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Formulation and Evaluation of Erythromycin-Loaded Ethosomal Gel for the Treatment of Acne

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ABSTRACT
Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed of mainly phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The present study was to investigate the effectiveness of Erythromycin ethosomal gel for the treatment of acne. Erythromycin is a macrolide antibiotic produced by Streptomyces erythreus, which is accountable for its effectiveness in the treatment of acne. Erythromycin ethosomes were prepared by cold method with six different formulations(F1-F6) containing different concentration of lecithin and ethanol. The best formulation was chosen based on their entrapment efficiency, which is then incorporated into the carbopol 934 gel. The prepared gel was then evaluated for its parameters like physical appearance, pH, drug content, spreadability, viscosity, in vitro diffusion studies and kinetic studies. Results revealed that the entrapment efficiency of the F2 was found to be highest i.e., 79.89% and showed cumulative drug release of 82.29±0.35%. The in vitro drug release of the ethosomal gel was found to be 76.33% in 8 hours and showed the first order release kinetics. The developed ethosomal gel found to be stable and the obtained results showed that erythromycin loaded ethosomal gel can be an effective strategy for acne management.

Key words: Erythromycin, ethosomes, zeta potential, Carbopol 934

I. INTRODUCTION

In comparison to oral drug delivery systems, transdermal drug delivery system (TDDS) shows promising result, eliminating gastrointestinal interferences and first pass metabolisms. However, the main advantage of TDDS is that it only works with lipophilic drugs due to the barrier properties of stratum corneum. Molecular weights of less than 500 Da can pass through it. TDDS have been created to augment the permeability of the skin or the driving power of drug diffusion.[1]

Acne

Acne vulgaris or simply known as acne is a human skin disease characterized by skin with scaly red skin (seborrhea), blackheads and whiteheads (comedones), pinheads (papules), large papules (nodules), pimples and scarring. Acne affects skin with dense oil follicles in areas such as the face, chest and back. [2,3,4]

Drug permeability across the stratum corneum has also been found to be improved by liposomes, niosomes, transferosomes and ethosomes. Enhancers of permeation makes the skin more permeable, allowing medications to pass through the skin more readily.[5]

Ethosomes can improve permeation through the stratum corneum barrier in contrast to traditional liposomes, which are known for delivering medications mostly to the skin's outer layers.[5]

It has been studied and reported that a vesicular lipid system containing ethanol at relatively high concentrations, called ethosome, is very effective in improving the skin penetration of certain drugs. It has been studied and reported that a vesicular lipid system containing ethanol at relatively high concentrations, called ethosome, is very effective in improving the skin penetration of certain drugs.[6]

Erythromycin is a macrolide antibiotic produced by Streptomyces erythreus. It inhibits the bacterial protein synthesis by binding to bacterial 50s ribosomal subunits; binding inhibits peptidyl transferase activity and interferes with translocation of amino acid during translation and assembly of proteins. Erythromycin may be bacteriostatic or bactericidal depending on the organisms and drug concentration. The drug in the form of topical gel avoids gastro intestinal irritation, to overcome first pass effect and to maximize the drug concentration at the site of action.[7]

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II. MATERIALS AND METHODS

Erythromycin, Soya lecithin, ethanol, cholesterol, and propylene glycol were purchased from Yarrow chemical, Mumbai, India. Triethanolamine was purchased from Loba chemical, Mumbai, India.

Preparation of ethosomes[8]

Ethosomal formulations were prepared by using cold method.Drug, phospholipid, cholesterol and propylene glycol were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. The mixture was heated to 30°C±1°C and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container.Mixing was continued for additional 5minutes, while maintaining the system at 30°C±1°C.The preparation was left to cool at room temperature for 30 minutes and it was subjected to ultra- sonication for an hour.

Table No.1: Composition of ethosomal formulations

Formulation code	Conc. Of drug (g)	Conc. Of ethanol (ml)	Conc. Of lecithin (ml)	Conc. Of cholesterol (mg)	Conc. Of propylene glycol (ml)	Distilled water up to (ml)
F1	1g	20	2	25	5	50
F2	1g	20	3	25	5	50
F3	1g	20	4	25	5	50
F4	1g	15	2	25	5	50
F5	1g	15	3	25	5	50
F6	1g	15	4	25	5	50

Fig. No.1: Prepared Ethosomal formulations

Evaluation of ethosomes[9,10,11]

Average particle size

Average particle size (in nanometer) of the ethosomes were measured using a Malvern nano zeta sizer instrument.

