SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 6 | Issue: 1 | January 2021 - Peer Reviewed Journal

FORMULATION AND EVALUATION OF MICROEMULSION BASED GEL OF POSACONAZOLE FOR TOPICAL DELIVERY

Dr.G.Jagadish[1]
[1]*Professor & Principal, Department of Pharmaceutics DBM College of Pharmacy, Janjgir Champa Chhattisgarh-495690*

Mrs.Rama Shukla[2]
[2]*Assistant Professor, Department of pharmaceutics,Lakshmi Narain College of Pharmacy, Raisen road,Bhopal-462022,M.P,India.*

Mrs.Purnima Shukla[3]
[3]*Assistant Professor, Department of pharmaceutics,Bansal College of Pharmacy,Bhopal*

ABSTRACT

Background: *Microemulsion is one of the promising sub-micron carriers for topical drug de- livery as it offers advantages like high drug-loading capacity and good skin penetration. Microemulsion based gel of posaconazole (broad-spectrum antifungal agent) could be an effective strategy for treatment of topical fungal infections.*

Objective: *To develop microemulsion based hydrogel of posaconazole was for effective treatment of skin mycosis.*

Methods: *Optimized microemulsion batches were selected through pseudoternary phase diagram (using oleic acid, tween 80 and IPA as oil, surfactant and co-surfactant, respectively) followed by stability stu- dies and characterization.*

Results: *The drug content and pH were found in the desired range. The droplet size of optimized formula- tions were found within the desired range (<200nm). The hydrogel prepared (from selected microemulsion batches) were found to have good spreadability and texture (adequate adherence). The in-vitro and ex-vivo studies exhibited effective VCZ flux from microemulsion based gel. The skin retention of the drug from F12-Me-Gel was significantly higher when compared with the microemulsion (F12-ME) as well as the con- ventional gel. Similarly, the diameter of the zone of inhibition (against Candida albicans) of F12-Me-Gel found to be higher than the microemulsion batch (F12-ME). Moreover, the skin irritation studies confirmed the benignity of the microemulsion based gel. Furthermore, the formulation was found to be stable at various temperatures (5 ± 3, 25 ± 2 and 40 ± 2 °C) as reported by the stability studies.*

Conclusion: *posaconazole loaded microemulsion based gel could be used effectively for the treatment of topical fungal infections.*

KEYWORDS: *Microemulsion, hydrogel, posaconazole, pseudoternary phase diagram, Candida albicans, sub-micron carriers, co-surfactant.*

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1. INTRODUCTION

Fungal infections are the most prevalent forms of skin in- fections and occur mostly in moist and humid climates. The leading cause of superficial infection is the opportunistic infection caused by *candida* spp [1].Though *Candida* is the leading cause of the superficial opportunistic infections but there is limited number of availability in the antimycotics therapy [2]. Azoles are the most commonly used antifungal agents in the treatment of both systemic and superficial infec- tion. Different types of azoles include itraconazole, flucona- zole, posaconazole and posoconazole. Among them posaconazole has exhibited higher potency in *in vitro* studies against broad range of yeast infections [3-4]. posaconazole (VCZ) is a broad spectrum triazole antifungal agent which is derived from fluconazole. It disrupts the fungal cell membrane by inhibiting 14α-methyl group of lanosterol which is an important step in the synthesis of ergosterol [5-6]. Recent clinical studies proposed topical VCZ as a new and promising therapy for fungal keratitis in combination with sys- temic VCZ [7-8]. As there is no topical formulation of voricon-azole available in the market, so the present study aims to de-velop effective topical delivery system of VCZ.

Topical delivery of the drug offers several advantages over other routes of administration like patient compliance, ease of administration, release of effective drug concentration at the target site, bypass of first pass metabolism and degra- dation of drug by gastric acid and juices as occurred in oral delivery as well as preventing contraindications of the drug with other drugs. Furthermore, the therapy can be withdrawn or removed when not desired or any adverse effect occurs, thus, increasing efficacy of the drug and reducing the side effects associated with intravenous and oral formulation such as nausea, headache, blurred vision, chills and convulsions [9].

However, the efficacy of drugs is adversely affected by the protective barrier of skin (statum corneum).To overcome the limited penetration of drugs through the stratum corneum, researchers have developed many formulation strategies (es- pecially *via* nanotechnology). Some of the drug delivery sys- tems includes microemulsions, liposomes, solid lipid nano- particles, microspheres, ethosomes, niosomes, dendrimers, microparticles and nanostructured lipid carriers [10].

Microemulsions are one of the interesting and promising sub-micron carriers for topical drug delivery. These are transparent, optically isotropic and thermodynamically stable dispersions of oil in water (o/w) or water in oil (w/o) in the presence of amphiphilic compounds (surfactant and co-surfactant) [11]. Microemulsions offer several advantages like high drug-loading and good skin penetration (by reduc- ing the diffusional barrier of the stratum corneum) with ac- ceptable biocompatibility (due to the presence of physiologi- cal lipids/oils) [12, 13]. Furthermore, microemulsions do not require any expensive sophisticated instruments for prepara- tion and therefore, the cost of preparation as well as time required for its preparation is less.

Recently, researchers successfully exploited microemul- sion based gels for transdermal delivery of several drugs like diclofenac, clobetasol, bifonazole, penciclovir, lacidipine, *etc*. [14-18]. Hence, this research deals with the development and characterization of VCZ microemulsion based hydrogel for improved transdermal delivery.

