{mix} ratio 1/9 and (b) the sizes of microemulsion zones were almost identical at the oil/S_{mix} ratio 2/8, which could be diluted by water to 43.9% content without causing the clouding of mixture. Moreover, the literature reports that, for dermal delivery, where enhanced skin permeation is the aim, the maximum flux is usually not obtained with formulations that contain the highest amount of surfactant (48,52). Based on these observations, the studied formulations were selected from the o/w microemulsion zone of the pseudo-ternary phase diagram constructed at S_{mix} 2:1, along the 2/8 water dilution line.

Preparation of Microemulsion-Based Hydrogel of Fluconazole

Different gelling agents, namely hydroxypropylmethylcellulose (Methocel K4M), Carbopol ETD 2020 and Gantrez AN 119, were evaluated for their thickening potential of the FZ microemulsions. Selection of the suitable gelling agent was made on the basis of compatibility with microemulsions components. It was observed that cellulose derivative was not able to gel the fluconazole-loaded microemulsions. This inefficiency could be attributed to

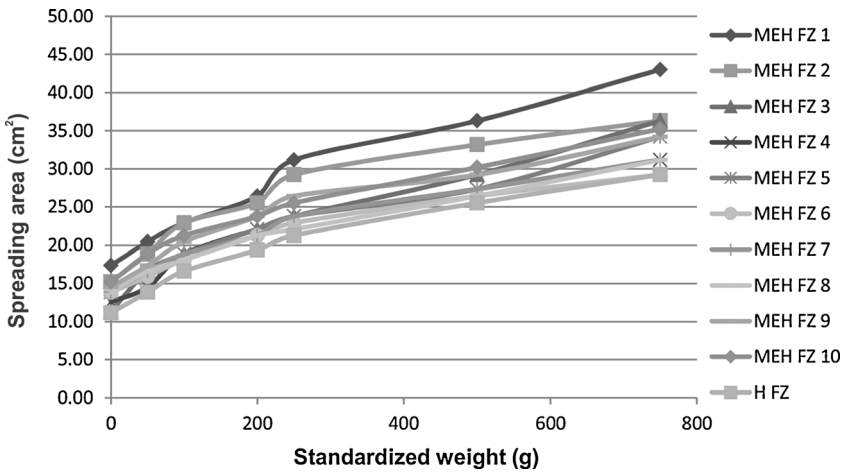

Fig. 3. Extensiometric curves of the studied fluconazole hydrogel formulations. Data shown as mean±SD, which was <2% and is not presented in the interest of clarity

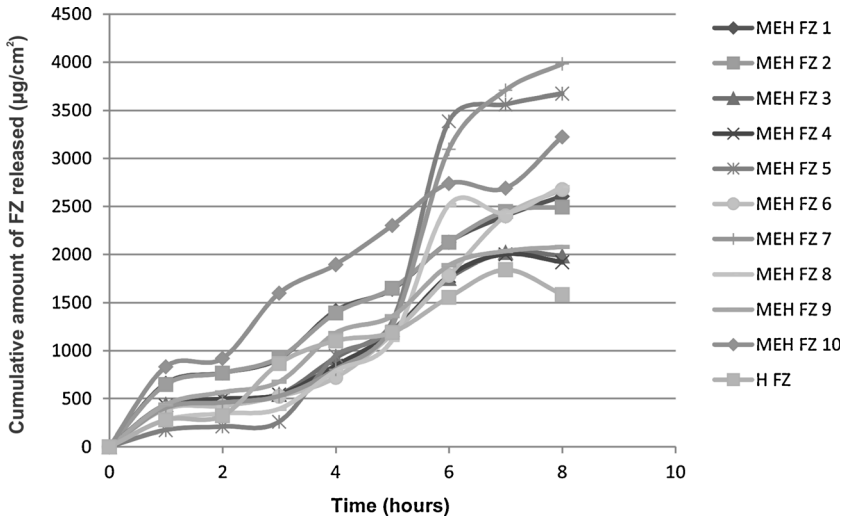

Fig. 4. *In vitro* fluconazole permeation profiles through synthetic membrane from microemulsion-based hydrogels and hydrogel (mean±SD, $n=3$)

its susceptibility to coagulate in the presence of high concentrations of surfactants. Similarly, in the case of Carbopol ETD 2020 and Gantrez AN 119, it was noticed that the microemulsions thickening could not be achieved after neutralisation, *i.e.* adding triethanolamine, as is generally recommended. It is necessary to remark that, in case of fluconazole hydrogel, the gelation occur after the neutralisation of Carbopol EDT 2020 with triethanolamine. Based on these observations, one can affirm that the abolition of gelling ability of the two acrylic polymers by neutralisation in microemulsions is most probably due to the large amount of surfactant, which determines the coagulation of the resulted salt. However, a clear gel could be obtained if the neutralisation was not performed.

