Formulation, evaluation and optimization of Piroxicam loaded ethosomes: A Factorial Study

Anasuya Patil[1], Anjali M. Wanegaonkar[2],Mahendra Vitthal Khairnar[3], Varsha Deva[4], Harshada Heramb Puranik[5], Deepshikha verma[6], Savita S.Patil[2*], Nagam Santhi Priya[7]

[1]Department of Pharmaceutics, KLE College of Pharmacy, II Block Rajajinagar, Bengaluru-560010, Karnataka, India.

[2]Bharati Vidyapeeth College of Pharmacy, 8, YMCA Marg, Sector 3A, CBD Belapur, Navi Mumbai, Maharashtra 400614

[3]Department of Pharmaceutical Chemistry,SVKM's Institute of Pharmacy, Dhule, Maharashtra 424001, India

[4]Glocal University pharmacy College, Delhi-Yamunotri Marg (State Highway 57), Mirzapur Pole, Saharanpur-247121, India

[5]Department of Pharmaceutical Chemistry, Rasiklal M Dhariwal Institute of Pharmaceutical Education and Research Chinchwad Pune, Maharashtra, India

[6]Department of pharmacy, Guru GhasidasVishwavidalaya, Bilaspur, Chhattisgarh- 495001, India

[7]Department of Pharmaceutics, Vignan Pharmacy College, Vadlamudi-522213, Guntur, Andhra Pradesh, India

***Corresponding author:**

Ms. Savita S. Patil (Pol)

Assistant Professor,

Bharati Vidyapeeth College of Pharmacy,

8, YMCA Marg, Sector 3A,

CBD Belapur, Navi Mumbai,

Maharashtra 400614

Email: savitabvcop@gmail.com

Abstract

The anti-inflammatory drug piroxicam (PXM) is used to treat inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and others. It is said that when taken orally, it might lead to peptic ulcers, edoema, ulcerative colitis, and gastrointestinal discomfort. So, the ethosome was developed as a substitute delivery mechanism. The present study describes the preparation, characterization, and optimization study of piroxicam-loaded ethosomes using 3^2 factorial designs. Ethosomes were produced using the cold technique and then examined for FTIR studies, zeta potential, % entrapment efficiency (%EE), and particle size. SEM was used to measure the size of the vesicles, which varied depending on the soy lecithin and ethanol concentrations from 140±2.03 to 432±1.02 nm. Measurements of drug retention in the skin, entrapment effectiveness, and vesicle size were used to optimise the formulation. The vesicular charge has changed from

positive to negative due to the high quantity of ethanol in ethosomes. The E1 and E5 formulations were found to have zeta potentials of -16.91 mV and -35.17 mV, respectively, and not aggregate quickly. It was revealed that the created ethosomes had the maximum elasticity and better stability across all responses compared to previous vesicular formulations. Similar findings were found while using its gel formulation.

Keywords: Piroxicam, soy lecithin, ethanol, ethosomes, factorial design etc.

Introduction

Due to hepatic first pass metabolism, the traditional oral dose forms suffer from the drawbacks of inadequate bioavailability. Transdermal delivery not only avoids first pass metabolism but also permits continuous infusion of medications with short biological half-lives, eliminates pulsed entry into systemic circulation, which frequently results in unfavourable side effects, and keeps clinically effective concentrations for a long time. The purpose of dose planning for transdermal medicines is to maximize the flux of the medication into systemic circulation through the skin while simultaneously minimizing drug retention and skin metabolism [1]. Despite the benefits of patient compliance, self-administration, and a decrease in systemic side effects, this approach is limited by the stratum corneum's sluggish diffusion[2].Therefore, a variety of techniques have been employed to reduce this skin barrier and improve medication delivery via the skin. Use of vesicle formulations as cutaneous dermal system [3] is one of these methods. Ethosomal vesicles have been discovered to be beneficial for the long-term administration of drugs via the skin among the numerous vesicular systems examined [4]. Ethosomes are soft, flexible vesicles made up of water, phospholipids, and ethanol (in a relatively high concentration). These aid in reducing the washout of the medication via the circulation, which occurs often when medications are administered topically through penetration enhancers, ionotophoresis, or electrophoresis processes. In addition to these benefits, elastic vesicles can deposit medication in the deeper skin layers by avoiding the stratum corneum and the capillary bed of the skin [5]. The synergistic interaction between phospholipid vesicles, skin lipids, and high concentrations of ethanol may explain why ethosome carriers have superior permeability. As a result of its interactions with polar head group-containing lipid molecules, ethanol may eventually improve membrane permeability via increasing fluidity. Additionally, it can provide the vesicles smooth, flexible qualities that make it simple for them to enter the deeper layers of the skin. When skin lipids and ethosome fuse, the medication may be released at different locations along the pathway of penetration [6].

Piroxicam is a long-acting, very effective NSAID having analgesic, antipyretic, and anti-inflammatory properties. It is used to treat rheumatoid arthritis and osteoarthritis pain, soreness, edoema, and stiffness. It reduces the concentration of PG in synovial fluid, slows platelet aggregation, and is a reversible COX inhibitor, all of which increase bleeding time. Additionally, it acts by reducing leucocyte chemotaxis and IgM rheumatoid factor production, which inhibits inflammation. Piroxicam is a class 2 medication with limited solubility and high permeability, according to Biopharmaceutic [7]. Its pharmacokinetic profile is characterized by slow, gradual absorption through the oral route and a long half-life of elimination, resulting in a prolonged

therapeutic action but also a delayed onset of anti-inflammatory and analgesic effect as well as trigger numerous adverse effects like ulcerative colitis, gastrointestinal irritation, edoema, and peptic ulcer [8]. Although piroxicam is readily absorbed after oral administration, there are some gastrointestinal issues that have been linked to its usage [9]. On the other hand, it causes severe pain and inflammation at the injection site when it is given parenterally. The medicine may be used topically to treat these adverse effects. When compared to NSAIDs with a log P value of 3.5, piroxicam with a log P 1.8 showed a greater plasma concentration following topical treatment in rats. It is recommended that drugs having a log P value of about 2 make suitable candidates for topical distribution [10].

