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RESEARCH ARTICLE

Formulation and Evaluation of Ethosomal Topical Gels of Etoricoxib

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ABSTRACT

The aim of the current investigation was to develop an Etoricoxib loaded ethosomal gel for better anti-inflammatory activity by sustaining the drug release and reduces adverse effects. Ethosomes are lipid vesicular carriers containing ethanol which provides better penetration of drug into the skin. Etoricoxib is a non steroidal anti-inflammatory drug which has shown many side effects when used orally. Etoricoxib ethosomes were prepared by hot method using soya lecithin, ethanol, cholesterol and drug in different ratios. They were evaluated for particle size, entrapment efficiency and *in vitro* drug release. Optimized ethosomal formulation showed an entrapment efficiency of 88.09% and drug release of 90.4% in 8hrs. The optimized formulation was incorporated into gel using carbopol 934, HPMC K4M; HPMC K100.Optimized ethosomal gel (EG3) showed the drug content of 93.36% and drug release of 75.5% in 8hrs.Ex Vivo studies were performed for the optimized gel and the drug release was found to be 73.5%in 8hrs respectively. Stability studies indicated that optimized formulations were stable for a period of 3months under refrigerated conditions. It was concluded that Etoricoxib loaded ethosomal gels were successfully formulated to increase the efficacy and reduce its side effects.

KEYWORDS

Ethosomes, Ethanol, Ethosomal gel, Etoricoxib, Soya Lecithin, Phospholipon 90H, Carbopol, HPMC K4M, and HPMC K100, *Ex vivo* studies

INTRODUCTION

Etoricoxib is a non-steroidal anti-inflammatory drug (NSAIDs) used for the symptomatic relief of pain and inflammation in osteoarthritis, rheumatoid arthritis and acute gout. Oral administration of this drug is associated with severe gastrointestinal side effects like ulceration and gastro intestinal bleeding. Further as it is required for chronic use in the condition like rheumatoid arthritis, these drawbacks become disabling factors of such therapy. In order to avoid these disadvantages, the ethosomal gel formulations have been proposed as topical applications.

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Ethosomes are soft, malleable vesicles used for delivery of drugs to reach the deep skin layers and/or the systemic circulation and are the modified forms of liposomes that are high in ethanol content.

They are capable of incorporate a variety of hydrophilic and hydrophobic drugs to enhance the accumulation of drug at the administration site and to reduce the side effects.[7,9]

Ethosomal drug delivery system has several advantages as compared to other transdermal and dermal delivery systems. These include enhanced permeation of drug through skin; ethosomes provide platform for the delivery of large and diverse group of drugs across the skin (peptides, protein molecules); ethosomes contain non-toxic

materials in formulation, ethosomal drug is administered in semisolid form (gel or cream) hence producing high patient compliance.

Ethosomal drug delivery system can be used widely in pharmaceutical, veterinary and cosmetic fields. The system is passive, non-invasive and is available for immediate commercialization. Also ethosomal drug delivery is very simple in comparison to iontophoresis and phonophoresis and other complicated methods.[9]

The objective of the present study was to develop Etoricoxib loaded ethosomal gels for better anti-inflammatory activity by improving permeation and sustaining the drug release.

MATERIAL AND METHODS

Etoricoxib was obtained as a gift sample from Cirex pharmaceuticals Ltd. Medak. Soya lecithin was purchased from Himedia, Phospholipon 90H was obtained as gift sample from Lipoid, Germany, Ethanol from SD fine chemicals limited and Carbopol 934P from Loba chemie, Mumbai, HPMC K4M and HPMC K100 were of commercial grade. All other chemicals used were of analytical grade.

Preparation of Ethosomes using Lecithin[6,7 9]

Hot Method

Ethosomes were prepared by hot method using different lipids like soya lecithin, Phospholipon 90H. Lipids and cholesterol in different ratios as shown in table 2 were accurately weighed and dissolved in water and kept for stirring using magnetic stirrer for 30 min with heating at 40^0C. Organic phase comprising of specified amount of Etoricoxib added to ethanol followed addition of propylene glycol was kept for stirring separately. Then, lipid solution was added drop by drop to the organic phase with continuous stirring on a magnetic stirrer for 1hr. The solution was subjected to Sonication using probe sonicator for 15 min to reduce the vesicle size.

Preparation of Ethosomes using Phospholipon 90H[1]

Etoricoxib ethosomes were prepared using lipid film hydration technique using Phospholipon 90 H. Lipid and cholesterol in different ratios as shown in table 1 were accurately weighed and dissolved in 15 ml mixture of chloroform and methanol (2:1v/v).

