Effect of Physicochemical and Rheological Properties of Ibuprofen Loaded Ethosomal Gel and its Optimization by 3^2 Factorial Design

B Sudhakar [1*], Atmaram pawar [1], N Madhavi [2]

[1]Department of Pharmaceutics, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune, Maharashtra-411030, India
[2] Department of Pharmaceutics, CMR College of Pharmacy, Affiliated to JNTUH, Hyderabad, Telangana-501401, India.

Corresponding author: sudhakarelixir@gmail.com

Abstract

The current research work aimed to develop and evaluate the ibuprofen loaded ethosomal gel for transdermal delivery. Ibuprofen is an anti-inflammatory drug. On oral administration, it undergoes extensive hepatic metabolism. It has short biological half-life (1.8-2hr) and low bioavailability (49-70%). To overcome the side effects, it would be preferable to apply via transdermal delivery. Ibuprofen loaded ethosomes were prepared by film hydration technique using 3^2 factorial design and characterized for various physicochemical parameters. The optimized ethosomes was incorporated into gel using carbopol 940NF as a gel base. The developed gels were tested against rheology,exvivo skin permeation studies, FTIR studies and stabilitystudies.The prepared ethosomal dispersion shown particle size in the range of 140 nm to 242nm, zeta potential was found in the range of -18 to -36mV, %EE was found in the range of 44 to 84% andin vitro permeation studies were found in the range of 71 to 94% respectively. Based on rheological properties, 1%w/v concentration was optimized among the prepared gels. The ethosomal gel flux was found to be 27µg/cm^2/hr which was found as better than other test products. FTIR studies revealed that there was no incompatibity between drug and excipients. Stability studies resulted that the optimum storage condition (2-8°C) doesn't effect on degradation of physicochemical properties. It was concluded that ethosomes alters physicochemical properties of drug and thus enhances its permeation across the skin. Hence, ethosomes are considered as promising tool for anti-inflammatory therapy via transdermal delivery.

Key words: Factorial design, Gel, Rheology, Permeation, Ethosomes

1. Introduction

Ibuprofen is an NSAID, which is believed to act by inhibition of COX-2; thereby inhibiting the prostaglandin synthesis. The most common adverse effects of ibuprofen are nausea, GIT ulceration, bleeding etc. To avoid such adverse effects, it has a possibility to apply through transdermal route. In spite of several advantages offered by the transdermal route, only a few molecules are suitable to administer through topical/transdermal delivery (Gillman G, 1995 and Rang H*et al.,* 2011).Skin is the largest external organ of human body.The stratum corneum (SC) is the first layer of the skin. The SC plays a rate limiting step to permeation of drugs across the skin. Most of the drugsdoesn't enter into SC due to their physicochemical properties and the type of dosage form. Therefore, it requires to improve the diffusion of drug across the skin (Barry BW, 2001) Hence, we are proposed to developsuitable dosage form to maximize the permeation across the skin. On transdermal delivery, ethosomes has a potential role for anti-inflammatory therapy due to intercalation propertyfor effective drug permeationacross deeper layers of theskin. The ethosomal vesicles may adhere to the skin surface and increases thermodynamic activity of the drug at the SC interface (Verma P*et al.,* 2010).

Ethosomes are soft malleable ethanolic bilayered vesicles. Huge research was available on ethosomes for transdermal delivery, in that few literature reports arementioned in current research.Ainbinder *et al*.,(2005) developed testosterone ethosomes for transdermal delivery.They reported that ethosomal formulation could enhance testosterone levels in systemic circulationas compared to the marketed product.Madhavi *et al*.,(2019)developed etodolac loaded liposomes and ethosomes and evaluated against commercial product (PROXYM®) for transdermal delivery. They reported that etodolac loaded ethosomes shown superior results against liposomes and commercial product respectively. Zeenat Iqbal *et al*., (2020) developed a naproxen sodium loaded ethosomal gel for amelioration of rheumatoid arthritis. The pharmacodynamic study reveals that ethosomal gel shown better inhibition of paw edema than marketed gel.Senshang Lin *et al*., (2015) developed diclofenac ethosomal gel using quality by design. The pharmacodynamic studies reveals that ethosomal hydrogel exhibited better anti-inflammatory activity than other testformulations.Shumilov *et al*., (2010) developed ibuprofen ethosomes gel for transdermal delivery using Phospholipon®90G by cold method. They reported that developed liposomes shown particle size around 200nm.They reveals that ethosomal gel has high relative bioavailability by transdermal route compared to that of the oral administration. Ibuprofen ethosomal gel had an efficient antipyretic effect in fevered rats. The rat body temperature decreased to normal value within 12 hrs duration of action than the 7 hrs after the oral treatment.Moghaddam *et al*., (2018) developed ibuprofen loaded nano-ethanolic liposomes (NEL) using phospholipon® 90G and Tween 80 by film hydration technique.They concluded that the presence of ethanol and flexibility of NEL leads to provide better permeation enhancement of ibuprofen via rat skin. The*in vivo* anti-inflammatory study of ethosomal gel shown significant reduction (41.18%) of edema in carrageenan induced rat paw edema than the ibuprofen conventional gel.

Based on the above literature, we are proposed to develop the ibuprofen loaded ethosomes for effective transdermal delivery using phospholipon 90H, which isa base for ethosomes and Carbopol 940NF as a base for gel respectively. Being a colloidal dosage form,

the conventional randomization procedure in the development cannot produce the formulation with desired properties. Hence, in the current study we have selected the 3^2 factorial design to develop the ibuprofen loaded ethosomes by film hydration technique.

2. Materials and Methods

2.1.Materials

Phospholipon 90H gift sample from Lipoid Pvt Ltd Germany, Cholesterol from Merck (India) Ltd. Ibuprofen purchased from Sigma-Aldrich, India. carbopol (940NF) gift sample from CP Kelco Ltd, India.

2.2.Methods

2.2.1. Analytical method development of ibuprofen by UV spectroscopy

The ibuprofen calibration curve was constructed using pH 7.4 phosphate buffer. The linearity range of ibuprofen concentrations was taken from 10 to 80mg/ml. The absorbance values were determined at 223nm using UV spectrophotometer against pH 7.4 phosphate buffer solution as a blank (Pentak D*et al.,* 2020).

