ORIGINAL ARTICLE

Investigation of microemulsion system for transdermal delivery of itraconazole

Arpan Chudasama, Vineetkumar Patel, Manish Nivsarkar[1], Kamala Vasu[2], Chamanlal Shishoo

Department of Pharmaceutics, [1]Pharmacology & Toxicology, and [2]Medicinal Chemistry, B V Patel PERD Centre, Ahmedabad, Gujarat, India

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ABSTRACT

A new oil-in-water microemulsion-based (ME) gel containing 1% itraconazole (ITZ) was developed for topical delivery. The solubility of ITZ in oils and surfactants was evaluated to identify potential excipients. The microemulsion existence ranges were defined through the construction of the pseudoternary phase diagrams. The optimized microemulsion was characterized for its morphology and particle size distribution. The optimized microemulsion was incorporated into polymeric gels of Lutrol F127, Xanthan gum, and Carbopol 934 for convenient application and evaluated for pH, drug content, viscosity, and spreadability. *In vitro* drug permeation of ME gels was determined across excised rat skins. Furthermore, *in vitro* antimycotic inhibitory activity of the gels was conducted using agar-cup method and *Candida albicans* as a test organism. The droplet size of the optimized microemulsion was found to be <100 nm. The optimized Lutrol F 127 ME gel showed pH in the range of 5.68±0.02 and spreadability of 5.75±1.396 gcm/s. The viscosity of ME gel was found to be 1805.535±542.4 mPa s. The permeation rate (flux) of ITZ from prepared ME gel was found to be 4.234 μg/cm/h. The release profile exhibited diffusion controlled mechanism of drug release from ME ITZ gel. The developed ME gels were nonirritant and there was no erythema or edema. The antifungal activity of ITZ showed the widest zone of inhibition with Lutrol F127 ME gel. These results indicate that the studied ME gel may be a promising vehicle for topical delivery of ITZ.

Key words: Antifungal, microemulsion, permeability, phase diagram, transdermal

INTRODUCTION

Recently, there have been an increasing number of fungal infections caused by fungi, such as those belonging to the genus *Candida*, *Aspergillus*, and *Cryptococcus*. This is a particular complication encountered in transplant patients, those administered a large quantity of antibiotics, anticancer drugs (carcinostatic), or a steroidal agents over a long period, AIDS patients, or those suffering from cancer in the terminal stage, and those with hematological malignancies undergoing intensive chemotherapy and/or bone marrow transplantation.[1] Invasive fungal infections are a major cause of morbidity and mortality in patients receiving bone marrow transplants for leukemia as well as in immunocompromised cancer patients.[2]

Itraconazole (ITZ), an azole antifungal agent, is widely used clinically for a variety of serious fungal infections in normal and immunocompromised hosts, including *Aspergillosis*, *Cryptococcus*, *Candida*, *Blastomyces*, disseminated *Penicillium mameffei* infections, and *Histoplasma capsulatum var. capsulatum*.[3,4] ITZ acts by impairing the synthesis of ergosterol, the essential component of the fungal cell membrane.[5] The log partition coefficient of ITZ is 5.66 in a system of n-octanol and an aqueous buffer solution at pH 8.1, which indicates the hydrophobicity of the drug. ITZ is a weak base with a pKa of 3.7, and relatively insoluble in water.[6] It has been used successfully in the treatment and prevention of *Aspergillus* infections with a lower toxicity than amphotericin B, indicating a better therapeutic index.[7-10] However, the bioavailability of ITZ from the

Address for correspondence

Prof. Chamanlal Shishoo,
B V Patel PERD Centre, Thaltej-Ganhinagar Highway, Thaltej, Ahmedabad, India. E-mail: cjshishoo2010@rediffmail.com