Zeta potential

Measurement of zeta potential of the ethosomal formulation were done by using Malvern zeta sizer instrument.Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion systems.

Entrapment efficiency

For entrapment efficiency, 1ml of the formulation was taken and centrifuged at 15000rpm for 2 hours in an ultracentrifuge. Sediment and supernatant were separated. The amount of erythromycin was estimated UV spectrophotometrically. The entrapment efficiency was determined by applying the following equation:

$$\mathrm{EE}(\%) = \frac{\text{total amount of drug taken} - \text{unentrapped drug} \times 100}{\text{total amount of drug taken}}$$

In vitro drug release from ethosomes

In vitro permeation studies were performed using bi chamber donor receiver compartment model (Franz diffusion cell). The formulation was taken in the donor compartment and phosphate buffer pH 6.8 was taken in the receptor compartment. The cellophane membrane, previously soaked overnight in the diffusion

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medium (phosphate buffer pH 6.8) was placed between the donor and receptor compartment. Ethosomal suspension was placed on the membrane. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37 ± 0.5^0 C. Samples were withdrawn from the receptor cell at specified time intervals of 1, 2, 3, 4, 5, and 6 hours. Each time immediately after the removal of the sample, the medium was compensated with fresh Phosphate buffer (pH 6.8). The cumulative amount of drug from ethosomes permeated through synthetic membrane was plotted against time.

Preparation of ethosomal gel[12]

1% Carbopol 934 gel was prepared by dispersing it in distilled water.Dispersed by constant stirring at 600 rpm with the aid of magnetic stirrer for 1 hr.To this, ethosomal formulation was added and mixed properly.Prepared gel was neutralized by the dropwise addition of triethanolamine.Glycerol was added to balance the viscosity and methyl paraben as a preservative.

Evaluation of ethosomal gel[13,14,15,16]

Measurement of pH of the ethosomal gel

1g ethosomal gel was mixed in 100 ml distilled water utilizing a homogenizer. Electrodes were then immersed in the developed gel solution and readings were documented from digital pH meter in three times and mean value was estimated.

Viscosity determination

Viscosity measurements were done on Brookfield viscometer by selecting spindle no 64 at 60 rpm. 1 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated. The procedure
was repeated three times and observations are recorded as mean.

Spreadability

Spreadability of the gel formulation was determined by taking two glass slides (14*5cm) of equal length. On one glass slide, 1gm gel was applied. To the other slide, weights (1000g) are added and the time taken for the second glass slide to slip off from the first glass slide was determined. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula,

$S=M*L/T$

Where, S = spreadability,
M = Weight kept on upper slide,
L = Length of glass slides,
T = Time taken to slip off the slides completely from each other.

Drug content

One gram of gel was dissolved in a 100 ml of phosphate buffer pH 6.8 stirred constantly for 10 minutes. From this 1 ml of solution was taken and diluted with 100ml of phosphate buffer pH 6.8. The resultant solution was analysed U.V spectrophotometrically.It can be calculated by applying following equation:

$$\%\ \text{drug content} = \frac{\text{concentration} \times \text{DF} \times 100}{1000}$$

In vitro diffusion study

In vitro diffusion studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 50ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. 1 gm of formulated ethosomal gel was placed over the membrane (In the donor compartment) and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 6.8. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5 ˚C by surrounding water in jacket. The samples were withdrawn at time interval of 1, 2, 3, 4, 5, 6, 7 and 8 hours and the receptor phase was replaced with an equal volume of phosphate buffer at each time of sample withdrawal. The samples were analyzed spectrophotometrically.

Stability studies

The success of an effective formulation can be evaluated only through stability studies. The prepared ethosomal gel was placed in plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 25±2°C and 60±5%RH for a period of 3 months.

III. RESULTS AND DISCUSSION

In the present study, six erythromycin-containing ethosomal formulations were prepared by cold method with different phospholipid and ethanol ratios and the best formulation was transformed into a gel.

