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FORMULATION, BOX-BEHNKEN OPTIMIZATION, AND EVALUATION OF LURASIDONE HCL-LOADED ETHOSOMAL GEL FOR TRANSDERMAL DELIVERY

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ABSTRACT

Background: Lurasidone HCl is an antidepressant drug, which is available in solid dosage form in the market. The drug lurasidone HCl in the tablet form shows poor oral bioavailability, which is only 9-19%. Therefore, an alternative dosage form, such as ethosomes, is preferred. **Methodology:** The thin film hydration technique was used to formulate ethosomes. A Box-Behnken design was used to optimize the formulation, with statistical and graphical analyses of the response surface plots. Ethanol (X1), phosphatidylcholine (X2), and penetration enhancer (X3) were selected as the independent variables, and the dependent variables were vesicle size (Y1), zeta potential (Y2), and entrapment efficiency (Y3). An optimized formulation was selected after characterization of ethosomes. The optimized ethosomal formulation was incorporated into a gel base. Various ratios of Pluronic F 127 and carbopol 934 were used to prepare gel formulations, which were then evaluated. **Results and Discussion:** Microscopic examination of formulated ethosomes shows results within the standards. After 24 hours of study, the percentage of drug permeated was 94.7 ± 0.153. A Box-Behnken design was used to prepare and optimize ethosomes. Optimized ethosomes exhibited a vesicle size of 276.8 nm, a zeta potential of -55.7 mV, and an entrapment efficiency of 90%. Carbopol gel base was used to prepare the gel and evaluated. **Conclusion:** The optimized ethosomal formulation was selected for preparing an ethosomal gel loaded with lurasidone HCl. The prepared gel formulations were evaluated, and all showed satisfactory results, indicating that ethosomes are the best alternative route for administering lurasidone HCl.

INTRODUCTION

Ethosomes are lipid vesicular carriers, also known as alcoholic liposomes, composed of phospholipids and ethanol at sufficiently high concentrations [1]. Ethosomes can be used to transport both hydrophilic and lipophilic drugs [2]. Ethosomes are small in size (microns to nanometers), which enables their potential for skin penetration and systemic drug delivery [3]. The ease of formulation, non-irritating character, ability to encapsulate a broad range of drug molecules, and increased stability compared with other vesicular systems indicate that ethosomes are the most preferred delivery vehicles for topical drug delivery [4]. The formulations may contain phospholipids

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ranging between 1 and 4 percent. Ethosomal formulations contain 22-40 percent alcohol, which may act as a softener, vehicle, and penetration enhancer, combined with glycol [5]. Several studies reported that drug administration can be adjusted by altering the mixture composition of alcohol and phospholipids, thereby increasing bioavailability [6].

Lurasidone HCl is an antidepressant drug that belongs to BCS class II with limited water solubility (0.224 mg/mL) and high permeation abilities [7]. The drug has poor oral absorption, with only 9-19% reaching the systemic circulation, because a large portion of the medication is broken down during the initial hepatic filtration phase and undergoes 76% first-pass metabolism [8].

Food affects lurasidone absorption when this drug is taken by mouth. Conventional dosage forms of lurasidone exhibit low bioavailability and adverse effects such as muscle tremor, orthostatic hypotension, and hyperprolactinemia [9]. Nanolipid carriers, such as ethosomes, have been developed to address the limitations of traditional dosage forms by enhancing bioavailability via transdermal drug delivery [10]. The objectives of the current research work are to formulate ethosomes loaded with lurasidone HCl using the solvent evaporation method and to characterize the ethosomal formulations in terms of size, shape, surface charge, and entrapment efficiency. A Box–Behnken design was used to optimize an ethosomal formulation. An ethosomal gel was prepared incorporating the optimized ethosomal formulation.

MATERIALS & METHODS

GBS Life Sciences Pvt. Ltd., Hyderabad, provided lurasidone HCl as a gift sample, and the remaining excipients, such as soya lecithin, ethanol, cholesterol, transcutol, chloroform, Rhodamine, Carbopol, and Pluronic F 127, were laboratory-grade chemicals procured from SD Fine Chemicals Ltd. in Chennai, India.

PRELIMINARY STUDIES

Construction of Standard Calibration Curve

Dilutions were prepared using standard methods. 20 µl of lurasidone HCl solution was injected into the HPLC system, and separation was performed using a UV detector at 228nm. The lurasidone HCl content in a sample of unknown concentration was measured by quantifying the peak volume on the chromatogram and comparing it with the standard calibration curve. The peak area reflects a specific concentration of lurasidone HCl; thus, the drug quantity can be determined [11].

Drug-excipient incompatibility studies

Potential interactions between lurasidone hydrochloride and the excipients in the formulated ethosomes were evaluated using Fourier transform infrared (FTIR) spectroscopy. All FTIR spectra of physical mixtures of pure lurasidone HCl and each of the excipients were acquired by the KBr pellet method on a Perkin-Elmer FTIR spectrophotometer ranging between 4000 and 400 cm-1. Comparing the spectra of the physical mixtures with those of the pure components could reveal shifts or variations in peak positions and indicate interactions between lurasidone and the excipients. To ensure no incompatible interactions had occurred that would affect the stability and performance of the ethosomal gel, FTIR was employed [12], [13].

Methodology- Box-Behnken design

Based on the literature review, it was suggested that the concentrations of ethanol, phospholipids, and penetration enhancers in ethosomes formulations are 10-50%, 1-4%, and 5-20%, respectively [14]. Based on this, the ideal concentrations were set to low, middle, and high levels to develop formulations using a Box-Behnken design. Ethanol, lipid, and penetration enhancer concentrations significantly impact vesicle size, zeta potential, and entrapment efficiency (EE) of ethosomes.

