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Original article

Development and Box–Behnken Optimization of Empagliflozin-Loaded Ethosomes for Enhanced Transdermal Delivery

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ABSTRACT:
Preventing type 2 diabetes, a metabolic condition, requires adjusting one's diet, exercising regularly, and keeping one's weight in check. People with type 2 DM want individualized care plans that improve their quality of life. We developed a new transdermal vesicular delivery method for Empagliflozin ethosomes in the current investigation. Box Behnken design using-response surface methods was used to improve the cold-prepared Empagliflozin ethosomes. Empagliflozin ethosomes were tuned and characterized for EE, VS, ZP, PDI, and shape using scanning electron microscopy. Empagliflozin was successfully incorporated into ethosomes, as shown by DSC and FT-IR tests demonstrating its amorphous condition in ethosomes. In vitro transdermal absorption and skin retention studies showed that ethosome-encapsulated Empagliflozin exhibited significantly higher cumulative penetration (Qn) and in vitro permeation compared to that of 25% pure drug solution, indicating not only improved transdermal absorption but also increased storage in the skin. Ethanol-phospholipid cholesterol ethosomes exhibited excellent features, including good skin permeability and adequate stability, and hence represent a promising approach for Empagliflozin.

Keywords: Empagliflozin, Ethosomes, Box Behnken design, Transdermal, in vitro permeation.

1. INTRODUCTION

Conventional drug delivery systems such as tablets, capsules, and injections often suffer from limitations including poor bioavailability, dose-related toxicity, frequent dosing, and reduced patient compliance. To overcome these drawbacks, novel drug delivery systems (NDDS) have been developed with the aim of improving therapeutic efficacy, minimizing side effects, enabling site-specific drug targeting, and enhancing patient adherence. These advanced systems mainly include controlled drug delivery and targeted drug delivery approaches, offering benefits such as improved safety, prolonged drug action, reduced toxicity, and better treatment of previously incurable diseases.

Among NDDS, vesicular drug delivery systems have gained significant attention due to their ability to encapsulate both hydrophilic and lipophilic drugs. Vesicles are microscopic, bilayered structures resembling biological membranes and play a vital role in cellular transport and communication. Their amphiphilic nature allows efficient drug loading, protection from degradation, and controlled release. Importantly, vesicular systems can overcome the stratum corneum, the major barrier to transdermal drug delivery.

The transdermal drug delivery system (TDDS) has emerged as a promising alternative to oral and parenteral routes, offering advantages such as avoidance of first-pass metabolism, reduced gastrointestinal side effects, sustained drug release, ease of termination, and improved patient compliance. Various vesicular carriers such as liposomes, niosomes, transferosomes, pharmacosomes, virosomes, cubosomes, and ethosomes have been explored for transdermal applications.

Among these, ethosomes represent a significant advancement in vesicular research. Ethosomes are soft, malleable lipid vesicles composed mainly of phospholipids, ethanol (20–45%), and water. The high ethanol content enhances skin permeability by fluidizing the lipid layers of the stratum corneum, allowing deep penetration into the skin and even systemic circulation. Compared to conventional liposomes, ethosomes exhibit superior deformability, higher drug entrapment, enhanced permeation, and improved stability.

Ethosomes offer several advantages, including non-invasive delivery, suitability for large and poorly permeable molecules (such as peptides and proteins), use of non-toxic excipients, and versatility in pharmaceutical, cosmetic, and

veterinary applications. They have been successfully investigated for the transdermal delivery of antivirals, antibiotics, hormones, anti-inflammatory agents, anticancer drugs, DNA, and vaccines. Several ethosomal products are already available in the market, confirming their clinical potential.

Despite their advantages, ethosomes face certain limitations such as low yield, formulation instability at high alcohol content, and possible skin irritation. Nevertheless, their safety profile is generally favorable, with studies reporting minimal skin irritation and good tolerability.

Overall, ethosomes represent a promising and efficient vesicular carrier system for transdermal drug delivery, capable of enhancing skin permeation, improving therapeutic outcomes, and addressing the limitations of conventional dosage forms.

2. MATERIALS & METHODS

Empagliflozin was obtained as a gift sample from **Hetero** Pharma Pvt. Ltd**.**Phospholipon® 90H (soya lecithin) was used as the phospholipid. Other excipients included cholesterol, triethanolamine, Carbopol 940, propylene glycol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, absolute ethanol (99.9%), methanol (HPLC grade), and purified water. All chemicals were of

Major instruments used included UV–Visible spectrophotometer, FT-IR spectrophotometer, DSC, electronic balance, mechanical and magnetic stirrers, sonicator, centrifuge, and Malvern Zetasizer for vesicle characterization.

