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PHARMACEUTICAL DEVELOPMENT OF ETODOLAC TRANSFERSOMAL GEL FOR TOPICAL DRUG DELIVERY SYSTEM IN RHEUMATOID ARTHRITIS

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ABSTRACT

Background: Transferosomes provide delivery of the drug into systemic circulation via the skin as a topical delivery system. So, this study started with the objective of formulating Etodolac transfersomal gel to enhance its skin permeation. **Methodology:** A total of nine transferosomes (ET-1 to ET-9) containing lecithin, different grades of span and tween, were successfully prepared using a rotary film evaporator. **Results and Discussion:** After primary evaluation, results were as particle sizes ranged from 222 to 421 nm, zeta potential shows results from –18.50 to –62.53 mV with PDI values 0.254 to 0.303, and the entrapment efficiency (EE%) of Etodolac in the transferosomes ranged from 54.15% to 80.25%. Additionally, the transfersomes formulations were included in carbopol 940 gels (ETC-1 to ETC-9 and EC-0 without transferosomes) and assessed for various characteristics like color, pH, homogeneity, spreadability, viscosity, and *in vitro* drug release study. Optimized formulation (ET4 and ETC4) underwent further analysis using SEM, TEM, DSC, FTIR, XRD, *ex vivo* skin permeation, skin irritation and *in vivo* studies. The in vivo results were compared. % edema inhibition maximum was observed with optimized transfersomal gel formulation (ETC4) as compared to the marketed formulation and plain Carbopol gel when the study was completed after 8 hrs. **Conclusion:** After this research, it is suggested that Etodolac Transfersomal gel (ETC4) can be considered as an alternate drug carriers system for topical delivery and it could be used to treat Rheumatoid Arthritis.

INTRODUCTION

The skin is the body's largest organ, accounting for approximately 16% of an adult's total body weight, and it is used as the site of application for topical routes. It mainly consists of the epidermis, dermis, subcutaneous layer, and auxiliary structures like hair follicles and sebaceous glands. Skin is a natural barrier, continuously protecting against harmful stimuli such as allergens, UV radiation, pathogens, and irritants [1,2]. The skin is a vital connection between the body's internal and external environments [3]. The stratum corneum layer primarily permits drugs by passive diffusion. But it should pass with

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certain conditions like physical and chemical characteristics like its molecular weight should be <500 Da, high lipophilic, and a low melting point near about <250°C, log P 1 to 5 [4]. Due to its unique properties, the skin presents an appealing alternative route for drug administration, with one significant method being Topical and transdermal Delivery Systems [5]. Both systems provide an excellent alternative for oral administration and hypodermic injections, popular and widely used dosage forms. However, both have some side effects, which can be avoided by using the topical route. This route bypasses the potentially harmful digestive and metabolic effects associated with oral delivery, such as enzymatic digestion, gastric emptying time, acidic pH of (Gastrointestinal tract) GIT, and the first-pass impact due to the liver. Furthermore, the topical route is beneficial for patients who are unconscious or nauseated, as it bypasses the gastrointestinal tract. Compared to injection delivery, Topical Drug Delivery Systems avoid pain, bruising, and bleeding; because of all these advantages, the topical route enhances patient acceptance and compliance. They also eliminate the risk of disease transmission and accidental injury due to needles and reduce the generation of hazardous medical waste generation like syringes, saline bottles, etc. [6]. For topical delivery, one of the best approaches is Nano vectors.

Nano vectors are superior in the field of dermal delivery for two main reasons: firstly, their general non-toxicity, and secondly, their ability to control drug bioavailability. With this, they can also serve as penetration enhancers, give a slow and uniform release of drugs through the skin, and prolong drug residence time in the stratum corneum and the epidermis. They have less systemic absorption, which is essential for topical delivery [7]. Among the numerous existing systems, we will selectively highlight the most advanced and successful vectors in topical delivery, i.e., vesicular systems [8]. Vesicular drug delivery systems offer significant advantages over other drug delivery methods. Some are listed as colloidal particulate vesicles that function as drug reservoirs, adjusting the release rate through modifications to their composition or surface properties. Traditional liposomes and niosomes are examples of these vesicles. However, many liposomes are reported to face stability issues and high costs. These stability problems include leakage of drugs, changes in the size of vesicles during storage, and chemical degradation of lipid components [9]. These problems could be overcome by using new vesicular systems like transferosomes, ethosomes, Proniosome, cubosomes, etc. [10].

When liposomes get modified with edge activators into their lipid bilayer membranes, we get Transferosomes with more advantageous properties, like reducing epidermis membrane stiffness by making them flexible. Because of this, vesicle rupturing can be avoided during drug delivery through the stratum corneum, and this is helpful for the transport of drugs from the epidermis to inner tissues. Transfersomes can deform (reduce their vesicle size) and reform when they pass through pores that typically confine other vesicular drug delivery systems. Because of all these reasons, transfersomes can enhance the in vitro and in vivo topical delivery of most drugs, achieving a therapeutic level of drug comparable to other delivery, including subcutaneous injections[11].

Etodolac is a (Nonsteroidal anti-inflammatory drug) NSAID selective cyclo-oxygenase-2 inhibitor drug. Etodolac treats symptoms like inflammation, swelling, stiffness, and joint pain, mainly in rheumatoid arthritis and osteoarthritis. Still, whenever it is taken by oral route, it causes severe side effects, which may include swelling of the face, fingers, feet, or lower legs, severe stomach pain, black, tarry stools, vomiting of blood or material that looks like coffee grounds, unusual weight gain, yellow skin or eyes, decreased urination, unusual bleeding or bruising, or skin rash. Also, signs of severe heart problems could occur, including chest pain or tightness, fast or irregular heartbeat, unusual flushing or warmth of skin, weakness, or slurring of speech.

To prevent these complications, an alternative route of administration is necessary. Maintaining a uniform concentration of Etodolac over a long period in the blood can be done using a topical dosage form [12]. For this study, spans and tweens were selected as Edge activators. These surfactants have many advantages: physical stabilizing agents and effects on the permeability characteristics of several biological membranes; they can enhance the skin penetration of other compounds in the formulation. Moreover, they have long been recognized as those with the least toxicity and irritant potential [13].

Rheumatoid Arthritis (RA) is a type of autoimmune disease. It is a chronic disease. Inflammation of the synovial membrane destroys articular cartilage, resulting in bone deformities [14]. RA has symptoms like warmth, swelling, and tenderness in the joints, which loss of motion and grip strength [15]. According to WHO, RA mainly occurs between the ages of 20 and 40,

adversely affecting people's quality of life. Recent epidemiological analyses indicate that the occurrence of RA in most of the countries is approximately 0.3–1%, which is more common in women (4%) than in men (2%). Because of this disease, 50% of patients lost employment within 10 years of RA onset [16]. A survey shows that annually, 41 people are diagnosed with RA out of 1,00,000, and it is most common in Americans[17]. The present study prepared transferosomes using the rotary evaporator technique (Thin film hydration) and different edge activators (Tweens and spans). After optimization, it was incorporated into Carbopol 940 gel. This transfersomal gel was evaluated for various parameters.

