



Formulation and Optimization of Zaltoprofen Loaded Ethosomal Gel by using 2³ Full Factorial Designs

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KA and SP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DSS, PN, PK and AK engaged the analyses of the study. Authors CLL and SPK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim:The objective of the present study is to design and characterize the ethosomal gel containing Zaltoprofen for sustained drug delivery and also to reduce the side effects. Zaltoprofen was chosen here as the drug candidate because of its short half-life and increased dosing frequency.

Methods: The ethosomes containing Zaltoprofen was prepared by using cold method. A 2³ full factorial design containing 10 experimental trails was used in order to obtain an optimized formulation. The prepared ethosomes were characterized by Scanning Electron Microscopy, PDI, zeta potential, vesicle size, and percentage entrapment efficiency. Optimized ethosomal formulation was incorporated in 1% carbopol gel to deliver the drug through topical route. *In vitro* drug permeation studies of ethosomal gel (EGL) and conventional gel (CGL) was conducted and flux and permeability coefficient were calculated.

Results:The vesicle size, zeta potential, and % entrapment efficiency of optimized formulation were found to be 124.33 nm, -45.2 mV and 70.03%, respectively. The surface of the vesicles was found to be spherical and smooth. The *in vitro* drug release studies of the ethosomal gel

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formulation showed sustained drug delivery when compared with the conventional gel containing the pure drug. In vitro permeability studies show that the flux of ethosomal gel was 2.5 fold higher than conventional gel, which may be the attribution of ethanol and flexible nature of ethosomes.
Conclusion: It was concluded that the ethosomal gel could be a better choice for the topical delivery of Zaltoprofen with improved bioavailability for its anti-inflammatory activity.

Keywords: Zaltoprofen; soyaphosphatidyl choline; ethanol; full factorial design; ethosomes.

1. INTRODUCTION

Rheumatoid arthritis is an autoimmune disorder which affects around 1% of Indian population. It is one of the common inflammatory diseases of unknown etiology categorized by inflammation of the synovial membrane which causes swelling of joint and thickening of synovial membrane due to increased synovial exudates [1,2]. The disease resulted in shortened life span and morbidity. The manner of development of the disease was mainly emphasized on immune complexes and auto-antibodies at the early stages and later it was found that the tumor like behavior of rheumatoid synovial membrane and T-cell-independent cytokine networks are also involved [3,4].

In the management of rheumatoid arthritis non-steroidal anti-inflammatory drug (NSAIDs) plays an important role. They are used to reduce the pain and inflammation. Most of the NSAIDs differ in side effects even though the efficacy is same at equivalent doses. The adverse effect includes gastrointestinal irritation and renal toxicity. Zaltoprofen is a propionic acid derivative of NSAIDs, which has excellent effects on post-surgery or post trauma chronic inflammation. It selectively inhibits COX-2 activity and prostaglandin E2 production. It is used in treatment of rheumatoid arthritis, osteoarthritis, and other chronic inflammatory pain conditions [5]. Although zaltoprofen is well tolerated orally compared to other NSAIDs, it has to be administered in three to four doses per day and was associated with ulcerogenicity, bellyache and indigestion. This makes administration of zaltoprofen unsuitable for patients with gastric ulcer and is also associated with drug interactions [6]. The peak plasma levels of Zaltoprofen are obtained after 1.6 hours of intake. It has lower oral half-life of 4 hours [7,8]. Zaltoprofen has poor aqueous solubility and poor oral bioavailability as it belongs to BCS Class II drug [9]. Further it undergoes rapid first pass metabolism after its oral administration and add on in lowering the bioavailability of drug, hence the drug is required higher doses to show the therapeutic efficacy. Therefore, it is important to

develop an alternative dosage form which is easier to administer and avoids first-pass metabolism. The transdermal route meets all the above advantages [10].

The topical drug delivery systems aids in the application of the drug on the surface of the skin for treating the skin diseases and inflammatory conditions. The topical delivery system by passing the first pass metabolism of drug. But one of the major challenges of the topical drug delivery system is to permeation of a drug through the stratum corneum barrier [11,12]. Various techniques such as sonophoresis, iontophoresis, etc. have been studied to enhance the permeation of drug through the skin. Even the lipid vesicular systems like liposomes, ethosomes, niosomes and transferosomes show better permeability [13].

Ethosomes are non-invasive drug delivery systems which are soft, malleable vesicles mainly consist of phospholipid, high ethanol concentration and water. Ethosomes have a size range from nanometers to microns. They are slight modifications of liposomes which contain high amount of ethanol that enables the penetration through stratum corneum [14,15]. And also higher concentration of ethanol makes the vesicles flexible, soft and improves stability. They enhance the delivery of the therapeutically active agents to the deep layers of skin and provide improved bioavailability [16,17].

Further these ethosomes are incorporated into gel for topical delivery of the drug. Gels are semisolid preparations consisting of small inorganic or large organic particles interpenetrated by liquid intended to be applied on skin. Gels are two phase system with solid like properties containing the presence of several form of continuous structure. A synthetic or natural polymer at a comparatively less amount forms a 3 dimensional matrix in a hydrophilic liquid [18].

Therefore in the present study, Zaltoprofen loaded ethosomes was prepared and their formulation parameters were statistically

optimized using 2^3 full factorial designs. The optimized formulation was incorporated into a suitable 1% carbopol gel to improve skin retention during transdermal application.

