Development And In Vitro Evaluation of Miconazole Ethosomal Gel

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***Abstract*—The present study was focused on formulating and evaluating Miconazole containing ethosomal gel formulation for in vitro studies. Ethosomal formulations were prepared by using cold method and were evaluated for in vitro characteristics, stability studies. Ethosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that ethosomal formulation was spherical in shape. Ethosomes containing lipid higher percentage of drug release after 8 h as compared to other formulations. F-3 formulation was found to be stable at the end of the study on storage condition. The present study suggested that ethosomal gel formulations provide sustained and prolonged delivery of drug with enhance bioavailability.**

***Index Terms*—Ethosomal gel, Miconazole, bioavailability, cold technique, in vitro drug release studies.**

I. INTRODUCTION

Ethosomes are vesicle lipid vesicle carriers that contain phospholipids and have a high concentration of ethanol and water. It is a system that has important characteristics related to the ability to increase skin permeability. The size of ethosomes will be in the range of tens of nanometers to microns (μ) which have a smaller size related to liposome.[1] Ethanol interacts with lipid molecules in the polar head group region resulting in increasing fluidity and may finally lead to increase membrane permeability. In addition, it may provide the vesicles with soft flexible characteristics, which will improve the drug distribution ability in the stratum corneum lipids of the skin, and also formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs[2,3] Ethosomal Gel is a semi-solid formulation in which ethosomal vesicles are dispersed in a suitable gel base to deliver drugs through the skin more efficiently than traditional topical systems. Ethosomal gel Enhanced skin penetration and Suitable for both hydrophilic and lipophilic drugs Miconazole nitrate which is used in the management of fungal infections has limited effectiveness owing to its poor aqueous solubility.[4] In the present study, Ethosomes formulation was prepared by cold technique by Further, the vesicular suspension was converted into a gel formulation by adding Carbopol for gelling into the vesicular suspension.[5] This study aimed to evaluate the drug-excipients interactions, gel rheological characteristics, vesicular shape and surface morphology, particle size distribution, zeta potential, and entrapment efficiency, spread ability and viscosity. The drug embedded ethosomes in the form gel may produce significant effects to alter the drug related pharmacokinetic characteristics and improve the efficacy of drug to treat tinea corporis disease.

II. MATERIALS AND METHODS

Materials: Miconazole procured from Hetero Labs, HYD. Phosphatidyl choline and Ethanol was obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

Methodology: Compatibility study (IR spectroscopy)

FTIR analysis was performed in order to study the compatibility of ingredients used in the preparation of nanoparticles, using a Shimadzu FTIR spectrophotometer (Prestige21, Shimadzu Corporation, Kyoto, Japan). Atomoxetine and Excipients their mixture with ratio (1:1) was evaluated

using FTIR spectrophotometer using potassium bromide disc technique where 1mg of the sample is mixed with 100 mg of dry powdered KBr; the mixture is pressed into a transparent disc and was inserted in the apparatus for IR scan.[6]

Formulation development

Table-1: Composition of Miconazole Ethosomes (F1 to F4)

S. No.	Ingredients (mg)	F1	F2	F3	F4
1	Miconazole (mg)	50	50	50	50
2	Phosphatidyl choline (mg)	250	500	750	1000
3	Ethanol(ml)	5	5	5	5
4	Water(ml)	10	10	10	10

Procedure

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation. Phospholipid and drug and other pharmaceutical ingredient listed in table were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to 300°C ± 10°C and a fine stream of distilled water was added slowly, with constant mixing at 100 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 300°C ±10°C. The preparation was left to cool at room temperature for 30 mins and then it was sonicated at 40°C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator. [7]

Formulation of Ethosomal gel

A predetermined amount of Carbopol 940 was introduced into distilled water, followed by the addition of Glycerin and subsequent stirring until complete dissolution was achieved Subsequently, the solution underwent neutralization and was rendered viscous through the inclusion of triethanolamine. The amalgamation was agitated incessantly for a duration of one hour until it coalesces into a lucid gel, as reported in reference. A 5ml ethanol solution containing Ethosomes was generated and subsequently introduced gradually into the pre- existing polymer gel. The ultimate amount was adjusted to utilizing distilled water. The gels that were produced were subsequently stored in appropriate receptacles at ambient temperature in order to facilitate subsequent investigations. [8]