In the current work, an effort has been made to create vesicles that contain ethanol and lipid in varying amounts. With the aid of a chosen experimental design, a systematic optimization of the formulation factors that may have an impact on the formulation characteristics of ethosomes has been screened and optimized. Furthermore, the gel for the transdermal administration of piroxicam has been effectively formulated using a designed carrier system.

Materials and Methods

Materials

Piroxicam (Wockhardt, India), Soybean phosphatidylcholine (Central Drug House, New Delhi), Propylene Glycol (Thomas Baker Chemicals Pvt. Ltd Mumbai), Ethanol (Chong Yu Hi-Tech Chemical, China)Triton® X-100

Solubility studies of PXM

Finding the best dispersion medium for the manufacture of PXM ethosome required evaluating the effects of pH and ethanol concentration on PXM solubility. An excessive quantity of PXM was added to glass vials holding 10 ml of the hydroethanolic solution with 10-30% v/v ethanol and the necessary media, which included buffer solutions pH 1.5, 2.0, 4.5, 5.5, 6.5, 7.0, 7.4, and 8.0 produced in accordance with USP 30. To make sure that the solubility of PXM had achieved equilibrium, the samples were subjected to 30 minutes of sonication and then left to stand at room temperature for 48 and 72 hours, respectively. PXM solubility in the samples was assessed using UV spectrophotometry at a wavelength of 365 nm after the surplus medication was filtered through a 0.45 m syringe filter membrane. Three copies of each determination were made. The paired t-test was employed in statistical analysis to compare PXM's solubility over the course of 48 and 72 hours. Significant results were defined as those with $p < 0.05$ [11].

Preparation of PXM ethosomal vesicles by 3^2 factorial designs

The effect of two independent variables or CPP, namely SOY concentration (X1) and ETH concentration (X2), on CQA (Y1: zeta potential, Y2: % entrapment efficiency, and Y3: size) (Table 1) was assessed. Nine model formulations were prepared in accordance with a 3^2-factorial design. PXM and PG dosages were predetermined at certain amounts. PG has reportedly been shown to prevent vesicles from freezing or melting at extreme heat. Additionally, it aids PXM in skin penetration. Soy lecithin, a surfactant, improves the look of dry or injured skin by decreasing flaking and restoring suppleness, while ethanol has been shown to boost the penetration of many medications through the skin by enhancing drug partition and diffusion

[10,12]. Soy lecithin (1-3%) and ethanol (10-30%) containing 10 mg PXM were used to create PXM ethosomes. To prevent ethanol from evaporating, the resultant mixture was covered and agitated at 700 rpm. To create the ethosomal colloidal suspensions, distilled water was gradually added while being stirred constantly. The final ethosome suspension was continuously stirred for 30 min at room temperature. Formulas were kept chilled and then sonicated at 40°C using a probe sonicator (SH 70G, Bandelin Sonopuls UW 2070, Berlin, Germany) while being stirred at 700 rpm for 15 min (3 cycles with a pause of 5 min). Prior to usage, the vesicle formulations were kept in sealed containers at 4°C [13].

Table 1: Variables and their levels in 3^2 Factorial design

Variables	**Levels**		
	Low	**Medium**	**High**
Transformed Values	-1	0	1
Independent variables			
X1: Concentration of SOY (%)	1	2	3
X2: Concentration of ETH (%)	10	20	30
Dependent Variables			
Y1: Zeta potential (-mv)			
Y2: Entrapment efficiency (%)			
Y3: Size (nm)			

Quality Target Product Profile (QTPP) and Risk Analysis of PXM

The term "QTPP" refers to the qualitative characteristics that a drug product must have in order to achieve the quantitative qualities set out in the target product profile (Figure 1). A quantitative surrogate to characterize the elements of clinical safety and effectiveness should be provided by QTPP. An anticipated list of a medication product's quality attributes that it should ideally possess to assure the intended quality, taking into account the safety and effectiveness of the medicinal product [12].

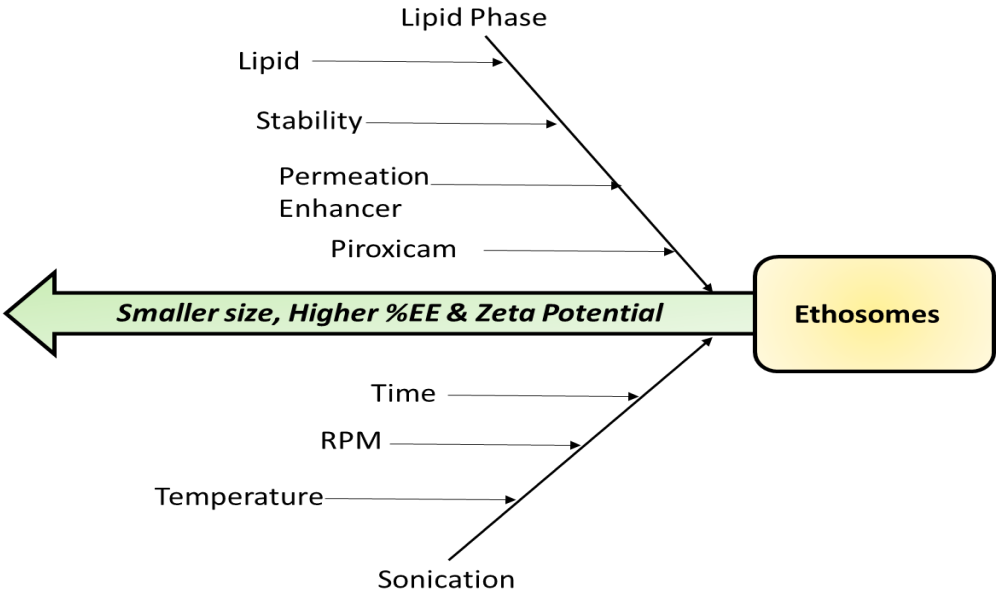


Figure 1. Quality Target Product Profile for development of PXM loaded ethosomes

Characterization of prepared ethosomes

Fourier Transform Infrared Spectroscopy (FTIR)

An FTIR research was conducted to determine if medications and excipients were compatible. After two weeks of storage (50^0C), the IR spectra of all physical combinations were measured using an FTIR Spectrophotometer from Perkin Elmer Spectrum [14]. At a scanning speed of one minute, the scanning range was 4000-400 cm^{-1}.

