ETHOSOMES CARRYING ETODOLAC-EFFECTIVE DRUG CARRIER AS TOPICAL DOSAGE FORM IN THE TREATMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: The priority-based objective of this research was to develop and evaluate the etodolac loaded Ethosomes as topical delivery in Rheumatoid Arthritis autoimmune disease.

Methods: The ethosomes (EE1-EE6) were prepared with varied concentrations (10%-60% v/v) of ethanol by using Rotary evaporator. The prepared Ethosomes evaluated for %EE (Entrapment Efficiency), Zeta potential, SEM (Scanning Electron Microscopy), TEM (Transmission Electron Microscopy), Vesicle size, *In vitro* release, *In vivo* study, Irritancy test, stability test etc.

Results: Prepared ethosomes analysed for zeta potential, vesicle size and % entrapment efficiency and ranges are found to be-18.50mV to-64.53mV, 166.66 nm to 848.97 nm and 53.15% to 89.35%, respectively. Furthermore, when these ethosomes were incorporated in Carbopol 940 gels (EEC1-EEC6), it evaluated for appearance, spredability, pH, viscosity study, drug content and *In vitro* drug release. All ethosomal gels show satisfactory results. After optimization of ethosomes (EE1-EE6) and ethosomal gels (EEC1-EEC6) on previous criteria, optimised formulations EE4 and EEC4 characterised for SEM, TEM, *Ex-vivo* permeation, Irritancy, *In vivo* and different analytical evaluation. The values of r² were found higher for the first-order model and Korsmeyer-Peppas model for all formulations. Irritancy test shows safe use of formulation and *in vivo* test shows that EEC4 had a significant inhibitory effect on edema, which was developed in the right paw of rat by injecting carrageenan when it compared with plain Carbopol gel and marketed formulation.

Conclusion: The present study has confirmed that the formulated Etodolac Ethosomal gel can be used as best vehicle for topical administration, which may be used for the management of rheumatoid arthritis.

Keywords: Etodolac, Ethosomes, Carbopol 940, Topical, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis is one of the diseases which is characterized by painful with swollen and stiff joints. The clear etiology for this disease is not known but, some study shows that it occurs when the body's own immune system attack on its own joints. Complete cure for this disease not known only symptomatic treatment is available to relieve pain and stiffness of joints to improve patient normal daily activity [1, 2]. Pain, fever and tenderness occurs with inflammation due to the release of important chemical in the body known as Prostaglandins [3].

Furtherly to treat pain and inflammation associated with prostaglandin, patients need to use anti-inflammatory drugs like etodolac. Unfortunately, etodolac have many side effects when it is taken by oral route like gastrointestinal disturbances, and it worsens it can cause gastric ulcers and bleeding [4]. With this etodolac also have poor solubility and less bioavailability. Repeated dosing is required because its half-life is 7 h. Thus, to avoid such complications, it is necessary to change the route of administration. There are multiple reasons to prefer Topical route like effectiveness, safety, bypass the first-pass metabolism, easy administration and organ dysfunction due to systemic adverse effects, but with these advantages there is a disadvantage like it delivers in adequate amount of drugs [5]. To avoid this disadvantage, encapsulate the drug in the different vesicles [6, 7]. These delivery systems are capable of carrying drug molecules and show its therapeutic effects at site of action. It never without interfere with the surrounding tissues and can cross hard layer of skin i. e. Stratum corneum [8-10].

Moreover, it not only delivers hydrophilic drugs but also it can deliver lipophilic drugs. The advantage of these vesicles that it has the same properties as skinny as it made from phospholipids, ultimately, it results in improving drug permeability through the skin layers, mostly stratum corneum layer, without barrier which

results in better therapeutic activity [11, 12]. But the therapeutic efficacy of drugs across the skin using first generation vesicular system (liposomes and niosomes) is less due to their stiff structure [13]. Thus, researchers are exploring next-generation vesicular system which are flexible in their structure, like transferosomes and ethosomes [14].

Ethosomes are vesicular drug carriers which have lipids and high concentration of ethanol in their structure. Because of high concentration of ethanol helps them to modify the highly compact structure of stratum corneum layer, which results in deep penetration of drugs into the tissue through skin [15, 16]. The high concentration of ethanol results in a net negative charge on the surface of ethosomes, which causes electrostatic repulsion which results in stability of vesicles [17]. Ethanol also responsible for high solubility of lipophilic drugs [18]. Ethosomes are less toxic, irritant and suitable for topical delivery of drugs [19]. Studies have also shown that the incorporation of vesicular carriers into another gel i. e. Carbopol gel can improve their stability and skin permeability [20]. This study, then, aims to formulate ethosomes and incorporate ethosomes vesicles into Carbopol 940 gel to improve skin permeation.

MATERIALS AND METHODS

Materials

Etodolac was procured from Yarrow Chem Ltd, Mumbai. Soya lecithin was procured from Modern chemicals, Nashik. Other chemicals and different solvents, including water were used of analytical grade and purchased from different suppliers.

Formulation of etodolac containing ethosomes

All ethosomes were prepared by using the thin-film hydration technique (Rotary Evaporation). First, oil phase prepared by

dissolving etodolac and lipids (Soya Phosphotidyl Choline and cholesterol) in ethanol and aqueous phase was a hydroethanolic solution. Both phases were separately sonicated at 60 °C for 30 min. It became homogenous. Then, this homogenous mixture was added in a round-bottomed flask and a thin lipid film was formed by removal of ethanol using a rotary evaporator (Rotary Vacuum Film Evaporator-Coslab-Model-CRE400) at 40 °C to obtain the thin lipid film after complete evaporation of the organic solvents. The film was hydrated with a phosphate buffer (pH 7.4) by rotation for 1hr. The vesicles were kept for swelling for 1 h. Both previous steps were carried out at room temperature. The resultant vesicles were sonicated for 30 min in a bath sonicator (Digital Ultrasonic Cleaner). The sonicated vesicles were stored at 4 °C [21, 22]. The composition of ethosomal vesicles is shown in the table 1.

Table 1: Formulation code and composition of preparation of Ethosomes

Formulation	Cholesterol (w/v)	SPC (% w/v)	Ethanol (% V/V)	Water (% V/V)
EE1	0.5	4	10	90
EE2	0.5	4	20	80
EE3	0.5	4	30	70
EE4	0.5	4	40	60
EE5	0.5	4	50	50
EE6	0.5	4	60	40

SPC: Soya Phosphatidylcholine

Preparation of ethosomal carbopol gel

In the first step, plain Carbopol gel was prepared by incorporating 1 g Carbopol 940 in 100 ml water at 50 °C by continuous stirring. Methyl paraben and propyl paraben were as preservatives. The Ethosomes equivalent to 2% of etodolac was mixed with mentioned Carbopol gel and simultaneously, one plain Carbopol gel also prepared with same concentration (2%). After 2 h swelling, triethanolamine was added to neutralize the prepared Carbopol 940 gel [23].
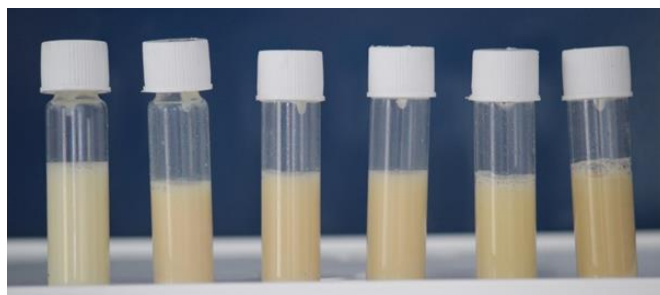

Fig. 1: Formulation batches of ethosomes

Optimization of prepared ethosomes

Optical microscopy

The prepared ethosomes were observed under light microscope, Metzer Biomedical Model VFM9003. This evaluation used to give an idea about the shape of the ethosomes [24].