The concentrations of ethanol, phospholipid (Soya lecithin), and penetration enhancer were taken as independent variables. In the Box-Behnken design, some formulations have the same factor values. These points are called center points and are repeated multiple times. Repeating center points helps estimate experimental error; repeating the same formulation also helps determine pure error and check reproducibility: if all 5 runs give similar responses, the experiment is reliable and consistent. The levels of independent variables used to develop an optimized ethosomal formulation via Box-Behnken design are shown in Tables 1 and 2 [15], [16].

Table 1: Dependent and independent variables at various levels used in the Box-Behnken design

Independent Variables	Levels			Dependent Variables
	Low	Medium	High	
Ethanol	10	20	30	Vesicle Size
Phospholipid	1	2.5	4	Zeta Potential
Transcutol	2.5	6.25	10	EE

Table 2: Formulation of lurasidone HCl ethosomes using Box-Behnken design (with independent variables and their responses)

Formulation run	Independent Variables			Lurasidone HCl (mg)	Cholesterol % w/v	Chloroform (ml)	Rhodamine
	Ethanol% v/v	Phospho lipid %w/v	Transcutol% v/v				
E1	30	2.5	2.5	20	1	10	0.05%
E2	20	1	2.5	20	1	10	0.05%
E3	10	2.5	10	20	1	10	0.05%
E4	20	4	2.5	20	1	10	0.05%
E5	10	1	6.25	20	1	10	0.05%
E6	30	1	6.25	20	1	10	0.05%
E7	20	2.5	6.25	20	1	10	0.05%
E8	20	2.5	6.25	20	1	10	0.05%
E9	20	2.5	6.25	20	1	10	0.05%
E10	20	1	10	20	1	10	0.05%
E11	20	2.5	6.25	20	1	10	0.05%
E12	10	2.5	2.5	20	1	10	0.05%
E13	30	2.5	10	20	1	10	0.05%
E14	20	2.5	6.25	20	1	10	0.05%
E15	30	4	10	20	1	10	0.05%
E16	10	4	6.25	20	1	10	0.05%
E17	20	4	6.25	20	1	10	0.05%

Preparation of Ethosomes Loaded with Lurasidone HCl

Thin Film Hydration Technique was used to prepare lurasidone HCl ethosomes. The combination of an evaporation temperature of 45 °C and a rotation speed of 90 rpm for 15 minutes proved to be optimal. A round-bottom flask received the required amounts of lurasidone HCl, Soya lecithin, and cholesterol, which were dissolved in 10 mL of chloroform with shaking.

The required amount of penetration enhancer should be added to this mixture. Rotary evaporation was used to create the thin film by operating the rotary evaporator at 45 °C, 400 mmHg, and 90 rpm for 15 minutes. The film required vacuum drying for a few minutes. The round-bottom flask was stored in a refrigerator before the hydration process. The thin film was hydrated with ethanol at the specified ratio while magnetic stirring was continuously run for 30 minutes, until the ethanol fully penetrated the film.

After magnetic stirring, the formulation was homogenized using a Thermo Scientific D 1000 hand-held 115 V high-speed hand homogenizer at 16000 rpm for 15 minutes. Homogenization was followed by sonication using a Labpro 114A 9 L ultrasonic sonicator for 15 minutes at 15°C. All prepared ethosomal dispersions were stored at 4 °C before characterization [17], [18].

CHARACTERIZATION OF FORMULATED ETHOSOMES

Microscopic examination of vesicle size and polydispersity index

Photon correlation spectroscopy (Zeta master, Malvern Instruments Ltd.) was used to measure the mean size of the ethosomal colloidal suspension [19]. The source of light used in the experiments was a 4.5 mW laser diode at 670 nm. Size measurements were performed at a scattering angle of 90 °. The third-order cumulant fitting correlation function was used to measure the mean diameter and polydispersity index of colloidal suspensions using a Malvern PCS sub-micron particle size analyzer [20]. The samples were appropriately diluted in a filtered water-ethanol mixture at the proportion used in the preparation of ethosomes to eliminate multiple scattering, and then transferred to a quartz cuvette [21]. As usual, ethosomal colloidal suspension size determination involves measuring the mean size of five batches, yielding the mean ± standard deviation [22].

Zeta Potential Analysis

Zeta potential is a numerical value of the overall electrostatic character, due to charge-repelling or attracting forces between particles. The zeta potential was measured using a zeta potential analyzer (nanoparticle analyzer SZ-100) operated at 25 °C. Mobility in electrophoresis and mean zeta potential could now be measured directly [23], [24].

Entrapment Efficiency

The measurement of lurasidone HCl entrapment in different ethosomes colloidal systems was carried out using the dialysis method with a cellulose membrane, and the procedure was performed for 24 h at 37 °C to separate the free drug solution from the formulation. After 24 h, the entrapped drug was collected from the dialysis bag. An HPLC method was used to determine the amount of drug present in the supernatant after removing the ethosomal pellet by ultracentrifugation [25], [26]. The entrapment efficiency can be obtained by using the following equation:

$$\textit{Entrapment efficiency} = \frac{\textit{Total Drug conc.} - \textit{Supernatant Drug conc.}}{\textit{Total Drug conc.}} \times 100\%$$

Scanning Electron Microscopy

A proper dilution of the ethosomal dispersion was subjected to sonication treatment. A small aliquot of the dispersion was placed on the grid for drying. After the samples dried completely, we recorded their images using a scanning electron microscope at 60× magnification with a 12.0kV accelerating voltage under 25±2°C temperature conditions [27], [28].

Transmission Electron Microscopic Studies

Transmission electron microscopy imaging required placing a sample drop onto a carbon-coated copper grid, followed by phosphotungstic acid staining as a negative agent for 15 minutes before drying. Observations were made after the grid had completed air-drying [29], [30].

