Original Article

Development And Formulation Of Microemulsion For Topical Drug Delivery Of Fluconazole

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Abstract

Microemulsions are thermodynamically stable transparent or translucent formulations which contain mainly oil, water, surfactants and co-surfactants. The aim of the present study was to develop and evaluate microemulsion for the topical drug delivery of fluconazole (FLZ). The solubility of fluconazole in different oils, surfactants and co-surfactants was selected to identify the components of the microemulsion. The pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature. The selected microemulsions were assessed for globule size, zeta potential conductivity, viscosity, spreadability, pH and stability study, Besides this, the microemulsion loaded fluconazole formulations were evaluated for and *in vitro* permeation studies and skin irritation studies .*In-vitro* permeation studies performed through wister rat skin membrane. The optimized microemulsion FLZ MEs-1 and FLZ MEs-6formulations consisting of FLZ 1%, castor oil 10%- 20% respectively, Smix(4:1, 2:1), 50%- 48% respectively and aqueous phase 39%-31% respectively. The *in vitro* permeation through excised Wister rat skin from the studied microemulsion was best described by the zero-order and first order models. Finally, the optimized FLZ MEs-1 formulations showed higher activity as compared to that of FLZ MEs-6 respectively. The results suggest the potential use of developed microemulsion as vehicles for topical drug delivery of fluconazole, encouraging further *in vitro* evaluation.

Keywords: Microemulsion, *In- vitro* permeation study, Pseudo-ternary phase diagram.

INTRODUCTION

Microemulsion is defined as a dispersion which has a quaternary composition consisting of oil, surfactant, co-surfactant and aqueous phase at appropriate ratios, which is a single optically isotropic, transparent, clear thermodynamically stable liquid solution with a droplet diameter usually within the range of 10–100 [1].It is believed that around 40% of newly developed chemical entities have solubility issue either in aqueous phase or oil phase which leads to the poor absorption, poor bioavailability and poor stability[2].Now a day's fungal infection of the skin is a major dermatological problem. Fungal skin infections are infections on the skin which is caused by a fungus. The fungal skin infection shows various symptoms include rashes with a variety of different appearances like red skin, scaly, itchy, and dry skin[3].Fluconazole, is chemically 2(2, 4difluorophenyl) 1, 3bis (1H1, 2, 4triazole1yl) 2propanol, an antimycotic agent, a hydrophilic bistriazole with a broad spectrum, approved by the Food and Drug Administration (US FDA) in 1990. The pharmacokinetic properties of fluconazoleis differ from the other azole derivatives due to the presence of two triazol rings in their structure that make it less lipophilic and with less affinity against proteins[4-8].Fluconazole shows higher bioavailability 90% due to the presence of two triazol rings and halogenated phenyl ring, lipophilicity *log P = 0.5*, itshows low protein binding ±12% and plasma pH (pKa = 2.03). After oral administration, fluconazole is delivered to the skin where it diffuses and accumulates quickly and extensively in the stratum corneum (SC)·Fluconazole are mainly used as primary treatment of all kind of susceptible *Candida* infections in both immunocompetent and immunocompromised hosts. It acts by blocking the synthesis of ergosterol, in cell membrane via inhibition of cytochrome P-450 dependent 14-αdemethylase[9-13].The aim of this present study was to develop and formulate new type of microemulsion formulations to be used as vehicles for topical drug delivery of fluconazole and to evaluate their potential characteristic.

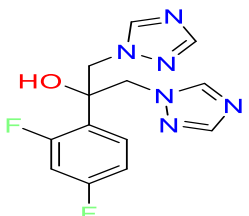


Figure 1: Structure of fluconazole

MATERIALS AND METHODS

Fluconazole was kindly donated as gift sample from Sun Pharmaceutical Industries Ltd. Dewas, MP, India. Castor oil was purchased from Moly Chem Pvt. Ltd, Oleic acid, cinnamon oil, sesame oil, soybean oil, sesame oil was purchased from Samar Chem Pvt.Ltd. India. Polyethylene glycol (PEG), propylene glycol (PG) was purchased from Moly Chem Pvt, Ltd, India. Tween 80(Polyoxyethylene sorbitan mono-oleate), dihydrogen phosphates (DHP) were purchased from Loba Chem Pvt.Ltd, India. All the other chemicals used were of analytical grade. Distilled water was used throughout the experiment.

