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Design and Development of NSAIDs loaded drug Ethosomal gel formulations for Rheumatoid Arthritis

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ABSTRACT
The goal of the current study was to create an ethosomal gel of CXB to treat rheumatoid arthritis. Different amounts of lipoid S100 (50 mg–200 mg), ethanol (20–50%), and water were used to create CXB ethosomes. The drug encapsulation efficiency, vesicle size, PDI, surface charge, and shape of the produced nanovesicles were identified. The optimisedethosomal formulation exhibited a zeta potential of -31.1 mV, a particle size of 161±0.01 nm, a polydispersity index (PDI) of 0.259, an entrapment efficiency of 89.21%, and a drug release of 97.87% in a 24-hour period. The optimised formulation's TEM investigation revealed somewhat smooth spherical formations. The drug's hydroalcoholic solution could only pass up to 75.5 µm, however ethosomes could readily infiltrate deeper dermal layers (up to 104.3 µm), according to Confocal laser scanning microscopy. Additionally, the optimisedethosomal formulation was added to a 1% carbopol 934 gel basis and further optimised, resulting in a transdermal flux that was discovered to be roughly ten times greater than that of the hydroethanolic solution. The ethosomal gel was created with success and has demonstrated that it could be a viable alternative to traditional rheumatoid arthritis treatments.
KEYWORDS: Ethosomal nanovesicles; rheumatoid arthritis; celecoxib

1. INTRODUCTION

An autoimmune condition called rheumatoid arthritis (RA) is characterised by symptoms such as pain, weakness, and joint swelling, redness, and persistent inflammation [1]. In the world, women are more likely than males to have arthritis (18%), according to the World Health Organisation. While the exact cause of arthritis remains unknown, pro-inflammatory cytokines like TNF-α and IL-1β are thought to be the cause of inflammation and cartilage deterioration. The use of glucocorticoids, disease-modifying anti-rheumatic medications, and NSAIDS are among the therapeutic modalities for the management of RA. Since the latter drug classes frequently impede the course of the disease but do not alleviate pain or inflammation, NSAIDS are considered to be cornerstone treatment regimens [2]. One of the cyclooxygenase-selective COX-2 inhibitors with strong analgesic and anti-inflammatory properties is celecoxib (CXB) [3]. Because of its effectiveness, CXB is frequently prescribed for inflammatory bone conditions such rheumatoid arthritis and osteoarthritis [4, 5]. Additionally, it has shown anti-cancer efficacy against several cancer types, including as lung and bladder tumours [9, 10], colorectal cancer [8], skin carcinoma [6], and breast tissue cancer [7, 10]. Oral administration of CXB raised numerous questions about its low bioavailability and poor absorption [11, 12], not to mention the cardiovascular issues linked to large dosages [13]. Therefore, topical administration may be advantageous in this context. One important barrier and rate-limiting step for medication diffusion through the skin is the stratum corneum (SC), the top layer of skin [14]. Over the past few years, lipid vesicles—which include liposomes and second-generation vesicles like transferosomes, ethosomes, and transethosomes—have demonstrated promise for the effective delivery of many medications [15, 16]. When compared to ordinary liposomes, the second-generation lipid vesicles' contents demonstrated substantial skin penetration performance [17].

According to the literature review, vesicle formulations like ethosomes are becoming more and more well-liked because of their lipid-based composition and improved skin penetration. Phospholipids, water, and a significant amount of ethanol make up etherosomes. Because of the elastic nature that ethanol imparts, they are often referred to as deformable vesicles. The flexible feature of nanosize, usually less than 200 nm, aids in improving the drug-loaded ethosome formulation's skin penetration. Because phospholipid and ethanol work together to increase lipid fluidity, ethers easily pass through skin lipids [18, 19]. The goal of this study is to create a topical gel solution loaded with CXB to increase the drug's skin permeability and facilitate better CXB delivery for the treatment of rheumatoid arthritis.

2. MATERIAL AND METHODS

2.1. Materials: Celecoxib was obtained as a gift sample from Sun pharmaceutical industries limited (India). Lipoid S100 was obtained from Lipoid® GmbH (Germany), ex gratia. Ethanol was purchased from Loba chemicals. All other reagents were of analytical grade and were purchased from Merck, Mumbai, India.