Scanning Electron Microscopy (SEM)

Using a scanning electron microscope (SEM), the surface morphology of the ready ethosome was examined. Aluminium stubs were covered with double-sided carbon tape. On tape, an ethosome sample was placed. A scanning electron microscope (JSM- 6490LV, JEOL, Japan) vacuum chamber was filled with aluminium stubs. For morphological characterization, the samples were examined [15].

Particle size and zeta potential analyzer

The laser diffraction particle size analyzer, made by Sympatec, Clausthal-Zellerfeld, Germany, was used to measure the ethosomes' vesicle sizes. The system included a small-volume sample holding cell (Su cell), a multi-element detector, and a He-Ne laser beam with a wavelength of 632.8 nm that was focussed with a minimum power of 5 mW using a Fourier lens (R-5). Prior to measuring the particle size, the sample was agitated. After diluting 10 PL of ethosome with 4 mL Milli-Q water, the ethosomes' zeta potential was measured using a Zeta Potential Analyzer model Zeta PALS (Brookhaven Instruments Co., NY, USA) at 25^0C and a scattering angle of 90 degrees [16].

Determination of PXM-Entrapment Efficiency

After disrupting the vesicles with Triton® X-100 (0.1% w/v) at a 1: 1 volume ratio and appropriately diluting with phosphate buffer solution (pH 7.4), the concentration of PXM in the formulation was measured. At 4°C and 10,000 rpm for 10 minutes, the vesicle/Triton® X-100

solution was centrifuged. Using a 0.45 µm nylon syringe filter, the supernatant was purified [17]. According to the following equation, the formulation's PXM loading entrapment efficiencies were determined:

$$\% \text{ Entrapment Efficiency} = \frac{\text{CL}}{\text{CI}} \times 100$$

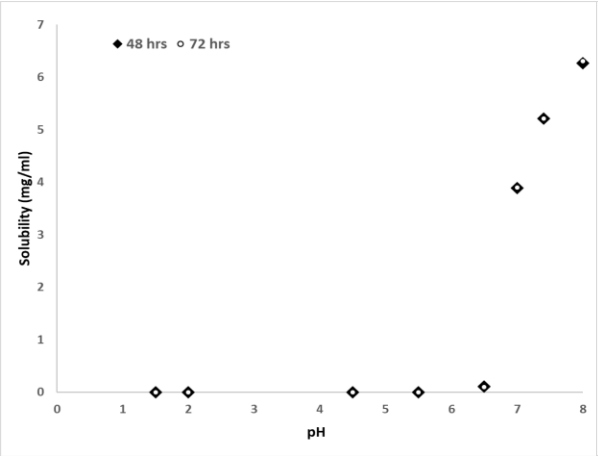
Where, *C*L is the concentration of PXM loaded in the formulation and *C*i is the initial concentration of PXM added to the formulation.

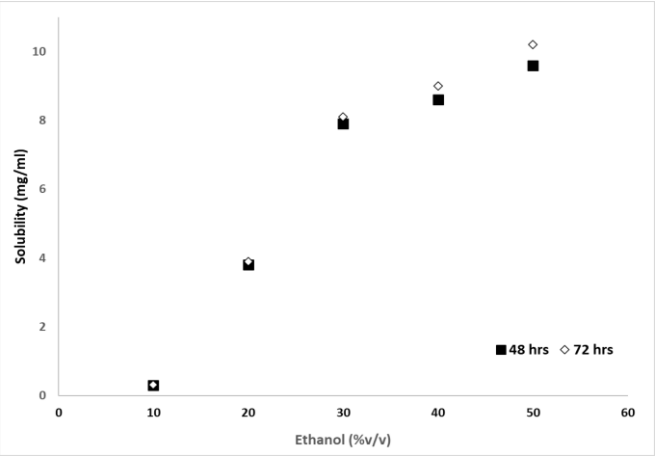
Results and Discussions

Effect of pH and ethanol on solubility of PXM

Because solubility properties have an influence on formulation and preparation of ethosome containing PXM,we need to find an appropriate dispersion medium that is able to avoid drug precipitation in the formulation. In this case, the effects of pH on PXM solubility were evaluated. As PXM is very slightly soluble in water (0.13 mg/ml) with an acidic pKa value of 6.3, its solubility increases with increasing the pH.At pH 7.0, 7.4 and 8.0, the solubility of PXMwas found to increase about 3.89, 5.21 and 6.27 mg/mL, respectively. PXM was found to be unstable at pH 8.0 due to hydrolysis catalyzed by basic ions. Therefore, pH 7.4 buffer was selected for further use in preparing ethosome formulations [18]. The solubility profile of PXM in various ethanol concentrations showed that the solubility of PXM increased as ethanol concentration increased, Figure 2. As the concentration of ethanol increased from 10, 20 and 30% v/v, the solubility of PXM increased to 0.3, 3.8 and 7.9 mg/mL, respectively. Ethanol is a solubilizing agent and thus assist in skin permeability. Thereby, in this study, 10-30% v/v ethanol was added in dispersion media of ethosome formulations (Figure 2).

