



DEVELOPMENT AND IN VITRO EVALUATION OF MUPIROCIN ETHOSOMAL GEL

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ABSTRACT

The present study was focused on develop and evaluating Mupirocin 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. F2 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.

Key words:

Ethosomal gel, Mupirocin, bioavailability, cold technique, in vitro drug release studies.

1.INTRODUCTION

Vesicles are the structures that are small in size having a bilayer arrangement similar to the natural lipid bilayer structure of our body membrane. They are highly efficient in encapsulating drugs having varied physico-chemical properties.¹ Stratum corneum is regarded as the major hindrance in attaining a good penetration of drugs transdermal and is easily overcome by these vesicular structures.¹ Amphiphilic nature of vesicles helps to deliver both hydrophilic as well as lipophilic drugs to their respective targets with relative ease.² Liposomes were developed earlier as pioneer model in vesicular delivery system. Vesicles contribute greatly in cellular communication as well as particle transport. Researchers have revealed their conclusion that the vesicular morphology helps them to deliver drugs in efficient manner and vesicles can be tagged for cell specificity, thus producing a targeted action.³ The liposomes were further modified for better features which lead to the discovery of ethosomes which is considered by many as one of the major advancement and advantage in vesicular research. Ethosomes are defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers or systemic circulation. These are soft, malleable vesicles

tailored for enhanced delivery of active agents.⁴ The vesicles have been well known for their importance in cellular communication and for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer period of time.⁵ Ethosomes, the lipid vesicular carrier systems, also called as alcoholic liposomes are composed of phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are suitable carrier systems for both hydrophilic and lipophilic drugs. The small particle size of ethosomes (microns to nanometer) facilitates their potentiality in carrying the drug through the skin into systemic circulation. The ease of preparation, non-irritant nature, efficiency to encapsulate wide range of drug molecules and higher stability than any other vesicular systems make the ethosomes the most opted carriers for topical delivery of drugs.⁶

2.MATERIALS AND METHODS

Mupirocin was collected as a gift sample from Hetero labs, Hyderabad and various excipients and polymers were purchased from Synpharma Research Lab, HYD.

FTIR study⁷

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.

2.1 METHODOLOGY

Formulation Development

Table 1: Formulation table

S.No.	Ingredients (mg)	F1	F2	F3	F4
1	Mupirocin	250	250	250	250
2	Phosphotidyl choline	250	500	750	1000
3	Methanol	5	5	5	5
4	Water	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 Methanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to $30^{\circ}\text{C} \pm 10^{\circ}\text{C}$ 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 $30^{\circ}\text{C} \pm 10^{\circ}\text{C}$. The preparation was left to cool at room temperature for 30 min 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.⁸

GEL PREPARATION

Table2: Composition of Mupirocin Ethosomal gel (F1 to F4)

S. No.	Ingredients (mg)	F1	F2	F3	F4
1	Ethosomal gel	5	5	5	5
2	Carbopol 934	250	500	-	-
3	HPMC	-	-	250	500

The incorporation of the Mupirocin loaded ethosomes into separate gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes. The optimized formulation was incorporated into different polymeric concentration.⁹

CHARACTERIZATION^{10,11,12}

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.

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 on top of the upper slide. Allow the weight to sit for a fixed time (usually 5 minutes).

Remove the weight carefully. Measure the diameter of the spread gel using a ruler or caliper in two perpendicular directions, and calculate the average.

$$\text{Spread ability (S)} = M \times L / T$$

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 min at the constant temperature ($25 \pm 10^\circ\text{C}$) before the measurements.

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 Mupirocin were separated from untrapped drug by centrifugation at 9000rpm for 45 min at 4°C . The supernatant was recovered and assayed spectrophotometrically using Spectrophotometer.

The encapsulation percentage of drug (EP) was calculated by the following equation

$$\text{EP} = \frac{[\text{Ct} - \text{Cr}]}{\text{Ct}} \times 100$$

Where, Ct, concentration of total Mupirocin, Cr, concentration of free Mupirocin

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 $37 \pm 5^\circ\text{C}$. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Mupirocin 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.