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existing market formulation like the pellet capsule form is very low in neutropenic patients,[11] and inadequate plasma concentrations are often found in patients receiving antineoplastic therapy. Topical drug delivery opens up a number of opportunities with regard to efficient drug therapy for fungal infection and would be more effective in these individuals. A topical application may be helpful for many neutropenic and other immunocompromised patients who have difficulty swallowing the oral capsule formulation. On the other hand, Oral Solution (Sporanox®) is forbidden to be used in patients with impaired renal function, not because of the toxicity of the drug itself, but the adjuvant hydroxypropyl β-cyclodextrin (HP-β-CD). Each milliliter of Oral Solution (Sporanox®) contains 10 mg of ITZ solubilised by 400 mg of HP-β-CD as an inclusion complex. Following a single dose of 200 mg Oral Solution (Sporanox®) to the subjects with severe renal impairment, clearance of HP-β-CD was six-fold reduced compared with subjects with normal renal function. Although its clinical relevance is unknown, it has been reported that HP-β-CD produces pancreatic adenocarcinoma in a rat carcinogenicity study.[12] Potential advantages of topical administration route include site directed delivery, which can obviate the need for oral and other systemic treatments and can reduce the total drug dose, thereby reducing nontarget site toxicities.[13,14] A useful case in point is the treatment of cutaneous fungal infections where many useful agents must be administered orally to achieve clinically relevant cure rates.[15-17]

Microemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant, and co-surfactant in appropriate ratios. Unlike coarse emulsions micronized with external energy, microemulsions are based on low interfacial tension. This is achieved by adding a co-surfactant, which leads to spontaneous formation of a thermodynamically stable microemulsion. The droplet size in the dispersed phase is very small, usually below 200 nm in diameter, which makes the microemulsions a transparent liquid.[18] In principle, microemulsions can be used to deliver drugs to the patients via several routes, but the topical application of microemulsions has gained increasing interest. The three main factors determining the transdermal permeation of drugs are the mobility of a drug in the vehicle, release of a drug from the vehicle, and permeation of a drug into the skin. These factors affect either the thermodynamic activity that drives the drug into the skin or the permeability of drug in the skin, particularly stratum corneum. It has been reported that microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations, such as emulsions[19,20] and gels.[21,22]

The aim of the present study was to investigate the potential of microemulsion-based gel formulation of ITZ to be delivered by transdermal route. Although the topical therapy for skin and mucous membranes with azoles is of limited value,[23] such topical therapy may serve as an adjunct to systemic treatment with azole.[24] The developed ITZ topical formulation may slow the spread of infection in patients who are unwilling or unable to take oral or intravenous formulations.[25]

MATERIALS AND METHODS

Materials

ITZ was a generous gift from Matrix Laboratories Ltd. (Hyderabad, India); Propylene glycol monocaprylate (Capryol™ 90), Isopropyl myristate (IPM), Propylene Glycol Dicaprylate/Dicaprate (Captex® 200), and Glycerol Caprylate Caprate (Captex® 355) were obtained from Subhash Chemical Industries (Pune, India); and PEG-8 glycol caprylate (Labrasol®) and diethylene glycol monoethyl ether (Transcutol P®) were provided by Gattefosse, France. 2-Pyrrolidone (Soluphor P®) and Polyoxyethylene polyoxypropylene block copolymer (Lutrol® F 127 or Poloxamer 407) were obtained from BASF Corp., Germany. PEG-20 sorbitan monolaurate (Tween 20) and Tocopherol acetate were purchased from Merck (Mumbai, India); Carbopol 934® and Xanthan gum were purchased from Astron Chemicals (Ahmedabad, India). *Candida albicans* ATCC10231 was purchased from National Collection of Industrial Micro-organism, NCL Pune and Sabouraud dextrose agar broth from Himedia, Mumbai. Buffer powders (pH 4.0, 7.0, and 9.2) for pH meter calibration were purchased from Rankem (New Delhi, India). All other chemicals and solvents used were of analytical grade.

Selection of oil and surfactants for microemulsion

For selecting different components for formulating microemulsion of ITZ, the solubility of ITZ was investigated in different oils and surfactants, such as Capryol 90, Captex 200, Captex 355, IPM, Tween 20, Labrasol, Transcutol P, and Soluphor P. An excess amount of ITZ was added to 5 mL of each selected oils and surfactants and was shaken reciprocally at 20°C for 24 h (model R100 Rotatest Shaker, Luckham Ltd., Burgess Hill, UK). The supernatant portion of the supersaturated solution was carefully withdrawn and suitably diluted with methanol, and solubility of ITZ was determined using HPLC (Jasco, Japan) at 263 nm.[26-28] The HPLC system consisted of RP column (LCGC Qualisil BDS C18; (250 × 4.6) mm, 5 μm) and acetonitrile:water (90:10) as a mobile phase. A 40 μL volume was injected into the column at a flow rate of 0.7 mL/min.