A paired sample t test was conducted to evaluate whether a statistically significant difference existed between the PXM solubilities in various pH and ethanol concentrations at 48 hrs and 72 hrs. The results of paired sample t test were not significant, ($t(7) = 0.798$, $p>0.05$ for different pH and $t(2) = 0.198$, $p>0.05$), indicating that there is no significant increase in solubilities from 48 hrs to 72 hrs, which showed that PXM reached its equilibrium.





(A) **(B)**

Figure 2: Effect of (A) pH and (B) Ethanol content on solubility of PXM

Assessment of produced liposomes

FT-IR spectra are illustrated in 3. PXM exhibits characteristic bands at 3330 cm^{-1} (-NH stretching), 2970 cm^{-1} (-OH stretching), and 1630 cm^{-1} (C=O group of the cubic form). The spectra of CDs present a profile without distinctly high peaks in the range of 3000–3700 cm^{-1} (–OH groups) and in the range of 2800–3000 cm^{-1} (C-H groups). Characteristic peaks attributed to the presence of PXM are clearly visible in all formulations. In the case of E6 formulation, broadened peaks are the dominating features in the spectra [19].

TEM was used to examine the ethosome morphology (Figure 4). The spherical form of the generated ethosomes, which was seen in negative-strain TEM images, may have an effect on the release of the medication. Regardless of the manufacturing method or the loaded drug, no drug crystals could be seen in TEM pictures [20]. The liposomes' vesicle sizes ranged from 140 to 432 nm on average.

For effective stability and to prevent aggregation, a £ value > 30 mV is required. No discernible change was discovered between the various drug-loaded batches since surface charge is mostly produced by ethosome constituent phospholipids, which were the same in all formulae (specific data are not presented) [21]. Zeta-potential values for all liposomes ranged between 16.42 to 26.61 mV, which is regarded as the ideal potential for guaranteeing particle stability. Ethosomes were all negatively charged. It was shown that the produced liposomes' £ potential has a significant charge to prevent vesicle agglomeration.

There was evidence of the compositions' entrapment effectiveness. Ethosomal formulations made with 36% alcohol (E5) and 3.5% phospholipid had a 75% entrapment efficiency. Increased ethosome size may be the cause of the higher entrapment efficiency with more soylecithin. Because the membrane is less flexible when the concentration of permeation enhancer (PG) is increased from 5 to 10%, entrapment decreases [22]. Due to the increase in membrane flexibility, PG from 3–5% exhibit increased entrapment. A further increase in PG content is likely to makethe vesicles leakier and reduce their ability to entrap molecules [23, 24].

Table 2: Table showing formulation factor and response variables

Formulations	**Formulation factors**				**Response variables**		
	Fixed factors		**Variable factors**		**Physicochemical characteristics**		
	PXM (mg/ml)	**PG (ml)**	**X1**	**X2**	**Y1**	**Y2**	**Y3**

E1	10	3	-1	-1	16±0.91	84±0.87	432±1.02
E2	10	3	0	0	19±1.34	77±0.47	356±1.63
E3	10	3	1	1	20±1.98	69±1.02	221±2.01
E4	10	3	-1	0	21±0.34	79±0.34	378±1.98
E5	10	3	0	1	35±1.75	75±1.93	140±2.03
E6	10	3	1	-1	17±2.01	78±2.98	441±1.22
E7	10	3	-1	1	26±0.84	72±2.45	249±1.87
E8	10	3	0	-1	16±1.83	82±1.84	398±2.09
E9	10	3	1	0	24±1.39	78±0.92	275±1.32