Table 1: Formulation of Ethosomes Using Phospholipon 90H

Formulation code	Drug concentration (mg)	Pc(mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol (ml)	Water (Up to 10ml)
FL1	25	90	45	20	3	q.s
FL2	25	100	45	20	3	q.s
FL3	25	200	45	20	3	q.s
FL4	25	300	45	20	3	q.s
FL5	25	400	45	20	3	q.s
FL6	25	500	45	20	3	q.s

To this mixture propylene glycol is added. It was then vortexed in a round bottomed flask at 60^0C for 30 minutes at a speed of 100 rpm and reduced pressure of 25 mm Hg to remove the solvent. The resulting film was hydrated with hydroethanolic drug solution and vortexed for 30 minutes. The obtained colloidal dispersion was sonicated using probe type sonicator.

Preparation of Ethosomal Gel[2, 5]

The gels were prepared by dispersion method using hydroxy propyl methyl cellulose (K4M, K100) and carbopol 934 in different ratios as shown in table 3. Gels were prepared by dispersing gelling agents in distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as preservative .The prepared gels were filled in glass vials and stored at 4 to 8^o C.

Preliminary Studies

Drug-Polymer Interaction Studies

The drug-excipient compatibility studies were determined by IR Spectrophotometer (Shimadzu 8400S).Samples of pure drug and physical mixtures of drug and excipients were scanned in the range between 400-4000 cm^{-1}.

Optimization of Formulation Variables

A. Optimization of Concentration of Lecithin

Ethosomal dispersions F1-F5 were prepared by varying the lecithin concentration. The dispersions were evaluated and based on rate of drug release the lecithin concentration was optimized.

Table 2: Formulations of Ethosomes using lecithin

Formulation Code	Drug (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol (ml)
F1	25	100	25	10	3
F2	25	200	25	10	3
F3	25	300	25	10	3
F4	25	400	25	10	3
F5	25	500	25	10	3
F6	25	300	25	10	3
F7	25	300	35	10	3
F8	25	300	45	10	3
F9	25	300	55	10	3
F10	25	300	65	10	3
F11	25	300	75	10	3
F12	25	300	85	10	3
F13	25	300	45	20	3
F14	25	300	45	25	3
F15	25	300	45	30	3
F16	25	300	45	35	3

Table 3: Formulations of Ethosomal Gel

Formulation code	Ethosomal suspension (ml)	Carbopol (%)	HPMC K4M (%)	HPMC K100 (%)	Triethanolamine (%v/v)	Water Up to 30ml
EG1	10	0.5	-	-	0.5	q.s
EG2	10	1	-	-	0.5	q.s
EG3	10	1.5	-	-	0.5	q.s
EG4	10	2	-	-	0.5	q.s
EG5	10	-	2	-	0.5	q.s
EG6	10	-	3	-	0.5	q.s
EG7	10	-	4	-	0.5	q.s
EG8	10	-	5	-	0.5	q.s
EG9	10	-	-	3	0.5	q.s
EG10	10	-	-	4	0.5	q.s
EG11	10	-	-	5	0.5	q.s
EG12	10	-	-	6	0.5	q.s

B. Optimization of Concentration of Cholesterol

Ethosomal dispersions F6-F12 were prepared by varying the cholesterol concentration. The dispersions were evaluated and based on rate of drug release the cholesterol concentration was optimized.

C. Optimization of Concentration of Ethanol

Ethosomal dispersions F13-F16 were prepared by varying the ethanol concentration. The dispersions were evaluated and based on rate of drug release the ethanol concentration was optimized.

D. Optimization of Concentration of Phospholipon 90H

Ethosomal dispersions were prepared by varying the phospholipon 90H concentration. The dispersions were evaluated and based on rate of drug release the phospholipon 90 H concentration was optimized.

Evaluation of Ethosomes

A. Size and Shape of Ethosomes

Surface morphology of ethosomes was determined by using Scanning Electron Microscopy (Hitachi S-3700N).SEM gives a three dimensional image of the globules.

B. Zeta Potential

The zeta potential of the ethosomes was determined using zeta sizer (HORIBA SZ-100). Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

C. Entrapment Efficiency(EE)[1,2,6]

The entrapment efficiency of ethosomes was estimated by using ultracentrifugation method using centrifuge where the ethosomal dispersions were centrifuged at 15000rpm for 45 minutes.

