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Research Article

Preparation and Evaluation of Fluconazole Topical Microemulsion

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ABSTRACT

A fluconazole w/o microemulsion was developed for topical application using isopropyl myristate as the oil phase. Pseudo-ternary phase diagrams were constructed to determine the microemulsion existence region using surfactant (tween 80) and co-surfactant (polyethylene glycol 400). Different formulations were prepared to evaluate the effect of oil content, surfactant/ co-surfactant concentration on in-vitro permeation rates. In-vitro transdermal permeability of fluconazole from the microemulsions was evaluated using Keshary Chien diffusion cells mounted with 0.45µ cellulose acetate membrane. The amount of fluconazole permeated was analyzed by HPLC. The permeability of the fluconazole incorporated into the microemulsion systems was 2.5 - fold higher than that of the marketed formulation. These results indicate that the microemulsion system studied is a promising tool for the percutaneous delivery of fluconazole.

Key words: Fluconazole, Topical Microemulsion, permeation

INTRODUCTION

Topical drug delivery is one for the suitable route of administration for drugs that undergo first-pass metabolism[1]. Recently delivery of drugs via skin using ointments, creams and gels have been extensively investigated[2-4]. The principle goal behind delivery of such drugs through the skin is to achieve better systemic absorption or for local treatment.

The stratum corneum of the skin acts as a principle barrier to the permeation of topically applied drugs. Researchers have investigated various permeation enhancers that modify the nature of the stratum corneum for better drug permeation[5]. Currently, however, there has been a growing interest in the development of topical drug delivery systems involving, solubilisation of insoluble drugs and the modification of stratum corneum. Various techniques have been developed for enhancing the penetration of drugs[6,7]. These include physical techniques, such as microneedle technologies[8,9], iontophoresis[10-13], electroporation[14-19] and ultrasound[20] and delivery systems like, microemulsions[21,22], liposome[23], solid lipid nanoparticle[24] etc.

Microemulsions system represents a pharmaceutically versatile formulation for various applications. Unlike coarse emulsions that are micronized with external energy, microemulsions are based on low interfacial tension achieved by adding a cosurfactant which leads to spontaneous formation of a thermodynamically stable formulation. The droplet size in the dispersed phase is very small, usually below 140nm in diameter, which makes the microemulsions transparent liquids[25] These systems can be used to deliver drugs via various route but topical applications have gained a considerable interest in the current decade.[26]

Fluconazole is a synthetic triazole antifungal drug used for the treatment of superficial and systemic fungal infections. It is available as tablets for oral administration, as a powder for oral suspension and as a sterile solution for intravenous use. The drug has a slight solubility in water (8 mg/mL at 37°C) and a melting point of 138°C to 140°C [27].

It is widely used in vaginal candidiasis, oropharyngeal and esophageal candidiasis and cryptococcal meningitis. It is also effective for the treatment of Candida urinary tract infections, peritonitis, and systemic Candida infections including candidemia, disseminated candidiasis, and pneumonia.

The present study aims at formulating and evaluating fluconazole in topical microemulsion (0.5% w/w) using Labrafil M 1944 CS as an oleaginous phase, Cremophor RH 40 as surfactant and ethanol as cosurfactant. The antifungal activity of fluconazole was evaluated using *Candida albicans* as a model fungus.

MATERIALS AND METHODS

Fluconazole USP was gifted by Dr. Reddy's Laboratory, Hyderabad, isopropyl myristate (IPM), tween 80, were gifted by Rita Corporation, Crystal Lake, USA, polyethylene glycol 400 was gifted by Signet Chemicals, Mumbai. All other chemicals used were of AR grade and used without further purification.

Screening of microemulsion components

For selecting different components for formulating microemulsion of fluconazole, the solubility of fluconazole was investigated in different oils, surfactants and cosurfactants like IPM, isopropyl palmitate, tween 80, plurol oleique, cremophor RH40, labrasol etc.