2. MATERIALS AND METHODS

2.1 Materials

Zaltoprofen was obtained from Ipca Laboratories (Mumbai). Soyaphosphatidyl choline (SPC) and cholesterol were procured from Hi media laboratories (Mumbai, India). Potassium dihydrogen phosphate and propylene glycol used were of analytical grade (Lobachemie, Mumbai). All other chemicals and reagents used were analytical grade unless otherwise indicated.

2.2 Methods

2.2.1 Formulation of ethosomes

2.2.1.1 Design of experiments

The DoE approach is mainly helps in determination of relationship between the input factors (independent variables) affecting the one or even more output responses (dependent variables), through the establishment of mathematical models. Based on initial studies taken place in-house and reports printed and published elsewhere, they recognized the key factors which influenced the formation of ethosomes [19,20]. The major factors (independent variables) were identified from literatures as the concentration of SPC(A), concentration of ethanol (B), and Concentration of cholesterol (C) which play major role in the formation of ethosomal vesicle size and entrapment efficiency [21,22]. Hence these three factors were considered for designing of experiment as shown in Table 1. To optimize their concentration 2^3 full factorial design with 2 centre points was adapted, wherein the dependent variables were entrapment efficiency, vesicle size, and zeta potential [23].

The response obtained of the 10 run was subjected for multiple regression analysis using Design Expert® software (version 11.0.3.0 64-

bit, Stat-Ease, Inc. Minneapolis, MN, USA). The model obtained from regression analysis is expressed in the form of following equation,

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 BC + \beta_5 AC + \beta_6 ABC$$

Where $\beta_0 - \beta_6$ are the standard beta coefficients for the observed experimental values of Y_i , and AB, BC, AC & ABC are the interaction terms. And they represent the polynomial terms of the independent variables. A is the concentration of soyaphosphatidyl choline, B is the ethanol concentration and C is the concentration of cholesterol. And BC, AC, ABC are the interaction terms. The positive and negative signs of the coefficient values in the equations obtained after data analysis represent the agonists and antagonist effect of the independent variables while the magnitude of the beta coefficients represents the extent of impact of corresponding independent variable [24]. While the magnitude of the beta coefficients represents the extent of impact of the corresponding independent variables. Polynomial terms were included in the model to account for the curvature effect of independent variables, if any. Statistically significant F ratio ($p < 0.05$) and adjusted coefficients of determination (adjusted R^2) between 0.8 and 1.0 were set as criterion for adequacy of the model [25].

2.2.1.2 Preparation of ethosomes

Preparation of ethosomes loaded with zaltoprofen was prepared by cold method as per Table 2. Ethosomal suspension was prepared by dissolving soya lecithin, drug and cholesterol in 3 ml of ethanol in a magnetic stirrer. This ethanolic mixture was heated at 30°C in water bath during which propylene glycol was added. Then 6ml of water was added to the ethanolic mixture while stirred at 700 rpm. The solution was continuously stirred for 15 min to allow the formation of ethosomal vesicles [26]. Then the final formulation were sonicated for 5 minutes time in a probe sonicator to obtained the nanosized of ethosomal.

Table 1. Factors and levels

Independent Variables	Levels	
	Low	High
SPC(X1, mg)	250	1500
Ethanol (X2, %)	10	40
Cholesterol (X3, mg)	25	250
Dependent Variables	Vesicle size (nm) Y1 Zeta potential (mV) Y2, Entrapment efficiency (%) Y3	

Table 2. Composition of ethosomes as given by 2³ full factorial designs for 10 ml formulation

Form. Code	Drug mg	SPC mg X1	Ethanol3ml of (% v/v) X2	Cholesterol mg X3	Propylene glycol ml	Water ml
F1	100	1500	40	250	1	6
F2	100	250	10	250	1	6
F3	100	250	40	25	1	6
F4	100	1500	10	25	1	6
F5	100	875	25	137.5	1	6
F6	100	250	10	25	1	6
F7	100	1500	10	250	1	6
F8	100	1500	40	25	1	6
F9	100	875	25	137.5	1	6
F10	100	250	40	250	1	6

2.2.2 Characterization of Zaltoprofen ethosomes

2.2.2.1 Vesicle size, size distribution and zeta potential

The average particle size and size distribution of ethosomes was determined by Zeta Sizer by dynamic light scattering (Nano ZS, Malvern Instruments, UK). Formulation was suitably diluted with distilled water and measured for the above parameters. The Zeta potential of a particle is the overall charge that the particles obtain in a particular medium. Zeta potential values help to assess the stability of the formulation [27].

2.2.2.2 Percentage drug entrapment efficiency

Take 10 ml of the ethosomal suspension in Tarsus centrifuge tube of 15 ml capacity and it is centrifuged by cold centrifugation at 12000 rpm for 1 h at 4°C. After centrifugation the supernatant and the sediment are separated. The concentrations of zaltoprofen present in the supernatant were analyzed by UV spectroscopic method at 338 nm [28] and amount of drug was calculated by using regression equation ($y = 0.0697x$, $R^2 = 0.9987$) which was obtained from the standard plot. The percentage entrapment efficiency was calculated using the following formula [29-31].

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{amount of drug in supernatant}}{\text{Total amount of drug}} \times 100$$

2.2.2.3 Formulation and characterization of optimized batch

The optimization of ethosomes was carried out by entering the responses with minimum particle size, zeta potential within a range and maximum

entrapment efficiency in DoE software. The software generated the solution with desirability ≥ 8 was selected as an optimized formulation. As per the solution generated by the software; 738.802 mg SPC, cholesterol 25.917mg and 10% ethanol were used to formulate the optimized formulation.