The clear supernatant from the resulting solution was diluted appropriately using methanol and analyzed using UV-Visible spectrophotometer at

235nm. The percent of encapsulation efficiency (EE %) was calculated using the following equation:

$$EE\% = \frac{[Total drug] - [Free drug]}{Total drug} X\ 100$$

D. In Vitro Drug Release

Studies were performed for all the formulations. The modified diffusion cell consisted of a hollow glass cylinder (length 14cm and internal diameter 2.5cm) made up of borosil glass. One end of the cylinder was covered with Himedia dialysis membrane, which was previously soaked in warm water and placed on the receptor compartment.

The temperature was maintained at 37^0C. Phosphate buffer pH 7.4 was placed in the receptor cell. Samples were withdrawn at predetermined time intervals over 8hours and the medium was replaced with fresh phosphate buffer (pH 7.4). The samples were analyzed to estimate the drug using UV-Visible spectrophotometer at 235nm.

Characterization of Ethosomal Topical Gel

Visual Appearance and pH

The formulations were visually observed for the presence of any particulate matter. The pH of ethosomal topical gels was measured using digital pH meter.

Drug Content

Drug content was estimated spectrometrically where the formulation containing 10mg equivalent of drug was taken and dissolved in suitable solvent and filtered.

The volume was made to 100 ml with the methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 235nm using UV-Visible spectrophotometer.

Viscosity

Viscosities of the formulations were determined using Brookfield synchroelectric viscometer (DV Pro II) fitted with S-64 spindle at 5, 10, 50 and 100 rpm.

In Vitro Drug Release Studies

In Vitro release studies were carried out using Franz diffusion cell containing two compartments (cells). Upper one is donor (diffusion) cell, consisting of two open ends and lower one is receptor cell, with one open end. One end of the donor compartment was covered with Himedia dialysis membrane, which was previously soaked in warm water and placed on the receptor compartment. The receptor cell contained a small magnetic bead and was rotated at a constant speed and the temperature was adjusted to 37 ± 0.5^0C. Samples were withdrawn at periodic intervals for 8hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible spectrophotometer using phosphate buffer (pH7.4) as blank at 235nm.

Ex Vivo Drug Permeation Studies[1]

After approval of protocol (IAEC/SVCP/ 2015/004) from Institutional Ethics Committee permission as per ICMR the study was conducted.

Ex vivo studies were carried out using skin of albino rat. Rats (male albino) 6 to 8 weeks old, weighing 120 to150 g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel.

The excised skin was placed on aluminum foil and the dermal side of the skin was gently tarred off for any adhering fat and/or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The *in vitro* study was approved by the institutional ethical committee.

The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 was taken in the receptor compartment. Temperature was maintained at 37 ± 0.5^0C. Optimized gel formulation was placed in the donor compartment. Samples were withdrawn at

predetermined time intervals over 8hours and replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed using UV-Visible spectrophotometer at 235nm using phosphate buffer (pH 7.4) as blank.

Drug Release Kinetic Modeling

The kinetics of etoricoxib release from the ethosomal vesicles and from the gels formulated was determined by finding the best fit kinetic model by fitting the release data into various kinetic equations such as Zero order, First-order, Higuchi, and Korsemeyer-Peppas and finding the R^2 values of the release profile corresponding to each model.

Accelerated Stability Studies

The optimized ethosomal dispersion which had higher entrapment efficiency was placed in vials and kept for a short term accelerated stability study at $40^0\pm 2^0C/75\pm5\%$ RH and 5 ± 3^0C as per modified international conference on harmonization guidelines. Samples were analyzed at periodic time intervals for 3months for the estimation of pH and drug content.

RESULTS AND DISCUSSION

Preliminary Studies

Drug Polymer Interaction Studies

FTIR spectrum of pure drug and mixture of drug and polymers are shown in Figure 1, 2 and 3.