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Preformulation studies

General descriptions like odor, melting point & solubility of erythromycin were performed and obtained results are shown in the table no. 2.

Table No.2: Preformulation studies of pure drug

PROPERTIES	REPORTED		OBSERVED	
Description	White or slightly yellow crystals or powders		White amorphous powder	
Odour	Odourless		Odourless	
Melting point	133-135˚C		133˚C	
Solubility	Water	2mg/ml	Water	1.3mg/ml
	Methanol	20mg/ml	Methanol	18mg/ml
	Phosphate buffer pH 6.8	2.3mg/ml	Phosphate buffer pH 6.8	2.1mg/ml

Drug- excipient interaction

Compatibility studies of Erythromycin with different polymers were carried out prior to the formulation of ethosomes. The peaks obtained in the spectra of physical mixture were correlated with the peaks of drug spectrum. It is represented in the Fig.no. 2 and 3.

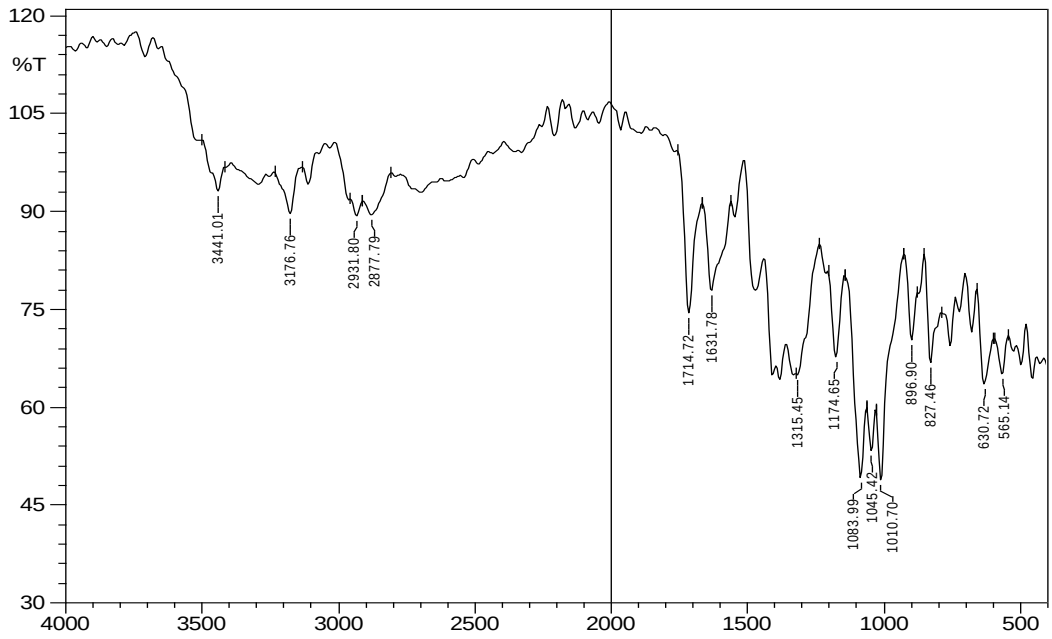

Fig. No.2: FTIR spectra of Pure drug

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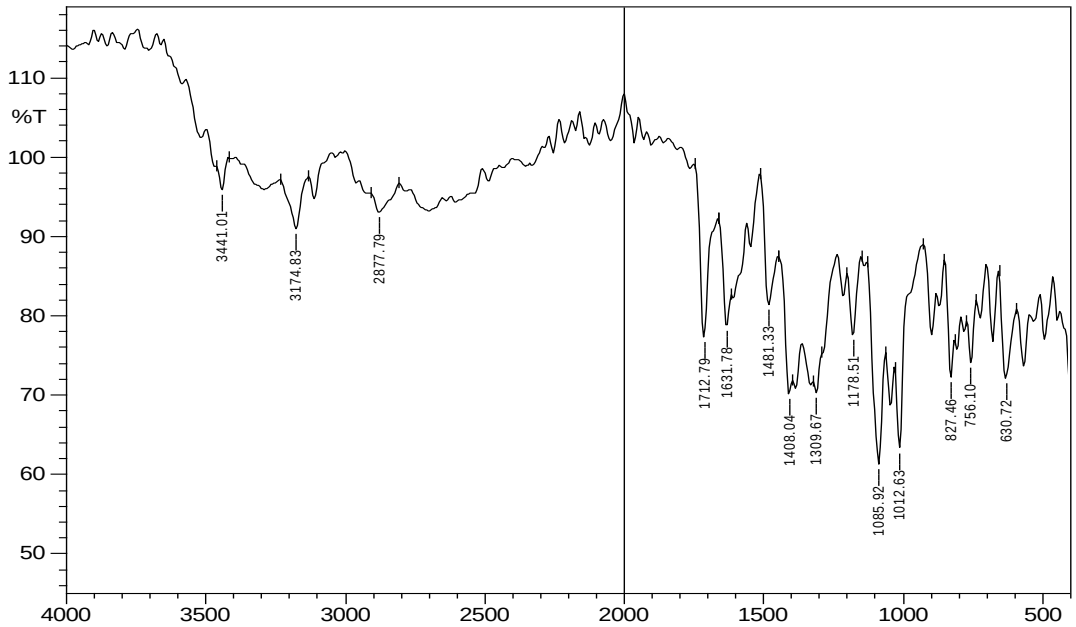