Characterisation of Fluconazole Microemulsions

The mean droplet size of fluconazole microemulsions was found in the range of 4.084–5.002 nm (Table III). For the formulations ME FZ 1–8 containing about 10–37% (*w*/*w*) water and 50–72% (*w*/*w*) S_{mix}, the mean droplet size ranged between 4.084 and 4.250 nm, with no significant differences. The mean droplet size was lowest (formulation ME FZ 3) when the concentration of S_{mix} was 3.2-fold higher than water concentration and increased 1.22-fold when the S_{mix} content was lower than 50% and water concentration was higher than 37%. Hence, the formulation ME FZ 10 containing 42% water, 10% oil and 46% S_{mix} presented the highest average droplet size (5.002 nm), followed closely by formulation ME FZ 9 (4.942 nm) having a similar composition (39.5% water, 10.5% oil and 48% S_{mix}). These results showed that the droplet diameter slightly increased with decreasing content of S_{mix}, most probably due to the reduction of surfactant effects (namely, condensation and stabilisation) on the interfacial film. However, in all formulations, the ratio between oil and S_{mix} remained constant. Due to the very small average droplet size of all studied microemulsions, their surface areas are assumed to be high; therefore, a better contact between the oil droplets and the skin can

Table V. The Permeation and Release Parameters of the Fluconazole-Loaded Formulations (Microemulsion-Loaded Hydrogels and Hydrogel) Through Synthetic Membrane

	Permeation parameters			Release parameters	
Formulation code	J_s (µg cm^{-2} h^{-1})	K_P (×10^{-6} cm/h)	t_L (h)	k (µg cm^{-2} h$^{-1/2}$)	D (×10^{-2} cm^2/h)
MEH FZ 1	343.80±0.76	171.90	–	1561.20±12.58	0.019
MEH FZ 2	350.36±0.16	175.18	–	1654.00±1.23	0.021
MEH FZ 3	377.86±0.53	188.93	1.58±1.80	1652.70±9.63	0.021
MEH FZ 4	385.24±0.56	192.62	1.70±1.50	1682.60±0.95	0.022
MEH FZ 5	904.72±1.51	452.36	2.91±1.60	3938.60±9.04	0.122
MEH FZ 6	482.23±1.03	241.12	2.26±1.37	2129.80±1.06	0.036
MEH FZ 7	865.66±0.50	432.83	2.83±0.76	3615.10±1.66	0.103
MEH FZ 8	510.48±0.12	255.24	2.26±0.36	2344.00±3.56	0.043
MEH FZ 9	341.82±0.02	170.91	0.82±0.85	1504.00±0.54	0.018
MEH FZ 10	312.99±1.43	156.49	–	1533.00±10.81	0.018
H FZ	263.39±0.50	131.70	–	1142.80±7.62	0.010

J_s steady-state flux, K_p permeability coefficient, t_L lag time, k release rate, D diffusion coefficient

Table VI. Results of Kinetic Analysis of the *In Vitro* Permeation Data Through Synthetic Membrane Obtained for Fluconazole Loaded Microemulsion-Loaded Hydrogels and Hydrogel

	Zero order		First order		Higuchi		Korsmeyer–Peppas		
Formulation code	K_0 (μg/h)	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-0.5}$)	R^2	K_P (h^{-n})	n	R^2
MEH FZ 1	9.0266	0.9821	0.1644	0.9600	22.661	0.8939	1.1993	0.6577	0.8838
MEH FZ 2	9.0089	0.9782	0.1631	0.9599	22.779	0.8975	1.1997	0.6659	0.8952
MEH FZ 3	7.4978	0.9561	0.1131	0.9322	17.254	0.8104	0.9884	0.7539	0.8035
MEH FZ 4	7.4893	0.9444	0.1129	0.9165	17.185	0.7985	0.9852	0.7541	0.7979
MEH FZ 5	15.1512	0.8633	0.3686	0.5072	26.487	0.573	0.4513	1.6046	0.7884
MEH FZ 6	9.6573	0.9235	0.1752	0.8429	19.416	0.6881	0.9307	0.8017	0.7775
MEH FZ 7	14.9207	0.8651	0.2456	0.5518	27.368	0.5932	0.897	0.9789	0.7041
MEH FZ 8	10.5008	0.8889	0.1918	0.8313	20.178	0.643	0.736	1.1217	0.7801
MEH FZ 9	7.7642	0.9722	0.1200	0.9613	18.674	0.8626	1.0179	0.8109	0.8979
MEH FZ 10	10.7857	0.9685	0.2702	0.9168	28.983	0.9406	0.9261	0.7058	0.9261
H FZ	6.4966	0.9373	0.0903	0.9309	15.866	0.8588	0.8194	1.0431	0.9111