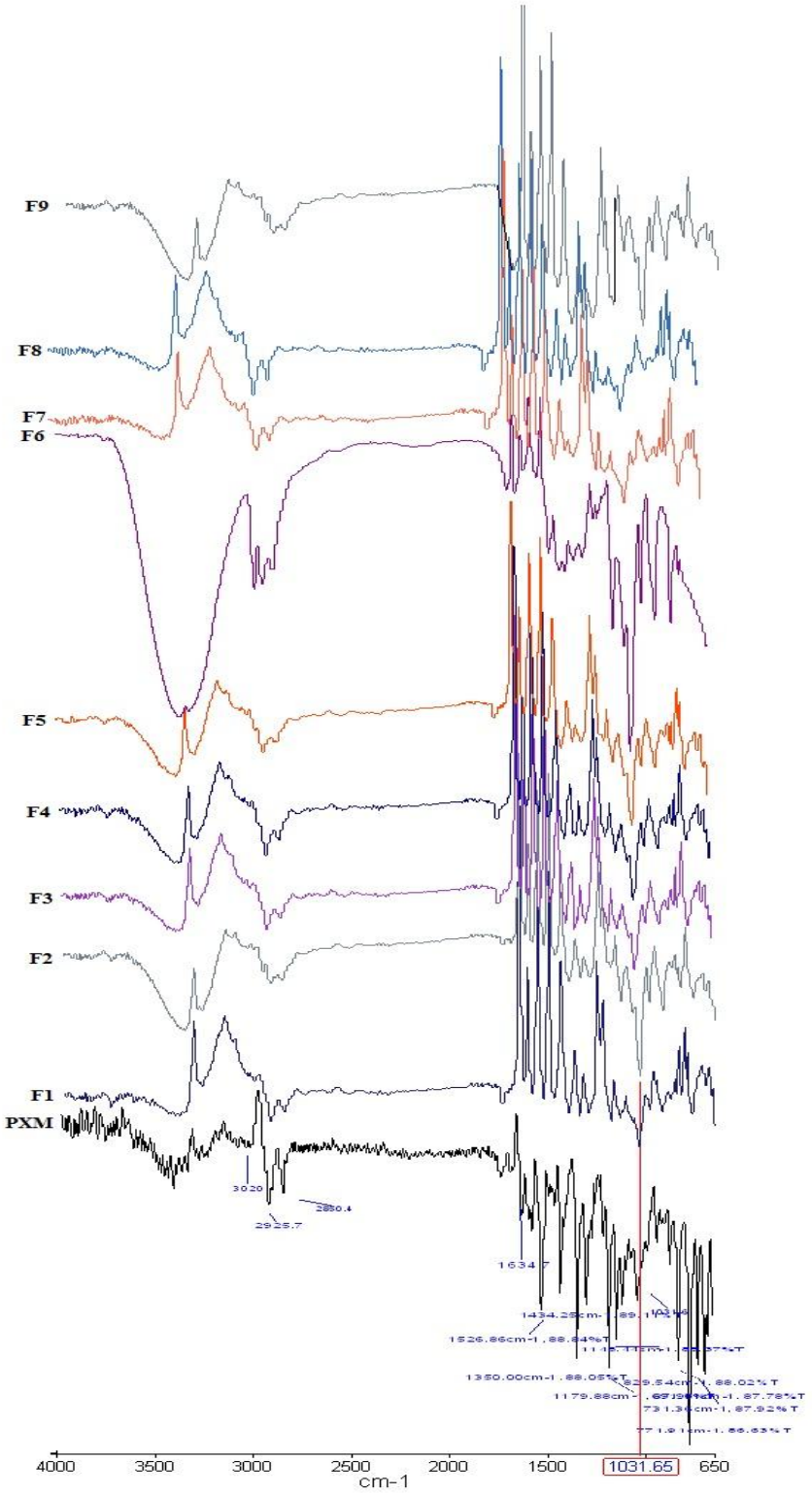


Figure 3. FTIR spectra of all ethosomes compared to PXM.

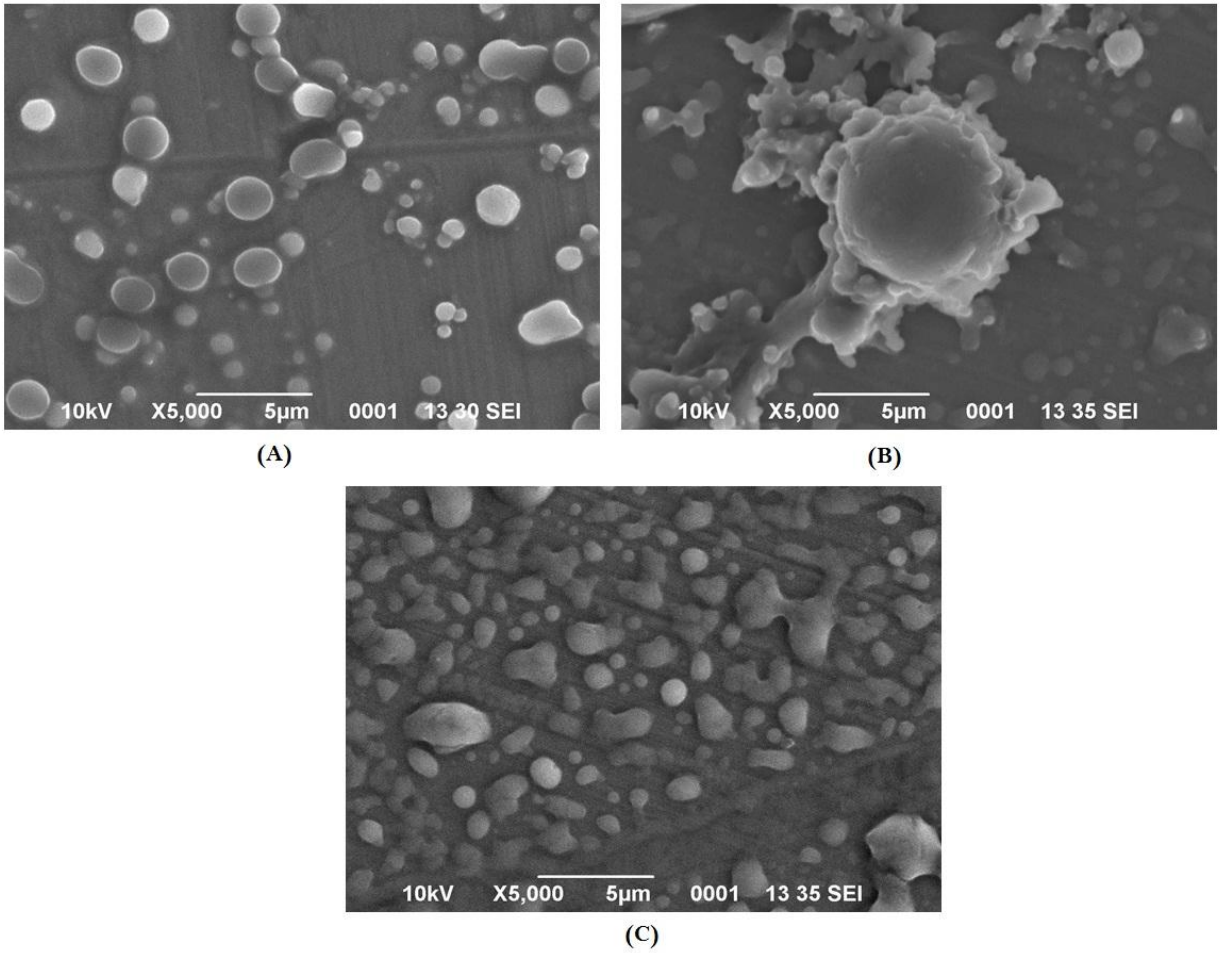


Figure 4. SEM images of (A) Ethosome (E5), (B) PXM and (C) Blank

Experimental design and statistical analysis

The link between the formulation parameters and the physical and chemical properties of the ethosomal formulations (as response variables) was assessed using Design Expert® software (Design Expert® version 8, Stat-Ease, Inc., MN, U.S.A.). Axially defining locations for the quadratic term were built into the primary composite experimental design. To counter the impact of noise and to assure dependability, the twin copies of the centre point were implemented. The core composite experimental design was displayed in Table 1 together with the formulation parameters (X1 and X2) and response variables (Yn), such as zeta potential (Y1), entrapment effectiveness (Y2), and vesicle size (Y3). Using statistical tools, the dataset's regression analysis was performed on the assumption that factors will interact in a quadratic fashion. The term of the quadratic polynomial model of the coded factor can be determined as:

$$Y = \beta_0 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2 + \beta_1X_1 + \beta_2X_2$$

