International Journal on Current Trends in Drug Development & Industrial Pharmacy
Vol. 3, Issue 1 - 2019

FORMULATION AND EVALUATION OF ETHOSOMAL GEL OF FLUCONAZOLE FOR TOPICAL DRUG DELIVERY

ROSHNI DHURVE[*], ASHWANI MISHRA[**]

ABSTRACT

Ethosomes has been used widely as a transdermal drug delivery system in past few years not only for improving bioavailability but also for the modified release property and compatibility as compared to the conventionally available topical formulations .In this work cold method was used to formulate Fluconazole entrapped Ethosomes for topical application .Prepared Ethosomes formulation were evaluated for Size, Percentage Entrapment, pH, Viscosity, In vitro Drug release study and interaction study .The best formulation was chosen on the basis of their release profile , entrapment efficiency and size, Then Optimized formulation RE4 and RE5 were uniformly dispersed in to the gel base of carbopol and Hydroxypropyl methyl cellulose for the proper and feasible application of the formulation on skin. These results obtained after determining Viscosity and pH of the Fluconazole ethosomes entrapped in gel were satisfactory, which confirmed that ethosomes could be efficient carrier for topical administration of fluconazole.

INTRODUCTION

Lipid-based systems has been widely used in the transdermal delivery due to their biocompatibility, ease of mixing with the skin lipids and devoid of irritation effect . There has been huge interest in the use of liposomes for transdermal drug delivery[1]

One of such drug delivery system is Ethosomes, They are innovative nano structured vesicles containing the drug in a matrix of lipids, or in hydrating medium it can be water or ethanol . The ethosomes are soft and a highly flexible vesicle efficiently and easily penetrates through the skin and increases the drug delivery of drug molecules. They are generally considered as elastic vesicles made up of Phospholipids containing 20-45% ethanol. Ethanol has one more characteristic of penetration enhancer by dissolving the skin lipids and increasing its fluidity. Theseethosomes have some more advantages over Liposomes and proliposomes such as more stability, less leakage of drugs, fusion of vesicles and breaking of vesicles. Ethanol is a well-known permeation enhancing drug delivery system .[2]

Ethosomes are formulated for Topical drug delivery which can be defined as the application of drug containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of general diseases with the intent of containing the pharamacological or other effect of the drug to the skin surface or within the skin.[3]

[*]Pharmacist at Community Health Centre, Department of Pharmacy, Barkatullah University, Bhopal.
[**]Assistant Professor, Department of Pharmacy, Department of Pharmacy, Barkatullah University, Bhopal.
Correspondence E-mail Id: editor@eurekajournals.com

 ISSN: 2581-7655

The aim of the present work was to design ethosomal drug delivery system of fluconazole, to overcome the disadvantages associated with conventional dosage forms and to achieve longer duration of action in order to improve topical bioavailability of the drug.

MATERIAL AND METHODS

Fluconazole was obtained as a gift sample, HPMC K4M, Soya lecithin, Cholesterol, Propylene glycol were purchased from CDH New Delhi, other chemicals were of analytical grade.

PREPARATION OF FLUCONAZOLE LOADED ETHOSOMES

Ethosomes of fluconazole were prepared by cold method. Phospholipid (soya lecithin), drug (Fluconazole) and other lipid materials (cholesterol) were dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mechanical stirrer at 1000 rpm for 2hrs. Propylene glycol was added during stirring. Stirring was continued for about 20-25 minutes. This mixture was heated to 30°C in a water bath. The water was heated to 30°C in a separate vessel is added to the mixture in the form of fine stream by using 22 guage syringe , which is then stirred for 5 min in a covered vessel at 700 rpm for 15 min in mechanical stirrer. The vesicle sizes of ethosomes can be reduced in bath sonicator (Systronics Naroda Ahemedabad). Finally, the formulation is stored under refrigeration. This method was used for different ratios of phospholipid, propylene glycol and ethanol.

OPTIMIZATION OF GEL BASE

Two gelforming polymers were taken for optimization they were HPMC K4M and Carbopol 934. It was visually observed that the consistency was not good and viscosity of HPMC K4M was not appropriate for ethosome whereas the consistency of Carbopol 934 was remarkably good. The pH of carbopol gel was maintained by using triethanolamine. As shown in Table no 1.