In Vitro Drug diffusion studies

The Franz diffusion cell was used to conduct in vitro diffusion studies of ethosomes loaded with the drug lurasidone HCl. The cellophane membrane was cut into pieces of the same size (6 cm x 2.5 cm) and kept in distilled water for 12 hours before use.

The drug-release analysis of the loaded ethosomes containing lurasidone HCl was conducted in 10 mL of pH 7.4 phosphate-buffered saline maintained at 37.5 ± 2 °C. The Franz diffusion cell was equipped with a magnetic stirrer for steady heating. A 2 mL ethosomes sample was added to the receptor compartment. At the standard reading positions, 1 mL of aliquots was removed and replaced with an equal volume of fresh buffer. Fresh media was added to the aliquots as needed to dilute them. Drug diffusion through the membrane was determined using HPLC. According to the diffusion study, the F17 ethosomal formulation was regarded as the optimized formulation [31], [32].

Preparation of Ethosomal Gel Incorporated with Optimized Ethosomal Formulation

Based on the results of characterization studies of ethosomes, the F17 formulation was considered the optimized formulation and incorporated into the gel base. Different concentrations of carbopol 934 and Pluronic F 127 were used to formulate five ethosomal gels, as shown in Table 5.

The carbopol 934 was soaked in water for 2 hours. The ethosomal formulation was centrifuged to obtain a 1% concentrate, which was added to the prepared gel base. Then Pluronic F 127 was added to the above gel with continuous magnetic stirring, and the remaining volume of the gel was made up with water [33], [34].

Table 3: Preparation of ethosomal gel

Formulation	Carbopol (%)	Pluronic F 127 (%)	Ethosomal formulation (%)
GF1	0.2	16	1
GF2	0.4	18	1
GF3	0.6	20	1
GF4	0.8	22	1
GF5	1	24	1

EVALUATION OF ETHOSOMAL GEL FORMULATIONS

FTIR Study

The ethosomal gel was studied using FTIR to assess drug-excipient incompatibility.

pH, visual appearance, viscosity, spreadability and drug content

The pH meter was used to determine the pH of all gel preparations. The physical stability of gels was observed for a period of three months in open and closed vials. During the 1st, 2nd, and 3rd months, samples were collected and observed visually for changes in color and appearance. Viscosity of gel preparations was determined by a Brookfield viscometer.

Spreadability studies were performed using the glass slide method. Accurately weighed 1 gm of gel was dissolved in 100 mL of 7.4 phosphate buffer & stirred continuously with a homogenizer for 2 h. After filtration, the resultant filtrate was analyzed for drug content at 228 nm using a UV Spectrophotometer [35–37].

Stability studies

Stability studies were carried out on the optimized ethosomal gel formulation in accordance with the International Conference on

Harmonization guidelines. A sufficient quantity of *in situ* gel in nasal spray bottles was stored in a desiccator containing a saturated sodium chloride solution (relative humidity (RH) of 75 ± 5%). The desiccator was placed in a hot air oven maintained at 40 ± 2 °C & the samples were withdrawn at 1, 3 & 6 months. Changes in pH, drug content, and viscosity of stored formulations were investigated. Mean values were recorded in Table 10.

Ex-vivo skin permeation studies

The *ex vivo* skin permeation studies of ethosomal gel loaded with drug and plain drug gel without ethosomes were performed using a Franz diffusion cell system, and 25 mL of pH 7.4 phosphate buffer was the dissolution medium, which was maintained at a temperature of 37± 1o C. Magnetic stirring at 100 rpm was applied throughout the procedure.

The goat skin was collected from a nearby slaughterhouse, thoroughly cleaned to remove adipose tissue, and used for the permeation study. The tissue was mounted so that the stratum corneum side faced the donor compartment. An accurately weighed 1 g of ethosomal gel was placed in the donor compartment. Samples were withdrawn at 24 h intervals, and an equal volume of fresh buffer was added to replenish the receptor phase [38], [39], [40].

RESULTS & DISCUSSION

Standard calibration curve for lurasidone HCl:

The HPLC technique was used to plot a linearity graph for lurasidone HCl over the concentration range of 10 to 50 µg/ml, with an r2 value of 0.999. The line was y = 49966x + 199.62, with a slope of 49966 and an intercept of 199.62. The calibration curve shown in Figure 1 was used to determine the unknown concentrations of lurasidone in plasma samples from the in vitro and ex vivo drug release study.

Drug excipient incompatibility studies

The interaction was measured by Fourier transform infrared (FTIR) spectroscopy. A comparison of FTIR spectra of the pure lurasidone drug and the formulation is shown in Figure 2.

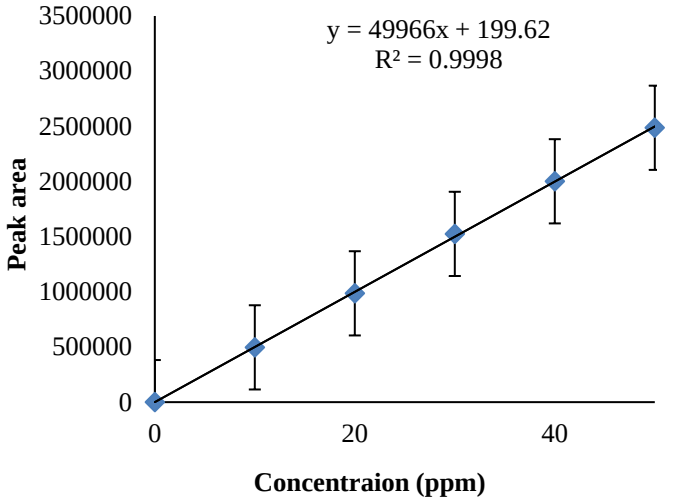

Figure 1: Standard Calibration Curve of drug lurasidone HCl by using HPLC method

Pure Drug (Lurasidone HCl) characteristic peaks in fingerprint region

- **Results of FTIR of Formulation mixture**

Major drug peaks are preserved

- Peaks at 1453, 1378, 1349, 1243, 934, 880, 839, and 664 cm^{-1} align with pure drug peaks
- No significant peak shifting or disappearance

FTIR results show compatibility between the drug and the excipients, with no significant interaction observed.

