Preparation and Evaluation of Fluconazole Topical Microemulsion

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ABSTRACT

A fluconazole o/w microemulsion was developed for topical application using isopropyl myristate as the oil phase. Pseudo-ternary phase diagrams were constructed for the determination of existence region of micro emulsion region using the surfactant (tween 80 & Cremophor RH-40) and co-surfactant (ethanol). Different formulations were prepared for the evaluation of oil content, surfactant/co-surfactant concentration on in-vitro permeation rates. In-vitro transdermal permeability of flucanazole from the micro emulsions was evaluated using Keshary Chien diffusion cells mounted with 0.45μ with cellulose acetate membrane. The amount of drug (Fluconazole) permeated was analyzed by HPLC.

Keywords: Fluconazole, Micro emulsion, In-vitro studies.

INTRODUCTION

The human skin is a readily accessible surface for drug delivery. Over the past three decades, developing controlled drug delivery has become increasingly important in the pharmaceutical industry. Transdermal drug delivery-the delivery of drugs across the skin and into systemic circulation-is distinct from topical drug penetration, which targets local areas. Transdermal drug delivery takes advantage of the relative accessibility of the skin.

Penetration of a Drug from Topical or Transdermal Drug Delivery System [1, 2, 3]:

There are two main pathways by which drugs can cross the skin and reach the systemic circulation. The more direct route is known as the transcellular pathway. By this route, drugs cross the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the phospholipid bilayer of the cell one more time. This series of steps is repeated numerous times to traverse the full thickness of the stratum corneum. Few drugs have the properties to cross via this method.

Microemulsions have been widely studied to enhance the bioavailability of the poorly soluble drugs. They offer a cost effective approach in such cases. Microemulsions have very low surface tension and small droplet size which results in high absorption and permeation. Interest in these versatile carriers is increasing and their applications have been diversified to various administration routes in addition to the conventional oral route. This can be attributed to their unique solubilization properties and thermodynamic stability which has drawn attention for their use as novel vehicles for drug delivery. The results obtained have been indeed very promising. In recent past, micro emulsion formulation of a poorly soluble immunosuppressant was marketed as a soft capsule which contains a mixture of drug dissolved in oil and surfactant [4-6]. It converts into an oil-in-water (o/w) micro emulsion in situ in an aqueous environment in the stomach and the small intestine. Microemulsion formulation made the bioavailability and plasma concentration profiles of the drug more reproducible which is clinically important in the case of drugs showing serious adverse effects. This is a significant step forward in the delivery of poorly soluble drugs. Microemulsion systems are also now being increasingly investigated for transdermal [7], ocular [8], nasal [9], pulmonary, vaginal [10], rectal and intravenous drug delivery.

Microemulsions have advantages over both colloidal systems under investigation and conventional emulsions, suspensions and micellar solutions and may provide alternative drug: carriers. They are promising delivery systems which allow sustained or controlled drug release for percutaneous, peroral, topical, transdermal, ocular and parenteral administration of medicaments. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, improved drug solubilization of hydrophobic drugs and bioavailability. Also microemulsions that have inverse micellar structure may be less

Components of Microemulsion Formulations

A large number of oils and surfactants are available which can be used as components of microemulsion systems but their toxicity, irritation potential and unclear mechanism of action limit their use. One must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that will result in mild and non-aggressive microemulsions.

The excipients of Microemulsions are

- Oil phase
- Surfactants
- Co-surfactants

MATERIALS AND METHODS

Fluconazole, Isopropyl myristate, Tween 80, Soyabean Oil, Cotton Oil, Olive Oil, Cremophor R 40, Ethanol was purchased from LEO CHEM Pvt. Ltd, Bangalore, India. All the reagents were of analytical grade.

Methods

Pre-Formulation Studies

Melting point determination

Melting point determination of the obtained drug sample was done by open capillary method. Drug was taken in glass capillary whose one end was sealed by flame. The capillary containing drug was dipped in liquid paraffin inside the melting point apparatus. Melting point is a good first indication of purity of the sample since the presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range.

Drug-excipient compatibility studies

The proper design and the formulation of a dosage form require consideration of the physical, chemical and biological characteristics of the drug and excipients used in fabricating the product.

The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, easy to administer and safe.

The compatibility studies provide the framework for the drugs combination with excipients in the fabrication of the dosage form. The study was carried out to establish that the therapeutically active drug has not undergone any changes, after it has been subjected to processing steps during formulation of tablets.

IR Spectroscopy:

The FT- IR spectrum of the obtained sample drug is determined by determining thefunctional groups present in it.

Differential Scanning Calorimetry (DSC) Study

Differential scanning calorimetry (DSC) experiments were performed with differential scanning calorimeter. Mainly these studies are performed for the calibration of the sample and the various excipients used.

Analytical methods used in the determination of Ketoconazole

The UV spectrophotometric method was developed for the analysis of the drug using the double beam Shimadzu 1800 spectrophotometer.

Preparation of O/W Microemulsion Loaded With Drug

Fluconazole containing microemulsions were formulated by appropriate quantities of oil Isopropyl myristate surfactant Tween 20, co-surfactant Ethanol with varying component ratio as seen below in the table. 0.5% w/w of fluconazole was dissolved in this mixture and then appropriate amount of water was added to the mixture drop by drop with constant stirring on magnetic stirrer, at room temperature. **Ref: Table 1.**

Table 1. Formulation of Fluconazole microemulsion

Ingredients	FTEI-1	FTEI-2	FTEI-3	FTEI-4
Fluconazole	500 mg	500 mg	500 mg	500 mg
Isopropyl Myristate	0.5 ml	0.70 ml	1 ml	1.25 ml
Tween 20	4ml	3.75 ml	3.34 ml	2.5 ml
Water	4 ml	3.75 ml	3.5 ml	3.25 ml

Evaluation Parameters

Melting point determination

Melting point of Fluconazole was determined by open capillary method.