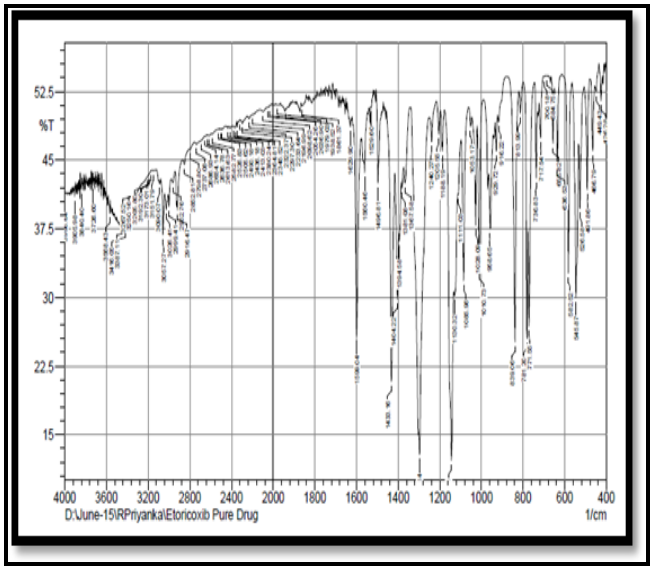

Figure 1: IR Spectrum of Drug

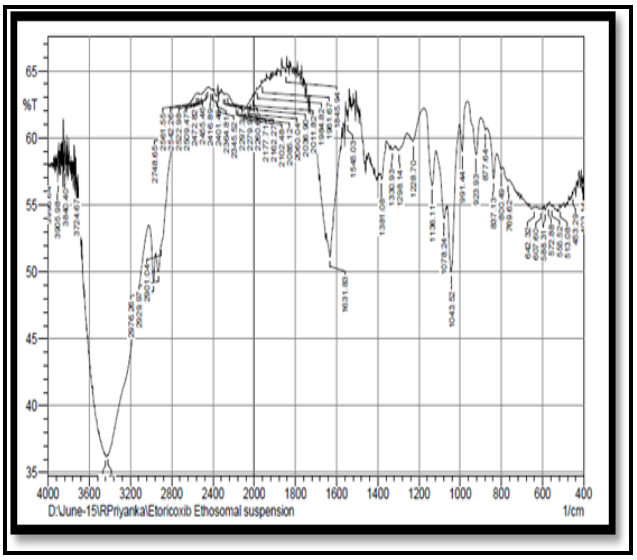

Figure 2: IR Spectrum of Drug and Excipients

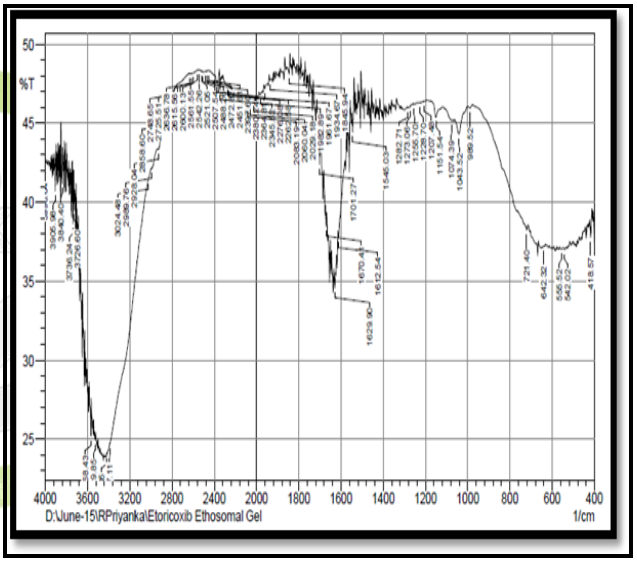

Figure 3: IR Spectrum of Etoricoxib Gel and Excipients

From the spectral study, it was observed that the FTIR spectrum of pure Etoricoxib peak was observed at 1433 cm^{-1} and 1599 cm^{-1} for amino stretching and saturated methyl group. There was no significant change in the peaks of pure drug and drug polymer mixture. Therefore, it can be inferred that there is no specific interaction was observed between the drug and the polymers used in the formulations.

Vesicle Shape and Size of Ethosomes

From SEM images Figure 4 and microscopic evaluation it was observed that most of the vesicles were spherical in shape and is smooth in

surface. The vesicular size of the ethosomes significantly increased with increase in phospholipids concentration and also decreased with increase in concentration of ethanol.

