An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DESIGN, OPTIMIZATION AND CHARACTERIZATION OF AN ETHOSOMAL GEL USING MICONAZOLE NITRATE FOR TRANSDERMAL DRUG DELIVERY

Pratima S. Kokare*

Department of Pharmacy,

TPCTs College of Engineering, Osmanabad-413501 (M.S), India.

***Corresponding author E-mail:** pratimakokare19@gmail.com

ARTICLE INFO

Key Words
Ethosomes, Miconazole nitrate, transdermal, Touitou hot method

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. The aim of current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes. Miconazole nitrate ethosomes were prepared using Touitou hot method. Miconazole nitrate loaded ethosomes were prepared using varying concentrations of phospholipid and ethanol, with the help of propylene glycol as penetration enhancer, were optimized and characterized for percent entrapment efficiency, zeta potential, particle size, vesicle morphology and *in-vitro* drug permeation studies. Among all formulations (H1 to H4 containing PC50 while H5 to H8 containing PC70), the formulations with PC70 showed better cumulative amount of drug release. Miconazole nitrate ethosomal gel was prepared using carbopol 940 and it was characterized for pH, spreadability, homogeneity, percent drug content and all the formulations showed fairly acceptable values. 2^2 full factorial design was applied for optimized method H5 to H8 prepared with Pc 70 and ethanol by using Design Expert which showed significant effect on the responses, entrapment efficiency (96.66%) and percent drug release (90.38%). The compositions of ethosomes and gels were manipulated to investigate their effects on the characteristics of final formulations. The miconazole nitrate ethosomal gels also characterized for Erythema and Edema on Albino rats which showed zero irritation score.

1. INTRODUCTION

Skin covers a total surface area of approximately 1.8m^2 and provides the contact between human body and external environment. Drug delivery through human skin has become important aspect of modern therapy. Dermal drug delivery is the topical application of drugs to the skin in the treatement of skin diseases and other infections. Transdermal delivery has become important delivery route that delivers very precise amount of drug through the skin for systemic action. The almost insurmountable nature of SC is a major challenge for systemic delivery of percutaneously applied drugs. Furthermore, it is even more difficult for anything to penetrate to the deeper strata of skin. To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier.[1, 2]

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. Ethosomes are well established drug

carrier than liposome with slight modification.[3] The size range of ethosomes may vary from tens of nanometers (nm) to microns (μ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux .[4,5,6,7]

In the present research work, Miconazole nitrate is used as anti fungal drug. It has low solubility which leads to inadequate therapeutics effect. Therefore, miconazole nitrate ethosomes were prepared for enhancing the drug penetration in skin.

2. MATERIALS AND METHODS:

2.1 Materials:

Miconazole nitrate was obtained from Cadila Pharmaceutical ltd. Ahemdabad as gift sample. Levic 50/70 grade phosphotidylcholine was supplied from VAV, Life sciences, Mumbai. Propylene glycol was supplied from Fine chemicals, Mumbai. All other chemicals used for analysis were of analytical grade.

2.2 Methods:

2.2.1 Prepararion of ethosomes: Miconazole nitrate ethosomes was prepared as described by Touitou et al. Hot method and was used for preparation of ethosomes given in **Table 1**. The ethosomes system of 2 % w/w Miconazole nitrate was comprised of 1-4% (w/v) phospholipid, 50% and 70% levic, ethanol 30-50% (v/v),propylene glycol, and water up to 100%w/w.

Hot method: Disperse phospholipid in water by heating in a water bath at 45°C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat up to 45°C. Add the organic phase into the aqueous phase. Dissolve the drug in water or ethanol depending on its solubility on magnetic stirrer (Mechanical stirrer, Remi equipments, Mumbai) at 1000 rpm in a closed vessel and mix properly.[5,6]

2.2.2 Prepararion of ethosomal gel: The specific amount of carbopol 940 powder was added to distilled water and kept at 100°C for 20 min. Triethanolamine was added to it drop-wise. Ethosomal suspensions equivalent to 2% of drug was then incorporated into gel base. Water q.s. was added with continuous stirring until homogenous formulations were achieved. Gel containing free ethosomes was prepared by similar using 2% carbopol 940.[8] Composition of ethosomal gel given below in **Table. 2.**

2.2.3 Characterization:

A) Characterization of ethosomes:

Ethosomes were characterized by optical microscopy for photographic images, then Miconazole entrapment efficiency calculated by using percentage entrapment formula. The particle size of ethosomes was analysed for homogenous size distribution. Zeta potential of ethosomes was checked for surface charges which are responsible for flocculation. The morphological characterization of ethosomes was carried out using Scanning Electron Microscopy (SEM). Also, turbidity of ethosomes was evaluated by using Turbidometer.

B) Characterization of ethosomal gel:

Physical parameters of gels: [8]

Various physical parametes of Ethosomal gel formulations (H1 to H4) were characterized for pH, spreadability, homogeneity and viscocity.

Drug Content Determination:

Drug concentration in Gellified ethosomes was measured by spectrophotometer. Miconazole nitrate content in Gellified ethosoms was measured by dissolving Known quantity of Gellified Ethosomes in solvent (methanol) by Sonication. Absorbance was measured after suitable dilution at 272nm in UV/VIS spectrophotometer.

***In- vitro* Drug Permeation Study: [8]**

In-vitro release of Miconazole nitrate from ethosomal formulation was studied using locally Franz diffusion cell (Dolpin-1366, Systronic Analytical Instrument, Ahmadabad). The effective permeation area of the diffusion cell and receptor cell volume was 2.50cm^2 and 200ml of pH 7.4 and was constantly stirred by magnetic stirrer at 100rpm. The skin of mice was mounted between the donor and receptor compartments. Ethosomal gel formulation (equivalent to 10 mg drug) was applied to the membrane. 2ml sample were withdrawn through sample port of the diffusion cell at predetermined time interval over 24 hours and diluted it to 10 ml with methanol. The samples were analyzed spectrophotometrically at 272nm. The receptor phase was immediately replenished with equal volume of distilled

water. Sink condition was maintained throughout the experiment.

Permeation data analysis:

Cumulative drug permeated through skin (µg/cm^2) was plotted as function of time (t) for each formulation. Drug flux at steady state (Jss) was calculated from the slope of linear portion of graph.

Release Kinetics:[9]

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into, zero order, first order, Higuchi matrix, and Peppas model. In this by comparing the r- values obtained, the best- fit model was selected. Studies were analysis by PCP disso 2.8v software.

Skin irritation studies:

The Rats were divided into III groups. On the previous day of the experiment, the hairs on the backside area of Rats were removed. The animals of group I was served as normal, without any treatment. One group of animals (Group II, control) was applied with ethosomal gel. A 0.8% v/v aqueous solution of formalin was applied as a standard irritant (Group III). The animal's studies were carried up to 3 days and finally the application sites were graded according to a visual scoring scale, always by the same investigator.

$$\text{Average score} = \frac{\sum Ertyhema\ grade\ at\ 72\ hr + \sum Edema\ grade\ at\ 72\ hr}{No.of\ subject}$$

…eq 3[10]

3. RESULTS AND DISCUSSION:

3.1 Preformulation studies [11]

3.1.1 Solubility studies: Miconazole nitrate was found to be freely soluble in methanol and warm propylene glycol.

3.1.2 Melting point determination: The Melting point of Miconazole nitrate was found to be 180-185°C which is within reported range. It complies with the standards thus indicating the purity of drug sample.