MATERIAL AND METHODS

Material

Etodolac was purchased from Yarrow Chem Products, Mumbai. Soya lecithin purchased from Modern Chemicals, Nashik. Purified water was used for the study & all other chemicals and solvents were purchased from different commercial suppliers, which were analytical grade**.**

Formulation of Etodolac containing Transferosomes-

Soya Phosphatidylcholine, edge activator (500 mg), and Etodolac were dissolved in chloroform, and the methanol mixture was in the ratio 2:1, v/v, and placed in a clean, dry round-bottom flask of a rotary evaporator. The chloroform and methanol were removed by rotary evaporation (Rotary Vacuum Film Evaporator - Coslab - Model-CRE400) at 40°C, 60rpm for 1hr. The film, which was deposited, hydrated with phosphate buffer by rotating for 1 hour. This step was carried out at room temperature. Vesicles formed were allowed to swell for 2 hrs at room temperature and then sonicated for 30 minutes in a sonicator (Digital Ultrasonic Cleaner) to reduce their size. The composition of these formulations is shown in Table 1[18].

Preparation of Carbopol gel

A plain Carbopol gel was prepared by dissolving 1 g of Carbopol 940 into 100 mL of distilled water. This step is carried out at 50°C, requiring continuous stirring. Methyl paraben and propyl paraben were added in small amounts as a preservative. The Transferosomes formulation, equivalent to 2% Etodolac, was thoroughly mixed with the prepared Carbopol gel, and another plain Carbopol gel was prepared with 2% w/w of Etodolac. The gel was allowed to set for 24 hours. Lastly, a measured quantity of triethanolamine was added [19].

Table 1: Formulation code of preparation of transferosomes

Formulation	EA	PC: EA	Chloroform: Methanol	Phosphate buffer (pH 7.4) ml
ET1	Tween 20	85:15	2:1	10
ET2	Tween 40	85:15	2:1	10
ET3	Tween 60	85:15	2:1	10
ET4	Tween 80	85:15	2:1	10
ET5	Span 20	85:15	2:1	10
ET6	Span 40	85:15	2:1	10
ET7	Span 60	85:15	2:1	10
ET8	Span 80	85:15	2:1	10
ET9	Span 85	85:15	2:1	10

EA: Edge activator; PC: Phosphatidylcholine

Optimization of prepared Transferosomes-

Optical microscopy: The prepared transfersomes were observed under a light microscope, Metzer Biomedical Model VFM9003. This evaluation gives an idea about the shape of the transfersomes[20].

Particle size and polydispersity index (PDI): Particle size and PDI of Eodolac transferosomes were determined by using Zetasizer (Diya Lab, Mumbai, India [21,22].

Zeta Potential: Zeta potential means levels of electrophoretic mobility of transferosomes were determined using a zeta sizer (Diya Lab, Mumbai, India), and the values were obtained at 25ºC [23].

Drug Loading (%) & Entrapment Efficiency (%):

Transferosomes were centrifuged at 12000 rpm for 30 minutes (Remi, Cε, NT 2178GK). The supernatant was separated. The absorbance of the supernatant was taken spectrophotometrically at 281 nm using a UV spectrophotometer (UV-2450, Shimadzu, Japan)[24,25]. The following formulas were used to calculate % EE and % DL:

$$\% \text{ EE} = \frac{\text{A Total Drug conc.} - \text{Supernatant Drug conc.}}{\text{Total Drug conc.}} \text{ X } 100$$

$$(\%) \text{ DL} = \frac{\text{A Total Drug Conc.} - \text{Supernatant Drug conc.}}{\text{Total weight of sample}} \text{ X } 100$$

Characterization of prepared transfersomal gel

The prepared transferosomes (ET1-ET9) batches were incorporated in Carbopol gel, and these gels were evaluated for the following characteristics. The batches were labeled from ETC-1 to ETC-9, respectively, and the batch that did not contain transferosomes was labeled as EC-0.

Physical appearance and homogeneity: All the formulated transfersomal gels (ETC1 -ETC9 and EC0) were assessed for homogeneity and texture. A small amount of the formulated gels was pressed between the two fingers. Their appearance and presence of any aggregates, particles, or fibers were examined[26].

Clarity and Color: All the prepared gels were tested for color and clarity [27].

Spredability: The spredability apparatus has two glass slides. 2 grams of gel was placed on one slide and kept on another slide the gel. Both slides should have the exact dimensions. A 100-gram weight was placed on top of the slides to create a uniform gel film between them and remove the air between them. The time (in seconds) required to detach both slides was recorded [27]. A time required less indicates good spreadability [28]. For calculating Spreadability, the following formula was used:

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

Where, L = Glass Slide length (cm), W = Weight Applied to upper slide (g), T = Time for separation of Slide (sec)

***pH*:** The pH of the developed gels (ETC1 -ETC9 and EC0) and the plain gel was measured by a pH meter (pH Cal Model). The pH measurement was conducted to assess the gel's potential for causing skin irritation. One gram of the gel was mixed with 20 mL of distilled water, and the pH was measured [29].

***Rheological study*:** The viscosity of Transferosomes was measured using the Brookfield viscometer LV DV-II +PRO model. T-95 Bar spindle was used. 50 g of each transferosome sample was dipped into the gel. The viscosity readings were taken at 10,20,30, 40, and 50 rpm at room temperature. The spindle was rotated for 5 min for every reading.[21] [30]

***Drug content*:** To determine the percentage of drug content in the gel formulation, approximately 1 gram of the gel was taken and lysed by sonication using solvent methanol for 15 minutes. Then, the solution was centrifuged (Remi, Cε, NT 2178GK) at 14,000 rpm for half an hour. The clear solution obtained was again diluted with methanol. Aliquots of the prepared solution were evaluated using a UV spectrophotometer (281 nm) to calculate the drug content of Etodolac. If necessary, the solution was further diluted with phosphate buffer [30,31].

***In vitro drug release study*:** This study was carried out by using a Franz diffusion cell. For this, an A-soaked cellophane membrane was placed between the two compartments of the Franz diffusion cell. Transfersomal gel was applied to the cellophane membrane and phosphate buffer was kept in the receiver compartment and stirred continuously with a magnetic bar at 50 rpm. 1 ml sample was taken at 0, 1, 2, 3, 4, 5, 6, and 12-hour intervals, the volume of the receiver compartment was replaced with 1 ml of buffer solution, and the sample was analyzed by using a UV spectrophotometer at 281 nm. The percentage of drugs released was then calculated [32,33].

Evaluation of Optimized formulations

***Scanning Electron Microscopy*:** The morphology of optimized transfersomal formulations, ET-4, was determined SEM at Cochin, SAIF, India. The sample was evaluated at 20 kV[34].

Transmission Electron Microscopy: The detailed morphology and structure of the transfersomes were also analyzed using TEM [35].