Table no 1.selection of gel base

S.no	HPMC K4M(%w/v)	Carbopol 934(%w/v)
1	0.5	0.5
2	0.75	0.75
3	1	1

INCORPORATION OF ETHOSOME IN GEL BASE

The prepared and optimized ethosomes having good entrapment efficiency were incorporated into the gel base 0.375g of Carbopol 934 was weighed and soaked in sufficient quantity of distilled water for 24 hr. After 24 hrCarbopol was dispersed in 50ml of distilled water by using magnetic stirrer at 500 rpm for 2 hr. It was then neutralized with sufficient quantity of Triethanolamine and stirred until a homogeneous gel was formed. As shown in Table no.2.

 ISSN: 2581-7655

Table 2.Different batches of drug entrapped Ethosomes

Batch No		COMPOSITION(%w/w)				
	Drug	Soya lecithin	Cholesterol	Propylene glycol	Ethanol	Water
RE 1	20mg	1	0.1	1	25	Upto 100ml
RE 2	20mg	1	0.1	1	30	Upto 100ml
RE 3	20mg	1.5	0.1	1	30	Upto 100ml
RE 4	20mg	1	0.1	1	35	Upto 100ml
RE 5	20mg	1.5	0.1	1	35	Upto 100ml
RE 6	20mg	1	0.1	1	40	Upto 100ml

EVALUATION AND CHARACTERIZATION OF ETHOSOMES

PARTICLE SIZE ANALYSIS

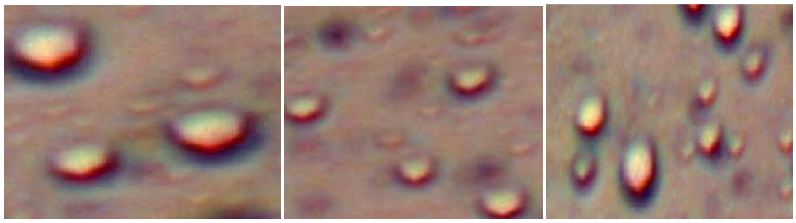
The particle size and particle size distribution of ethosome was evaluated using optical microscope. The prepared slide of ethosome and it was examined by an optical microscope and size of the ethosome was measured using the pre-calibrated occularmicrometer at 40x magnification. About 25 ethosomes of each formulation were observed and average particle size was determined,as shown in Table no.3.

Table 3.Ethosome size of different batches

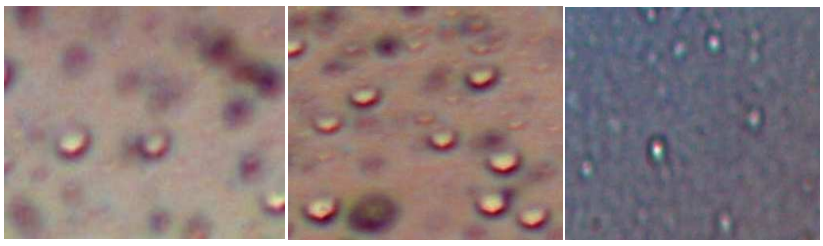
Formulation code	Vesicle size(µm)
RE 1	5.98±1.03
RE 2	5.13±0.73
RE 3	5.45±0.89
RE 4	3.89±0.34
RE 5	4.62±0.46
RE 6	3.46±0.35

SIZE AND SURFACE MORPHOLOG

Shape and surface morphology of prepared ethosomes were observed in Leica EC3. The prepared slide was observed at 10 x magnification,as shown in Fig no.1.