K_0 zero order release constant, K_1 first order rate constant, K_H Higuchi release constant, K_P Korsmeyer-Peppas release rate constant, n diffusion coefficient in Korsmeyer–Peppas model, R^2 squared correlation coefficient

be accomplished, thus providing high concentration gradient and improved permeation of fluconazole.

The values of polydispersity index observed for all the formulations (Table III) were very low (0.051–0.219) and closed to zero, indicating that the microemulsion droplets were homogenous and had narrow size distribution.

The viscosity of microemulsion formulations (Table III) tends to decrease with increasing water content, but the differences between the different formulations were very small. Moreover, the viscosity of all formulations was low, which is expected as one of the properties of microemulsions is low viscosity.

The refractive index indicates the isotropy of the microemulsions, the values of refractive index ranged between 1.4069±0.05 and 1.4409±0.02 (Table III). As water content was increased from 10 to 42%, the refractive index decrease from 1.4409 to 1.4069 due to the lower refractive index of water compared with that of other components of the formulations, *i.e.* oil or S_{mix}.

Zeta potential values of the studied microemulsion formulations were negative in the range of −0.010±0.13 to −0.950 ±0.04 mV (Table III). These very small values indicated the stability of systems, as the globules aggregation is not expected to take place, due to the presence of polysorbate 20, which acts through sterical stabilisation. Furthermore, it is known that ethoxylated surfactants give slightly negative zeta potential value, which is assigned to ion adsorption.

The pH values of all formulations were found in the range of 5.09±0.06 to 5.25±0.04 (Table III), being similar to the natural skin surface pH.

Characterisation of Fluconazole Hydrogels

Determination of Drug Content and pH. The drug content of the studied formulations was evaluated considering the requirements of most pharmacopeial monographs of

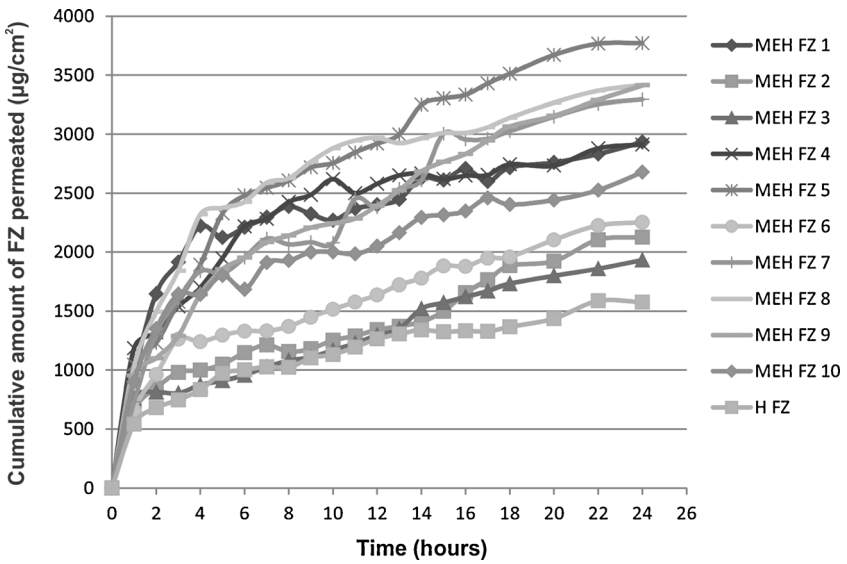

Fig. 5. *In vitro* fluconazole permeation profiles through pig ear skin from microemulsion-based hydrogels and hydrogel (mean±SD, n=3)