Optimized formulation was also characterized as the factorial batches.

Further shape and surface morphology of optimized formulation was performed by Scanning Electron Microscopy and Transmission Electron Microscopy.

2.2.3 Formulation of ethosomal gel incorporated with zaltoprofen

The ethosomal and conventional gels of Zaltoprofen were prepared as per Table 3, by incorporating optimized ethosomal suspension and pure drug in the 1% carbopol 934 gel. 1g of carbopol was dissolve in 100ml distilled water and it was stirred for 2 hours and it was left out for overnight to swell. Ethosomal suspension (equivalent to 100 mg of drug) and 100 mg of drug were added to 10 g of 1% carbopol 934 gels respectively to get 1 % ethosomal and conventional gel. It was stirred continuously by introducing triethanolamine to achieve the skin pH [32].

2.2.4 Evaluation of gel incorporated with zaltoprofen ethosomes

2.2.4.1 Measurements of pH

Here 100 mg of approximately weighed quantity of the ethosomal gel was diluted with 10 ml distilled water. Then pH meter was placed or immersed in the prepared gel solution. The experiment was conducted in three times and the average value was calculated [33].

Table 3. Composition of conventional gel (CGL) and Ethosomal gel (EGL)

Form Code	Drug	carbopol 934	Glycerine	Triethanolamine	Water
CGL	100 mg drug	1%	0.2 ml	q.s.	q.s 10 g
EGL	Optimized ethosomal suspension equivalent to 100 mg drug	1 %	0.2 ml	q.s.	q.s 10 g

2.2.4.2 Viscosity

Viscosity measurements were done by the Brookfield viscometer, DV-II+pro D220 by selecting the spindle number 94 at different rpm. Weigh approximately 30 g of gel and placed in 50 ml beaker; such that the spindle groove must be dipped in the formulation and different rpm was set (5, 10, 50, and 100) and the dial reading was measured [34].

2.2.4.3 Spreadability

It is the term which determines the extent of area to which the gel spreads on application to the surface of the skin. "Spreadability was done by two slides to slip off from the portion placed in between the slides under the direction of certain load. The spreadability is better when the separation of gel between two slides takes lesser time. It is calculated by using the formula.

$$S=(M \times L) / T$$

Where M= weight tied to the upper slide, L= length of glass slides , T= time taken to separate the slides.

Weigh approximately 0.1 g of ethosomal gel and placed between the two slides which is left for about 5 min where no more spreading was expected. The diameter of the spreaded circles was measured in cm and was taken as comparative values for spreadability. The standardized weight of 125 g was tied on upper slide with the help of string. The results obtained are average of three dimensions [31].

2.2.4.4 Drug content determination

500 mg of the formulation was taken and dissolved in 10ml methanol and filtered. The volume was made up with phosphate buffer. Where 1ml of the above solution was withdrawn and made up to 10ml with phosphate buffer. Hence absorbance was measured at

338 nm using UV-Visible spectrophotometer [35].

2.2.4.5 In vitro drug release studies of ethosomes

The *in vitro* drug release studies were carried out using the Franz diffusion cell. The donor compartment consists of two open ends where one end is covered with the diffusion membrane, which was previously soaked in the phosphate buffer of pH 7.4. The 200 mg (equivalent to 2mg of drug) of gels was placed on the diffusion membrane. Reservoir compartment was filled with 30 ml phosphate buffer of pH 7.4 which contained a small magnetic bead rotated at constant speed of 50 rpm, and the temperature was adjusted to 37±0.5°C. The sample was withdrawn at the periodic intervals for 8 h and thus it is replaced with the fresh buffer solution to maintain the perfect sink condition. The absorbance of the sample was measured at 338 nm using UV-Visible spectrophotometer after proper dilutions using phosphate buffer pH 7.4 [36].

2.2.4.6 Drug release kinetics

The data obtained from drug release profile studies was subjected to kinetic analysis for first order (log cumulative percentage of drug v's time), and zero order kinetics (cumulative amount of drug released v's time). Mechanism of drug release was determined by fitting to korsmeyer-peppas model (log cumulative percentage of drug released v's log time) and Higuchi's matrix model (cumulative percentage of drug release v/s square root of time) for *in vitro* kinetic study.

2.2.4.7 In vitro skin permeation studies

In vitro skin permeation studies were performed through goat ear skin by using Franz diffusion cell in similar way to *in vitro* drug release studies. Here the goat ear skin was collected from the slaughter house; hair was removed from the skin and was placed in the phosphate buffer pH 7.4. 200 mg (equivalent to 2mg of drug) of gels were

applied on dermal side of the skin in donor compartment [37].

The steady state flux (J_{ss}) was calculated against a time (h) plot from the slope of the linear part of the cumulative amount of drug permeated per unit area ($\mu\text{g}/\text{cm}^2$). The permeability coefficient (K_p) of extract through the goatskin was determined using the following equations:

$$\text{Permeability coefficient } (K_p) = \frac{J_{ss}}{C}$$

Where; C is the concentration of gel. The findings were analyzed using GraphPad software, by using paired T-test.

3. RESULTS AND DISCUSSION

The ethosomes containing Zaltoprofen were prepared by Cold method by applying 2^3 full factorial designs to understand the effects of the ethosomal constituent. Nanosized ethosomes were attained by sonicating the final formulation for 5 minutes through probe sonication and formulation are shown in Fig. 1. Responses of factorial batches are shown in Table 4 and 5.