Where Y represents response variables associated with each formulation factor levelcombination, $X12$and $X22$ are the quadratic factors, $X1X2$ represents the binary interactionsamong factors, and $X1$ and $X2$ are the formulation factors concentration of soy lecithin and concentration of ethanol, respectively. Analysis of variance (ANOVA) was executed to identify thesignificance of the quadratic factors ($Xn2$), binary interactions ($XnXm$) and single factors (Xn)in relation to the influence on the response analysis. The responses were consideredsignificant at a coefficient p value < 0.05.

Statistical analysis was done by Design expert software version 8.0.7.1 (Stat-Ease, Inc., Minneapolis, USA) and the second order polynomial equations were derived. A response surface was constructed to study the effect of independent variables viz (Figures 5,6,7). concentration of soy lecithin (X1) and ethanol (X2) on dependent variables viz. zeta potential (Y1), Entrapment efficiency (Y2) and Particle size (Y3) of ethosomes. A variety of polynomial equations, response surfaces, and contour plots were produced after analyzing the data from all nine formulations. Multiple regression analysis and the F-statistic were used to analyze the replies and find statistically significant variables. The quadratic model's polynomial equation is fitted to all the variables, and an ANOVA analysis was run to identify the important variables. The relative importance of the components and their interaction were determined using the size of coefficients and the mathematical sign they carry. When two factors are altered at once, the response changes, as seen by the interaction terms (X1, X2). Plots are then created using the complete Equation (an equation with just statistically significant elements) to quickly see the effects of altering variables. The optimum point may be identified from the plot. The model equation for zeta potential was given below:

$Y1= 21.56 + 0.47\ X_1^2 + 0.47\ X_2^2 + 0.67\ X_1X_2 + 0.47\ X_1 + 0.47\ X_2$

$Y2=77.11 + 4.22\ X_1^2 - 0.89\ X_2^2 + 0.95\ X_1X_2 + 0.89\ X_1 + 0.89\ X_2$

$Y3=17.68 + 2.90\ X_1^2 - 0.62\ X_2^2 + 0.45\ X_1X_2 + 0.62\ X_1 + 0.62\ X_2$

The coefficient of X1 and X2 was found to be positive indicated that predicted values of zeta potential could be obtained when concentration of SOY (X1) and ETH (X2) were increased i.e. 3% and 30 % respectively.

The response Surface linear model generated for X1 and X2 was found to be significant with an *F*-value of 2.50 and 6.80 (P<0.0500) respectively. The Model F-value of 10.42 (p<0.0001) implies the model is significant."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 6.358 shows a strong indication. The design space may be explored using this paradigm. According to the coefficient table, the dependent variable was significantly impacted by variables X1, X3, and X12, X22. The D/P ratio had a beneficial impact on particle size, according to the positive sign of the X1 coefficient. Low D/P ratios resulted in smaller particle sizes. Both factor X2 and its interaction terms were determined to be negligible. The biggest effect was shown by X3, where a positive value denoted a favourable effect of solvent on ethosomes, i.e., a higher ethanol concentration resulted in lower particle size.

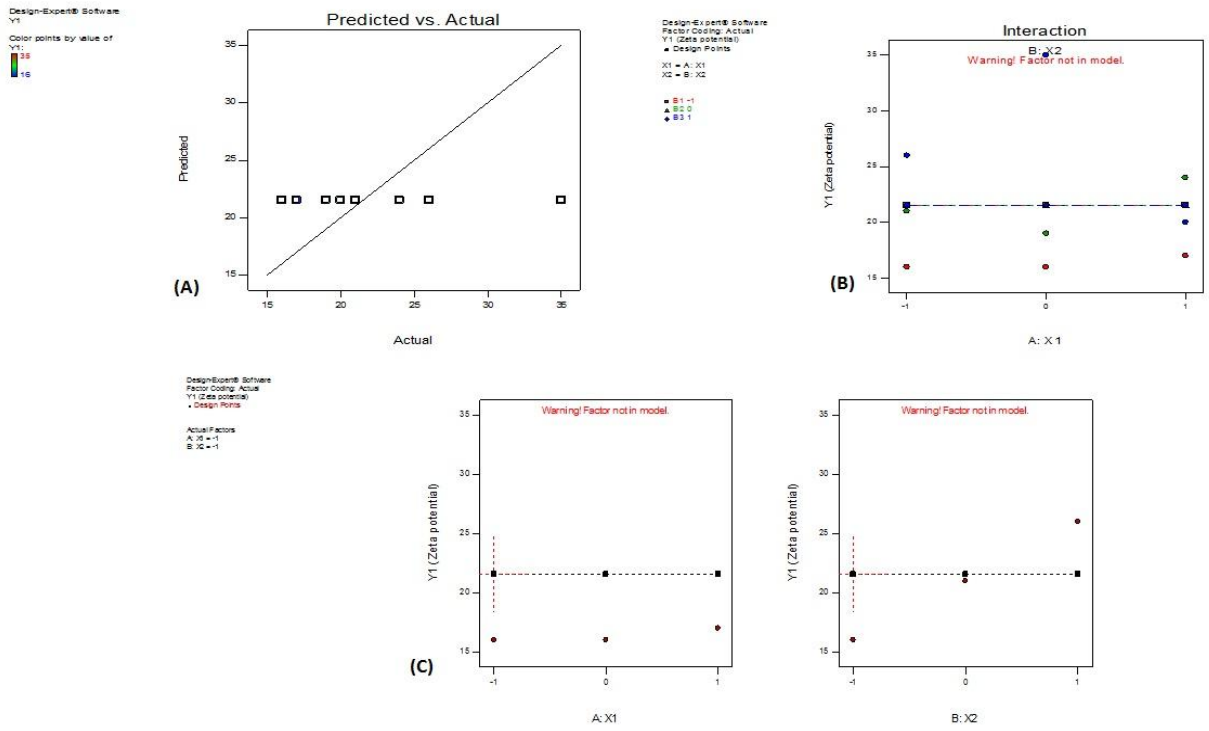


Figure 5. Predicted versus actual values of dependent variable i.e. Zeta potential (Y1)