Empagliflozin is a selective SGLT-2 inhibitor used in the management of type-2 diabetes mellitus. It reduces renal glucose reabsorption, leading to increased urinary glucose excretion and improved glycemic control.

Drug Identification and Characterization

Empagliflozin was authenticated by physical appearance, melting point, solubility, FT-IR, DSC, UV spectroscopy, and partition coefficient determination.

Drug–Excipient Compatibility

Compatibility was assessed using FT-IR, DSC, and UV spectroscopy for the drug, excipients, physical mixtures, and optimized ethosomal formulation under accelerated stability conditions.

Analytical Method

A calibration curve of Empagliflozin was prepared using methanol and phosphate buffer (pH 7.4). The maximum absorbance was determined at **224 nm** using UV spectrophotometry.

Preparation of Empagliflozin-Loaded Ethosomes

Ethosomes were prepared by the **cold method**. Empagliflozin, lecithin, and cholesterol were dissolved in ethanol and propylene glycol. The aqueous phase was slowly added under mechanical stirring, followed by probe sonication to reduce vesicle size. The formulation was stored at 4–8 °C.

Experimental Design

A Box–Behnken design was employed using Design-Expert® software to optimize lecithin (X_1), cholesterol (X_2), and ethanol concentration (X_3). Responses evaluated were entrapment efficiency (Y_1), vesicle size (Y_2), zeta potential (Y_3), and drug release (Y_4).

Characterization of Ethosomes

- **Entrapment Efficiency (%EE):** Determined by centrifugation and UV analysis
- **Vesicle Size & Zeta Potential:** Measured using Malvern Zetasizer
- **In-vitro Drug Release:** Conducted using dialysis bag method in phosphate buffer

In-Vitro Skin Permeation Study

Skin permeation was studied using Franz diffusion cells with rat/goat skin. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically. Flux and permeability coefficient were calculated.

Formulation of Ethosomal Gel

Optimized ethosomes were incorporated into Carbopol 940 gel. pH was adjusted to 6.0–7.5 using triethanolamine. The gel was filled into aluminum tubes.

Evaluation of Ethosomal Gel

The gel formulations were evaluated for:

- Physical appearance
- pH
- Viscosity
- Spreadability
- Drug content
- In-vitro drug release and permeation

Stability Studies

Stability studies were conducted in accordance with ICH Q1A (R2) guidelines at refrigerated and accelerated conditions for a period of three months. Samples were evaluated for physicochemical parameters and drug release.

3. RESULTS & DISCUSSION

Identification and characterisation of Empagliflozin. Empagliflozin was successfully identified and characterised physicochemically, yielding the following findings: 5.2 FTIR (Fourier Transform Infrared Spectroscopy): A drug's molecular structure and fingerprint can be determined via FTIR, a type of vibrational spectroscopy. Pure Empagliflozin displayed the signature bands of O-H, C=C, aromatic C-O, O-H, C=O, and C=C groups. Absorption peaks were seen in the FTIR spectra of pure Empagliflozin at 3367.10 cm-1 (OH stretching), 1613.16 cm-1 (C=C, aromatic), and 1246.70 cm-1 (C-O ester stretching). Peaks for the C-Cl bond at 1018 cm-1, the O-H elastic response at 3375 cm-1, and the C-C bond at 1614 cm-1 were all produced by Empagliflozin in all of the compounds.

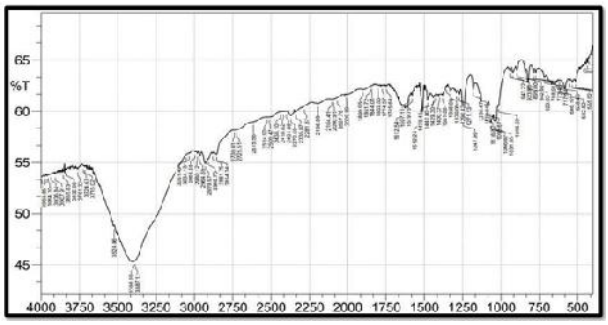


Figure 1: FTIR spectral studies of Pure Drug (Empagliflozin)

Differential scanning calorimetry

Since this is one of the most useful methods for assessing drug and excipient mix compatibility, the thermodynamic compatibility of empagliflozin with different polymers was investigated by measuring their crystalline melting and glass transition temperatures. The DSC curve's departure and subsequent return to the baseline indicate the melting endotherm. By extending the baseline from the point where the tangent hits the steepest slope on the peak's major side, one can determine the melting point, also known as the onset temperature. In Figure 12, the melting endotherm of empagliflozin is displayed at a heating rate of 20C min-1. The sample's onset temperature is 80.14 degrees Celsius; its peak temperature is 74.90 degrees Celsius, and its endset temperature is of 83.19 degrees Celsius (DHm = -19.4 J/gm).