Fig. No.3: FTIR spectra of Drug- Polymer mixture

Evaluation tests for ethosomes containing erythromycin

Entrapment efficiency (EE)

Entrapment efficiency of all the formulations was done by centrifugation method. Results obtained are shown in the table no. 3.

Average particle size and size distribution

Particle size of ethosomal formulations were performed and it is represented in the table no. 3.

Table No.3: Percentage entrapment efficiency (%EE) and particles size of Ethosomes

Sl No	Formulation code	% EE (%)	Particle Size (nm)
1	F1	71.48±0.27	486.6
2	F2	79.89±0.36	291.7
3	F3	67.12±0.65	784.6
4	F4	62.24±0.43	774.1
5	F5	57.99±0.78	849.6
6	F6	53.80±0.21	852.4

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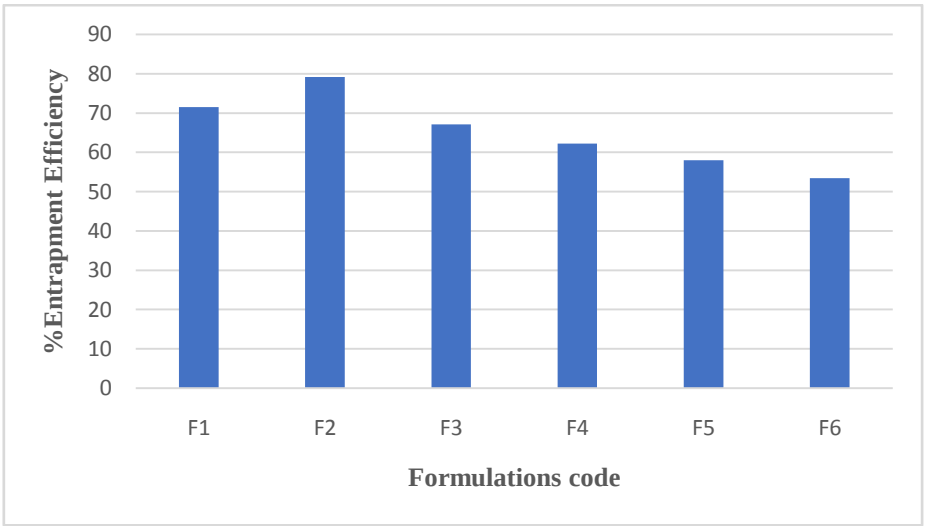

Fig. No.4: Entrapment Efficiency of F1-F6

The %EE of all the formulations (F1-F6) was in the range of 53.80±0.21– 79.89±0.36% as shown in table No.3 and figure No.4. The highest entrapment efficiency was found in the batch F2, consisted 3ml of soya lecithin and 20 ml of ethanol.