2.2.2. Optimization of the formulation through factorial design

Ethosomes were prepared using 3^2 factorial design. Amount of phospholipid (phospholipon 90 H) and % of ethanol were found to be critical in the preparation and stabilization of ethosomes. Hence selected as independent variables. Vesicle size and % entrapment efficiency and % drug release were selected as dependent variables.

To study the effect of variables on characterization performance of ethosomes, different batches were prepared using 3^2 factorial design by using Design Expert® 8.0.7.1 Trail Version. Amount of lipid (X_1) and % ethanol (X_2) was selected as two independent variables which were varied at three levels, low level (-1), medium level (0), high level (+1).Amount of stearic acid (20mg), 20ml of pH 7.4 phosphate buffer and ibuprofen (20mg) were kept constant. Vesicle sizes (Y_1), % entrapment efficiency (Y_2) and % drug release (Y_3) was selected as dependent variables. Values of variables and batch codes are shown in the Table 1 **(**Varsha B*et al*., 2006**).**

Table 1: Composition of ibuprofen loaded ethosomes by 3^2 factorial design

Batch Codes	Variable-X_1 (Lipid)	Variable-X_2 (%v/v Ethanol)
E_1 40:15	40	15
E_2 40:30	40	30
E_3 40:45	40	45
E_4 60:15	60	15
E_5 60:30	60	30
E_6 60:45	60	45
E_7 80:15	80	15
E_8 80:30	80	30
E_9 80: 45	80	45

2.3.Preparation of ethosomes

Ethosomes were prepared by thin film hydration technique using a rotary flash evaporator. 20mg of ibuprofen loaded ethosomes was prepared as different concentrations of the phospholipid, stearic acid (20mg) and cholesterol (20mg) were weighed and dissolved in (1:3) mixture of methanol: chloroform in a clean, dry round bottom flask at 60°C using a rotary vacuum evaporator to form a thin lipid film on the walls of the flask by rotating speed at 120rpm. It was drying in a vacuum desiccator overnight to remove the solvent. Furthermore, the dried film was hydrated with different % of ethanol embodied pH 7.4 phosphate (20ml) for 1hr at 60°C to form ethosomes. The ethosomal dispersion was let to cool to room temperature. Finally obtained ethosomes were stored at 2-8°C in refrigerator (Gajanand Sharma*et al*., 2016, Touitou E*et al*., 2000, Mustafa MA Elsayed*et al*., 2006).

2.4.Characterization of ethosomes

2.4.1. Percentage drug content

One milliliter of suspension was pipetted from the ibuprofen-loaded ethosomes and lysed with methanol. It was further diluted with pH 7.4 phosphate buffer and the samples were analyzed spectrophotometrically at 223nm.For gel drug content analysis, the required quantity of ethosomal gel was taken and lysed with methanol. It was further diluted with pH 7.4 phosphate buffer and the samples were analysed spectrophotometrically at 223nm (Madhavi N*et al*., 2019).

2.4.2. Determination of vesicle size, PDI and Zeta potential (*ζ*)

The ethosomes after dilution (1:100) with distilled water was taken in the cuvette. The cuvette was placed inside the sample holder of the instrument (Malvern Nano ZS90, Malvern, UK) for measurement of size. The principle of photon correlation spectroscopy was used for determining the hydrodynamic diameter of the vesicle via Brownian motion. The observations of globule size were recorded at 90° light scattering angle and at 25°C. The zeta potential was measured based on the electrophoretic mobility of vesicle which used the Helmholtz–Smoluchowski equation (Rudhrabatla VSAP*et al*., 2020).

2.4.3. Determination of percentage entrapment efficiency

To determine % EE, the ethosomal system was kept overnight at 4°C and centrifuged in (Eppendrof centrifuge 1999 model) at 4°C at 20000 rpm for 3hrs. Supernatant containing unentrapped ibuprofen was withdrawn and the concentration measured using UV spectrophotometer at 223nm (Madhavi N*et al*., 2019 and Rudhrabatla VSAP*et al*., 2020).% EE was calculated by equation 1

$$\% EE = \frac{T-C}{T*} X\ 100 \qquad \text{Equation 1}$$

Ψ*: Where T is concentration of total ibuprofen and C is concentration of free ibuprofen

2.4.4. *In vitro* percent drug release

In vitro drug release studies were performed using vertical Franz diffusion cells with an effective diffusional area of 4.52cm^2. 2 ml volume of different formulations was placed in

the donor compartment. 28ml of phosphate buffer pH 7.4 was used as receptor medium. The receptor compartment was maintained at 32°C and stirred by a magnetic bar at 100rpm. In between the donor and receptor compartment cellulose dialyzing membrane (Membra–Cel MD 34-14, cutoff 14 kD) was placed which was previously soaked in the suitable medium for overnight. At predetermined time intervals, required sample aliquots were withdrawn and to maintain a constant volume of the medium in receptor compartment, an equal volume of the fresh buffer was added. The samples were analyzed spectrophotometrically at 223nm (Madhavi N *et al*., 2019 and Bonacucina G*et al.,* 2006).

2.5.Preparation of gels

Optimized ethosomes was selected to prepare ethosomal gel. Twenty ml of ethosomal dispersion was added with sufficient quantity of carbopol 940 NF powder to obtain 1% w/v and 2% w/v gel strength and soaked overnight for complete swelling of carbopol 940 NF and mixed thoroughly to obtain a final concentration of 1% w/v and 2% w/v respectively. Similarly, plain carbopol 940 NF gel loaded with pure drug ibuprofen (1% w/v and 2% w/v) was prepared by dissolving pure drug and carbopol 940NF in phosphate buffer pH 7.4 and soaked overnight (Bonacucina G*et al.,* 2006).

2.5.1. Percentage drug content

Required quantity of ethosomal gel and plain gel was taken and lysed with methanol. It was further diluted with pH 7.4 phosphate buffer and the samples were analysed spectrophotometrically at 223nm.

2.5.2. Rheology of ethosomal Gel

Rheology is one of the important parameter of gel and it is used to calculate the spreading and adhesion property of the gel. Rheological analysis of ethosomal gel was performed using a stress control rheometer: Viscotech Rheometer (Rheologica Instruments AB, Lund, Sweden), equipped with the Stress Rheologic Basic Software, version 5, using cone-plate geometry with the diameter of the cone being 25mm and a cone angle of 1°, operating in the oscillation and static mode. The gap was maintained at 0.5mm. Rheological analysis of gelatin solutions was performed at 25°C. All experiments were performed in triplicate (Bonacucina G*et al.,* 2006).