Table VII. The Permeation and Release Parameters of the Fluconazole-Loaded Formulations (Microemulsion-Loaded Hydrogels and Hydrogel) Through Pig Ear Skin

	Permeation parameters		Release parameters	
Formulation code	J_s (μg cm^{-2} h^{-1})	K_P (×10^{-6} cm/h)	k (μg cm^{-2} h$^{-1/2}$)	D (×10^{-4} cm^2/h)
MEH FZ 1	43.14±4.50	21.57	280.37±4.36	1.54
MEH FZ 2	46.96±5.42	23.48	286.71±5.11	1.61
MEH FZ 3	56.66±6.05	28.33	420.29±6.18	3.47
MEH FZ 4	74.48±3.73	37.24	717.62±3.25	10.11
MEH FZ 5	86.60±4.08	43.30	716.14±4.15	10.06
MEH FZ 6	59.90±6.23	29.95	354.91±5.93	2.47
MEH FZ 7	90.44±5.62	45.22	579.48±5.26	6.59
MEH FZ 8	94.98±3.33	47.49	503.83±4.09	4.98
MEH FZ 9	85.20±2.85	42.60	649.42±3.02	8.28
MEH FZ 10	56.91±4.42	28.45	335.55±4.35	2.21
H FZ	43.51±6.28	21.76	281.59±5.97	1.56

J_s steady-state flux, K_p permeability coefficient, t_L lag time, k release rate, D diffusion coefficient

dosage forms (including topical semisolid preparations) for the range of the claimed drug content, namely 90–110% (53). The fluconazole content of microemulsion-loaded hydrogels and conventional hydrogel (Table IV) ranged from 98.90±0.53 to 107.12±0.34% and 100.14±0.33% respectively, of the theoretical value (2%, *w*/*w*), which complies with the pharmacopeial specifications for drug content. The obtained data indicated the uniform distribution of drug within the hydrogels.

The developed microemulsion-loaded hydrogels had pH values varying from 4.69±0.12 to 4.73±0.08, slightly lower than those of fluconazole microemulsion formulations. This decrease in the pH can be attributed to the presence of the gelling agent Carbopol EDT 2020, a compound with acidic character. On the other hand, the FZ hydrogel showed a neutral pH (7.16±0.42), hence higher than those of fluconazole microemulsion-loaded hydrogel formulations because it was obtained by neutralising the carbomer with triethanolamine.

Rheological Characterisation. The viscosity values of microemulsion-loaded hydrogels were in the range from 1.37 ±0.66 Pa s to 1.95±0.45 Pa s, as shown in Table IV, indicating a slight increase with the water content. It was also observed that the viscosities of microemulsion-loaded hydrogel formulations increased significantly compared with those of microemulsions, due to the addition of 1.5% Carbopol EDT 2020, which made the preparations more suitable for topical administration.

Formulations MEH FZ 6, 8, 9 and 10 presented lower penetration values, indicating a higher consistency; contrastingly, formulations MEH FZ 2, 5 and 7 had the highest penetration values, therefore the lowest consistency (Table IV).

Among the studied formulations, the fluconazole hydrogel presented the highest viscosity value (1.97±0.13 Pa s) and consequently the lowest penetration level (100.4 ±4.85 mm).

Spreadability is a very important property of topical semisolid formulations since it indicates the facility of applying the formulations on the skin or mucosa. It was found that higher spreading area was obtained for MEH FZ 1, whereas the spreading areas of all other tested formulations were slightly

Table VIII. Results of Kinetic Analysis of the *In Vitro* Permeation Data Through Pig Ear Skin Obtained for Fluconazole Loaded Microemulsion-Loaded Hydrogels and Hydrogel