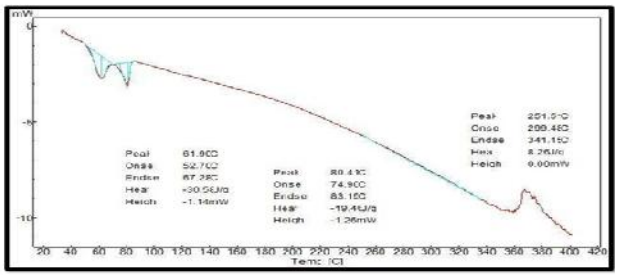


Figure 2: DSC thermogram of Pure Drug

Powder X-ray diffraction (P-XRD) analysis

The XRD spectrum of empagliflozin is shown in the graphic. The reference data supplied by Savi Pharma (India) is consistent with the XRD spectra of empagliflozin. The same 21° frequency marked the peak for both. The strong peak in the XRD spectra indicates that the medication is crystalline. The molecular structure of powdered empagliflozin was examined using an XRD pattern. It is evident from this picture that there were ten peaks in the range of the tenth and thirtieth places.

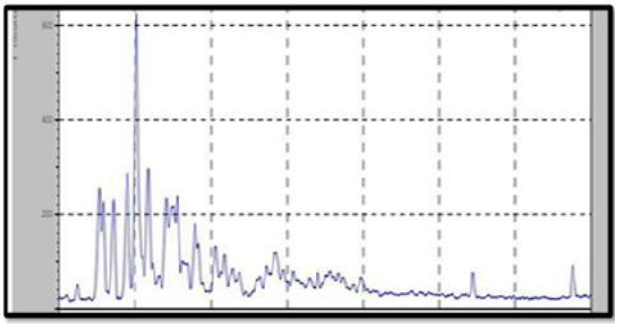


Figure 3: Powdered X-ray diffraction studies of Empagliflozin

The previously described tests verified the validity and purity of the obtained Empagliflozin sample. Empagliflozin exhibits some crystalline behaviour, as seen by a large, sharp peak in DSC thermograms and XRD spectra.

Physicochemical Characterizations

Organoleptic properties

Colour – White to off-white solid.

Taste – Slightly bitter taste,

Odour - odourless

Preformulation studies of Empagliflozin.

Preformulation experiments have established the nature of the medicine. Every range observed in the preformulation trials closely resembles the ranges observed with regular Empagliflozin.

Melting Point

The drug's melting point was ascertained by capillary fusion. Measuring the drug's melting point revealed that it was within the usual range. You can see the findings in Table 6 below. 74.09±1.59°C was found to be the melting point of empagliflozin utilising the glass capillary technique and melting point equipment. This value was found to be compatible with the 74–78°C melting point of empagliflozin that is described in the scientific literature.

Table 1: Melting Point of Empagliflozin

Apparatus	Observedvalue	Reference Value
Meltingpoint apparatus	74.09±1.59 ^{0}C	**74 -78°C**

Solubilitystudies

Acyclonemixer(REMICM101,India)wasusedtotestthesolubilityofempagliflozin in a range of solvents using the equilibrium solubility technique. pharmaceutical solubility experiments in the buffer mediums and solvents given below. When dissolvedinmethanol(27.36±0.12mg/mL)andpolyethyleneglycols(>25mg/mL)at 25 degrees Celsius, empagliflozin dissolves much more readily than it does in water (0.173±0.23 mg/mL). According to the solubility investigation, the drug is soluble in methanol but nearly insoluble in water. It has been reported in the literature that empagliflozin dissolves in water at a rate of 0.8 mg/mL.

Table 2:Solubility determination in different mediums

S. No	Medium	Solubilitydetermination Concentration(mg/mL)
1	Methanol	27.36±0.12
2	Dichloromethane	21.34±3.45
3	PEG200	16.36±3.21
4	PEG400	19.39±2.46
5	PEG600	13.84±1.27
6	Ethanol	6.39±1.25
7	Chloroform	17.43±0.83
8	0.1N HCl pH 1.2	19.37±0.07
9	BufferpH6.8	3.35±0.023
10	BufferpH7.4	1.17±0.053
11	Water	Insoluble

Ultraviolet(UV) spectrum

Preformulation research necessitates the creation of an analytical technique for assessing a drug's effectiveness. C-glycosylation As an alternative to the anomeric hydroxygroup, the beta-D-glucose derivative empagliflozin contains a 4-chloro-3-(4- ethoxybenzyl) phenylgroup. In conjunction with a balanced diet and frequent exercise, propanediol monohydrate helps people with type 2 diabetes better control their blood sugar levels. Figure 14 plots the UV absorption spectra of empagliflozin in methanol and buffers containing 1% sodium lauryl sulphate (SLS). In methanol with SLS 1% buffer, the maximum absorption (max)of empagliflozinwasobservedtooccurat235.5 nm.