Preformulation Studies

The preformulation studies of fluconazole drug are identified by using the FTIR-spectrophotometer technique, λ-max determination, calibration curve, solubility study.

Screening Of Formulations Components

Screenings of oils were carried out by using various types of oil such as castor oil, oleic acid, cinnamon oil, soybean oil and sesame oil. The solubility of fluconazole was determined at 258 nm by using UV-Visible spectrophotometer[14].Screening of surfactants were done using different types of surfactants such as Tween 80, span 20 and tween 20.The solubility of fluconazole was determined by using UV-Visible Spectrophotometer[15].

Screening of co-surfactants were done using different types of surfactants such as polyethylene glycol (PEG) and propylene glycol (PG). The solubility of fluconazole was determined by using UV-Visible spectrophotometer[16].Construction of pseudo-ternary phase diagrams were constructed using Pro-Sim ternary diagram (STRATEGE Batiment A BP- 2738).The pseudo-ternary phase diagrams of oil (Castor oil), surfactant (Tween 80), co-surfactant (Propylene glycol) and water were constructed using water titration method. The ratios of oil to Smix were varied as 0.5:4.5, 1.0:4.0, 1.5:3.5, 2.0:3.0, 2.5:2.5, 3.0:2, 3.5:1.5, 4.0:1.0, and 45:0.5[17],[18].

Preparation Of Fluconazole Microemulsion Formulations

According to microemulsion regions in the phase diagrams, six microemulsion formulations were selected at different component ratios. The composition of microemulsion loaded fluconazole formulations was given in table 1.

Table1: Compositions of microemulsion loaded fluconazole formulations

Formulation	Fluconazole (%w/w)	Oil Phase (%w/w)	Aqueous Phase (%w/w)	Surfactants : Co- surfactants (Smix in %w/w)		
				4:1	**3:1**	**2:1**
FLZ MEs-1	**1**	**10**	**40**	**50**	**-**	**-**
FLZ MEs-2	**1**	**20**	**35**	**45**	**-**	**-**
FLZ MEs-3	**1**	**10**	**38**	**-**	**52**	**-**
FLZ MEs-4	**1**	**20**	**34**	**-**	**46**	**-**
FLZ MEs-5	**1**	**10**	**36**	**-**	**-**	**54**
FLZ MEs-6	**1**	**20**	**32**	**-**	**-**	**48**

FLZ MEs=Fluconazole microemulsions, Smix=Mixture of surfactant and co-surfactant.

Evaluation Parameter Of Microemulsion Loaded Fluconazole

This was carried out by usingTransmission Electron Microscope (TEM) analysis[19],viscosity analysis[20],ph measurement[21], zeta-sizer analysis (globule size analysis)[22], zeta- potential[23],conductivity analysis[24], spreadability measurement[25], thermodynamic stability (freeze thaw cycle, heating cooling cycle and centrifugation)[26, 27], *in vitro* skin permeation studies, skin irritation studies.

In Vitro Skin Permeation Studies

The *in vitro* skin permeation study was carried out under the guideline compiled by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, Delhi, and Government of India). The abdominal skins obtained from male wistar rats weighing 240±20gm (age, 6-8 weeks) was used for *in vitro* permeation experiments of 6 prepared formulations. After hair was shaved carefully with an electric clipper, the skin was excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues were removed without damaging the epidermal surface. The excised rat skins were washed and examined for integrity, and then stored at 4°C for 24 h in phosphate buffered saline pH 6.8 (PBS), and then used for the permeation experiments. The permeation experiments were performed using Franz diffusion cells fitted with excised rat skins having epidermal surface outward. The effective diffusion area was 3.14 cm (20 mm diameter orifice), and the receptor compartment was filled with 10 ml of PBS. The diffusion cell was maintained at 37±1°C using recirculating water-bath and the solution in receptor chamber were stirred continuously at 600 rpm throughout the experiment. The formulation (1 g) was gently placed in a donor chamber. At 1, 2, 4, 6, and 8 h aliquot of 2ml of samples were withdrawn from the receptor compartment for Spectrophotometric determination and replaced immediately with an equal volume of fresh PBS. Average values of three readings of *in vitro* permeation data were calculated and the average cumulative amount of drug permeated per unit surface area of the skin was plotted *versus* time [28, 29].