The following tests were carried out using Rheometer

2.5.2.1. ***Oscillation stress sweep:*** The samples were exposed to increasing at a constant frequency. This test allows the determination of the linear viscoelastic region (LVR) of the samples, and therefore the consequent choice of the stress value to use in the other oscillation tests.

2.5.2.2. ***Oscillation frequency sweep:*** The samples were exposed to stepwise increasing frequency at a constant stress in the field of LVR and elastic moduli (G′) as well as viscous modulus (G″) were recorded against frequency.

2.5.2.3. ***Creep-recovery:*** The test was carried out at stress of 100Pa, which was maintained constant for 100s. It was then instantly removed and the recovery.

2.5.3. ***Ex-vivo comparative permeation studies***

Ex vivo skin permeation studies of test formulations were conducted on the Franz diffusion cell with an effective diffusional area of 2.52cm^2 and 28ml of receptor compartment capacity. The rat abdominal skin was used as a permeation membrane. The excised rat skin abdominal hairs were removed using a hand razor and it was surgically removed from the animal and adhering subcutaneous fat was carefully cleaned with isopropyl alcohol to remove adhering fats.The excised rat skin was washed with pH 7.4 phosphate buffer and stored in the deep freezer at -21°C until further use.

The skin was brought to room temperature and it could be placed between the donor and receptor compartments of the Franz diffusion cell. The skin stratum corneum side was facing towards the donor compartment while dermal side was facing the receiver compartment.The test samples (ethosomes dispersion (2ml), plain gel (1gm) and ethosomes gel (1gm))were applied and samples were collected periodically after 0.5,1,2,3,4,5,6,8,10,12,24 hrs and replaced by an equal volume of fresh buffer solution. The receptor compartment was maintained at 32°C with magnetic stirring at 100rpm. The collected samples were analyzed using UV spectrophotometer at a 223nm (Madhavi N *et al*., 2019 and Choi M J *et al*., 2005).

2.5.4. FTIR studies

Appropriate amounts of ibuprofen, phospholipon 90H, cholesterol, stearic acid and ethosomes were studied for interaction studies with the functional groups of the drug and other excipients. KBr pressed pellet technique was used and the mixtures of all the excipients IR spectra were recorded on a Bruker FTIR spectrophotometer equipped with Opus software (Madhavi N *et al*., 2019 and Choi M J *et al*., 2005).

2.5.5. Stability studies

The optimized batch was further selected for stability studies with respect to percent drug entrapment at different temperature conditions, such as 2-8°C and 25°C/60%RH. The percent drug retained after every 2, 4, 6 and 8 weeks at both temperatures was determined (Entjurcb MS*et al*., 1999).

3. Results and Discussion

Ibuprofen is the drug of choice used in the treatment of pain, but it is extensively undergoing first-pass metabolism and slightly water soluble. Thus, the objective of the present work is to formulate ethosomal dispersion and incorporate into the carbopol 940 NF gel base to apply transdermal delivery to avoid first pass metabolism. Elka Touitou*et al*., (2005) invented the ethosomes and proved that, the film hydration technique appears to be a suitable and rapid technique for production of multilamellar ethosomes. Hence, in the current study, we have adopted film hydration technique to develop the ibuprofen ethosomes. The calibration curve was constructed using pH 7.4 phosphate buffer. From the calibration curve of ibuprofen, the values of regression coefficient ‘r’were found to be 0.9991, Slope 22.76, Intercept -0.009 respectively.

Table 2: Physicochemical properties of ibuprofen loaded ethosomes

Batches	Drug content (%)	Size (nm)	PDI	%EE	Zeta potential	% Drug release at 24hrs
E_1	99±1.2	242±0.1	0.841	56±0.1	-18±0.9	76±0.2
E_2	101±0.2	213±0.2	0.452	61±1.2	-24±0.1	81±1.5
E_3	98.9±1.4	230±0.2	0.365	44±0.8	-21±0.2	74±1.2
E_4	99.5±0.2	210±0.1	0.524	64±0.5	-24±0.5	71±0.6
E_5	101±0.5	196±0.2	0.336	71±0.1	-27±0.3	90±0.2
E_6	98±0.2	226±.2	0.248	52±1.0	-19±0.9	81±0.1
E_7	97.5±0.1	174±0.8	0.368	76±0.2	-30±0.6	78±0.6
E_8	101±0.1	140±1.2	0.415	84±0.5	-36±0.3	94±0.1
E_9	102±0.9	163±0.1	0.524	68±0.2	-28±0.2	73±0.4

Each value represents means of three determinations ±SD (n=3)

The percentage drug content of ethosomal formulations were found in the range of 97.5% to 102%. Based on the observation all the formulations are within the standard limit i.e 90 to 110% as per USP norms. It indicates that the uniform distribution of drug in each ethosomal formulation. The mean vesicle size of prepared ethosomes was found in the range of 140 to 242 nm. The polydispersity index (PDI) was found in the range of 0.248 to 0.841. All the prepared vesicles were in the nanometer range and having low PDI, indicate the homogeneity of the particle size. It was strongly affected by the selected variables. Zeta potential values of prepared ethosomes dispersions were ranged from -18mV to -36mV. The E8 batch ethosomal dispersion exhibited a maximum zeta potential value of -36mV due to the surface charge imparting nature of stearic acid. The values of zeta potential showed that prepared ethosomes have sufficient charge to inhibit aggregation of vesicles due to electric repulsion. The values are shown in Table 2.

The %EE of ethosomes was determined after separating entrapped and unentrapped drug by ultra-centrifugation. It varied from 44% to 84% for all the formulations. The highest % entrapment efficiency of 84% was observed for E8 formulation. The *in vitro* release profile of all formulations was studied using cellulose membrane. The range of percentage drug release found to be 71% to 94%. The study revealed that the release of the drug from the formulations depends on the relative amounts of lipid and % of ethanol. These study states that E8 showed a maximum drug release of 94% after 24 hrs. The percentage release of the drug increased with increased concentration of lipid and at a certain level of lipid, in spite the drug release decreased at higher levels of ethanol. This is because ethanol at higher levels makes the lipid bilayer more deformation and retards the release of the drug. As the formulation E8 showed an optimum particle size, highest entrapment efficiency and maximum drug release, it was optimized and used for further studies (Kincl M*et al*., 2005 and Fu X*et al*., 2019).