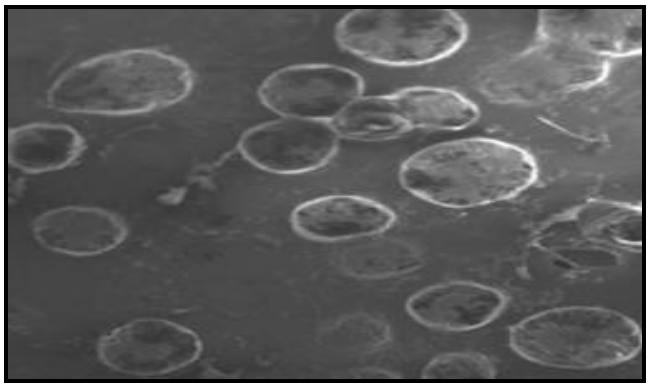

Figure 4: Scanning Electron Microscopic Images of Etoricoxib Ethosomes

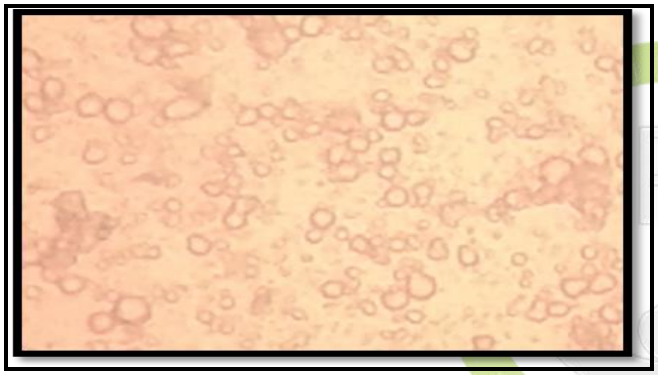

Figure 5: Photomicrograph of Etoricoxib Loaded Ethosomes

From Figure 6(a) it was observed that the diameter of the optimized ethosomal formulation F14 was found to be in the range of 100 to 1000 nm. The average sizes of ethosomes were found to be 213.1 nm.

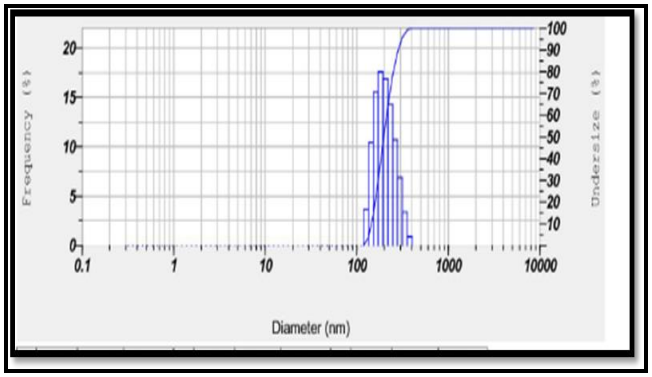

Figure 6(a): Particle size analysis of Etoricoxib loaded ethosomes

Zeta Potential

The zeta potential of the ethosomes was determined using zeta sizer. From the Figure 6 (b) the value of the optimized ethosomal formation F14 was found to be -6.6 mV which indicates that ethosomes were stable.

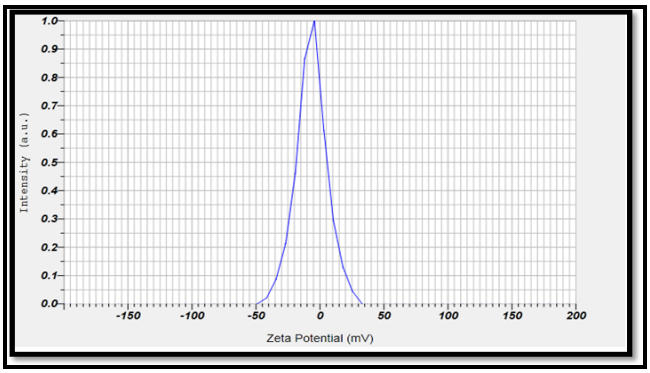

Figure 6(b): Zeta potential of Etoricoxib loaded Ethosomes

Entrapment Efficiency

Percentage entrapment of Etoricoxib ethosomes was found to be in the range of 74-82% as shown in Figure 7. The entrapment efficiency was found to be higher for the formulation F14 (88.03%), prepared using lecithin. Amounts of ethanol, lecithin and Phospholipon 90H, used for ethosomes preparation seemed to influence the entrapment efficiency. Of all factors examined, the concentration of ethanol was found to influence the entrapment efficiency to a significant extent. Increase in the amount of ethanol increased entrapment efficiency and the reason could be possibly due to the formation of thinner membrane.

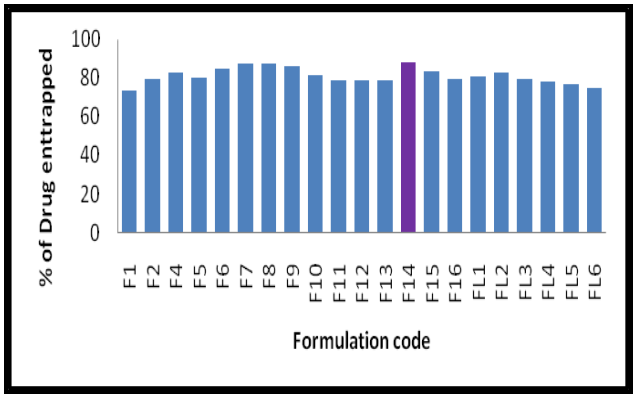

Figure 7: The Entrapment Efficiency of the Ethosomes prepared using Lecithin

In Vitro Drug Release

The cumulative percentage drug release from various ethosomal formulations is shown in Figure 9-11. Formulation F14 showed higher cumulative drug release of 90.4% in 8 hrs. It showed higher entrapment efficiency and drug release than the other formulations, therefore F14 has been selected for formulating the ethosomal gel.