2.2. Preparation of CXB Loaded Ethosomes: With a small modification, ethosomes were produced using the cold technique [20]. Different ratios of phospholipids, ethanol, and water were used to generate different batches of ethosomes. Using a mixer, phospholipid and CXB were weighed and dissolved in ethanol at 30°C in a water bath. In a covered vessel, the ethanolic mixture was stirred and water that had been heated to the same temperature was added dropwise. To minimise the size of the vesicles, it was then sonicated for one cycle at 4°C using a sonicator (PRAMA Ultrasonicator, Mumbai, India). At last, the ethosomal suspension that had developed was refrigerated.

2.3. Optimization Using Experimental Design: Design Expert Software v.11 (Stat-Ease, USA) and three-factor two-level Box Behnken experimental design (BBD) were used to optimise the ethosomal vesicles loaded with celecoxib. Because BBD produces fewer runs

than central composite design, it provides a more accurate and economical response prediction. This is why it was chosen. A non-linear quadratic model was created using the data, and the equation is as follows:

$$Y= \beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{12}X_1X_2+ \beta_{13}X_1X^3+ \beta_{23}X_2X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33}X_3^2 \quad (1)$$

Encapsulation efficiency, EE (Y1), and vesicle size (Y2) were utilised as dependent variables, and the amounts of ethanol (X1), lecithin (X2), and sonication duration (X3) were employed as independent factors. Lecithin and ethanol concentrations ranged from 20–50% and 50–200 mg, respectively. Additionally, the third factor's sonication time was set for 15 to 60 seconds. The coefficients β0, β1, β2, and β3 are constants in the equation, whereas β12, β13, and β23 are interaction coefficients among the three factors. The quadratic coefficients β11, β22, and β33 are derived from the observed experimental values of Y in the experimental runs. Vesicle size (Y1) and EE (Y2), the dependent variables, had minimum and maximum goals, respectively. ANOVA was used to analyse the responses from the 17 runs that were produced. Response surface plots were generated and the effect of different variables on the responses was interpreted. [21]

2.4 Vesicle Size and Size Distribution of the Ethosome Vesicles: Using the Malvern Zeta-sizer Nano-ZS (Malvern Instruments, Worcestershire, UK), a dynamic laser light scattering (DLS) approach was used to assess the particle size distribution of the produced lipid vesicles at 25.5 ± 0.5 °C. [22] The lipid vesicle solution was vortexed for at least a minute prior to each measurement after dispersing in deionized water at a concentration of 1 mg/mL. Through the use of electrophoretic mobility data obtained in deionized water, the surface charge of the generated CXB-lipid vesicles was ascertained. Three duplicates of each measurement were estimated.

2.5 Entrapment Efficiency: The ultracentrifugation method was used to indirectly calculate the amount of CXB that was entrapped. The ethosomal formulation was centrifuged (Stratos centrifuge, Germany) for one hour at 20,000 revolutions per minute and 4°C. After centrifugation, the supernatant was carefully collected and filtered through a 0.2µm Millipore membrane filter. Appropriate dilution with ethanol-water (30% v/v) was then performed. Using a 1:1 water-ethanol solution as a blank, the amount of unentrapped medicine in the supernatant was measured at 240 nm using a UV spectrophotometer (Shimadzu, UV-2401 PC, Japan). After three iterations of the experiment, the mean value and standard deviation (SD) were computed. After all, the EE% was determined using the formula below: [24]

EE % = (Total drug amount - (unentrapped) drug in the supernatant)/(Total drug amount)×100….3

2.6 Transmission Electron Microscopy (TEM): A transmission electron microscope (TEM) (JEOL 100 CX, Tokyo, Japan) operating at an accelerating voltage of 80 kV was utilised to photograph the optimised CXB-TES. To treat the sample, 20 µL of the TES suspension was added to a 300 mesh grid covered with Formvar and stabilised with evaporated carbon film for a duration of one minute. The extra sample was taken out using filter paper. The vesicles were negatively stained with an aqueous solution of uranyl acetate (2% w/v) by adding 20 µL and letting it sit for a few seconds. It was then allowed to dry overnight at room temperature. The AMT-700 camera (Advanced Microscopy Techniques, Woburn, MA, USA) was used to take the images. [25]

2.7 Zeta Potential Determination: Malvern Zetasizer (Nano-ZS, Malvern, U.K.) determined the optimised formulation's zeta potential. The same technology used for size analysis was also used to quantify the zeta potential [26].