For determining the solubility excess of Fluconazole was added to 5 ml each of oils, surfactants, and cosurfactants in screw capped bottles and shaken on orbital flask shaker (Model RS-24, Remi Sales and Engg. Ltd., Mumbai.) at 100 RPM for 48 hours at ambient temperature. The suspension was centrifuged at 5000 RPM (Model R-6C, Remi Sales and Engg. Ltd., Mumbai) and clear supernatant liquid was decanted and filtered through 0.45µ nylon membrane filter (Whatmann). The solubility of fluconazole was estimated by HPLC method.

Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed using titration method to determine existence region for microemulsion formation and to determine the possibility of making different possible compositions of oil surfactant/cosurfactant and water.

A ratio of surfactant to cosurfactants was fixed as 1:2, 1:1, 2:1 and such mixtures were prepared. These mixtures (S/CoS) were mixed with oil phase to give weight ratio of 9:1, 8:2, upto 1:9, and titrated with water drop by drop and stirred using magnetic stirrer until homogeneous dispersion or solution was obtained. The system was examined for appearance and flow property after each addition. The point at which the solution becomes cloudy or turbid was the end point. The quantity of aqueous phase required to make the mixture turbid was recorded. The percentages of the different incorporated pseudo phases were then calculated and the same procedure was followed with other S/CoS ratio.

Preparation of Fluconazole Microemulsion

0.5 % w/w of Fluconazole was added to the mixtures of oil, surfactant, and cosurfactant with varying component ratio as described in Table 1. Appropriate amount of water was added to the mixture drop by drop followed by stirring the mixtures at ambient temperature to obtain fluconazole microemulsion. All microemulsion formulations were stored at ambient temperature.

In-vitro permeation study [28-32]

The in-vitro permeation rates of fluconazole from various microemulsion formulations were determined to evaluate the effect of oil content & surfactant/co-surfactant.

The permeation studies were performed using Keshary Chien diffusion cells fitted with 0.45µ cellulose acetate membrane (Sartorius Inc.) at 37 ± 0.1°C using a thermostatic water bath with pump (Cyberbath, CB 2000, Cyberlab Inc., USA). The effective diffusion area was 2.54cm^2 (18mm orifice diameter), and the receptor compartment was filled with 13.5 ml of phosphate buffer pH 7.4, which was constantly stirred by externally driven teflon coated star head magnetic bars. Accurately weighed 1gm of Fluconazole microemulsion was placed on 0.45µ cellulose acetate membrane in the donor compartment. 1.0 ml sample were withdrawn from the receptor fluid at predetermined time interval for upto 6 hrs after the application. An equal volume of the fresh phosphate buffer was immediately replenished after each sampling. All the collected samples were stored at -20°C until analysed by HPLC. Permeation studies were done in triplicate.

HPLC analysis of Fluconazole

The amount of fluconazole in the receptor compartment was determined by HPLC method. The HPLC system consisted of a intelligent HPLC pump (model Jasco PU-2080 plus) with 20 µl loop sample injector per injection (#7725i, Rheodyne, USA) and UV-Vis Detector (model Jasco UV-2075) was used. The equipment was operated through software Borwin version 1.5, LC-Net II/ADC system. The column used was Inertsil ODS, C_{18} column having dimensions 4.6mm x 250mm i.d. 5µm particle size (GL. Sciences Inc., Japan.)

The samples were analysed using an isocratic mobile phase consisting 45:55v/v mixture of acetonitrile and 25mM TRIS hydroxyl-methyl amino methane in phosphate buffer pH 7.0. The pH of Mobile phase was adjusted to 7.0. The flow rate was 1.5 ml/min and the detection wavelength was 261 nm. All operations were carried out at ambient temperature.

Determination of pH

The pH values of the samples were measured by a pH meter (model DPH – 500 Global Instruments, Mumbai, India), at 20 ± 1^{0}C.

Determination of Viscosity [33-38]

The viscosities of microemulsions were measured with a Brookfield rotational viscometer (LV2, Brookfield Inc., USA) equipped with spindle no. 4. The measurement was done at ambient temperature. Viscosities were determined in triplicate.

Optical Birefringence [34,39]

Birefringence is a light scattering phenomenon. The formulations were examined under polarized light microscopy (Neoplan-Pol, polarizing microscope, Kyowa-Getner) in order to determine optical isotropy of the samples.

