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Advances in Bioresearch

ORIGINAL ARTICLE

Dapagliflozin -Loaded Ethosomes as Transdermal Drug Delivery Carriers: Statistical Design, Formulation And Evaluation

Vankayala Devendiran Sundar[1*], Magharla Dasaratha Dhanaraju[1], Anilkumar Vadaga[1], N V Sairahul[1]
[1]Department of Pharmaceutical Technology. GIET School of Pharmacy, Rajahmundry, AP, India.
*Corresponding Author's Email: sundarvd@yahoo.co.in

ABSTRACT
The purpose of this research was to create and refine Dapagliflozin nano vesicular ethosomal gel for use in the treatment of patients with diabetes and cardiovascular disease. For ethosome improvement, we used a 3^3-level factorial design with three factors. The entrapment efficiency (Y1), vesicle size (Y2), zeta potential (Y3), and % CDR (Y4) were selected as the dependent variables, whereas phosphatidylcholine (X1), cholesterol (X2), and ethanol (X3) were selected as the independent factor. By incorporating this drug inside lipid nanocarriers, we were able to generate ethosomes, where the vesicular size and lipid used for formulation controlled the sustained release of drugs. Following the incorporation of the optimized ethosomes into Carbopol® 940 gel, their rheological behaviour, in-vitro release, and ex-vivo skin permeation studies were characterized. When compared to drug solutions, in vitro and ex vivo permeation studies yielded more positive results.
Key words: *Dapagliflozin, Diabetes, SGLT2, in-vitro release, and ex-vivo, phosphatidylcholine, topical administration, 3^3-level factorial design.*

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INTRODUCTION

In 2014, the Food and medication Administration (FDA) authorized dapagliflozin, a novel oral hypoglycemic medication. Dapagliflozin (DFG) works by blocking the sodium-glucose co-transporter-2 in the kidneys, preventing glucose from being reabsorbed [1-2]. Maximum plasma concentration was recorded after 1.5 h, clearance was 4.9 mL/min/kg, and water solubility was 0.173 mg/mL. In a 24-hour urine glucose excretion study, DFG showed a linear pharmacokinetic profile between 2.5 and 500 mg/d. tmax ranged from 0.5 to 1.3 hours, and the half-life was only around 17 hours [3-6]. By blocking sodium-glucose transport proteins (SGLT2), it lowers blood sugar and flushes it out of the body through urine.

Transdermal route is, therefore, a better alternative to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens. The use of lipid vesicles as drug delivery systems for skin treatment has attracted increasing attention in recent years. However, it is generally accepted that conventional liposomes are of little value for this purpose [7,8]. Liposomes remain confined to the upper layer of stratum corneum (SC) and, hence, are suitable for topical drug delivery. Only specially designed vesicles were shown to deliver drugs across the skin layers.

Ethosomes contain phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Unlike classical liposomes, ethosomes were shown to permeate through the stratum corneum barrier and were reported to possess significantly higher transdermal flux in comparison to liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear [9,10]. The synergistic effects of the combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers.

MATERIAL AND METHODS

Materials

Dapagliflozin was obtained from Hetero pharm Pvt. Ltd. Soya Lecithin, Phospholipon® 90H and Triethanolamine was purchased from Sisco Research Laboratories Pvt .Ltd. Carbopol, Cholesterol was purchased from Ozone chemicals, Mumbai. Propylene glycol was purchased from S.D.Fine Chemicals.

Methods

Preparation of Ethosomes

The ethosomes were made using a cold technique. In a clean, dry round bottom flask, dapagliflozin was dissolved in ethanol. The cholesterol and soy-lecithin were added to the ethanol after being precisely measured [11]. A mechanical stirrer was used to combine the ethanol components. The aforementioned lipid combination was then added to propylene glycol while being stirred. The ethanolic mixture was heated in a water bath to 30 ℃. After that, the aqueous phase was gradually introduced to the lipid phase in a very thin stream while the mechanical stirrer was constantly mixing at a speed of 70 rpm. Mixing was maintained for 5 minutes and ethosomal suspension was produced after the entire inclusion of the aqueous phase. The size of the ethosomes was decreased by utilizing a probe sonicator to sonicate the ethosomal solution for 0.5 to 3 minutes. To get stable ethosomes, the sonicated ethosomal solution was then agitated continuously for 30 minutes. The mixture was kept at 4-8 ℃ until it could be used.