	Zero order		First order		Higuchi		Korsmeyer-Peppas		
Formulation code	K_0 (μg/h)	R^2	K_1 (h^{-1})	R^2	K_H (h$^{-0.5}$)	R^2	K_P (h^{-n})	n	R^2
MEH FZ 1	2.1288	0.6304	0.0535	0.8434	19.722	0.6328	1.5412	0.2933	0.8296
MEH FZ 2	1.8951	0.895	0.0311	0.9316	11.991	0.9259	1.2873	0.2725	0.9751
MEH FZ 3	1.7936	0.9107	0.0278	0.9547	11.234	0.9427	1.2599	0.2725	0.8478
MEH FZ 4	2.3831	0.6861	0.0603	0.8569	20.043	0.7692	1.5026	0.3405	0.9614
MEH FZ 5	3.6014	0.8427	0.1808	0.9130	23.874	0.958	1.4564	0.4405	0.9705
MEH FZ 6	1.9712	0.8498	0.0345	0.9372	13.756	0.9195	1.3223	0.3261	0.9335
MEH FZ 7	3.1356	0.8598	0.1011	0.9591	20.407	0.9527	1.4012	0.4191	0.9247
MEH FZ 8	2.8197	0.7038	0.1149	0.9443	22.981	0.8021	1.5232	0.3796	0.9426
MEH FZ 9	3.2370	0.8936	0.1114	0.9379	20.346	0.98	1.4066	0.4064	0.9772
MEH FZ 10	2.1054	0.7428	0.0448	0.8980	17.245	0.7919	1.47	0.2934	0.923
H FZ	1.3668	0.8248	0.0193	0.8869	9.9013	0.9108	1.178	0.3393	0.9884

K_0 zero order release constant, K_1 first order rate constant, K_H Higuchi release constant, K_P Korsmeyer-Peppas release rate constant, n diffusion coefficient in Korsmeyer–Peppas model, R^2 squared correlation coefficient

Table IX. Antifungal Activity of the Selected Gels and Commercial Cream Against *Candida albicans* ($n=3$)

Formulation code	Inhibition zone (mm)
MEH FZ 5	20
MEH FZ 7	25
MEH FZ 8	26
H FZ	26
Nizoral® cream	23

lower and almost the same (Fig. 3). However, all formulations presented good spreadability, proved by relatively high values of spreading areas.

The differences in consistency of the studied systems were most likely due to formulation variables, namely the concentration of oil, S_{mix} and water, which modifies the gelling potential of Carbopol EDT 2020. Thus, high concentrations of oil and S_{mix} and consequently low water content, loosened the gel matrix nature of microemulsion-based hydrogel formulations (case of formulations 2, 5 and 7), while the increase in water content improved the gelling ability of carbomer, particularly in the case of formulations 6, 8, 9 and 10. Furthermore, this assumption was confirmed by the fact that fluconazole hydrogel, without oil and S_{mix} in composition, presented the highest consistency, indicating that the gelling ability of polymer was not affected.

In Vitro Drug Release Studies. In a developmental topical drug preparation process, the *in vitro* diffusion study through an artificial synthetic membrane using the Franz diffusion cell is considered at present the most appropriate method for assessment of drug release from the vehicle and, consequently, the formulation performance. This technique is of great importance as it allows ascertaining that the drug release from the vehicle has occurred and was not the rate-limiting step for penetration and partition into the skin.

In vitro drug release studies were performed to compare the release and permeation of fluconazole from ten different microemulsion-loaded hydrogel formulations (MEH 1–10) and a fluconazole hydrogel, all containing same quantity (2%, *w*/*w*) of FZ. Comparing the total amount of FZ released from the studied MEHs after 6 h, it was observed that formulation 5 released the maximum amount of FZ (95.42±1.27%), followed closely by formulations 7 and 10 (94.35±0.42% and 91.48±1.03%, respectively). Futhermore, higher total drug release (71.61±0.52% to 77.12±0.85%) produced the compositions 1, 2, 6 and 8. The total amount of FZ released after 6 and also 8 h from all MEHs was higher than that observed from the conventional hydrogel as shown in Fig. 4.

The plots of cumulative amount of FZ released per surface area of membrane *vs*. time (Fig. 4) showed two linear portions: prior the steady state (the first 3 h) and during the steady state (from 3 to 7 h). A considerably higher and faster drug transfer can be observed during the first 3 h for MEH FZ 2 and 1, followed by the formulation 9. In turn, the flux and release rate of FZ prior to steady state of the formulations 6, 7, 8, 4 and 3 were significantly lower ($P<0.05$) than that of formula 2; the MEH FZ 5 produced the lowest flux and release rate in this period.

During the steady state, among the microemulsion-loaded hydrogels, the highest permeation flux of 904.72± 1.51 μg cm^{-2} h^{-1} was observed in case of formulation MEH FZ 5, followed closely by formulation MEH FZ 7 (865.66± 0.50 μg cm^{-2} h^{-1}). As shown in Fig. 4 and Table V, lower permeation flux values (2.3- to 2.6-fold) were observed not only in case of formulations 1, 2, 3 and 4, which contained higher amounts of oil (13–16%) and S_{mix} (58–72%) and lower amount of aqueous phase (10–27%), but also in case of MEH FZ 9 and 10 containing less oil and S_{mix} (10–10.5% and 46–48%, respectively) and more water (39.5–42%). The values of transfer rate across the membrane for MEHs 6 and 8 were very close (482.23±1.03 μg cm^{-2} h^{-1} and 510.48±0.12 μg cm^{-2} h^{-1}, respectively) and were only 0.5 times lower than that of the formulation MEH FZ 5.