Stability Studies

The formulations stored in glass vials covered with aluminum foil were kept at 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 Mupirocin.¹³

3.RESULTS & 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.

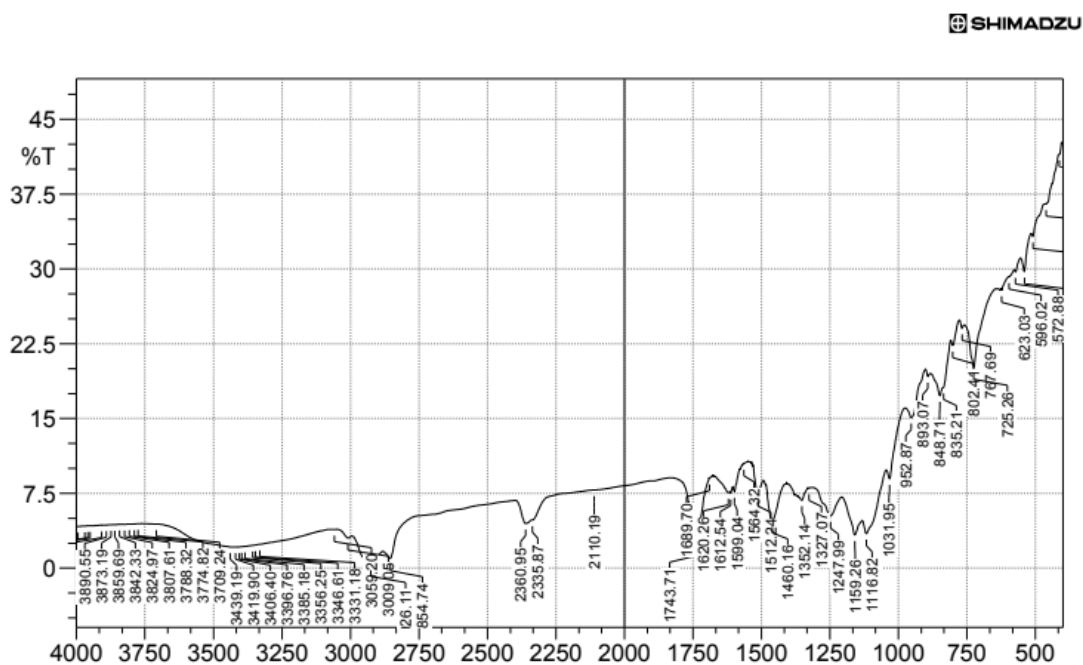


Fig 1: FTIR Studies of Mupirocin

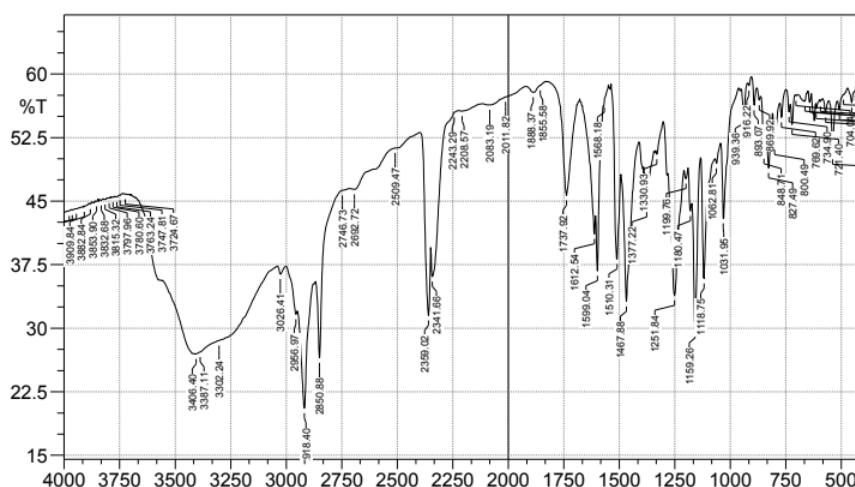


Fig 2: FTIR Studies of optimized formulation

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 ($\pm 100 \text{ cm}^{-1}$) the drug is compatible with excipients.