The average particle size of ethosomal formulations was found between 291.7nm-852.4nm. The F2 formulation showed significantly smaller vesicle size i.e., 291.7nm than other formulation.

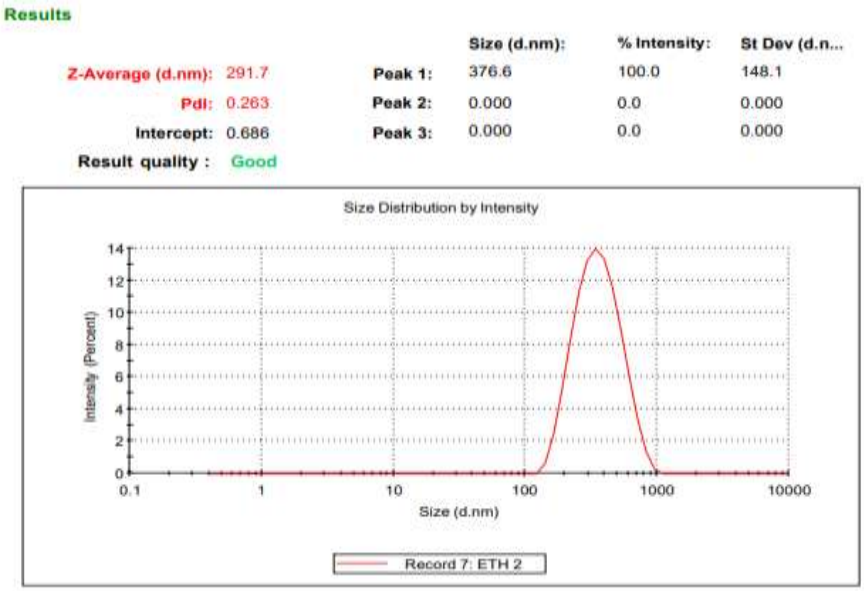

Fig. No.5: Particle size of F2

Zeta Potential

Zeta potential of the formulation was determined by Malvern nano zeta sizer instrument. Obtained result is shown in figure below. It was found that zeta potential of the formulation F2 was negative i.e, -6.51 Mv. Negative potential indicates that the particles have no charge as a whole system is stable.

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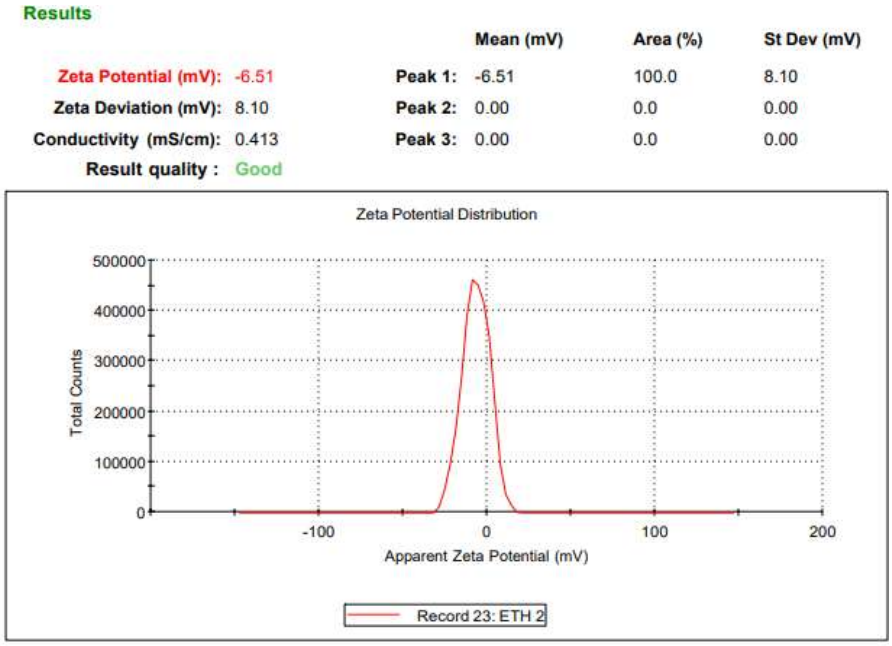