Box–Behnken Experimental Design

The data was analyzed using Design-Expert®software and a three-factor, three-level factorial design was utilized to find the optimal Dapagliflozin loaded ethosomal formulations. The % EE (Y1), vesicle size (Y2), zetapotential (Y3), and %CDR (Y4) were selected as dependent variables, while the phospholipon® 90G (X1), Cholesterol (X2), and ethanol (X3) were used as independent factors. Linear regression, two-factor interaction (2FI), and quadratic models were used inside the Design-Expert® program to fit the collected data [12,13]. The results were then used to build a second-order polynomial equation with interaction and quadratic components; this allowed us to assess the impact of the independent factors on each answer. This is the standard notation for a polynomial equation.

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

where Y1 and Y2 are linear coefficients, Y12 is the interaction coefficient between two factors, X1 and X2 are coded levels of independent variables, X0i is the arithmetic average response of the nine runs, X1X2 and Xi2(i = 1-2) denote the interaction and quadratic terms, and Yi(i = 1-2) are the two dependent variables.

The model and its terms were tested for statistical significance using ANOVA. If the p-value (significant probability value) is less than 0.05, then the model and/or model terms are considered significant at the 5% level of assurance. Goodness of fit is indicated by high values of R2 (>0.9000) between observed and predicted values for all answers. Based on the software's point prediction algorithm, the optimal formulation was chosen. Parameters of the formulation were optimized with the help of a quadratic model.

Characterization of the Dapagliflozin-Loaded Ethosomes

Determination of entrapment efficiency (%)

Separation of the free drug was achieved by spinning the mixture at 16,000 rpm and 4 °C in a cooling centrifuge (Sigma cooling centrifuge, Sigma Laborzentrifugen GmbH, Germany) [14]. Dilutions of 10 mL of distilled water (3 minutes) were made in the supernatants.

$$EE\% = \text{Total amount of drug} - \text{Free drug} / \text{Total amount of drug} \times 100$$

Fourier Transform Infrared Spectroscopy

The pellets were made by combining 100 mg of dry potassium bromide powder with 10 mg each of cholesterol and soy-lecithin [15]. The compound was then pelletized by being compressed in a hydraulic press at a pressure of less than 10 tons. Fourier transform infrared spectroscopy (Alpha-II, Bruker, USA) was used to record the infrared spectra after the thin pellet was placed on the pellet plate.

Differential Scanning Calorimetry (DSC) Study

Cholesterol and soy-lecithin were heated in a nitrogen environment at a rate of 20 degrees Celsius per minute in a differential scanning calorimeter (DSC - 8500, Perkin Elmer) for a study of their thermal stability [16].

Vesicle Size Analysis

The Malvern Zetasizer (Nano ZS, Malvern, UK) uses dynamic light scattering (DLS) to measure vesicle size. All formulations were diluted with distilled water and shaken before testing. After transferring the samples to glass cuvettes, the particle size was determined [17].

Zeta Potential Analysis

Electrophoretic mobility-based computerized Malvern Zetasizer (Instrument at Manipal University, Manipal, India) to determine the ZP. The stability of an ethosomal suspension depends on a number of factors, one of which is the particle charge [18].