RESULTS AND DISCUSSION

Screening of oils, surfactants and co-surfactants for microemulsion

Inorder to develop a topical microemulsion for poorly water soluble drug keen attention should be given on solublising capacity of oil, surfactant & co-surfactant. The solubility of fluconazole in the various oils, surfactant & co-surfactant are listed in Table No. 2

The solubility of fluconazole was found to be highest in isopropyl myristate (0.60 ± 0.044) amongst the oils investigated and followed by soya been, olive oil and cotton seed oil. Tween 80 (14.08 ± 0.532) showed maximum solubility followed by cremophor RH40 among the surfactants, while polyethylene glycol 400 (44.38 ± 1.52) showed highest solubility among the co-surfactant followed by Labrafac Lipofile, Plurol Oleique.

Isopropyl myristate, tween 80, polyethylene glycol 400 were selected for formulating microemulsions based on the solubil-

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Table 1. Formulation of Fluconazole microemulsion

Ingredients (in % w/w)	FITP – A	FITP – B	FITP – C	FITP – D	FITP – E	FITP – F
Isopropyl Myristate	60	50	40	30	20	10
Tween 80 /PEG 400	35	40	45	50	55	60
Water	5	10	15	20	25	30

(Mean ± S.D., n=3)

Table 2. Solubility of fluconazole in different oils, surfactant and cosurfactant

Phase type	Excipient	Solubility (mg/ml)
Oil	Oleic acid	0.65 ± 0.016
	IPM	0.60 ± 0.044
	Soy bean Oil	0.35 ± 0.08
	Cotton seed Oil	0.15 ± 0.03
	Olive Oil	0.2 ± 0.05
	Triacetin	0.55 ± 0.11
Surfactant	Tween80	14.08 ± 0.532
	Cremophor RH – 40	4.98 ± 0.197
cosurfactant	Labrafac Lipofile	30.40 ± 1.98
	Plurol Oleique	6.89 ± 0.350
	Polyethylene Glycol 400	44.38 ± 1.52
water	Distilled water	5.5 ± 0.5