EVALUATION PARAMETERS:

pH and Viscosity

Table 3: PH and Viscosity values of all formulations

F. Code	pH	Viscosity (cps)
F1	5.6	181
F2	5.1	175
F3	5.3	179
F4	5.5	180

Spread ability

Table 4: Spread ability values of all formulations

F. code	Spread ability (g.cm/sec)
F1	3.96
F2	4.12
F3	4.05
F4	4.50

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 Eppendorf tubes and centrifuged at 17000 rpm for 30 min. 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 230 nm.

Table-5: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	78.46
F2	80.18
F3	77.56
F4	79.20

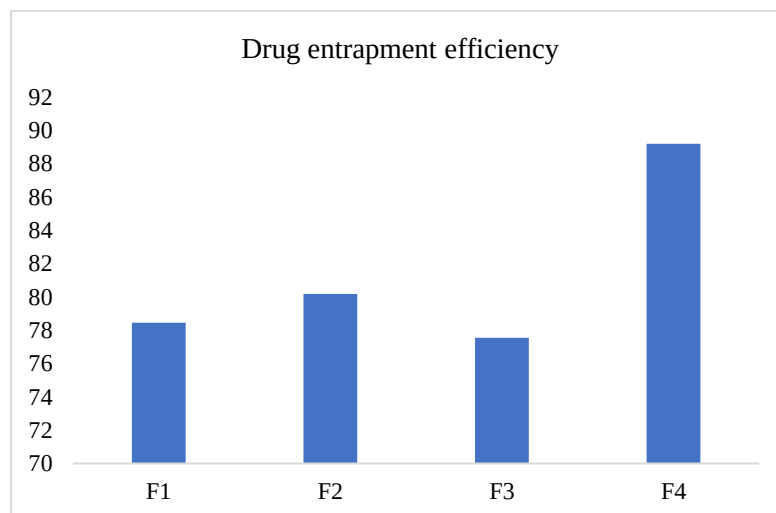


Fig 3: Drug entrapment efficiency of all formulation

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 min to sublime the surface moisture. Finally, the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120°C and voltage of 5kV.

