Asian Journal of Pharmaceutics

Original Research Articles

MICROPARTICULATE DRUG DELIVERY SYSTEM FOR TOPICAL ADMINISTRATION OF ITRACONAZOLE

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ABSTRACT

The purpose of the present study was to formulate microemulsion based gel for topical delivery of water insoluble antifungal agent Itraconazole with an aim to increase its penetration through skin and thereby its flux. Pseudoternary phase diagrams were constructed to identify microemulsion existing region using various combinations of surfactant and cosurfactant with oil and water. Solubility of drug in various oils was determined to select the suitable oil having maximum solubilizing capacity for Itraconazole. Different formulations so formed were evaluated for phase separation, isotropic nature, clarity and particle size analysis and optimized formulations were selected for formulating microemulsion based gel using 0.75% w/w of carbopol 934P NF. The microemulsion-based gels were evaluated for rheological behavior, in-vitro permeation studies and in-vitro antifungal activity. The in-vitro permeation studies was carried out on human cadaver skin, mounted on Keshary-Chien diffusion cell using 10% v/v methanolic solution of pH 1.2 phosphate buffer as diffusion medium and Candida albicans as a model fungus to evaluate the antifungal activity of Itraoconazole through the optimized formulations using cup plate method. Statistically significant increase in in-vitro permeation rate was found among the laboratory microemulsion based gel formulated when compared with conventional cream formulation. The rheological behavior of the prepared systems showed pseudoplastic (shear thinning) flow pattern. The in-vitro antifungal activity of Itraconazole was found to be significant with microemulsion based gel. Thus it can be concluded that microemulsion based gel is better choice of vehicle for delivery of Itraconazole as topical drug delivery system.

Keywords: Itraconazole, Microemulsion based gel, In-vitro permeation, Antifungal activity

INTRODUCTION

Most pharmaceutical drug substances are lipophilic compounds, which are practically insoluble in water[1]. For skin care and the topical treatment of dermatological disease, a wide choice of vehicles ranging from solids to semisolids and liquid preparations is available to clinicians and patients[2]. Topical application of antimicrobial agents is a useful tool for the therapy of skin and soft-tissue infections[2]. Itraconazole is synthetic triazole and 1:1:1:1 racemic mixture of four diastereoisomers (two enantiomeric pairs), each possessing 3 chiral centres. The structural formula is closely related to the imidazole, ketoconazole. Itraconazole is drug of choice for patients with indolent, non meningeal infections due to B. dermatitidis, H. capsulatum, P. brasiliensis and C. immitis. Approximately half of the patients with distal subungual onychomycosis respond well to Itraconazole. Itraconazole is often the best choice for the treatment of pseudallescheriasis, an infection not responding to the amphotericin B therapy., as well as cutaneous or extra cutaneous sporotrichosis, tinea corporis, and extensive tinea versicolor. Itraconazole is used in the treatment of toenail onychomycosis with terbinafine as one week per month for three months.

Microemulsion has been recognized as a good vehicle for the transdermal delivery of drugs[4]. It is defined as an O/W or W/O emulsion producing a transparent prod-

uct that has a droplet size <0.15 nm and does not have a tendency to coalesce[5, 6, 7]. Hoar and Schulman[8] were the first to introduce the word microemulsion, which they defined as a transparent solution obtained by titrating a normal coarse emulsion with medium-chain alcohols. The short to medium-chain alcohols are generally considered as cosurfactants in the microemulsion system[9]. Several mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of drugs. First, a large amount of drug can be incorporated in the formulation due to the high solubilizing capacity, with increased thermodynamic activity towards the skin. Second, the permeation rate of a drug from microemulsion may be increased, since the affinity of the drug to the internal phase in microemulsion can be easily modified, to favor partitioning into the stratum corneum, using different internal phases and changing the composition of the microemulsion. Third, the surfactant and cosurfactant used in the microemulsion may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers[10].

In this study, an optimum O/W microemulsion containing Itraconazole was developed after screening various oils to improve the drug solubility and the skin permeability.