Deformability index: In this process, the optimized transfersomal formulations, ET-4, were passed through a membrane filter and recorded in milliliters. A membrane filter with a pore size of 0.20 μm (200nm) was used, and the formulation was passed through it. The amount of transferosome formulation was passed, it measured, and the size of the vesicle after extrusion was measured with a Malvern Zetasizer (Diya lab, Navi Mumbai)[35] [36]. The deformability index formula is as follows: –

$$D = J \times \left(\frac{rv}{rp}\right)^2$$

Where, D = Deformability index, J = Amount of transferosome suspension extruded, rv= Vesicle size of formulation after extrusion, rp = Pore size of the membrane filter

***Ex vivo permeation study*:** For this technique, the 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. A sample transfersomal gel was placed in the receptor compartment. Aliquot samples of 1ml were withdrawn at regular intervals and replaced with the same volume of fresh buffer. The amount of drug diffused through the membrane was measured using a U.V. spectrophotometer at a wavelength of 281 nm against phosphate buffer (pH 7.4 saline) as the blank. [36,37].

***Irritancy test*:** The skin irritation potential of Etodolac-loaded transfersomal gel (ETC-4) was assessed using species Wistar Albino (200-300 g). The selected rats were acclimatized for one week before the study began. Approximately 4 hours before 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 transfersome with Etodolac
Group IV-Received transfersomal gel with Etodolac.

The formulations (100 mg containing 2%) were applied to a shaved skin area of 1 cm^2. After application, the rats were observed for irritation at 24, 48, and 72 hours. 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 [38].

In Vivo Study (Anti-inflammatory Activity): The anti-inflammatory study of optimized transfersomal gel (ETC4) formulations was evaluated by inducing carrageenan into the hind paws of rats with permission of the ethical committee (Biotox/IAEC/03/2024/RP-14). For this, 24 rats were divided into four groups. Each group had 6 rats. Carrageenan solution is prepared by dissolving in saline solution. The preparations contain a dose of 20 mg/kg of Etodolac. Formulations were topically applied to the surface of the left hind paw of the rats and gently massaged before 30 minutes of intraplanar injection of 100 µL of a 1% w/v carrageenan solution into the left hind paw. The right paw was considered as a control for each animal. The digital vernier caliper 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 hours 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 (right paw).

Group Design was as follows-

Group 1: rats with induced inflammation but who received no treatment (right paw)

Group 2: rats with induced inflammation treated with an Etodolac Carbopol gel

Group 3: rats with induced inflammation treated with a marketed etodolac gel

Group 4: rats with induced inflammation treated with etodolac-loaded Transferosomes dispersed in a Carbopol gel [39].

Fourier Transform Infrared Spectroscopy Study: The FTIR of etodolac, Carbopol gel without drug, etodolac Carbopol gel, optimized etodolac transferosomes, and optimized etodolac transfersomal Carbopol gel were recorded by FTIR spectroscopy (Thermo Nicolet iS50, SAIF, Cochin, India)[40].

Differential Scanning Colorimetry Study: Etodolac, drug-free Carbopol gel, etodolac Carbopol gel, optimized etodolac transferosomes, and optimized etodolac transfersomal Carbopol gel were used for DSC (Model: DSC 204 F1 PHOENIK NETEZCH, SAIF, Cochin, India) analysis. The samples were crimped, placed, and placed on an aluminum pan and were crimped and scanned. Afterward, the scan was recorded and plotted on the graph, representing temperature and heat flow (w/g) on the X-axis and Y-axis, respectively [41].

X-ray diffraction (XRD): The drug and selected formulations were analyzed using an X-ray diffractometer (Bruker D8 Advance, SAIF, Cochin, India)[42].

Ultraviolet Visible Analysis: Absorption spectrum of etodolac, drug-free Carbopol gel, etodolac Carbopol gel, optimized etodolac transferosomes, and optimized etodolac transfersomal Carbopol gel were measured in the wavelength range of 200–400 nm to determine its maximum absorption wavelength. The solvent that demonstrated the best characteristics for this method was methanol. A standard stock solution was prepared by dissolving 2 mg of an accurate amount of formulations and drug into 20 mL of methanol[43,44].

Stability study: Samples from the optimized transfersomal batch (ETC4) were stored in tightly closed containers and kept at three different conditions: 5°C ± 3°C; 25±2ºC, 60±5%RH; (40±2ºC,75±5%RH). A stability study was carried out for 6 months (180 days). At the following time interval, the sample was withdrawn after 30 days, 90 days, and 180 days, and evaluations were done. The optimized transfersomal gel (ETC4) formulation was evaluated for its properties, including appearance, color, pH, viscosity, % Drug content, and % Entrapment Efficiency [45,46].

RESULT AND DISCUSSIONS

Optimization of prepared Transferosomes-

A drug-excipient study was conducted. It was concluded that all excipients were compatible with the Etodolac drug, which was confirmed by the FTIR study. After formulating the transferosomes, different evaluations were done.

***Optical Microscopy*:** Imaging with an optical microscopy showing the spherical vesicles of transferosomes. The outer layers of the vesicles appeared clear and dark. The well-defined

spherical and sealed structure of the transfersome vesicles was confirmed with multiple numbers.

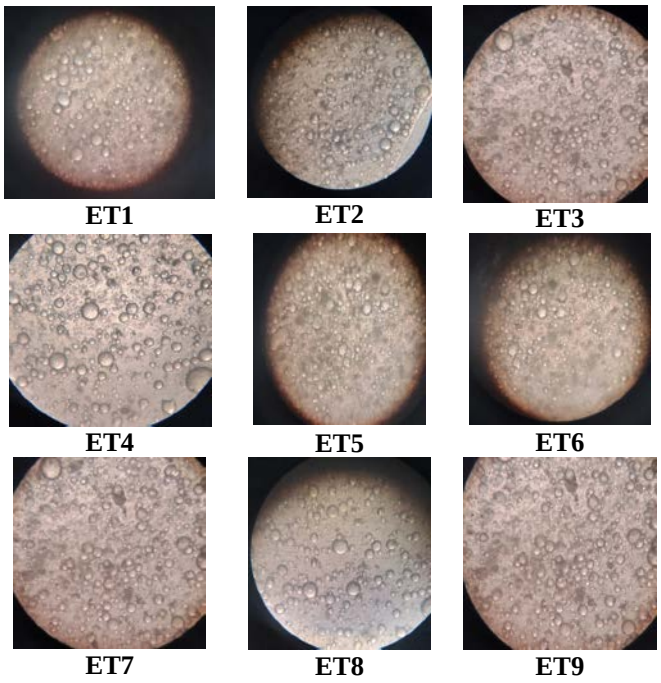


Figure 1: Optical microscopy of Transferosomes

Particle size and polydispersity index (PDI): Particle size of vesicle is helpful for drug permeation to the site of action and the therapeutic effect of a drug. It also influences the percentage encapsulation efficiency, biological distribution of drugs, drug release, and uptake of drugs from cells. Vesicles with diameters > 600 nm are not capable of penetrating the skin. Therefore, a particle size < 300 nm is ideal for topical drug delivery. The particle size of prepared transfersomes with Span 20 (ET5) have a small particle size of 185.85±5.94 nm, and transferosomes with Span85 (ET9) have a large particle size of 421.50±17.24.