Vesicle size, PDI and zeta potential

Vesicle size, PDI and zeta potential of etodolac ethosomes were determined by using Zetasizer (Diya Lab, Mumbai, India), and the values were obtained at 25 °C [25, 26].

Drug loading and entrapment efficiency

Ethosomes were centrifuged at 12000 rpm for 30 min (Remi, Cε, NT 2178GK). The absorbance of supernatant liquid was taken spectrophotometrically after separation from total mixture at 281 nm by using a UV spectrophotometer (UV-2450, Shimadzu, Japan) [27, 28]. The following formulas were used to calculate % EE and % DL.

$$\% \ EE = \frac{\text{A total Drug Conc.-Supernatant Drug Conc.}}{\text{Total Drug Conc.}} \times 100$$

$$\% \ DL = \frac{\text{A total Drug Conc.-Supernatant Drug Conc.}}{\text{Total Weight of Sample}} \times 100$$

Characterization of prepared ethosomal gel

The prepared ethosomes (EE1-EE6) batches incorporated in Carbopol gel and these gels evaluated for following characteristics.

The batches were labelled as EEC-1 to EEC-6, respectively, and the batch was contained only plain Carbopol gel was labelled as EC-0.

Physical appearance, homogeneity, clarity and colour

All the prepared gels (EEC-1 to EEC-6) were evaluated for appearance, homogeneity, colour and clarity [29].

Spreadability

Spreadability of gels was measured by using wooden block apparatus, on the wooden base, one glass slide was stuck and another glass slide was attached to the pulley, of which another end has weighed pan. The 2 gm of gel pressed between 2 slides for uniform thickness. The 100 gm of weight was removed, and times (seconds) require by movable slide getting separated from stationary slide were noted [30]. Spreadability was calculated as:

$$\text{Spreadability} = \frac{L \times W}{T}$$

Where,

L = Length of glass slide (cm),

W = Weight applied (g)

T = Time required for separation of slide (sec)

PH

The pH of the developed gels (EEC1-EEC6 and EC0) was determined in triplicate with pH meter (pH Cal Model). 1 gm gel was dissolved in

100 ml water after 2 h pH was measured [31]. Average pH is reported in table 6.

Rheological study

For viscosity determination a Brookfield viscometer LV DV-II+PRO model was used. T-95 Bar spindle was used and whole study done at room temperature. 50 g of each ethosomes sample was taken in was dipped. The Readings for viscosity were taken at 10, 20, 30, 40, 50 rpm at room temperature. Spindle was rotated for 5 minute for every reading. Evaluations were done in triplicates, and mean±SD viscosities were calculated [32].

Drug content

An accurately 1 g formulated gel was dispersed in a specific volume of phosphate buffer with vigorous shaking. The dispersion was then filtered and drug amount in the filtrate was analysed spectrophotometrically at λmax equal to 281 nm [33]. Equation was used to calculate drug content (%) as follows:

$$\text{Drug Content (\%)} = \frac{\text{Actual Amount of Etodolac}}{\text{Theoretical Amount of Etodolac}} \times 100$$

In vitro drug release study

A soaked cellophane membrane was placed between donor and receptor compartments of the Franz diffusion cell. Ethosomal gel was applied to the cellophane membrane and phosphate buffer was kept in receiver compartment and it stirred continuously with a magnetic bar at 50 rpm. 1 ml sample was taken at 0, 1, 2, 3, 4, 5, 6, and 12 h intervals, volume of receiver compartment replaced with 1 ml of buffer solution, and the sample was analysed by using Ultra-Violetviolet (UV) spectrophotometer at 281 nm. The percentage of drug released was then calculated [34].

Evaluation of optimized formulations

Vesicle morphology

The optimized ethosomes (EE4) formulation was characterized morphologically using SEM and TEM at Cochin, SAIF, India [35-37].

Ex vivo permeation study

For this technique, abdominal skin of albino rats was used. This study was carried out at 37± 2 °C. The Franz diffusion cell was placed on a magnetic stirrer with constant heating equipment. The gels were placed in receptor compartment. Aliquot samples of 1 ml were withdrawn at the regular intervals and replaced with the same volume of fresh buffer. Amount of drug diffused through the membrane was measured by using U. V. spectrophotometer at a wavelength of 281 nm against phosphate buffer (pH 7.4 saline) as the blank. The results were replicated three times as mean±SD [38].

Irritancy test

The skin irritation potential of etodolac-loaded ethosomal gel (EEC-4) was assessed with permission of ethical committee (Biotox/IAEC/03/2024/RP-14) by using rats from species Wistar Albino (200-300 g) sourced from Biotox Laboratory, Naskik, India. The selected rats were acclimatized for one week before the study began. Approximately 4 h prior to the experiment, the dorsal surface of each rat was shaved. Four groups were prepared by dividing rats. Each group contains 3 rats.

Group I-Received plain Carbopol gel (without Etodolac)

Group II-Received Carbopol gel with Etodolac

Group III-Received Ethosomes with Etodolac

Group IV-Received ethosomal gel with Etodolac.

The formulations (100 mg containing 2%) were applied onto a shaved skin area of 1 cm². After application, the rats observed at 24, 48, and 72 h for irritation. The severity of irritation was scored as follows: 0 for no erythema or edema, 1 for slight erythema or edema, 2 for moderate erythema or edema, and 3 for severe erythema or edema [39].

In vivo study (Anti-inflammatory activity)

Anti-inflammatory study of optimised ethosomal gel (EEC4) formulations was evaluated inducing carrageenan into hind paw of rats with permission of ethical committee (Biotox/IAEC/03/2024/RP-14). For this, 24 rats were divided into four groups. Each group had 6 rats. Formulations were topically applied 20 mg/kg of dose to the surface of the left hind paw of the rats and gently massaged before 30 min of intraplanar injection of 100 μl** of carrageenan solution (1% w/v) into the left hind paw. The digital vernier calliper was used to measure the difference in volume between the right and left paws (Baker, DDS Series) at ½, 1, 2, 4, 6, and 8 h after the carrageenan injection. The percentage inhibition of edema was calculated by using the following formula,

$$\text{Inhibition of edema} = \left(1 - \left(\frac{Vt}{Vc}\right)\right) \times 100$$

Where, Vt: the paw edema volume of the groups treated with the formulations in rats,

Vc: the mean edema volume of the control group.