Fig.1. Ethosomes after sonication

3.1 Characterization of Ethosomes

3.1.1 Vesicle size of ethosomes

Vesicles size plays a major role in skin permeation of ethosomes as per literature reported. The effect of ethanol, cholesterol and SPC concentration on the vesicle size of the formulation were obtained from the 2^3 full factorial designs. Results shown in (Table 4 and 4), depicts that increase in concentration of ethanol from 10% to 40% v/v, there was a decrease in the vesicle size at all concentration of SPC concentration. This may be due negative charge provider by ethanol for the surface of ethosomes, thereby preventing the aggregation of the vesicular system due to electrostatic repulsion and resultant shows reduce vesicle size. It may also be at high concentrations of ethanol causes interpenetration of the ethanol hydrocarbon chain, which leads to a reduction in

vesicular membrane thickness and hence reduces vesicular size [38]. As the concentration of cholesterol increases more cholesterol molecules will be distributed in the phospholipid bilayer, causing an increase in the ethosomal mean diameter at a particular SPC & ethanol concentration. It has been reported that higher cholesterol concentrations interferes with the close packing of the phospholipid bilayer by contributing to an increase in membrane fluidity which results in an increased distribution of aqueous phase within the liposomal vesicles [39,40].

Regression analysis was then applied to further understand the effect of formulation variables simultaneously on the vesicle size. Polynomial model implied significant with model f-value of 24.14. The Predicted R^2 of 0.7353 is in rational agreement with the Adjusted R^2 of 0.9278; i.e. the difference is less than 0.2. The results are summarized in (Table 5). The polynomial equation obtained from the results of the analysis:

$$\text{Vesicle size} = +150.94 - 7.36(B)^* + 21.08(C)^* + 7.91(AB)^* - 8.95(BC)^* + 5.89(ABC)$$

Where A is the SPC, B is the ethanol concentration and C is the cholesterol concentration, the coefficient in this equation represents the standardized beta coefficient and the asterisk sign indicates significance of the variable. The obtained regression model was found to be statistically significant ($p < 0.05$) with a high adjusted R^2 value of 0.9278. The significance of the polynomial terms on the vesicle size indicates that the model has a curvature at higher levels of formulation variables. The effect of linear and interaction terms on the vesicle size is also represented simultaneously in response surface graph in (Fig. 2).

3.1.2 Zeta potential

Zeta potential is an essential parameter that can influence vesicular stability. Aggregation of vesicles during storage is prevented by electrostatic repulsive force generated by charged particles. Effect of ethanol and cholesterol on zeta potential was shown in (Table 4). At all concentration of SPC & cholesterol and the ethanol provides a concentration dependent surface negative charge to polar head region of SPC, which prevent, or at least delaying the formation of vesicle aggregates, due to the electrostatic repulsions [29]. And at respective

ethanol concentration, the increase in produce the significant effect on the zeta potential I.
 concentration of SPC and cholesterol did not

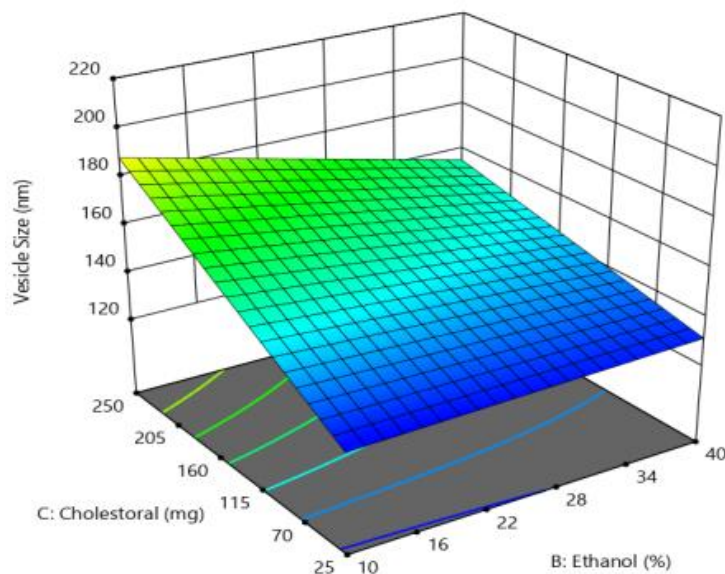


Fig. 2a

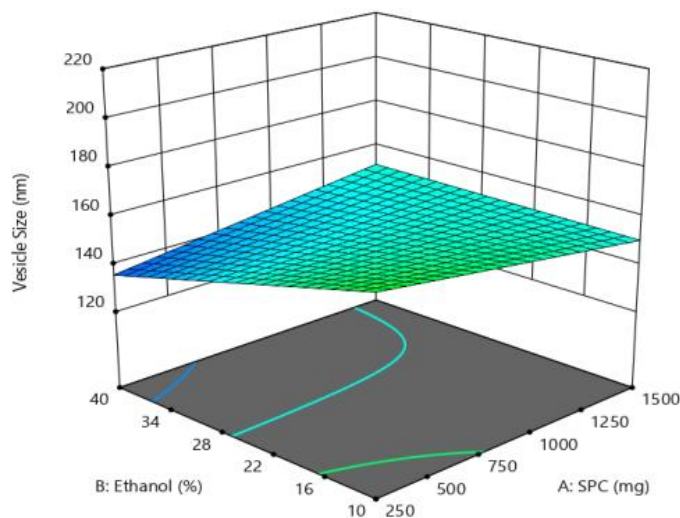


Fig. 2b

Fig. 2. Response surface curve representing the effect of a) cholesterol and ethanol & b) the effect of ethanol and SPC on the vesicle size of ethosomes