The term "polydispersity" describes the degree of nonuniformity of a size distribution of particles in the same medium. It is also called Dispersity, which IUPAC recommends. Generally, the numerical range for PDI is 0.0 to 1.0. where 0.0 indicated the uniform particle size of the sample while 1.0 indicated the non-uniform particle size of the sample. In prepared transferosomes, the lower PDI value of 0.260±0.01 indicated a uniform, narrow, and homogeneous particle distribution throughout the prepared formulations. All other formulations also exhibited good PDI.

***Zeta Potential*:** It was used to measure the surface properties of the developed transfersomes, as the surface charge of these vehicles plays a crucial role in formulation stability. The charge present on vesicles produces a repulsive energy barrier that prevents aggregation. 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. The zeta potential values of the transfersomes in the study ranged from -18.50± 0.78 to -62.53±0.39 mV, indicating considerable stability. Higher zeta potential values suggested greater stability of the transfersomes, with minimal, attractive forces, thereby achieving a more electrically stable colloid system. Due to the repulsion between similar charges, the negative charge was considered favorable for stability and enhanced penetration of the transfersomes. Particles with a zeta potential greater than ± 30 mV are stable[47]. The Vesicle size, PDI, and zeta potential values of optimized formulations are presented in Table 2.

Table 2: Vesicle size, PDI, & zeta potential of transfersomes

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
ET2	253.15±10.81	0.287±0.01	-34.07±0.74
ET3	295.40±12.43	0.254±0.02	-47.77±0.57
ET4	**222.97±6.50**	**0.271±0.02**	**-62.53±0.39**
ET5	185.85±5.94	0.252±0.00	-37.47±4.22
ET6	305.93±27.11	0.261±0.03	-45.73±0.60
ET9	421.50±17.24	0.260±0.01	-18.50±0.78

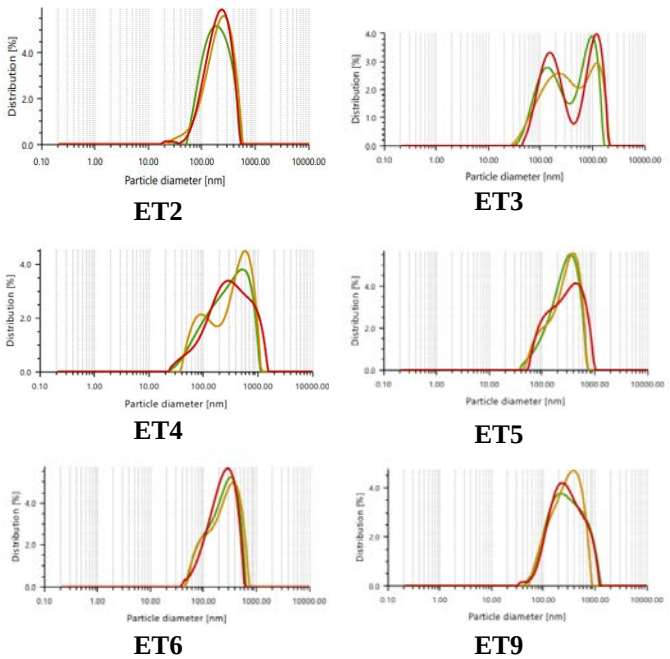


Figure 2: Particle size distribution and polydispersity Index of Transferosomes

***Drug Loading and Entrapment Efficiency*:** Loading capacity refers to the total amount of drug loaded in the nanoparticle, indicated in percentage (%). Entrapment efficiency (%EE)

represents the proportion of drug successfully entrapped in the formulation. Table 3 shows the drug loading (%) and entrapment efficiency (%) for all transferosomes. Drug loading was obtained in between the range of 17.52% to 35.84%, while entrapment efficiency was between 54.15% and 80.25%.

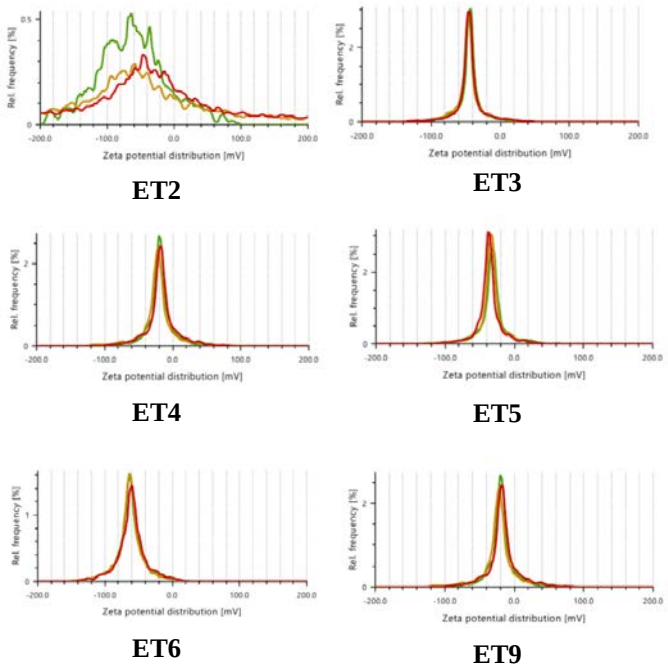


Figure 3: Zeta potential of Transferosomes

Table 3: Drug Loading and Entrapment Efficiency

Formulation	Shape	EE% (± S.D.)	D.L.% (± S.D.)
ET1	Spherical	59.99±1.16	35.84±0.60
ET2	Spherical	77.84±1.33	30.92±0.81
ET3	Spherical	72.61±1.43	21.96±0.85
ET4	**Spherical**	**80.25±2.85**	**31.18±1.44**
ET5	Spherical	78.29±2.16	27.35±0.83
ET6	Spherical	76.56±1.49	28.75±0.79
ET7	Spherical	54.15±1.35	17.52±0.46
ET8	Spherical	63.43±0.33	21.12±0.13
ET9	Spherical	78.72±1.09	32.69±0.60

Characterization of prepared transfersomal carbopol gel

Physical appearance and homogeneity: The plain Carbopol gel and the Transferosomes containing Carbopol gels exhibited good homogeneity, showing no lumps. This indicated that the prepared gels were uniform and smooth in consistency, with no particulate matter present.

***Clarity and Color*:** Visual inspection against a white background revealed that all formulations were clear. The color of all formulations was observed, and each formulation acquired a distinct color. This may be due to the color of the Edge activators used in the formulation.

Spreadability: Spreadability results from the structural and viscoelastic characteristics of the material that are related to rigidity, strength, elasticity, and viscous behavior. Good spreadability shows the effortless application of gels and more contact time at the application site. It also indicates how the formulation will behave when it comes from the tube. Spreadability depends mainly on the polymers' viscosity and physical and chemical properties. An inverse relation is present in the viscosity and spreadability. The effectiveness of topical therapy relies on the ability of a semisolid formulation to spread on the skin. The spreadability of the plain Carbopol gel was 5.76 g.cm/sec, while the spreadability of the transferosome-containing gels ranged from 7.25 to 15.23 g.cm/sec [48].

pH: The pH of the formulated gels was obtained from 5.83 to 7.13, as shown in Table 5. The pH range required for topical preparations should be between 4 and 7. Therefore, these gels are suitable for safe and non-irritant applications.