RE 1 RE 2 RE 3



RE 4 RE 5 RE 6

Figure 1.The size of formulation RE 1,RE 2,RE 3,RE 4,RE 5,RE 6 at 10x magnification

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DRUG ENTRAPMENT EFFICIENCY

The entrapment efficiency of ethosomes was measured by the ultracentrifuge method .Entrapment efficiency of ethosomal formulations was determined by separating the unentrapped drug from Vesicular preparations (2ml) containing fluconazole .Un-entrapped drug was separated by ultracentrifugation method. This vesicular suspension was diluted upto 10 ml with distilled water. Centrifugation was carried out at 2000rpm for 20min.The supernatant liquid was analyzed for an un-entrapped drug by UV spectrophotometer at 261nm. Again centrifugation was carried out at 12000 rpm for 30 min for analyzing total amount of drug in sample,as shown in Table no.4.

Table 4.Entrapment efficiency of different formulations

Batch No	Percentage Entrapment Efficiency
RE1	44.04%
RE2	55.71%
RE3	52.57%
RE4	73.53%
RE5	64.45%
RE6	45.60%

Amount of drug entrapped= Total amount of drug - Amount of unentrapped drug

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped (sample)}}{\text{Total amount of drug (sample)}} \times 100$$

IN VITRO DRUG RELEASE STUDY

The *invitro* skin permeation of fluconazole from ethosomal formulation was studied using locally modified diffusion cell. The *invitro* diffusion of the drug through semi permeable membrane was performed by incorporating saline phosphate buffer saline pH 7.4 as the release medium. The semi permeable membrane was soaked in a saline buffer for 6-8 hours. It was clamped carefully to one end of the hollow glass tube of 17 mm (area 2.011 cm^2) (dialysis cell). This acted as donor compartment. 50 ml of saline PBS 7.4 pH was taken in a beaker of 250 ml which was used as a receptor compartment. A weighed amount of ethosomal gel containing drug equivalent to 8 mg of Fluconazole, was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37 ± 2 ºC. The solutions of the receptor side were stirred by externally driven magnetic bars using magnetic stirrer at 50 rpm. 1 ml sample was withdrawn at every 1 hr interval and replaced by fresh saline PBS 7.4 pH to maintain the simulated condition. These samples were diluted upto 5 ml with saline PBS 7.4 pH .The drug concentrations in the aliquots were determined at 261 nm using UV spectrophotometer

PHOF THE ETHOSOMAL GEL

The pH measurements of Optimized batch RE4 and RE5 of the gel was carried out by using a digital pH meter by dipping the glass probe completely into the formulation as to cover the probe. Results are shown in Table no 6

VISCOSITY OF THE ETHOSOMAL GEL

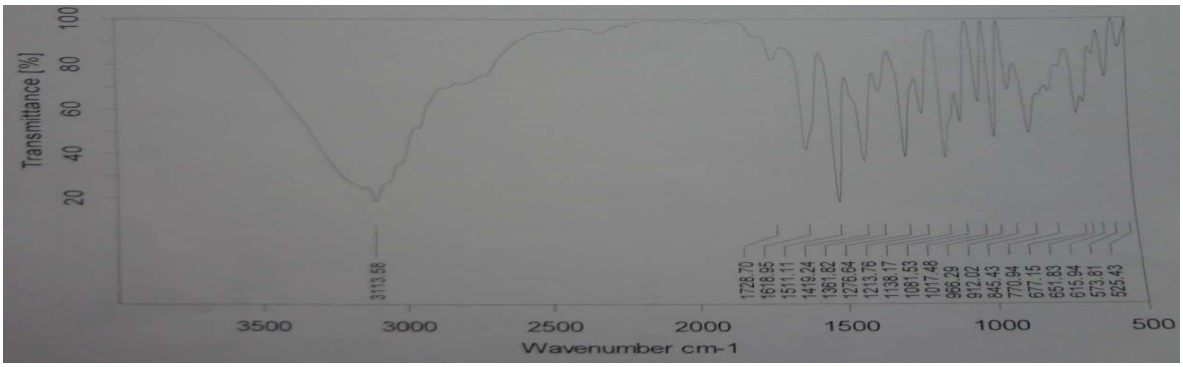
The viscosity ofthe gel was determined by using a Brookfield viscometer DVII model with T-Bar Spindle T 95 .The viscosity was measured using 50 gm of gel filled in a 100ml beaker. The Temperature probe was kept in to the formulation and recorded the temperature then rotation speed of the T shape spindle was