Construction of pseudoternary phase diagram

The pseudoternary phase diagrams were constructed using water dilution method.[29] Capryol 90 was used as the oil phase, Soluphor P as the surfactant and Transcutol P as the co-surfactant. Phase diagrams were constructed with 9:1 to 1:9 v/v ratio of oil to surfactant and various ratios of surfactant/co-surfactant (4:1, 3:1, 2:1, and 1:1 v/v). For each phase diagram at specific surfactant/co-surfactant,

mixtures of the oil, the surfactant and the co-surfactant were prepared, and the mixture was diluted with water by sequential addition of 10 μL of water using a micropipette (Accupipet, Tarson, India). Water was added drop by drop while mixing on a magnetic stirrer (Remi Instruments Ltd., Mumbai, India) at room temperature, and the samples were marked as being optically clear or turbid. The microemulsion regions were identified as transparent and isotropic mixtures. The percentage of three different phases, that is, oil, water, and the mixture of surfactant and co-surfactant were calculated.

Preparation of O/W microemulsion loaded with drug

Appropriate quantities of surfactant Soluphor P, co-surfactant Transcutol P, and oil Capryol 90, were weighed into a screw-capped glass vial. ITZ was dissolved in a concentration of 1% w/w in the oil being used and then the mixture of surfactant and co-surfactant was added. The mixture was stirred with a magnetic bar on magnetic stirrer, at room temperature with continuous addition of weighed amount of water, until the formation of a transparent system.

Characterization of microemulsion systems

Dispersion stability studies

To overcome the problem of metastable formulation, dispersion stability tests were performed. Selected formulations were centrifuged (Sorvall Biofuge Primo R Centrifuge, Thermo Electron Corp., USA) at 3000 rpm for 30 min. The formulations that showed no phase separations were taken for the heating and cooling cycle (freeze thaw cycle). Six cycles between the refrigerator temperature (4°C) (Electrolux, India) and 45°C in a hot air oven (Cintex, Mumbai, India) with storage at each temperature for not less than 48 h were done. The formulations which were stable at these temperatures and survived the stability tests were selected for further studies.

Transmission electron microscopy analysis

Morphology and structure of the microemulsion were studied using transmission electron microscopy (TEM) (Technai 20, Philips, Holland) at an acceleration voltage of 200 kV and typically viewed at a magnification of ×43,000. The size of the colloidal structures was determined using AnalySIS® software (Soft Imaging Systems, Reutlingen, Germany). In order to perform the TEM observations, a drop of the microemulsion was directly deposited on the holey film grid and observed after drying.

Measurement of droplet size

The average size and polydispersity index of the microemulsion droplets were determined by photon correlation spectroscopy (Nano ZS90, Malvern Instruments, UK). The measurement was performed using a He–Ne laser at 633 nm. Light scattering was monitored at 25°C.

Formulation of microemulsion-based gel

The formulation that showed stability for the above parameters was selected for further development of microemulsion-based gel. As microemulsions have low viscosity and are difficult to apply on the skin as such, they should be gelled with suitable gelling agents. Gels were prepared using three different polymers: Lutrol F127 (15.14% w/w; F1), Xanthan gum (1% w/w; F2), and Carbopol 934 (1% w/w; F3) were used as gelling agents. Required quantities of gelling agents (Xanthan gum and Carbopol 934) were dispersed in the aqueous phase under continuous stirring. Neutralization was performed using triethanolamine in the case of Carbopol 934 to attain gelling. A weighed amount of Lutrol F127 was solubilized in the microemulsion system with gentle heating on a water bath (Equitron, Chennai, India).