In vivo experimental group design

Group 1: Control group, No treatment, (n = 6),

Group 2: Traditional group Etodolac Carbopol gel, (n = 6),

Group 3: Conventional group Marketed etodolac gel (n = 6),

Group 4: Novel group Etodolac loaded ethosomal (n = 6). 40, 41

FTIR study

The FTIR (Fourier Transform Infra-red) of etodolac, Carbopol gel without drug, etodolac Carbopol gel, optimized etodolac ethosomes and optimized etodolac ethosomal Carbopol gel were recorded by FTIR spectroscopy (Thermo Nicolet IS50, SAIF, Cochin, India) [42].

Differential scanning colorimetry study

Etodolac, drug-free Carbopol gel, etodolac Carbopol gel, optimized etodolac ethosomes and optimized etodolac ethosomal Carbopol gel, were analysed by using DSC (Model: DSC 204 F1 PhoenikNetezch, SAIF, Cochin, India) [43].

X-ray diffraction (XRD)

XRD for Etodolac, drug-free Carbopol gel, etodolac Carbopol gel, optimized etodolac ethosomes and optimized etodolac ethosomal Carbopol gel were analysed using X-ray diffractometer (Bruker D8 Advance, SAIF, Cochin, India) [44].

UV analysis

Absorption spectrum of etodolac, drug-free Carbopol gel, etodolac Carbopol gel, optimized etodolac ethosomes and optimized etodolac ethosomal Carbopol gel were analysed in the wavelength range of 200–400 nm to determine its maximum absorption wavelength. A standard stock solution was prepared by measuring 20 mg of formulations and dissolved in 20 ml of methanol [45].

Stability study

The optimised ethosomal formulations were stored at 5 °C±3 °C; 25±2 °C, 60±5%RH; (40±2 °C, 75±5%RH) in stability chamber (Thermolab ISO 000090S) for 6 mo. Then stability of formulations was evaluated by determining % entrapment efficiency at respective temperature [46].

RESULTS AND DISCUSSION

Optimization of prepared transferosomes

Optical microscopy

Optical microscopy shows small spherical vesicles of Ethosomes for batches EE1 to EE5 at the magnification power of 45X. But, in Ethosomes EE5, the vesicles quantity is less as compared to other previous four formulations and in EE6 clearly damaged vesicles were observed, it may due to increased concentration of alcohol.
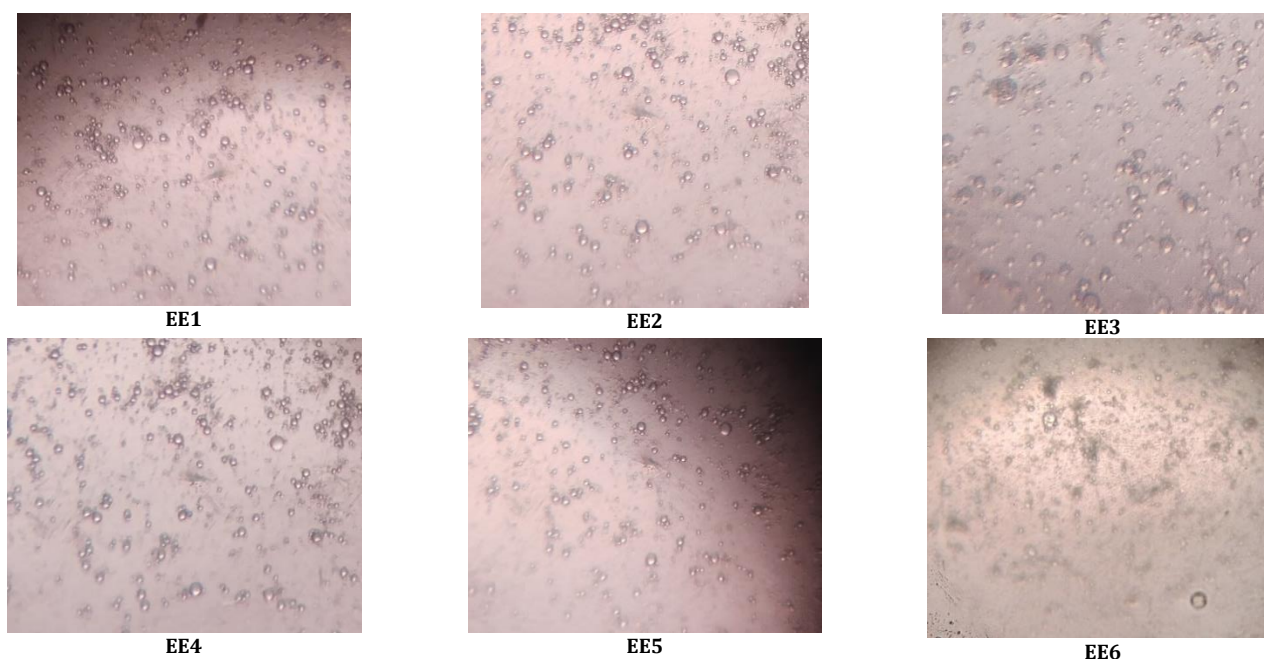
Fig. 2: Optical microscopy of ethosomes (45x)

Vesicle size, PDI and zeta potential

The vesicles sizes ≥600 nm cannot penetrate to deeper into skin layers, but vesicle sizes ≤300 nm can penetrate deeply into the skin [47]. Ethosomes vesicles were found to be in the range of 166.66 nm to 848.97 nm. In case of vesicle size, it was noticed that as the concentration of ethanol increases, the size of the vesicle also increases, which contrast observation than the previous study. PDI found in the range of 0.244 to 0.301 [48]. It indicates that formulated ethosomes have a uniform particle size as it is numerically near to number 0.

Zeta potential indicates stability of vesicles. A value of zeta potential of ±30 mV charge indicates that the vesicles have repulsion are more which results in stability of formulations. Ethanol is responsible for negative charge to the surface of ethosomes. The repulsive force is not only dependent only on the zeta potential but also on dispersion. Classification for degree of stability and corresponding zeta potential shown in table 2. It shows that a zeta potential of more than ±30 mV provides good stability and obtains excellent stability when the ZP reaches toward ±60 mV [49]. The Vesicle size, PDI, and zeta potential values of optimized formulations are presented in table 3 and fig. 3 and 4.

Table 2: Stability of nanofluids at different zeta potentials

Zeta potential (mV)	Stability degree
0	Slight to no stability
15	Less stability with light settling
30	Moderate stability
45	Decent stability with possible settling
60	Excellent stability with lesser possibility of settling

Table 3: Vesicle size, PDI, and zeta potential of ethosomes

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
EE1	166.66±7.99	0.271±0.01	-34.07±0.74
EE2	185.59±04.05	0.244±0.01	-47.77±0.57
EE3	249.00±20.70	0.251±0.01	-37.47±4.22
EE4	287.17±10.68	0.265±0.01	-64.53±0.39
EE5	372.10±09.39	0.270±0.01	-45.73±0.66
EE6	848.97±40.42	0.301±0.03	-18.50±0.78

Data are expressed as mean±SD (n = 3)

Drug loading and entrapment efficiency

Drug loading (%) indicates the quantity of drug present in net weight of the formulation. Entrapment efficiency (%) indicates the only quantity of drug entrapped in vesicles compared to net weight of the drug added in the formulation. Entrapment efficiency (%) for all formulations ranged between 53.15±0.64 to 89.35±1.19 and Drug loading (%) ranged between 18.96±0.18 to 43.51±0.47. Results are displayed in table 4.