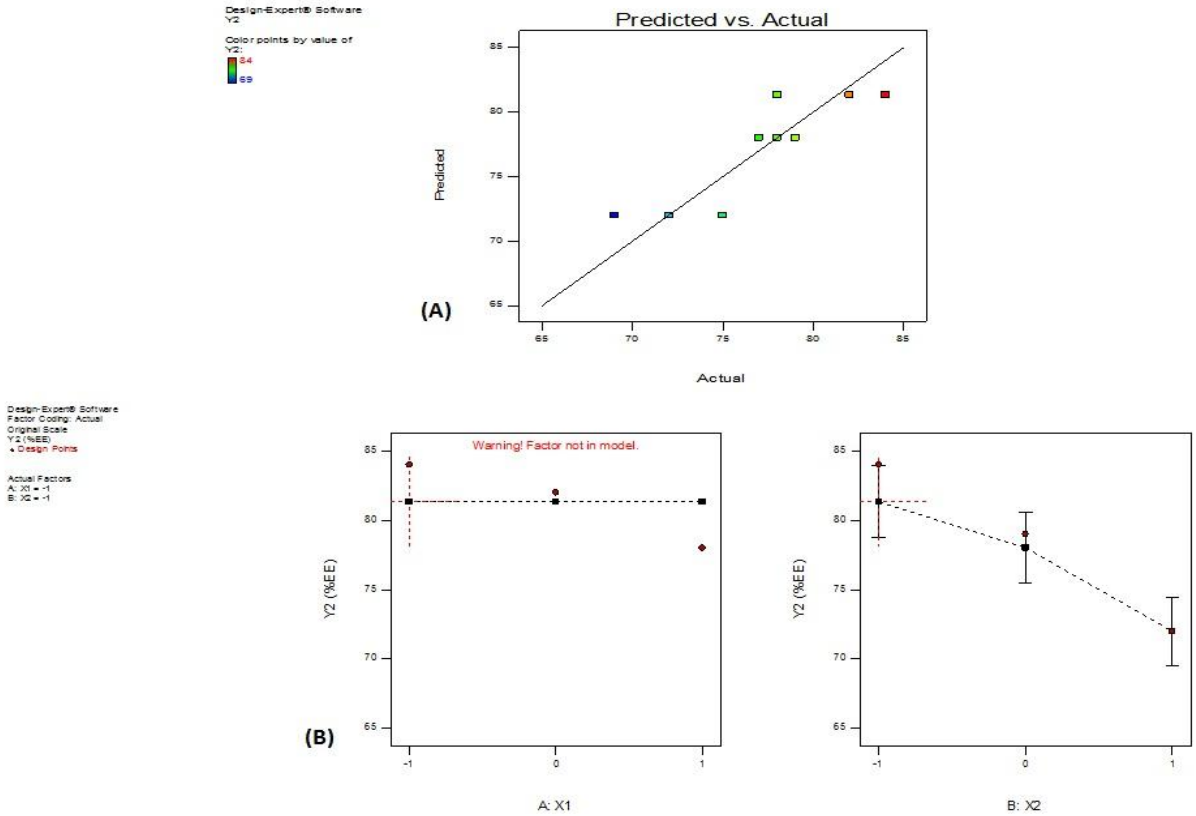


Figure 6. Predicted versus actual values of dependent variable i.e. Entrapment efficiency (Y2)

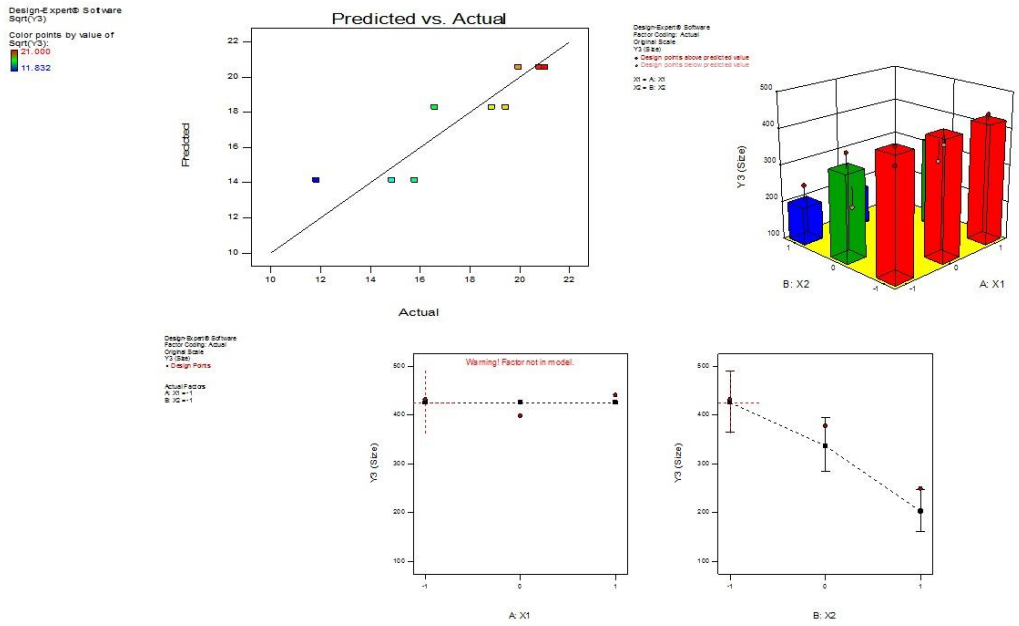


Figure 7. Predicted versus actual values of dependent variable i.e.Ethosome size (Y3)