Evaluation of Ethosomal Topical Gel

Visual Appearance

All formulations were opaque, light yellow in color, odorless, semi solid in nature and had smooth appearance.

pH

The pH results for all formulation exhibit in the range of 6.8-7.2 which demonstrate that the prepared gels are irritation free to the skin. The optimized formulation (EG 3) pH was found to be 6.9.

Drug Content

The formulations were analyzed for drug content spectrophotometrically at 235nm. All the formulations exhibited fairly uniform drug content. Results revealed that drug content of all developed formulations were in the range of 87-94% as shown in Table 4.

Table 4: % of Drug content

S.No	Formulation code	% of Drug content
1	EG1	94.52
2	EG2	94.75
3	EG3	93.36
4	EG4	92.14
5	EG5	91.78
6	EG6	90.62
7	EG7	91.52
8	EG8	89.49
9	EG9	91.96
10	EG10	90.78
11	EG11	88.46
12	EG12	87.18

Viscosity

The viscosity of the all gel formulations ranged from 874 to 7965cps as shown in Figure 8. The viscosity of the formulations decreased on increasing the shear rate i.e. pseudoplastic behaviour was noted.

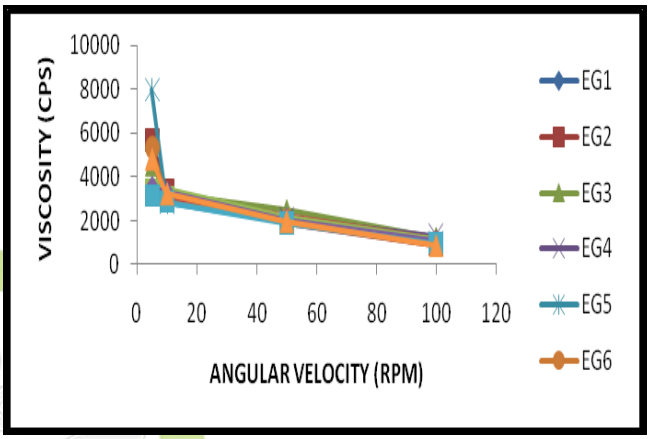

Figure 8: Viscosity of Ethosomal Gels

In Vitro Release

The results of *in vitro* release after incorporation of ethosomes in hydrogels are shown in Figure 12, 13, 14 and 15. The cumulative percentage drug release for 8hours was highest for formulation EG 3 formulated using 2% carbopol 934.

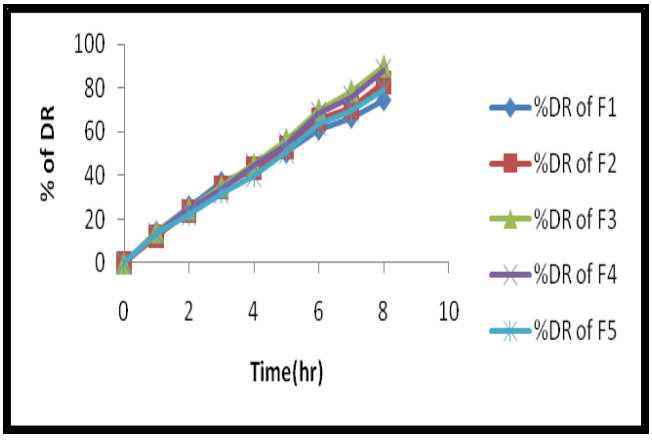

Figure 9: *In vitro* percentage drug release of ethosomes prepared by different lecithin concentrations

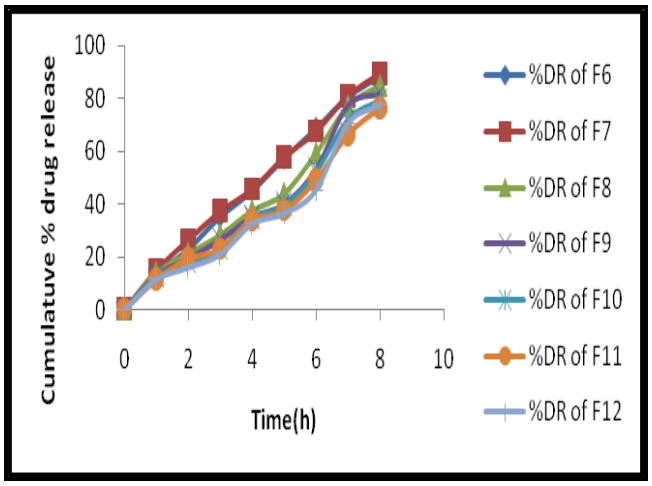

Figure 10: *In vitro* percentage Drug Release of Ethosomes prepared by different Cholesterol Concentrations