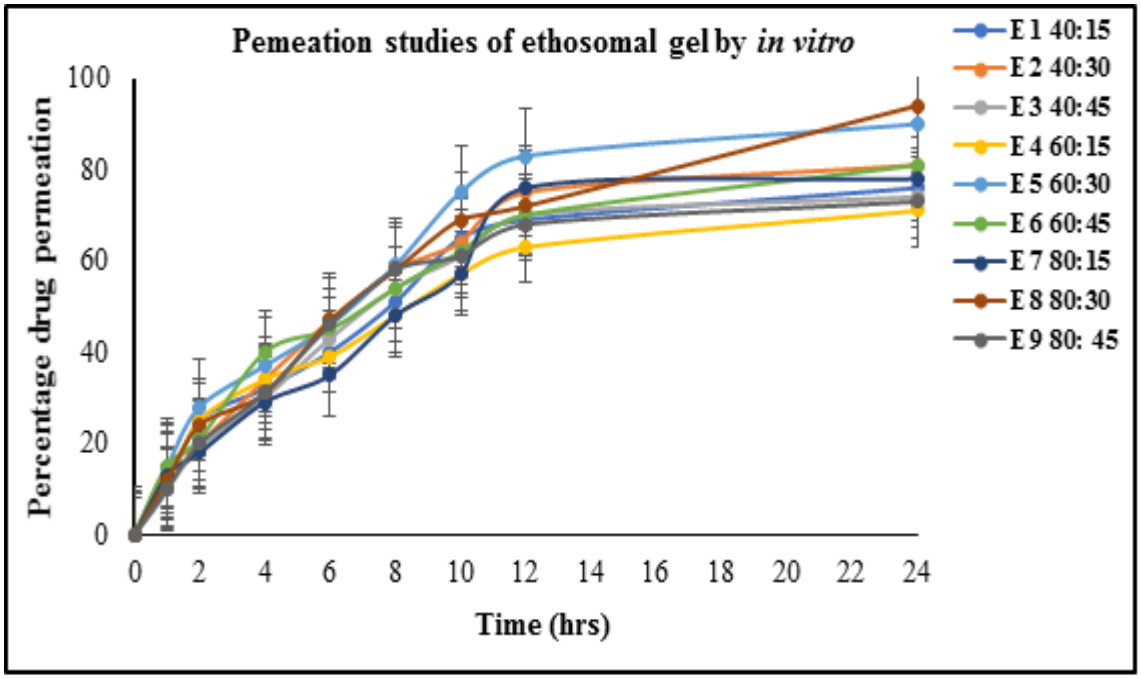

Figure 1. Cummulative perecentage of drug permeation of test formulations

3.1. Statistical data

The purpose of 3^2 factorial experimental designs was to conduct a comprehensive study of the effect of lipid (X_1) and % of ethanol (X_2) and their interactions using a suitable statistical tool by applying one-way ANOVA at 0.05 levels (Kincl M*et al*., 2005). Statistical analysis of the data and optimization of polynomial models including linear, interaction and quadratic terms were generated for all the response variables using Design Expert software. The best fitting model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the coefficient of determination (R^2), Adj R-Squared and the Pred R-Squared, (PRESS) which were provided by the Design Expert software. In addition, statistical analysis like analysis of variance (ANOVA) to identify significant effects of factors on response, regression coefficients, F test and P value were also calculated with the software. The relationship between the dependent and independent variables was further elucidated by using response surface plots **(Figure 2a-2c).**

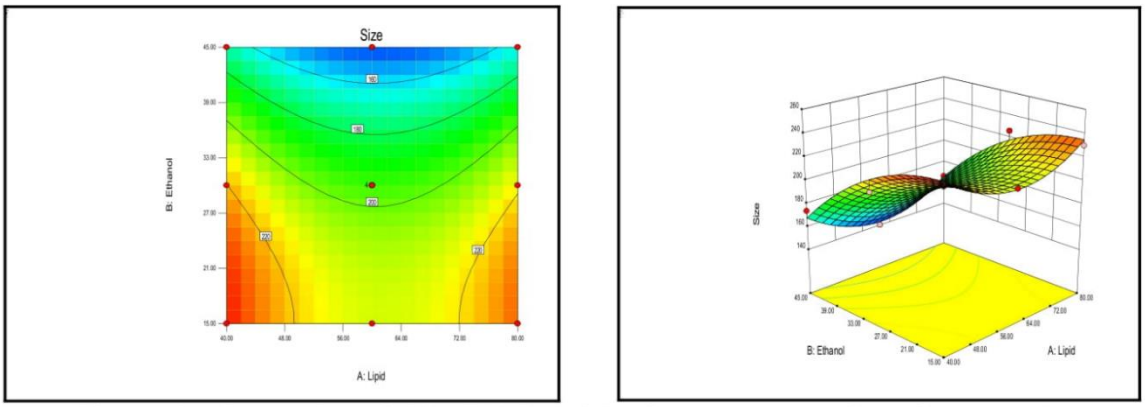

Figure 2a. Depend variable (Y_1) Particle size contour and surface graphs

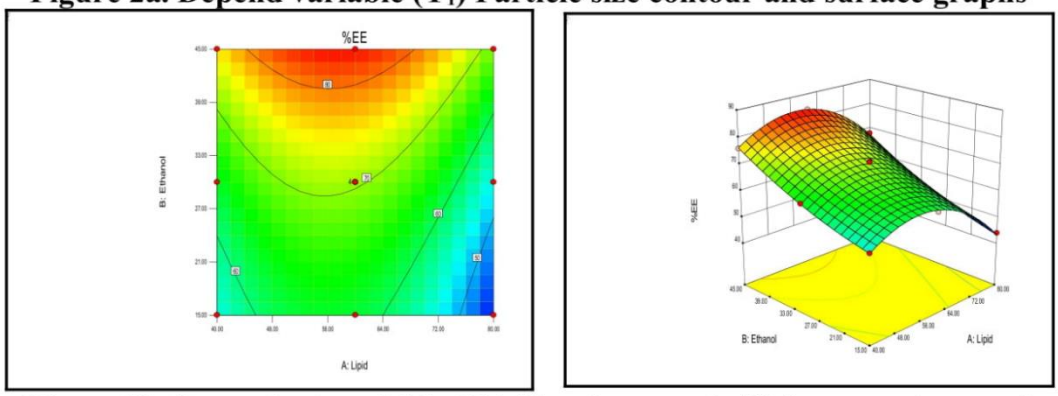

Figure 2b. Dependent variable (Y_2) % entrapment efficiency contour and surface graphs