2.8 In-vitro Drug Release Studies: The dialysis bag approach was used to carry out the investigation. We used a cellulose membrane dialysis bag (Sigma, USA) for our investigation, which only permits the free medication to diffuse. The membrane employed in

the investigation had a 60 ml per foot capacity and a cutoff molecular weight of 12000 g/mol. [27] Before being used, the dialysis bag was immersed in release medium for a duration of 12 hours. The hydroalcoholic solution and ethosome dispersion were used in the in-vitro release investigation. The dialysis bag was filled with the formulations and submerged in 100ml of release media (PBS pH 7.4 and ethanol; 8:2) that was contained in a beaker. An incubator shaker with a 200 rpm speed and a 37±0.5 °C temperature control was used to hold the beaker. To maintain sink conditions, three millilitre aliquots were taken out at regular intervals (0.5, 1, 2, 4, 6, 8, and 24 hours) and an equivalent volume of buffer was supplied [19]. The CXB in aliquots was measured at 232 nm using a UV spectrophotometer. Every measurement was made three times. To evaluate the release pattern from the formulations, the release data were fitted to a variety of kinetic models. To find the best match, the cumulative percent release was displayed in a number of models using the in vitro release graph for both formulations.

2.9 Preparation of CXB Ethosomal Trans gel: To prepare the gel, the optimal ethosomal formulation was chosen. To prepare the gel, several concentrations of carbopol 934 (1%, 1.5%, and 2%) were mixed with enough distilled water in a separate beaker and continuously stirred at 300 rpm. Following the full dispersion of carbopol in water, it was given an overnight swell period and sonicated for ten minutes to eliminate any remaining air bubbles. Drug-loaded ethosomal suspension was then gradually added to this while being constantly stirred to create a homogenous mixture. Triethanolamine was then used as a final neutralising agent [20].

Evaluation of CXB Ethosomal gel Formulation: [28-30]

Homogeneity: By visually evaluating the gel's appearance and the existence of aggregates, the homogeneity was evaluated.

Viscosity Measurement: With the use of a Brook-field viscometer with spindle number C-50-1, the viscosity of the prepared gel was measured.

Spreadability: We tested the formulation's spreadability by sandwiching around 1g of gel between two glass slides. The upward slide was secured to weights and let to move in the weight's direction. It was noted how long it took for the upper slide to move. The following formula was used to determine the spreadability: S=M.L/t, where M= mass tied to upper slide, L= length of the glass slide, t= time taken to separate the slide.

Drug Content: One gramme of drug-loaded vesicular gel was mixed with ten millilitres of ethanol and swirled for thirty minutes in order to determine the drug content of the gels. After centrifuging the mixture for 20 minutes at 3000 rpm, the supernatant was removed and filtered through a membrane filter with a pore size of 0.45 mm. A UV spectrophotometer was used to analyse it after it had been properly diluted.

pH Evaluation: The apparent pH of the gel was measured by pH meter (Accumet AB 15, Fisher Scientific, USA) at 25± 1 °C.

Extrudability (Tube Test): The weight in grammes needed to extrude a 0.5 cm gel ribbon from a collapsible tube in 10 seconds is known as extrudability. The prepared gel was put into a collapsible tube and tightly compressed at the crimped end, causing the gel to extrude when the cap was removed. This is how the extrudability was determined:

Extrudability = applied weight to extrude gel from tube (in gm)/ area (in cm^2) (3)

Ex vivo Permeation Studies: CXB ethosomal formulation was permeated ex vivo using a Franz diffusion cell, which has a receptor volume of 16 ml and a diffusion surface of 3.142 cm2. Rat skin that had just been excised was cleaned with buffer and placed between the donor and recipient compartments of the Franz diffusion cell with the dermal side in contact with the diffusion medium and the stratum corneum facing upward. The diffusion medium, phosphate buffer (pH 7.4) and ethanol (8:2), were agitated at 100 rpm and 37±0.5ºC. A

volume of 2 millilitres, which is equal to 5 milligrammes of CXB, was added to the donor compartment. To maintain sink condition, samples were taken out at regular intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours) and replaced with an equivalent volume of buffer. Using a UV spectrophotometer, the amount of drug in the samples was measured, and the cumulative percentage of drug that permeated was computed [25]. The transdermal flux (Js) values were computed by dividing the permeability coefficient (Kp) by the slope of the steady portion of the plot between the cumulative amount of drug penetrated vs. time and a diffusion area of Franz diffusion cell (µg/cm²/h). [31, 32]

$$\text{Permeability coefficient } (K_p) = \frac{\text{Flux}}{\text{Initial drug concentration in donor compartment}} \quad (4)$$