Table 4: Physical appearance, texture, homogeneity, clarity, and color of transfersomal gel and plain carbopol gel

Formulation	Physical Appearance	Color	Texture	Homog-eneity	Clarity
ETC1	Opaque	Brown	Smooth	Homogenous	Clear
ETC2	Opaque	Brown			
ETC3	Opaque	Light Brown			
ETC4	Opaque	Light Brown			
ETC5	Opaque	Brown			
ETC6	Opaque	Light Brown			
ETC7	Opaque	Brown			
ETC8	Opaque	Brown			
ETC9	Opaque	Brown			
EC0	Transparent	Colorless			

Rheological study: The viscosity study indicates that adding transferosomes decreases the viscosity of conventional Carbopol gel. It may be due to the addition of transferosomes to gels. Added transferosomes may interfere with the Carbopol networks, which promote a weakened interaction connection between the molecules present in polymers of Carbopol gel and hence show their thinning effect [37,49].

Table 5: Spreadability, pH, and drug content of transfersomal gel and plain carbopol gel

Formulation	Spreadability g.cm/sec	pH	Drug Content(%)
ETC1	09.97±0.21	6.36±0.02	97.92±0.65
ETC2	12.22±0.14	5.85±0.05	97.55±0.60
ETC3	07.25±0.11	6.12±0.04	96.17±0.60
ETC4	12.69±0.20	6.94±0.09	100.41±0.57
ETC5	15.23±0.24	7.13±0.04	104.74±0.45
ETC6	10.14±0.21	6.63±0.05	101.33±0.69
ETC7	14.64±0.20	6.86±0.01	100.55±0.58
ETC8	8.80±0.13	6.32±0.07	92.71±0.41
ETC9	07.92±0.14	6.02±0.04	88.52±0.79
EC00	5.76±013	7.05±0.02	98.43±0.58

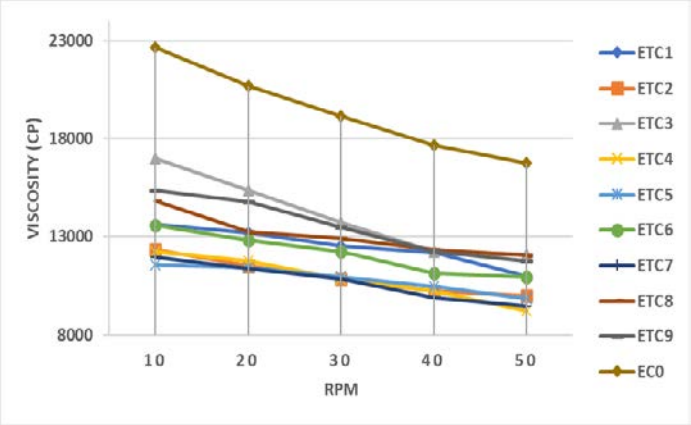


Figure 4: Rheological study of transfersomal carbopol gel

Drug content: Formulated Transfersomal gel formulation shows drug content between 88.52±0.79% to 104.74±0.45 %. It indicated a good capacity of formulation to hold the drug. All formulations show satisfactory results except ETC8 and ETC9.

***In-vitro* release study:** The diffusion study results of transfersomal Carbopol gels (ETC1 to ETC9) and plain Carbopol gel (EC0) formulations are presented in Table 6 and Figure 5. The order of release was ETC4 > ETC9 > ETC5 > ETC6 > ETC2 > ETC3> ETC8 > ETC7 > ETC1> EC0 release was observed. The maximum percentage of drugs released was determined 93.51 for ETC4. There is a relation observed that those formulations have better entrapment efficiency that shows a better release rate [50]. All transfersomal gels show better results than plain Etodolac Carbopol gel and among them best results are shown by ETC 4 formulations.

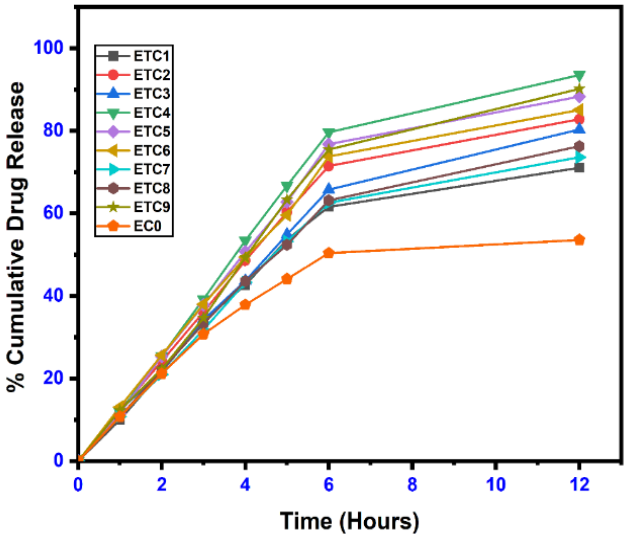


Figure 5: Cumulative percentage of drug released (%)

Table 6: Cumulative drug released, flux. permeability coefficient and enhancement ratio of Transfersomal Gel

Code	Q_{12hr}(µg/cm2)	Flux (µg/cm^2/hr)	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	1.00
ETC1	142.09±15.98	12.16±1.36	6.08±0.68	1.38
ETC2	165.60±18.50	14.20±1.57	7.10±0.78	1.61
ETC3	160.70±13.87	13.66±1.27	6.83±0.63	1.55
ETC4	187.03±11.31	16.22±0.99	8.11±0.49	1.84
ETC5	176.53±13.36	15.27±1.15	7.64±0.57	1.73
ETC6	170.13±16.90	14.48±1.42	7.24±0.71	1.64
ETC7	147.14±13.96	12.61±1.18	6.31±0.59	1.43
ETC8	152.53±22.35	12.97±1.95	6.49±0.95	1.47
ETC9	180.31±21.10	15.69±1.94	7.8±0.95	1.78

Evaluation of Optimized Formulations

Based on the results obtained from the above evaluation, formulations ET4, and ETC4 were selected for further evaluation, especially based on entrapment evaluation, particle size, zeta potential, in-vitro release study, etc.

SEM: Scanning electron microscopy of the optimized formulation (ET4) confirmed the morphology and size details of the transfersomes. The vesicles appeared uniform, with distinctive, smooth surfaces and loose aggregates. The mean size of the transfersomes ranged from approximately 121nm to 197nm. Figure 6 shows the resolution of transferosomes vesicles at 1500X, 3500X, 7000X, & 10000X [51].

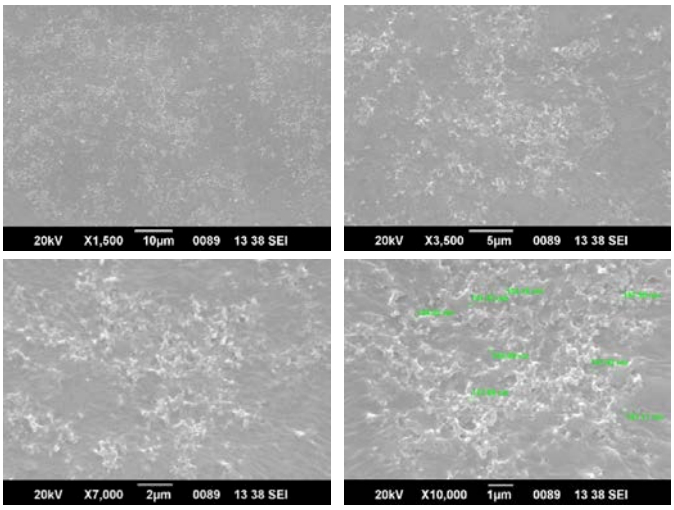


Figure 6: SEM images of Optimized Transferosomes

TEM: TEM images of vesicles show almost spherical vesicles with a clear and dark outline. The images showed that developed vesicles are in nm size. Outline and core of vesicles confirmed vesicular characteristics, indicating the integrity of closed structures. The vesicles are smaller unilamellar vesicles with a more homogenous size distribution [52].