2. MATERIALS AND METHODS

2.1. Materials

VCZ was a gift sample from Lifecare Innovations Pvt. Ltd. (Gurgaon, India). Oleic Acid (OA), Isopropyl Myristate (IPM), tween 80, propylene glycol, cotton seed oil, castor oil, liquid paraffin, and span 80 were purchased from S.D. Fine Chemicals (Mumbai, India). Plurol oleique was a gift sample from Gattefosse India (Mumbai, India). All other chemicals used in the study were of analytical reagent grade.

2.2. Methods

2.2.1. Equilibrium Solubility Studies (Selection of Micro- emulsion Components)

The various components of microemulsion such as oils (captex 200, castor oil, sesame oil, oleic acid, cottonseed oil, liquid paraffin and IPM), surfactants (Tween 80 and Plurol oleique) and co-surfactants (IPA, butanol, propylene glycol) were selected to perform solubility studies of VCZ. The drug was added in excess to the excipients in tightly closed *via*ls and was kept at 37±2ºC under agitation in the water bath shaker for 72 hours for equilibration. The dispersions were centrifuged at 3000 rpm for 15 min and the supernatant was filtered through 0.45µm membrane filter. The amount of VCZ solubilized was analyzed using UV–visible spectrophotometer at 256 nm.

2.2.2. Pseudoternary Phase Diagram and Stability Studies

2.2.2.1. Construction of Pseudoternary Phase Diagrams

For construction of each phase diagram, oil and specific S_{mix} ratio (surfactant:co-surfactant) were mixed carefully in diverse weight ratios from. Initially, 5% w/w oil was added to the surfactant mixture with varied concentration (5% to 90% w/w) and the remaining concentration of water was added to make the dispersion 100%. This process was repeat- ed for the different oil concentration varied from 5-90 % w/w. The visual appearance of the homogenous mixture was noted down for clear and easily flowable o/w microemul- sions.

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2.2.2.2. Stability Evaluation of Microemulsion

2.2.2.2.1. Thermodynamic Stability

Thermodynamic stability of the prepared microemulsions at different concentration of Oil mixture and Smix was per- formed to overcome the problem of metastable formulations. Initial screening of microemulsions was performed on the basis of thermodynamic stability.

2.2.2.2.2. Heating Cooling Cycle

It was performed by keeping the microemulsions at two different temperatures *i.e.*, refrigerated temperature (4°C) and at the higher temperature (45°C). Six cycles were studied with storage at each temperature not less than 48 h. Those formulations, which were stable at these temperatures, were subjected to further analysis.

2.2.2.2.3. Centrifugation

Those formulations that passed the heating cooling cycle were then subjected to centrifugation test. The microemul- sions were centrifuged at 3500 rpm for 30 min. Those formu- lations that did not show any phase separation were taken for the freeze thaw stress test.

2.2.2.2.4. Freeze Thaw Cycle

Three freeze thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 h were per- formed for the microemulsions. This test was performed to see whether the microemulsions were stable at very low tem- perature and whether it comes back to the stable form after freezing it.

3. CHARACTERIZATION OF MICROEMULSION

3.1. Preparation of Drug Incorporated Microemulsion

The drug was accurately weighed (1%w/w) and dissolved in the mixture of oil and surfactant using vortex mixture and then the required water was added. It was left for 24 h to equilibrate and observed for appearance of visible changes (if any).

3.2. Globule Size and Polydispersity Index (PDI)

The average globule size and PDI of microemulsions were determined by photon correlation spectroscopy. Meas- urements were made using Zetasizer 1000 HS (Malvern In- struments, Worcester-shire, UK), wherein light scattering was monitored at 25°C at a 90° angle.

3.3. Transmittance and Conductivity

Transparency of microemulsion formulation was deter- mined by measuring the percentage transmittance at 650 nm with reference to purified water (taken as blank) using UV spectrophotometer (Shimadzu 1700, Japan). The electric concdutivity (σ) awsmeasruedby meansof a digital conduc- tivity meter (ELICO, CM 180) at 25 ± 0.5 °C.

4. FORMULATION AND CHARACTERIZATION OF MICROEMULSION BASED GEL

The selected microemulsions were incorporated in 1% w/v of gel Carbopol 934 to make their hydrogels formulation. Carbopol gel was prepared by adding carbopol hot boiling water. The gel was allowed to swell for 6-8 hours and thus kept overnight. The pH of the gel was made around 5.8 by adding triethanolamine dropwise. Then equal amount of mi- croemulsion (containing 2%w/v drug so that the final drug concentration becomes 1%w/v) and gel (1:1) was mixed thoroughly using glass rod.

4.1. pH and Drug Content

The pH of the microemulsions was recorded using a pH meter (CyberScan pH 51, Eutech Instruments). Drug content of microemulsions was analyzed by UV–visible spectropho- tometer (Shimadzu 1700, Japan) at 256 nm.

4.2. Rheology

The rheology of the prepared formulations was measured using cone and plate viscometer of Anton Par Rheoplus, d is0.149. Change in shear stress and viscosity with change in shear rate was recorded.

4.3. Spreadability

Spreadability of the sample was studied using glass plates (20 x 20 cm) of known weights with a graph paper of known diameter pasted on the back of one of the plates. 0.5 g of the formulation was kept on the centre of a glass plate below which a graph paper was kept. Another glass plate was placed over the formulation and the change in diameter was noted. Similarly, more plates were carefully placed on it to avoid sliding (that could lead to false result interpretations) and the change in diameter was measured again. The increase in diameter was observed after addition of each plate [19].

4.4. Texture Analysis

The texture (firmness and work of shear) of the developed formulations was evaluated using Texture Analyser TM (sta- ble micro systems ltd. UK) equipment equipped with a 5 kg load. It consists of two conical probes, upper and lower probe. Sample was placed into the lower cone carefully to eliminate the air pockets.