RESULTS AND DISCUSSION

Optimization of Ethosomes: For the runs produced by design expert software, the responses were examined. The responses were fitted into a number of models, including cubic, quartic, linear, and quadratic, with the quadratic model providing the best match ($p<0.0001$).

Effect of Independent Variables on Size: Table 1 provides a succinct overview of the replies and their outcomes. After obtaining the response surface graphs (Fig. 1), the interaction effects between the independent and dependent variables were detected. The graph shows that the size of the vesicles rose considerably to roughly two times their original size when the amounts of lecithin were raised from 50 to 200 mg. On the other hand, the size of the vesicles decreased as the ethanol content increased. A polynomial equation produced by the software was used to determine the mathematical relationship after a response surface analysis was carried out.

Table 1. The experimental runs for ethosomal formulations and their responses as predicted by Box-Behnken design.

Runs	Independent Variables			Dependent Variables			
	Et (%)	LC (mg)	ST (sec)	EE (%)		Size (nm)	
				AV*	PV	AV*	PV
1	35	125	37.5	89.21	86.53	126.02	130.21
2	50	125	15	69.45	69.78	223.01	223.45
3	35	200	15	81.21	80.12	362.21	371.24
4	50	125	60	67.45	68.98	253.30	254.45
5	35	125	37.5	88.45	86.01	131.98	130.01
6	35	125	37.5	83.76	85.98	121.42	130.34
7	50	50	37.5	64.05	62.87	159.09	169.49
8	50	200	37.5	78.34	77.58	343.45	330.50
9	35	200	60	76.43	74.05	392.03	402.28
10	35	125	37.5	83.00	85.20	135.00	129.80
11	35	50	60	64.45	64.87	297.05	286.53
12	20	125	15	71.00	84.20	242.00	240.45
13	20	125	60	64.98	64.33	262.01	259.43
14	35	125	37.5	86.42	69.45	140.02	130.07
15	20	50	37.5	63.00	65.55	219.00	231.54
16	35	50	15	64.05	86.45	279.06	267.34
17	20	200	37.5	72.65	62.57	298.76	290.11

Sonication Time-ST; Ethanol-Et; Lecithin LC; Actual Value-AV; Predicted Value-PV

*The actual values denote the practical outcome of the experiment.

A positive number in the equation indicates a diminishing effect, while a negative value indicates a synergistic effect. Whereas factors X2 (lecithin) and X3 (sonication duration) have an inverse association, factor X1 (ethanol) has an augmentative impact and hence increases

the vesicle size. The polynomials' regression coefficient of 0.9901 further indicates a strong model fit, and a linear association between the actual and expected responses was discovered. Furthermore, as seen in Table 1, the actual values closely match the expected values. With a difference of less than 0.2, the adjusted R2 value of 0.9775 and the projected R2 value of 0.8693 are reasonably in accord. The model can be utilised to navigate the design space because the sufficient precision ratio of 26.839 suggests an adequate signal.