Regression analysis was then applied to further understand the effect of formulation variables simultaneously on the Zeta potential. Polynomial model implied significant with model f-value of 15.48. The Predicted R² of 0.7670 is in rational agreement with the Adjusted R² of 0.8894; i.e. the difference is less than 0.2. The results are summarized in (Table 5). The polynomial

equation obtained from the results of the analysis:

$$\text{Zeta potential} = -54.19 - 6.57(A)^* - 2.90(B)^* - 2.13(C) - 0.6963(AC)^* + 2.92(BC)^*$$

Where A is the soya phosphatidyl choline, B is the ethanol concentration and C is the

cholesterol concentration, the coefficient in this equation represents the standardized beta coefficient and the asterisk sign indicates significance of the variable. The obtained regression model was found to be statistically significant ($p < 0.05$) with a high adjusted R^2 value of 0.8894. The significance of the polynomial terms on the % entrapment efficiency indicates that the model has a curvature at higher levels of formulation variables. The effect of linear and interaction terms on the zeta potential is also represented simultaneously in response surface graph in Fig. 3 and Table 5.

3.1.3 Percentage entrapment efficiency

The effect of ethanol and SPC concentration on the entrapment efficiency of formulations

obtained from the 2^3 full factorial design showed in Table 2; that with the increase in ethanol concentration at all SPC concentration, the entrapment efficiency of ethosomal formulations decreased significantly. This is due to the partial fluidization bilayers of lipid by ethanol, resulting in leakage of entrapped. As the concentration of SPC increases at all ethanol concentration, the entrapment efficiency decreases since at higher concentration, chances of aggregation of vesicles increase. As the aggregation increases, the ability to form a stable film surface also decreases. As a result of this, drug leaching occurs and thereby entrapment efficiency decreases accordingly. And results show that there is no much effect by cholesterol on the entrapment efficiency.

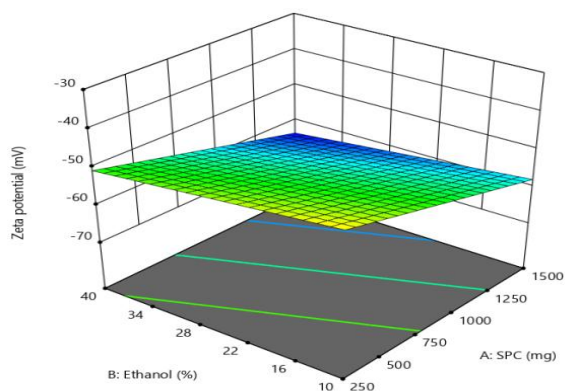


Fig. 3a

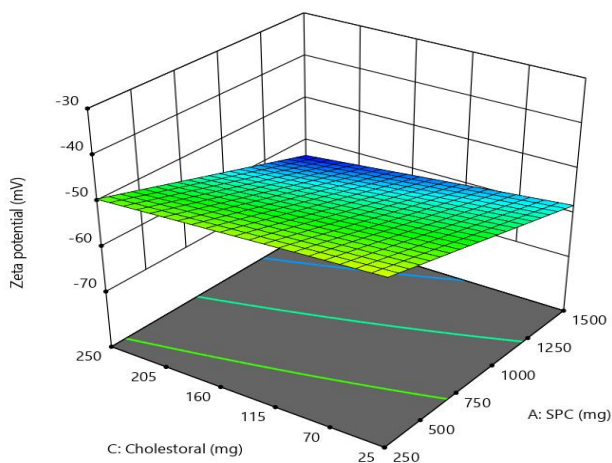


Fig. 3b

Fig. 3. Response surface curve representing the effect of a) SPC and ethanol & b) SPC and cholesterol (b) on the zeta potential of ethosomes

Regression analysis was then applied to further understand the effect of formulation variables simultaneously on the % entrapment efficiency (%EE). Polynomial model implied significant with model f-value of 13.29. The Predicted R² of 0.7416 is in rational agreement with the Adjusted R² of 0.8452; i.e. the difference is less than 0.2. The results are summarized in (Table 4). The polynomial equation obtained from the results of the analysis:

$$\% EE = +59.42 - 9.40(A)^* - 9.13(B)^* - 4.70(AB) - 2.15(ABC)$$

Where A is the SPC, B is the ethanol concentration and C is the cholesterol concentration, the coefficient in this equation represents the standardized beta coefficient and the asterisk sign indicates significance of the variable. The obtained regression model was found to be statistically significant (p<0.05) with a high adjusted R² value of 0.8452. The significance of the polynomial terms on the % entrapment efficiency indicates that the model has a curvature at higher levels of formulation

variables. The effect of linear and interaction terms on the entrapment efficiency is also represented simultaneously in response surface graph (Fig. 4 & Table 5).

3.1.4 Formulation and characterization of optimized batch

The optimization of ethosomes based on all the responses with minimum particle size, zeta potential within a range and maximum entrapment efficiency was carried out. The optimized formula was selected based on the desirability more than 0.8. The selected formulation was prepared according to the software by incorporating 738.802 mg SPC, cholesterol 25.917 mg and 10% ethanol, and observed vesicle size, zeta potential, and % entrapment efficiency were found to be 124.33 nm, -45.2 mV and 70.03%, respectively. The observed value was found to be 95% confidence interval of predicted value i.e., below 5% error which is acceptable as show in Table 6 and Fig. 5.