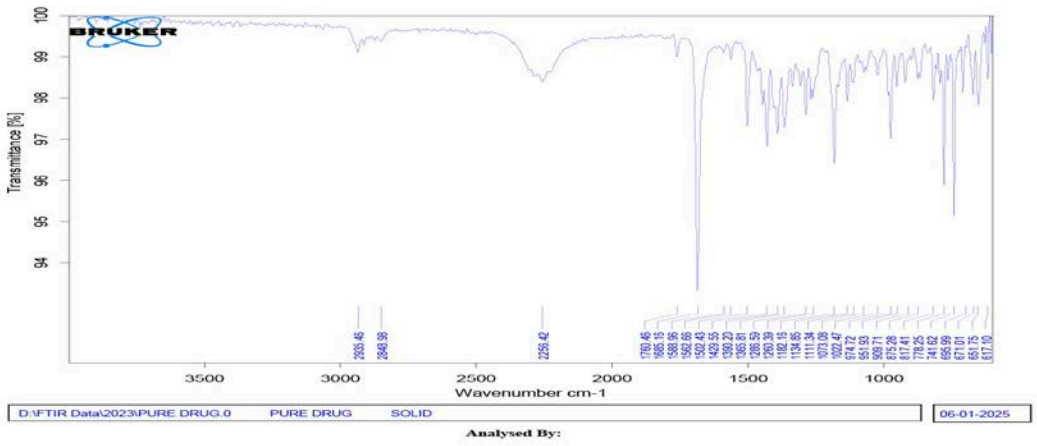

Figure 2a: FTIR of pure drug

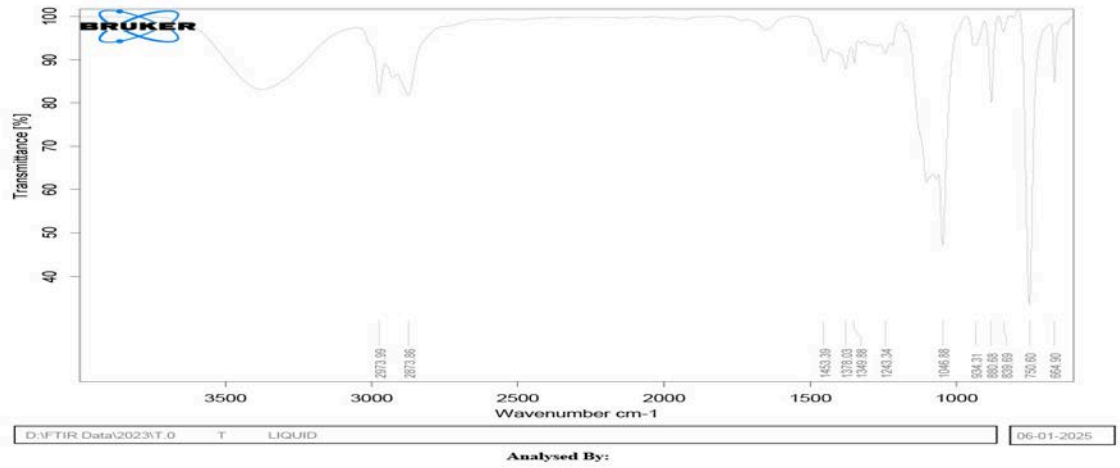

Figure 2b: FTIR of Ethosomes Formulation

Table 4: Results of evaluation parameters of formulated ethosomes by using Box-Behnken design

Formulation code	Vesicle size	Polydispersity index	ZetaPotential	Entrapment Efficiency
E1	284.9±0.27	0.184±0.36	-59.6±0.12	83.4±0.21
E2	278.9±0.18	0.301±0.45	-59.6±0.57	83.8±0.32
E3	286.3±0.35	0.159±0.57	-56.9±0.23	81.6±0.54
E4	277.6±0.38	0.214±0.26	-56.2±0.36	85.4±0.24
E5	288.5±0.18	0.236±0.39	-56.0±0.32	79.6±0.37
E6	283.4±0.29	0.261±0.48	-57.4±0.45	86.5±0.45
E7	278.2±0.35	0.183±0.27	-58.9±0.27	87.1±0.39
E8	278.2±0.47	0.162±0.49	-58.1±0.35	87.5±0.29
E9	278.2±0.34	0.238±0.32	-59.6±0.36	84.6±0.49
E10	279.9±0.31	0.354±0.47	-57.9±0.47	88.5±0.58
E11	279.6±0.45	0.251±0.26	-59.6±0.39	82.8±0.26
E12	285.7±0.18	0.158±0.39	-59.8±0.24	80.3±0.37
E13	285.4±0.24	0.205±0.45	-59.6±0.45	86.3±0.65
E14	278.2±0.61	0.196±0.28	-59.3±0.28	83.8±0.28
E15	289.3±0.47	0.231±0.59	-56.7±0.32	82.6±0.19
E16	282.4±0.36	0.184±0.19	-56.3±0.24	85.3±0.36
E17	276.8±0.24	0.154±0.18	-55.7±0.45	90±0.25

The predicted value of vesicle size is 306.81 for the optimized formulation. The predicted value for zeta potential is -56.61 for the optimized formulation. The predicted entrapment efficiency for the optimized formulation is 89.44.