Characterization of prepared ethosomal carbopol gel

Physical appearance and homogeneity, clarity and colour

All ethosomal Carbopol gel (EEC1-EEC6) and plain Carbopol gel without ethosomes (EC0) show smooth, clear and homogeneous visual appearance. All ethosomal formulations appeared opaque and off white in colour while EC0 appearance was transparent and colorless. As the concentration of alcohol was increased, the yellowish coloration also increases (fig. 1). Results are displayed in table 5.
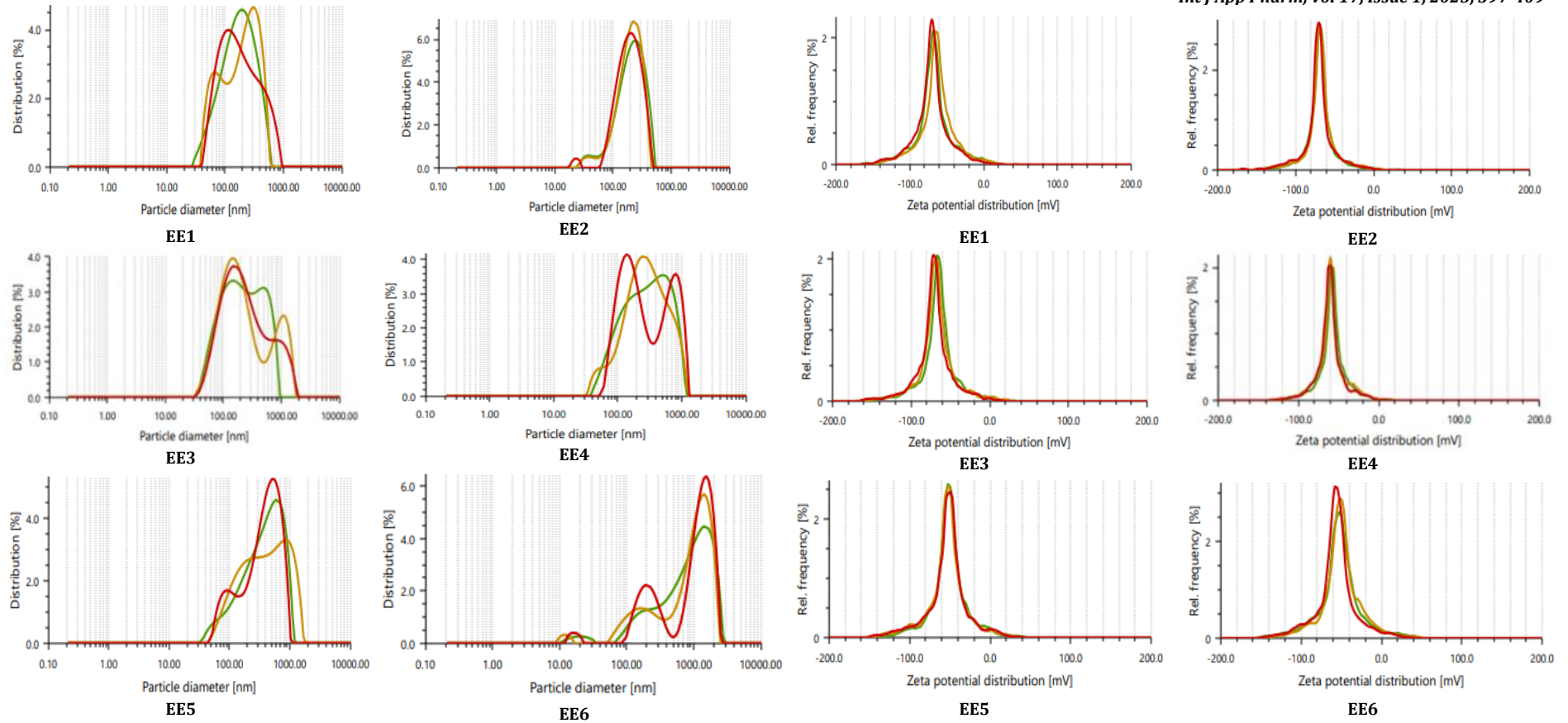
Fig. 3: Particle size distribution and PDI of Fig. 4: Zeta potential of ethosomes ethosomes

Table 4: Entraptment efficiency and drug loading

Formulation	EE% (±SD)	D.L.%(±SD)
EE1	74.70±0.90	43.51±0.47
EE2	77.84±1.33	30.92±0.81
EE3	80.96±0.66	29.40±0.31
EE4	89.35±1.19	36.22±0.55
EE5	56.38±0.47	18.96±0.18
EE6	53.15±0.64	23.20±0.25

Data are expressed as mean±SD (n = 3)

Table 5: Physical appearance, texture, homogeneity, clarity, colour, of ethosomal gel and plain carbopol gel

Formulation	Physical appearance	Texture	Homogeneity	Clarity	Colour
EE1	Opaque	Smooth	Homogenous	Clear	Off white
EE2	Opaque	Smooth	Homogenous	Clear	Off white
EE3	Opaque	Smooth	Homogenous	Clear	Off white
EE4	Opaque	Smooth	Homogenous	Clear	Off white
EE5	Opaque	Smooth	Homogenous	Clear	Off white
EE6	Opaque	Smooth	Homogenous	Clear	Off white
EC0	Transparent	Smooth	Homogenous	Clear	Colourless

Spreadability

Spreadability evaluation is important to indicate contact time of formulation with the site of action, effortless application to the site and easy and proper extrusion of formulation from container. All ethosomal gel shows good results regarding spreadbility. Plain Carbopol gel shows a spreadability 5.76±013. When ethosomes were added into plain Carbopol gel, spreadability increases. The concentration of ethanol is directly proportional to spreadability, and results are shown in table 6.

PH

pH of the formulated gels were obtained from 6 to 7.05, which is near to the neutral pH and determined pH shown in table 6. Therefore, these gels are suitable for safe and non-irritant topical application as it is compatible with human skin.

Rheological study

The viscosity study indicates that the concentration of ethanol increases in ethosomes decreases the viscosity of plain Carbopol gel.

This may be due to ethanol decreases the interactions between the molecules present in Carbopol gel and leading to their thinning effect. Rheological behavior of all formulations shown in fig. 5.

Drug content

The drug content of all formulations obtained in a range of 96.68 to 102.25 %, which confirmed that % drug content was within the limit of United States Pharmacopeia (100±10 %), which indicates uniform distribution of drugs in formulation (except EE2, which have drug content (%) is 87.92). This study confirmed that procedures and protocols followed in current research are suitable for formulation.

***In vitro* release study**

The results of Cumulative drug release flux. Permeability coefficient and enhancement ratio of Ethosomal Gel were determined and displayed in table 7 and fig. 6. It shows optimized results for EEC4 formulations. All other ethosomal formulations also show better results than plain Carbopol gel (EC0). Fig. 6 gives a clear idea about enhanced diffusion of etodolac drug from ethosomal formulations as compared to plain etodolac Carbopol gel.