3.1.3 Determination of λ max:

Miconazole nitrate pure drug solution in methanol was scanned between 200 nm to 400 nm using UV spectrophotometer exhibited in **Fig. 4.1**.The peak was observed at 272 nm and same was selected as λmax for further analysis of drug.[12]

3.1.4 Calibration curve of Miconazole Nitrate in methanol:

From the standard stock solution, a series of dilutions were prepared using methanol. The absorbance of these solutions was measured against blank methanol at 272nm. Calibration graph was plotted against absorbance Vs drug concentration given in **Fig. 2.**

3.1.5 Compatibility studies with FTIR:

FTIR Spectrum of pure drug was found to be similar to the reference standard IR Spectrum of Miconazole nitrate which indicates that obtained sample was pure.[13] An infrared spectrum of Miconazole nitrate ethosomes was found to have shown peaks at 3406.0 cm^{-1} due to CN stretching, aromatic C-H stretching at 2974.6cm^{-1}, aliphatic C-H_2 stretching at 2932.0cm^{-1}, C=C aromatic stretching at 1646.7cm^{-1}, C-N stretching at1335.8cm^{-1} and C-C stretching 1078.3cm^{-1} which are characteristics of Miconazole nitrate **Fig. 3** observation further supports from **Table 3**. FTIR spectroscopy results, indicated that there is no interactions between drug and additives used in the preparation.[14]

3.1.6 Compatibility studies with DSC:

DSC studies of pure drug were carried out and endotherm was found to be at 188°C. In study revealed that it is similar to the reference standard DSC of Miconazole nitrate which indicates that obtained sample was pure.[14] Phase transition from crystalline to amorphous is indicated by DSC thermogram *i.e.* pure Miconazole nitrate shows the endothermic peak at its melting point at 188.0°C. DSC curves of selected formulation (H5) observed at 179°C .The thermogram showed the slight shifting of melting endotherm of Miconazole nitrate, which could indicate the complete amorphization of drug as well as loss of its crystalline nature. The DSC of pure Miconazole nitrate and ethosomes formulation (H5) is as given in **Fig. 4 A** and 4 **B** respectively. This result further confirmed

that there is no any interaction between pure drug and phosholipid PC 70.

3.2 Preparation of ethosome: Ethosomal formulations composed of phospholipid, drug, propylene glycol and ethanol were prepared using the hot method in 2^2 factorial design and formulation of different batches were prepared by using Ultra Turrex.

3.3 Characterizations of ethosomes:

3.3.1 Microscopic study:

The vesicular structure was confirmed by, visualizing the ethosomes formulation of Miconazole nitrate under optical binocular microscope (Digi-2, Labomed, USA) in **Fig 5** microscopic prepared differently with two grades of (Pc) 50/70.Images below showed that spherical vesicles formed by hot method. [15]

3.3.2 Determination of percent entrapment efficiency: As shown in **Table 4** ethosomes prepared by using 50% (w/w) ethanol (H5) showed the maximum entrapment percentage of 96.66%. It was observed that ethosomes were more stable at higher ethanol concentrations. Ethanol may exert a stabilizing effect in the formulation, preventing or at least delaying the formation of vesicle aggregates, because of the electrostatic repulsions. One of important factors governing the stability and the entrapment efficiency of the vesicles is the ethanol concentration; the vesicles containing high ethanol concentrations have thinner membranes, corresponding to the formation of a phase with interpenetrating hydrocarbon chains. **Fig. 6** gives an idea about higher the concentration ratio of Pc and ethanol gives larger vesicle and when ratio of Pc and ethanol is less small vesicle had been observed. [15,16, 17]

3.3.3 Particle size and size distribution analysis: Formulation H5 was analysed for Particle size due to high percent entrapment efficiency and good vesicular structure given in **Fig. 7**. Formulation H5 contains was having largest Pc 70 and ethanol concentration i.e. 4%w/w Pc and 50%w/w ethanol. Particle size of H5 was found to be 181.4nm and polydispersity index (PI) was found to be 0.23 and it remained in all cases <0.1, indicating that the ethosomal suspensions of Miconazole nitrate showed a homogeneous size distribution in H5 formulation.[18]

3.3.4 Zeta potential: Zeta potential of formulation H5 was found to be -47.2mv in **Fig. 8** which shows good stability for ethosomes formulations.

3.3.5 Scanning Electron Microscope: Futher investigation of formulation by scanning electron microscope showed that the ethosomes have a lamellar vesicular structure, and this confirms the existence of vesicular structure of lipid bilayer, spherical structure of vesicles with a smooth surface **Fig. 9**.[19]

3.3.6 Turbidity studies: It was observed that turbidity measured in Nephalometric Turbidity Units (NTU) increased with decreasing ethanol concentration.[8] **Fig. 10** shows difference in turbity with difference in ethanol concentration.

3.4 Preparation of ethosomes into gel and its evaluation:[8]

Ethosomal suspension is then formulated in to gel using carbapol 940.

3.4.1Evalution of topical gel formulation:-

a) **Physical examination**
The prepared Miconazole nitrate Gellified suspension formulations were white viscous creamy with a smooth and homogeneous appearance.

b) **Measurement of pH**
The pH values of all prepared formulation ranged from 6.4 to 6.7, which are acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 6.5.

c) **Homogeneity**
All developed gel showed good homogeneity with absence of lumps.

d) **Spreadibilty**
The spreadability of various gel formulations shgows that spreadabilty of H5 is 22 (g.c.m/sec.) This is higher than other formulations. The values of Spreadability indicate that the gel is easily spreadable by small amount of shear as shown in **Table 5**.

e) **Viscosity**
The measurement of viscosity of the prepared Gellified ethosomes was done with Brookfield viscometer. The Gellified ethosomes were rotated at 10 (min.) and 100 (max.) rotation per minute with spindle 61. At each speed, the corresponding dial reading was noted in **Table 6.**

Table 1: Hot method with phosphotidylcholine 50% and 70%

Ethosomes formulations	Drug (gm) w/w%	Pc w/w%	Ethanol w/w%	Propylene glycol w/w%	Water w/w%
H1	2	4	50	20	q.s
H2	2	4	30	20	q.s
H3	2	1	50	20	q.s
H4	2	1	30	20	q.s
H5	2	4	50	20	q.s
H6	2	4	30	20	q.s
H7	2	1	50	20	q.s
H8	2	1	30	20	q.s