Measurement of Globular Size

The average droplet size and zeta potential of the microemulsions were measured using a Zetasizer Nano- ZS (Malvern Instruments, UK). The measurement was

done at 25°C. **Ref: Table 2.**

Table 2. Globular size of microemulsion

Sl No:	Formulation	Droplet Size(nm)	Zeta Potential (mv)
1	FTEI-1	418	-0.232
2	FTEI-2	274	-0.114
3	FTEI-3	187	-0.535
4	FTEI-4	122	-0.596

Determination of pH

The pH values of the samples were measured by a pH meter at 20 ± 1 _C. **Ref Table 3.**

Table 3. pH of Microemulsion

Sl No:	Formulation	pH
1	FTEI-1	3.96
2	FTEI-2	3.77
3	FTEI-3	3.48
4	FTEI-4	3.37

Drug Content Studies

10 mg of prepare Fluconazole microemulsions was taken in 10 ml volumetric flask containing 5 ml methanol and stirred for 30 minutes. Volume was made up to 10 ml with methanol. From the above solution, 0.1 ml was further diluted with 10 ml methanol to get 10 µg/ml. The resultant solution was filtered through Whatman filter paper and absorbance of the solution was measured at 260 nm using UV spectrophotometer.

Skin Irritation Test

Microemulsion should not produce skin irritation when applied topically. Hence, skin irritation test study was performed. The skin irritation test was performed on healthy white rabbit of average weight 1.75 to 2.25 Kg. About 9 cm^2 area on the dorsal surface of the rabbits in each group was shaved and cleaned with spirit.

Rabbits were divided into three groups (n=3) as follows:

Group- I (Control): There was no application on the surface of the rabbit skin.

Group-II (negative control): An aqueous solution of 1ml containing 0.8% formalin soaked om 9 cm^2 cotton wool (standard irritant) was placed in the back of the rabbit as negative control. The cotton wool was secured firmly in the place with adhesive plaster.

Group-III (Test): 1ml of microemulsion containing 20 mg of Fluconazole was applied to 9 cm^2 area on the dorsal surface of the rabbit. The visual inspection was observed for 3 days to check any evidence of skin irritation (sign of edema and erythrema). The scoring system of Draize et al was followed in grading the severity of the effect.

In-vitro- Permeability Studies

The in-vitro permeation rate of fluconazole from various microemulsion formulations was determined to evaluate the effect of the formulation variables. The permeation studies were performed using Keshary- Chien diffusion cells fitted with 0.45µ cellulose acetate membrane (Sartorius) at 37 ± 0.1°C using a thermostatic water pump. (Cyberbath, CB 2000, Cyberlab Inc. USA.) The effective diffusion area was 2.54 cm2 (18mm orifice diameter), and the receptor compartment was filled with 13.5 ml of phosphate buffer pH 7.4. The receptor fluid was constantly stirred by externally driven teflon coated star head magnetic bars. Accurately weighed 1gm of fluconazole was placed in the donor compartment. Samples (0.5ml) 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 20^0C until analyzed by HPLC. The permeability study was done in triplicate.

Determination of Viscosity:

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. **Ref: Table 4.**

Table 4. Viscosity of microemulsion formulations

Sl No:	Formulation	Viscosity
1	FTEI-1	93.3
2	FTEI-2	85
3	FTEI-3	85
4	FTEI-4	85

RESULTS AND DISCUSSION

MELTING POINT DETERMINATION

The melting point of the obtained drug sample was found to be116◦C which is within the reported range of 138◦C to 140◦C. it complies with the standards thus indicating the purity of the drug sample.

COMPATIBILITY STUDIES

Differential Scanning Calometry (DSC)
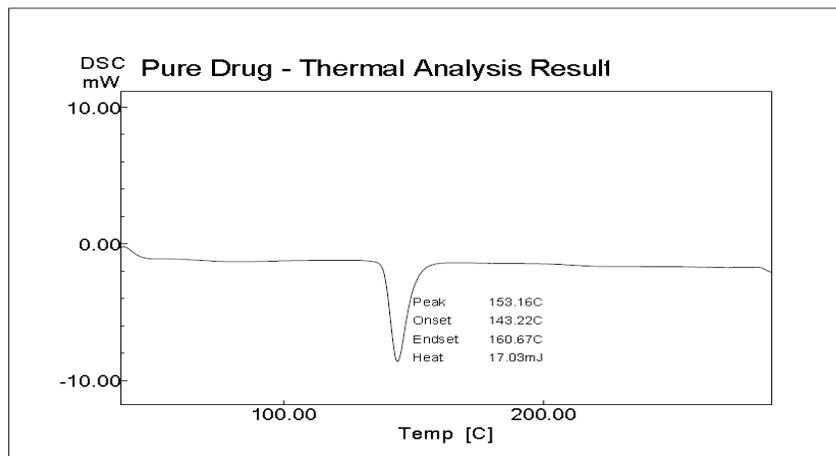
Ref: Fig 1, Fig 2, Fig 3.