Table 6: Spreadability, pH and drug content of ethosomal gel and plain carbopol gel

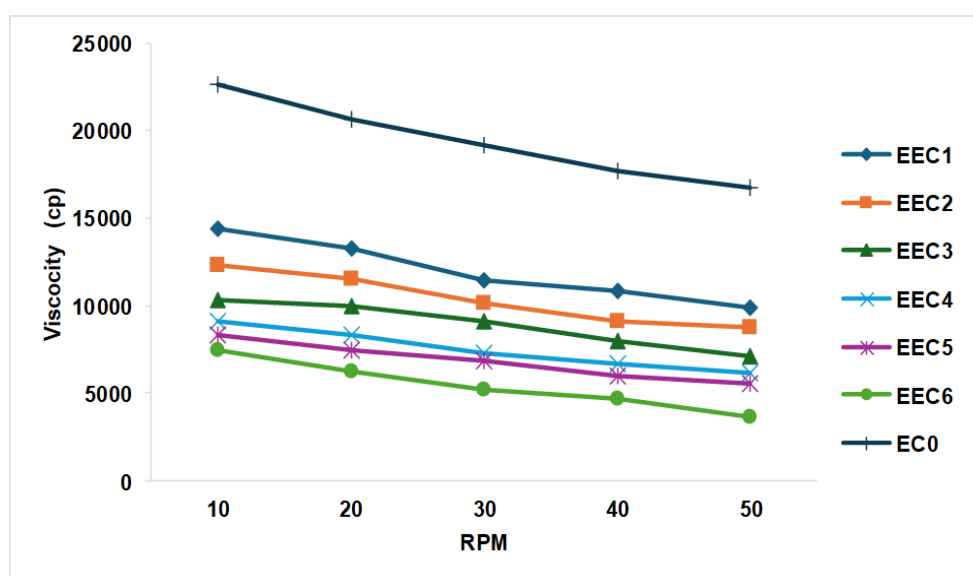| Formulation	Spreadability g. cm/sec	pH	Drug content (%)
EEC1	07.89±0.16	6.45±0.09	98.06±0.24
EEC2	12.88±0.34	6.13±0.05	87.92±0.17
EEC3	14.67±0.18	6.78±0.07	102.25±0.30
EEC4	16.09±0.33	6.00±0.05	99.67±0.35
EEC5	20.41±0.30	6.90±0.04	98.89±0.53
EEC6	24.60±0.38	6.22±0.02	96.68±0.58
EC0	5.76±013	7.05±0.02	98.43±0.58

Data are expressed as mean±SD (n = 3)

Fig. 5: Rheological study of ethosomal carbopol gel, data is expressed as mean of triplicates.
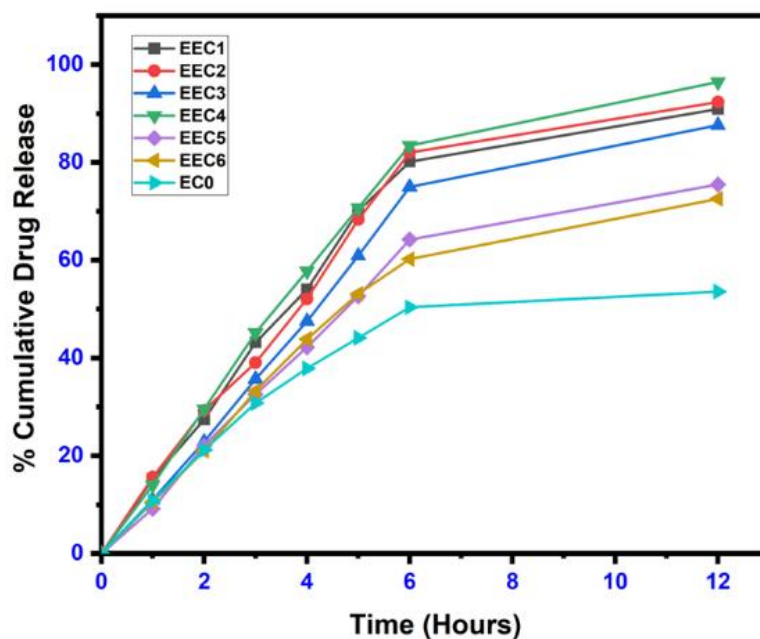
Fig. 6: Cumulative percentage of drug released (%)

Table 7: Cumulative drug release, flux, and permeability coefficient and enhancement ratio of ethosomal gel

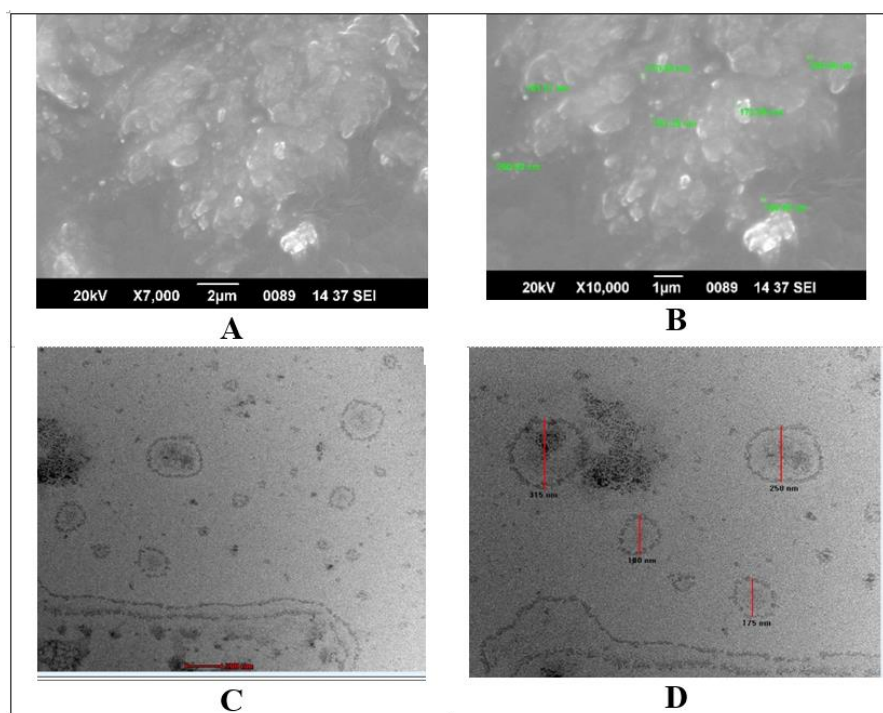| Code	Q_{12h} (µg/cm2)	Flux (µg/cm^2/h)	Permeability coefficient X 10^{-4} (Kp) (cm^2/h)	Enhancement ratio (ER)
EC0	107.12±11.14	8.83±0.94	4.42±0.47	1
EEC1	181.83±26.62	15.49±2.33	7.75±0.11	1.75
EEC2	184.64±30.76	15.76±2.42	7.88±0.12	1.78
EEC3	175.19±18.28	15.26±1.7	7.63±0.85	1.73
EEC4	192.95±18.44	16.45±1.44	8.23±0.72	1.86
EEC5	150.95±14.02	13.01±1.16	6.51±0.58	1.47
EEC6	145.09±12.12	12.34±1.06	6.17±0.53	1.40

Data are expressed as mean±SD (n = 3)

Fig. 7: SEM (A and B) and TEM (C and D) images of optimized ethosomes

Evaluation of optimized formulations

Based on results obtained from above evaluation, formulation EE4 and EEC4 were selected for further evaluation. Specially, results obtained from entrapment evaluation, particle size, zeta potential, *In vitro* diffusion study etc.