In Vitro Release Study

One milliliter of each formulation (containing 10 milligrams of dapagliflozin) was inserted into a dialysis bag (Mw cut-off = 14,000 Da). For the release studies, we utilized 40 mL of a phosphate buffer with a pH of 6.5 . The dialysis bag was then placed in a dissolving equipment (SR8, Hanson Research, Chatsworth, CA, USA) at 32±0.5 °C and 100 rpm, where it was submerged in the prepared release media. At 1, 2, 4, 8, 12, and 24 hours,1 mL samples replaced them with the same volume of fresh medium. Spectrophotometric analysis at 224 nm was used to calculate the sample concentrations [19].

$$DR\% \frac{\text{Amount of drug released at time t}}{\text{Initial amount of entrapped drug}} X100$$

In Vitro Skin Permeation

The skin from the abdomen area of rats was used to get the diffusible membranes at the Faculty of Pharmacy in Hyderabad, India. As has been described before, fresh rat skin was utilized. Each donor compartment was a vertical diffusion cell (5 cm^2) with a diffusion membrane attached. In order to isolate the receptor, a 40 mL volume of phosphate buffer (pH = 6.8) was utilized [20]. At 37±0.5 °C, 1 mL of each formula was placed on a diffusion membrane and submerged in the receptor compartment, which was then agitated at 600 rpm in a water bath. After that, after 1, 2, 4, 8, 12, and 24 hours, we removed 1 mL of medium and replaced it with an equivalent amount of fresh media. Finally, a spectrophotometer reading was taken at a wavelength of 235 nm for each sample.

The trans-epidermal electrical resistance (TEER) test was used to examine the condition of the skin. The donor and receptor chambers of the diffusion cell were filled with an aqueous NaCl solution (0.9%). Electrodes were submerged in each well, and resistance was measured at a frequency of 1 kHz using an LCR bridge (LCR400, Thurbly Thandar Instruments, Cambridge, England).In the study, 1 kΩ was chosen as the reference value [21].

Analysis of Permeation Study Data

Using the slope of a straight line representing the total quantity of penetrated drug per unit area and time ($g/cm^2/h$) [22], steady-state flux (j_{ss}) was determined. By dividing j_{ss} by the main Dapagliflozin concentration in the donor compartment (Co), one may get the permeability coefficient (Kp) of dapagliflozin from each preparation (1/cm.h).

$$Kp = j_{ss}/Co$$

Formulation of Dapagliflozin Ethosomal gel

Dapagliflozin ethosomal gels were made by adding 1 to 4% w/w Carbopol 940 P to a vortex of distilled water while swirling at room temperature [23]. Propylene glycol was added to the formulation together with sodium methyl hydroxybenzoate 0.2% w/w and sodium propyl hydroxybenzoate 0.02% w/w. The gel formulation includes a dapagliflozin ethosomal formulation with a concentration of 10 mg of the drug (depending on potency). Translucent gel bases were made by gradually adding an aqueous solution of disodium hydrogen orthophosphate to slightly acidic formulations in order to neutralize them and keep the pH in the range of 6.0 to 7.5. The gel formulation was mixed for 15 minutes, and the weight was adjusted to 100% w/w.

Table 1: Formulation of Ethosomal gel

Ingredients	G1	G2	G3	G4	G5	G6	G7
Carbopol 940	0.5	1	1.5	2	2.5	3	4
Propylene Glycol	2	4	6	2	4	6	8
Sodium methyl hydroxy benzoate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium propyl hydroxy benzoate	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Disodium hydrogen orthophosphate	5	5	5	5	5	5	5
Purified water (Q.S)	100	100	100	100	100	100	100

Characterization of Ethosomal gels

pH Determination

Each batch of ethosomal gel had its pH evaluated using a pH meter (Orion star A211, Thermo Scientific, Singapore).

Viscosity determination

Viscosity (cp) was estimated using the Plate and Cone Brookfield Viscometer (Brookfield, USA DV-II + Pro). The ethosomal samples were separated from one another in the plate's center and secured with the cone's side handle [24]. Verify that the indicated calibration temperature was maintained within

25±0.1°C using the circulating bath. The sample cup was filled with 2 mL of ethosomal gel. A shear rate of 2.00 N sec^{-1} was applied.