These differences in fluconazole transfer from *MEHs* through synthetic membrane could be attributed to the combined effect of various factors such as the solubility of the drug in the microemulsion components, the distribution of the drug among the three different phases (the internal oil phase, the external aqueous phase and the surfactant micelles), the proportion of components in the microemulsion and the viscosity of the microemulsions. As it was pointed out/demonstrated by numerous previous studies, the drug release from an oil-in-water microemulsion is limited by the drug diffusion from the oil and micellar phases to the surrounding aqueous phase where the drug molecules are free to be released. Further, the drug transfer from the micellar phase may determine a partial micelle disruption and the consequent solubilisation of surfactant molecules in the microemulsion aqueous phase. Besides that, because the drug micelle-aqueous phase transfer is much faster than that of oil-aqueous phase, the decrease in drug concentration in the external aqueous phase due to drug permeation through the membrane determines firstly the drug transport from the micellar to aqueous phase and consequently the alteration of micelle structure. Therefore, the micelle disruption leads to the increase in the drug solubility in the microemulsion aqueous phase, which is the driving force for enhanced release (19,54). Moreover, the drug solubility in the external phase of an oil-in-water microemulsion is correlated with the water content of the system. Hence, the solubility at saturation of the incorporated drug decreases with the increase in water proportion in the microemulsion, so that a temporary oversaturated solution with a high thermodynamic activity is formed.

Because the solubility of fluconazole in the S_{mix} is higher compared with that in the oil and aqueous phases, the drug molecules would be mainly located at the oil–water interface, from where would diffuse faster to the external aqueous phase. Therefore, the increase in the flux values from formulation MEH 1 to MEH 8 may be due to the presence of an increasing amount of soluble fluconazole in the aqueous phase with the decreasing in oil and S_{mix} content and the increase in water proportion. In this group of MEH, the much higher flux values produced by formulations 5 and 7 can be attributed not only to a greater instability of drug-surfactant micelle aggregates, but also to their viscosity (the MEH 5 and MEH 7 presented the lowest viscosity values). In turn, the formulations 9 and 10, containing higher amounts of aqueous phase and lower proportions of oil phase and S_{mix}, did not show higher flux values than the first eight formulations as

expected, most probably due to a greater stability of surfactant micelles and to their increased viscosity.

Comparison of cumulative permeation between MEHs and conventional hydrogel demonstrated that all MEH FZ preparations enhanced drug permeation significantly ($P<0.05$). Fluconazole transmembranar flux from MEH 5 and MEH 7 was, respectively, 3.4- and 3.3-fold higher than that from the *H FZ*, while the flux enhancement obtained for all the other formulations was less (1.2- to 1.9-fold).

The calculated release rates of drug during the steady state for all MEHs were found to range between 1504.00±0.54 μg cm^{-2} $h^{-1/2}$ (for MEH FZ 9) and 3938.60±9.04 μg cm^{-2} $h^{-1/2}$ (for MEH FZ 5), being considerably higher than that of the corresponding fluxes, as it can be observed (Table V). The lowest release rate of FZ was produced by the conventional hydrogel (1142.80±7.62 μg cm^{-2} $h^{-1/2}$). Ranking the studied formulations according to this release parameter, a similar hierarchy with that based on flux values was obtained, indicating once more that apart from the contribution of water content in enhancing FZ release, the varying oil and surfactant content might be responsible for improved drug release.