 ISSN: 2581-7655

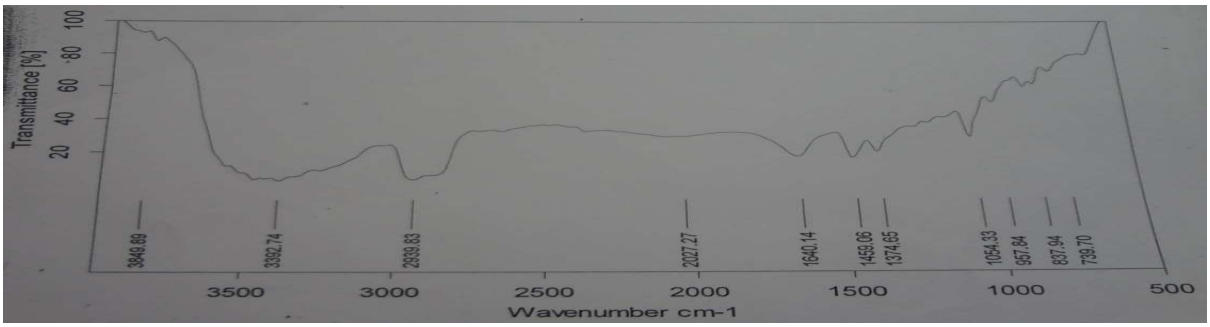
set at 100 rpm and it was then dipped in to the gel formulation and readings were recorded, are shown in Table no 6.

INTERACTION STUDY USING FTIR

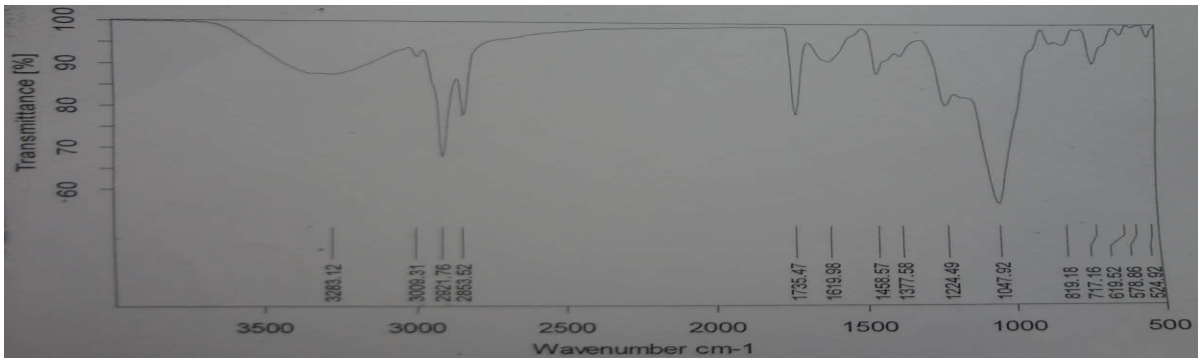
FTIR study was carried for the evaluation of physicochemical compatibility and possible interaction between drug and phospholipid. FTIR spectrum of fluconazole ,soya lecithin and cholestrol was taken by using KBr disc and spectra was recorded in the wavelength region between 4000 to 400 cm^1 FTIR study was also carried out for studying the possible interaction in the final formulation, as shown in Fig no 2.