4.5. In Vitro Release Studies

In vitro release studies of the posaconazole incorporated in microemulsion based gel were performed using cellophane membrane (14000 Da) as diffusional barrier on the Franz diffusion cell assembly. The diffusional cross-sectional area of Franz diffusion assembly was 2.83 cm^2 and 30 mL

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was the volume of the receptor. The temperature of the assembly was maintained at 32 ± 1 °C with the help of thermo regulated outer jacket, while the diffusion medium was stirred continu- ously with the help of magnetic stirrer [20-21]. The receptor compartment contains media compromising 1% tween 80 phosphate buffer (pH 5.8). Tween 80 was added to maintain the sink conditions. 1g of the prepared formulations was ap- plied uniformly onto the membrane in the donor compart- ment. Stirring rate was controlled *via* magnetic stirrer and an aliquot of 1 mL sample was withdrawn at suitable time inter- vals and was replaced with the same amount of media to maintain the receptor medium volume to 30 mL. The samples were diluted with an equal volume of the diluents and were analysed UV spectrophotometrically at a λ_{max} of 256 nm.

4.6. Ex Vivo Study (Drug Permeation and Retention)

The *ex vivo* studies of the drug were also performed on the Franz diffusion cell. Drug permeation studies were car- ried out using excised abdominal skin of Wistar rats obtained from the Central animal house, Panjab University, Chandi- garh. The shaved abdominal skin was wiped off using disin- fectant (ethanol) to remove the adhering fat material and was further washed with lukewarm water to completely remove fatty material. The removed intact skin was mounted on the Franz diffusion cells with the stratum corneum side facing towards donor compartment and dermis side towards the receptor compartment. The receptor compartment having 30 ml of phosphate buffer saline was stirred continuously at ambient temperature. After specific intervals, 0.5 ml of sam- ples were withdrawn through sampling port of the receptor compartment and was replenished with the same volume of fresh phosphate buffer solution. All the withdrawn samples were filtered through 0.45 µ membrane filter and the amount of drug was analysed by HPLC at 256 nm. The amount of drug retained within the skin was determined using HPLC after rinsing and homogenizing the tissue.

4.7. *In Vitro* Antifungal Activity

4.7.1. Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the drug was de- termined by dilution method. The fungal inoculum was standard- ized by McFarland reagent having cell count of 10^6 cells/mL. The final concentration of inoculum should be 5×10^2 to 2.5×10^3 cells/mL for determining MIC and therefore it was diluted 10 times with sterile 0.85% saline (NCCLS). Then 100µL of the inoculum was inoculated in sterilised test tubes containing 1 mL of the drug solution and 8.9 mL of the Sabouraud Dextrose Broth (SDB). It was visually observed after 24 h and 48 h. The mini- mum concentration at which the growth of the fungus was inhib- ited was taken as MIC of the drug.

4.7.2. Determination of Zone of Inhibition by Agar Well Method

Sabouraud Dextrose Agar (SDA) was made and when the temperature reached around 40°C; the standardized inoculum was added so that the final concentration in the plate remained to be 10^3cells/mL. (SDA) was poured into the plates in the ster- ile conditions and was allowed to solidify. After solidification,

Fig. (1). Equilibrium solubility studies of VCZ in various non-aqueous components

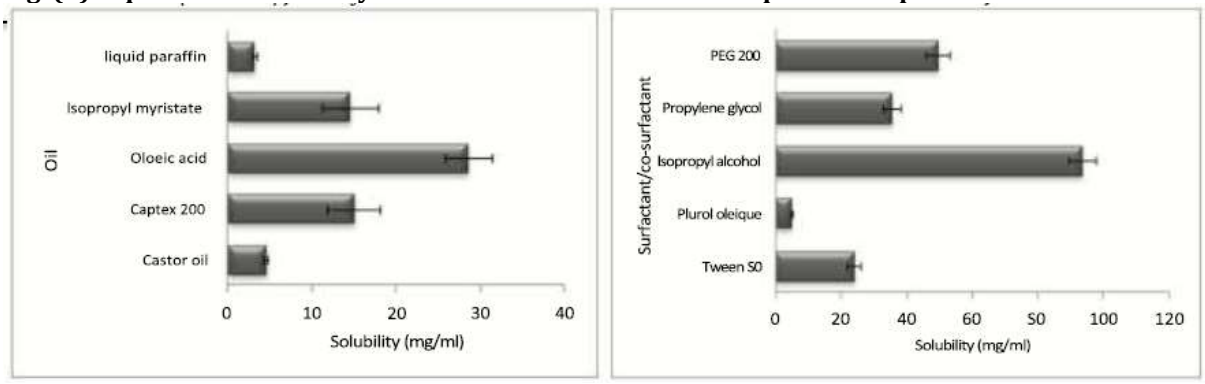

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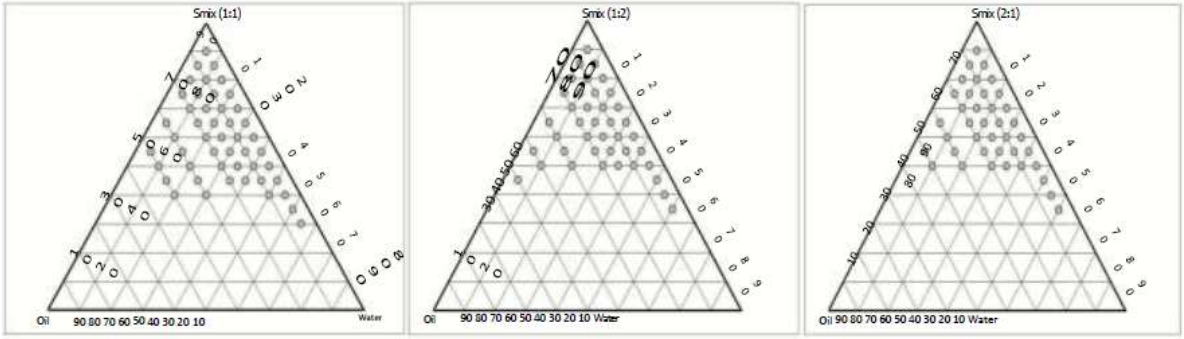
Fig. (2). Optimization of microemulsion region using pseudoternary phase diagram.