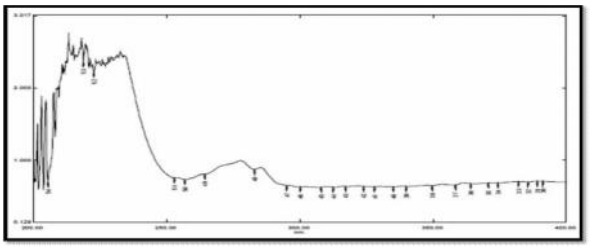


Fig 4: UV Spectrum analysis

Partition coefficient determination

The range of expected log P values for empagliflozin is 2.11±0.06 to 2.52±0.02,which suggests that it is hydrophobic and accounts for its affinity for lipid membranes, interactions with protein hydrophobic domains, and translocation across the blood-brain barrier, among other experimental findings. Empagliflozin may be easily recognized and its concentration precisely measured because to the previously described spectrum properties. In octanol:water, empagliflozin's log P value is 2.13±0.03.The estimated log Pvalue of 3.4 coincided with the number that had been reported earlier, 3.4.Drugsare considered hydrophobic if their logP value is greaterthan1.

Experimental design for the preparation and optimization of Empagliflozin ethosomes

Design-Expert® software was used to analyse the data, and a three-factor, three-level factorial design was employed to determine the best Empagliflozin loaded ethosomal formulations. The dependent variables in this study were the percentage EE (Y1), vesiclesize(Y2),zetapotential(Y3),and%CDR(Y4),whereasthei ndependentfactors were the phospholipon® 90G (X1), cholesterol (X2), and ethanol (X3). Following a thorough literature search and analysis of the early test results, we determined the independent variables and their corresponding values. Table 12 presents the results of 17 formulations made using the factorial design in terms of % EE, vesicle size, zetapotential, and %CDR. The gathered data was fitted using quadratic models, two- factor interaction (2FI), and linear regression. We were then able to evaluate the influence of the independent factors on each answer by using the results to construct a second-order polynomial equation with interaction and quadratic components. This is
how a polynomial equation is often written.

$$Yi = \beta 0i + \beta 1iX1 + \beta 2iX2 + \beta 12iX1X2 + \beta 11iX12 + \beta 22iX22 \quad (1)$$

In thiscase,X1 X2 and Xi2(i=1-2)represent the interaction and quadratic terms, Yi(i
= 1-2) are the two dependent variables, X1 and X2 are coded levels of independent variables, and X0i is the arithmetic average response of the nine runs. Calculating the coefficient magnitudes and their corresponding positive and negative mathematical signs allowed decision-makers to use the polynomial equation. The more significant themodel components, the closer the coefficient value is to one,themoreessentialthe model variables are. Positive and negative signs in polynomial equations indicate that increasing the amount of one variable causes the associated response to rise, and vice versa. Using ANOVA, the statistical significance of the model and its terms was evaluated.A5%levelofassuranceisappliedtothemodeland/ormodeltermsifthep- value (significant probability value) is less than 0.05. To help show and make clearer the relationships between the explanatory and analytical variables, Design-Expert®

software was utilised to create perturbation graphs, 3D response surface plots, and 2D contour plots. The linear correlation graphs between the predicted and observed data further demonstrated the model's dependability. High R2 values (>0.9000) for all answers show a good fit between the observed and anticipated values. The best formulation was selected using the software's point prediction technique. Using a quadratic model, the formulation's parameters were optimised.

Table 3: Independent variables, their levels and experimental runs of Box- Behnken design for ethosomes formulations.