MATERIAL AND METHODS

Itraconazole was obtained as gift sample from Glenmark Pharmaceuticals Ltd, Nashik, Captex 500, 355, 200 and Capmul MCM were obtained as gift samples from Abitec corporation, US, Oleic acid, Isopropyl alcohol, Tween 20, Tween 80, were purchased from S.D. Fine chemicals and all the other chemicals used were of analytical grade.

Selection of oils for microemulsions

To find out appropriate oils that have good solubilizing capacity of Itraconazole, the solubility of Itroconazole in various oils was measured. The oils investigated were soybean oil, olive oil, oleic acid, Captex 200, 355, 500 and IPM. An excess amount of ketoconazole was added to 5 ml of each selected oil and was shaken reciprocally at 20°C for 24 hrs. The supernatant portion of the supersaturated solution was carefully withdrawn and suitably diluted, and solubility of Itraconazole was determined using UV-VIS spectroscopy at 225 nm.

Preparation of Pseudoternary Phase Diagram for Determining Microemulsion Existing Region

The pseudo-ternary phase diagrams were constructed using water dilution method. Captex 200 and Captex 355 were used as the oil phase, tween 80 as the surfactant and Capmul MCM as the cosurfactant. Phase diagrams were prepared for different surfactant/cosurfactant ratios (S/C_oS) at 2/1, 3/1, 1/1 and 1.5/1. For each phase diagram at specific S/CoS, mixtures of the oil, the surfactant and the cosurfactant were prepared, and the mixture was diluted with water by sequential addition of 10µl of water using a micropipette. Water was added drop by drop while mixing on a magnetic stirrer 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 i.e. oil, water and surfactant and cosurfactant were calculated.

Preparation of O/W microemulsions loaded with Itraconazole

Appropriate quantities of surfactant, cosurfactant and oil, were weighed into a screw-capped glass vial Itraconazole was dissolved in a concentration of 2% w/w in the oil being used. The mixtures were stirred with a magnetic bar, at room temperature with continuous addition of weighed amount of water, until the formation of a transparent system.

Characterization of microemulsion systems

Freeze thaw cycle

This test places stress on the microemulsion, at temperature below freezing. Six heating/ cooling cycles between 45°C and refrigeration temperature with storage at each temperature for not less than 48 hours, were carried out for the selected microemulsions.

Centrifugation

Microemulsion systems were subjected to centrifugation at 3000 rpm for 30 minutes and then examined for any phase separation.

Measurement of droplet size

The mean diameter of the microemulsions were measured, at 20°C, using a Zeta sizer Nano- ZS (Malvern Instruments, Worcestershire, UK).

Formulation of microemulsion based gel

The formulations showed stability for the above parameters were selected for further formulation of microemulsion based gel. Microemulsions have low vis-

cosity and are difficult to apply on the skin they should be gelled with suitable gelling agents. A weighed amount of carbopol 934P was soaked in the microemulsion system, stirred to disperse the polymers in the microemulsion and left over night for gelling. To this the required quantity of triethanolamine was added till pH of formulation is adjusted to 7 and a clear gel is formed, and for neutralizing the carboxylic acid groups in carbopol.

Evaluation of microemulsion based gel

Rheological studies

The different gel bases were tested for their rheological characteristics at 25°C using Brookfield viscometer (Model DV-III, Brookfield Engineering Laboratories, USA). Viscosity measurements were carried out using a Brookfield Digital Rheometer at different shear rates. 5 ml of microemulsion based gel was filled in the cylindrical type of spindle SC4-16.

In vitro skin permeation study

The in-vitro permeation studies for the formulations were carried out using Keshary-chein diffusion cell. The human cadaver skin was washed and soaked for 24 hrs in 0.9% NaCl solution and then mounted on the Keshary-chein diffusion cell. The assembly was thermostated by circulating warm water at $37+1^0$ C in the external jacket of Keshary-chein diffusion cell to simulate the body temperature. The surface area available for diffusion was calculated and was found to be 1.77 cm^2. The formulation equivalent to 30 mg of drug from marketed as well as laboratory formulations were placed uniformly on the epidermal surface of skin in the donor compartment, while the receptor compartment contained 10% v/v of methanolic 0.1 N HCl solutions. 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 hours and replaced immediately with the same volume of the diffusion medium.