Table-2: Formulation development of Ethosomal gel

Ingredients	F1	F2	F3	F4
Ethosomes (ml)	5	5	5	5
Carbopol 934 (mg)	100	200	300	400
Methyl paraben (ml)	0.01	0.01	0.01	0.01
Glycerin (ml)	1	1	1	1
Water	q.s	q.s	q.s	q.s

III. CHARACTERIZATION

Particle size: All the prepared batches of ethosomal gel were viewed under microscope to study their size. Size of Ethosomes from each batch was measured at different location on slide by taking a small drop of ethosomal gel on it and average size of ethosomes were determined. [9]

SEM analysis: The morphology of Ethosomes was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater.
The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.10

Determination of pH: The value of pH of Ethosomal gels was measured by using digital pH meter (Lab India Sab 5000 pH meter) at the room temperature.[11]

Spread ability: Place a fixed amount of ethosomal gel (usually 0.5 g) in the center of the bottom glass

slide. Gently place the second glass slide on top of the gel, ensuring no air bubbles are trapped. Place a weight (500 g or 1 kg) on top of the upper slide. Allow the weight to sit for a fixed time (usually 5 minutes). Remove the weight carefully.[12] Measure the diameter of the spread gel using a ruler or caliper in two perpendicular directions, and calculate the average.

Spreadability(S)=M×L/T where:
M = weight tied to the upper slide (g)
L = length moved by the slide(cm) T = time taken (sec)

Viscosity: The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25°C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 mins at the constant temperature (25±/10°C) before the measurements. [13]

Zeta Potential: The zeta potential means the charges which are present on the surface of Ethosomal gel. The many time the charge is present on the surface of Ethosomal gel. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all transferosome present in formulation, due to some charge all Ethosomal gel particle repeal to each other and coagulation of particle are avoided. The zeta potential of Ethosomal gel was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25°C with the angle of detection 90°.The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of Ethosomal particle. [14]

Entrapment efficiency: To 0.2 g of Ethosomal, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 were added. The aqueous suspension was then sonicated. Ethosomal gel containing Miconazole were separated from unentrapped drug by centrifugation at 9000rpm for 45 mins at 4°C. The supernatant was recovered and assayed spectrophotometrically using Spectrophotometer. [15] The encapsulation percentage of drug (EP) was calculated by the following equation

$$EP = [(Ct - Cr)/ Ct] * 100$$

Where, Ct = concentration of total Miconazole, Cr = concentration of free Miconazole

In Vitro Drug release study
The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5°c. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Miconazole Ethosomal was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.[16]

Kinetics of drug release studies [17]
The quantitative elucidation of the values obtained in the dissolution study is facilitated by the usage of a generic equation that mathematically translates the dissolution curve in function of some parameters related to the ethosomal gel. For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the Invitro drug dissolution data of optimized formulations obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix and Korsmeyer-Peppas models.

Zero order kinetics
Drug diffusion from pharmaceutical dosage forms that do not disaggregate and release the drug slowly can be represented by the following equation:

$$Q0 - Qt = K0t$$

Arrangement of equation yields: $Qt = Q0 + K0t$
Where Qt is the amount of drug dissolved in time t,
Q0 is the initial amount of drug in the solution (most times, Q0 = 0)
and K0 is the zero-order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

First order Kinetics

The equation for first order release is given below

$$\text{Log } Qt = \log Q0 + K1t/2.303$$

Were,

Qt is the amount of drug released in time t,

Q0 is the initial amount of drug in the solution

and K1 is the first order release constant.

A graph of the decimal logarithm of the released amount of drug versus time will be linear. Ethosomal gel following this diffusion profile release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

Higuchi model

Higuchi described drug release as a diffusion process based on the Fick's law, square root time dependent. The simplified Higuchi equation is represented as

$$Qt = Kt^{1/2}$$

Were,

Qt = amount of drug released in time t,

K = Higuchi's constant

A linear relationship between amount of drug released (Q0 versus square root of time ($t^{1/2}$) is observed if the drug release from the ethosomal gel is diffusion controlled.