A pareto chart was produced to show that the formulations on the left side of the intersection have a significant impact on the dependent variables, whilst the formulations on the right have a negligible impact. In light of this, it was discovered by Pareto analysis that E5, E7, E9, E4, E3, E2; E1, E8, E4, E6, E9, E2, E5 and E6, E1, E8, E4, E2, E9 (Vital Few) had beneficial effects on zeta potential, %EE, and vesicle size, respectively. Results showed that 20% of causes (Formulations on the left side of the intersection) account for 80% of effects (Zeta potential, %EE, and vesicle size). Important reasons can be found via Pareto analysis, which can then be used to improve formulations. The E5 formulation had superior results in terms of zeta potential, %EE (right side of pareto chart), and vesicle size (left side of pareto chart), as shown in Figure8, because these are necessary conditions for an ethosome to be stable.

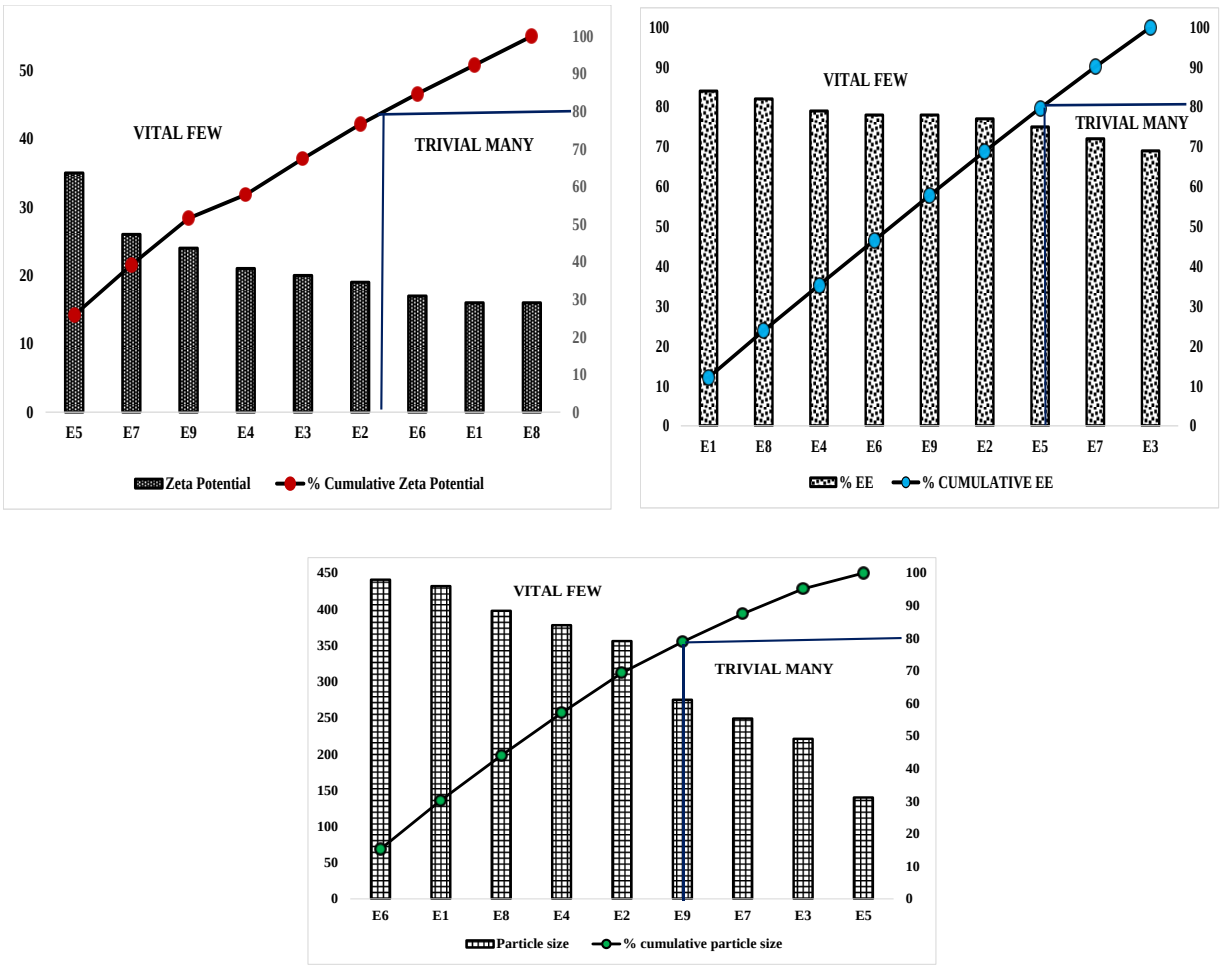


Figure 8. Pareto chart for cause-and-effect analysis

Conclusion

With better entrapment effectiveness, liposomal carriers were shown to be stable at room temperature. Furthermore, it's possible that the phospholipid-rich vesicle domains contributed to the development of the depot effect for pharmacological molecules. With liposomal formulations, the latter has been demonstrated by a greater level of medication retention within the epidermal layers. Thus, ethosome formulations with desired characteristics of drug loading and particle could be successfully prepared and optimization was done by 32 factorial design and maximum desirability value. E5 ethosome was optimized and have shown appreciable zeta potential, drug loading and particle size.

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