H1-H4 hot method with PC 50%, H5-H8 hot method with PC 70%

Table 2: Composition of ethosomal gel form

Gel Ingredients	**Ethosomal Gel**			
	H1	**H2**	**H3**	**H4**
Ethosomes	Eqv. To 2% of drug	Eqv. To 2% of drug	Eqv. To 2% of drug	Eqv. To 2% of drug
Miconazole Nitrate	-	-	-	-
Carbopal 940	2%	2%	2%	2%
Triethanolamine	0.5%	0.5%	0.5%	0.5%
Distilled water	q.s	q.s	q.s	q.s

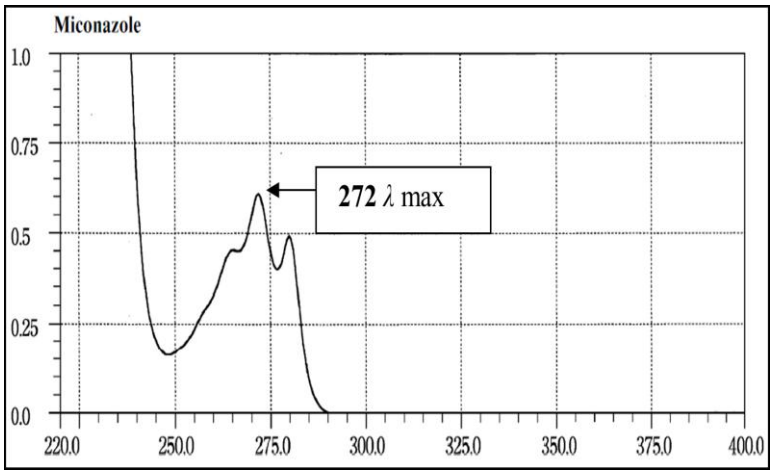


Fig.1 λmax of Miconazole nitrate

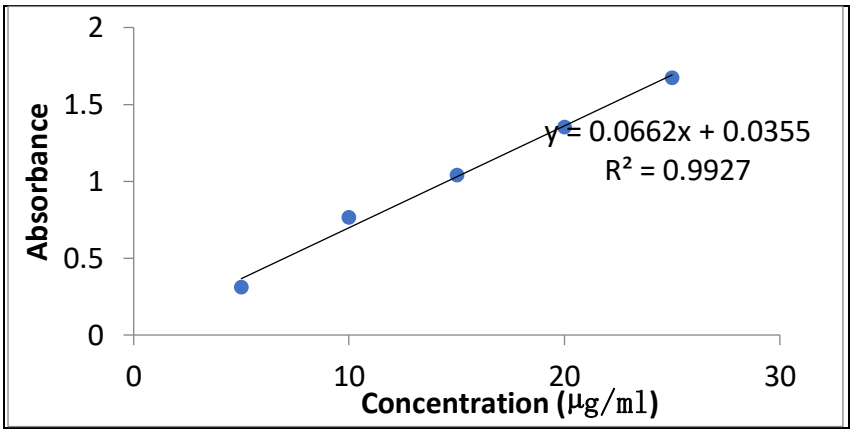


Fig. 2 Calibration curve of Miconazole nitrate

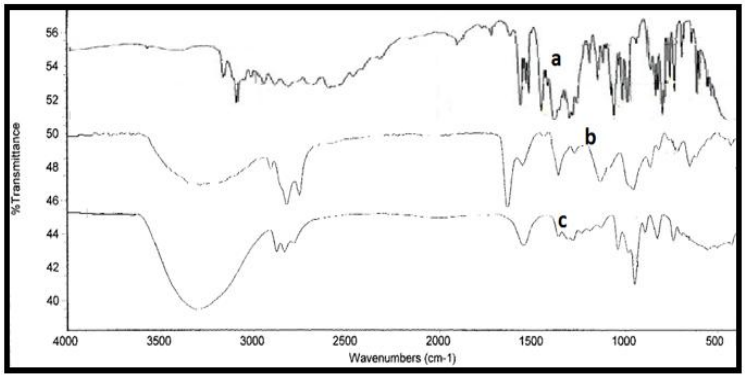
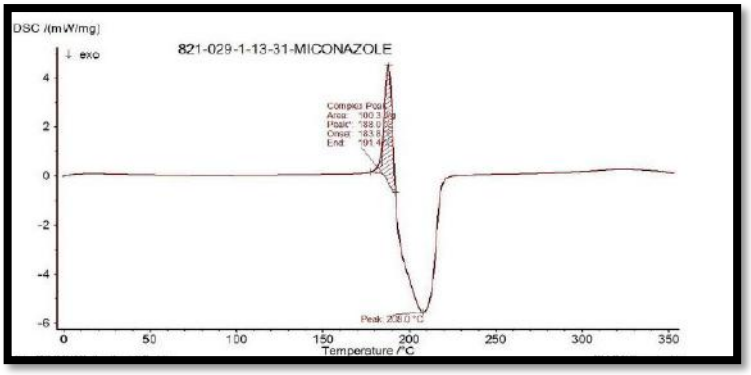


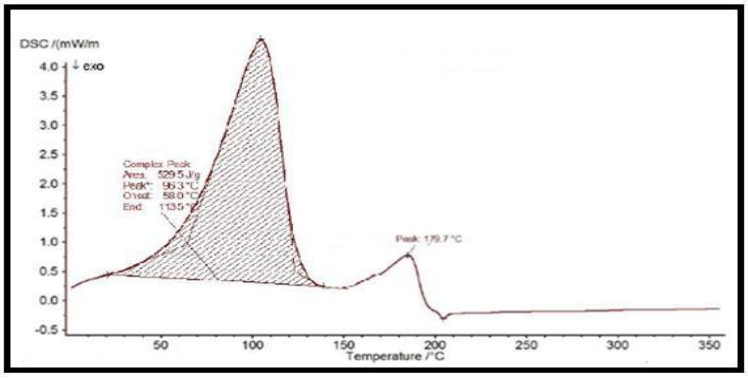
Fig. 3 Compatibility studies with FTIR (a) pure Miconazole drug, (b) phoshotidylcholine Levic 70 (c) complex drug phosholipid

Table 3: Frequencies of drug+ phospholipid

Frequency (cm^{-1})	**Miconazole nitrate**	**Frequency (cm^{-1}) Complex (drug+ phospholipid)**
3140	Imidazole C–N stretch	3406.0
3070	Aromatic CH stretch	2974.6
2995	Aliphatic CH_2 stretch	2932.0
2920	Aliphatic CH stretch	2880.4
1566	C=C aromatic	1646.7
1525	C=C aromatic	1454.3
1445	$–CH_2–$ bending	1405.2
1385	C–H bending (aliphatic)	1381.4
1310	C–N stretch	1335.8
1070	C–C stretch	1078.3