Evaluation of microemulsion-based gel

Determination of pH

The observed pH values of the dispersions were measured by a pH meter (Eutech, pH Tutor, Singapore), at 20°C±2°C. Gel (2.5 g) was accurately weighed and dispersed in 25 mL of purified water. The pH meter was calibrated before each use with buffered solution at pH 4.0, 7.0, and 9.0.

Drug content studies

Mmicroemulsion-based gel equivalent to 25 mg of ITZ was taken in 25 mL volumetric flask containing 15 mL methanol and stirred for 30 min. The volume was made up to 25 mL with methanol. From the above solution, 0.2 mL was further diluted with 4.8 mL methanol and 5 mL of 0.1 N HCl to get 20 μg/mL. The resultant solution was filtered through 0.45 μm membrane filter. The absorbance of the solution was measured spectrophotometrically (Shimadzu UV-2450, Japan) at 258 nm.[30]

Spreadability study

Spreadability was determined using apparatus suggested by Mutimer *et al*,[31,32] which was suitably modified in the laboratory. It consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of "slip" and "drag" characteristics of gels.[33] A ground glass slide was fixed on this block. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A weight of 500 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull off 125 g weight with the help of a string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm can be noted. A shorter interval indicates better spreadability.[31-35]

The spreadability was calculated by using the following formula:

$S = (m \times l)/t$

Value S is spreadability, m is the weight tied to the upper slides, l is the length of glass slide, and t is the time taken.

Viscosity measurement

The viscosity of formulation was determined at 25°C using Brookfield viscometer (Model DV-II+, Brookfield Engineering Laboratories, USA). The viscosity was measured using 175 g of gels filled in a 250 mL beaker. Spindle T 95 was used for the measurement of viscosity of all the gels.

Primary skin irritation test

The primary skin irritation test of developed microemulsion-based gels was carried out using Draize patch test on rabbits.[36-38] The experimental protocol was approved by the Institutional Animal Ethical Committee of B V Patel PERD Centre, Ahmedabad. White New Zealand rabbits weighing 2.5–3 kg were acclimatized before the beginning of the study. Animals were divided into four groups (n=3) follows:

- Group 1: No application (control)
- Group 2: F1 – Microemulsion-based Lutrol F127 gel
- Group 3: F2 – Microemulsion-based Xanthan gum gel
- Group 4: F3 – Microemulsion-based Carbopol 934 gel

The back of the rabbits were clipped free of hair, 24 h prior to application of the formulations. Formulations, 0.5 g were applied on the hair-free skin of rabbits by uniform spreading within the area of 4 cm^2. The skin was observed for any visible change, such as erythema (redness) or edema (swelling) after 24 and 72 h. The results of skin irritation study were evaluated using the Draize scale.[36] Scores between 0 and 4 were used to grade erythema and edema, which range from no response to a severe response.

In vitro *skin permeation study*

The *in vitro* permeation studies for the formulations were carried out using Franz diffusion cell (Hanson Research Co., USA), using dehaired abdominal skin of Wistar albino rat. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. All efforts were made to minimize animal suffering and to limit the number of animals used. Rats were euthanized in a carbon dioxide vacuum chamber.

The full thickness of rat skin was excised from the abdominal region and hairs were removed with an electric clipper. The subcutaneous tissue was removed surgically and the dermis side was wiped with isopropyl alcohol to remove the adhering fat. The cleaned skin was washed with distilled water and stored at –21°C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartments of the Franz diffusion cell having a capacity of 12.5 mL and surface area of 1.77 cm^2. The assembly was thermostated by circulating warm water at 37±0.2°C in the external jacket of Franz diffusion cell to simulate the body temperature. The formulation equivalent to 5 mg of drug was placed uniformly on the epidermal surface of skin in the donor compartment, while the receptor compartment contained 0.1 M Citrate–Phosphate buffer pH 5.5 containing 30% methanol. The diffusion medium in the receptor compartment was constantly stirred by means of teflon-coated magnetic bead on a magnetic stirrer. An aliquot of 2 mL was removed from the receptor medium at intervals of 1, 2, 4, 8, 12, 16, 20, and 24 h and replaced immediately with the same volume of the receptor medium. The samples were immediately analyzed directly for drug concentration spectrophotometrically at 258 nm.[39] Three replicates of each experiment were performed.