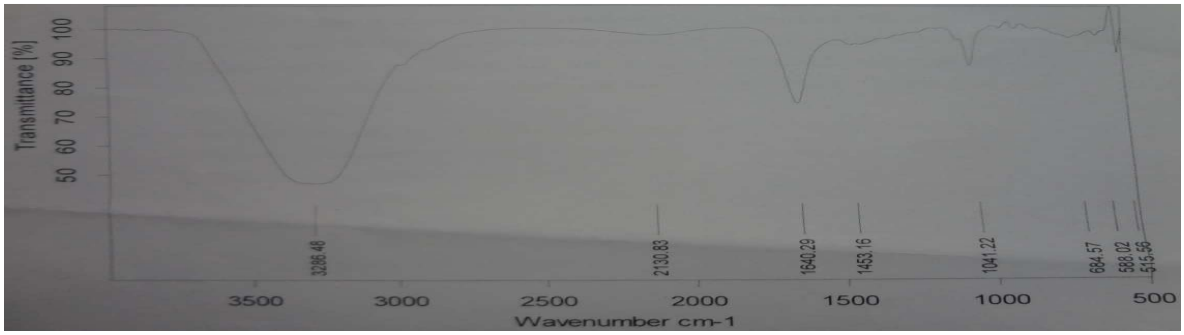
FTIR of Fluconazole



FTIR of Cholesterol



FTIR of lecithin



FTIR of final formulation

Fig no 2 FTIR Spectra of Fluconazole, Cholesterol, Lecithin and final Formulation

 ISSN: 2581-7655

RESULT AND DISCUSSION

PARTICLE SIZE

The size range of ethosomes were in the range of 3.46-5.98µm. The particle size was found to be increased with increase in soya lecithin concentration but as the ethanol concentration was increased the size range of ethosome was decreased, ethanol probably caused an alteration of the net charge of the system and conferred it some extent of steric stabilization that might finally lead to a reduction in the vesicle size. Depending upon the size range the order for the formulation was RE 1>RE 3>RE 2>RE 5>RE 4>RE 6. As shown in table no 3.

SHAPE AND SURFACE MORPHOLOGY

The shape and morphology was studied using Leica EC 3.The prepared slide was observed at 10x. It was observed that when the soya lecithin concentration was increased the size of ethosome also increased. The size of ethosome was also affected by ethanol concentration as ethanol concentration was lower the size was larger than the batch containing higher ethanol concentration. As shown in fig no 1.

ENTRAPMENT EFFICIENCY

The values were obtained for entrapment efficiency from the formulations and concluded that the entrapment efficiency was affected by phospholipid and ethanol concentration. By increasing phospholipid concentration it was observed that the entrapment efficiency decreases whereas on keeping phospholipid concentration constant and increasing ethanol results in increasing entrapment efficiency. Entrapment efficiency (average) of the formulations containing 1.5%w/w of soya lecithin and ethanol 30%w/w (i.e, RE 3) was lower than that of the formulations having 1%w/w soya lecithin and ethanol 30%w/w (i.e, RE 2).where as batch RE 5 having 1.5%w/w soya lecithin and 35%w/w shows better entrapment efficiency due to presence of high ethanol concentration. As shown in table no 4.

IN-VITRO DRUG RELEASE STUDY

In vitro drug release study was carried out for RE 4 and RE 5 formulation as their entrapment efficiency was better than RE 1 ,RE 2, RE 3,RE and RE 6.RE 4 showed maximum release. *In vitro* drug release study showed that the maximum drug release of 54.5% was obtained for RE 4 in 7 hours. As shown in table no 5.

INTERACTION STUDY BY FTIR

FTIR studies revealed that there was no interaction was observed between the drug and phospholipids used in the formulation i.e.Soya lecithin and cholesterol as shown in fig no 2 and table no 6-10-. FTIR study also revealed that due to presence of ethanol there was a slight shift in the triazole ring peak 1618.94 cm^{-1} to 1640.29 $cm^{-1}$and the C-N stretching vibration was observed in peak 1041.21 cm^{-1}.

PH THE ETHOSOMAL

Final batches RE 4 and RE 5 were incorporated into the gel and the pH was calculated. It was observed that batch RE 4 showed pH at 6.8 whereas RE 5 showed pH 6.6 due to presence of 1.5%w/w soya lecithin. As shown in table no 5.

VISCOSITY OF THE ETHOSOMAL GEL

The viscosity of the batch RE 4 and RE 5 were calculated. The viscosity was maintained so that it could spread easily. The viscosity of batch RE 4 was 3200 cps and RE 5 was 3100 cps at shear rate of 100 rpm as shown in table no 6.