Evaluation of Ethosomal formulations developed by Box-Behnken design (From E1 to E17)

The evaluation results for the prepared ethosomal formulations are shown in Table 4.

Vesicle size

The vesicle sizes of formulated ethosomes range from 276 to 786 nm. Formulations with high and low concentrations of ethanol show an increase in vesicle size, and ethanol at moderate concentration shows a smaller vesicle size. Formulations with higher concentrations of phospholipids and penetration enhancers also show smaller ethosomes vesicles.

Entrapment Efficiency

At moderate ethanol concentrations, entrapment efficiency is higher than at low or high concentrations. As the phospholipid concentration increases, the entrapment efficiency also increases. The entrapment efficiency obtained is in the range of 79-90%.

Zeta Potential

The zeta potential values range from 55.7 to 59.8 mV for the ethosomes. An optimal zeta potential was obtained at low and moderate ethanol concentrations. As the concentration of ethanol increases, the zeta potential also increases. At lower phospholipid concentrations, the zeta potential is higher; as the phospholipid concentration increases, it decreases.

Fitting data to the model

The quadratic model best fitted the given dependent variables such as vesicle size, zeta potential, and entrapment efficiency,

which were expressed in a multiple linear regression equation by fitting the data against the observed responses to many models.

Effect of independent variables on response 1- vesicle size. The vesicle size obtained at three different levels of factors A, B, and C was fitted to a multiple regression analysis to obtain a second-order polynomial equation.

$$\text{Vesicle Size} = 278.48+0.0125A-0.5750B+0.1625C+3.00AB-0.0250AC - 0.4500BC -7.35A^2+0.0725B^2-0.2525C^2$$

The positive sign in the equation represents the synergistic effect, and the negative sign represents the antagonistic effect of variables on the given response. Variables A and C show the required positive effect, indicating that increasing concentrations of ethanol and penetration enhancer increase vesicle size, and B shows the negative effect, as increasing vesicle size decreases.

Table 5: Fit statistics for vesicle size

Std. Dev.	0.8788	**R^2**	0.9802
Mean	281.85	**Adjusted R^2**	0.9548
C.V. %	0.3118	**Predicted R^2**	0.7664
		Adeq Precision	17.8419

The Predicted R^2 of 0.7664 is in reasonable agreement with the Adjusted R^2 of 0.9548; i.e., the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 17.842 indicates an adequate signal. This model can be used to navigate the design space.

Effect of independent variables on response 2- Zeta potential

$$\textbf{Zeta potential} = -59.10-0.5125A+0.7250B+0.6375C+0.2000AB-0.7250AC-0.4625A^2+2.09B^2-0.3375C^2$$

The two variables B & C show that an increase in their concentrations has a positive effect on the response. This indicates that as the concentrations of the phospholipid and penetration enhancer increase, zeta potential also increases, whereas variable A shows a negligible negative effect.

Table 6: Fit statistics for zeta potential

Std. Dev.	0.8773	**R^2**	0.8562
Mean	-58.06	**Adjusted R^2**	0.6713
C.V. %	1.51	**Predicted R^2**	-0.6921
		Adeq Precision	6.4093

A negative Predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher-order model may also predict better. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 6.409 indicates an adequate signal. This model can be used to navigate the design space.

Effect of independent variables on response 3- Entrapment efficiency

$$Z= +85.16+1.50A+0.6125B+1.69C-2.40AB+0.4000AC-0.0250BC-2.84A^2+1.18B^2+0.5825C^2$$

The equation indicates that all three variables positively affect entrapment efficiency. The ethanol concentration should be optimized to maximize entrapment efficiency; as the phospholipid concentration increases, entrapment efficiency also increases.

According to past literature, entrapment efficiency results show that the ethanol concentration should be optimal to maintain good entrapment efficiency and should not be too high or too low.

Table 7: Fit statistics for entrapment efficiency

Std. Dev.	1.78	**R^2**	0.8291
Mean	84.65	**Adjusted R^2**	0.6094
C.V. %	2.10	**Predicted R^2**	0.1644
		Adeq Precision	7.5017

The Predicted R^2 of 0.1644 is not as close to the Adjusted R^2 of 0.6094 as one might normally expect; i.e., the difference is more than 0.2. This may indicate a large-block effect or a problem with your model and/or data.

Things to consider include model reduction, response transformation, and outliers. All empirical models should be tested by doing confirmation runs. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.502 indicates an adequate signal. This model can be used to navigate the design space. Based on the results obtained from ethosomes formulations designed using a Box-Behnken design, the F17 formulation was considered the optimized formulation.

The responses of ethosomes with respect to concentrations of independent variables can be obtained by the initial design; the best concentrations of ethanol 23.824%, phospholipid 3.999%, and penetration enhancer 9.986 % were selected, which were considered as the design-suggested formulation, and at this optimum concentration of ethanol, i.e., 23.824%, the ethosomes maintain vesicle fluidization and stability.