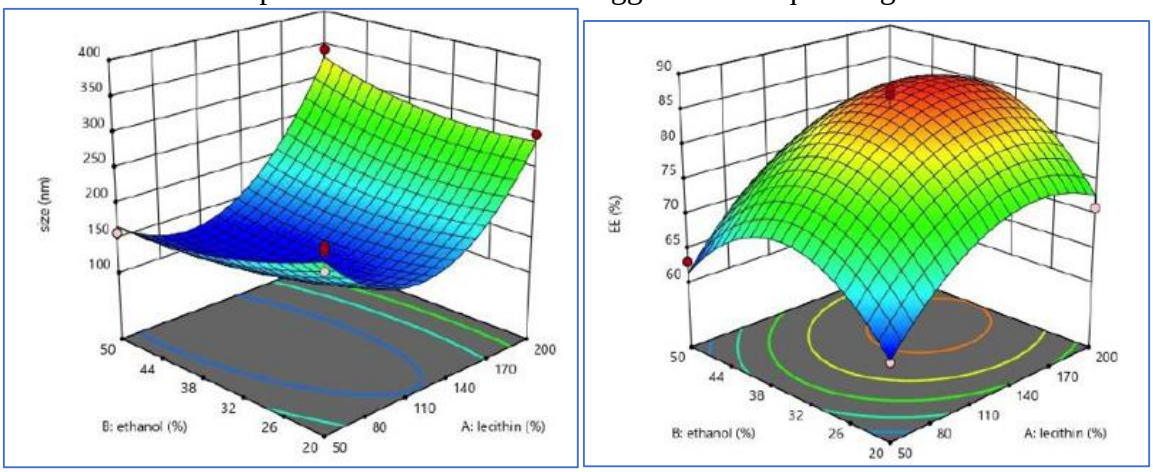

Figure (1). 3-D surface response graph for the effects of lecithin and ethanol on the particles size and drug entrapment (%EE).

Preparation of Optimized Formulation: The software-generated solution variables were used to produce the optimised formulation following extensive evaluation. The best formulation parameters, as determined by the experimental design, were 128.4 mg lecithin, 35.8% ethanol, and a 36-second sonication period. This would potentially result in particles with the smallest particle size (131 nm) and the highest entrapment efficiency (88.8%). The desirability function, which should be near to 1, was used to choose the program's solution.

Effect of Independent Variables on EE: Evaluating a formulation's ability to contain drugs and the dosage form's capability for distribution is crucial. A formulation that is successful will have a high drug load since it will decrease drug waste and enhance therapeutic efficacy. With a sequential p-value <0.0001 and a lack of fit value of 0.5041, indicating the model's relevance, a quadratic model was employed for prediction.

Vesicle Size and Size Distribution: The vesicles were prepared using formulation factors provided by BBD. The size of the measured length of the ethosome was 161.1 nm, which is rather near to the software's estimated size of 131 nm. This verifies the optimization's usage of the BBD architecture (Fig. 2A). Since the PDI measures how homogeneous the particles are, it was also evaluated for the formulation. The optimised formulation's PDI of less than 0.3 (0.295) indicates that the particle sizes in the ethosomal dispersion are homogenous..

Zeta Potential: Zeta potential evaluation is crucial since it provides insight into the formulation's physical stability. It was discovered that the optimised formulation's zeta potential was -31.1 mV. A formulation with a high zeta potential has reportedly been shown to be stable over time. The vesicular formulation's colloidal stability may be explained by the particles' propensity to reject one another [12]. Furthermore, the ethosomes' net negative charge promotes stability and improves the vesicle's capacity to interact with the skin. As such, our formulation is regarded as physically stable. Fig. (2B) displays the size, PDI, and zeta potential analysis plots.

Fig. (2). Vesicle size and zeta potential of ethosome measured by zetasizer.

Vesicular Shape and Morphology: The optimised formulation's TEM investigation revealed spherical ethosomal formulation structures with dimensions ranging from 97 to 130 nm (Fig. 3). The sizes found via TEM analysis and the size analysis produced by the Malvern Zeta Sizer agree.

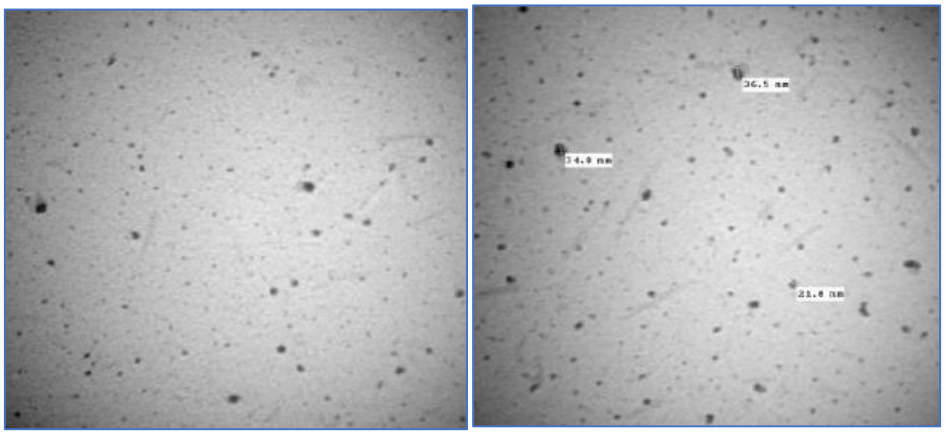

Figure 3: TEM images of optimized ethosome formulation.