Vesicle morphology

Morphology of Ethosomal vesicles (EE4) was studied by using two techniques namely SEM and TEM. These methods indicated that vesicles were present in the nanometer range. Ethosomes vesicles have loose aggregates and smooth surfaces. TEM images (fig. 7C and 7D) shows almost spherical vesicles with a clear and dark outline. Outline and core of vesicles confirmed vesicular characteristics indicating the integrity of closed structures with drug entrapped in vesicles.

Ex vivo permeation study

The linear curves and their corresponding equations for kinetics models with correlation coefficient (r^2) for etodolac Carbopol gel, Marketed gel and Ethosomal gel using different kinetics are shown in fig. 8 and table 8. The r^2 values were found higher for the first-order model and Korsmeyer-Peppas model for all formulations. The release profile of with Korsmeyer-Peppas equation revealed the value of "n" predicts the mechanism of the drug release. If "n" value<0.45 indicates Fickian diffusion, 0.45 to 0.89 denotes non-Fickian transport, n = 0.89 suggests Case II transport, and n>0.89 indicates super Case II transport for a cylindrical system [50]. Super Case-II transport is associated with stresses and transition present in hydrophilic glassy polymers which swell in presence of water or different biological fluids. These three formulations are shown value>0.89, it means a release is super Case II transport [51].

Table 8: Kinetics model data of ethosomal gel formulation

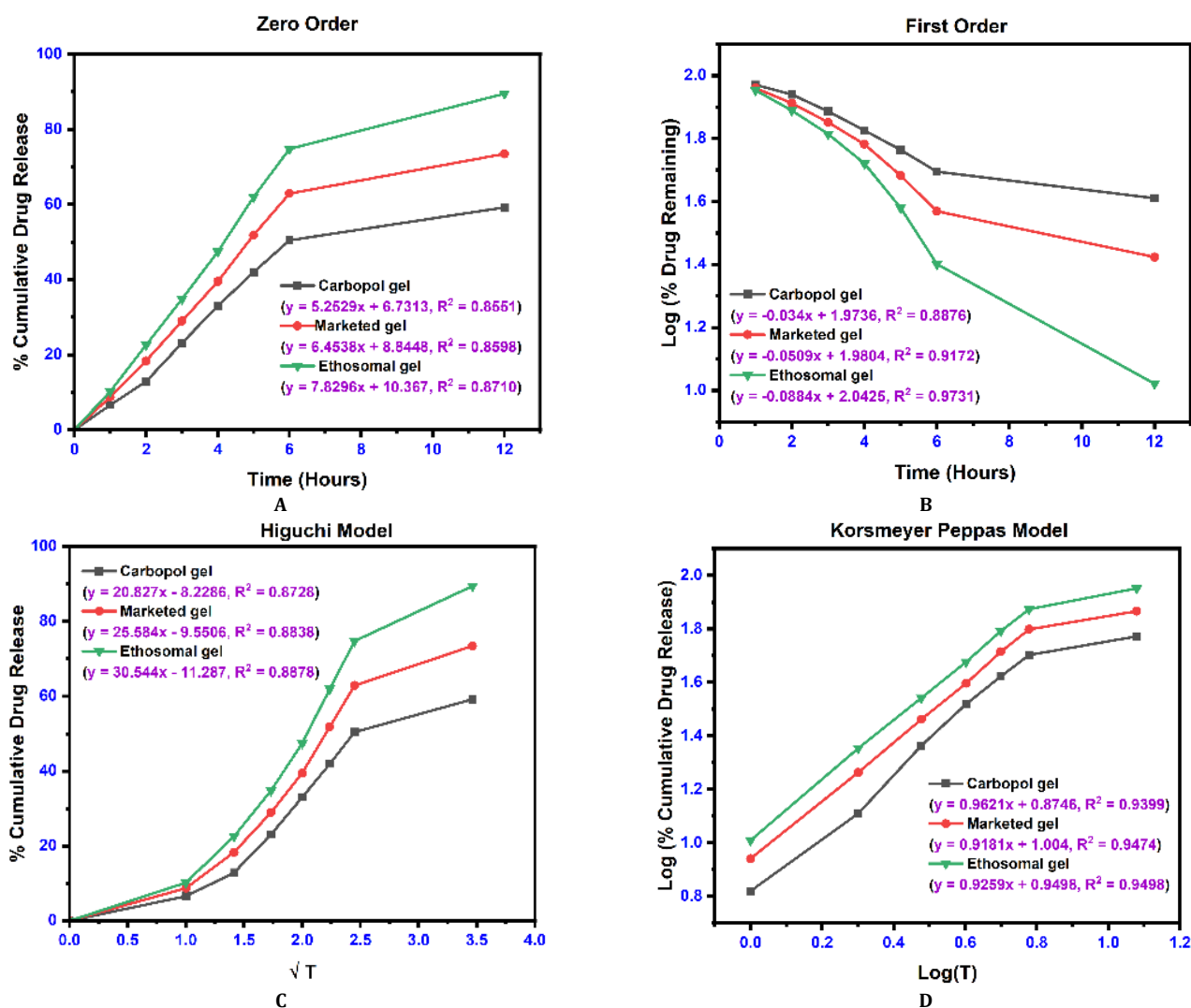| Formulation	Zero-order	First order	Higuchi's model	Korsmeyers peppas equation	
	r^2	r^2	r^2	r^2	n
Plain carbopol gel	0.8551	0.8876	0.8728	0.9399	0.9621
Marketed formulation	0.8598	0.9172	0.8838	0.9474	0.9181
Ethosomal gel (EEC4)	0.8710	0.9731	0.8878	0.9498	0.9259

Fig. 8: The release kinetics model fitting curves: (a) zero-order release kinetics; (b) first-order kinetic model; (c) Higuchi model; and (d) Korsmeyerspeppas model

Irritancy test

All gels had no edema, erythema or any irritation on rat skin. These results indicated that topical application of these gels is safe to use and no harmful signs on the skin.

In vivo test

In vivo for anti-inflammatory activity of (EEC4) formulation showed 36.58% reduction significantly (<0.05) of carrageenan-induced paw edema at the end of 8 hr. This result was superior as compared to Plain Etodolac Carbopol gel and Marketed formulation. These formulations show 20.10 % inhibition and 24.92 % inhibition, respectively. Edema produced after administration of carrageenan was observed in fig. 9. % Inhibition of rat edema graphically shown in fig. 10.