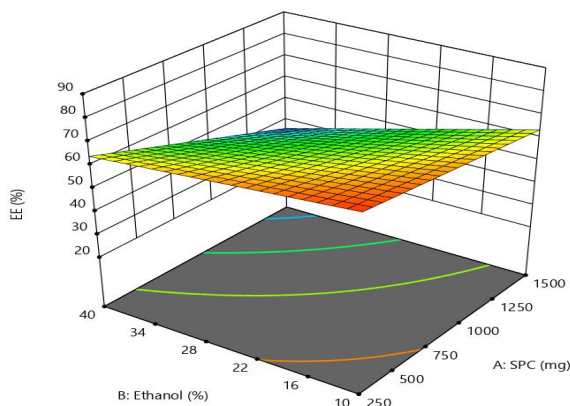


Fig. 4. Response surface curve representing the effect of SPC and ethanol on the % entrapment efficiency of ethosomes

Table 4. Result of response of ethosomes as per 2³ factorial designs

Formulation Code	Vesicle size (nm) Y1	Zeta potential (mV) Y2	% EE Y3	PDI
F1	166.3±3.43	-62±2.21	32.08±1.32	0.46±0.50
F2	208.03±2.16	-47.5±1.06	74.08±1.45	0.45±0.33
F3	129.4±4.23	-51.7±1.67	65.5±3.56	0.27±0.22
F4	128.96±3.75	-51.8±2.43	63.39±2.44	0.28±0.52
F5	142.7±1.54	-56.28±1.24	56.96±3.21	0.45±0.12
F6	130.26±3.05	-39.2±3.55	75.39±1.12	0.22±0.62
F7	171.33±3.56	-63.7±4.56	67.3±2.32	0.41±0.32
F8	136.2±2.44	-62.6±2.34	43.3±4.30	0.39±0.21
F9	148.4±1.65	-58.03±3.45	49.9±2.15	0.42±0.32
F10	147.8±2.14	-49.13±3.23	66.26±2.05	0.39±0.31

*Mean of 3 replications ± SD

Table 5. Summary of regression analysis and ANOVA

Sl. No	Factor	Vesicle size (Adjusted R ² =0.9278)		% EE (Adjusted R ² =0.8452)		Zeta potential (AdjustedR ² =0.8894)	
		Estimated beta coefficient	P value	Estimated beta coefficient	P value	Estimated beta coefficient	P value
1.	Intercept	+150.94	0.0044*	+59.42	0.0071*	-54.19	0.0101*
2.	A-SPC	-	-	-9.40	0.0046*	-6.57	0.0020*
3.	B- ethanol	-7.36	0.0359*	-9.13	0.0052*	-2.90	0.0340*
4.	C- Cholesterol	+21.08	0.0009*	-	-	-2.13	0.0811
5.	AB	+7.91	0.0287*	-4.70	0.0592	-	-
6.	BC	-8.95	0.0194*	-	-	+2.92	0.0334*
7.	ABC	+5.89	0.0676	-2.15	0.3163	-	-
8.	AC	-	-	-	-	-0.6963	0.04902*

Table 6. Comparison of vesicle size, zeta potential and % EE between experimental and theoretical values for the validation of model established for formulation optimization

Independent Factors			Responses		
SPC (mg)	cholesterol (mg)	Ethanol (%)	Vesicle size (nm)	Zeta Potential (mV)	EE (%)
738.802	25.917	10	Predicted Mean		
			128.96	-45	70.0312
			Observed Mean		
			124.33	-45.2	69.35
% Error			3.72	0.44	0.98

Results

Z-Average (d.nm): 123.0
Pdl: 0.272
Intercept: 0.914
Result quality Good

	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1:	151.1	97.0	61.54
Peak 2:	4834	3.0	708.1
Peak 3:	0.000	0.0	0.000