In-vitro Release Studies: The optimised formulation was used for the in vitro release research, and its results were compared with those of the drug-loaded hydroethanolic solution. The medication from ethosome first had a burst release impact that lasted up to five hours. This was followed by a regulated release that lasted for twenty-four hours. Conversely, the medication was suddenly liberated from the hydroalcoholic solution. Additionally, it was noted in the release protocol that about 100% of the drug was liberated from the hydroethanolic solution in around 8 hours, while the drug-loaded ethosomal formulation

demonstrated a 24 hour release of 95.87% of CXB (Fig. 4). The formulation's in vitro drug release study results were fitted to various release models, allowing the best-fitting model to link the mechanism of drug release from a drug delivery system. The release protocol of the CXB ethosomal formulation, on the other hand, appeared to follow Higuchi kinetics, as indicated by higher regression coefficient values (R2=0.976) (Table 2), which point to diffusion-controlled release.

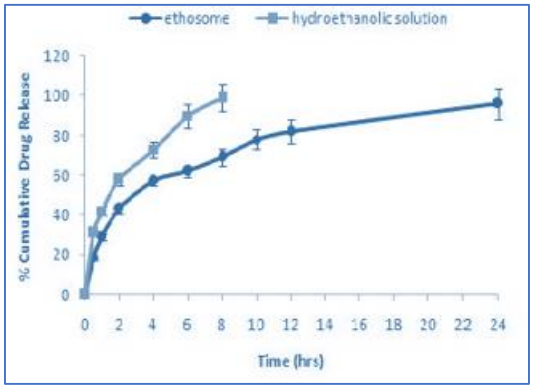

Figure 4: In-vitro drug release profile of optimized ethosome formulation.

Table 2. Drug release kinetics of optimized formulation.

Zero Order	First Order	Higuchi Model	Korsmeyer Peppas Model
0.732	0.998	0.976	0.945

Characterization of Ethosomal Gel: Different concentrations of carbopol 934, 0.5%, 1%, and 2% were employed to make CXB ethosomal trans-gel and evaluated based on various characterisation criteria mentioned in Table 3 in order to prepare a rheologically acceptable formulation. In contrast to the 0.5% and 2% gel, the produced gel containing 1% carbopol displayed good homogeneity and was lump-free. The formulation's viscosity and spreadability were determined to be 6.43 ± 0.32 Pas and 7.65 ± 0.24 gcm/sec, respectively. These results indicate that the formulation is easily applied and has adequate spreadability.

Table 3. Evaluation parameters of drug loaded ethosomaltransgel formulations with different concentrations of carbopol.

Carbopol Concentration	**Homogeneity**	**Mean Viscosity (Pa.s)**	**Mean Drug Content (%)**
0.5%	Presence of lumps, too thick	5.12±0.12	94.54±5.43
1%	Good	6.43± 0.32	97.45 ± 3.16
2%	Satisfactory, Flowy	12.86±1.37	96.76±4.76
Carbopol Concentration	**Mean pH**	**Mean Spreadability (gcm/sec)**	**Mean Extrudability (gm)**
0.5%	6.5±0.34	6.87±0.28	163±4.94
1%	6.3±0.43	7.65±0.24	164±3.74
2%	6.6±0.34	5.76±0.16	131±3.65

Ex vivo Studies: One crucial metric for assessing how much of a medication permeates the skin from the vesicles is the ethosomal formulation's skin penetration. Plotting the cumulative amount of CXB permeated per unit area of excised rat skin against time (Fig. 5) revealed a significant difference ($p<0.05$) between the amount of drug permeating from the ethosome formulation and the hydroalcoholic solution. after contrast to the 54µg that permeated the hydroalcoholic solution after 24 hours, about 350µg of the medication infiltrated the ethosome formulation.