A. DSC for pure Miconazole Nitrate



B. DSC for drug+ exciepient

Fig. 4 DSC compatibility studies

A

B

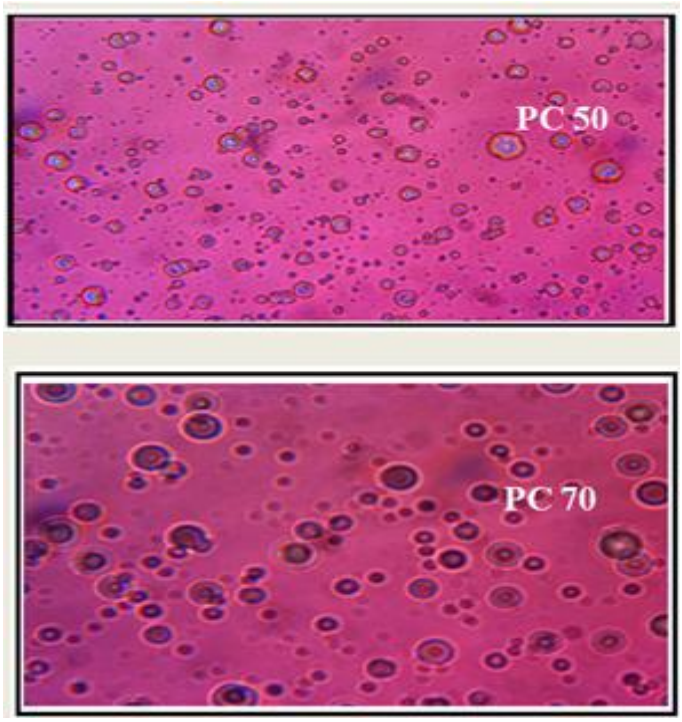


Fig. 5 Photomicro images ethosomes (A) Ethosomes formulated by using hot method with Pc 50

(B) Ethosomes formulated by using hot method with Pc 70

Table 4: Percent Entrapment efficiency for comparative studies

thosomes formulations	%Drug entrapment efficiency
H1	81.88%
H2	78.67%
H3	74.615
H4	69.445
H5	96.66%
H6	90.24%
H7	86.245
H8	84.34%