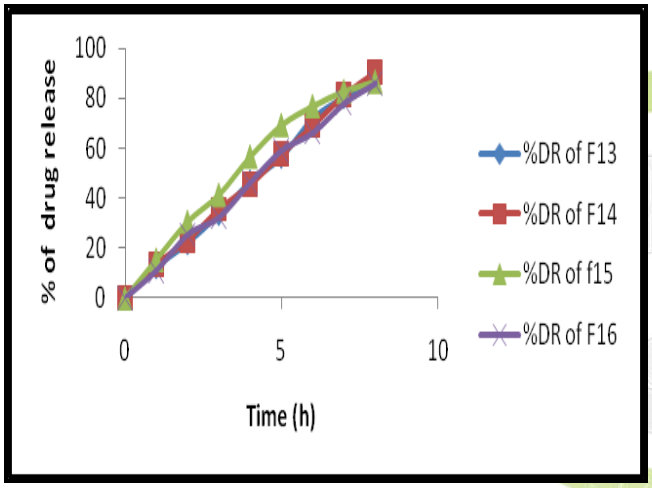

Figure 11: In vitro percentage Drug Release of Ethosomes prepared by different Ethanol Concentrations

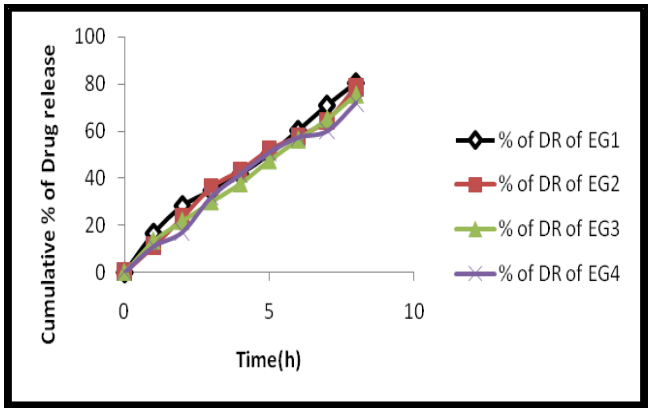

Figure 12: Cumulative Percentage Drug Release of Ethosomal Gels by using Carbopol 934

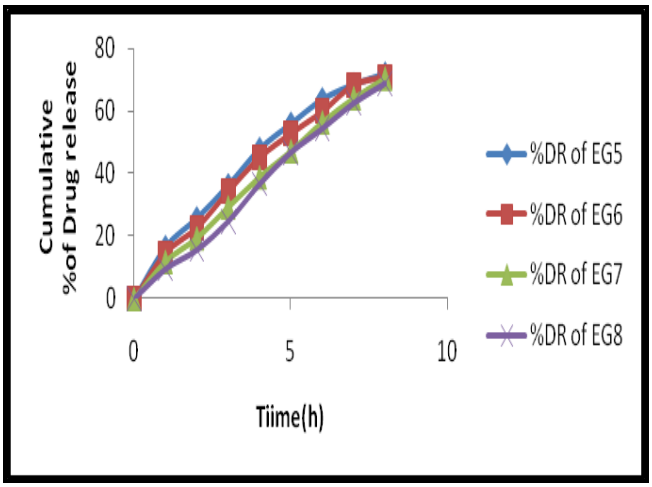

Figure 13: Cumulative Percentage Drug Release of Etoricoxib from Ethosomal Gels by using HPMC K4M

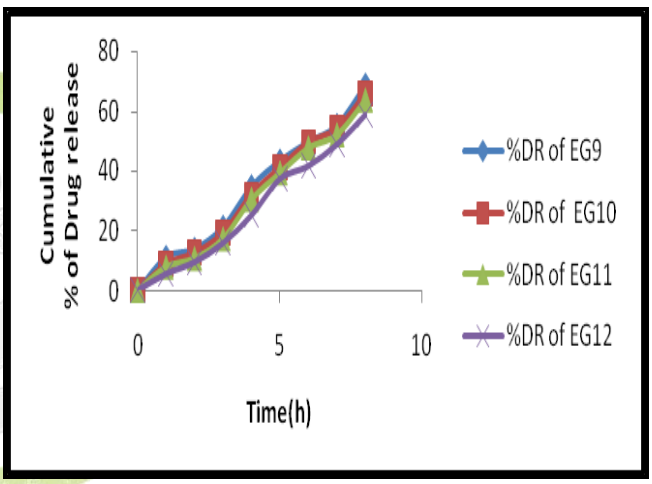

Figure 14: Cumulative percentage drug release of Etoricoxib from ethosomal gels by using HPMC K100