Skin Irritation Studies

Irritation studies were carried out by using six wister rats. The hair of rats was removed by electrical shaver on dorsal side and microemulsion (4 ml) was applied on the area and kept under observation for seven days. Each day the area was observed for irritation and redness[30].

RESULTS AND DISCUSSIONS

Identification of fluconazole using FTIR spectrophotometer

The procured sample of fluconazole was identified by using FTIR spectrophotometer. Thus we can say that the given sample is fluconazole (Table 2).

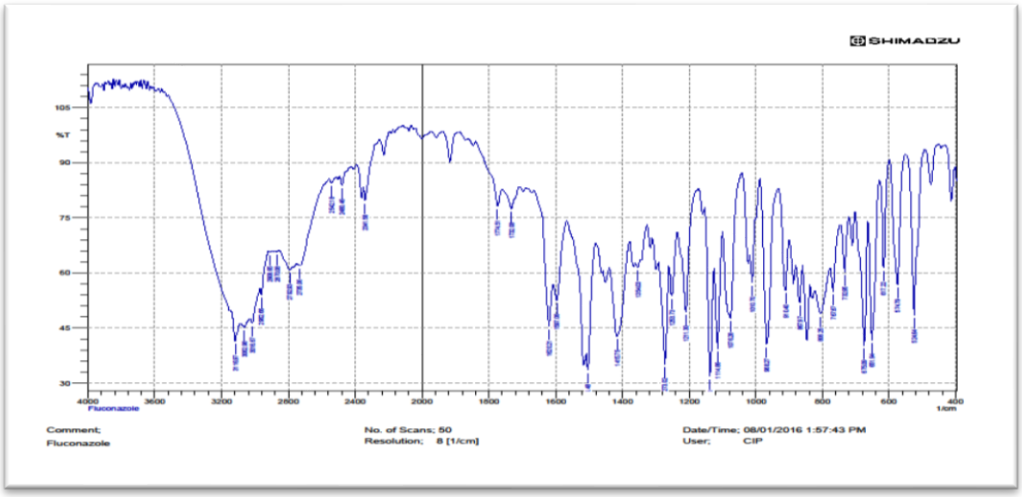


Figure 2: FTIR spectra of fluconazole

Table 2: Interpretation of IR spectra of fluconazole

S/no.	Wave Number(cm^{-1})	Assignments
1.	3116.97	Broad band due to hydrogen bond O-H stretching vibrations.
2.	3016.67	Aromatic C-H stretching vibrations
3.	1774.51	Combination bands consists with1, 2, 4-trisubstitutions of phenyl group.
4.	1557.05	Aromatic C=C and C=N stretching vibrations
5.	1211.30	Aromatic C-F stretching vibrations.
6.	968.27	Out of plane C-H deformation vibration for two adjacent aromatic hydrogen

DETERMINATION OF ABSORPTION MAXIMA (Λ-MAX)

The absorption maxima (λ-max) of drug sample was found to be 258 nm determined by using UV- Visible spectrophotometer (Shimadzu 1800).

DEVELOPMENT OF CALIBRATION CURVE

Standard calibration curve in table show the absorbance of fluconazole at different concentration range of 2,4,6,8, and 10µg/ml. in methanol. Figure 3shows the standard curve of fluconazole, which was found to be liner in the range of 2,4,6,8 and 10 µg/ml at 258 nm. The regression value was found to be $r^2 = 0.996$. (Table-3)

Table 3: Standard curve of fluconazole in methanol

S. No.	Sample	Concentration (µg/ml.)	Absorbance	Statistical parameter
1.	Fluconazole	2	0.109	Equation of line Y= 0.036x + 0.036. $r^2 = 0.996$
2.		4	0.187	
3.		6	0.245	
4.		8	0.319	
5.		10	0.404	