Table 8: Summary of ANOVA

Factors	P values obtained from screened responses		
	Vesicle size	Zeta potential	Entrapment efficiency
Percentage of Ethanol	0.9690	0.1424	0.0482
Percentage of Phospholipid	0.1067	0.0520	0.3616
Percentage of transcutol	0.6171	0.0789	0.0311
Lack of fit	0.1416	0.1445	0.7617

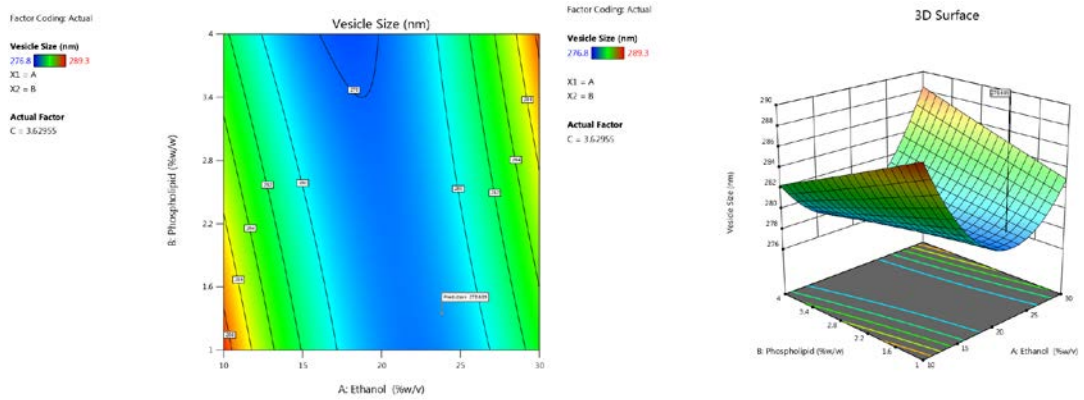

Figure 3a: Effect of variables on vesicle size

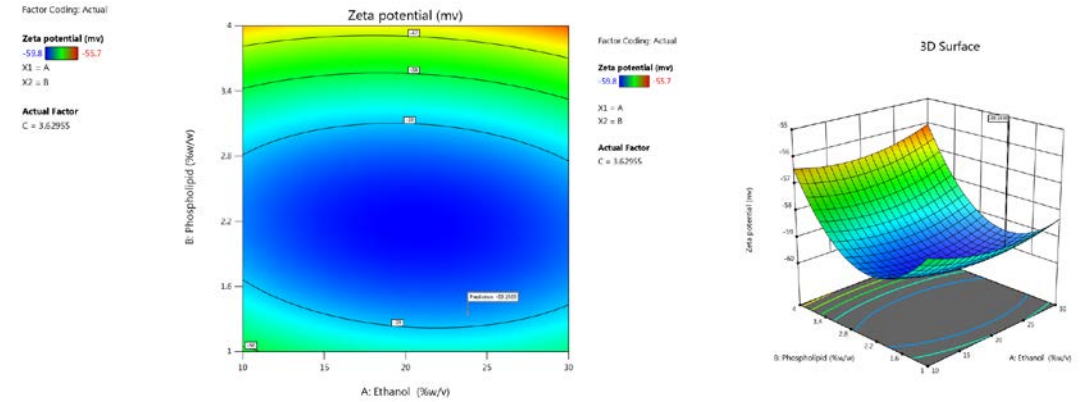

Figure 3b: Effect of variables on zeta potential

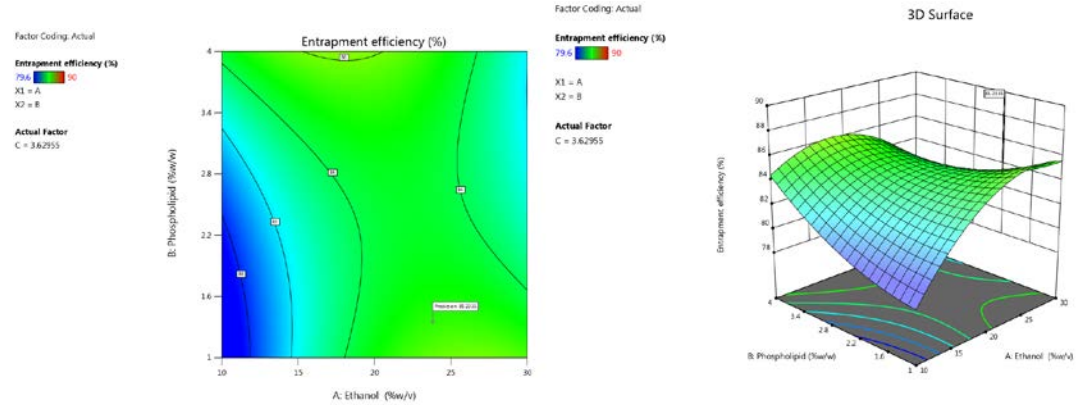

Figure 3c: Effect of variables on entrapment efficiency

Scanning Electron Microscopy

The ethosomes in the optimized formulation were predominantly spherical, as shown in Figure 4. The images suggested that round, smooth ethosomes with no crystalline drug structure and a multilamellar structure were formed. The compact particles observed in the SEM images indicated that the lipids were highly dense, thereby contributing to controlled drug release.

Transmission Electron Microscopy

Transmission electron microscopy, as shown in Figure 5, also helps confirm the surface morphology of the optimized formulation, and its observations clearly indicated that ethosomes were round and smooth.

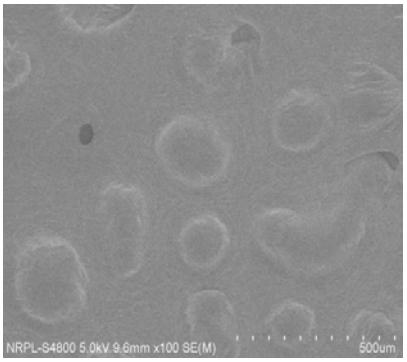

Figure 4: SEM Image of optimized ethosomes

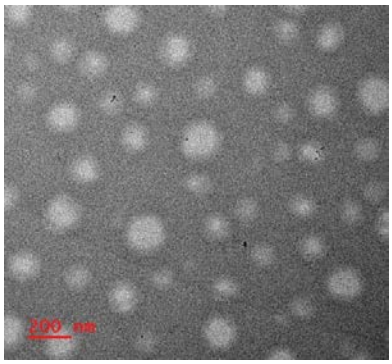

Figure 5: TEM Image of optimized ethosomes

In vitro drug diffusion studies

Based on the physical analysis results, formulation F17 was selected as the optimized formulation. Peak percent drug diffusion after 10 hours was 97.2% in the optimized formulation, compared with 52.6% in the drug suspension, as shown in Figure 6. When comparing the ethosomal formulations with the drug suspension, the ethosomal preparations exhibited a higher diffusion rate, likely attributable to the vesicular systems. The relationship between trapping ability and diffusion rate was observed in the ethosomal preparations. Formulations with high entrapment efficiency exhibited higher drug diffusion rates, implying the importance of the vesicular systems (the outer lipid membrane) in drug diffusion.