Korsmeyer-Peppas model

This mathematical model, also known as the Power Law, has been used, very frequently; to describe the drug release from several different pharmaceutical modified release dosage forms. The Korsmeyer-Peppas model relates drug release exponentially to time. It is described by the following equation

$$Mt/M\infty = atn$$

Where 'a' is a constant incorporating structural and geometric characteristics of ethosomal gel, 'n' is the release exponent, indicative of the drug release mechanism, and the function of 't' is $Mt/M\infty$ (fractional release of drug).

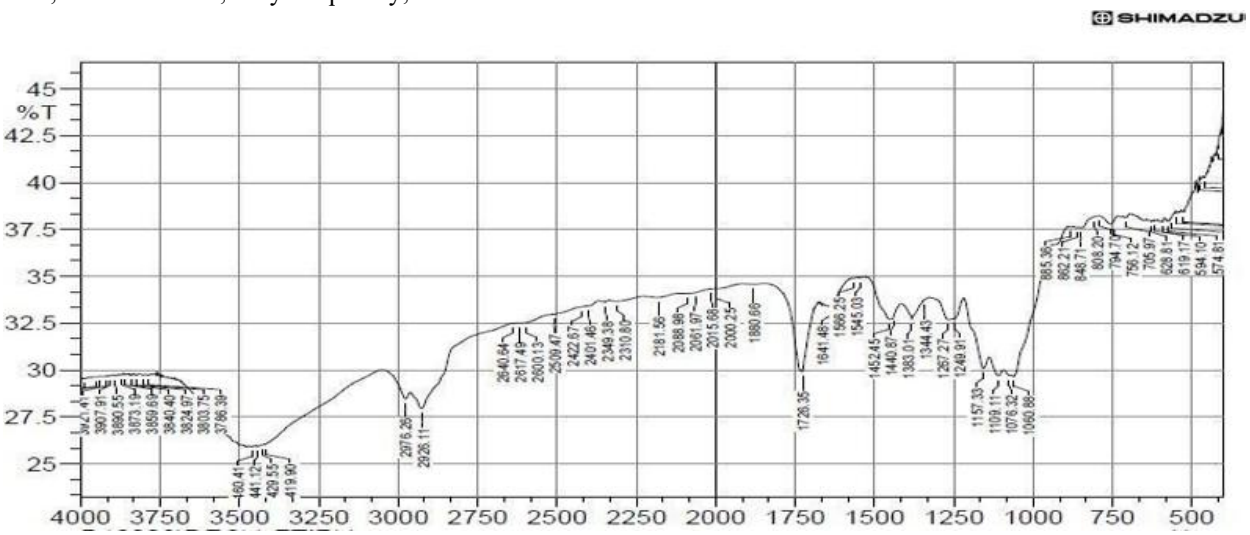
Stability Studies

The formulations stored in glass vials covered with aluminum foil were kept a t room temperature and in refrigerator (4°C) for a period of 90 days, samples were withdrawn and hydrated with phosphate-buffered saline (pH 7.4) and observed for any sign of drug crystallization under optical microscope. Furthermore, the samples were also evaluated for particle size and percent retention of Miconazole.[18]

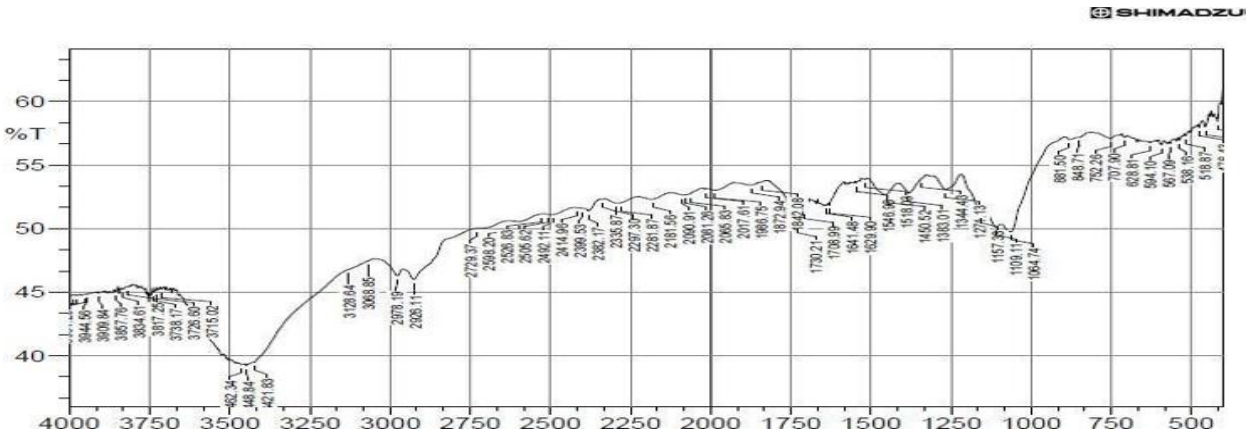
IV. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR):