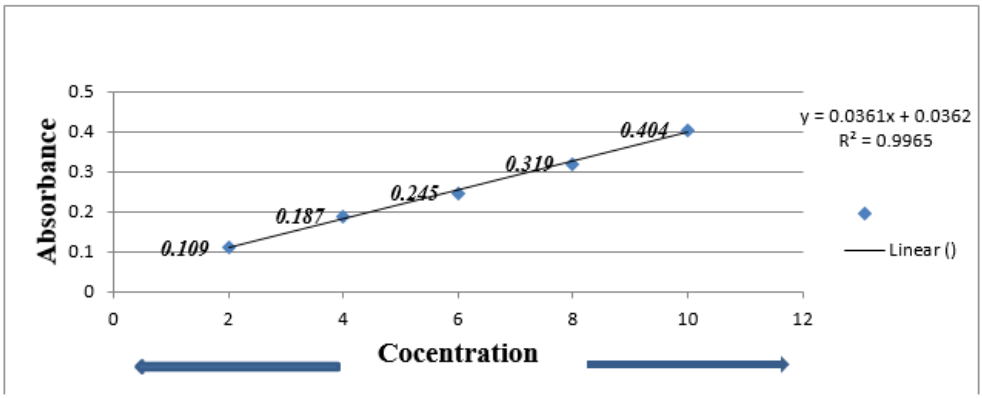


Figure 3: Standard calibration curve of fluconazole at 258 nm

Screening of oils for microemulsion

In the present work, solubility of fluconazole in different oils were determined and found to be highest in Castor oil(1.424 ±0.044 mg /ml), as compare to the Oleic acid (0.796 ± 0.023 mg /ml), Cinnamon oil (0.717±0.019 mg /ml), Soybean oil (1.290±0.008 mg/ml) and Sesame oil (1.274±0.042 mg /ml). Thus, Castor oil was selected as oil phase for the development of microemulsion formulation.

Screening of surfactants for microemulsion

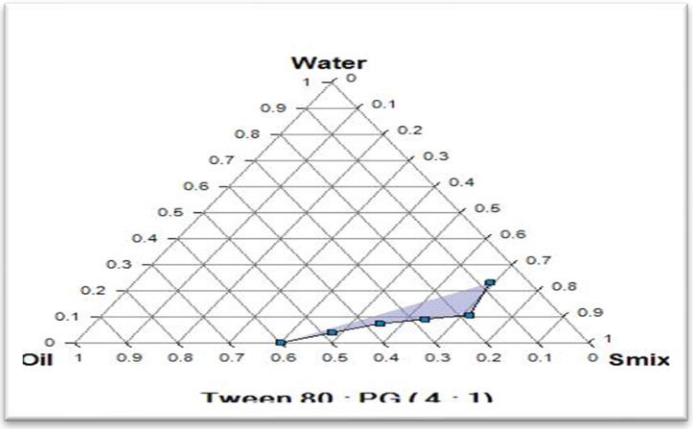
In the present work, solubility of fluconazole in different Surfactants were determined and found to be highest in Tween 80 (9.126±0.129 mg /ml), as compare to the other surfactants such as Tween 20 (7.254±0. 1.10 mg/ml) and Span 80 (5. 233±1.321). Thus, Tween 80 was selected as surfactants for the development of microemulsion formulation.

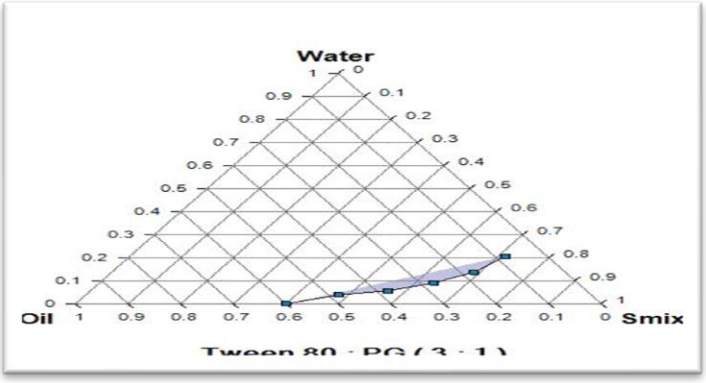
Screening of co-surfactants for microemulsion

In the present work, solubility of fluconazole in different co-Surfactants were determined and found to be highest in Propylene Glycol (4.665±1.453 mg/ml) as compare to the Polyethylene Glycol (3.449± 0.876 mg /ml). Thus, Propylene glycol (PG) was selected as co- surfactants for the development of microemulsion formulation.

Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams for microemulsion along with the ratios of surfactant and co-surfactant, as 2:1, 3:1, and 4:1 shown in Figure 4, the phase diagram at 4:1 (S/Cos) weight ratio was obtained the highest area of emulsification and so it was selected for further formulation.





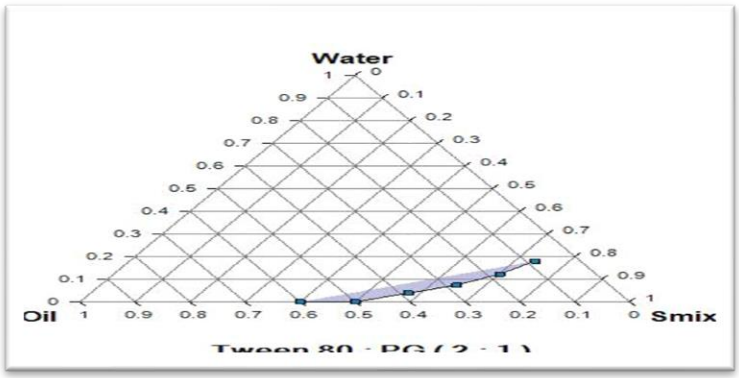
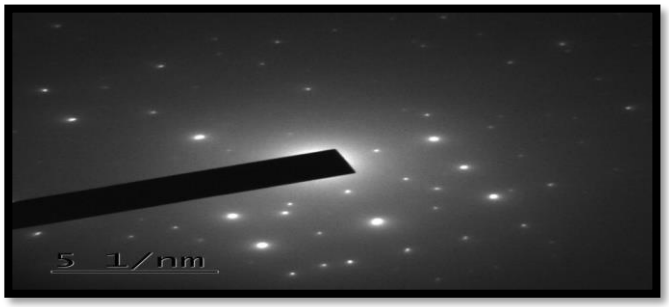


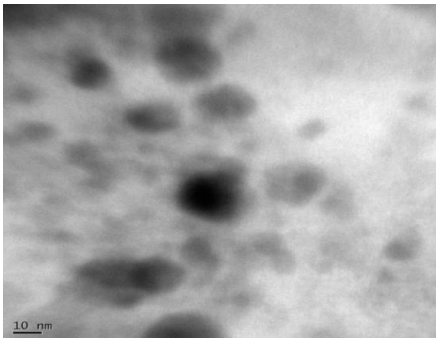
Figure 4: Pseudo-ternary phase diagram of microemulsion composed of oil phase (Castor oil), surfactant (Tween 80), co- surfactant (Propylene Glycol) and aqueous phase (Distill water)

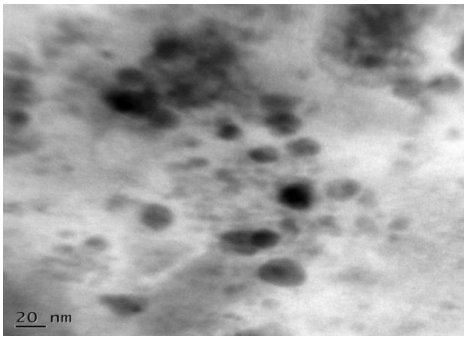
Transmission electron microscopy (TEM) analysis

The surface morphology of the prepared fluconazole microemulsion was characterized by TEM studies. The results of TEM pictures reveal that fluconazole micro-droplets were almost spherical in shape. The TEM images of the formulation are shown in Figure 5.