agar wells were made with a sterilised borer and the known and equal amount of formulations were added to each plate. The zone of inhibited growth by the formulation was measured after 24 h at 30°C.

4.8. Skin Irritancy Studies

Modified Draize tests were used to access the skin irritan- cy potential of the prepared formulation. The protocol of the experiment was approved by institutional animal's ethical committee, Panjab University, Chandigarh. The animals were kept under standard laboratory conditions, with temperature of 25 ± 1°C and relative humidity of 55 ± 5%. The animals were divided into three groups (each group contains 5 ani- mals). The rats were shaved 48 h prior to the treatment and
0.5 g of the optimised formulations was applied daily for seven days on hair free skin of rats by uniform spreading within the area of 4 cm^2. The skin was observed for any visi- ble changes such as erythema (redness) for four weeks after application of saline (group I), irritant (group II) and opti- mized formulation (group III). The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema [22].

4.9. Stability Studies

The optimized microemulsion based gel formulations were subjected to stability studies at different temperatures for a period of three months. Formulations were kept at different tempera- tures, 5 ± 3°C, 25 ± 2°C and 45 ± 2°C. All the formulations were tested for spreadibilty, colour, grittiness, pH and drug content after one month, two months and three months

4.10. Statistical Analysis

Simple analysis of variance (one-way ANOVA, GraphPad Prism 5) was used to determine statistically significant differ- ences between the results and values with $p<0.05$ were consid- ered statistically significant as analyzed by the Dunnett test.

5. RESULTS AND DISCUSSION

5.1 Solubility and Pseudoternary Phase Diagram

Based on the solubility studies (Fig. **1**), oleic acid (28.61±2.77 mg/mL), tween 80 (23.86±2.11 mg/mL) and IPA (93.68±4.22 mg/mL) were selected as oil, surfactant and co-surfactant, respectively. The o/w microemulsion was pre- pared by drop-wise addition of oil in the pre-mixed disper- sion of water, surfactant and co-surfactant.

Hydrophilic-Lipophilic Balance (HLB) of surfactant (non- ionic) plays an important role in the formation and stabilization of microemulsions as the value ranges from 3-6 for w/o micro- emulsion and 8-18 for o/w microemulsion. However, a blend of low and high HLB surfactants has been usually preferred for microemulsion preparation. Besides, addition of co-surfactant (like short chain alcohols) further enhances the stability as well as fluidity of the microemulsions by preventing formation of rigid structures like liquid crystals [23-24].

The phase diagrams were constructed comprising differ- ent S_{mix} ratio (tween 80: IPA) *i.e.*, 1:1 1:2 and 2:1 (Fig. **2**). These phase diagrams helped in determination/selection of microemulsion region with desired concentration range of the components (Oil, S_{mix} and water. Microemulsion points that passed thermodynamic stability tests were selected for fur- ther characterization and analysis.

5.2 Selection and Characterization

5.2.1 Selection of Microemulsion

Formulations that passed the thermo stability and dispers- ibility tests were selected for further evaluations. From these stable microemulsions, batches with lower Smix (surfactant and co-surfactant) concentration were selected from each pseudoternary diagram. These microemulsion batches were further characterized for globule size, drug content, visual evaluation and pH.

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5.2.2 Characterization of Microemulsion

The globule size was in the range of 33.29±3.42 to 317.3±4.83 nm as shown in Table **1**. The globule sizes were found to increase with increasing oil content and/or decreasing S_{mix} content (for each phase diagram). The PDI of microemulsion batches ranged from 0.296±0.132 to 0.844±0.068 *i.e.* consisted of batches having both narrow as well as broad particle distribution.

Table 1.Composition and characterization of microemulsions.

S.No.	Components (%v/v)				Zetasizer		Transmission (%)	Conductivity (µS)
	Smix	Oil	water	Smix	Size(nm)	PDI		
F1	1:1	5	65	30	33.29±3.42	0.729±0.123	96.83±2.23	115±2.31
F2		10	45	45	39.77±2.54	0.844±0.068	95.36±1.16	98±1.66
F3		15	35	50	91.76±1.92	0.795±0.12	82.5±2.25	82±3.21
F4		20	40	40	125.4±4.52	0.589±0.141	86.9±1.06	87±2.11
F5		30	25	45	145.8±3.23	0.532±0.073	20.46±2.33	112±3.44
F6	1:2	5	60	35	85.84±2.11	0.496±0.054	98.99±0.46	98±2.16
F7		10	40	50	104.9±3.15	0.82±0.021	92.46±1.34	90±3.22
F8		15	35	50	143.1±3.56	0.342±0.021	92.75±2.17	82±1.06
F9		20	25	55	317.3±4.83	0.85±0.131	86.92±0.78	76±1.33
F10		30	20	50	236.5±3.42	0.791±0.153	29.65±2.23	72±2.31
F11	2:1	5	65	30	124.5±2.54	0.531±0.048	99.34±0.16	106±2.22
F12		10	45	45	69.85±3.72	0.296±0.132	99.26±0.15	88±3.37
F13		15	35	50	113.7±3.51	0.436±0.121	93.92±1.36	84±2.46
F14		20	20	60	163.8±2.23	0.745±0.063	96.44±2.33	75±3.22
F15		30	20	50	143.4±2.47	0.479±0.044	67.31±2.46	70±2.26

Table 2.Characterization of microemulsion based gel.