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.



Graph-1: FTIR spectra of Miconazole



Graph-2: FTIR Spectra of physical mixture of drug and excipient

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm- 1) the drug is compatible with excipients.

Evaluation parameters:

Fig-3: Ethosomal Gel Formulations

Fig-4: Four Formulations of Ethosomal Gel

PH and Viscosity

Table-3: PH and Viscosity values of all formulations

F. code	pH	Viscosity (cps)
F1	6.5±0.27	180±7.50
F2	7.1±0.32	175±5.63
F3	6.8±0.25	181±4.90
F4	6.7±0.07	176±5.61

Spreadability

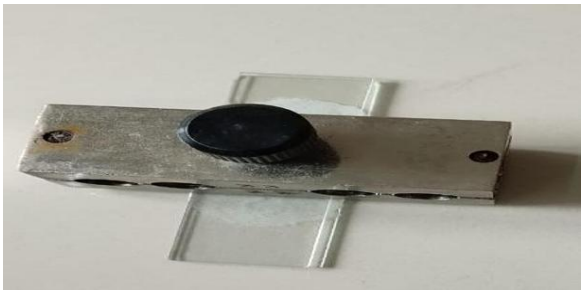


Fig-5: Spreadability Test for Ethosomal gel

Table-4: Spreadability values of all formulations

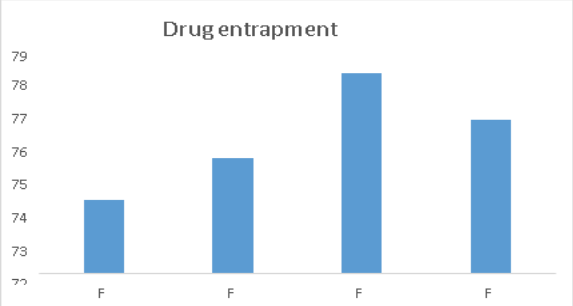
F. code	Spread ability (g.cm/sec)
F1	4.86±0.18
F2	5.02±0.02
F3	5.18±0.13
F4	5.11±0.06

Entrapment Efficiency:

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 mins. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 245 nm.

Table-5: Drug entrapment efficiency

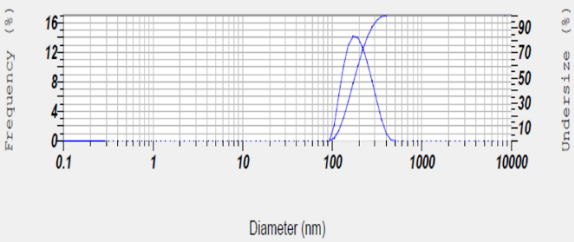
F. code	Drug content
F1	68.30±5.35
F2	72.39±1.26
F3	78.10±4.44
F4	75.82±2.16



Graph-6: Drug entrapment efficiency of all formulation

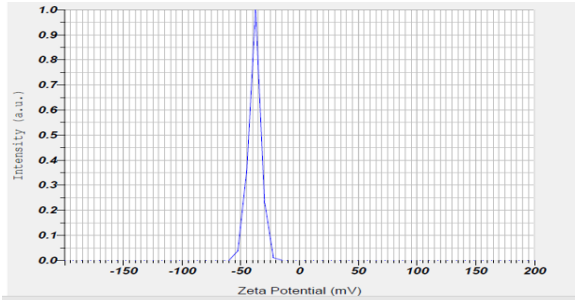
Determination of Vesicle morphology and Size

The morphological characteristics of formulated ethosomes were carried by using Scanning electron microscopy (SEM). A small drop of Ethosomal gel was placed between two rivets fixed on a gold-plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85°C for 30 mins to sublime the surface moisture. Finally, the sample was coated with gold and allowed the SEM to capture the images at a temperature of -1200°C and voltage of 5kV.