At 51nm







At 10nm **At 20nm**

Figure 5: Transmission electron micrographs of the formulation FLZ MEs-1 taken at 30,000 ×magnification. (At 200nm, 100nm, 51nm, 50nm, 20nm, 10nm) with accelerating voltage (200kv), Camera length (499.6) and resolution (0.24nm)

Zeta-sizer analysis (globule size analysis)

In this study, the droplet size of FLZ MEs 1 was found to be (153.9± 0.915nm) as compare to the other formulation like FLZ MEs 2 (312.2 ±0.418nm), FLZ MEs 3 (195.0 ± 0.931nm), FLZ MEs 4 (250.1± 0.120 nm), FLZ MEs 5 (294.8 ± 0.250 nm) and FLZ MEs 6 (193.0±0.351nm). ThusFLZ MEs1 (153.9± 0.915 nm) was found to be lowest droplet size on this basis FLZ MEs1 is good (Table 4).

Table 4: Zeta-sizer (nm) of fluconazole microemulsion

S.No.	Formulations	Droplet size (nm) Mean ± SD, n= 3
1.	FLZ MEs 1	153.9± 0.915nm
2.	FLZ MEs 2	312.2 ±0.418nm
3.	FLZ MEs 3	195.0 ±0.931 nm
4.	FLZ MEs 4	250.1± 0.120 nm
5.	FLZ MEs 5	294.8 ± 0.250 nm
6.	FLZ MEs 6	193.0±0.351nm

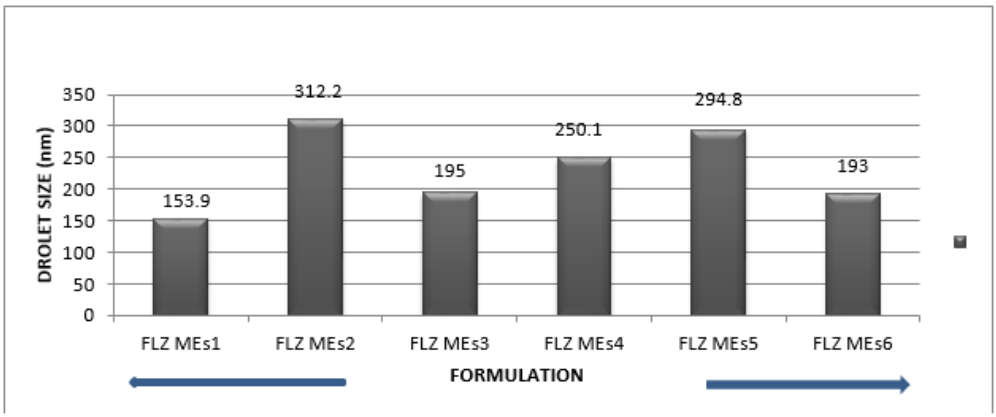


Figure 6: Droplet size (nm) of fluconazole microemulsion

Figure7: Droplet size report of FLZ MEs 1

Zeta potential analysis

In this study, the droplet size of FLZ MEs 1 was found to be (-10.9± 0.80 mV) as compare to the other formulation like FLZ MEs 2 (-15.9± 0.75mV), FLZ MEs 3 (−14.9 ± 0.34 mV), FLZ MEs 4 (-13.4 ± 0.95mV), FLZ MEs 5 (-13.0 ± 0.65 mV) and FLZ MEs 6 (-13.9 ± 0.70mV).Formulation FLZ MEs1 has lowest zeta potential (-10.9± 0.80 mV) on this basis FLZ MEs1 is good (Table 5).

Table 5: Zeta potential (mV) of fluconazole microemulsion

S/no.	Formulations	Zeta potential (mV) Mean ± SD, n= 3
1.	FLZ MEs 1	-10.9± 0.80 mV
2.	FLZ MEs 2	-15.9± 0.75mV
3.	FLZ MEs 3	-14.9 ±0.34 mV
4.	FLZ MEs 4	-13.4 ±0.95mV
5.	FLZ MEs 5	-13.0 ± 0.65 mV
6.	FLZ MEs 6	-11.9 ± 0.70mV