Run	X1	X2	X3	Y1	Y2	Y3	Y4
1	4	0.5	40	64.51±0.24	328.54±5.34	-33.12±0.59	76.56±0.69
2	2	0	30	72.72±0.13	119.34±2.16	-28.61±1.24	89.23±1.34
3	3	0.5	30	74.94±0.26	128.48±4.18	-26.54±1.03	66.73±0.25
4	3	0	40	90.89±0.01	109.46±2.17	-27.41±1.26	94.49±1.37
5	3	1	20	51.82±0.35	329.64±2.01	-36.45±3.05	70.41±0.34
6	3	1	40	53.69±0.14	385.15±1.06	-32.15±1.08	63.28±0.26
7	3	0.5	30	78.93±0.28	127.64±3.25	-28.35±2.14	66.72±0.59
8	3	0.5	30	70.84±0.16	159.28±5.37	-26.48±1.22	72.62±1.52
9	4	1	30	49.19±0.37	321.51±4.13	-30.12±1.34	72.27±2.04
10	2	1	30	48.76±1.25	299.86±2.01	-21.03±1.03	66.81±1.06

Fitting of data to the model

To find the optimal model,the observed responses for each of the 17 formulations were concurrently matched to the various mathematical models using Design-Expert® software. The predicted residual sums of squares (PRESSs), standard deviations, coefficientsofvariation,projectedmultiplecorrelationcoefficients,modifiedmultiple correlation coefficients, mean values, and multiple correlation coefficients for each model are shown in Table 3. With low SD, CV, and PRESS values and high R2, adjusted R2, and expected R2s values,the quadraticmodel was foundto havethebest fit for all four answers (Y1, Y2, Y3, and Y4). The PRESS statistic will demonstrate if themodelandthedataareagoodfit.Withrespecttothealternatives,therecommended model's PRESS will be lower.

Effect of independent variables on % entrapment efficiency(Y1)

The percentage of the fullmedicine contained in nanovesicular formulations is known as their entrapment effectiveness. The system's capacity for drug storage and subsequent distribution is determined by the entrapment efficiency, which makes its computation essential. The results of the ethosomes formulations of empagliflozin providing efficient trapping are shown in Table 13. A maximum of 90.89±0.01 for F4 and a minimum of 48.76±1.25 for F10 were the ranges of entrapment efficacy. This quadratic equation shows how the independent variables and the % EE are related to each other:

EE=+74.34+0.6813A-14.61B+2.31C-0.0575AB+5.86AC-0.4100BC-13.99A2-1.34B2- 3.56C2

whereY1representstheentrapmentefficiencyinpercentageandX1,X2,andX3stand for the phospholipid, cholesterol, and ethanol, respectively. The equation shows that increased phospholipid and ethanol concentrations lead to a higher proportion of EE, while increased cholesterol concentrations have the reverse effect. This implies that while the ethosomes' percentage EE decreases with increasing cholesterol level, it increases with increasing phospholipid content. The greater X2 coefficient value indicates that cholesterol had a more significant effect on the Empagliflozin ethosome EEthanphospholipid.Themodelcanberegardedasstatisticallysignificantwithan F- valueof80.54.Lessthan0.01%ofcaseshaveanF-valuethishighasaresultofrandom chance. If the p-value of a model term is less than 0.0500, it is considered statistically significant. In this case, the model's essential elements are B, C, AC, A2, and C2. The Lack of Fit is statisticallysignificant with an F-valueof 8.52. It is quiteunlikely(only 3.28percent)thata LackofFitF-value this high is the product of random chance. signal-to-noise ratio is 28.439.

The 3D-response surface graphs and corresponding 2D-contrast plots in Figure 16 illustrate the impact of independent factors on the percentage of energy efficiency. As thephospholipidconcentrationwasincreasedfrom2%to4%,figure16 shows that the ethosomes' ability to entrap empagliflozin was more effective. When lipophilic drugs are encapsulated in ethosomes, it has been demonstrated that their EE is significantly higher than that of hydrophilic drugs. Drug EE is improved by increasing the phospholipid concentration in the vesicle because hydrophobic medicines are better able to interface hydrophobically with the vesicle membrane. An increase in phospholipid concentration may potentially improve medication molecules' access to the lipid phase and their subsequent accommodation in lipid bilayers. But it was discovered that a substantial rise in the percentage of EE occurred when the ethanol levelwasraisedfrom30%to40%;this couldbebecauseofethanol'sco-solventaction. Because of its lipophilic molecular nature, empagliflozin is soluble in ethanol. Thus, raisingtheethanolcontentwouldencouragedrugsolubilizationand entrapmentinside the hydro-ethanolic core and lipid bilayers of the vesicle. The phospholipid bilayer in ethanolispartiallydissolvedbyethanol,whichcausesthevesicleslo leakwhenethanol