H1-H4 hot method with PC 50%, H5-H8 hot method with PC 70%

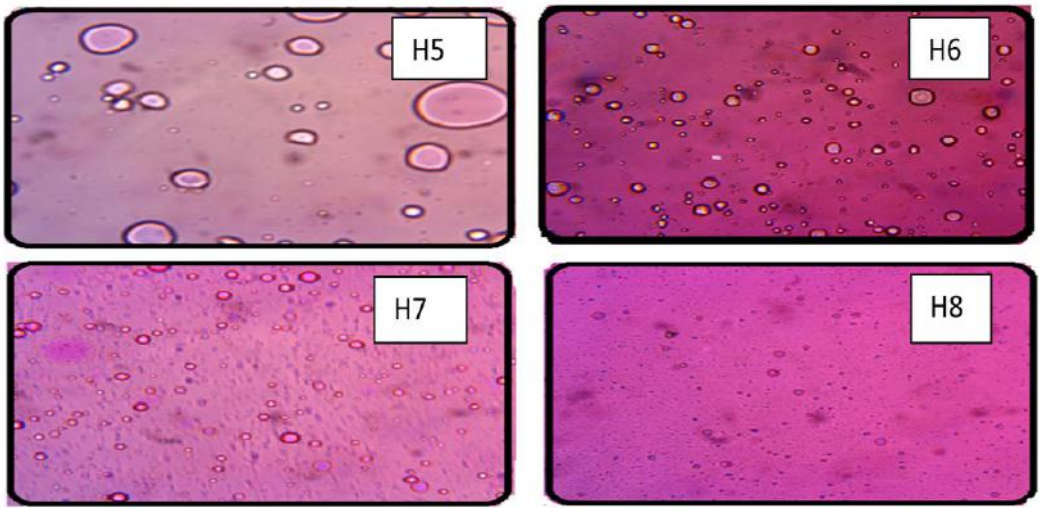


Fig.6 Photomicro images observed under microscope for optimized formulation

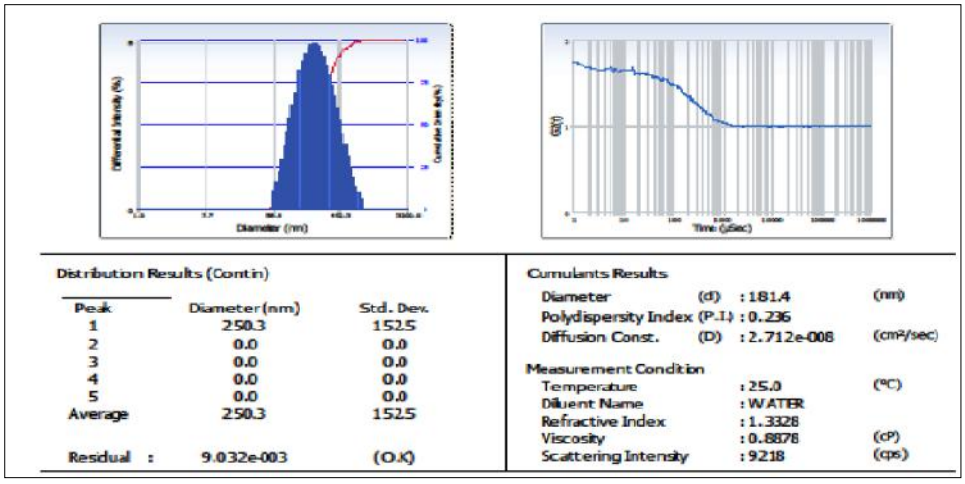


Fig. 7 Size distribution of optimized formulation H5

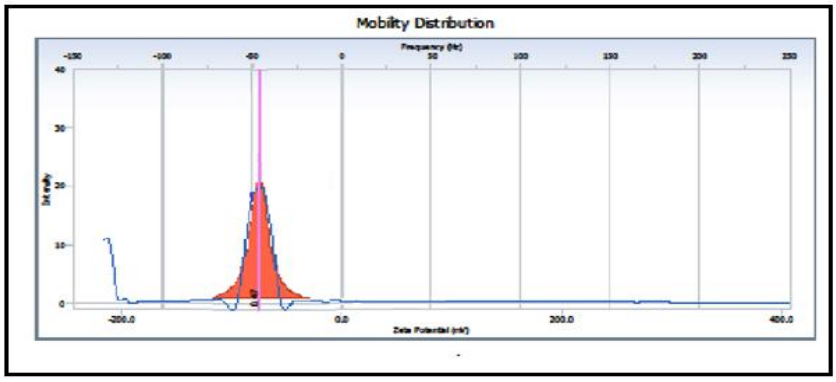


Fig. 8 Zeta potential of optimized formulation H5

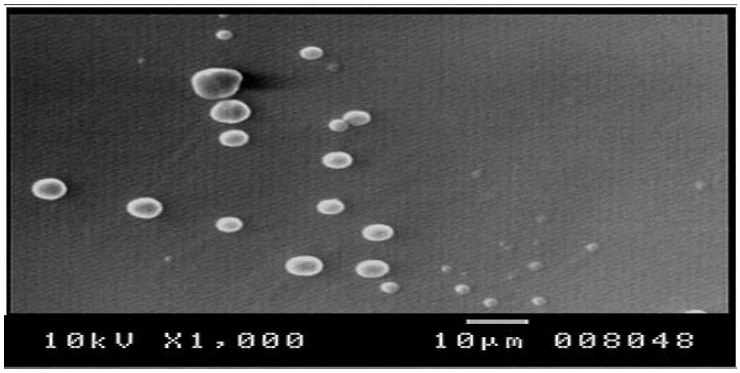


Fig. 9 Photographs of ethosomes observed by SEM of optimized formulation H5

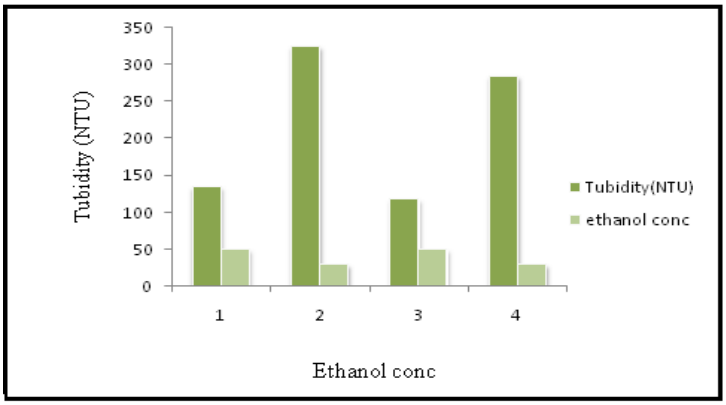


Fig. 10 Turbidity for Formulation with PC 50/ 70 (hot method)

Table 5: Spreadability studies (hot method)

Formulation with PC 50	Time (g.c.m/sec.)	Formulation with PC 70	Time (g.c.m/sec.)
H1	20	H5	22
H2	17.99	H6	19.5
H3	15.45	H7	18
H4	14.77	H8	16

Table 6: Viscosity Studies (hot method)

Formulation with PC 50	Viscosity cps		Formulation with PC 70	Viscosity cps	
	Max 100 Spindle	Min 10 Spindle		Max 100 Spindle	Min 10 Spindle
H1	5322	780	H5	5500	700
H2	5177	515	H6	5106	530
H3	4024	423	H7	4032	430
H4	3078	405	H8	3095	400

Table 7: Studies for Percent Drug content

Formulation with PC 50	% Drug content	Formulation with PC 70	% Drug content
H1	88	H5	90
H2	85.56	H6	88.27
H3	83.66	H7	86.89
H4	82.93	H8	85.76

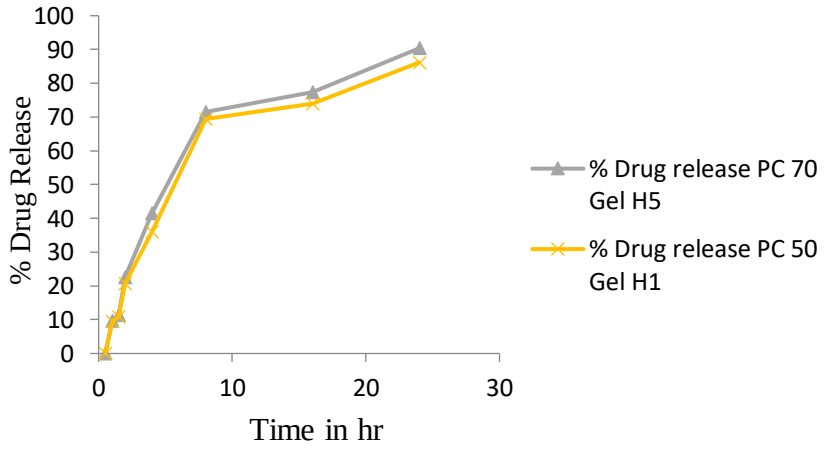


Fig. 11 *In vitro* drug release of H1and H5 formulations

Table 8: Percent Drug release

Time in hr	Percent Drug release PC 70 Gel - H5	Percent Drug release PC 50 Gel H1
0.5	0	0
1	9.733	9.458
1.5	11.43	10.901
2	22.548	20.69
4	41.634	36.039
8	71.428	69.436
16	77.428	74.022
24	90.38	86.175

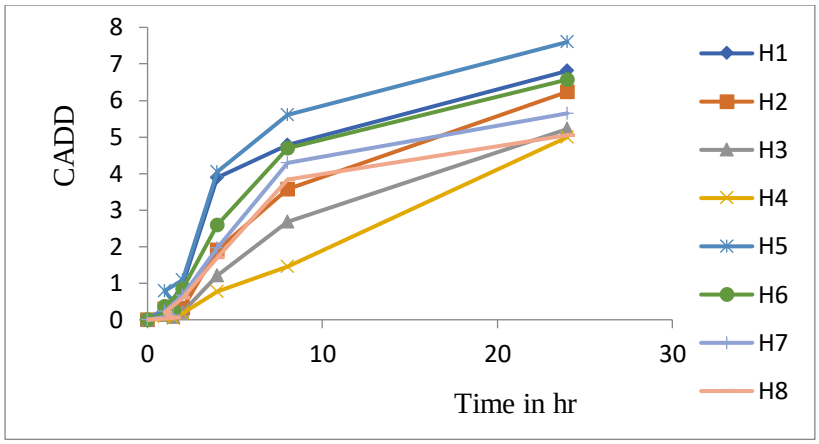


Fig. 12 *In vitro* drug release formulations H1 to H8

Table: 9: Cumulative amount of drug release (H1 to H8)

Time in hr	H1	H2	H3	H4	H5	H6	H7	H8
0	0	0	0	0	0	0	0	0
1.5	0.4598	0.21	0.07	0.039	0.47	0.25	0.13	0.06
1	0.1976	0.2935	0.127	0.08113	0.792	0.377	0.229	0.185
2	0.9573	0.321	0.205	0.178	1.087	0.845	0.671	0.597
4	3.891	1.915	1.203	0.763	4.052	2.591	1.958	1.705
8	4.778	3.57	2.678	1.459	5.598	4.698	4.294	3.845
24	6.809	6.237	5.218	4.997	7.601	6.572	5.649	5.05

Table 10: Comparisons of permeability parameters for H5 to H8 and marketed formulations

Formulation	CADD µg/cm^2	Jss µg/cm^2/hr	Kp cm/hr	Enhancement ratio
H5	7601	12.708	0.31	1.329
H6	6572	11.66	0.29	1.219
H7	5649	10.25	0.25	1.072
H8	5050	9.54	0.23	0.997
Marketed	5306	9.56	0.24	-

CADD-Cumulative amount of drug diffuses, Jss- Flux, Kp-permeability coefficient, ER- Enhancement ratio.