The steady state flux (J_{ss}, μg/cm/h) of ITZ and the lag time (T_{lag}, h) were calculated from the slope and the intercept, respectively, of the straight line obtained by plotting the cumulative amount of ITZ permeated versus time in steady conditions. Permeability coefficient (kP, cm/h) was calculated by dividing the flux obtained by the initial concentration of drug in the donor compartment. Statistical significance of the results was ascertained using ANOVA and Student's t test.

Release kinetic studies of microemulsion-based gels

The kinetic data for the *in vitro* release of ITZ from microemulsion-based gels was estimated using different kinetic orders, such as zero, first, and Korsemeyer–Peppas equations and a system, such as Higuchi's diffusion model.[40,41] The criterion for selecting the most appropriate model was chosen on the basis of goodness-of-fit test.[42-44]

Microbiological assay of itraconazole

In vitro antifungal studies were performed against *Candida albicans* in Sabouraud's agar medium by the agar-cup method.[45-48] Suspension of *C. albicans* was inoculated in Sabouraud dextrose broth and then poured into a sterile petridish (15 cm in diameter), and allowed to solidify. Wells were done in plate using borer of size 8 mm and 1 g each of developed formulations containing 1% of ITZ were poured into the wells. These plates were kept at 4°C for 1 h. After 1 h, plates were incubated at 37°C±1°C for 24 h. The mean zone of inhibition of ITZ released from prepared microemulsion-based gels was calculated in millimeters. Statistical analysis using one-way ANOVA test was used to compare difference in antifungal activity within the formulations.

RESULTS AND DISCUSSION

Solubilities of Itraconazole in Various Oils

The physicochemical properties of ITZ suggest that it has good potential for topical drug delivery. The important criterion for selection of materials for the nanoemulsion formulation development is that the components are pharmaceutically acceptable, nonirritant, and nonsensitizing to the skin and fall under the Generally Regarded as Safe (GRAS) category. Among the selected oils that were screened [Table 1], maximum solubility of ITZ was found in Capryol 90 followed by Captex 200, IPM, and Captex 355. Among the surfactants [Table 1], Soluphor P followed by Transcutol P and Tween 20 showed reasonable solubilizing potential for ITZ. Transcutol P proved to be the best solubilizer for ITZ.

Construction of Pseudoternary Phase Diagrams

The construction of pseudoternary phase diagrams is used to determine the concentration range of components in the existence range of microemulsion. The pseudoternary phase diagrams with various weight ratios of Soluphor P to Transcutol P are depicted in Figure 1. The translucent microemulsion region is presented in phase diagrams with no distinct conversion from water-in-oil (W/O) to oil-in-water (O/W) microemulsions were observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. The area of microemulsion isotropic region changed slightly in size with the increasing ratio of surfactant to co-surfactant.

The phase study revealed that the maximum proportions of oil were incorporated in microemulsion systems when the surfactant-to-co-surfactant ratio was 1:1 and the same ratio was selected for microemulsion formulation. All the formulations were evaluated for clarity, flowability, dispersion stability, and particle size analysis. The optimized microemulsion formulation [Table 2], showed consistent stability for the parameters evaluated and was selected for further studies.

Table 1: Solubility of itraconazole in various oils and surfactants at 25°C (n =3)

Oil / Surfactant	Solubility (mg/mL)
Isopropyl myristate	0.206±2.07
Captex 200	1.008±2.08
Captex 355	2.127±1.97
Tocopherol acetate	5.22±1.87
Capryol 90	22.132±1.58
Tween 20	3.709±1.04
Labrasol	7.147±1.65
Transcutol	4.6±1.48
Soluphor	29.733±1.57

Table 2: Composition of microemulsion system

Active/ingredients	% w/w
Itraconazole	1
Capryol 90	9.44
Transcutol P	27.14
Soluphor P	27.14
Distilled Water	q.s. to 100

Characterization of Microemulsion

The microemulsion appeared dark with bright surroundings and a positive image [Figure 2]. The droplet size ranged between 19 and 100 nm and was in agreement with the droplet size distribution measured using photon correlation spectroscopy.