Batch	Spreadability (%)	Texture		pH	Drug content (%)	*In vitro* flux (µg/cm^2.h)
		Firmness (g)	Work of shear (g.sec)			
F6	233.33±6.32	1482.43±6.23	305.43±3.45	5.45 ±0.04	98.61± 0.58	5.43±0.39
F8	227.78±4.55	1514.16±5.55	328.61±4.22	5.58 ±0.13	98.65 ±0.90	3.69±0.34
F12	233.33±3.74	1596.32±4.39	358.85±3.21	5.68 ±0.24	99.50 ±0.17	5.93±0.77
F13	250±5.22	1428.17±5.67	302.75±2.78	5.69 ±0.12	98.82 ±0.72	3.46±0.54

The transmittance of the formulations ranged from 20.46±2.33 to 99.34±0.16% while the conductivity was found between 70±2.26 and 115±2.31 (suggesting the pres- ence of o/w microemulsions).

With respect to the various formulation aspects, lower size (< 200 nm), narrow size distribution (< 0.5) and higher clarity (%T > 90) were considered as the primary criteria for the selection of batches (*viz*. F6, F8, F12 and F13) for prepa- ration of microemulsion based gel.

5.2.3 Characterization of Microemulsion Based Gel

The pH of the microemulsion based hydrogels was be- tween 5.68 and 5.87 which is similar to the pH of skin sur- face (pH=5.5) that might prevent the occurrence of any irrita- tion or caustic reaction. The rheogram of the formulations indicates the pseudoplastic (shear thinning) behaviour as the consistency/viscosity of the formulation decreases with in- creasing shear rate (Fig. **3a**). Such properties are highly bene- ficial for transdermal system as they facilitate easy applica- tion of the formulation on the skin. Further, the high spreada- bility values (Table **2**) supported the pseudoplastic properties of microemulsion based gels.

The firmness values of the formulations were in the range of 1482.43±6.23 to 1596.32±4.39 g and work of shear was found between 302.75±2.78 to 358.85±3.21 g.sec (Table **2**). The high value of firmness indicates that the formulation will be adhered to the skin appreciably and for longer period which would help to deliver the drug to the skin for longer duration (Fig. **3b**).

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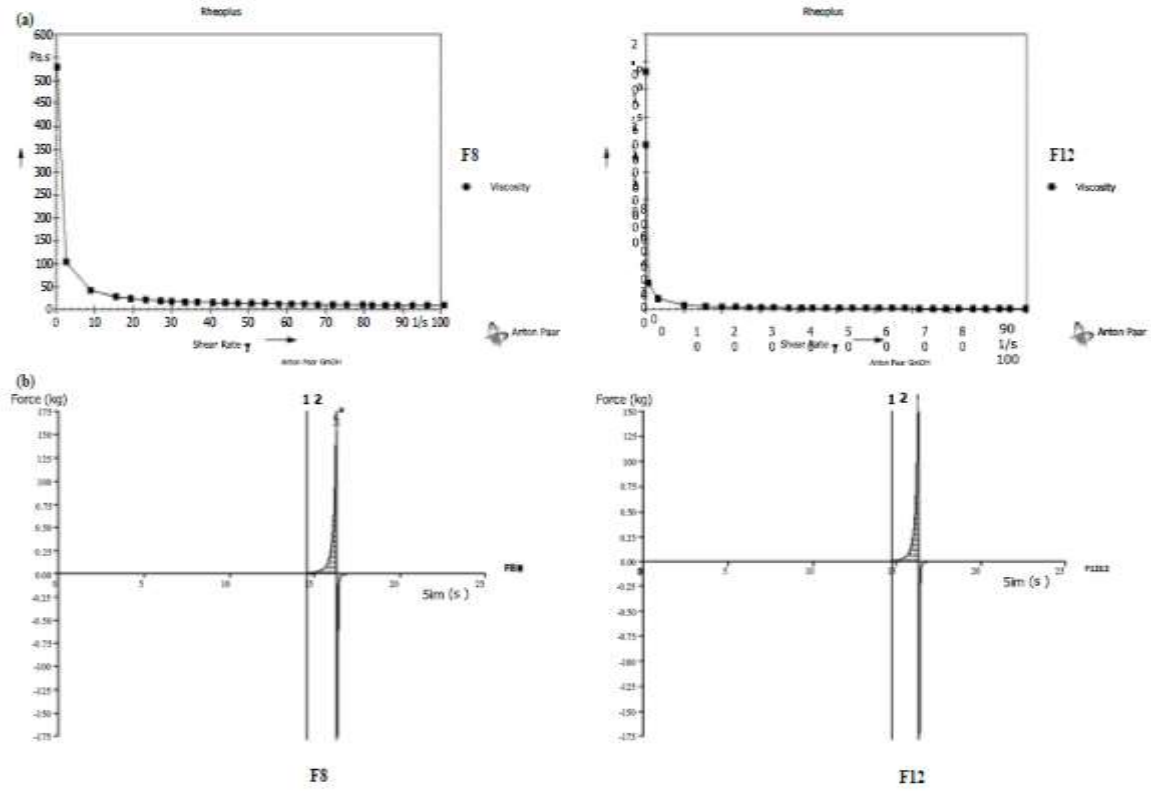

Fig. (3). (a) Pseudoplastic behaviour (b) Texture analysis of microemulsion based gels (F8 & F12).