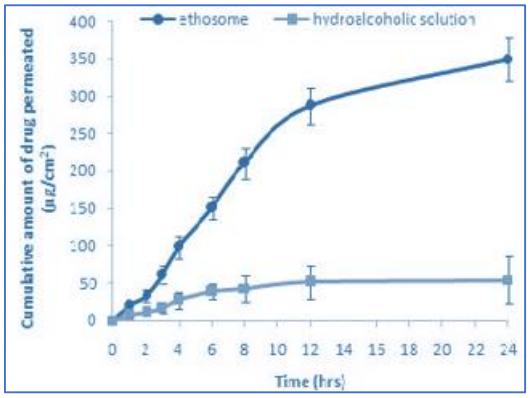

Figure 5: Ex-vivo permeation profile of optimized ethosome formulation.

The graph's linear portion's slope was used to get the steady-state flux value. The transdermal CXB flow from ethosomes (19.90 µg/cm2/h) was substantially ($p<0.05$) higher than that of the hydroalcoholic solution (2.92 µg/cm2/h), indicating that the drug reaches the deeper layers of the skin and that the formulation has a reasonably long residence time on the skin. Furthermore, the hydroalcoholic and ethosome gels showed permeability coefficients of 0.001461 cm2/h and 0.009952 cm2/h, respectively, indicating a better penetration profile of our formulation.

Confocal Microscopy Study: After applying an ethosomal gel formulation and a hydroalcoholic solution containing Rhodamine B for eight hours, the skin of a killed rat was examined using confocal laser scanning microscopy. Getting medication into the stratum corneum is the hardest part of topical administration. It is simple for the medication or formulation to absorb through deeper skin layers once it has passed through the stratum corneum. The penetration of Rhodamine B ethosome and hydro-alcoholic formulations through the skin at varying intensities is depicted in Fig. 6.

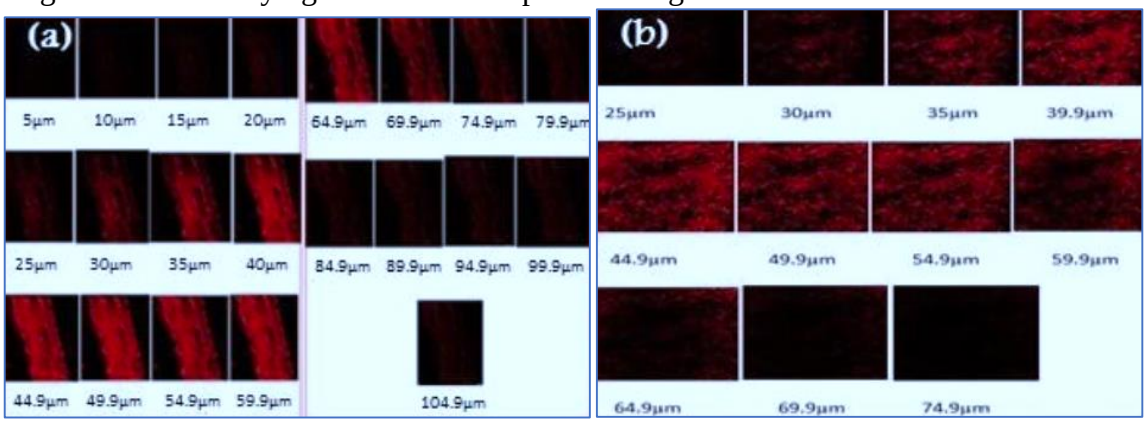

Figure 6: Penetration of Rhodamine B from (a) hydroalcoholic solution (b) optimized ethosome analyzed by confocal microscopy.

CONCLUSION

In conclusion, the ex-vivo release experiments verified that the ethosome formulation had an ideal size range for skin penetration. When compared to its hydroalcoholic solution, the confocal investigations demonstrated greater promise and efficacy. The optimisedethosomal formulation was effectively added to a carbopol gel and characterised by rheological analysis, drug content, homogeneity, extrudability, and spreadability. Thus, the formulation of CXB ethosome shows promise as a therapeutic strategy for arthritis. The study's findings suggest that our formulation, which has advantages over the traditional formulation in terms of dose reduction, frequency of administration, and patient compliance, may be a good substitute.

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