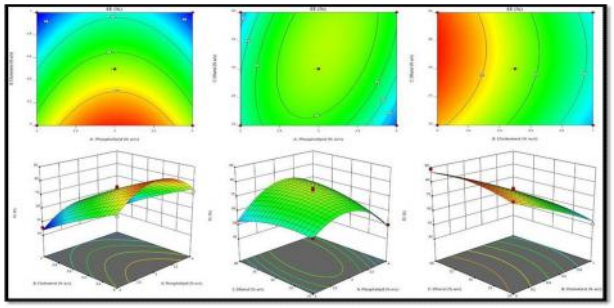


Fig 5: Response surface study

The model appears to be significant based on its model F-value of 80.54. An F-value this enormous could only be the result of noise in 0.01% of cases. Model terms are significant if the P-value is less than 0.0500. Significant model terms in this instance are B, C, AC, A2,and C2. It is implied that thelack offit is substantial bytheF-value of8.52.Thelikelihoodofasignificant LackofFit F-valueoccurringowingtonoiseis only 3.28%.

ThereislessthanO.2discrepancybetweentheAdjustedR2of0.9781 andthePredicted
R2of0.8657,indicatingasatisfactoryagreement.Thesignaltonois eratioismeasured
byAdeqPrecision.Aratiothatishigherthan4ispreferred.Thesignal strengthof
28.439 is sufficient.

Effectofindependentvariablesonvesiclesize(Y2)

For the transdermal or topical administration of medications, vesicle size is essential. Thisissothatitsencapsulateddrugcanreachthe skin'sdermallayers;onlyextremely small vesicles, 300 nm in diameter or less, are capable of doing this. Furthermore, particle size influences both physical stability and vesicle absorption into cells. The diameters of the various Empagliflozin ethosome compositions' vesicles are provided inTable12.Thelargestvesiclesmeasured385.15±1.06nm,wherea sthesmallestwere F4 vesicles (109.46±2.17 nm). The quadratic equation that follows can be used to describe how the independent factors affect vesicle size.

ParticleSize=+142.41+4.72A+103.94B-
1.61C+5.21AB+38.49AC+32.44BC
+59.28A2+13.02B2+91.56C2

where Y1 stands for vesicle size, X1 for phospholipid percentage, X2 for cholesterol percentage, and X3 for ethanol percentage. The link between vesicle size and phospholipid and cholesterol concentrations is positive, but the association between ethanol concentration and vesicle size is negative, as the equation illustrates. The size of ethosome vesicles increases with rising phospholipid and choleterol percentages, while vesicles decrease with increasing ethanol percentage. The big X2 figure 17 suggests that the ratio of cholesterol to ethanol has a discernible impact on the size of ethosomevesicles.WithanF-valueof67.97,the modelcanbeconsidered statistically significant. Less than 0.01% of cases have an F-value this high as a result of random chance.

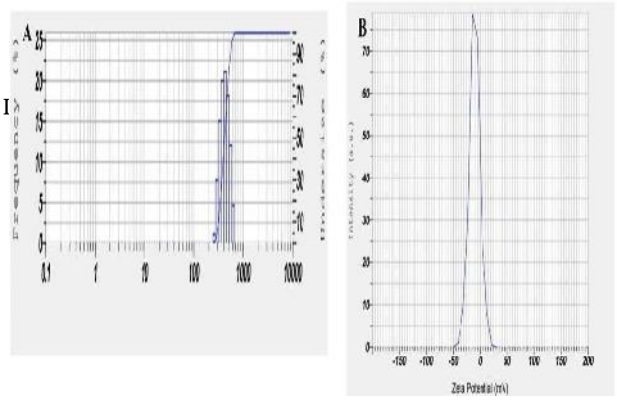
If the p-value of a model term is less than 0.0500, it is considered statistically significant. Theterms AB, CA, BC, AA, and C2 aresignificant in this model. F=1.54 indicates that there is no significant difference between the pure mistake and the lack offit.Itis33.53percentlikelythataLackofFitF-valuethislargecouldbetheproduct
ofpurechance.TheadjustedR2of0.9741andtheprojectedR2of0.8 949arerelatively
neartoeachother(thedifferenceislessthan0.2).AdeqPrecisionisut ilisedinthe
evaluation of the signal-to-noise ratio. A ratio greater than 4 is ideallydesired. With a ratio of 22.602, your signal is acceptable. The effect of changing the independent parameters on the vesicle size is shown by 2D contour plots and 3D response surface graphs (Figure 17). Accordingto these graphs and plots, thereis aconsiderablerise in vesicle size when the phospholipid concentration is increased from 2 to 4%, but there is a large decrease in vesicle size when the ethanol concentration is increased from 30 to 50%. Because higher ethosome viscosity hinders ethosomes from diffusing throughoutthesystem,increasesinphospholipidconcentrationres ultinlargervesicles. The substantial decrease in phospholipid bilayer thickness observed in ethosomes containing high concentrations of ethanol may be attributed to the creation of a phase
containinginterdiffusinghydrocarbonchains.AccordingtoChene tal.(2010),ethanol contributes a net negative charge to the ethosomal systems and gives some steric stability to the lipid vesicles, both of which may cause the ethosomes' vesicle size to decrease. These results are in good agreement with those of Ahad et al. (2013), who described a similar trend in the shrinking vesicle size of valsartan-loaded nanoethosomesinresponsetoincreasingethanolconcentrations.It wasdiscoveredthat the phospholipid percentage had a considerable impact on the vesicle size, as seen by the perturbation graph's modest bend in factor B (percentage ethanol) and steep slope of factor A (% phospholipid) (Figure 17)