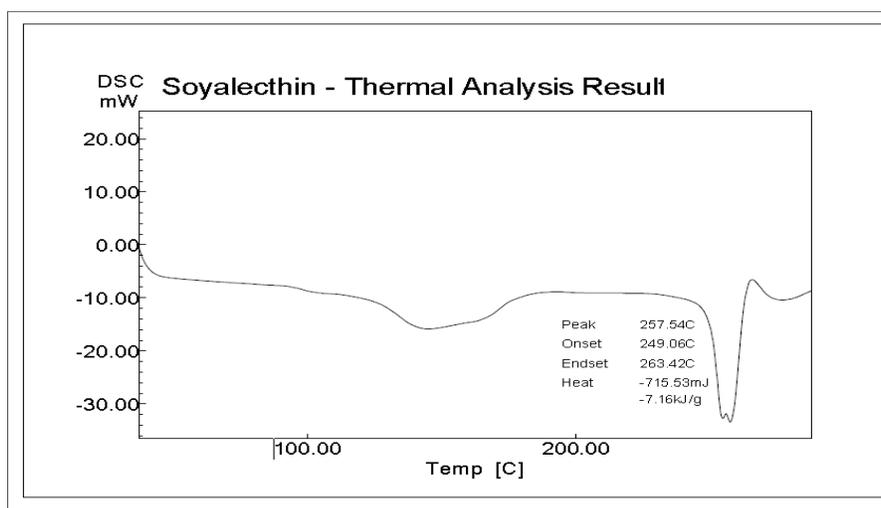**Figure 1. DSC Thermogram of Fluconazole**

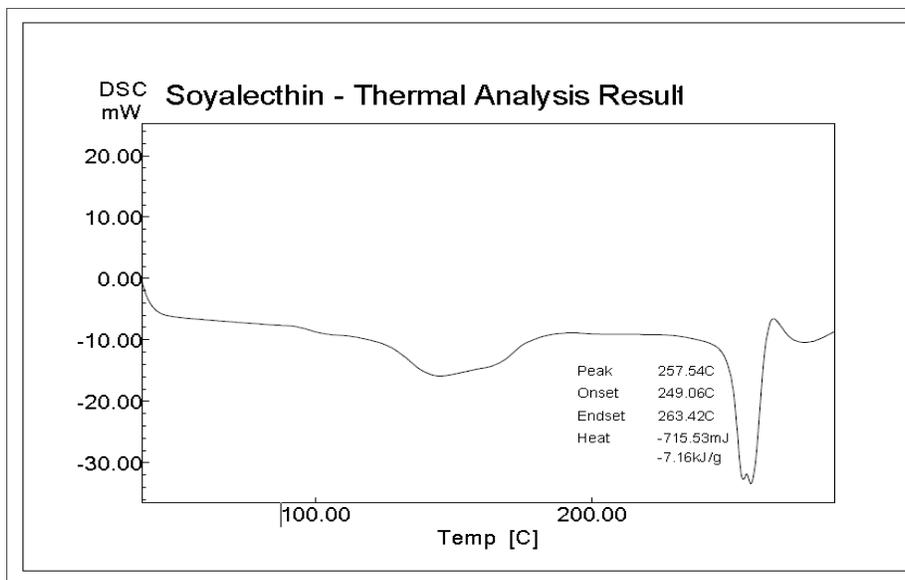**Figure 2. DSC Thermogram of physical mixture of Fluconazole and Isopropyl myristate**

Figure 3. DSC Thermogram of Isopropyl myristate

Identification Test

a) IR Spectroscopy

The IR spectrum of the pure Fluconazole sample recorded by FT-IR spectrophotometer is shown in figure (4), which was compared with the standard functional group frequencies of Fluconazole shown in **figure 4. Ref: Table 5. Fig 5, Fig 6, Fig 7**.

Table 5. Interpretation data of FTIR spectrums

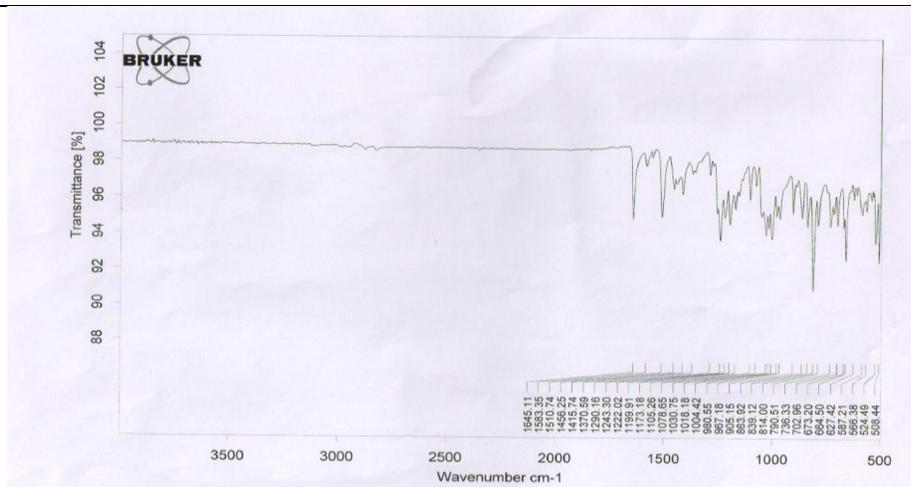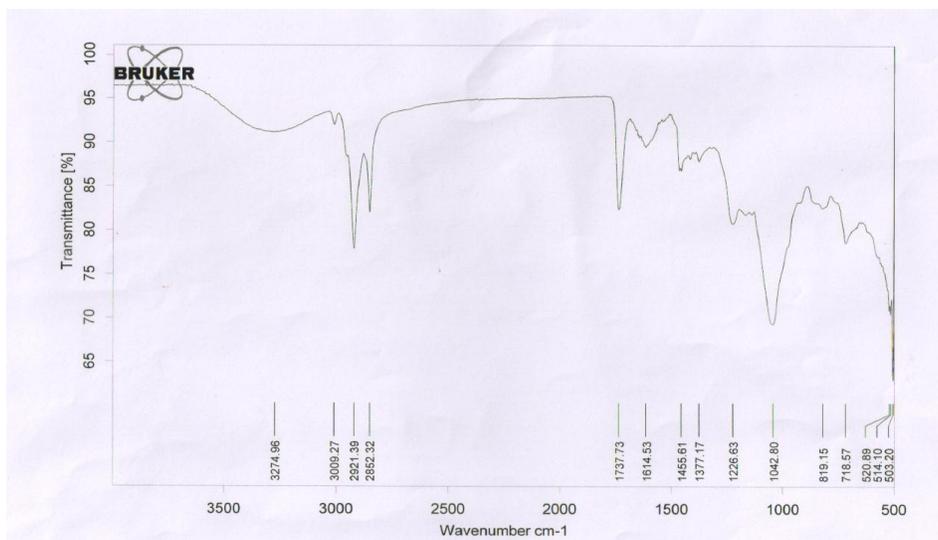| Sl No:	Functional Group	Fluconazole Peak area (cm⁻¹)	Fluconazole + Isopropyl myristate Peak area(cm⁻¹)	Fluconazole microemulsion	Streching / Deformation (cm⁻¹)
1	O-H	3434	3434.03	3434.56	Stretching
2	C=N	1620	1620.65	1619.38	Stretching
3	C=C	1516	1516.10	1516.20	