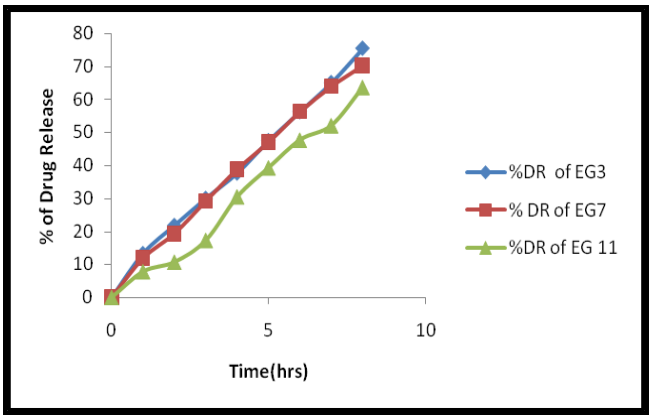

Figure 15: Comparison of Cumulative Percentage Drug Release of Etoricoxib from Ethosomal Gels by using Carbopol, HPMC K4M and HPMC K100

Ex Vivo Studies

Ex vivo drug permeation study was performed for optimized formulation (EG3), and the drug release was shown in Figure 16.

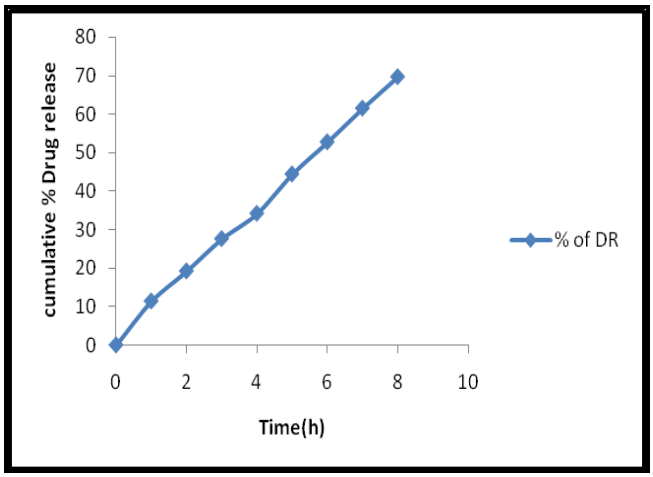

Figure 16: Ex vivo Percentage Drug Release of Etoricoxib from Ethosomal Gel prepared using Carbopol 934

Analysis of Drug Release Mechanism

On drug release kinetic modeling and comparison of the release profile, it was found that there was not much difference in the release pattern of drug from all formulation of ethosomes vesicles and gels. All of them were found to release the drug in accordance to zero order kinetics and the regression value (R^2) for the optimized formulation EG3 was found to be 0.997.

Table 5: Drug release kinetics

Type of release order	Regression value (R^2)
Zero order	0.997
First order	0.988
Higuchi	0.943
Kros-peppas	0.938

Stability Studies

The stability studies of ethosomal gel were performed at $40^0C\pm2^0C$/ 75±5% RH for 3months. The formulations were examined visually for precipitation. The drug content, pH and gelling capacity were determined for every 30 days for 3months. It was observed that there was no change in the physical appearance of the formulation. The drug content was analyzed and there was marginal difference between the formulations stored at different temperatures as shown in the table 6. Ethosomal topical gel formulations retained good stability throughout the study.

Table 6: Stability Studies

Storage condition	pH			
	Initial	1 Month	2 Month	3 Month
	6.9	6.94	6.97	6.99
$40^0C\pm2^0C$/75±5% RH	Drug content			
	Initial	1 Month	2 Month	3 Month
	93.36%	93%	92.23%	91.89

CONCLUSION

Etoricoxib loaded ethosomal gels were successfully formulated. Ethosomes have been considered as a possible vesicular carrier for transdermal delivery of Etoricoxib an analgesic anti-inflammatory agent. The study confirmed that ethosomes are very promising carrier for the transdermal delivery of Etoricoxib which was revealed from the higher entrapment efficiency and better stability profile.

Finally, it can be concluded that ethosomes offers advantages of rapid onset and maximum release of drug with reduction of side effects.

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