PDI and ZP

Table 12 displays the PDI and ZP values for ethosomes of empagliflozin. With PDI valuesrangingfrom0.12to0.35,theproducedvesiclepopulationex hibitedremarkable consistency. ZP, which is connected to the charge on the nanovesicle surface, could have an impact on the vesicle skin connections and the physical stability of the formulation. An elevated ZP value, whether positive or negative, enhances the long- term stability of vesicular formulations by decreasing the probability of vesicles with the same charge aggregating due to electrostatic repulsion. ZP values ranged from - 21.03±1.03(F10)to-39.82±3.02mV(F12)forallethosomalpreparations.Theaddition of ethanol results in a charge shift on the vesicles from positive to negative, which explains whyethosomal formulations have a negative ZP value (Touitou et al., 2000). Theaqueouscoreandthelipidbilayermembraneofethosomesboth containedethanol molecules. Hydrogen bonding between the hydroxyl groups in the ethanol molecules orbetweentheethanolandwatermoleculescanproducenegativeZP (Zhaietal.2005; Mbah et al. More ethanol causes the vesicles to become more negatively charged.

Figure 6: :Particle size distribution and zeta potential of optimized formulation

Evaluation of Ethosomalgel
Physical description



Basedonphysicalexamination,theEmpagliflozinethosomalgelis clear,odourless, and has a pale yellow to off-white colour spectrum.

pH determination

The results of measuring and comparing the pH of ethosomal gels are shown in Table.

Viscosity

The Brookfield Viscometer was used to test the thickness of the gels using a plate and cone. The following is a list of the outcomes of the HADV-II+ pro model ran on the cone spindle CPA-41Z.

Spreadability

Theresults of assessing the ethosomal gel's spreadability are presented in Table.

Drug content

The vesicular gel formulation's drug content analysis was characterised using a UV spectrophotometertoascertaintheamount(%)ofdrugpresentinthe formulation.The results obtained about the drug content of ethosomal gel is presented in Table 25.

Table 4:Characterization of gel formulations

Formulation	pH	Viscosity	Drug content	Spreadability
G1	6.12±0.12	15894±12.3	96.54±0.02	10.32±1.02
G2	6.95±0.35	13328±15.2	98.32±0.04	16.59±1.32
G3	6.58±0.11	16852±10.4	96.31±0.05	11.24±1.24
G4	6.37±0.02	14326±13.2	98.02±0.01	13.51±1.36
G5	6.75±0.43	15982±16.5	97.42±0.03	14.27±1.05
G6	6.84±0.03	16023±12.4	96.18±0.01	12.46±1.26
G7	6.51±0.02	15483±11.0	95.34±0.02	13.28±1.03

Vesicleshapeand morphology

SEMImages:SEMimagingdemonstratedthatoptimisedethosomesandethosomalgel wereunilamellar vesicles, with sizes varying from a few nanometers to a few microns (Figure21).Theirregularshapesareattributedtothepresenceofethanol,whichconfers significant flexibilityto the bilayer membrane (Faisal et al., 2018). Photomicrographs taken with scanning electron microscopyfrequentlydisplaysizes that are smallerthan those found by dynamic light scattering. Scanning electron microscopy imaging requires the dehydration and immobilisation of the nanovesicles on a solid substrate. During this dehydration stage, hydrophilic surfaces could constrict and vesicle shapes couldbecomeabnormal.AccordingtoZhaoetal.(2015),stainingethosomes'outer hydrophilic surface is a famously challenging task. Using dynamic light scattering (DLS),the hydrodynamicvolumeofhydratednanovesicleswasfoundtobegreaterdue to solvent effects.