Graph-7: Particle size analysis of Optimized ethosomal gel

Zeta potential



Graph-8: Zeta potential of ethosomal gel

Table-6: Evaluation Studies of particle size and Zeta potential Ethosomal gel

F. No	Particle size (nm)	Zeta potential
F1	122.3±32.7	-29
F2	182.1±27.0	-31
F3	176.7±21.6	-25
F4	139.2±15.8	-30

SEM Analysis

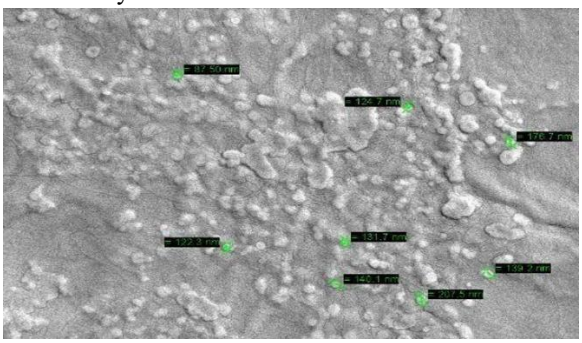


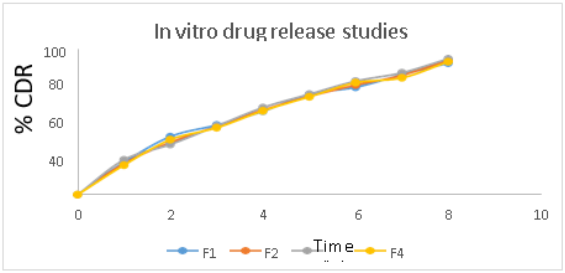
Fig-9: SEM Analysis of Ethosomal gel

In vitro drug release studies:

Table-7: In vitro drug release studies of (F1-F4) formulation

Time	F1	F2	F3	F4
0	0	0	0	0
1	22.20±53.3	21.36±56.4	23.98±58.1	20.25±55.5
2	39.86±31.3	35.86±3.15	34.45±2.7	37.81±3.1
3	47.82±26.8	46.93±26.9	47.10±25.1	45.89±27.8
4	57.15±5.7	58±6.3	59.82±6.9	57.54±7.5
5	68.97±3.56	67.42±3.15	68.97±2.67	67.18±2.30
6	73.68±20.9	75.20±19.3	77.95±17.8	76.89±16.7
7	82.36±28.7	81.96±2.52	83.69±2.38	80.16±2.45
8	90.25±36.6	92.34±3.71	93.35±4.23	91.25±3.50