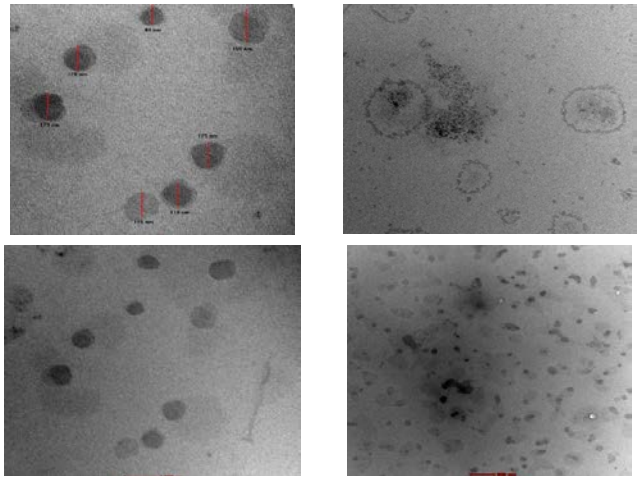


Figure 7: TEM images of Optimized Transferosomes

Deformability index: The transferosomes should possess deformability to facilitate their passage to pass through tiny pores of the epidermis. The prepared optimized transfersomal gel formulations underwent a deformability study by extrusion method. Transfersomes dispersion fluid effortlessly traversed the polycarbonate membrane. Deformability serves as a crucial parameter reflecting the transferosomes' capability to navigate the narrow channels of the skin without changing their shape and size [53,54].

The values to calculate the deformability index is $D=J \times \left(\frac{rv}{rp}\right)^2$

Where, D = Deformability index, J = Amount of transferosome suspension passed through membrane= 12.8 ml, rp = Pore size of the membrane filter = 200 nm

The optimized transferosomes (ET4) showed a % deformability index of 13.7±1.77.

Table 7: Deformability of Optimized Formulations (ET4)

Sr. No.	Vesicle Size nm (rv)	Deformability Index	Mean ± S.D. (n=3)
01	221.7	10.93	13.7±1.77
02	241.7	12.99	
03	262.0	15.27	

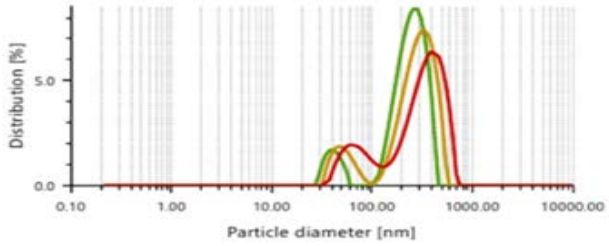


Figure 8: Vesicle size for Deformability Index (ET 4)

***Ex vivo* permeation study:** The drug amount released from optimized transfersomal formulations (ETC4) at predetermined time intervals was determined. These drug release data fitted to different kinetic models e.g. zero order, first order, Higuchi model, Korsmeyer-Peppas model etc. These models were used to find out the drug release mechanism. For this evaluation, correlation coefficient (r^2) values were used. The linear fit curves and their corresponding equations with correlation coefficient (r^2) for Carbopol gel, Marketed gel and Transfersomal gel using different kinetics are shown in Figure 9 and Table 8. The values of r^2 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 the "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.

Case II transport related to the absorption is entirely controlled by stress-induced relaxations at a sharp boundary of a swollen shell from an unpenetrated glassy core[55]. Super Case-II transport is associated with stresses and transitions in hydrophilic glassy polymers that swell in water or different biological fluids. These three formulations show a value > 0.89, which means the release is super Case II transport [40].

Table 8: Regression analysis data of transfersomes 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
Transferosomal Gel(ETC4)	0.8648	**0.9487**	0.8998	**0.9523**	0.8686

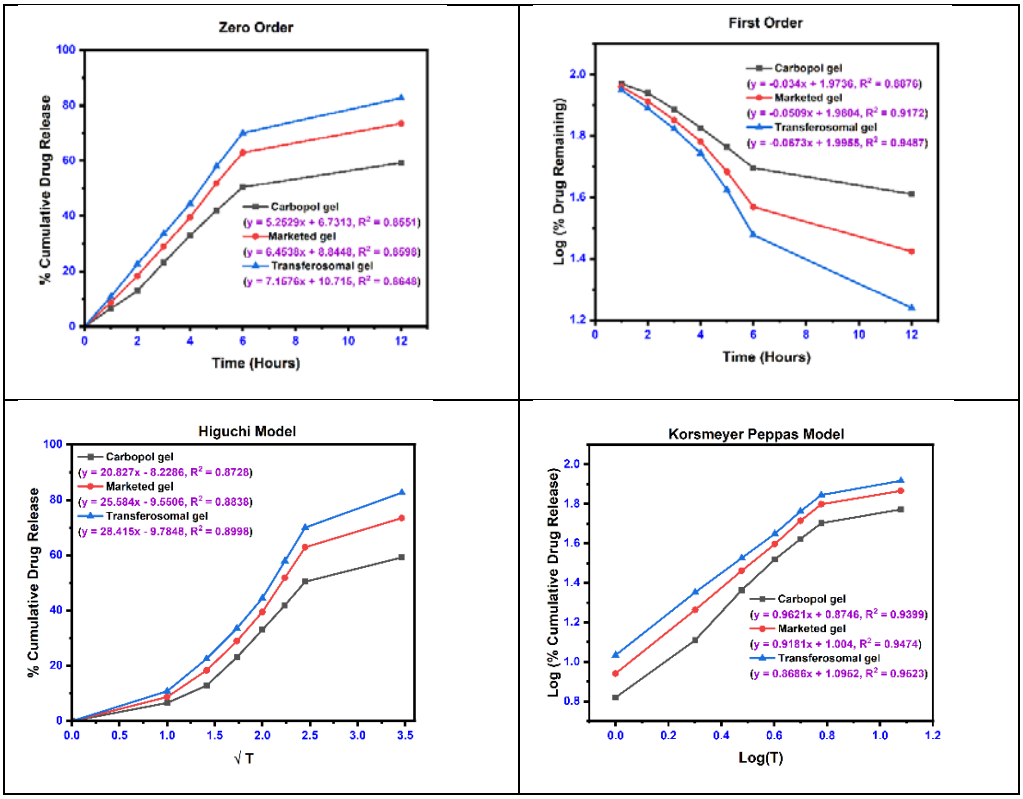
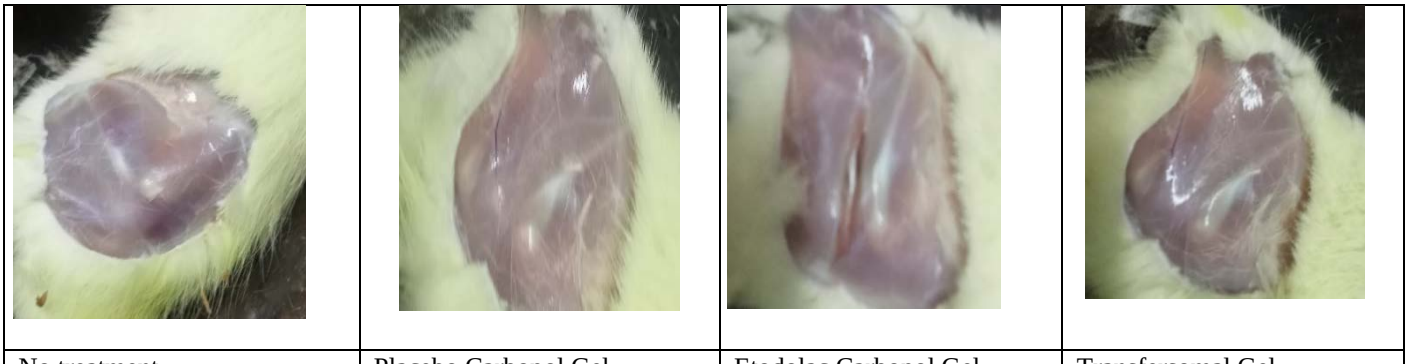