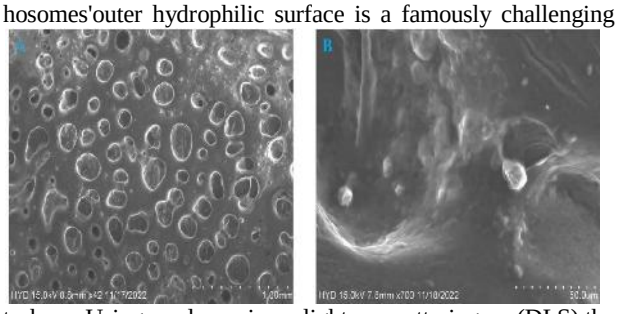


Figure 7: SEM images of Optimized formulations of Ethosomes and ethosomal gel Solid state characterizations

InVitroStudiesto EvaluateSkin Permeation

In the drug solution, the amount of drug penetrated was 1054±18.1 µg/cm2, the cumulative permeation percentage was 89%, and the steady-state flux was 1.93 µg/cm2/h. Empagliflozin's steady-state flow was 4.26 g/cm2/h, but when the optimal ethosomal suspension formulation was employed, the cumulative penetration was 97.6%. 2.95 µg/cm2/h was the steady-state flow, and 78% of the total permeation was achieved; additionally, the optimized ethosomal gel showed 1348±21.7 µg/cm2 of penetrated empagliflozin. At last, the ethosomal gel exhibiting the maximum on centration of ethanol, the lowest concentration of lecithin, and the intermediate concentration of cholesterol demonstrated the best permeability at significant intervals ($p \leq 0.05$). Trans epidermal resistance was greater than 30±1.5 kΩ as a result of the electrostatic repulsion that was found. It was a sign that the skin was doing well [26].

The medication penetration rate increased in our formulations as the phospholipid concentrationdecreased.Whentheconcentrationofcholesteroldropped,sodidtherate at which the drugpermeated the body. A previous studyalso found that therigidityof the ethosomal vesicle bilayer increased with increasing quantities of lecithin and cholesterol. By interacting with the polar head group of SC lipid molecules, ethanol enhanced drug diffusion across the membrane. This was achieved by lowering the melting point of SC lipids and raising the permeabilityand fluidityofthe lipidbilayer. The drug's permeability through the vesicles to its target was increased by thecombined action of ethanol, vesicles, and SC lipid molecules. Because carbopol has the best bufferingcapacityfeaturesandananionicpolymer,itcanmaintainthe properpHwhile preventing skin irritation. The optimal viscosity and bio-adhesion properties are achieved by combining carbopol with ethosomes [49,50].

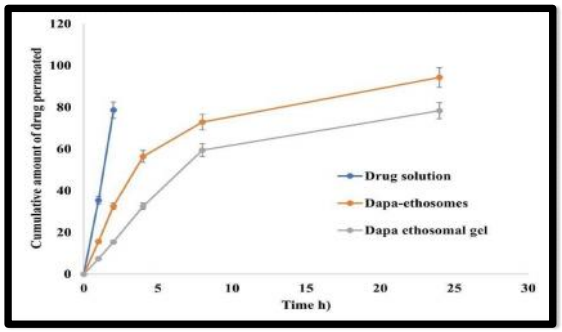


Figure 8: Ex-vivo permeation studies of optimized ethosomal suspension and gel

Stability Studies

The ethosomal formulation (F4) kept at 4°C was found to have greater drug retention capacity,entrapmentefficiency,size,andCDRwhencomparedto25±2°C.Apossible explanation for the decrease in entrapment efficiency is drug leakage from the ethosomesathighertemperatures.Therefore,ethosomesreducedthedrugrentiontime in response to higher temperatures. Ethosomecharacterisation is altered by the accelerated stability investigations.

4. SUMMARY AND CONCLUSION

Diabetes mellitus continues to increase globally, largely due to inadequate early detection. Type 2 diabetes, a preventable metabolic disorder, can be managed through lifestyle modification and public awareness, yet no definitive cure exists. This study developed a novel transdermal vesicular delivery system of empagliflozin ethosomes to improve therapeutic outcomes. Empagliflozin ethosomes were prepared by the cold method and optimized using a Box–Behnken design. The formulations were characterized for vesicle size, entrapment efficiency, zeta potential, PDI, and morphology. DSC and FT-IR studies confirmed successful drug encapsulation in an amorphous state. In vitro skin permeation and retention studies demonstrated significantly enhanced transdermal absorption and cumulative drug permeation compared to a 25% pure drug solution. These findings indicate that ethanol–phospholipid–cholesterol ethosomes are a promising and stable transdermal delivery system for empagliflozin.

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