In vitro antimicrobial activity[11, 12, 13, 14]

Suspension of Candida 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 8mm and one gram each of laboratory and marketed formulations containing 2 % of Itraconazole were poured into wells. These plates were kept at 4^0 C for 1 hour. After one hour plates were incubated at $37 + 1^0$ C for 24 hrs. the mean zone of inhibition of Itraconazole released form laboratory and marketed formulations was calculated in mm. Statistical analysis using one way ANOVA test followed by Dunnet's comparison was used to compare difference in permeation within the formulations.

RESULTS AND DISCUSSION

Solubilities of Itraconazole in various oils

The solubilities of Itraconazole in various oils at room temperature are presented in Table 1. Oils showing satisfactory solubility were chosen as the oil phase for formulating microemulsion systems.

Phase behaviour

A pseudoternary phase diagram as shown in figure was constructed to determine the composition of an aqueous phase, an oil phase, and surfactant, cosurfactant systems that will yield a stable isotropic microemulsion. Using various ratios of surfactant to cosurfactant microemulsion existing region on phase diagram was obtained, and all the formulations obtained were evaluated for clarity, flowability, phase separation, and particle size analysis. The optimized microemulsion formulations, which showed consistent stability for the parameters evaluated, were selected for further studies. The % w/w concentration of each component in microemulsion systems selected for further studies is given in table

Measurements of droplet size

The droplet size for the formulations was found to be in the range of 65-200nm range.

Rheological behaviour of all microemulsion based gel systems

Viscosities for all microemulsion based gel systems were measured at seven different shear rates (rpm) at room temperature. The shear viscosity decreases with shear rate for all the formulations. This indicates that the samples undergo shear thinning. The rheological behavior of thixotropic pseudoplastic systems generally typical for pharmaceutical gel systems was observed in all the laboratory formulations. However it is needless to say that thixotropy is a desirable characteristic of pharmaceutical gels, no thixotropic studies were carried.

In vitro skin permeation study

Drugs can permeate through the stratum corneum but two micro pathways; one is intracellular and other transcellular. Of these routes the intracellular route plays a major role in the percutaneous absorption of the drugs. To explain the probable mechanism by which microemulsion based enhances release and percutaneous absorption rate efficiently, the physicochemical properties of the microimulsion and that of stratum corneum must be taken into consideration. Most probably the nanosize oil globules embedded with the drug freely enter the strata of skin and alter both lipid and polar pathways. The lipophilic domain of the Microemulsion can interact with the stratum corneum in many ways. The drug dissolved in the lipid domain of the microemulsion can directly partition into the lipids of stratum corneum, thereby destabilizing its biayer structure. On the other hand the lipophilic domain of Microemulsion can hydrate the stratum corneum increasing the intracellular volume, resulting in the disruption of its interfacial structure. Swelling of the proteins cause disruption of the lipid layers resulting in the enhanced permeation of the lipophilic drugs.

Interestingly, in-vitro release data studied using percutaneous absorption of formulations through human cadaver skin mounted on the Keshary-chein diffusion cell were found to be superior in microemulsion formulations, compared to the marketed formulations. Maximum drug permeation and several times improvement in the drug release profile of laboratory formulations were achieved in comparison to marketed formulations.

***In vitro* antimicrobial activity**

The anti-fungal activity for Itraconazole manifested that the mean zone of inhibition of the laboratory microemulsion formulations was larger than that of the reference marketed formulations.

Table 1: Solubility of Itraconazole in various oils at 20^{0}C

Oil	Solubility (mg/ml)
Oleic acid	46.32
Isopropyl myristate	2.117
Captex 200	4.712
Captex 355	1.121
Captex 500	11.517
Olive oil	16.308

Table 2: Compositions of microemulsion systems selected for further studies (%w/w)

Ingredients	Formulation code	
	K_1	K_2
Itraconazole	2	2
Captex 200	25	-----
Captex 355	-----	20
Tween 80	30	41.25
Capmul MCM	15	13.75
Carbopol 934P	0.8	0.8
Triethanolamine	0.75	0.65
Disodium edetate	0.15	0.15
Water	Upto 100	Upto 100