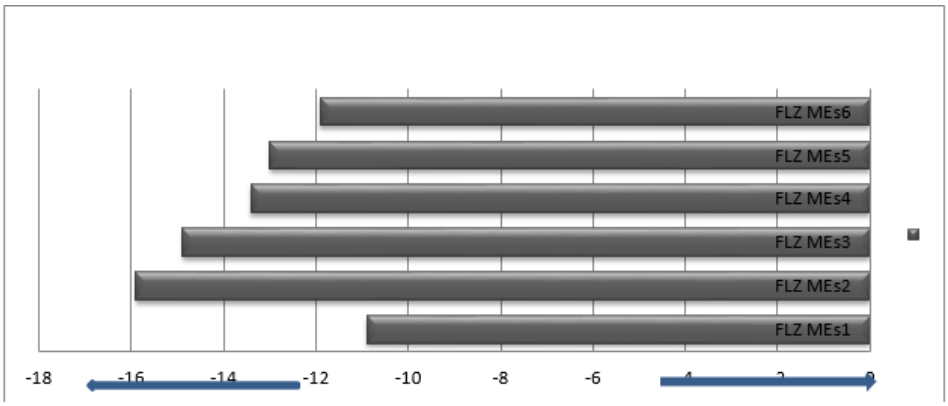


Figure 8: Zeta-potential (mV) of fluconazole microemulsion

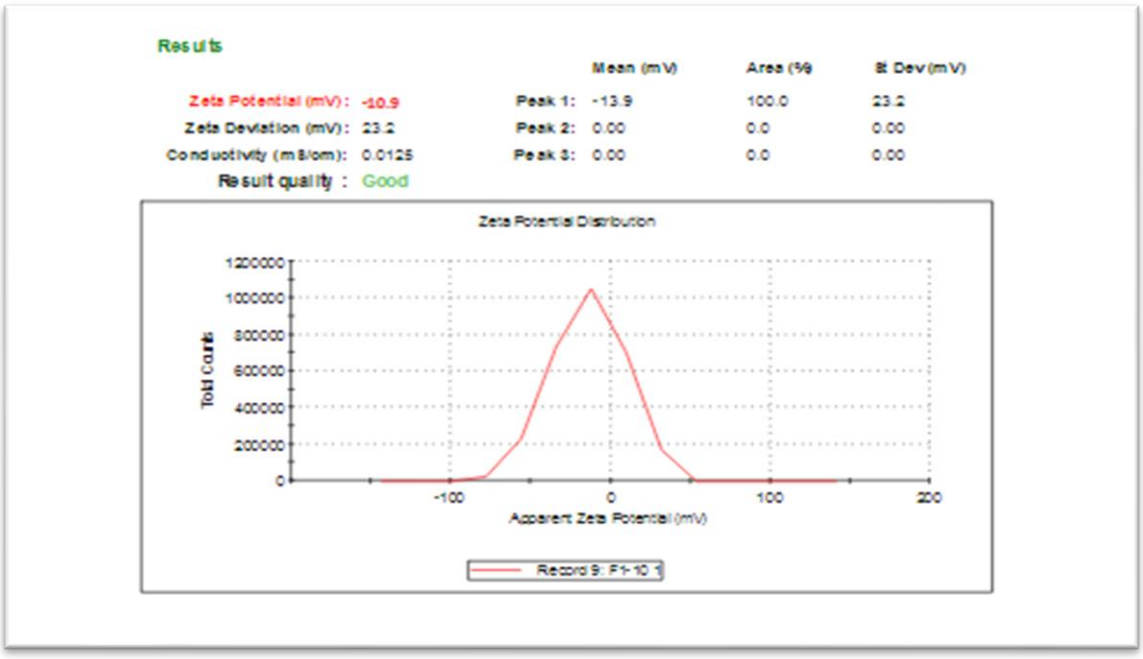


Figure 9: Zeta-potential (mV) of FLZ MEs1.