Fig. No.6: Zeta Potential of F2

In vitro drug release study

TableNo.4: In vitro drugdiffusion studiesoftheformulations

Time(hrs)	Formulations code					
	%CDR					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	22.73±0.23	20.60±0.22	18.32±0.11	19.60±0.11	19.96±0.22	20.57±0.11
2	35.01±0.34	41.82±0.46	34.94±0.73	29.90±0.38	31.72±0.65	29.48±0.29
3	51.29±0.72	64.04±0.37	49.63±0.82	38.20±0.92	41.28±0.29	36.69±0.36
4	64.64±0.52	71.43±0.61	58.92±0.49	47.86±0.72	52.49±0.61	48.05±0.84
5	72.89±0.21	77.36±0.42	70.06±0.13	61.49±0.57	59.83±0.73	53.71±0.37
6	78.82±0.36	82.29±0.28	75.85±0.59	68.04±0.73	64.49±0.24	60.95±0.51

The results obtained for all formulations (F1 –F6) are shown in table No.4 and fig.No.7. The cumulative percent drug release after 6 hours was found to be in the range of 60.65±0.21-82.04±0.35%. Formulation F6 showed least percentage cumulative drug release value 60.65±0.21% at 6 hrs and formulation F2 showed highest percentage of drug release value

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82.04±0.35% at 6 hrs. As expected, all formulations (F1 –F6) were showed controlled drug release for 6 hours.

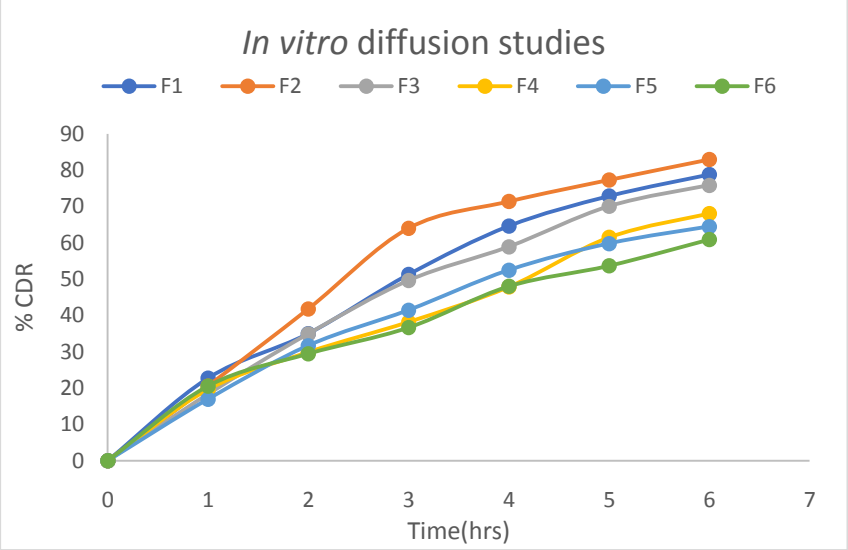

Fig. No.7: In vitro diffusionstudy

Evaluation test for formulated erythromycin loaded ethosomal gel

Based on the entrapment efficiency, particle size and in vitro studies, F2 was found to show better results which was incorporated into Carbopol gel and subjected for further studies as shown in the table no. 5.

Physical characteristics of Erythromycin loaded ethosomal gel

Table No.5: Physical characteristics of Erythromycin ethosomal gel

Formulation code	Appearance	pH	Spreadability g cm/sec	Drug Content	Viscosity (cps)
F2	Creamish brown	5.3±0.05	16.08±0.103	90.84%	4657

Fig. No.8: Prepared Ethosomal gel formulation

Physical examination

The prepared ethosomal gel formulation was creamish brown in color with a smooth and homogeneous consistency and there was no phase separation. Results of physical examinations are shown in the table No.5.

Measurement of pH

pH of the formulation is shown in the table No.5. The pH values of the prepared

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formulation was found to be 5.3±0.05, which is considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Viscosity Study

Viscosities of the formulation was evaluated by using Brookfield viscometer at 27^{o}C using spindle no.64 at 60 rpm. The viscosity of the ethosomal gel was found to be 4657cps.