Spreadability

The spreadability apparatus (Spreadability Fixture Texture Analyzer TA-SF, CT3, Brookfield) consisted of a female perspex cone anchored to a wooden board and a male perspex cone, which could be moved, placed on top of the female perspex cone. The male cone of moveable perspex weighed 20 grams [25]. A female perspex cone was used to hold a 5 g gel sample, which was then smoothed down with a knife to create a uniform surface. The male perspex (weight) was then inched closer to the female perspex cone across a distance of 6 centimeters, with the elapsed time between each sample being recorded. The values were obtained independently three times, and then computed using the following method:

$$\text{Spreadability (g.cm/sec)} = \frac{\text{Weight of male perspex cone X Length moved on fixed female perspex cone}}{\text{Time taken to move towards female perspex cone}}$$

Drug content

Carefully measure out 500 mg of ethosomal gel into a 10 ml volumetric flask. Each sample's flask was filled to the brim with a solvent combination consisting of methanol and phosphate buffer pH 7.4 (70:30 ratio). The solutions were combined and filtered using a 0.45 m membrane; then, 1 ml of the filtered solution was transferred to a volumetric flask with a 10-ml capacity, and the remaining 9 ml was filled with a solvent combination of methanol and phosphate buffer (70:30). UVSpectrophotometer UV-1800, Shimadzu, Japan) at max 224 nm was used to examine these solutions [26].

In vitro drug release study (IVRT)

Franz diffusion cells (Teledyne Hanson Research, 6 cells) were used for the in vitro drug release analysis. The egg membrane was used as a model for a semi-permeable membrane to study the diffusion of drugs. The receptor compartment has a permeable surface area of 1.76 square centimeters and an effective volume of around 7 milliliters. Between the donor and the receptor compartment, the egg membrane was attached [27]. Each donor compartment received 100 mg of a Dapagliflozin vesicular gel formulation, with a weighted average of 0.1% w/w. Each receptor compartment was then filled with a 7.4 pH phosphate buffer solution for solubilization, and its temperature was maintained at 32±0.5 °C in a circulating water bath. Using a magnetic stirrer, the dissolution medium was continuously stirred at 400 rpm during the experiment. Every half an hour, an hour, two hours, four hours, and six hours, samples were taken and replaced with the same volume of new samples. The removed sample was evaluated using a UV-Spectrophotometer (UV-1800, Shimadzu, Japan) set to max 224 nm after passing through a 0.45 m filter.

In vitro drug permeation study (IVPT)

The dermis and subcutaneous fat layer of goat skin were removed, and the skin was then sterilized in hot water before being utilized in an in vitro skin permeation investigation with the use of a Franz diffusion cell (Teledyne Hanson Research, 6 cells). The receptor compartment had a surface area of 1.76 cm2 and a capacity of 7 ml. Phosphate buffer solution (pH 7.4) was used to soak skin membrane skin for 1 hour. Each donor chamber of the Franz diffusion cell was treated with 100 mg of the gel formulation [28]. Each receptor compartment was then filled with a 7.4 pH phosphate buffer solution for solubilization, and its temperature was maintained at 32±0.5 °C in a circulating water bath. Using a magnetic stirrer, the dissolution medium was continuously stirred at 400 rpm during the experiment. Every half an hour, an hour, two hours, four hours, and six hours, samples were taken and replaced with the same volume of new sample. The withdrawn sample was examined using a UV spectrophotometer (UV1800) after being filtered via a 0.45 m membrane. Using the equation

$$\text{Flux} = K_p C_0,$$

where K_p is the permeability coefficient and C_0 is the starting concentration of drug in the gel applied to the donor compartment.

Stability study as per ICH

As recommended by the ICH guideline Q1A (R2), stability experiments were conducted on selected batches of optimized ethosomal packaged in lacquer-coated aluminum collapsible tubes. For three months (0, 1, 2, 3 Months), batches were stored at 5±3^{0}C (2^o to 8 ^{o} C), 25°C/60%RH, and 30°C/75% RH to test the product's stability with the primary pack. At predetermined intervals, samples were taken from the stability chambers and analyzed for color, pH, viscosity, drug content, and drug diffusion [29].

RESULTS AND DISCUSSION

Experimental design

Table 2: Box-Behnken design for ethosomes formulations.