FTIR study

FTIR graph of pure Etodolac with all other formulations are summarised in fig. 11. Etodolac and all formulations were show characteristics peaks in the same range because Etodolac drug and carbapol 940 have same functional groups. FTIR spectrum of etodolac revealed a characteristic sharp peak at 3344.07 (-NH stretching), 2971.33 (-OH stretching), 1745.44 (-C=O stretching), 1412.16 (C-H stretching), 1034.08 (C-O-C stretching) and Carbopol 940 show characteristics peaks at 3000-2950 (O-H stretching vibration) intramolecular H-bonded, 1750-1700 (C=O group of acids (C=O stretching vibration) 1450-1400 (C-O stretching vibrations) 1250-1200 (C-O-C stretching vibration) and 850-800 (C-H out of plane bending vibration) [52].
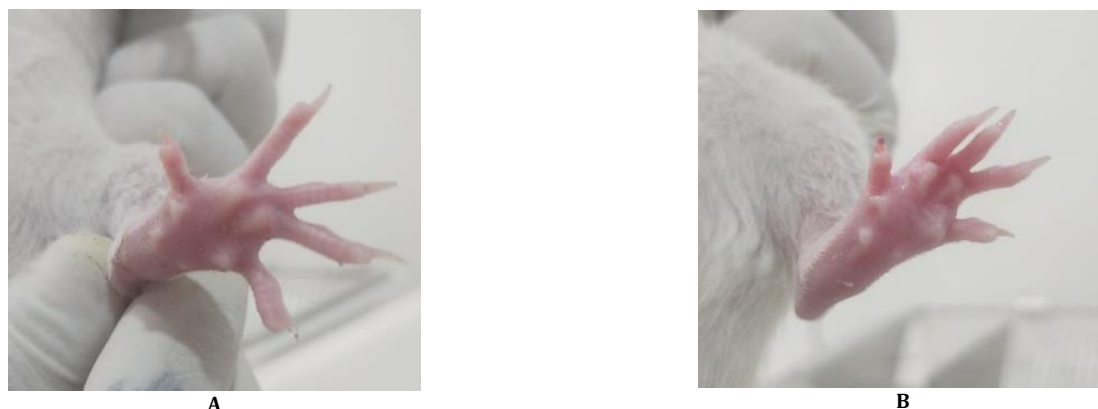

Fig. 9: Picture of left paw of rat A. Before carrageenan injection B. After carrageenan injection
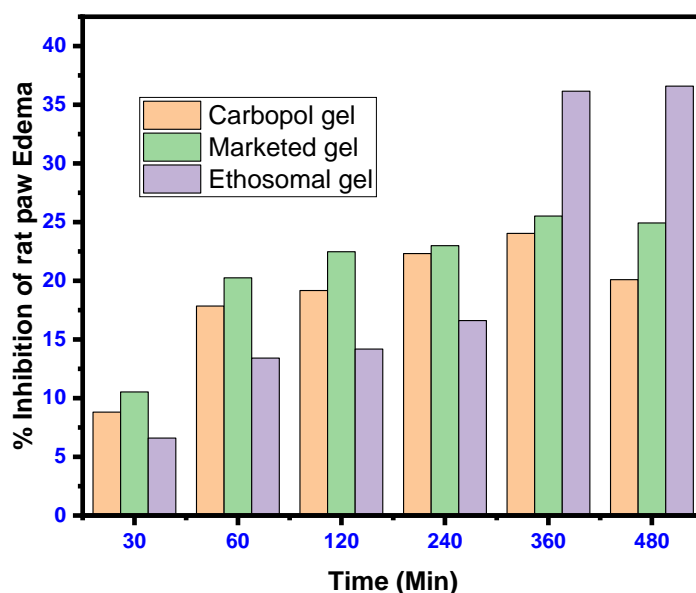

Fig. 10: % Inhibition of rat paw edema, data are expressed as mean

DSC study

The DSC thermogram of etodolac drug showed a sharp endothermic peak at 154.5 °C temperature, which indicates crystalline nature of etodolac pure drug. The thermogram of etodolac transferosomes showed transition temperature 107.1 C, whereas as plain Carbopol gel (without drug) shows at 96.2 °C,

etodolac Carbopol gel showed 138.2 °C and etodolac Ethosomal Carbopol gel shows at 120.7 °C. The lower transition temperatures of gels indicate fluidity. Sharpness of peak was very law in fig. 12-D because of double gel system one is ethosomes and another was Carbopol gel. These results suggest that in formulation, the crystalline nature of etodolac was changed from crystalline state to amorphous state [53].
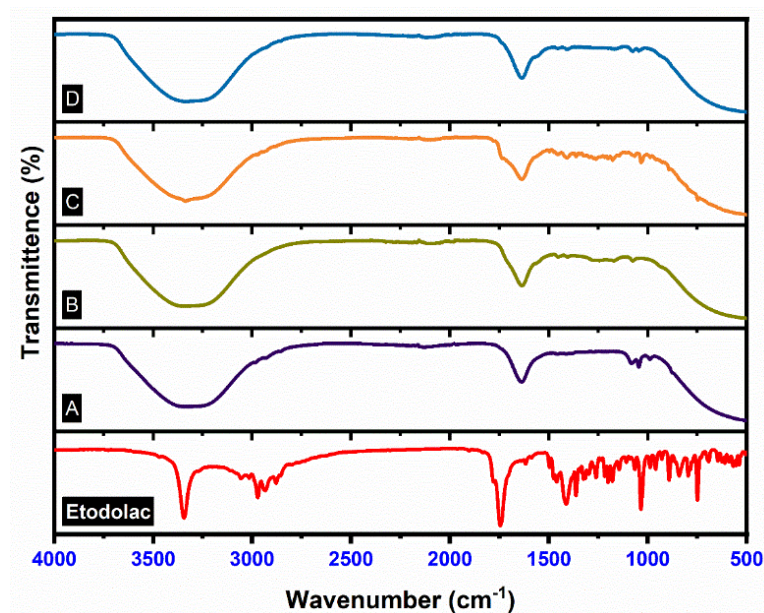
Fig. 11: FTIR spectra of etodolac; A: Etodolac transferosome, B: Plain carbopol gel, C: Etodolac carbopol gel, D: Etodolac ethosomal gel
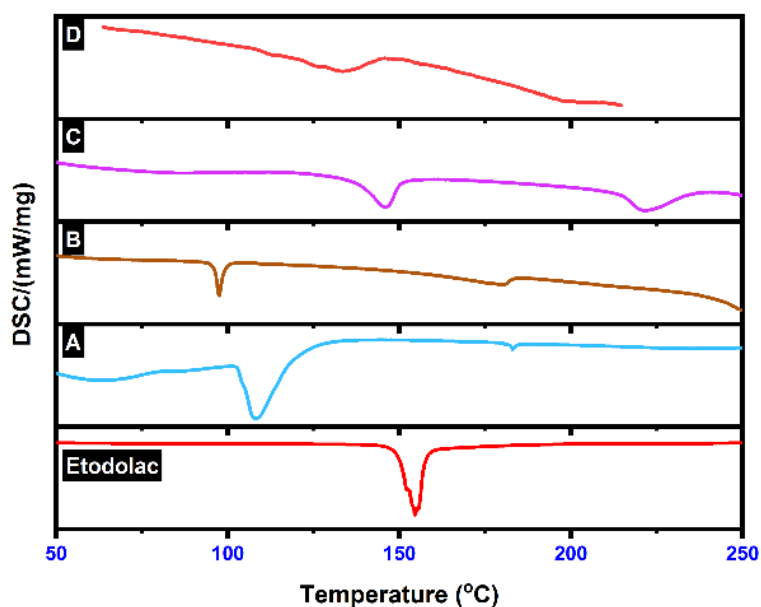