Figure 4. IR spectrum of Fluconazole

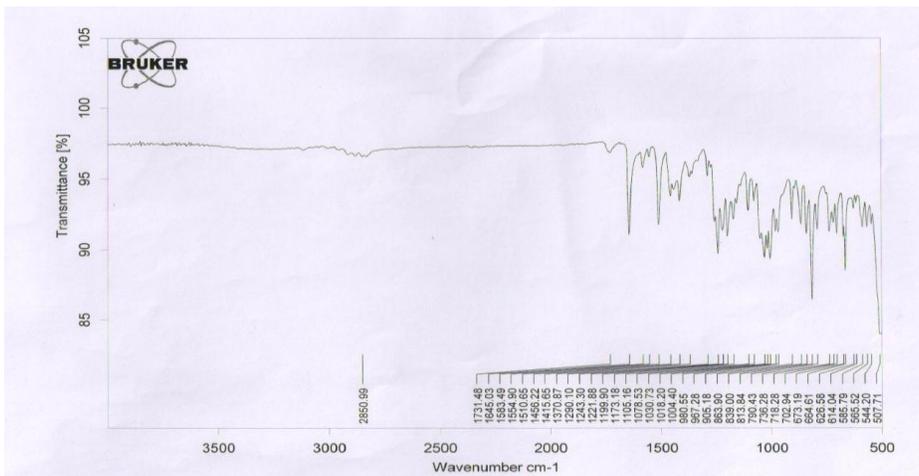**Figure 5. IR spectrum of Isopropyl myristate**

Figure 6. IR spectrum of physical mixture of Fluconazole and Isopropyl myristate
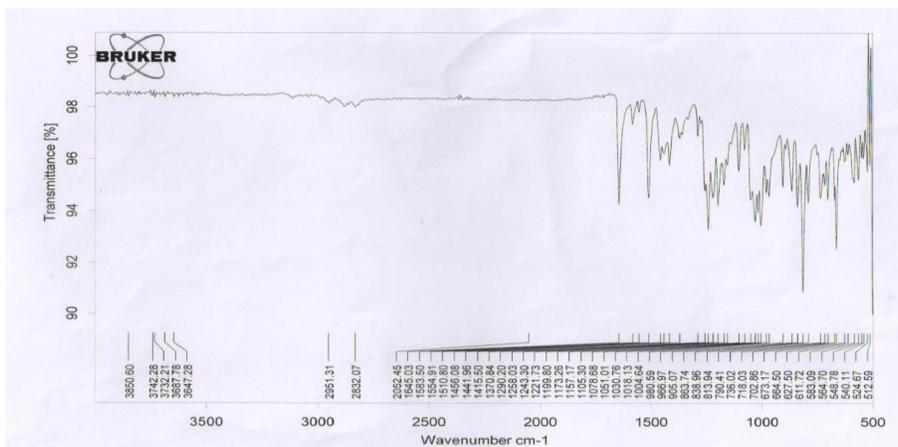

Figure 7. IR spectrum of Fluconazole microemulsion

Spectroscopic Studies

Standard calibration curve of Fluconazole using phosphate buffer pH 7.4. Ref Table 6. Fig 8.
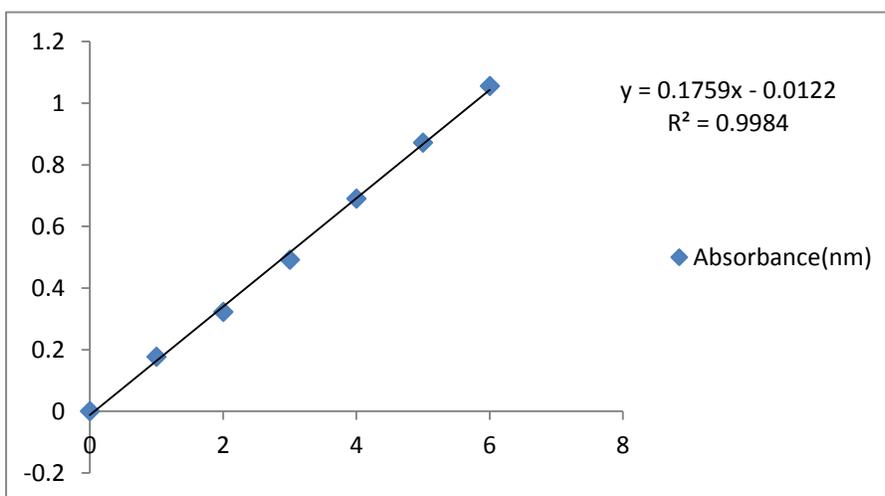

y = 0.1759x - 0.0122
R^2 = 0.9984

Figure 8. standard calibration curve of Fluconazole in pH 7.4 phosphate buffer

Table 6. Absorption data for the calibration curve of Fluconazole in pH 7.4 Phosphate buffer