Table 3: *In vitro* permeation parameters for Itraconazole

Formulations	Permeation parameters			
	Flux (J_{ss})	Lag time (L_t)	Permeation coefficient	Diffusion coefficient
K1	0.51+0.015	0.365	0.017	6.07E-06
K2	0.516+0.013	0.439	0.017	7.31E-06
Marktd form	0.399+0.018	0.416	0.013	6.93E-06

Table 4: ANOVA table for the flux values of different formulations of Itraconazole

ANOVA	Sum squares	Degree of freedom	Mean sum squares	F - value
Treatment(between columns)	0.00005234	2	0.00002617	25.52
Residual (within columns)	0.000006154	6	0.000001026	
Total	0.00005850	8		

Table 5: Antimicrobial activities for Itraconazole

	Zone of inhibition (mm)			
Formulation	1	2	3	Mean
K_1	9	8	9	8.66
K_2	10	10	9	9.66
MF (Keto)	4	6	5	5

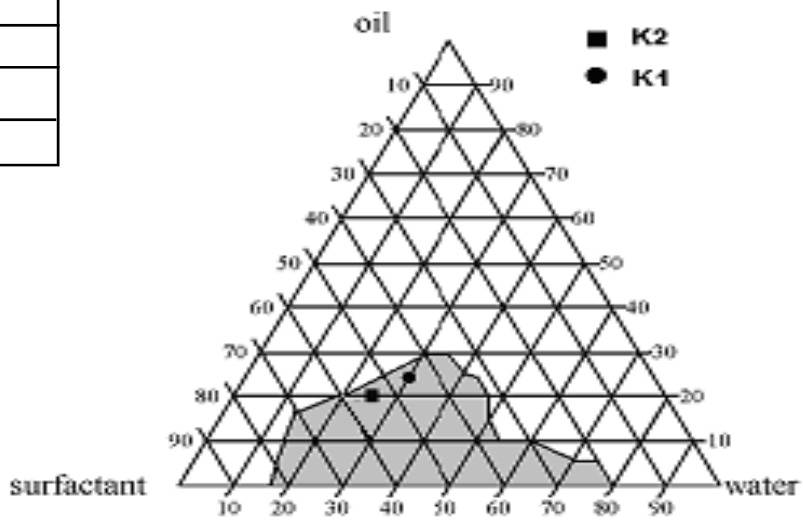

Figure1: Pseudoternary phase diagram for determining microemulsion existing region