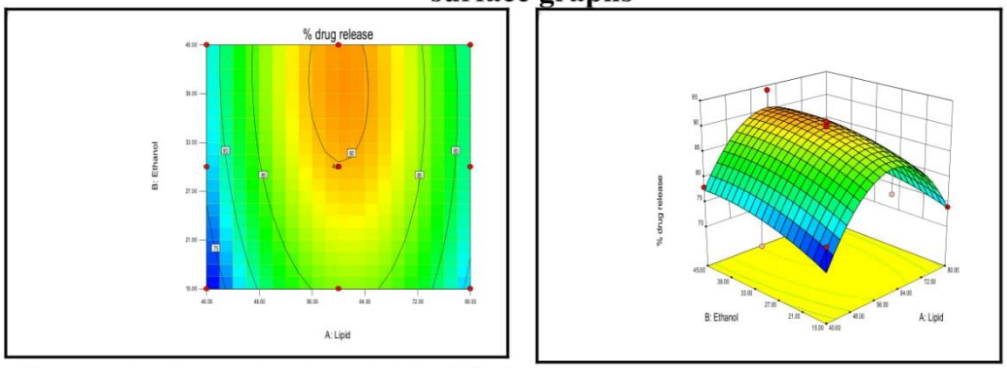

Figure 2c. Dependent variable (Y_3) *in vitro* permeation release contour and surface graphs

These plots are useful in the study of the effects of factors on the response at one time and predict the responses of dependent variables at the intermediate levels of the independent variables. Subsequently, a numerical optimization technique by the desirability approach and graphical optimization technique by the overlay plot were used to generate the new formulations with the desired responses.To validate the chosen experimental design, the resultant experimental values of the responses were quantitatively compared with those of predicted values and % relative error was calculated by the following Eq.

$$\%\ Relative\ error = \frac{predicted\ value - experimental\ value}{predicted\ value} X\ 100$$

Nine batches of ethosome formulations were prepared using experimental design which has been evaluated for particle size, %EE, % drug release. From the ANOVA data, the F-value of all three response factors (39.9, 459.8 and 6.3) respectively indicates that the model was significant. The Prob value > F value is less than 0.05 for all the response factors indicate that the models were significant. The response observation for particle size is B, A^2, B^2, %EE A, B, AB, A^2, B^2 and % drug release are found to be model significant terms. The lack of fit F value for three factors were found to be significant. Similarly, the R-squared value was calculated and found to be near to one which indicates a significantly good model. The "Pred R-Squared" value for particle size and % EE values are in reasonable agreement with "Adj R-Squared" value. But for % drug release "Pred R-Squared" does not agrees with the "Adj R-Squared" value. In all the cases, Adeq Precision values are in the range of 6-75.98 which shows adequate signal. The application of response surface methodology yielded the following regression equations which are an empirical relationship between the logarithmic values of size(Y_1), % EE(Y_2) and % drug release(Y_3).

Size (Y_1) = +195.17-1.17* A-34.67* B+0.25* A * B +24.00 * A2 -17.50* B^2

% EE (Y_2) = +70.54-5.33 * A+11.17* B+1.00 * A * B-12.12 * A^2+2.38 * B^2

% Drug releae (Y_3) = +89.92+0.50 * A+2.33 * B-0.75* A * B -13.25* A^2 -1.75 * B^2

The % EE was found to be increased with an increase in the amount of lipid and ethanol (from positive coefficients of X_1 and X_2). More amount of lipid accommodates more drugs in its bilayers. From the data, it was observed that very high ethanol content had a lowering effect on drug entrapment. This is explained by the fact that % ethanol beyond a certain level disrupts the regular bilayer structure leading to loss of entrapped drug. This effect of ethanol was also not found to be statistically significant. The coefficient of X_2X_2, that is, ethanol-ethanol interaction effect pointed out a curvilinear relationship with entrapment efficiency. AR. Mohammed*et al*., (2004) investigatedibuprofen loadedliposomes, they stated that, based on the composition of phospholipid and cholesterol ibuprofen molecules have been interacted with bilayered liposomes and thus enhances permeation. In the current study, we prepared different batches of ethosomes using ethanol and phospholipid ratios by 3^2 factorial design. The ultracentrifugation method for measuring entrapment capacity gives quantity of drug in three regions of the vesicular system. The quantity adsorbed on to the vesicular membrane, the quantity incorporated into the vesicle membrane bilayer. And quantity incorporated in the internal core phase. Lamellarity and solubility of drug in the medium both influence the entrapment capacity of vesicles. Assuming that the core and membrane of ethosomes are saturated with medium, this would allow for ibuprofen to distribute throughout the vesicle. The current research work is well correlated with above mentioned literature.

Table 2: Summary of ANOVA results in analyzing lack of fit (LOF) and pure error

Parameters	Sum of squares	Df	Mean square	F value	P-value	Remark
Size distribution						
Model	9025.8	5	1805.1	39.9	0.0002	Significant
Residual	271.0	6	45.1			
Lack of Fit	270.3	3	90.1	360.4	0.0002	Significant
Pure error	0.75	3	0.25			
% EE						
Model	1323.1	5	264.6	459.1	<0.0001	Significant
Residual	3.46	6	0.58			
Lack of Fit	2.71	3	0.90	3.6	0.1598	Not significant
Pure error	0.75	3	0.25			
% Drug release						
Model	618.6	5	123.7	6.3	0.0215	Significant
Residual	116.2	6	19.3			
Lack of Fit	115.5	3	38.5	154.0	0.0009	Significant
Pure error	0.75	3	0.25			

Table 3: Statistical parameters of responses of variables

Parameters	Size	% EE	% Drug release
Std.Dev	6.7	0.7	4.4
Mean	198.4	65.6	82.4
C.V%	3.39	1.1	5.3
PRESS	2769.0	25.5	1186.7
R-Squared	0.9708	0.9974	0.8418
Adj R-Squared	0.9465	0.9952	0.7100
Pred R-Squared	0.7022	0.9808	-0.6147
Adeq Precision	19.9	75.9	6.1