Run	X1	X2	X3	Y1	Y2	Y3	Y4
1	4	0.5	40	65.51±0.24	326.54±5.34	-33.12±0.59	78.56±0.69
2	2	0	30	71.72±0.13	115.34±2.16	-28.61±1.24	90.23±1.34
3	3	0.5	30	73.94±0.26	126.48±4.18	-26.54±1.03	68.73±0.25
4	3	0	40	88.89±0.01	103.46±2.17	-27.41±1.26	96.49±1.37
5	3	1	20	50.82±0.35	325.64±2.01	-36.45±3.05	69.41±0.34
6	3	1	40	55.69±0.14	394.15±1.06	-32.15±1.08	60.28±0.26
7	3	0.5	30	75.93±0.28	129.64±3.25	-28.35±2.14	68.72±0.59
8	3	0.5	30	73.84±0.16	156.28±5.37	-26.48±1.22	70.62±1.52
9	4	1	30	46.19±0.37	324.51±4.13	-30.12±1.34	70.27±2.04
10	2	1	30	45.76±1.25	294.86±2.01	-21.03±1.03	68.81±1.06
11	2	0.5	20	59.79±3.02	336.95±1.03	-35.61±2.11	70.34±2.35
12	3	0	20	82.38±2.48	164.72±2.35	-39.82±3.02	65.24±1.08
13	3	0.5	30	74.49±0.97	143.26±1.36	-27.43±2.06	68.34±1.04
14	2	0.5	40	51.61±0.58	249.88±0.46	-24.15±0.15	66.13±2.13
15	4	0	30	72.38±1.24	124.13±3.29	-23.06±2.34	88.69±2.46
16	3	0.5	30	73.49±1.03	156.39±3.51	-29.46±1.05	69.76±2.31
17	4	0.5	20	50.25±1.05	259.64±4.26	-36.42±0.43	48.98±3.15

Effect of independent variables on % entrapment efficiency (Y1)

Entrapment effectiveness ranged from a high of 88.89±0.01 for F4 to a low of 45.76±1.25 for F10. The following quadratic equation describes the relationship between the independent variables and the % EE:

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

It can be seen from the equation that higher concentrations of phospholipids and ethanol result in a higher percentage of EE, whereas higher concentrations of cholesterol have the opposite effect.

Figure 1 demonstrate that the entrapment effectiveness of dapagliflozin in the ethosomes improved when the phospholipid concentration was raised from 2% to 4%. An increase in the ethanol content from 30% to 40% was found to significantly increase % EE; this may be due to the co-solvent effect of ethanol. Ethanol at high concentrations renders the vesicles leaky because ethanol partially solubilizes the phospholipid bilayer in ethanol. This resulted in a decrease in % EE when the ethanol concentration was increased up to 50%.

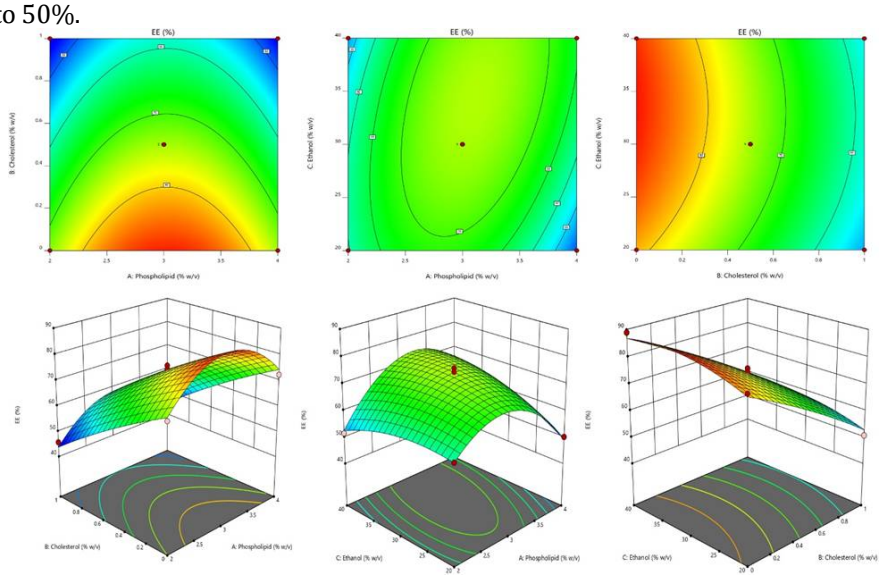

Fig. 1: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on ethosomal Entrapment Efficiency (Y1)