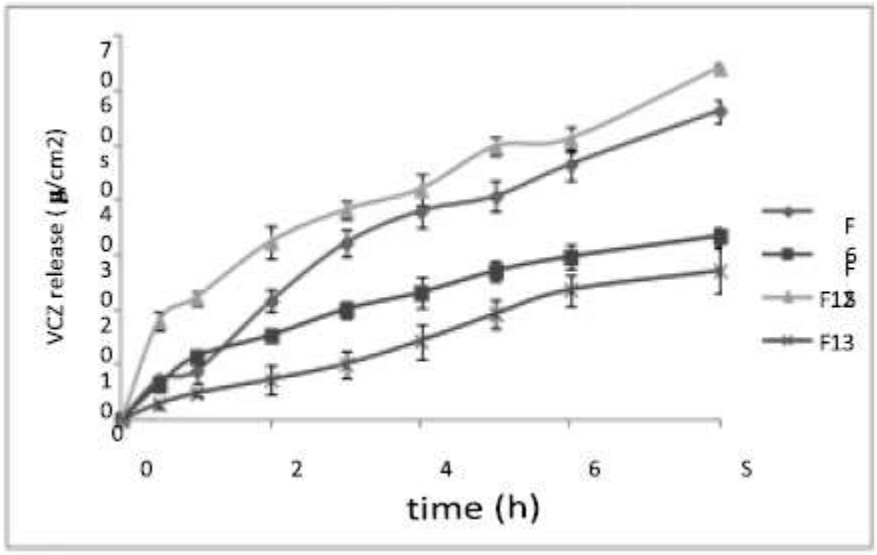

Fig. (4). In vitro release studies of microemulsion based gels

The content of the drug in the microemulsion based gels formulations were found in the range of 97.81 ± 1.53 % to 99.08 ± 0.63 (Table **2**). This indicates a higher loading of drug in prepared formulations.

5.3 In Vitro Drug Release Studies

The permeation flux was calculated from the slope of the linear graph plotted between drug released per unit area vers- es time (Fig. **4**). The area of contact between the membrane and the formulation was 2.8 cm^2. The formulation F12 (5.93±0 .77 µg/cm^2.h) showed comparatively higher permea- tion flux in contrast to F6 (5.43±0 .39 µg/cm^2.h), F8 (3.69±0 .34µg/cm^2.h) and F13 (3.46±0 .54 µg/cm^2.h).

Hence, based on good spreadability, texture, drug

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content and *in vitro* permeation flux, formulation F12 (microemul- sion based gel) was selected as the optimized batch for per- forming *ex-vivo* and irritation studies.

5.4 Ex Vivo Skin Permeation Studies

Atsatitsically isgnificant ($p \leq 0$ .0 5) iksn permeation flux of F12-ME-Gel (12.61±0 .53µg/cm^2.h and F12-ME (15.0 1±0 .85 µg/cm^2.h) was found when compared with con- ventinal gel (0 .62±0 .23 µg/cm^2.h) of VCZ (Fig. **5**). The lower flux of microemulsion based gel in comparison to the ME could be due to the good adhesive property of the gel that resulted in tighter contact with the skin and hence, re- leased lesser extent of the drug [21]. Further, the meagre flux of the conventional gel may be due to the poor penetration of the drug into the stratum corneum.

The skin retention of VCZ was found significantly higher ($p \leq 0.05$) for F12-ME-Gel (41.83±0.49%) followed by F12-ME (25.7±0.68%) and conventional gel (5.83±0.11%). For the treat- ment of topical infections, the retention of drug is an important parameter for better efficacy of the drug as it ensures higher con- centration of drug in the skin for longer duration.

Sahoo and co-workers exhibited improved skin retention of sertaconazole from the hydrogel of microemulsion [13]. Similar studies were conducted by Hashem and co-workers wherein microemulsion gel form displayed better retention of clotrimazole as compared to the liquid microemulsion [21].

5.5 In Vitro Antifungal Activity

The MIC of VCZ against *candida albicans* was found to be 4µg/mL. It is the minimum concentration of the drug in which the growth of the microorganism is inhibited.

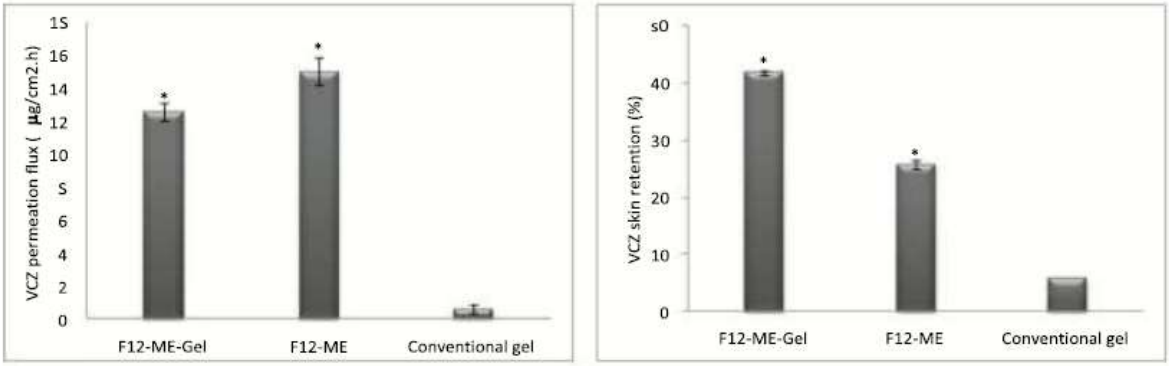

Fig. (5). *Ex vivo* studies of microemulsion based gels (a) permeation flux (b) skin retention (*P ≤ 0.05, statistically significant as analyzed by Dunnett test).