SL No	Concentration (µg/ml)	Absorbance
1	0	0
2	1	0.177
3	2	0.322
4	3	0.4922
5	4	0.6899
6	5	0.8722
7	6	1.056

Regression coefficient (R^2) = .998

Absorbance = 0.175x

Measurement of Droplet Size of Microemulsion:

The droplet size and zeta potential for the formulations are represented in given below table. The result shows that the droplet diameter decreases with increasing ratio of oil: surfactant/co-surfactant. These results are in accordance with the report that the addition of surfactant to microemulsion system causes the interfacial film to condense and to be stable, while the co surfactant causes the film to expand. **Ref: Table 7.**

Table 7. Globular Size of Fluconazole

Sl No:	Formulation	Droplet Size(nm)	Zeta Potential (mv)
1	FTEI-1	418	-0.232
2	FTEI-2	274	-0.114
3	FTEI-3	187	-0.535
4	FTEI-4	122	-0.596

Skin Irritation Test:

Best formulation FTEI3 was subjected to skin irritation test and showed no edema, erythema and redness after 3 days. **Ref: Table 8.**

Table 8. Dermal response of skin irritation studies conducted on rabit skin with the prepared Fluconazole microemulsion (FTEI3)

Formulation Number	Score gained by rabbits during 72 hours study		
	24hrs	48 hrs	72 hrs
FTEI3	0	0	0

IN VITRO DRUG RELEASE STUDIES

In vitro drug release data of Fluconazole Microemulsion Ref; Table 9. Fig 9.

Table 9. *In vitro* drug release data of Fluconazole microemulsion

TIME(HRS)	% CUM.DRUG RELEASE			
	F1±SD	F2±SD	F3±SD	F4±SD
0.5	19.05 ± 0.58	20.91 ±0.35	18.79 ± 0.23	18.26 ± 0.43
1	23.77 ± 0.32	24.20 ± 0.23	23.13 ± 0.53	22.60 ± 0.62
2	30.41 ± 0.21	29.89 ± 0.21	27.76 ± 0.34	27.75 ± 0.24

|				
3	36.93 ± 0.31	35.88 ± 0.43	32.41 ± 0.64	32.93 ± 0.14
4	41.10 ± 0.65	40.58 ± 0.64	36.56 ± 0.35	36.56 ± 0.72
5	48.74 ± 0.51	47.42 ± 0.45	41.52 ± 0.42	41.26 ± 0.23
6	53.51 ± 0.43	51.12 ± 0.20	45.72 ± 0.13	74.83 ± 0.43
7	58.56 ± 0.54	55.10 ± 0.58	50.73 ± 0.53	52.59 ± 0.45
8	64.17 ± 0.46	60.16 ± 0.91	54.71 ± 0.42	56.85 ± 0.34
9	67.96 ± 0.65	66.58 ± 0.35	59.77 ± 0.55	62.45 ± - 0.23
10	73.09 ± .34	70.91 ± 0.14	63.27 ± 0.19	69.41 ± 0.64
11	78.51 ± 0.61	74.46 ± 0.25	66.52 ± 0.26	73.22 ± 0.35
12	83.96 ± 0.43	78.04 ± 0.34	71.93 ± 0.56	77.05 ± 0.23

SD=Standard Deviation (n±3)
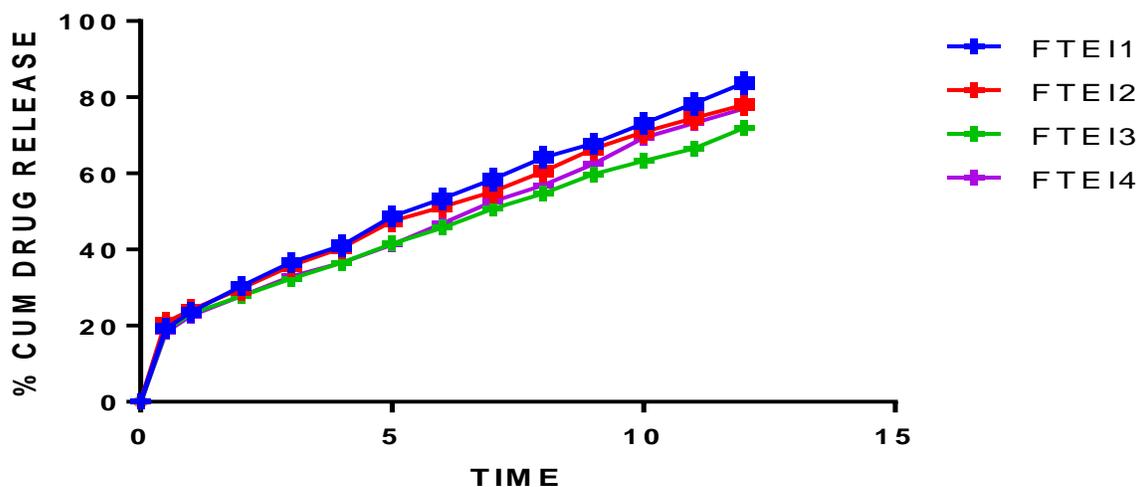
Figure 9. *In vitro* dissolution profiles for microemulsion

DRUG RELEASE KINETICS OF FLUCONAZOLE MICROEMULSION

Zero order kinetics Ref: Table 10, Fig 10.