Spreadability

The value of spreadability was shown in table No.5. The ethosomal gel formulation showed 16.08±0.103g cm/ sec. It was revealed that the therapeutic efficiency of ethosomal gel is substantially determined by its spreading value. The formulation showed good spreadability.

In vitro diffusion study

Table No.6: In vitro diffusion study of Ethosomal gel

Time (hrs)	% Cumulative drug release (%)
0	0±0.00
1	18.14±0.21
2	31.95±0.17
3	42.51±0.15
4	53.75±0.29
5	62.48±0.52
6	68.26±0.71
7	72.83±0.33
8	76.33±0.46

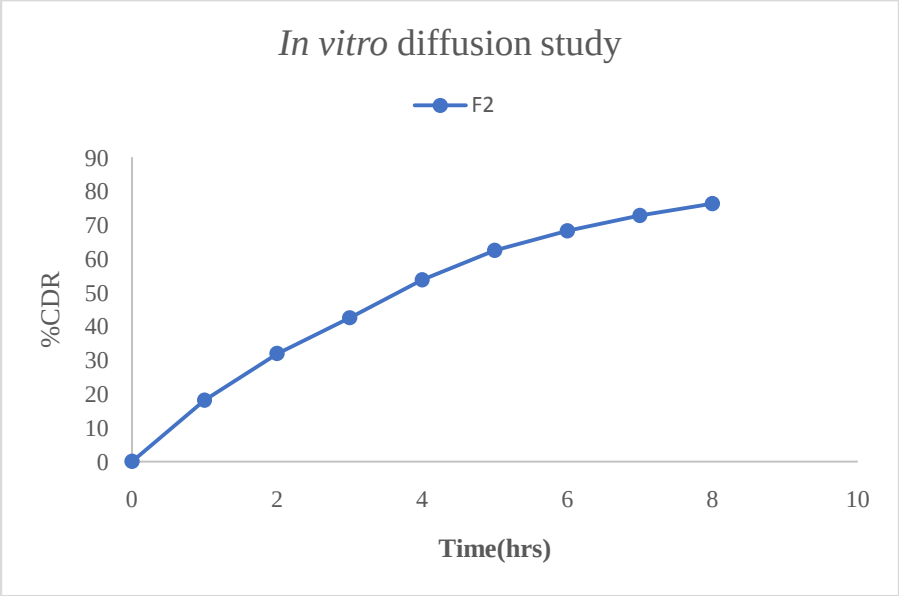

Fig. No.9: In vitro diffusion study of ethosomal gel

In vitro diffusion study of ethosomal gel

The in vitro diffusion study of the drug is given in fig no.9. The ethosomal gel formulation with 3 ml of soya lecithin and 20 ml ethanol showed 76.33±0.46% of drug release within 8 hrs.

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Stability studies

Table No.7: Evaluation of F2 for stability study

Evaluation Parameters	Time (days) Accelerated condition 25±2°C at 60±5%RH			
	0 day	30 days	60 days	90 days
Colour	Creamish brown	Creamish brown	Creamish brown	Creamish brown
Drug content (%)	90.84%	89.12%	88.71%	88.59%
In vitro diffusion in 8 hr	76.33%	75.87 %	74.62 %	73.04 %

Stability study of the vesicles is the major determinant for the stability of the formulations. The study was carried to evaluate physical appearance, drug content and in vitro diffusion studies at accelerated condition 25±2°C at 60±5%RH. The results of stability study of the formulation are depicted in table No.7.

IV. CONCLUSION

In the present work, effective erythromycin ethosomal gel formulation was successfully formulated. The purpose of the study was to avoid the side effects of orally taken erythromycin, by its prolonged use as topical formulation. From the reproducible results of executed experiments, the following conclusions are made;

- Erythromycin entrapped ethosomal gel for transdermal drug delivery was prepared by using various concentrations of phospholipids and ethanol.
- The prepared formulations showed good entrapment efficiency, particle size and drug release. From the results obtained, the formulation F2 was concluded as the best formulation, because of the spherical vesicular shape(291.7nm) and good drug entrapment efficiency (79.89%).
- It was observed that ethosomal gel formulation followed zero order drug release kinetics, hence it can be suggested that ethosomes could be superior drug carrier for topical delivery of erythromycin.

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