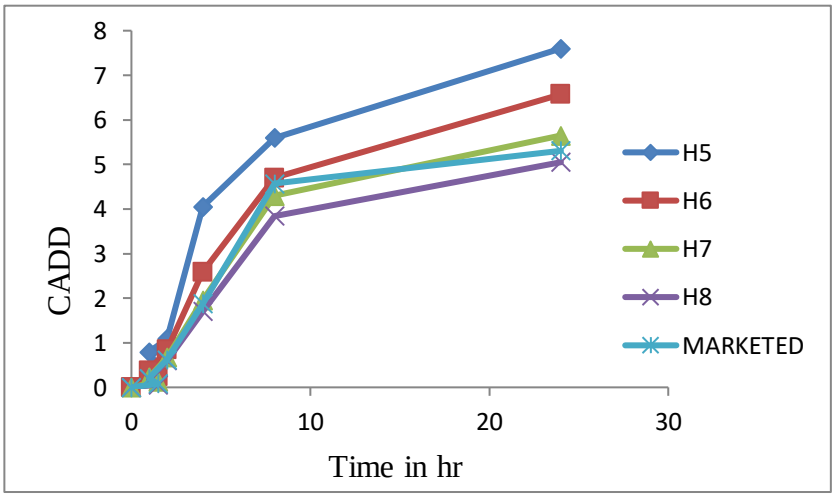


Fig. 13 Cumulative amount of drug diffused Vs time for H5 to H8 and Marketed formulation

Table 11: Kinetics release studies for best fit model

Ethosome Formulation Code	Zero Order Kinetics	First Order Kinetics	Higuchi Kinetics	Peppas		Best fit model
	R	r	r	r	N	
H5	0.9025	-0.9685	0.9737	0.8669	0.8381	Higuchi
H6	0.9012	-0.1169	0.9675	0.96720	0.795	Higuchi
H7	0.8816	-0.8924	0.8924	0.694	0.620	Higuchi
H8	0.8228	-0.8407	0.8703	0.7201	0.680	Higuchi
Marketed Gel	0.8177	-0.8188	0.8418	0.6572	0.0103	Zero order

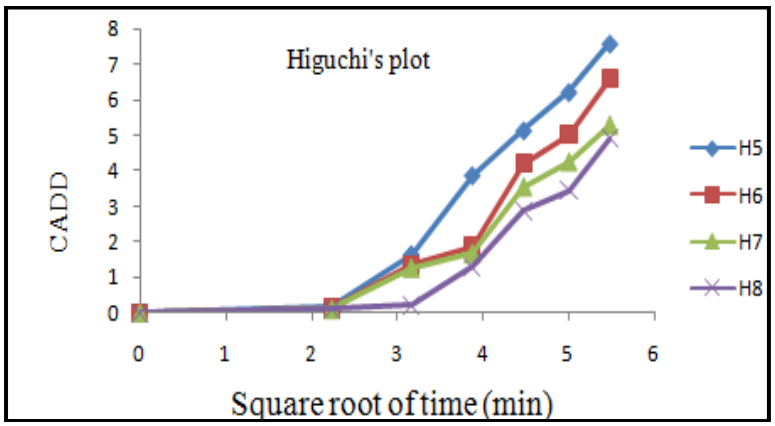


Fig. 14 Higuichi's plot

Table 12: Higuichi's plot square root of time Vs various Formulation

Square root of time (min)	H5	H6	H7	H8
0	0	0	0	0
2.23606	0.156	0.129	0.102	0.086
3.16227	1.65	1.35	1.275	0.198
3.87298	3.88	1.87	1.68	1.29
4.47213	5.148	4.22	3.56	2.86
5	6.23	5.03	4.26	3.45
5.47722	7.6	6.6	5.3	4.9

Table 13: Skin irritation tests of ethosomal gel on rat skin

Treatment	Irritation index		
	24 hr	48 hr	72 hr
Without treatment	0	0	0
Ethosomal gel	0	0	0
0.8%v/v aq. Solution of formalin.	4.3	4.9	5.1

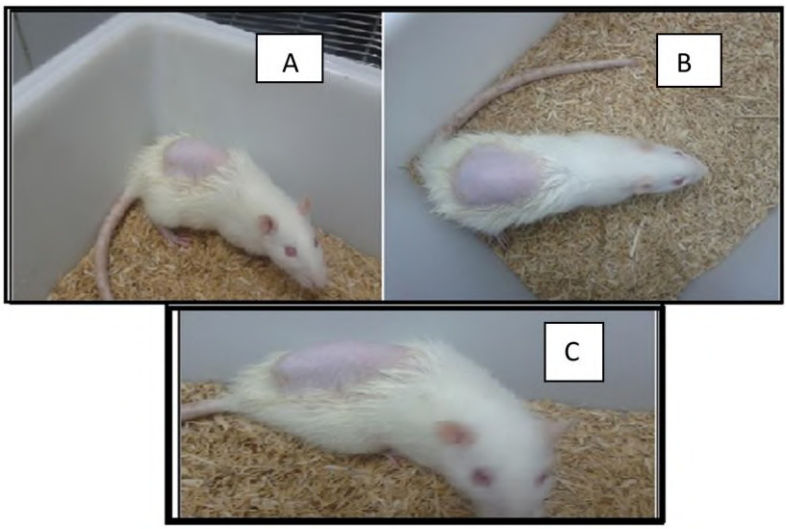


Fig. 15 Effect of Miconazole nitrate gel on erythma and edema with ethosomal gel in rat skin. (A-after 24 hr , B- after 48hr , C- after 72hr)

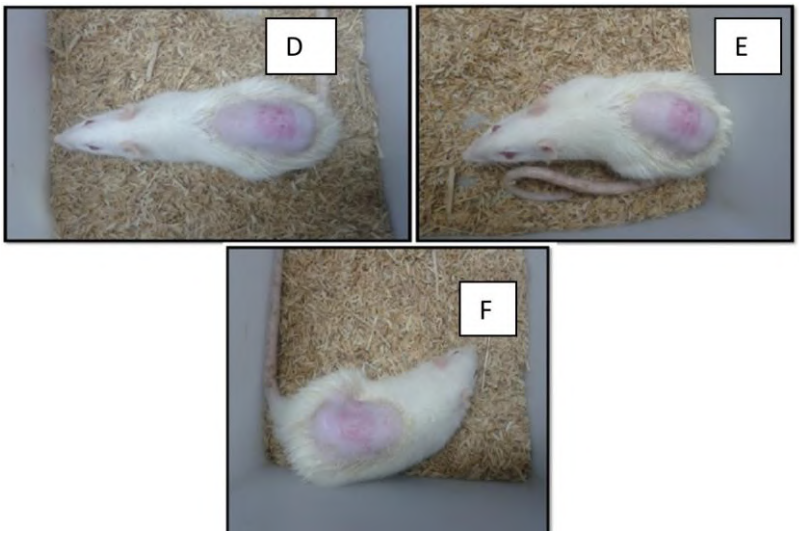


Fig. 16 Effect on erythma and edema with formalin solution application in rat skin (D-after 24 hr , E- after 48hr , F- after 72hr)

Table 14: Factor significance estimated by ANOVA for entrapment efficiency

Response 1 ertrapment efficiency

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	28.0075	2	14.00375	148.765	0.0492	significant
Residua	0.05188	1	0.05188			
Cor						
Total	28.5188	3				