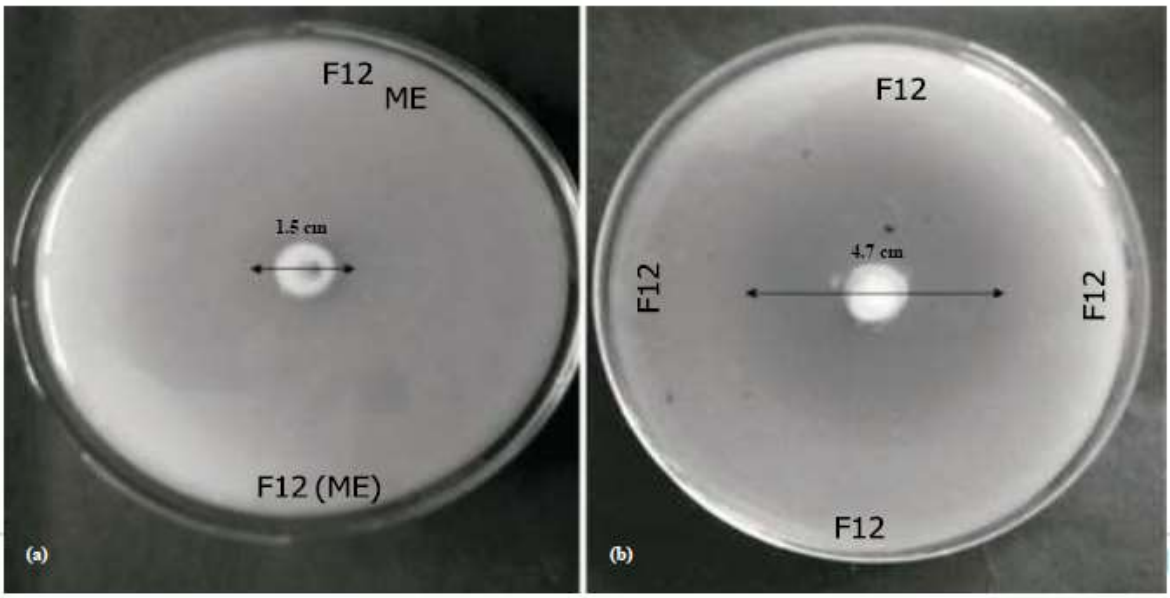

Fig. (6). Zone of inhibition of (a) F12-ME (b) F12-ME-Gel against Candida albicans.

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Table 3. Mean erythema scores of Wistar rat skin on various groups (n=5).

Days	Mean erythema score		
	Group I	Group II	Group III
1	0	0	0
2	0	0	0
3	0	1	0
4	0	1	0
5	0	2	0
6	0	2	0
7	0	3	0

0 (No erythema); 1(Light erythema); 2 (Moderate erythema); 3 (Moderate to severe erythema); 4 (Severe erythema)