Effect of independent variables on vesicle size (Y2)

F4 vesicles were the smallest (103.46±2.17nm), while F6 vesicles were the biggest (394.15±1.06 nm). It is possible to characterize the impact of the independent factors on vesicle size using the following quadratic equation:

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

The equation demonstrates a positive relationship between phospholipid and cholesterol concentrations and vesicle size, and a negative relationship between ethanol concentration and vesicle size. These graphs and plots show that increasing the phospholipid concentration from 2 to 4% results in a significant increase in vesicle size, but increasing the ethanol concentration from 30 to 50% results in a significant drop in vesicle size. Increases in phospholipid content cause an increase in vesicle size because increased ethosome viscosity makes it harder for ethosomes to diffuse throughout the system.

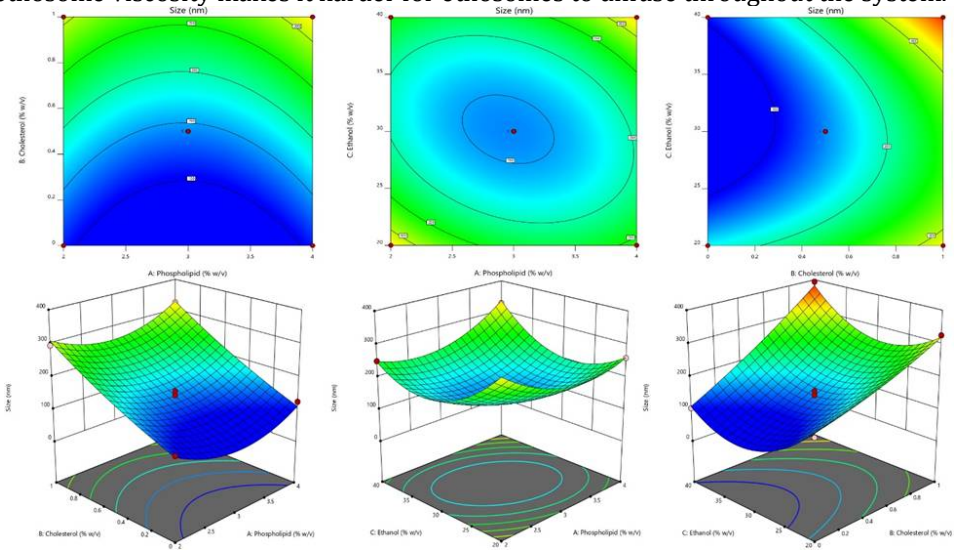

Fig. 2: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on Vesicle size (Y2)

Effects of Experimental Variables on ZP (Y3)

Zeta potential (ZP) values for Dapagliflozin-loaded ethosomes varied from -21.03±1.03 (F10) to -39.82±3.02mV (F12). The electric repulsion between vesicles is strong enough at these zeta potential levels to avoid aggregation and preserve the physical stability of the dispersion.

ZP= -27.65 -1.67A -0.1062B +3.93C -3.66AB -2.04AC -2.03BC +1.79A2 +0.1573B2 -6.46C2

It has been shown that the negative charge carried by the vesicles improves medication absorption through the intestinal lymphatic transport channel and increases drug uptake by M-cells in the Peyer's patch. Ethmosomal vesicles may be transported more easily through the intestinal membrane because of the negative charge's destabilizing effect on membranes.