From Table V, it can be observed that, in all the cases, the lag time values did not vary as expected, namely longer lag time values in case of slow diffusion. Thus, longer lag time values (from 2.26±0.36 h to 2.91±1.60 h) were observed in case of formulations 5, 6, 7 and 8, characterised by faster diffusion (Table V); the highest lag time values were obtained for the formulations MEH FZ 5 and MEH FZ 7 (2.91±1.60 h and 2.83±0.76 h, respectively). In contrast, in case of formulations 3, 4 and 9, which presented lower flux values, significantly shorter lag time values ($P<0.05$) were calculated (0.82±0.85 h to 1.70±1.50 h). Moreover, the transmembranar permeation profiles of MEH FZ 1, MEH FZ 2, MEH FZ 10 and H FZ were slightly different, but without lag time. Correlating these unexpected results obtained for the steady-state period of permeation with the values of flux and release rate obtained prior the steady state, it is clear that the lag time (a permeation parameter) of studied formulations depends not only on the diffusivity of the drug through the membrane but also on the drug release, both phenomenon being influenced by the systems composition and their viscosity.

The permeation profiles of all the studied fluconazole formulations (microemulsion-loaded hydrogels and conventional hydrogel) were in accordance with the zero-order model equation (R^2 ranged from 0.86 to 0.98).

In Vitro Skin Permeation Studies. In vitro drug permeation through skin is an alternative technique to *in vivo* studies in humans for evaluating the bioavailability of a newly developed topical formulation. It is well known that, unless the drug is highly lipophilic, the diffusion through stratum corneum is the limiting step for transdermal absorption. Accordingly, it is of great importance to measure the amount of compound that can be transferred through the stratum corneum into the upper layers of the skin. Although the human skin is the best model to be used in the *in vitro* skin permeation studies, due to the lack of its availability, the animal skin is an alternative model extensively used. Of all the species examined, the pig appears to be most representative as it has been shown that the histological and biochemical properties of the porcine ear skin are similar to the human skin and the results of permeation studies through pig ear skin are comparable to human skin (55–58).

Therefore, in the present study, the skin permeation experiments employed excised pig ear skin. Additionally, it is important to point out that there are few previous published studies regarding the *in vitro* fluconazole permeation through pig ear skin from topical dosage forms (45).

Unlike the plots of cumulative amount of FZ released per surface area of synthetic membrane *vs.* time, the permeation profiles of fluconazole, through excised pig ear skin over a 24-h period (Fig. 5) showed only one linear portion, during the steady state. Statistical comparison of the flux values calculated for MEHs showed that the formulations 8 and 7 provided the highest rates of permeation (about 90–95 μg cm^{-2} h^{-1}), followed closely by MEH FZ 5 and MEH FZ 9 (about 85–87 μg cm^{-2} h^{-1}). Lower flux values, ranged between 56.66±6.05 and 74.48±3.73 μg cm^{-2} h^{-1}, produced the MEH FZ 3, 4, 6 and 10, while the formulations 1 and 2 provided the lowest flux values (43.14±4.50 and 46.96±5.42 μg cm^{-2} h^{-1}, respectively). These results indicated a 2- to 2.2-fold improvement in fluconazole permeation through pig ear skin achieved with formulations containing 10.5–12.5% oil, 48–56% S_{mix} and 29.5–39.5% water (MEH FZ 5, MEH FZ 7, MEH FZ 9 and MEH FZ 8) in comparison to conventional hydrogel (without oil and S_{mix}). The flux values were similar to that resulted from conventional hydrogel (43.51±6.28 μg cm^{-2} h^{-1}) when the oil and S_{mix} content was higher (15–16% and 68–72%, respectively), and the percentage concentration of water was only 10–14.5% (formulations MEH FZ 1 and MEH FZ 2). These results were consistent with previous reports demonstrating that the skin permeation rate of a hydrophobic drug from an oil-in-water microemulsion would increase with the decreasing oil and S_{mix} content, which resulted in decreased drug solubility (increased thermodynamic activity of the drug) in the vehicle and increased partition into the skin (23). Additionally, the values of skin permeation rates were not in accordance with these assumptions in all the cases, most probably due to the viscosity differences between the systems, indicating that this parameter did not governed the skin permeation of fluconazole from the studied MEHs formulations.

Apart from the important role of the microemulsions composition, alternative mechanisms were proposed in the literature to explain their enhanced efficiency as skin drug delivery systems (*i.e. vs.* conventional hydrogel). One mechanism is the possibility of direct drug partition from the microemulsion droplets to the stratum corneum, which, combined with the very small droplet size, creates a very large surface area for drug transfer to the skin. The second posibility is the penetration enhancing effect of the microemulsion components. In the present case, it must be considered not only the penetration enhancing effect of the employed surfactant (polysorbate 20) and cosurfactant (propyleneglycol) but also that of the oil phase (Transcutol P has been shown to be an effective permeation enhancer), which could significantly reduce the barrier of stratum corneum and increase the diffusion coefficient of drug in skin.