In-Vitro skin permeation studies

Results of Kinetic Analysis of the *In Vitro* Permeation Data through Wister rat Skin Obtained for fluconazole Loaded microemulsion were kinetically evaluated by the same mathematical models, namely zero-order, first order, Higuchi and Korsmeyer-Peppas model.The result are presented in (Table 6 & 7)

Table 6: % Cumulative drug release of FLZ MEs1 (4:1) and FLZ MEs3 (3:1)

TIME (hrs.)	% CUMULATIVE DRUG RELEASE(%CDR)	
	FLZ MEs1(4:1)	FLZ MEs3 (3:1)
1.	11.651±0.015	8.322±0.056
2.	29.964±0.036	23.132±0.032
3.	46.892±0.302	40.322±0.194
4.	56.702±0.403	51.526±0.029
5.	63.405±0.102	57.632±0.139
6.	72.408±0.016	67.540±0.049
7.	89.318±0.324	86.341±0.092
8.	95.215±0.215	92.504±0.103

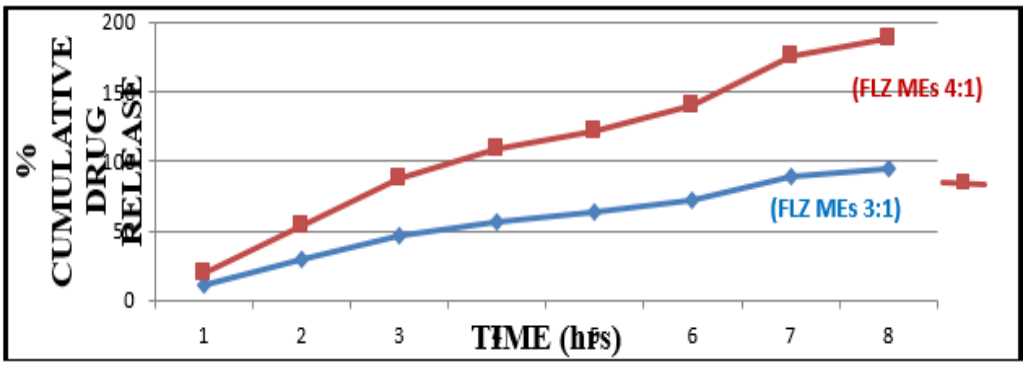


Figure 10: %Cumulative drug release of FLZ MEs (4:1 red line) and FLZ MEs (3:1 blue line)

Table 7: Results of kinetic analysis of the *in vitro* permeation data through Wister rat skin membrane obtained for fluconazole loaded microemulsion

Formulation	Zero Order (r^2)	First Order (r^2)	Higuchi (r^2)	Korsmeyer Peppas		Best fit model
				n	(r^2)	
FLZ MEs1	**0.993**	**0.966**	**0.925**	**0.889**	**0.912**	**Zero order**
FLZ MEs3	**0.980**	**0.976**	**0.958**	**0.743**	**0.929**	**Zero order**

Skin irritation study

In the skin irritation study only two groups were used. The results of skin irritation study revealed no irritation from microemulsion formulation of FLZ MEs1 and FLZ MES 3 as it produce a score of 0 revealed no irritation. (Table 8)

Table 8: Score of irritation study

FORMULATION	SCORE	
	Day 1	DAY 7
FLZMEs1	0	0
FLZ MES3	0	0

Where:0 = No irritation.

CONCLUSION

The microemulsion containing fluconazole was studied for topical delivery. Various type of components such as Castor oil, Tween 80, Propylene Glycol ((PG) and distill water used to preparation of microemulsion. All the components were selected through the screening method and the different microemulsion were prepared by using pseudo-ternary phase diagram. The microemulsion (4:1) FLZ MEs -1, containing 10% Castor oil, 54% Surfactant mixture (Smix) and 36% water was considered optimum. The FLZ MEs 1 showed the optimum value of all the parameter such as pH (5.41), Conductivity ($0.023\pm0.0015\mu s/cm$), Viscosity (200± 0.0067 cps), Centrifugation (Clear), Dilution analysis (Stable), Spreadibility (0.805± 0.005g/cm/s),Zeta-Sizer ($153.9\pm0.915nm$), Zeta-Potential ($-10.9\pm0.80mV$). Finally, the Microemulsion formulation (FLZ MEs-1) was found to be better result, may be effective for developing commercially viable formulation. From among all the developed formulation, FLZ MEs1 shows better drug diffusion for a period of 8 hrs, did not produced skin irritation. Therefore, it was selected as the best formulation. The release rate of drug from FLZ MEs1 formulation is best fitted to Zero- Order matrix model.

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