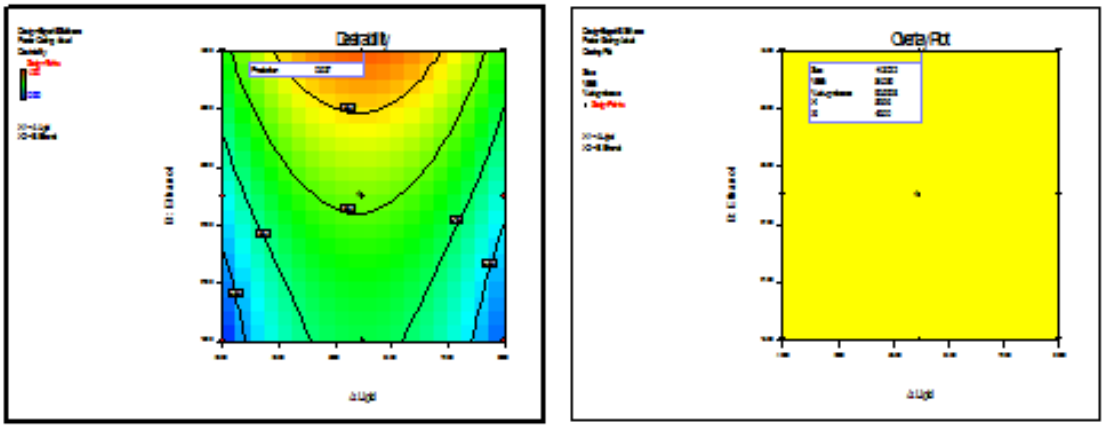

Figure 3. Desirability and overlay plots for optimization of ethosomal dispersion

Evaluation and validation of optimized formulations

The high desirability value indicates that more suitability of the formulation and the optimized formula can directly obtained from the desirability function response surface plots and (or) overlay plots. The desirability function as shown in Figure**3**, was found to be higher (near to 1) for the optimized formula indicating the suitability of the formulations. The optimal values of independent test variables were obtained from the overlay plots and the optimized formulation of contained 59.96 mg of lipid, 45% of ethanol.The optimized formulation successfully fulfilled with the physicochemical properties such as Size, %EE and %drug release studies were carried out for the verification of theoretical prediction. Observed responses and predicted values of size,% EE and % drug release were estimated respectively. The % relative error between the predicted values and experimental values of each response was calculated and the values were found to be 141nm size, 85% EE and 91% for drug release respectively. From the result, it was concluded that these experimental findings are in close agreement with the model predictions which confirmed the predictability and the validity of the model (Ismail TA*et al*.,2021).

3.2. Rheological studies for test gels

The ethosomal gels were formulated using carbopol940NF by considering its ease of application. The percentage drug content of the ethosomal gels (1% w/w, 2% w/w) was found 97.23%, 95.1% respectively. It indicates that the ethosomal suspension wasuniformly distributed throughout gel matrix.

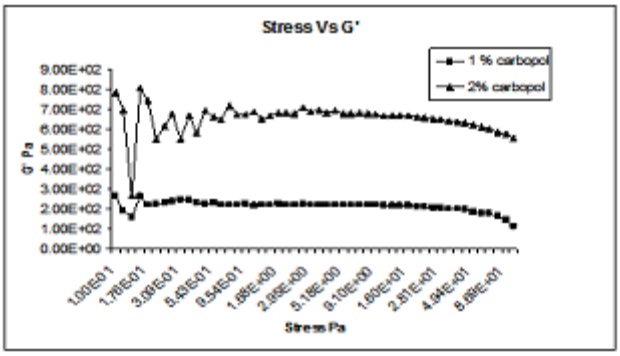

Figure 4. Oscillation stress sweep

The effect of shear stress on elastic modulus (G′) of carbopol gels at varying concentrations (1% and 2%) at 25°C temperature is shown in Figure 4.The 1%of drug-carbopol system has shown maximum G’ and LVR as compared to 2% drug carbopol system and it was comparatively more stable over applied stress range. Hence, it was concluded that 1% carbopol system was more stable and viscoelastic.