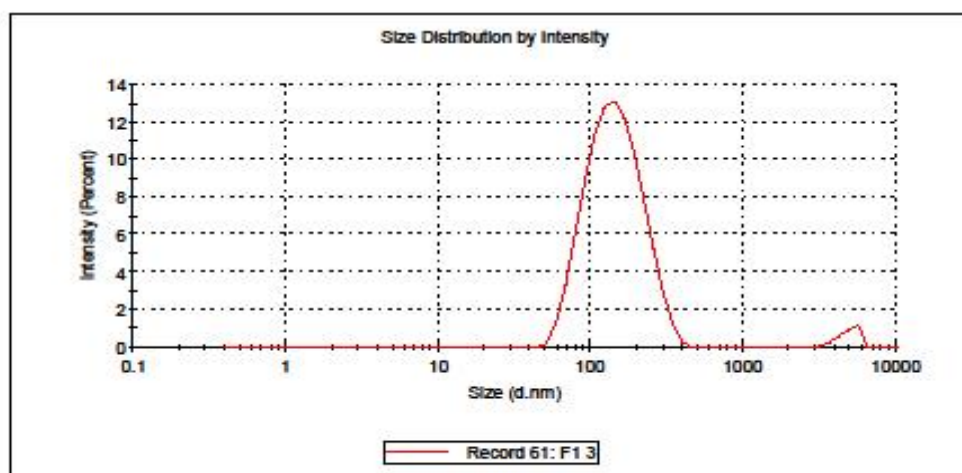


Fig. 5a

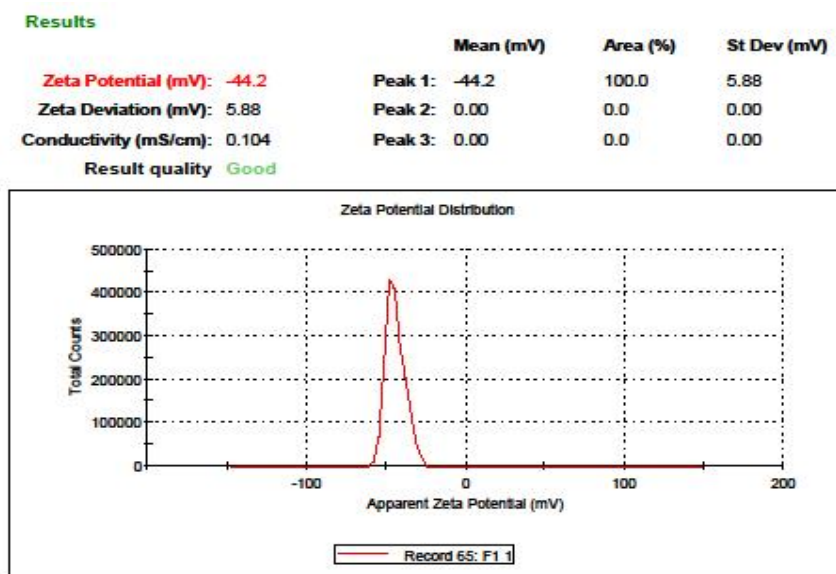


Fig. 5b

Fig. 5. a: Size distribution 5b: Zeta potential of optimized formulation of ethosomes.

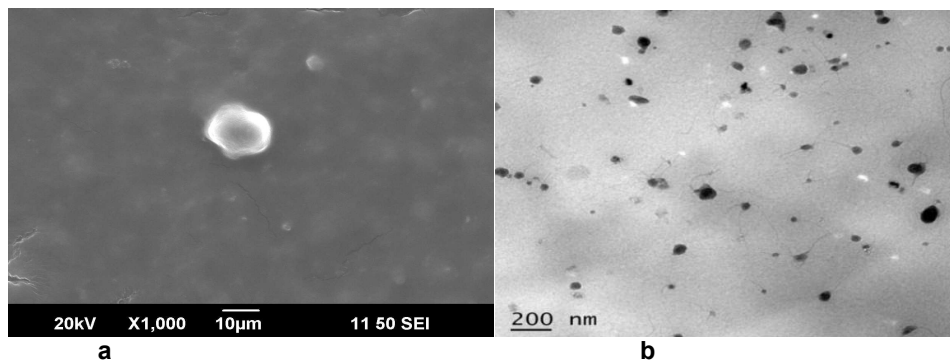


Fig. 6. a) SEM of optimized ethosomal vesicle b) TEM of optimized ethosomal vesicle

3.2 Surface Morphology

The vesicle shape and surface morphology of the formulations was determined by the SEM analysis and Transmission electron microscopy (TEM). The vesicles observed were spherical, smooth, and uniform in size with less than 200nm vesicle size.

3.3 Formulation and Characterization of Ethosomal Gel Incorporated with Zaltoprofen

Ethosomal (EGL) and conventional (CGL) Gels were homogenous, off white in color, smooth, and free from grittiness. Both gel formulations were characterized by the measurement of pH, spreadability, drug content and viscosity, and the

results are shown in Table 7. The pH of the both gel formulations was measured thrice and the mean was noted. Thus pH of the Ethosomal (EGL) and conventional (CGL) gels were found to be 6.83 and 6.9 respectively, which lie in normal pH range of skin. This signifies that the formulation does not cause any irritation on the skin. The spreadability of the ethosomal gel EGL was found to be 13.51g/cm². Thus it indicates that the prepared gel is easily spreadable by small amount of shear which specifies good spreadability. The viscosity of the formulation EGL and CGL was determined by Brookfield viscometer at 5, 10, 50, 100 rpm. The viscosity of the formulation is decreased with increase in the shear rate i.e pseudoplastic behavior was noted. Percentage drug content was found to be more than 85%.

3.4 *In vitro* Drug Release from Ethosomal Gel

In vitro drug release profile of EGL and CGL was performed and result show that drug release from EGL was slow and less when compared to CGL (shown in Fig. 7) .From this, we can conclude that the decreased percentage of drug release was due to more compact wall around the drug by biodegradable lipid and it showed a sustained release pattern for prolonged time period.

The maximum amount of drug release after 480 min from ethosomal gel (EGL) was found to be 80.14%, whereas 100 % of drug released from CGL within 360 min. The release kinetics of the ethosomal gel (EGL) and conventional gel (CGL) was studied by various kinetic models shown in Table 8. Both EGL and CGL gels followed zero order release kinetics with high regression coefficient (R^2) of 0.931 and 0.854 respectively when compared to zero order kinetic models which has a regression coefficient (R^2) of 0.690 & 0.797 respectively. The mechanism of drug release from gel formulations were studied by fitting the data in korsmeyer Peppas and Higuchi model. Higuchi model showed higher the regression coefficient (R^2) value when compared to the korsmeyer Peppas model. It showed drug

release by swelling of the polymer and diffusion through the matrix.