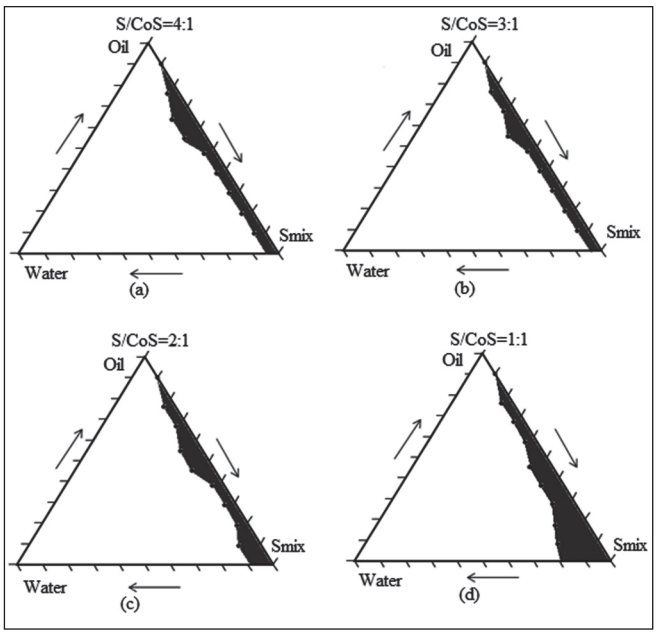

Figure 1: Pseudoternary phase diagrams showing the O/W microemulsion (shaded area) regions of Capryol 90 (Oil), Soluphor P (Surfactant), Transcutol P (Co-surfactant) at different Smix ratios (a) Smix 4:1; (b) Smix 3:1; (c) Smix 2:1; and (d) Smix 1:1.

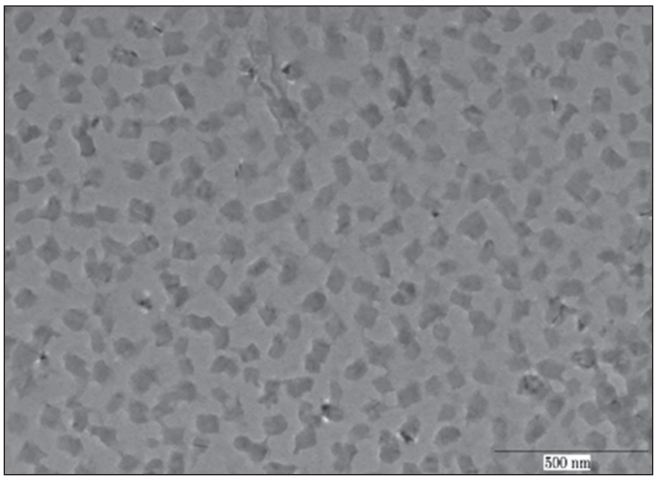

Figure 2: Transmission electron microscopic positive image of itraconazole microemulsion

The average droplet size of microemulsion was found to be 78.03±2.36 nm with low value of polydispersity index of 0.392. Polydispersity is the ratio of standard deviation to the mean droplet size. This signifies the uniformity of the droplet size in the formulation. Our obtained value of polydispersity index of the microemulsion formulation indicates uniformity of droplet size within the formulation.

Evaluation of Microemulsion-based Gel

The pH of the microemulsion-based gel formulations were found to be in the range of 5.68–7.0 [Table 3], which was within the acceptable limits for topical application. The drug content was in the range of 98.64%–104.09% for the developed gel formulations. The spreadability plays an important role in patient compliance and helps in uniform application of gel to the skin. The spreadability of formulated gels [Table 3] indicates that the gel will take less time to spread at the site of application.

The viscosity of the microemulsion was found to be 19.57±1.99 mPa s, which was very low as expected.[49] Recently, different hydrogel matrices, such as Carbopol 934 and carrageenan have been used to increase the viscosity of microemulsion for dermal application.[50-53] The addition of gel matrix into the microemulsion resulted in the formation of the microemulsion-based gel, which was more suitable for dermal application when compared with microemulsion.