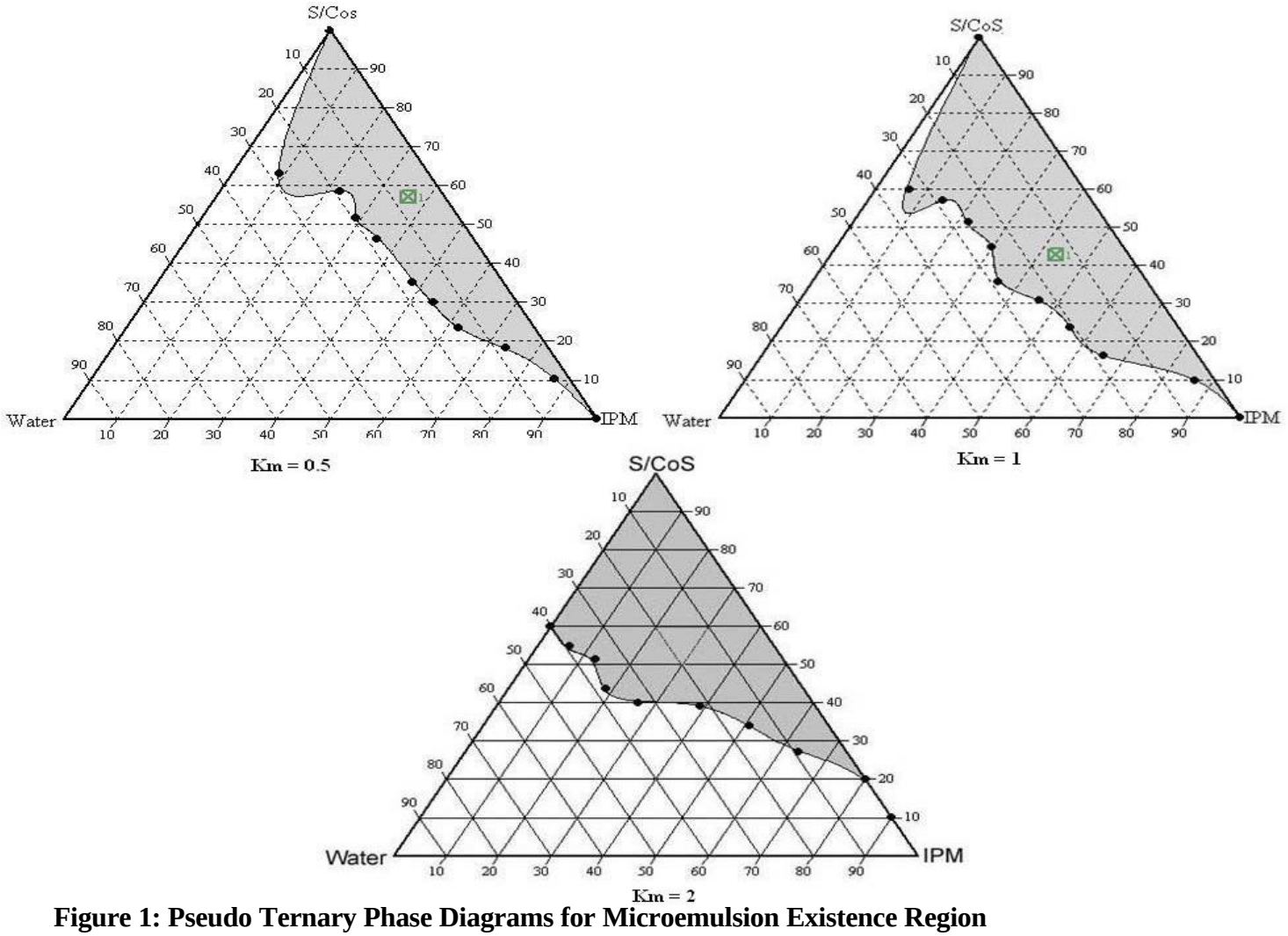

Figure 1: Pseudo Ternary Phase Diagrams for Microemulsion Existence Region

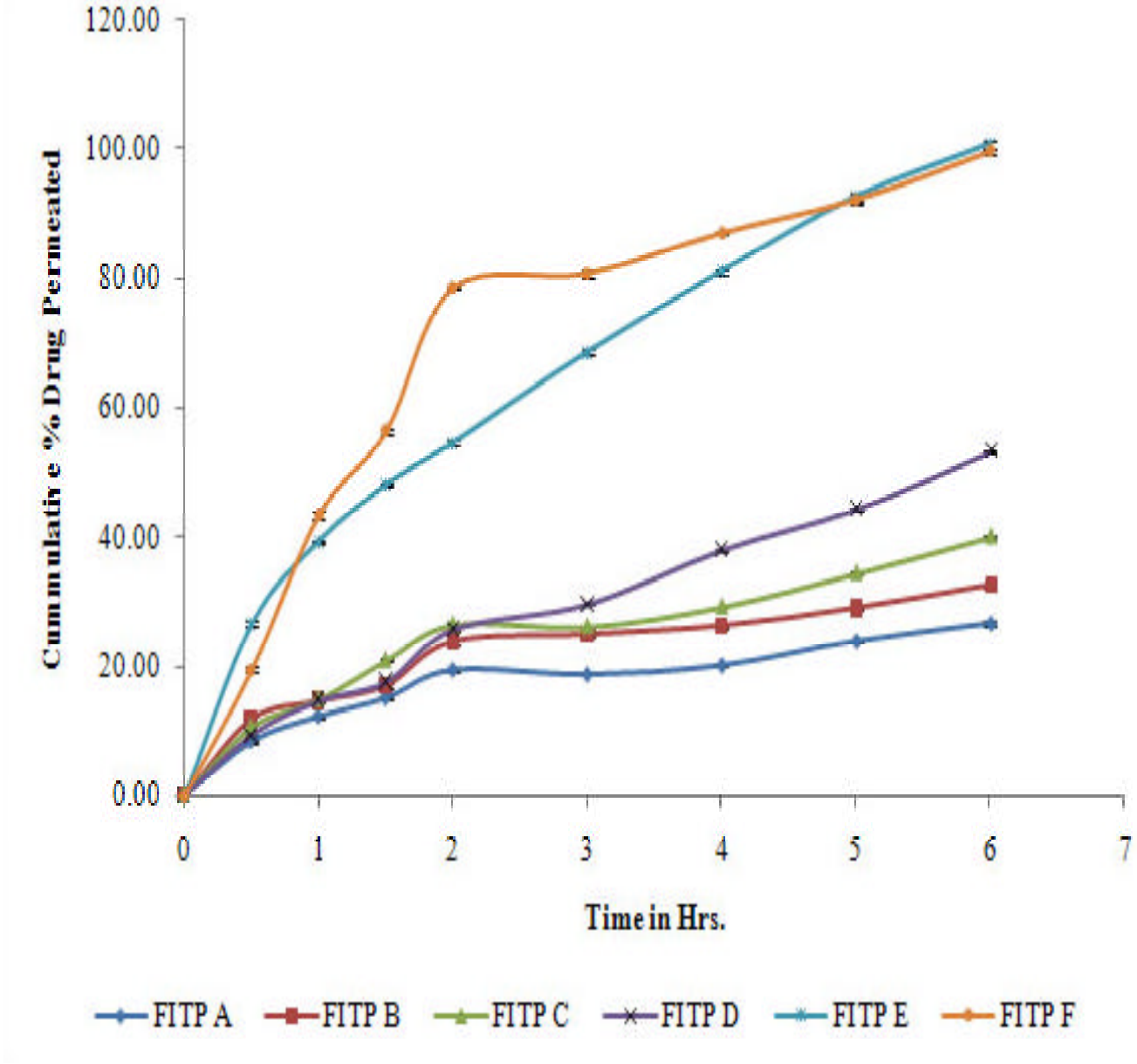

Figure 2: In-vitro Permeation studies for different Microemulsion Formulations

ity studies and the preformulation studies as oil, surfactant and co-surfactant.

Construction of pseudo-ternary phase diagrams and microemulsion formulation

Pseudo ternary phase diagrams were constructed to determine the microemulsion existence region. The pseudo ternary phase diagrams of various weight ratios of tween 80/ polyethylene glycol 400 are depicted in Fig. 1. The translucent region presented in phase diagram represents microemulsion existence region. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsion was observed. The rest of region on the phase diagram represents the turbid and conventional emulsions based on visual inspection.

The phase study clearly reveals that as surfactant:co-surfactant increases the existence area of microemulsion enlarge, reaching a maximum at S/CoS of 2.

Based on the phase diagram varying proportions of surfactant - co-surfactant (35-60%), oil (60-10%) and water (5-30%) were selected for formulation.

In-vitro permeation study

The drug permeation rates through various microemulsion formulations are illustrated in Fig 2. Amongst the formulations tested the batch FITP – E and FITP – F showed highest permeation rate.

The high permeation rate of microemulsion might attribute to several factors. Firstly the high concentration of fluconazole in microemulsion resulted in high concentration gradient which might be the main mechanism of drug permeation through the membrane from microemulsion. Microemulsions acts as a drug reservoir where drug is released from dispersed phase to the continuous and then further on to membrane [40].

The fluconazole permeation rate was affected significantly by the content of surfactant mixture in microemulsion. This may be due to increase in thermodynamic activity of the drug in the microemulsion at the lower content of surfactant, as fluconazole is poorly water soluble and yet solubilised in the surfactant mixture.

As the surfactant concentration increases drug permeation also increases due to increased contact of drug and membrane. This might be due the reduction in surface tension and increased wetting of drug.

From the permeation studies, it clearly reveals that the permeation rate increases (26% to 100 %), as the concentration S/CoS increases.

CONCLUSION

Microemulsions containing fluconazole were formulated for topical application. The components and their concentration ranges for the formation of microemulsion were obtained using the construction of pseudoternary phase diagrams. Their concentrations were optimised by evaluating in-vitro permeation rates of fluconazole. The optimum formulation of the microemulsion consisted of IPM 20 %, Tween 80/ PEG-400 55% (2:1) and water.

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