ETHOSOMAL GEL

FTIR study of ethosomal gel

FTIR studies were performed on the formulated ethosomal gel, as shown in Figure 7. The FT-IR spectrum of the ethosomal gel also displayed characteristic functional group bands.

Comparative Interpretation

- The major functional peaks of the drug are retained in the ethosomal gel, indicating no chemical degradation.
- Minor shifts and broadening of O–H, C=O, and C–N bands suggest hydrogen bonding and physical entrapment of drug within ethosomal vesicles.
- No new peaks were observed, confirming the compatibility of the drug with ethosomal excipients and successful entrapment without chemical interaction.

pH, visual appearance, viscosity and drug content

All gel formulations were kept at room temperature and evaluated for physical appearance; changes in color and lump formation were observed at different time intervals. The ethosomal gels were clear and transparent, with no lump formation, indicating stable compositions. The pH of the gels was measured with a pH meter, and it ranged from 6.2 to 6.8, indicating they are nonirritating during long-term exposure. The observed drug content ranged from 85.81 ± 0.5 to 95.13 ± 0.5. The viscosity of the formulations was observed to be in the range of 5000 to 10000 cPs, which is optimal for transdermal delivery. The spreadability test was performed for all gel formulations, and the results are within limits. Based on the physical evaluation, formulation GF4 is considered the best and most stable, with a pH of 6.8 ± 0.5 and a drug content of 95.13 ± 0.5. Further ex vivo permeation studies were conducted on the GF4 formulation.

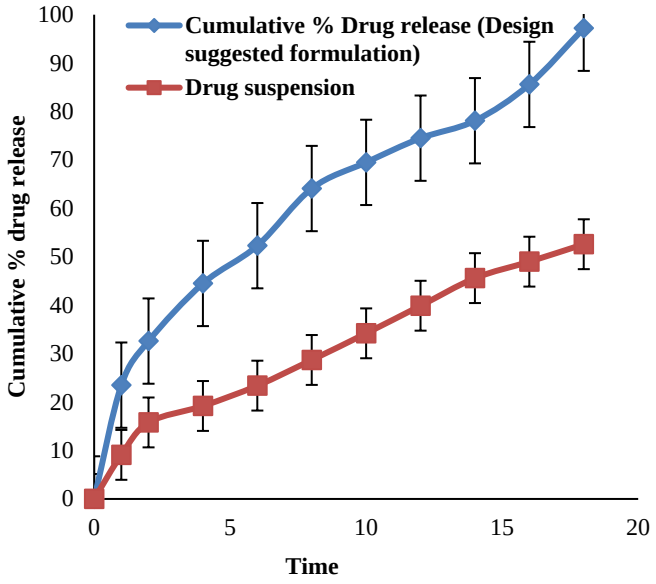

Figure 6: In vitro drug diffusion profile of optimized formulation compared with that of the in vitro drug diffusion profile of drug suspension.

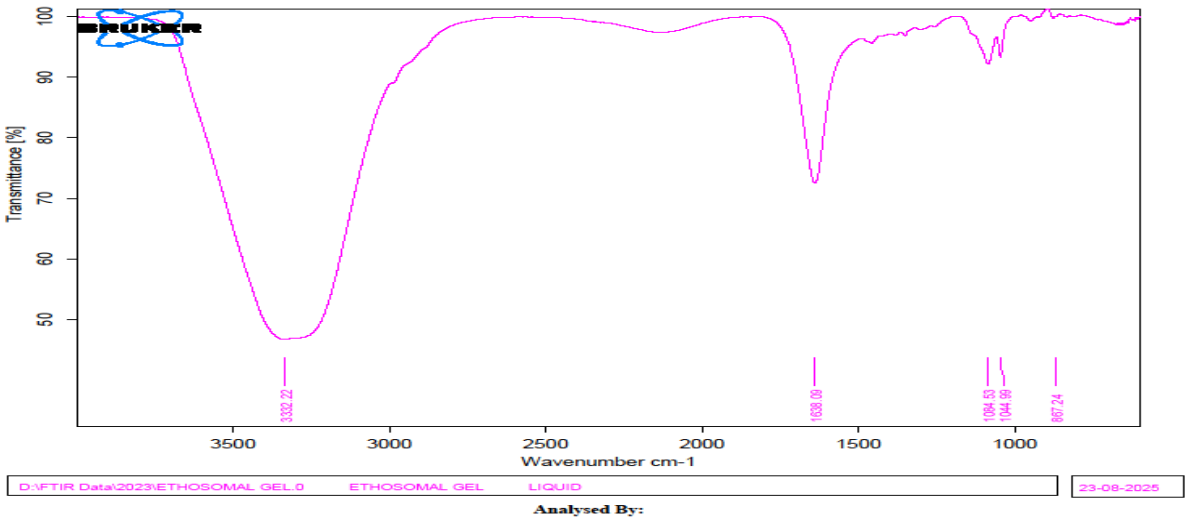

Figure 7: FT-IR of formulation excipients of Ethosomal gel

Table 9: pH, Visual appearance, viscosity, and drug content of ethosomal gels

Formulation	pH	Visual appearance	Drug content	Viscosity	Spreadability (g.cm/sec)
GF1	6.7±0.5	No change in color and no lump formation	87.42±0.5	5789cP	12.56±0.32
GF2	6.4±0.5		85.81±0.5	6135cP	13.87±0.21
GF3	6.2±0.5		93.32±0.5	7120cP	15.58±0.35
GF4	6.8±0.5		95.13±0.5	7816cP	15.92±0.12
GF5	6.7±0.5		91.40±0.5	8122cP	16.23±0.22