Comparison of correlation coefficients obtained for each studied formulation after the curve fitting into various kinetic models (Table VIII) indicated that MEH FZ 1, MEH FZ 3, MEH FZ 6, MEH FZ 7 and MEH FZ 8 followed the first order model (R^2>0.9, excepting MEH FZ 1 with R^2>0.8), whereas all other MEH FZ and H FZ followed the Korsmeyer–Peppas model (R^2>0.9) over a period of 24 h. Moreover, in the case of the second group of formulations (fitting to Korsmeyer–Pepas model), the analysis of the first 60% of drug release data using this model was performed to determine the values of diffusion exponent (n), an indicative of drug release mechanism: Fickian diffusion when $n \leq 0.5$, non-Fickian transport when $0.45<n<0.89$, case II transport when $n=0.89$ and super case II transport when $n>0.89$. According to the calculated values of diffusion exponent, n, ranged between 0.2725 and 0.4405 (Table VIII), the fluconazole permeation from the respective systems followed the Fickian (non-steady) diffusional mechanism.

In Vitro Antifungal Activity. The results obtained for all tested preparations were satisfactory in terms of antifungal activity expressed as the mean of inhibition zone, which was in the range of 20–26 mm, while the marketed Nizoral® cream produced a 23-mm inhibition zone (Table IX). The similar mean diameter of inhibition zone showed by MEH FZ 7 and MEH FZ 8 might be determined by similar diffusion process of FZ from these semisolid systems. Although the microemulsion-loaded hydrogels selected based on *in vitro* skin permeation results (*e.g.* formulations 5, 7 and 8) have been shown to be a suitable/appropriate dosage form for fluconazole dermal penetration in comparison with conventional hydrogel, their antifungal effect was not enhanced most probably because of the influence of the excipients used in formulation. It was reported that *in vitro* antifungal activity of fluconazole in microemulsions was reduced by the presence of propyleneglycol in the formulation (45). However, it is necessary to remark that propyleneglycol is frequently used in the formulation of topical products containing azole derivatives (*e.g.* marketed product Nizoral® cream) as it presents high solubilisation potency for these drugs and penetration enhancer effect. At present, to our knowledge, there are no investigations regarding the possible interfering effect of propyleneglycol with antifungal activity of azole derivatives.

CONCLUSION

Our results pointed out that the content of microemulsion-loaded hydrogel components (oil, S_{mix} and water) had significant effect on their physical, rheological and *in vitro* drug release characteristics. The experiments proved that the solubility of fluconazole and the partition into different phases of these systems influence the *in vitro* drug permeation through pig ear skin, a less used model in this kind of studies implying fluconazole, although it can be related to the human skin.

According to the results of the characterisation and *in vitro* permeation studies, the most desirable formulations for topical delivery of fluconazole were considered the microemulsion-loaded hydrogels 7 and 8 containing Transcutol P (11.5% and 11%, respectively) as oil phase, S_{mix} (2:1) Lansurf SML 20-propyleneglycol (52% and 50%, respectively) as surfactant–cosurfactant, Carbopol EDT 2020 (1.5%) as gelling agent (%, *w*/*w*) and water (34.5% and 37%, respectively). These exhibited the highest flux values, higher release rate values and lower surfactant content among the tested variants. However, the antifungal effect of these formulations was not much higher than that of the reference hydrogel and the marketed preparation, due to the presence of propyleneglycol as cosurfactant in their composition.

Thus, further investigations will be performed by us, regarding the composition of microemulsions and the corresponding *in vitro* and *in vivo* stability, safety and therapeutic efficacy in order to develop a commercially viable topical microemulsion-loaded hydrogel formulation for fluconazole.

ACKNOWLEDGEMENT

The authors gratefully acknowledge to Synevo Medical Laboratory, Timisoara, Romania, for providing help in carrying out antifungal studies. In addition, the authors would like to thank Professor Zoltan Szabadai for his support and technical discussion on some quality assessment tests of the studied formulations. The authors from 'Ilie Murgulescu' Institute of Physical Chemistry gratefully acknowledge the support of EU (ERDF) and Romanian Government allowing for acquisition of the research infrastructure under POS-CCE O2.2.1 project INFRANANOCHEM, No. 19/2009.03.01 and the support from PN-II-ID-PCE-2011-3-0916 grant.

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