The viscosities of different microemulsion-based gels are recorded in Table 3. The gel prepared with Xanthan gum gave less viscosity with sticky texture and the gel was unclear. On the other hand, Carbopol 934 yielded turbid gel due to agglomeration of microemulsion. Lutrol F127 at the concentration of 15.14% w/w was able to produce microemulsion-based gel with desired rheological property.

Table 3: Physicochemical characterization of microemulsion-based gels

Formulation	pH	Spreadability (gcm/s)	Viscosity (mPa s)
F1	5.68±0.02	5.75±1.396	1805.535±542.4
F2	6.38±0.03	4.32±1.205	1234.262±357.3
F3	7.0±0.09	4.88±2.30	1533.827±436.5

Table 4: Primary irritation index of microemulsion-based gels at the end of 24 and 72 h (n=3)

Formulation	Irritation index	
	24 h	72 h
Control	0	0
F1	0	1
F2	0	0
F3	0	1

Lutrol F127 is a polyoxypropylene–polyoxyethylene, nonionic, surface-active block copolymer composed of ~70% ethylene oxide and 30% propylene oxide with an average molecular weight of 1,15,000 Da.[54]

Primary Skin Irritation Test

The results of skin irritation test of both 24 and 72 h are shown in Table 4. The intensity criterion of skin irritation followed protocol that scores of <0.5 meant no irritation, 0.5–3 for slight irritation, >6 for severe irritation. The scores given in table indicate that there is no serious sensitivity reaction in either of the developed microemulsion-based gels.

In Vitro Skin Permeation Study

The concentration of Lutrol F127, Xanthan gum, and Carbopol 934 were kept minimum. The decreased drug release encountered by increasing the concentration of the gel may be attributed to the difference in the viscosity of the polymers.[55-57] As shown in Figure 3, among the three gels, after 24 h of permeation study, Lutrol F127 gel showed the highest drug release followed by Xanthan gum and Carbopol 934 gels. Agglomeration and turbidity of Carbopol 934 gel may be the obvious reason for least drug release. ITZ is soluble at acidic pH and while neutralizing carbopol gel the drug may have precipitated. The release profile of Xanthan gum gel was similar to that of Lutrol F 127 gel up to 8 h. A similar viscosity profile between both the gels could be the reason for it. The fall in drug release after 8 h from Xanthan gum gel may be due to pH-dependent solubility of ITZ as apparent from haziness of the Xanthan gum gel, but the same needs further evaluation. The *in vitro* release of ITZ from microemulsion-based gels could be arranged in descending manner as follows: 15.14% Lutrol F 127 (F1) > 1% Xanthan gum (F2) >1% Carbopol 934 (F3). The calculated flux of gels, lag time (T_{lag}) and the permeability coefficient (kP) are as shown in Table 5.

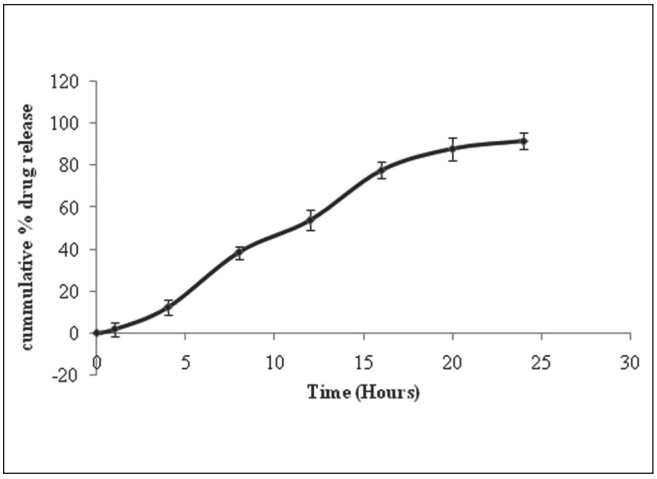

Figure 3: Permeation profile of itraconazole through excised rat skin from microemulsion-based gels (n=6)