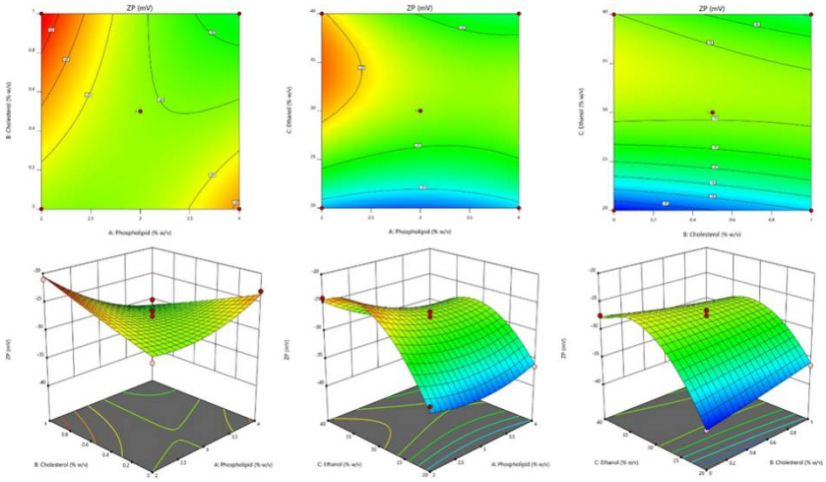

Fig. 3: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on Zeta potential (Y3)

Increases in X1 would cause a rise in the absolute value of zeta potential when X3 was reduced from 30 to 20 (i.e., ethanol was added). Therefore, the appearance of negative charge on the ethosomal surface is facilitated by the incorporation of lipid within the vesicular structure of the ethosomal.

Effect on In Vitro Drug Release

As can be shown in Figure 4, free dapagliflozin suspension had a much faster drug release rate than the studied ethosomal forms. In addition, a fast initial drug release in the first 3 h (20.03–44.67%) was seen with all ethosomal formulations, followed by a steady release profile until 24 h. After 24 hours, the cumulative percentage of dapagliflozin released by ethosomes varied from 48.98±3.15% (F17) to 96.49±1.37% (F4).

%DR= +69.23 -1.13A -8.98B +5.94C +0.7500AB +8.45AC -10.09BC +1.71A2 +8.56B2 -4.94C2

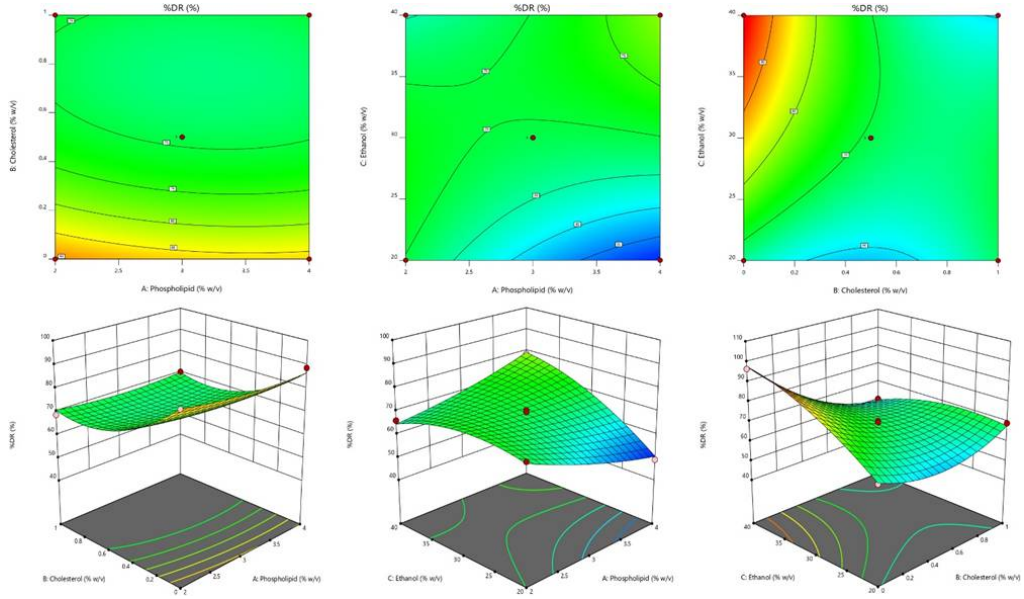

Fig. 4: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on % CDR (Y1)

Characterization of ethosomes

PDI and ZP

The prepared vesicle population was quite consistent, with PDI values between 0.12 and 0.35. All of the ethosomal preparations had ZP values between -21.031.03 and -39.823.02 mV.