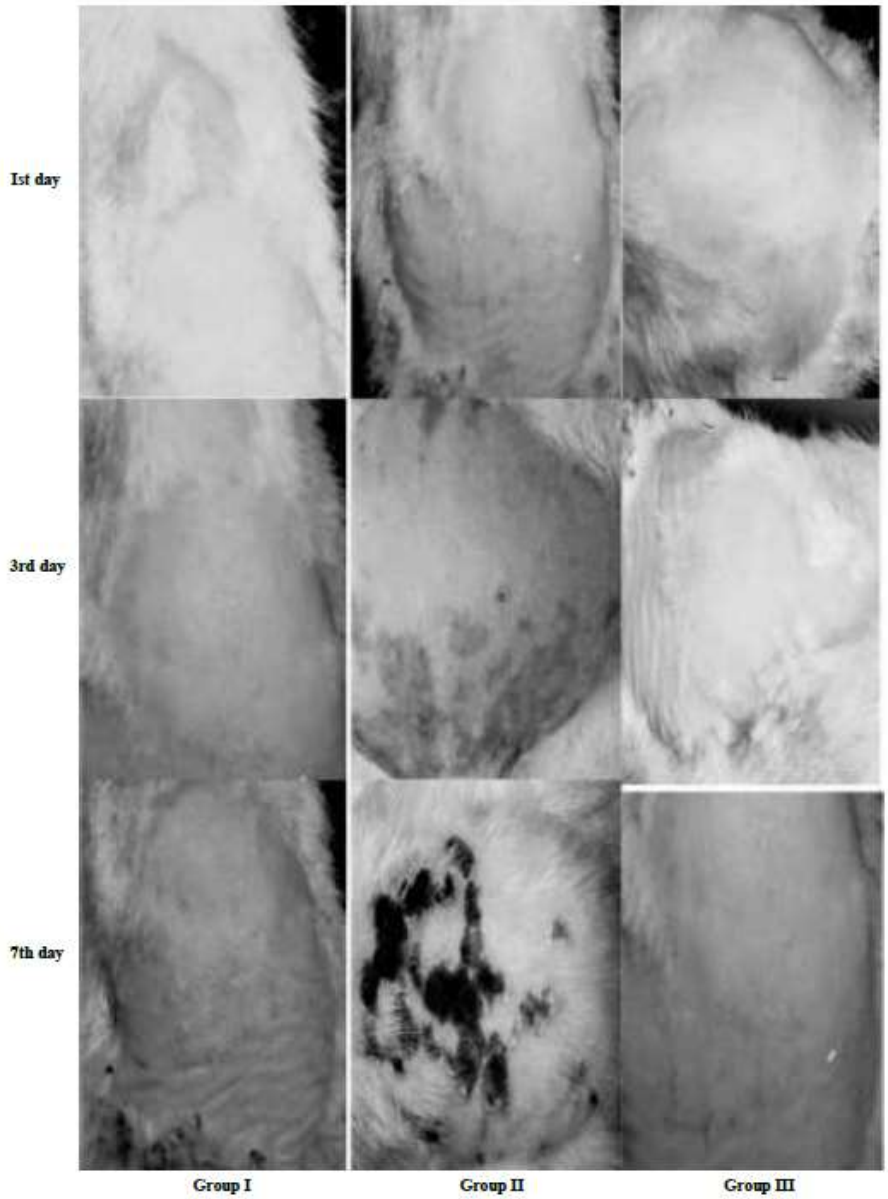

Fig. (7). Skin irritancy studies on Wistar rats.

The comparison of the efficacy of F12-ME- Gel and F12- ME was done by measuring the zone of

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inhibition of fungal growth as shown in Fig. (**6**). The formulations were poured in the well made in SDA plates and was allowed to diffuse for an hour and different inhibition zones were observed after 24 hours incubated at 30°C. A larger zone of inhibition of F12-ME-Gel (4.73±0.23 cm) was found in comparison to the F12- ME (1.53±0.06 cm) that indicates a higher efficacy of the microemulsion based gel formulation. It could be attributed to the poor diffusion of the drug into the agar plate from the microemulsion, while on incorporation into gel enhances its tendency of diffusion which makes it more effective.

Table 4.Stability studies of optimized formulation (F12-ME-Gel).

Temperature (^{O}C)	Time (month)															
	Spreadability (%)				pH				Drug content (%)				Colour & grittiness			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
5 ± 3	233.33 ± 3.74	222.22 ± 4.21	222.22 ± 2.75	216.67± 3.21	5.68 ± 0.24	5.64 ± 0.22	5.63 ± 0.21	5.63 ± 0.18	99.50 ± 0.17	98.84 ± 0.36	98.68 ± 0.23	98.37± 0.04	W & N	W & N	W & N	W & N
25 ± 2	233.33 ± 3.74	233.33 ± 3.22	244.44 ± 5.06	255.55± 2.77	5.68 ± 0.24	5.68 ± 0.32	5.68 ± 0.12	5.63 ± 0.22	99.50 ± 0.17	98.83 ± 0.68	98.73 ± 0.43	98.34± 0.91	W & N	W & N	W & N	W & N
45 ± 2	233.33 ± 3.74	244.44 ± 2.54	250.11 ± 3.22	261.11± 4.56	5.68 ± 0.24	5.61 ± 0.11	5.52 ± 0.21	5.45 ± 0.34	99.50 ± 0.17	98.25 ± 0.78	98.15 ± 0.38	98.06± 0.43	W & N	SY & N	SY & N	SY & N

W = White; SY = Slightly yellow; N = Non-grittiness

5.6 Skin Irritation Studies

Unlike irritant (group II), no irritation was observed on the rats treated with topical application of control (group I) F12-ME-Gel (group III) until 7 days (Fig. 7). The erythemat- ic score of prepared formulation was zero as compared to the irritant (score=3) (Table 3). This confirmed the non-irritant nature of the formulation (F12-ME-Gel).

5.7 Stability Studies

Stability studies of the F12-ME-Gel were carried for three months at three different temperatures i.e., 5 ± 3oC, 25 ± 2oC and 45 ± 2oC. The formulations were tested after 1, 2 & 3 months for various parameters (Table 4). The formulations were found to be stable at different temperatures for three months.

There was no significant difference in the spreadibility as well as pH of the formulation. Also, the drug content of the formulation was found almost similar at all temperatures which suggested the absence of any drug degradation at these conditions.

The formulations were opaque and white at refrigerated temperature and room temperature after 3 months while a slightly yellowish colour of the formulation was observed at 45oC. It may be attributed to the change in the physical- chemical properties of any excipients at higher temperature.

CONCLUSION

In the present study, the application of microemulsion systems in the gel form for the topical delivery of posaconazole was studied. Pseudoternary phase was used to optimize the formulations and selected formulations were evaluated. The results suggested that the skin permeation/retention properties of microemulsion have been increased by incorpo- rating microemulsion into gel form. Compared with conven- tional gel as well as microemulsion (F12-ME) of the posaconazole, the prepared formulation (F12-ME-Gel) has better permeation as well as retention properties. The results of *in vitro* antifungal activity of posaconazole against *candida albicans* inferred that the microemulsion based gel is more efficacious in contrast to microemulsion and conventional gel. Furthermore, the formulations were found to be non-irritant. Hence, the prepared formulation (microemulsion based gel) could be used effectively for the treatment of cu-taneous fungal infections effectively.

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HUMAN AND ANIMAL RIGHTS

No humans were used in this study, all animal reported experiments were in accordance with the standards set forth in the 8th Edition of Guide for the Care and Use of Laborato- ry Animals (http:// grants.nih.gov/grants/olaw/Guide-for-the- care-and-use-of-laboratory-animals.pdf) published by the National Academy of Sciences, The National Academies.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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