3.5 *In vitro* Drug Permeability Study

Fig. 8, demonstrated the permeation profile of drug from EGL and CGL. The total quantity of drug delivered from EGL was found to be 1810.4 μg , which was substantially higher than the quantity found that the delivered by extract solution, which was 799.2 μg ($p < 0.0001$). The enhanced permeation of drug from EGL may be the presence of ethanol in the core of Ethosomes which helps to solubilize the lipid in the stratum corneum, allowing high vesicles penetration. Parameter of permeability, as shown in Table 9, in the case of EGL the steady-state flux was greater than the CGL. The steady-state flux and permeability coefficient of EGL was found to be 3.688 $\mu\text{g}/\text{cm}^2\cdot\text{min}$ & 0.0379×10^{-3} cm/min respectively whereas for CGL was shown to be 1.479 $\mu\text{g}/\text{cm}^2\cdot\text{min}$ & 0.0148×10^{-3} cm/min respectively after 360 minutes. Results indicated that the flux and permeability coefficient of EGL is 2.5 fold higher than CGL It was found that there is a direct relationship between steady-state flux and permeability of coefficients. The previous results could be attributed to ethanol content in the ethosomal core which dissolve the lipid of skin and overcome the skin barrier properties [41].

Table 7. Characterization of physicochemical properties of gel

Form. Code	pH	Viscosity (cps)				Spreadability (g/cm ²)	% Drug content
		10	20	50	100		
CGL	6.83	449.6±3.2	279.8±2.4	102.8±2.2	59.4±3.2	13.51	89.50±1.3
EGL	6.9	508.5±2.3	248.4±2.6	106.4±2.3	67.3±2.2	15.64	85.28±2.4

Average \pm SD (n=3)

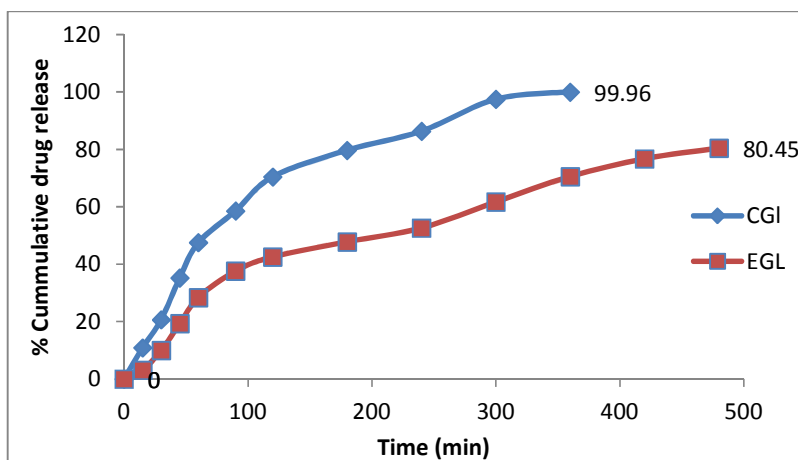
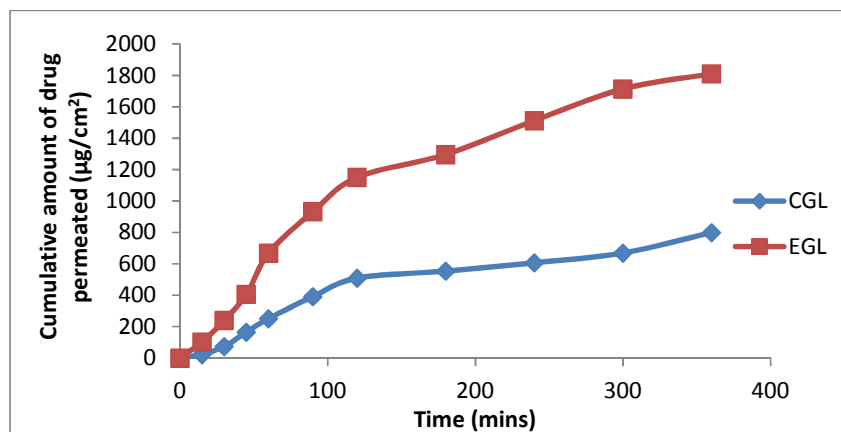


Fig. 7. Comparative *in vitro* drug release studies from CGL and EGL

Table 8. Drug release kinetics of prepared gels obtained from *In vitro* release studies

Form. Code	Kinetic models								
	Zero order		First order		Higuchi		Korsmeyer-peppas		
	R2	K	R2	K	R2	K	R2	K	n
CGL	0.865	-0.254	0.797	-0.072	0.961	-0.174	0.363	2.68	0.412
EGL	0.931	-0.214	0.690	-0.024	0.988	0.056	0.909	0.746	0.544

Fig. 8. Comparative *in vitro* drug permeability study of ethosomal gel (EGL) with conventional gel of ZaltoprofenTable 9. Comparative *in vitro* permeation parameter of prepared gels

Form. Code	Permeated amount at 360 mins (µg/cm ²)	Flux (µg/cm ² .min)	Permeability constant (Kp) × 10 ⁻³ (cm/min)
CGL	799.2	1.479	0.0148
EGL	1810.4	3.688	0.0369

4. CONCLUSION

The present study revealed that skin permeation of Zaltoprofen loaded ethosomes was essentially dependent on the interaction of formulation and physicochemical properties. The vesicle size and zeta potential plays a crucial role in skin permeation of ethosomes. Based on the regression analysis, it was established that vesicle size and zeta potential are significantly affected by the concentration of ethanol. *In vitro* permeability study supported that ethosomal gel has more permeability than conventional gel.

DISCLAIMER

There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for

the advancement of knowledge. Also, the research was not funded.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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