First order release kinetics

Ref: Table 11. Fig 11.

Higuchi Model

Ref: Table 12. Fig 12.

KORSMEYER-PEPPAS MODE

Ref: Table 13. & Table 14. Fig 13.

Table 10. Zero order release kinetic data of Fluconazole microemulsion

TIME(HRS)	% CUM.DRUG RELEASE			
	F1±SD	F2±SD	F3±SD	F4±SD
0.5	19.05 ± 0.43	20.91 ± 0.54	18.79 ± 0.23	18.26 ± 0.42
1	23.77 ± 0.21	24.20 ± 0.63	23.13 ± 0.14	22.60 ± 0.14
2	30.41 ± 0.64	29.89 ± 0.34	27.76 ± 0.54	27.75 ± 0.74

3	36.93 ± 0.45	35.88 ± 0.25	32.41 ± 0.35	32.93 ± 0.24
4	41.10 ± 0.36	4.58 ± 0.63	36.56 ± 0.72	36.56 ± 0.59
5	48.74 ± 9.24	47.42 ± 0.21	41.52 ± 0.23	41.26 ± 0.68
6	53.51 ± 0.54	51.12 ± 0.12	45.72 ± 0.12	47.83 ± 0.25
7	58.56 ± 0.23	55.10 ± 0.43	50.73 ± 0.43	52.59 ± 0.64
8	64.17 ± 0.27	60.16 ± 0.67	54.71 ± 0.64	56.85 ± 0.32
9	67.96 ± 0.56	66.58 ± 0.58	59.77 ± 0.22	62.45 ± 0.63
10	73.09 ± 0.33	70.91 ± 0.23	63.27 ± 0.12	69.41 ± 0.31
11	78.51 ± 0.14	74.46 ± 0.42	66.52 ± 0.77	73.22 ± 0.77
12	83.96 ± 0.26	78.04 ± 0.53	71.93 ± 0.82	77.05 ± 0.26

Table11. First order release kinetic data of Fluconazole microemulsion

S.NO	TIME (HRS)	Log% Cumulative Drug Remaining To Be Released			
		F1±SD	F2±SD	F3±SD	F4±SD
1	0.5	1.90 ± 0.45	1.89 ± 0.62	1.91 ± 0.16	1.91 ± 0.88
2	1	1.88 ± 0.22	1.87 ± 0.35	1.88 ± 0.54	1.89 ± 0.23
3	2	1.84 ± 0.75	1.84 ± 0.74	1.85 ± 0.30	1.85 ± 0.51
4	3	1.79 ± 0.34	1.80 ± 0.12	182. ± 0.29	1.82 ± 0.12
5	4	1.77 ± 0.21	1.77 ± 0.52	180. ± 0.87	1.78 ± 0.54
6	5	1.70 ± 0.74	1.72 ± 0.85	1.76 ± 0.15	1.75 ± 0.22
7	6	1.66 ± 0.38	1.68 ± 0.21	1.71 ± 0.64	1.72 ± 0.34
8	7	1.61 ± 0.56	1.65 ± 0.63	1.67 ± 0.72	1.65 ± 0.58
9	8	1.55 ± 0.31	1.60 ± 0.28	1.63 ± 0.46	1.60 ± 0.21
10	9	1.50 ± 0.73	1.52 ± 0.42	1.57 ± 0.32	1.55 ± 0.68
11	10	1.42 ± 0.25	1.46 ± 0.56	1.48 ± 0.72	1.51 ± 0.23
12	11	1.33 ± 0.42	1.40 ± 0.29	1.42 ± 0.16	1.41 ± 0.64
13	12	1.20 ± 0.63	1.34 ± 0.33	1.36 ± 0.32	1.39 ± 0.27

Table 12. Higuchi's release kinetic data of Fluconazole microemulsion

S.NO	√T	% CUM.DRUG RELEASE			
		F1±SD	F2±SD	F3±SD	F4±SD
0.5	0.707	19.05 ± 0.32	20.91 ± 0.58	18.79 ± 0.12	18.26 ± 0.05
1	1	23.77 ± 0.19	24.20 ± 0.09	23.13 ± 0.54	22.60 ± 0.44
2	1.41	30.41 ± 0.83	29.89 ± 0.35	27.76 ± 0.73	27.75 ± 0.84
3	1.73	36.93 ± 0.41	35.88 ± 0.67	32.41 ± 0.21	32.93 ± 0.21
4	2	41.10 ± 0.72	40.58 ± 0.80	36.56 ± 0.46	36.56 ± 0.55
5	2.23	48.74 ± 0.18	47.42 ± 0.37	41.52 ± 0.80	41.26 ± 0.41
6	2.449	53.51 ± 0.23	51.12 ± 0.50	45.72 ± 0.29	47.83 ± 0.69
7	2.646	58.56 ± 0.65	55.10 ± 0.01	50.73 ± 0.58	52.59 ± 0.05
8	2.828	64.17 ± 0.29	60.16 ± 0.55	54.71 ± 0.81	56.85 ± 0.51
9	3	67.96 ± 0.72	66.58 ± 0.22	59.77 ± 0.34	62.45 ± 0.29
10	3.162	73.09 ± 0.89	70.91 ± 0.83	63.27 ± 0.67	69.41 ± 0.46
11	3.316	78.51 ± 0.42	74.46 ± 0.56	66.52 ± 0.55	73.22 ± 0.31
12	3.464	83.96 ± 0.81	78.04 ± 0.04	71.53 ± 0.72	77.05 ± 0.86