Release Kinetic Studies of Microemulsion-Based Gels

In order to develop an ideal kinetic model to interpret the diffusion data in terms of meaningful parameters, various kinetic models, including zero-order, first-order, Korsemeyer–Peppas and the Higuchi diffusion model were applied. As shown in Table 6, it was found that *in vitro* release of ITZ microemulsion-based gel formulae followed zero-order for all the three investigated gelling agents. Formulation F1 with Lutrol F127 also followed Higuchi diffusion order. This indicates that the rate-controlling stage in the release process was diffusion of the dissolved drug through the gel network of Lutrol F127 to the external medium, which, in turn, explains the prolonged release of drug. ANOVA showed that there was statistically significant difference ($P<0.05$) in cumulative percent drug release due to three different gelling agents. The Student's *t* test ascertained that the drug release from Lutrol F127 and Xanthan gum gels till 8 h showed no significant difference ($P<0.05$) and then after there was a significant difference ($P<0.05$) among all the three formulations.

Microbiological Assay of Itraconazole

Table 7 manifested that the developed microemulsion-based gel with Lutrol F127 showed maximum zone of inhibition (the antifungal activity) among the tested gelling agents. ANOVA showed that there was significant difference among the formulations with three different gelling agents at $P<0.05$.

CONCLUSION

Microemulsion is a promising transdermal drug delivery vehicle. Microemulsion-containing ITZ was formulated for topical application. The components and their concentration ranges of oils, surfactants, and co-surfactants for the formation of microemulsion were screened using the construction of pseudoternary phase diagrams. Various gel bases containing ITZ microemulsion were studied for drug release, viscosity, primary skin irritation potential, and antimycotic activity of the topical formulations. Lutrol F127 gel showed the highest release of ITZ after 24 h (91.77%) followed by Xanthan gum gel (84.2%) and Carbopol 934 gel (61.95%). The optimum formulation of the microemulsion-based gel consisted of Capryol 90 9.44 %, Soluphor P/Transcutol P 54.28% (1:1), Lutrol F127 15.14%, and water. The addition of Lutrol F127 to the microemulsion resulted in the increase of the viscosity, which also acts as permeation enhancer. The results suggest that the studied microemulsion system may be an appropriate vehicle for the topical delivery of lipophilic antifungal agent ITZ. The developed system prolonged drug release up to 24 h and could, therefore, produce some benefits, such as reduction in total dose, frequency of administration, and dose-related systemic side effects. Considering *in vitro* release, appearance and esthetic attributes, skin irritancy, and microbial activity, the Lutrol F127 gel base containing 1% ITZ in microemulsion formula was the best among the studied formulations and it can be concluded that the developed microemulsion-based gel with Lutrol F127 has a great potential for the delivery of drug by transdermal route. Nevertheless, significant work still needs to be carried out to confirm these results and explore the tolerance of the vehicles in healthy patients and those with fungal infection.

Table 5: Skin permeation parameters

Parameters	F1	F2	F3
Permeation rate (J_{ss}) (μg/cm/h)	4.234	3.478	2.480
Lag time (T_{lag}) (h)	0.564	1.986	1.687
Permeability coefficient (kP) (cm/h)	0.0847	0.0696	0.05

Table 6: Kinetic parameters of itraconazole released from various microemulsion-based gels

Formulation	Kinetic order or model	Correlation (r^2)
F1-Lutrol F127	Zero order	0.973
	First order	0.970
	Koresemeyer–Peppas	0.892
	Higuchi diffusion	0.974
F2-Xanthan gum	Zero order	0.984
	First order	0.943
	Koresemeyer Peppas	0.886
	Higuchi diffusion	0.978
F3-Carbopol 934	Zero order	0.989
	First order	0.941
	Koresemeyer–Peppas	0.925
	Higuchi diffusion	0.932

Table 7: *In vitro* antimycotic activity of 1% itraconazole in different microemulsion-based gel formulations using Agar-cup method and *C. albicans* as test organism

Formulation	Inhibitor zone diameter (mm)			
	Test 1	Test 2	Test 3	Mean±SD
F1	15	12	14	13.667±1.527
F2	10	9	11	10±1
F3	9	8	6	7.667±1.527

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