Fig. 12: DSC graph of etodolac; A: Etodolac transferosome, B: Plain carbopol gel, C: Etodolac carbopol gel, D: Etodolac ethosomal gel

XRD analysis

Fig. 14 shows the changes in the XRD peaks of etodolac drug after encapsulation into the ethosomes and plain Carbopol gel. Pure etodolac showed characteristic crystalline peaks, indicating the crystalline nature of the pure drug. Etodolac ethosomes (fig. 13-A) showed low-intensity characteristic peaks as compared to plane pure drug. The change in peak height and intensity confirms that etodolac was soluble in the used lipids. The formation of vesicles shows that the etodolac drug was entrapped in the lipid of vesicles and lost its crystalline nature. The absence of a sharp etodolac peak may be due to the Etodolac-phospholipid complex formation [54].

UV analysis

The Maximum absorption spectra of pure etodolac, plain Carbopol gel, etodolac Carbopol gel, and Etodolac ethosomal gel are shown in fig. 14. All formulations (fig. 14-A, C, D) shows the same spectra because of the presence of the Etodolac drug. Only fig. 14-B shows different spectra may be due to the absence of the drug.

Stability study

Stability study of the optimized ethosomal gel (EEC4) was carried out at different conditions like 5 °C±3 °C, 25 °C±2 °C, 60±5% RH and 40±2 °C, 75±5% RH for 180 d and results are shown in table 9. It indicates that the formulation has been shown different stability behaviour related percentage drug entrapment efficiency, it reduced significantly when this formulation was stored at 40±2 °C, 75±5%RH at more stable at 5 °C±3 °C. These results show that storage condition should maintain for ethosomal formulation to achieve stability.
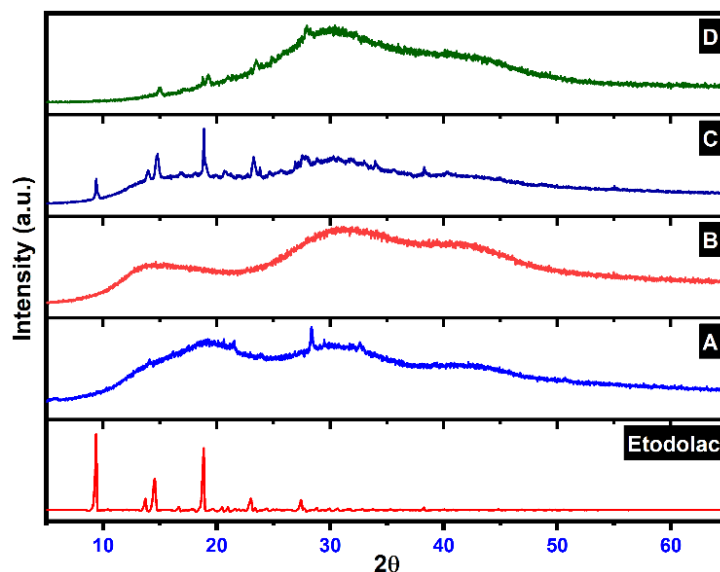
Fig. 13: XRD graph of etodolac; A: Etodolac transferosome, B: Plain carbopol gel, C: Etodolac carbopol gel, D: Etodolac ethosomal gel
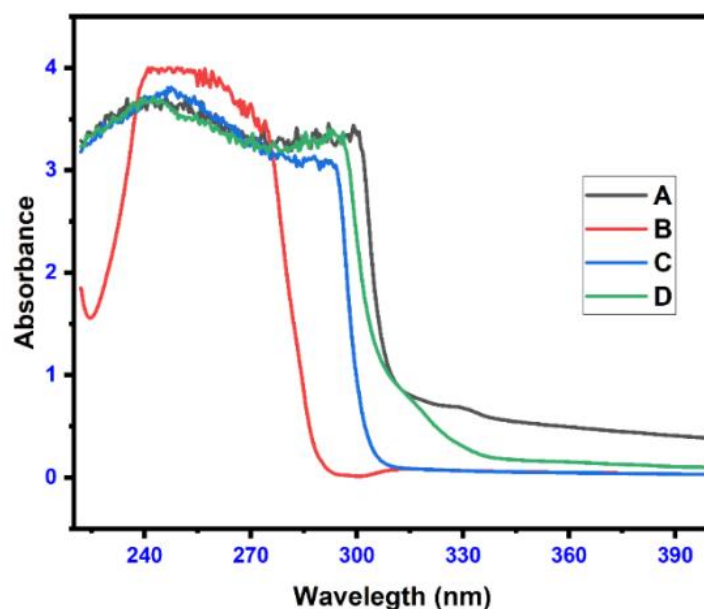

Fig. 14: Maximum absorption spectra of etodolac; A: Etodolac transfer some, B: Plain carbopol gel, C: Etodolac carbopol gel, D: Etodolac ethosomal gel

Table 9: Stability study of optimised transfersomal gel (EEC4)

Months	0	1	3	6
Storage	% Entraptment Efficiency			
5 °C±3 °C	84.51±0.42	81.41±0.60	78.60±0.68	75.68±0.53
25±2 °C, 60±5%RH	84.51±0.42	76.96±0.89	70.84±1.74	66.44±0.71
40±2 °C, 75±5%RH	84.51±0.42	65.62±0.90	66.44±0.71	55.96±0.79

Data are expressed as mean±SD (n = 3)

CONCLUSION

This research suggests that Ethosomes are an effective drug delivery system through topical route with less side effect. The formulations of ethosomes with different ratios of alcohol (EE1-EE6) were successfully manufactured by thin-film hydration method. The results obtained from vesicle size, zeta potential, polydispersity index (PDI) and entrapment efficiency of etodolac ethosomes were in acceptable ranges. Furthermore, ethosomal formulations were incorporated into carbopol-940 gels. These ethosomal gels were subjected to colour, pH, spreadability, viscosity, homogeneity, and *In vitro* drug release for different models. The optimized formulation (EE4) was further evaluated for SEM, TEM, *Ex vivo*, Irritancy, *In vivo* and different analytical techniques. Finally, concluded that Etodolac

Ethosomal gel could become stable at specific condition successful topical dosage form for symptomatic relief in Rheumatoid Arthritis.

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AUTHORS CONTRIBUTIONS

Ashwini A. Bachhav-Conducted the experiments, performed data analysis, and contributed to the interpretation of results. Dr. Prashant L. Pingale-Conceptualized the study, designed the experiments, and wrote the initial draft of the manuscript. Dr. Chadrashekhar D. Upasani- oversaw the statistical analysis, helped with manuscript editing, and supervised the research project.

CONFLICT OF INTERESTS

Declared none

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