Table 10: Stability studies

Testing period	pH	Drug content	Viscosity
1 month	6.6±0.5	95.30±0.5	7820cP
3 months	6.5±0.5	94.63±0.5	7820cP
6 months	6.8±0.5	95.13±0.5	7820cP

Ex-vivo skin permeation studies of ethosomal gel

Based on the drug content results, gel formulation GF4 has a high drug content and is considered the optimized gel formulation. Ex-vivo permeation studies were carried out using goat skin, and the percent drug permeation was found to be 94.7 ± 0.15% over 24 hours, as shown in Figure 8.

Kinetic model fitting

Different release characterization models have been attempted to describe permeation profiles. The R2 value of the ethosomal gel was found to be 0.966 and 0.915 at 0 to 30min and 30 min-24 hrs, respectively, by zero order; 0.965 and 0.989 at 0 to 30min and 30 min- 24 hrs, respectively, by first order; and 0.745 and 0.934 by the Higuchi and Peppas model. The best fit was indicated by the correlation coefficient (R2). Based on the kinetic model fitting analysis, the ethosomal delivery systems were found to follow first-order kinetics, and the higher R2 values associated with the Korsmeyer-Peppas model (R2=0.934, n=0.54) suggest that the encapsulating polymer plays the major role in regulating drug release. The value of the release exponent, n, in the Peppas model showed non-Fickian diffusion.

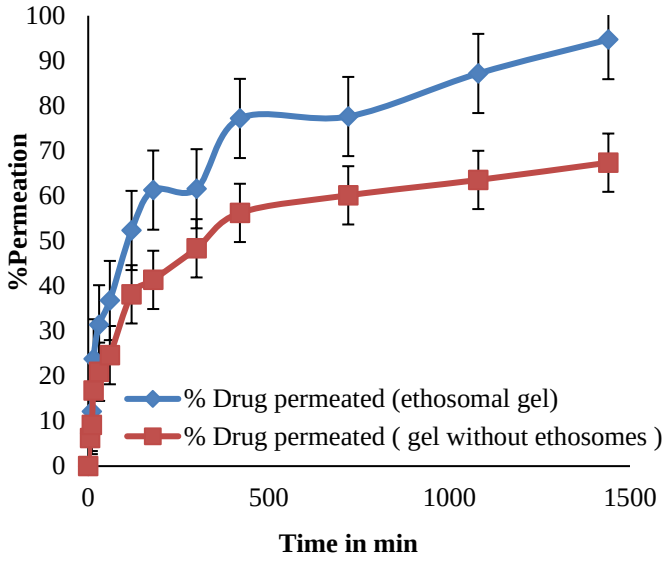

Figure 8: Ex vivo skin permeation studies of optimized ethosomal gel formulation

Calculation of steady state flux

Table 11: Steady state flux for the given ex vivo permeation data

Name of the gel	Steady state flux	Enhancement Ratio
Gel loaded with ethosomes	130µg/ml .cm3	3.25
Gel (control without ethosomes	40 µg/ml .cm3	

Results of steady-state flux indicate that transdermal delivery of ethosomes is the best route of administration, with an enhancement ratio compared with gel-loaded ethosomes in plain gel. The threefold increase in flux indicates a good enhancement ratio.

CONCLUSION

The current research has been effective in developing and optimizing ethosomal formulations of lurasidone HCl for transdermal drug delivery. To understand the effect of critical variables in the formulation, the Box-Behnken design was used. The Independent variables, namely ethanol concentration, phospholipid content, and the penetration enhancer, showed significant effects on vesicle size, zeta potential, and entrapment efficiency. Ethanol 23.824 %, phospholipid 3.999 %, and penetration enhancer 9.986 % were the concentrations of the design-suggested formulation that displayed desirable physicochemical properties. This is an efficient way to identify formulations with the desired properties.

The optimized ethosomal formulation was incorporated into a gel base, and five gel formulations were prepared at different concentrations of Pluronic F127 and carbopol 934. The fabricated gels were evaluated for their properties such as pH, viscosity, drug content, and visual appearance. Based on the results for drug content, pH, and viscosity, the G4 formulation was considered the best and was evaluated in ex vivo skin permeation studies. The ex vivo permeation studies found that the ethosomal gel exhibits first-order kinetics. The release of lurasidone HCl from the drug-loaded ethosomal gel demonstrated the potential of ethosomes to deliver the drug through the transdermal route. This method is more efficient than conventional dosage forms like tablets. Further studies, such as in vivo evaluation, may demonstrate the efficiency of ethosomes in improving the bioavailability of lurasidone HCl.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Shailaja Pashikanti provided overall supervision throughout the research work, and Sesha Sai Durga Manyam carried out the remaining work, including literature review, Investigation, Methodology, sample analysis, and writing the original manuscript, ensuring accuracy and reliability in the experimental work.

DECLARATION OF USE OF AI TOOLS

The authors did not use any AI tools to draft this manuscript. All content, analysis, and conclusions were reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work.

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