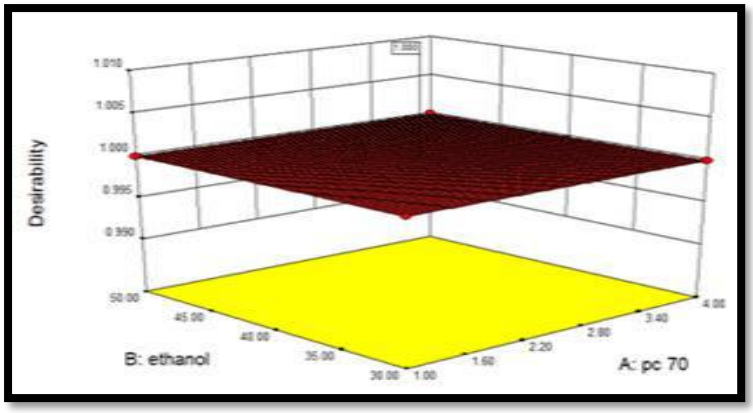


Fig. 16 Response surface plot of variables (pc 70 and ethanol) on entrapment efficiency

Table 15: Factor significance estimated by ANOVA drug release

Response 2 drug release

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	22.056	2	11.028	139.56	0.0407	significant
Residual	0.045675	1	0.045675			
Cor Total	22.84568	3				

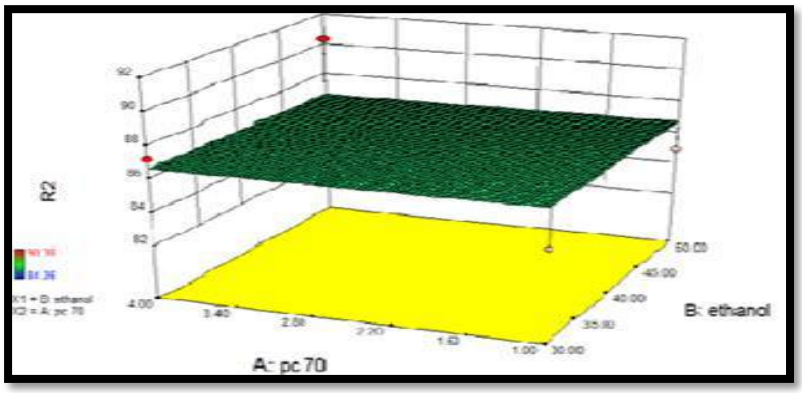


Fig. 17 Response surface plot of variables (pc 70 and ethanol) on drug release

Table 16: Effect of storage on percent entrapment efficiency

Days	Percent entrapment % (5±3ºC)	Percent entrapment % (25±2ºC)
0	96.66	94.23
15th	95.63	92.59
30th	93.01	91.22
45th	91.86	89.03
60th	90.02	87.56

Table 17: Effect of storage on % Residual drug content on storage at different intervals of time

Formulation	% Residual drug content on storage at different intervals of time				
	Initial drug concentration	15th days	30th days	45th days	60th days
Ethosomes suspension at refrigerator temp (5ºC ± 3ºC)	100%	98.66±0.5	97.56±0.4	96.45±0.7	93.03±0.3
Ethosomes suspension at room temp(25ºC ± 2ºC)	100%	97.73±0.4	95.78±0.6	94.22±0.5	89.86±0.8

f) Drug Content uniforminity

The drug content of all Gellified Ethosome formulation is given below in Table 7. As concentration of ethanol increases drug percent uniformity also increases H1 (88%) and H5 (90%) were as in H4 (82.93 %) and H8 (85.76%).

g) *In- Vitro* drug permeation studies [8]

The drug release from H5 and H1 formulation prepared by hot method was 90.38% and 86.175% respectively shown in **Fig. 11 and Table 8**.

The data obtained during studies also suggest that value of percentage drug released depend on the ethanol concentration. As concentration of ethanol increased, percentage drug release of Miconazole nitrate increased up to 90% and further increase in the ethanol concentration significantly decreased the percentage drug release. The possible reason for this may be the detoriating effect of ethanol on lipid bilayers at higher concentrations. The significant difference in percentage drug release between ethosomal formulations containing different concentrations of ethanol (30& 50%) clearly indicates that the ethosomal system with 50% ethanol concentration has better permeability through rat skin than formulation containing lower concentrations of ethanol.

h) Cumulative amount of drug release

Cumulative amount of drug release was done with all formulations as shown in **Fig 12 and Table 9** for Pc 70 interpreted formulation gives more drug release in 24 hr which interpreted that ethosomes made by Pc 70 had better cumulative amount of drug release as compare to ethosomes made by Pc 50.

i) Transdermal flux: For different formulation across rat skin was calculated. The flux from ethosomal gel H5 12.708 µg/cm$^{2/}$hr found higher than marketed formulation .Data indicates that the ethosomal system was more effective in delivering Miconazole nitrate then Marketed formulation.

j) Release Kinetics:

The drug release was analyzed by PCP Disso Version 2.08 software to study the kinetics of Drug release mechanism. On comparison of kinetic modeling and release profile data it was evident all the ethosomal formulations were found to release the drug in accordance to Higuchi kinetics, the regression coefficient was not found to be exactly near to 1, which could be due to influence of some other factors. Amongst all, formulation H5 was found to have highest regression coefficient value of (0.9737) in Higuchi kinetic model and was found to show sustained release pattern given **Table 11**.[9,20]

The study of drug release kinetics showed that majority of the formulations governed by Higuichi‘s model. The curve was obtained after plotting the cumulative amount of drug released from each formulation of hot method with Pc 70 *i.e* formulation vs. time given in **Table 12** and **Fig. 14** below.

k) Skin irritation studies

No sign of erythema and edema were observed on the skin of albino rats after 72 hr, when a primary skin irritation test of ethosomal gel on rat was studied. Irritation score (primary skin irritation index) for ethosomal gel was zero, which exhibited that it is safe and acceptable shown in **Table 13** and **Fig. 15, Fig16**[10]

l) Statistical Analysis using ANOVA

ANOVA was used to establish the statistical validation of the factorial model. A total of 4 runs were generated by 2^2 full factorial design applied for optimized method H5 to H8 (Hot method) prepared with Pc 70 and ethanol by using Design Expert. All the responses observed were simultaneously fitted into the models and were evaluated in terms of statistically significant coefficients and R^2 values. The ANOVA results are indicated in Table 7.15 -7.16 of the examined formulation factors, according to the 2-level full factorial design. It is seen that both the factors (concentration of Pc 70 and ethanol) exert a significant effect on the responses, entrapment efficiency and percent drug release ($p < 0.0500$). P-value for entrapment efficiency response depicts 0.0492 and whereas percent drug release found that 0.0407 both the responses found to be significant shown in **Table 14 and 15**. Three dimensional responses surface plots drawn also studied for graphical **Fig. 16 and 17** which were useful for study of interaction effects of the independent variables on the responses, the plots demonstrate that both X_1 and X_2 affects entrapment efficient and % drug release shows linear relationship.

m) Stability Studies

Stability studies of optimized ethosomal formulation H5:

Physical appearance and % entrapment efficiency –

The studies showed that optimized gel kept for 15th, 30th, 45th and 60th days under 5ºC ± 3ºC as well as 25ºC ± 2 ºC temperature conditions showed no change in their physical appearance. The optimized ethosomal formulation (H5) was kept in sealed vials (10mL) at 5±3ºC and at 25±2ºC for 2 months to study the effect of storage conditions on percent entrapment. It was observed that the ethosomal vesicular suspension was more stable at 5±3ºC as compared to 25±2ºC as shown in **Table 16**. This could be due to degradation of lecithin at higher temperature. This suggests that formulation should be stored at low temperature conditions.