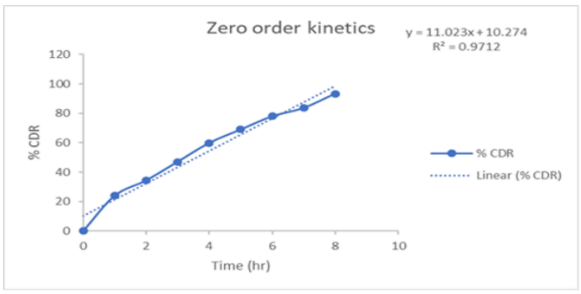
Fig-10: Franz Diffusion cell



Graph-11: In vitro drug release studies of (F1-F4) formulation

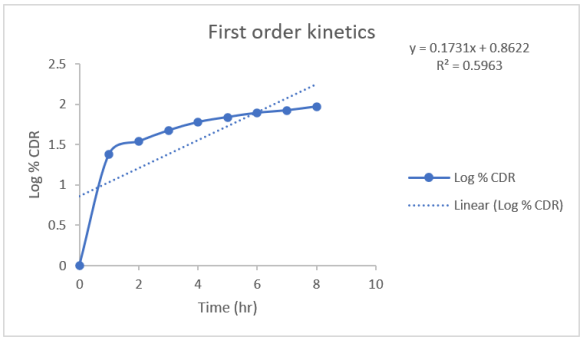
Drug release kinetics

Zero order kinetics



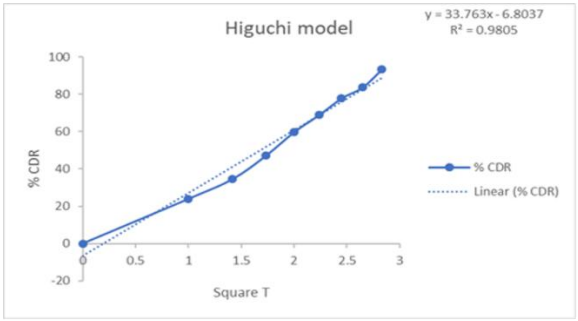
Graph-12: Zero order kinetics of optimized formulation

First order kinetics



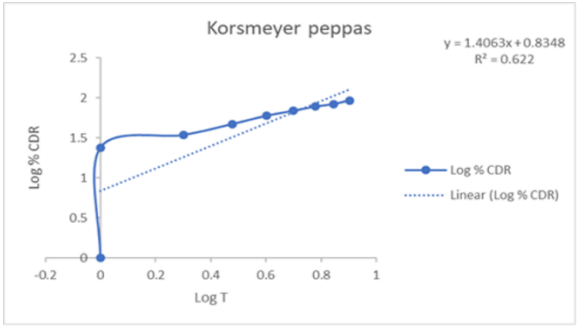
Graph-13: First order kinetics of optimized formulation

Higuchi model



Graph-14: Higuchi model of optimized formulation

Korsmeyer peppas



Graph-15: Korsmeyer peppas of optimized formulation

Stability studies:

There was no significant change in physical and chemical properties of the Ethosomal gel formulation F3 after 3 months. Parameters quantified at various time intervals were shown. Optimized formulations F3 was selected for accelerated stability studies as per ICH guidelines.

Table-8: Stability studies of optimized formulations

S.NO	Time in days	Physical changes	Mean % drug release		
			Miconazole		
			250C/60%	300C/75%	400C/75%
1	01	No Change	93.35±4.23	92.36±3.67	91.36±3.25
2	30	No Change	93.35±4.23	92.58±3.15	91.23±2.46
3.	60	No Change	93.35±4.23	92.15±2.79	91.02±3.18
4.	90	No Change	93.35±4.23	92.14±2.56	91.10±3.55

V. DISCUSSION

Particle size and zeta potential of the ethosomes were measured by photon correlation spectroscopy using a Malvern Zeta sizer and entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium by UV spectrophotometer. Zeta potential of optimized ethosomal formulation was found -25 mV. Prepared gel of ethosomes loaded with Miconazole was prepared and evaluated for viscosity, pH, % drug content, spreadability and drug release study. It was found that viscosity of prepared gel was 181±4.90 cps, pH was 6.8±0.25%, drug entrapment efficiency was 78.10±4.44 and spreadability was found as 5.18±0.13 (g.cm/sec), respectively. In vitro drug release from ethosomes was examined using Franz diffusion cell method and found to be 93.35±4.23 in 8hrs. It was due to the release of free drug present in bag after leaching from ethosomes. Drug release from ethosomal formulation was found in very sustained and controlled manner.

VI. CONCLUSION

The present study was aimed for preparation of Miconazole ethosomal gel, the ethosomal gel was prepared by cold method. Ethosomes were prepared by and optimized on the base of average vesicle size and % drug entrapment. The optimized formulation was further incorporated with gel base (Carbopol gel) and characterized for their viscosity, pH, % drug content, spread ability, drug release study and kinetics. The prepared four formulations were evaluated for various parameters. Among the various formulation, the combination F3 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F3 comprising phosphatidylcholine fulfills the requirement of good ethosomal gel formulation. In vitro drug release up to 8 hrs. and more than 93.35±4.23 % drug released. A higher Miconazole entrapment was observed. The role of these Miconazole ethosomal gel, obtained in this study, can only be settled following future in vitro drug release studies. It can be concluded that prepared gel containing miconazole-loaded ethosomal formulation was optimized and successfully formulated in the gel form can be of use for topical preparation for its antifungal infection.

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