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Table 5.*In vitro* percentagedrug release profile of ethosomal formulation code RE 4 and RE 5

Time (hours)	% Drug release	
	RE 4	RE 5
1	31.25	31.37
2	35	33.75
3	38.37	35.62
4	41.75	37.25
5	45.62	39.75
6	50.12	41
7	54.5	42.12

Table 6.pH of batch RE 4 and RE 5

S. no	Batch name	pH	Viscosity(cps) at 100rpm
1	RE 4	6.8	3200
2	RE 5	6.6	3100

In batch 1 HPMC K4M had very poor consistency and viscosity of 1100cps whereas carbopol 934 of viscosity 2100 cps was better than HPMC K4M. In batch 2 HPMC K4M was better than batch 1 but still poor consistency and viscosity of 1200 cps but carbopol showed good consistency and viscosity of 3200cps. In batch 3 HPMC K4M was still not up to the mark whereas Carbopol had became thick and viscous from previous batches with viscosity of 4100cps so,0.75%w/v Carbopol was selected for incorporating ethosomes, as shown in Table no.2.

Table 7.Interpretation of IR spectra of fluconazole

S no	Wavenumber(cm^{-1})	Characteristic Absorbtion	Nature of peak
1	3113.58	C-H aromatic stretching	Strong
2	1618.95	C=N (Triazole ring)	Strong
3	1511.11-1419.24	C=C stretching	Strong
4	1276.64	Aryl Fluorides	Strong
5	1213.75-1017.47	C-N stretching vibration	Strong

Table 8.Interpretation of IR spectra of cholestrol

S no	Wavenumber(cm^{-1})	Characteristic Absorbtion	Nature of peak
1	3849.88-3392.74	OH stretching inter molecular H bonding	Weak
2	2939.82	C-H bonding	Medium
3	1374.64	Di methyl group	Strong
4	957.84-739.70	C-H out of plane bending polynuclear aromatic	Strong

Table 9.Interpretation of IR spectra of soya lecithin

S no	Wavenumber(cm^{-1})	Characteristic Absorbtion	Nature of peak
1	3009.30-2853.52	C-H streching	Weak
2	1735.47-1619.97	C=O stretching	Strong
3	1458.57-1377.58	$CH_2$and CH_3 deformation	Strong
4	1224.48-1047.92	P=O bond	Medium

 ISSN: 2581-7655

Table 10.Interpretation of IR spectra of final formulation showing peaks of functional groups of Fluconazole

S. no	Wavenumber cm^{-1}	Characterstics absorption	Nature of peak
1	1640.29	C=N(Triazole ring)	Strong
3	1041.21	C-N stretching vibration	Strong

CONCLUSION

In the present work a potential drug delivery system of fluconazole was developed and characterized.

Fluconazole loaded ethosome was successfully prepared by using cold method and incorporated in the gel base. The prepared ethosomes were evaluated for particle size, surface morphology, entrapment efficieny. The particle size was found to be in the range of 3.46-5.98µm. it was observed that the size of ethosome was directly affected by the ethanol concentration and soya lecithin concentration, as the ethanol concentration was increased the size of ethosomes decreased and on keeping ethanol constant and increasing soya lecithin concentration the size was found to be increased. The ethosomal batch RE 4 with 1% w/w soya lecithin and 35%w/w ethanol shows entrapment efficiency of 73.53%. At optimum ethanol concentration the entrapment efficiency was increased while increasing its concentration leads to disturb the bilayer structure of ethosome and decreased entrapment efficiency.

The ethosomal formulation RE 4 and RE 5 was finally incorporated into gel and *in vitro* drug release was performed. The release rate was dependent on ethanol and phosholipid concentration. The pH and viscosity of the final formulation was observed to be 6.8 and 6.6, 3200 cps and 3100 cps respectively for batch RE 4 and RE 5. The pH was affected due to the soya lecithin concentration.

The interaction study by using FTIR between the phopholipid and drug shows no change in characteristic peak of the drug. Whereas the interaction study of final formulation showed a very slight difference in the characterstic peak of drug**.** But the intensity of peak was strong same as of the pure drug.

These results are very satisfactory which confirmed that ethosomes are a very efficient carrier for topical administration of fluconazole.

ACKNOWLEDGEMENT

Author is thankful to Dr A.K. Pathak Head Department of Pharmacy to provide research facility and Dr Ashwani Mishra for constant support and guidance .

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 ISSN: 2581-7655