Figure 9: The release kinetics model of Etodolac drugs: (a) zero-order kinetics model; (b) first-order kinetics model; (c) Higuchi model; and (d) Korsmeyers peppas model



No treatment	Placebo Carbopol Gel	Etodolac Carbopol Gel	Transfersomal Gel

Figure 10: Images of skin irritation studies

Table 9: Stability study of Optimised Transfersomal gel (ETC4)

Months	Parameters					
Storage Condition	5°C ± 3°C					
	Appearance	**Color**	**pH**	**Viscosity**	**Drug Content %**	**Entrapment Efficiency %**
0	Opaque	Light brown	6.94±0.09	9244 ±22	100.41±0.57	78.72±1.09
1	Opaque	Light brown	6.97±0.03	9204±11	99.44±1.86	78.10±0.83
3	Opaque	Light brown	6.92±0.04	9220±13	100.73±0.38	75.84±0.60
6	Opaque	Light brown	6.95±0.03	9214±09	100.55±0.39	75.52±1.09
Storage Condition	25±2ºC,60±5%RH					
	Appearance	Color	pH	Viscosity	Drug Content %	Entrapment efficiency %
0	Opaque	Light brown	6.94±0.09	9244±22	100.41±0.57	78.72±1.09
1	Opaque	Light brown	6.98±0.07	9242±17	100.64±0.43	76.47±0.79
3	Opaque	Light brown	7.01±0.09	9238±17	101.56±1.22	76.36±0.32
6	Opaque	Light brown	6.96±0.05	9237±20	100.78±0.51	75.15±1.25
Storage Condition	40±2ºC,75±5%RH					
	Appearance	Color	pH	Viscosity	Drug Content %	Entraptment Efficiency %
0	Opaque	Light brown	6.94±0.09	9244 ±22	100.41±0.57	78.72±1.09
1	Opaque	Light brown	7.02±0.08	9182±12	100.23±0.59	74.97±0.81
3	Opaque	Dark Brown	6.93±0.02	9136±10	101.10±0.93	68.70±0.63
6	Opaque	Dark Brown	6.94±0.04	9085±11	100.69±0.71	65.95±0.48

Irritancy Test: A skin irritancy test was performed on optimized transfersomal gel (ETC 4), and a picture of the dorsal skin area of one rat from each group was shown in Figure 10. Etodolac-loaded transfersomal gel caused no edema, erythema, and irritation. These results indicated that topical Etodolac transfersomal gel is safe to use and has no harmful signs on the skin [56].

***In vivo* Anti-inflammatory test:** The carrageenan-induced rat paw edema method was used to determine *In vivo* anti-inflammatory activity. The percentage inhibition of edema was calculated; the results are shown in Figure 11. Maximum % edema inhibition was observed with transfersomal gel formulation compared to the marketed and plain gel after 8 hrs. Although the difference observed is narrow at the endpoint, improvement is consistent at all time points, presenting the outcome's significance.

According to ex vivo and in vivo studies, transfersomal gel (ETC4) showed better permeation and effectiveness than other formulations. This may be due to the effect of the edge activator, the size of vesicles, and the total amount of drug entrapped [42].

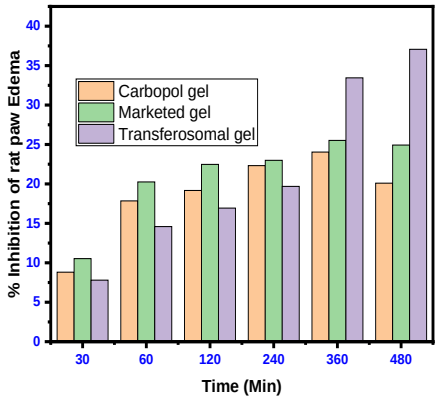


Figure 11: % Inhibition of Rat paw edema

FTIR Study: The pure Etodolac shows bands 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). Characteristic peaks observed in the FTIR spectra of Etodolac also appeared clearly in the Etodolac-loaded formulations. A small but distinct change was noted in these spectra compared to the pure Etodolac drug. Therefore, it can be concluded that Etodolac was successfully entrapped into the formulations without significant changes in its properties. The FTIR of both the blank (Figure 12-B) and other Carbopol gel

formulations (Figure 12-C, D) are identical, showing the same band intensities for identified functional groups occurring at the same wave numbers [57,58].

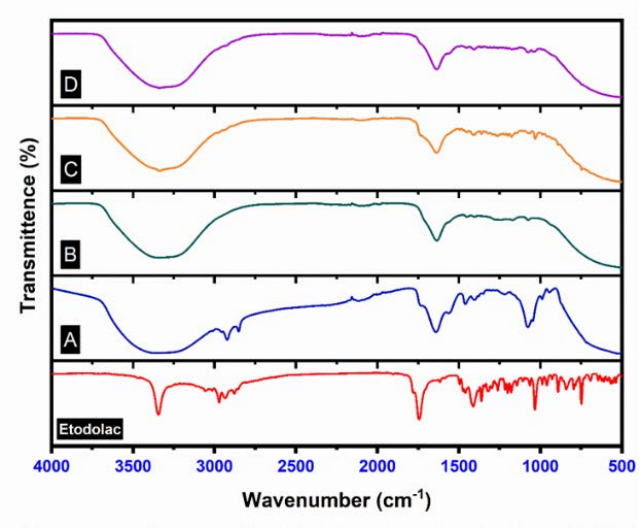


Figure 12: FTIR spectra of Etodolac; A: Etodolac Transferosome, B: Plain Carbopol gel, C: Etodolac Carbopol gel, D: Etodolac Transfersomal gel

DSC study: DSC thermograms are used to assess the crystallinity, melting point, and crystallization of formulations. Etodolac powder exhibited an endothermic melting peak at 154.5°C, which indicates its highly crystalline nature. This suggested that Etodolac had transitioned from a crystalline to an amorphous state in all other formulations (Figure 13-A, C, D). Figure 13-B shows a small endothermic sharp peak because of Carbopol. The complete loss of crystallinity was observed in Figure 13-D, as in this Etodolac drug, which was first present in a transferosome and then incorporated in Carbopol gel [59].