Table 13. Peppas release kinetics data of Fluconazole microemulsion

S.NO	Log T	Log% CUM.DRUG RELEASE			
		F1±SD	F2±SD	F3±SD	F4±SD
0.5	-0.301	1.28 ± 0.23	1.32 ± 0.27	1.27± 0.51	1.26 ± 0.29
1	0	1.37 ± 0. 65	1.38 ± 0.59	1.36 ± 0.82	1.35 ± 0.81
2	0.301	1.481 ± 0.09	1.47 ± 0.11	1.44 ± 0.63	1.44 ± 0.43
3	0.477	1.56 ± 0.51	1.55 ± 0.49	1.51 ± 0.41	1.51 ± 0.62
4	0.602	1.61 ± 0.26	1.60 ± 0.73	1.56 ± 0.65	1.5 ± 0.39
5	0.698	1.68 ± 0.82	1.67 ± 0.44	1.61 ± 0.38	1.61 ± 0.84
6	0.778	1.72 ± 0.61	1.70 ± 0.26	1.66 ± 0.71	1.67 ± 0.11
7	0.845	1.76 ± 0.22	1.74 ± 0.51	1.70 ± 0.22	1.72 ± 0.80
8	0.903	1.80 ± 0.73	1.77 ± 0.88	1.73 ± 0.84	1.75 ± 0.07
9	0.954	1.83 ± 0.41	1.82 ± 0.27	1.77 ± 0.37	1.79 ± 0.49
10	1	1.86 ± 0.60	1.85 ± 0.69	1.80 ± 0.51	1.84 ± 0.58
11	1.041	1.89 ± 0.29	1.87 ± 0.22	1.82 ± 0.86	1.86 ± 0.05
12	1.079	1.92 ± 0.88	1.89 ± 0.75	1.85 ± 0.59	1.88 ± 0.46

Table 14. Regression coefficients fit to different drug release kinetic models for Fluconazole microemulsion

Formulation code	Zero order	1st order	Higuchi	Peppas	
	r^2	r^2	r^2	r^2	N
FTEI1	0.995	0.968	0.984	0.980	0.4757
FTEI2	0.995	0.984	0.982	0.968	0.4365
FTEI3	0.997	0.988	0.979	0.969	0.4279
FTEI4	0.993	0.970	0.967	0.961	0.4639
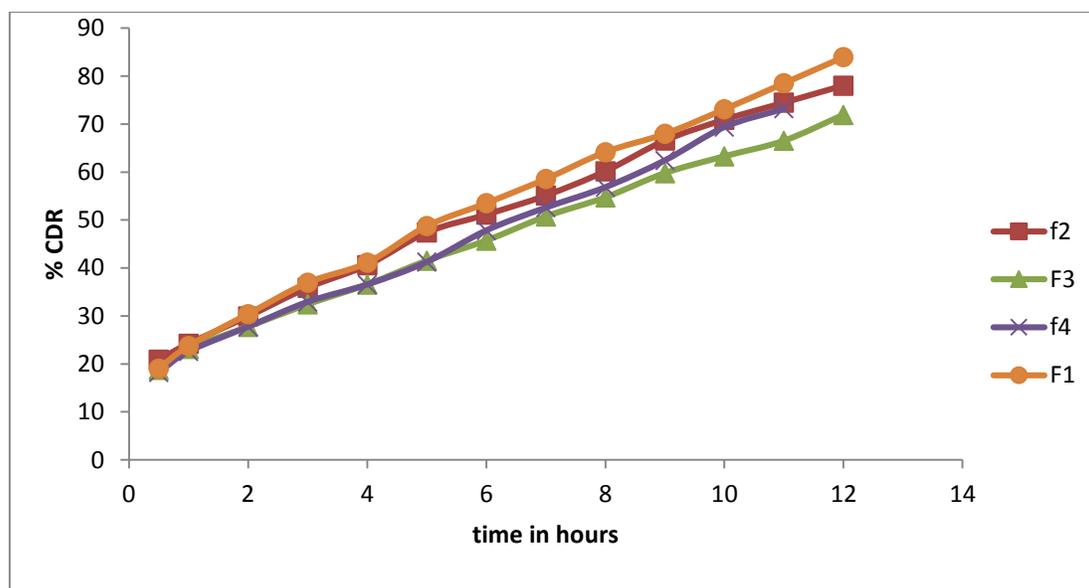
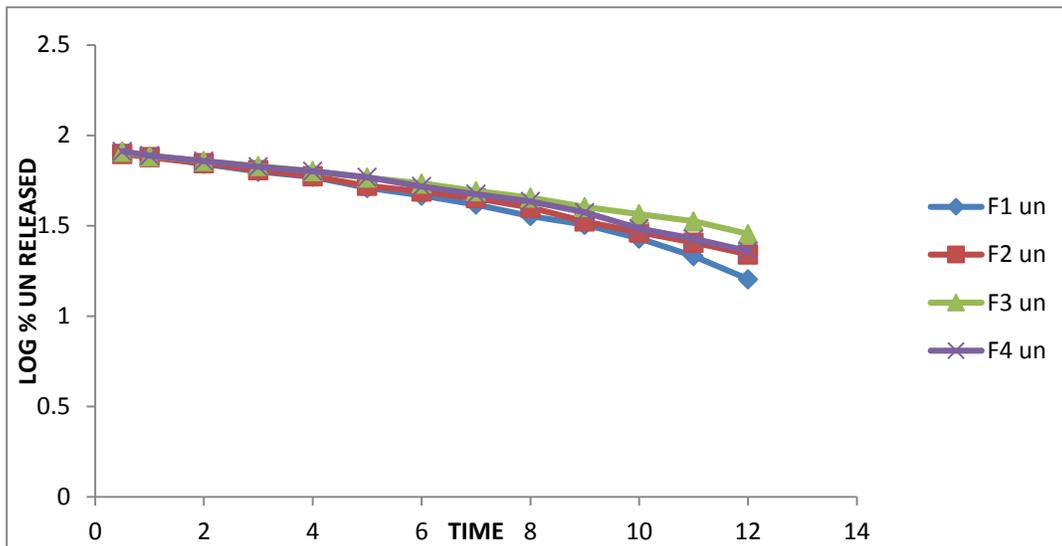**Figure 10: Zero order release kinetic profile of Fluconazole microemulsion**