Content uniformity-

Optimized ethosomal gel kept for 15 th,30th ,45th and 60th under 5ºC ± 3ºC as well as 25ºC ± 2ºC temperature conditions were studied for uniformity of content. The results showed in **Table 17** no significant changes in content uniformity at 5ºC ± 3ºC and 25ºC ± 2ºC after 60 th day. As evident from table 7.20 at 5ºC ± 3ºC content uniformity was found to show approximately 5% decrease (98.66 ± 0.5% to 93.051± 0.3%)and at 25ºC ± 2ºC content uniformity 8% decreased from (97.73 ± 0.4% to 89.86 ± 0.8%). Studies revealed good stability of ethosomal formulation, the above result shows formulation stored at refrigerated conditions was more stable than room temperature because greater drug loss was observed from formulation stored at room temperature.[21, 22]

4. CONCLUSION

Ethosomes of miconazole nitrate were prepared successfully by using Touitou hot method alongwith different concentrations of Phospholipid as well as the incorporation of the ethosomes into carbopol 940 base gel to obtain ethosomal gel formulations. Among all formulations (H1 to H4 containing PC50 while H5 to H8 containing PC70), the formulations with PC70 showed better cumulative amount of drug release. The prepared formulations were characterized for various parameters. 2^2 full factorial design applied for optimized method H5 to H8 (Hot method) prepared with Pc 70 and ethanol by using Design Expert which showed significant effect on the responses, entrapment efficiency and percent drug release. The compositions of ethosomes and gels were manipulated to investigate their effects on the characteristics of final formulations. The miconazole nitrate ethosomal gels also characterized fo Erythema and Edema on Albino rats which showed zero irritation score. From the results of the study, it can be concluded that miconazole nitrate ethosomes can be integrated as transdermal drug delivery systems with enhanced drug delivery efficiency and therapeutic efficacy, suggesting an approach to overcome the higher dose of miconazole nitrate required in conventional topical administration, frequent application and systemic adverse effects.

5. REFRENCES:

1. Surver, C. and Davis, F.A., Bioavailability and Bioequivalence, In Walter, K.A. Ed. 3rd, *Dermatological and Transdermal Formulation,* Marcal Dekker, INC. New York: 119, 2002, 403,323,326,327,403.
2. Mithal B and Saha R. A hand book of cosmetics, Vallabh Prakashan Delhi: 2003,(1):11-17,21-22,37-38,61-89,90-93,177,214-215.
3. Swarnlata S, Rahul R, Chanchal D. K, Shailendra S. Colloidosomes an advanced vesicular system in drug delivery. *Asian J.Sci.Res* 2011; 4(1): 1 –15.
4. Nikalje A and Tiwaris S. Ethosomes; A novel tool for transdermal drug delivery. *Int J Res Pharm Sci* .2012; 2(1):1-20.
5. Touitou E, Godin B, Weiss C. Enhanced delivery into and across the skin by ethosomal carries. *Drug Dev Res* 2000; 50:406-445.
6. Tautiou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes- novel vesicular carrier for enhanced delivery: characterization and skin penetration properties. *J Control Release*.2000; 65:403-418.
7. Touitou E, inventor; Composition of applying active substance to or through the skin. United states patent 5 540934, 1996, July 30.
8. Sheer N and Chauhan M. Ethosomes as vesicular carrier for enhanced transdermal delivery of ketoconazole - formulation and evaluation . *J Pharm Cos*. (2011);1(3):1-14.
9. Jain A, Deveda P, Vyas N, Chauhan j, Hemant Khambete, Dr.Sanjay Jain. Development of antifungal emulsion based gel for topical fungal infection(s). *Int J Pharm Res Dev*.2011;2(12):18-25
10. Jain S, Goswami M, Bhandari A , Arora V .Skin irritation study of intradermal patch of chitosan containing trazodone-hcl on rat skin. *Int J Res Pharm Biomed Sci* .2011; 2 (3):1082-1084.
11. Lachman L., Lieberman H.A., Kanig J.C. "The theory and practice of industrial pharmacy", 3rd edition, 1991, Varghnese Publishing House, Bombay: 479,492-494, 502,526-531, 548,564,584-585,589,615-618.
12. Singh A, Rathore P, Shukla M and Nayak S. Comparative Studies On Skin Permeation Of Miconazole Using Different Novel Carriers. *Int J Pharm Sci Res*. 2010; 1(9): 61-66.
13. Indian Pharmacopoeia, Government of India. Ministry of Health and Family Welfare. Ghaziabad: The Indian Pharmacopoeia Commission.2007; 2:771-772.
14. Negi L, Chauhan M, Garg A. Gel containing ethosomal vesicles for transdermal delivery of aceclofenac. *Int J Pharm Pharm Sci*. 2010 ;(2):2102-108.
15. Ehab R, Bendas L, Mina I, Tadros, Enhanced Transdermal Delivery of Salbutamol Sulfate via Ethosomes, *Am Asso Pharm Scientists*. 2007; 8 (4):107.
16. Chourasia M, Lifeng, K, Sui C. Nanosized ethosomes bearing ketoprofen for improved transdermal delivery, *Results Pharma Sci* . (2011); 1: 60–67.
17. Jain S, Tiwary A, Sapra B, Jain K. Formulation and Evaluation of Ethosomes for Transdermal Deliveryof Lamivudine , *Am Asso Pharm Scientists*. 2007; 8 (4): 111.
18. Chen J, Jiang Y et al., Preparation of triptolide ethosomes, *Afr J Pharm Pharmacol*. 2012; 6(18):1341-1347.
19. Shaik. R, Tirumooorthy N, Girhepunje K, Enhanced transdermal delivery of ketoconazole via ethosomes formulation and evaluation. *World J Pharm Pharm Sci*. 2012; 1(1):238-249.
20. Brahmankar DM, Jaiswal SB. Biopharmaceutics and pharmacokinetics a treatise. 1st edition. vallabh Prakashan. 2002. 337.
21. International Conference of Harmonization topic: Q1A (R2) Stability testing of new drug substances and products. European Medicines Agency (document on internet).2006; 7-8. Available from: URL:http://www.emea.eu.int.cited on 15th December 2013.
22. Bhalerao S, Harshal A. Preparation, Optimization, Characterization, and stability studies of salicylic acid liposomes. *Drug Dev Ind Pharm*. 2003; 29(4):457-67.