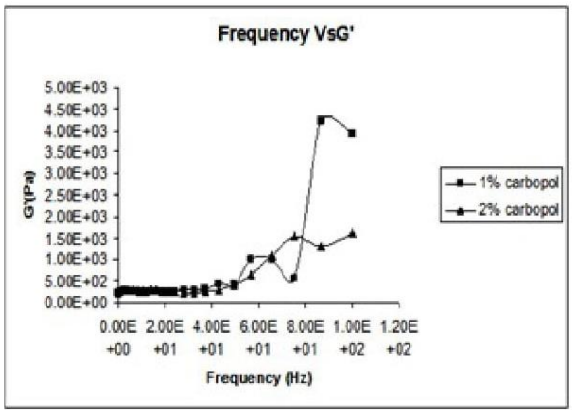

Figure 5a. Oscillation frequency sweep

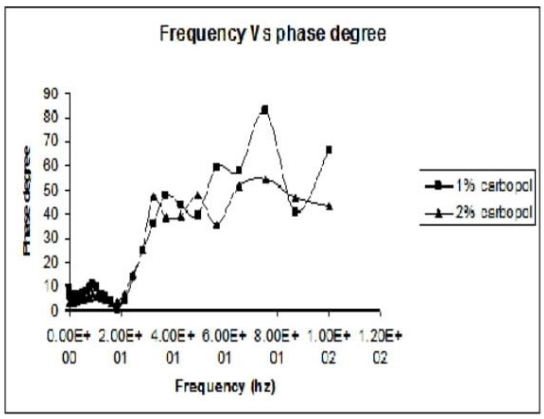

Figure 5b. Effect of frequency sweep on G' of drug loaded ethosomes gel

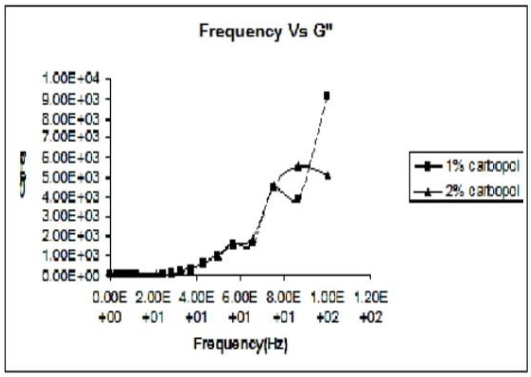

Figure 5c. Effect of frequency sweep on G" of drug loaded ethosomes gel

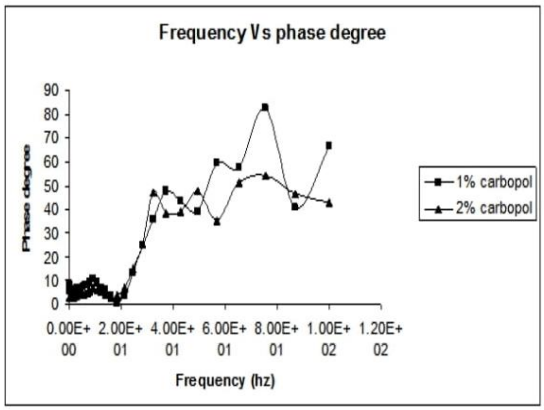

Figure 5d. Frequency Vs Phase degree

Degree of dependency of G' and G'' is examined over the applied frequency range. The G' will be large if a material is predominantly elastic. The G'' will be large when sample is predominantly viscous. Figure5a-5d represents the behaviour of G' and G''over applied frequency range. From graphs it was concluded that 2% carbopol system did not exhibited a little monotonous increase in solid component showing stable nature of 2% carbopol gel system. Trend of phase degree and loss tan as shown in Figure 6 also described the viscoelastic nature of gel.

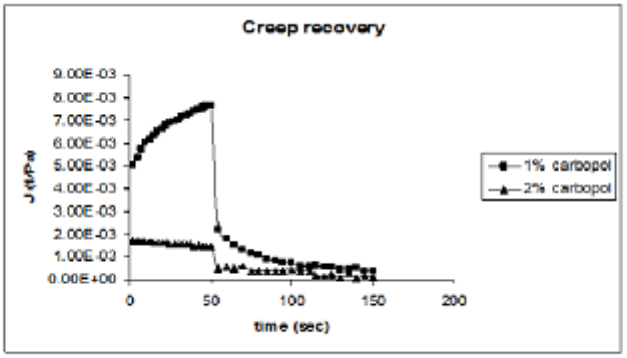

Figure 6. Creep Recovery

The creep recovery diagram corresponding to 1% and 2% at 25°C temperature. As material starts to flow, the strain increased constantly as long as the stress is applied and when the stress is set to zero deformation is reversible and recovered. This indicates the viscoelastic characteristics of the gels. The order of J values from higher to lower is 1%>2%. Smaller the J values more elastic the material is. Therefore, the order of elasticity present in samples is 2%>1% (Cristiano MC*et al*., 2020 and Yang J*et al*., 2018).

Table 4: *Ex vivo* permeation parameters of test formulations

Formulation	Cumulative amount permeated (µg/cm^2)	Transdermal flux (µg/cm^{2}h^{-1})	Permeability coefficient (cm/hr)
Ethosomal dispersion	787.70	30.79	7.7 X 10^{-3}
Ethosomal gel	667.97	27.10	6.7 X 10^{-3}
Plain gel	529.94	21.07	5.2 X 10^{-3}

Each value represents means of three determinations ±SD (n=3)

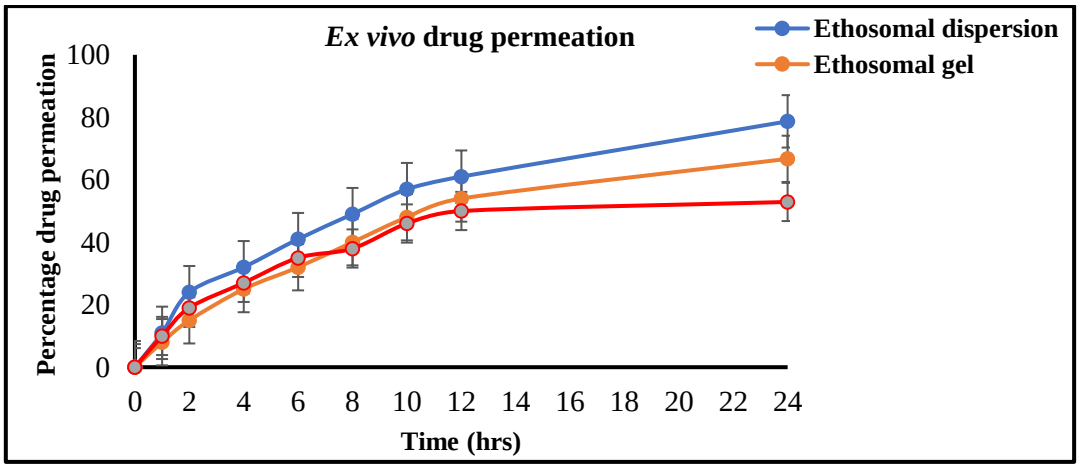

Figure 7. Comparative*ex vivo* permeation profiles of test formulations

The *ex-vivo* skin permeation study was performed on the excised Wistar rat skin using Franz diffusion cell. Figure 7shows the values of cumulative drug release for 24 hrs. Cumulative drug release was found to be more for ethosomes dispersion (89±0.22%) owing to less release of drug when compared with ethosomal gel (EG) 76.01±1.05% and plain gel (PG) 59±1.02%. From Table 4, indicated that vesicles, which are in a liquid state, they can easily deliver the drug into/across stratum corneum, but in a gel state vesicle, couldn't penetrate or permeate comparatively. Because of the increased viscosity of formulation due to carbopol 940 NF that retards the release of vesicles from its cohesive mass.The above *ex vivo* comparative studies confirm the ability of ethosomes to alter the therapeutic effect of ibuprofen due to the synergetic effect of lipid and ethanol. Ethosomal vesicles increase the fluidity of skin barrier by interacting with a lipid portion of the stratum corneum, it leads to decrease the phase transition temperature. It helps the ethosomal vesicles to partition easily and penetrate deeper into the skin from ethosomes. Higher cumulative amount of drug permeated and steady state transdermal flux from the ethosomal gel formulation can be explained by solubilisation, penetration and permeation enhancement effect of amphiphiles of the ethosomal bilayer and ethanol.