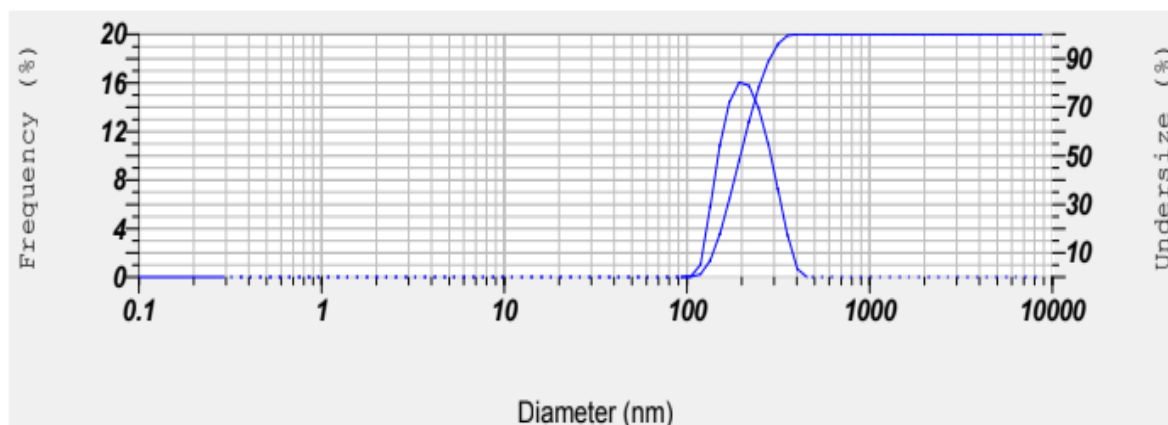


Fig 4: Particle size analysis of Optimized ethosomal gel

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

F. No	Particle size (nm)	Zeta potential
F1	226	-22
F2	196	-26
F3	219	-30
F4	210	-28

SEM Analysis

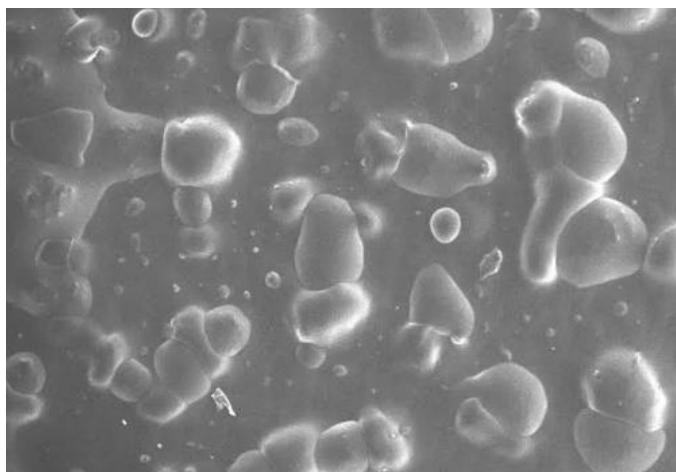
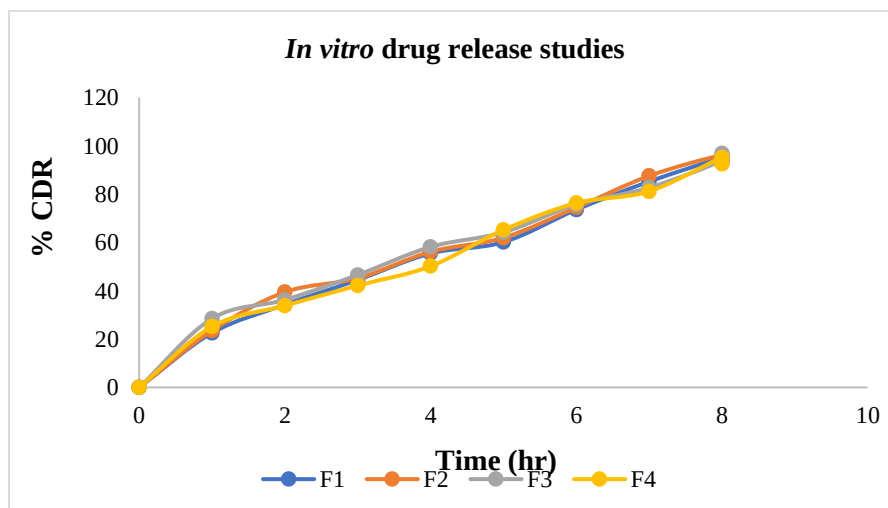


Fig 5: SEM Analysis of Ethosomal gel

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

Time (hr)	F1	F2	F3	F4
0	0	0	0	0
1	26.15	23.51	28.52	25.19
2	33.19	39.46	36.26	33.86
3	47.45	45.19	46.59	42.16
4	55.55	56.16	58.18	50.19
5	67.84	61.96	64.19	65.20
6	78.76	74.45	75.75	76.32
7	81.90	87.57	82.68	81.16
8	92.79	96.15	93.93	95.21

Fig-6: *In vitro* drug release studies of (F1-F4) formulation

Stability studies:

There was no significant change in physical and chemical properties of the Ethosomal gel formulation F-2 after 3

months. Parameters quantified at various time intervals were shown. Optimized formulations F2 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		
			Mupirocin		
			25°C/60%	30°C/75%	40°C/75%
1	01	No Change	96.15	95.86	94.78
2	30	No Change	96.15	95.48	94.36
3.	60	No Change	96.15	95.16	94.28
4.	90	No Change	96.15	95.20	94.16

4. CONCLUSION

The present study was aimed for preparation of Mupirocin 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, Extrudability, spread ability, and drug release study. The prepared four formulations were evaluated for various parameters. Among the various formulation, the combination F2 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F2 comprising phosphatidylcholine fulfills the requirement of good ethosomal gel formulation. In vitro drug release up to 8 hr. and more than 96.15 % drug released. A higher Mupirocin entrapment was observed. The role of these Mupirocin 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 Mupirocin-loaded ethosomal formulation was optimized and successfully formulated in the gel form can be of use for topical preparation for its antifungal infection.

5. REFERENCES

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