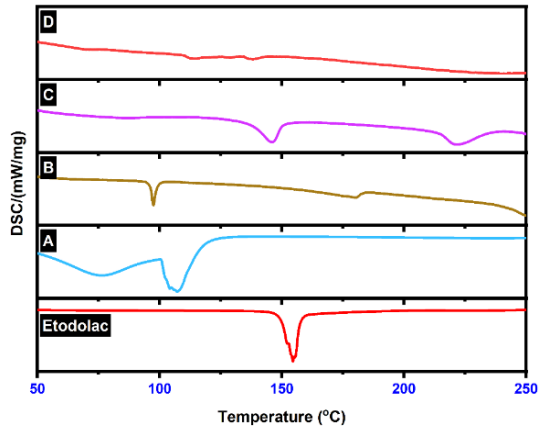


Figure 13: DSC graph of Etodolac; A: Etodolac Transferosome, B: Plain Carbopol gel, C: Etodolac Carbopol gel, D: Etodolac Transfersomal gel

***XRD analysis*:** The XRD patterns of the etodolac drug, plain Carbopol gel, Etodolac Carbopol gel, and Etodolac transfersomal gel are taken, and results are shown in Figure 14. The etodolac showed specific sharp peaks, indicating its crystalline nature. All other formulations (Figure 14-B, C, D) did not show sharp peaks like the Etodolac drug, indicating the material's amorphous state. The XRD spectra of the Etodolac Transferosome (Figure 14-A) showed a reduction of peak intensity, which demonstrated decreased crystallinity or conversion into an amorphous phase of the drug [60]. Plain Carbopol gel without the drug also shows the amorphous nature of the gel (Figure 14-B). In contrast, the Carbopol gel with the drug (Figure 14-C) shows a mixed nature of crystalline peaks because of the drug present in the gel and loss of crystallinity in the amorphous gel. Again, in (Figure 14-D) the XRD graph of Etodolac transfersomal Carbopol gel shows an amorphous nature as Carbopol gel, but there is a presence of one peak that could be because of the presence of the drug.

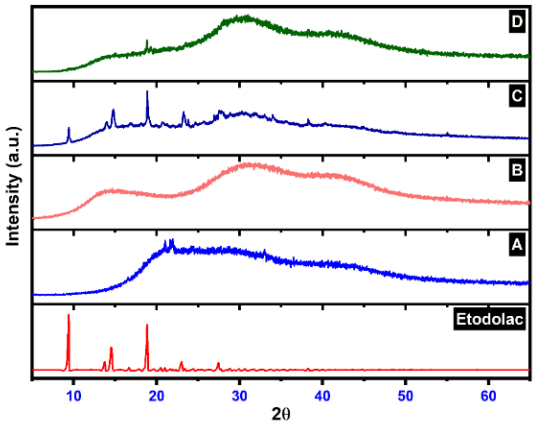


Figure 14: XRD graph of Etodolac; A: Etodolac Transferosome, B: Plain Carbopol gel, C: Etodolac Carbopol gel, D: Etodolac Transfersomal gel

UV Analysis: The maximum absorption spectra of pure etodolac, plain Carbopol gel, Etodolac Carbopol gel, and Etodolac transfersomal gel are shown in Figure 15. All formulations (Figure 15-A, C, D) show identical spectra because of the presence of the Etodolac drug. Only Figure 15-B shows different spectra, which may be due to the absence of the Etodolac drug.

Stability study: The stability study of the optimized transfersomal gel (ETC4) was carried out at different conditions like 5°C ± 3°C, 25°C ± 2°C, 60±5% RH, and 40±2ºC, 75±5%

RH for 180 days, and results are shown in Table 9. It indicates the formulation has good stability behavior related to pH, appearance, color, and % drug content. But, in the case of viscosity and percentage drug entrapment efficiency, it reduced significantly when this formulation was stored at 40±2ºC,75±5%RH. These results show that storage conditions should be maintained for transfersomal formulation to achieve stability.

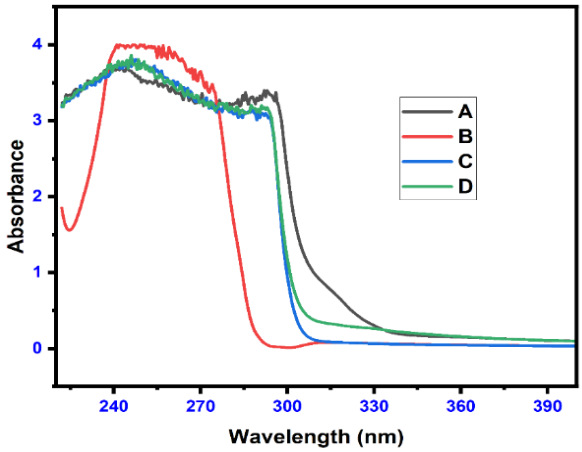


Figure 15: Maximum absorption spectra of Etodolac; A: Etodolac Transferosome, B: Plain Carbopol gel, C: Etodolac Carbopol gel, D: Etodolac Transfersomal gel

CONCLUSION

Etodolac Transfersomes successfully deliver into the skin through the topical route, and it could become one of the best approaches. The different formulations of transferosomes containing SPC, various grades of Spans, and Tweens (ET-1 to ET-9) were successfully prepared by the rotary evaporator method. The vesicle size, zeta potential, PDI values, and Entrapment efficiency (%) were within acceptable range. Meanwhile, transfersomes were incorporated into carbopol-940 gel and evaluated for color, homogeneity, pH, viscosity, spreadability, and in vitro drug release. Optimized formulation was selected mainly based on vesicle size, Zeta potential, entrapment efficiency (%), and in vitro release study, as these characteristics affect the permeation of dosage form. The optimized transferosomes (ET4) were further evaluated for SEM, TEM, and deformability index, which showed satisfactory results. *Ex vivo* study shows that the first-order and Korsmeyer-Peppas models were more suitable for formulations based on values of r^2. Etodolac-loaded transfersomal gel caused no skin edema, erythema, and irritation, indicating the safe use of this formulation. Transfersomal gel formulation shows Maximum % edema inhibition compared to the marketed and plain Carbopol gel. Stability study results show that storage conditions should be maintained for transfersomal formulation to achieve stability.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Ashwini A Bachhav conducted the experiments, analyzed data, and contributed to interpreting results. Prashant L Pingale conceptualized the study, designed the experiments, and wrote the initial draft of the manuscript. Chandrashekhar D Upasani oversaw the statistical analysis, helped with the manuscript editing, and supervised the research project.

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