3.3. FTIR Studies

Fourier-transform infra-red (FTIR) spectra of pure drug, optimized ethosomes dispersion and ethosomal gel were taken on Jasco V-5300 FTIR. The pellet was prepared on KBr press (Spectra Lab, India). The spectra were recorded over a wave number range of 4000-400cm^{-1}.From Figure 8, FTIR spectra of ibuprofen shows characteristic peaks of carbonyl at 1720cm^{-1} and hydroxyl group at 2922cm^{-1} Overlay spectra of ibuprofen and ibuprofen ethosomal shows that both groups are shifted to slightly lower wave numbers carbonyl at 1633 cm^{-1} and hydroxy at 2908cm^{-1} These characteristic groups are intact in ethosomal system, showing that ibuprofen shows no chemical interaction with the ethosomal system. Similarly, overlay spectra of ibuprofen. The FTIR spectra are shown in Figure8(Syed Ahmed Iizhar*et al*., 2016).

Stability studies revealed that, the vesicles size and entrapment efficiency of the ethosomes up on storage for 8 weeks at room temperature (25-30ºC) were affected and for 12 weeks at 2-8°C were slightly affected. The stability studies indicated that only cold conditions favoured the ethosomes because there was minor effect on vesicles size and entrapment efficiency, but in the room temperature more drastic changes were observed. The ethosomal size was increased and % entrapment efficiency was decreased. The drug leakage from the vesicles was less at lower temperature. This may be attributed to the phase transition of the phospholipid at higher temperature causing vesicle leakage during storage. Hence, it can be concluded that the optimum storage condition for the ethosomes was found to be between 2-8°C.

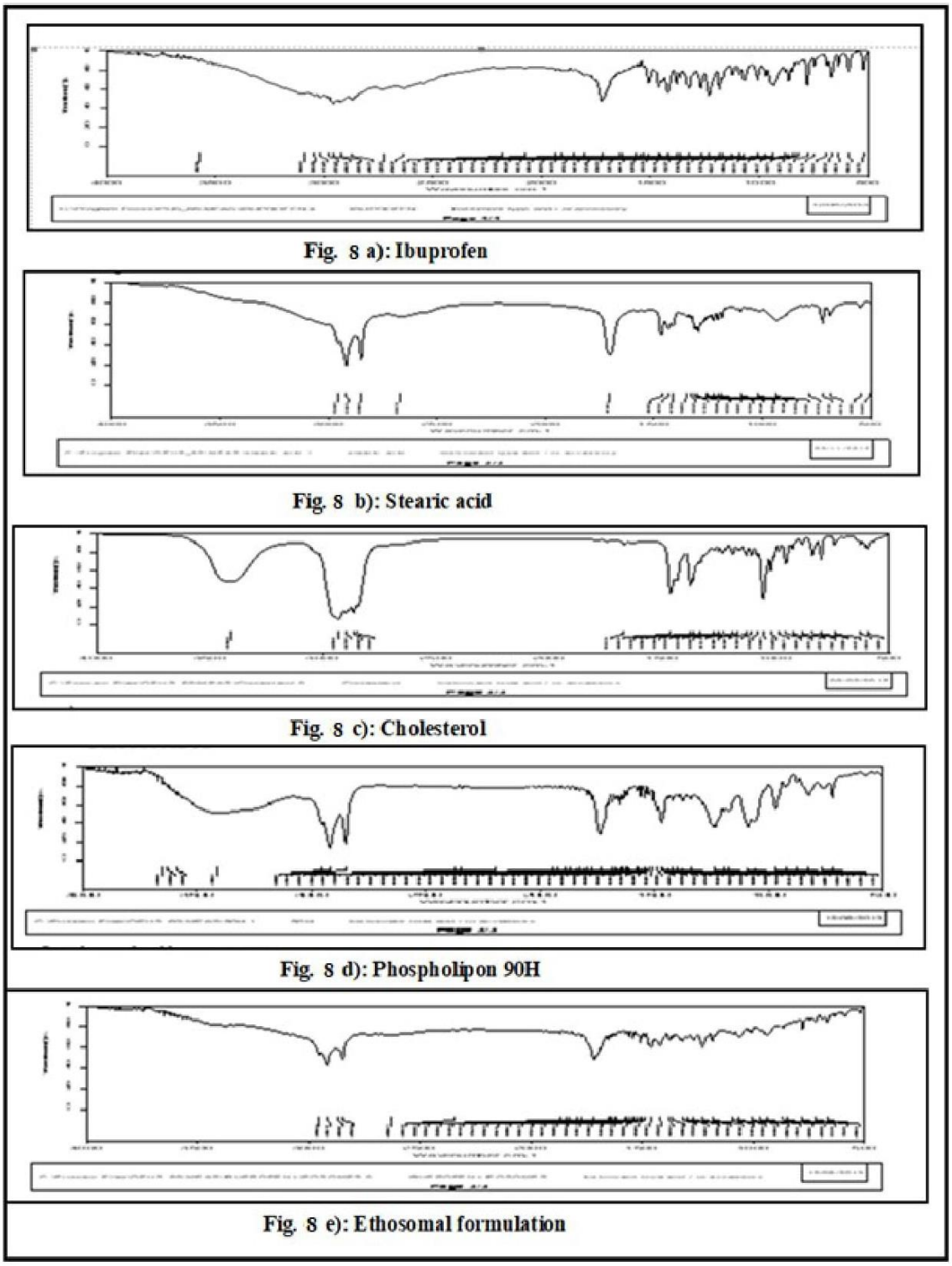

Fig. 8 a): Ibuprofen

Fig. 8 b): Stearic acid

Fig. 8 c): Cholesterol

Fig. 8 d): Phospholipon 90H

Fig. 8 e): Ethosomal formulation

Figure 8. FTIR spectra of 8 a) ibuprofen 8 b) stearic acid 8 c) cholesterol 8 d) Phospholipon 90H 8 e) ethosomal formulation

Conclusion

Ibuprofen loaded ethosomes were developed successfully using 3^2 factorial design. The prepared ethosomes and ethosomal gel shown superior properties compared to plain gel. *Ex-vivo* permeation studies confirm the ability of ethosomes to alter the therapeutic effect of ibuprofen due to the synergetic effect of lipid and ethanol. The ethosomal vesicles increase the fluidity of skin barrier by interacting with a lipid portion of the stratum corneum due to the intercalation action of ethanol. The maximum cumulative amount of drug permeated and steady state transdermal flux from the ethosomal gel formulation can be explained by solubilisation, penetration and permeation enhancement effect of amphiphiles of the ethosomal bilayer and ethanol. Based on the results, it was concluded that the ibuprofen ethosomal gel was effectively penetrated through the deep layers of the skin. Thus, it was suitable to treat pain/inflammation by transdermal delivery.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

Declared None.

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