Figure 11: first order release kinetics profile of Fluconazole microemulsion
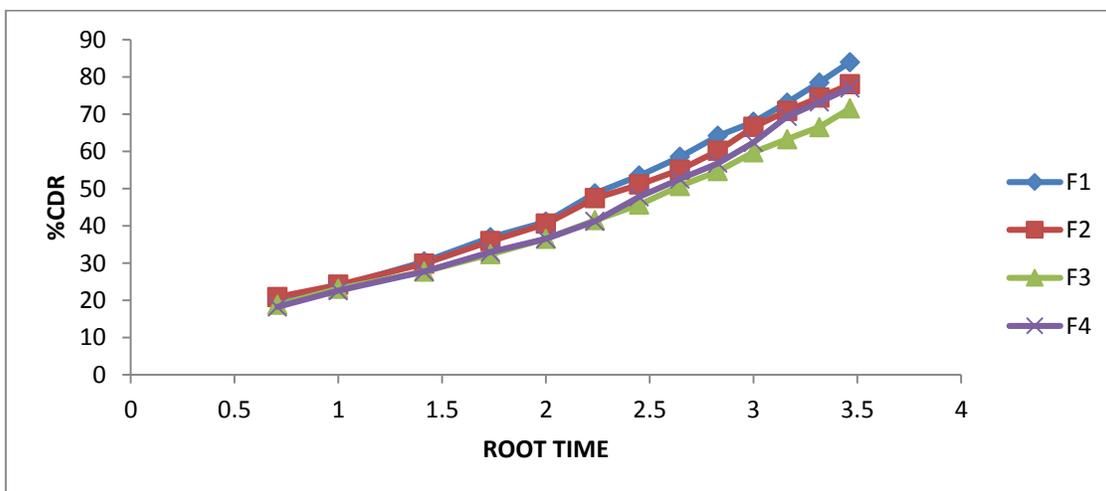
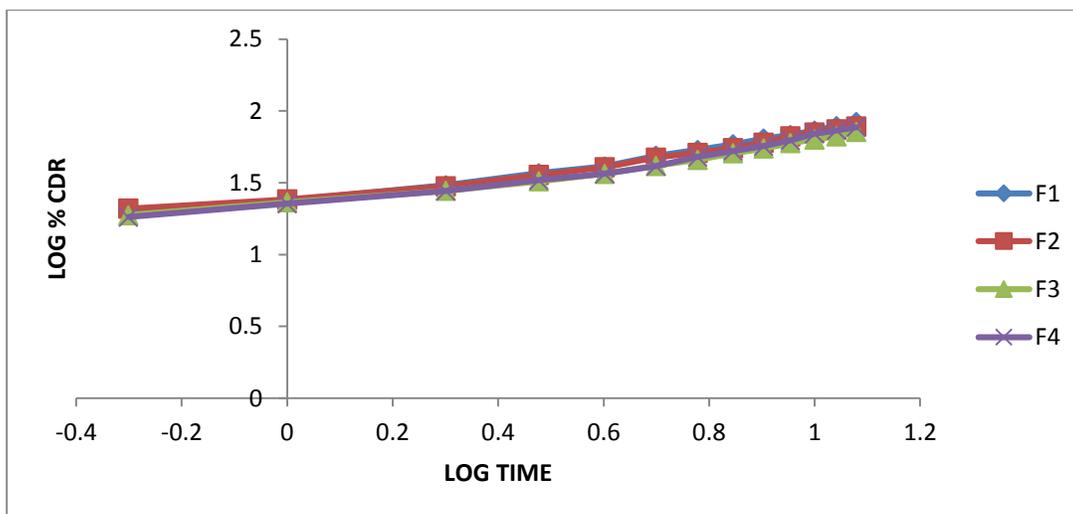**Figure 12: Higuchi's release profile of Fluconazole microemulsion**

Figure 13:Peppas release kinetics of Fluconazole

CONCLUSION

Preparation of microemulsions was found to be well suited and sound approach to obtain skin permeation. From the solubility studies, it was concluded that Fluconazole was more soluble with Isopropyl myristate and thus suitable for the formulation of Fluconazole. The present studies suggest that the studied microemulsion system is an appropriate vehicle for the topical delivery of lipophilic antifungal agent Fluconazole. 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. *In vitro* permeation studies were performed for all the formulations, FTEI1 to FTEI4 by using Keshary-Chein diffusion cell at 37^{0}C. Increase in Fluconazole permeation with decrease in surfactant mixture was signified as the drug being poorly soluble in water and yet solubilised may have been solubilised in surfactant mixture. Increase in percutaneous absorption of drug might also be affected by the globule size of microemulsion. As the droplet size is very small the number of vesicles that interact on fixed area of stratum corneum also increased, thereby increasing the efficiency of percutaneous uptake. Finally, it was concluded that Isopropyl myristate can be successfully used in the formulation of Fluconazole control release topical drug delivery system. The developed topical microemulsions of Fluconazole may be used in clinic for prolonged drug release for at least 12th thereby improving the bioavailability and patient compliance.

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