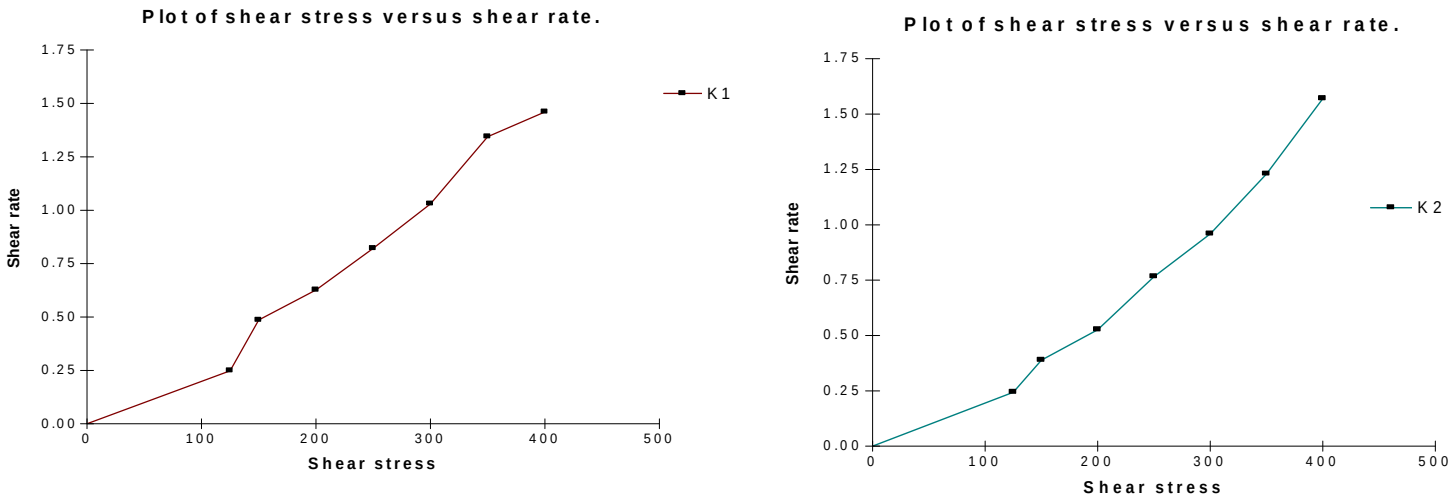

Figure 2: Rheological parameters of different formulations at room temperature

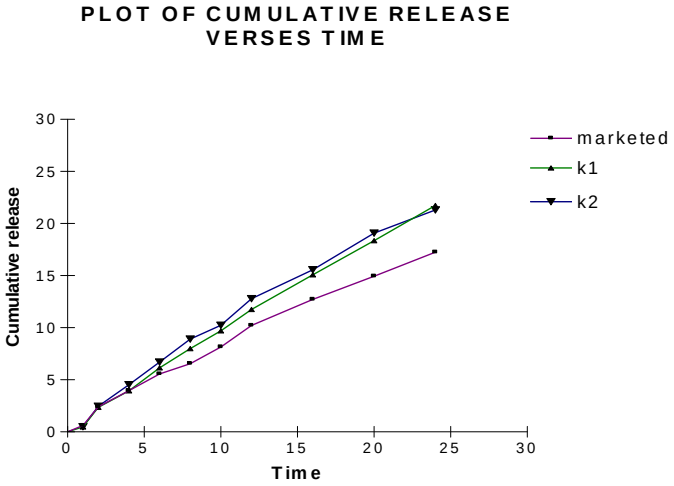

Figure 3: Cumulative Release per unit area of various formulations of Itraconazole through microemulsion and marketed formulation

REFERENCES

1. Puranajoti P, Patil RT, Sheth PD, Bommareddy G, Design and Development of Topical Microemulsion for Poorly Water-Soluble Antifungal Agents. J. Of Applied Research, 2002, 2, 1-12.

2. El laithy HM, El-Shaboury KMF, The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole. AAPS Pharm Sci Tech, 2002, 3 (4), 35.

3. Hardman JG, Limbird LE, Goodman and Gilman's The Pharmacological Basis of therapeutics, 10th Edn, 2001, 1301, 1302, 1812.

4. Swarbrick I, Boylar JC, Encyclopedia of Pharmaceutical Technology, Marcel Dekker, Inc. New York, 1992, 9, 375-471.

5. Lalwani AN, Shah TJ, Parmar NS, In: Jain NK, (Ed.), Progress in Controlled Drug Delivery Systems, CBS Publishers, 2004, 309-341.

6. Shaji J, Reddy SM, Pharma Times, 2004, 36, 17-23.

7. Swarbrick I, Boylar JC, Encyclopedia of Pharmaceutical Technology, Marcel Dekker, Inc. New York, 1992, 9, 375-471.

8. Hoar, TP, Schulman JH, Transparent water-in-oil dispersions: The oleopathic hydromicelle. Nature, 1943, 102, 152.

9. Lalwani AN, Shah TJ, Parmar NS, In: Jain, N. K., (Ed.), Progress in Controlled Drug Delivery Systems, CBS Publishers, 2004, 309-341.

10. Park ES, Yun BL, Ko In-Ja and Chi SC, Transdermal Delivery of Piroxicam Using Microemulsions, Arch. Pharm. Res., 2005, 28 (2), 243-248.

11. The Indian Pharmacopoeia. Vol. I, Ministry of Health and Family Welfare, Govt. of India, Controller of Publications, Delhi, 1996, A-100 to A-110.

12. Foroutan SM, Hossein-Ali E, Torabi HR, Formulation and In Vitro Evaluation of Silver Sulfadiazine Spray. Iranian Journal of Pharmaceutical Research, 2002, 1, 47-49.

13. Bou-Chacra NA, Gobi SS, Ohara MT, Antimicrobial activity of four different dental gel formulas on cariogenic bacteria evaluated using the linear regression method. Brazilian J. Pharm. Sci., 2005, 41 (3), 323- 331.

14. El laithy HM, El-Shaboury KMF, The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole. AAPS Pharm Sci Tech, 2002, 3 (4), 35.