Evaluation of Ethosomal gel

Table 3: 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

Vesicle shape and morphology

Images obtained using scanning electron microscopy (SEM) revealed optimized ethosomes and ethosomal gel to be unilamellar vesicles. The presence of ethanol, which gives the bilayer membrane considerable flexibility, gives the irregular forms. Sizes shown in scanning electron microscopy photomicrographs are often smaller than those determined using dynamic light scattering.

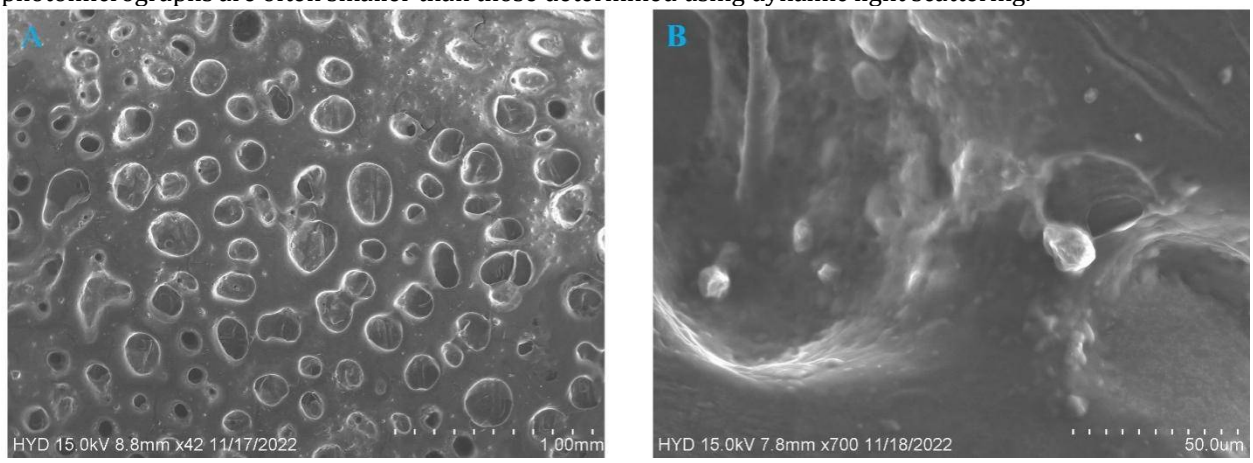

Fig.5 : SEM images of Optimized formulations of Ethosomes and ethosomal gel

Fourier Transform Infra-Red Spectroscopy (FTIR)

Absorption peaks were seen in the FTIR spectra of pure dapagliflozin 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 dapagliflozin in all of the compounds.

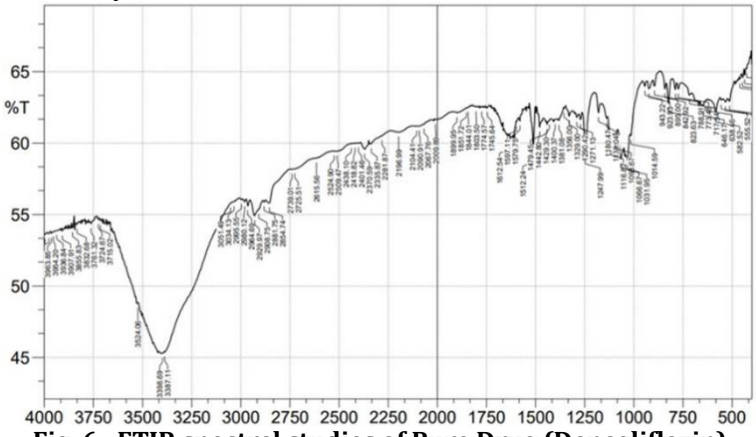

Fig. 6 : FTIR spectral studies of Pure Drug (Dapagliflozin)

Both ethosomes and ethosomal gel, which are used as medication excipients, revealed a high peak at 3743 cm^{-1} in their IR spectra, indicating the existence of an O-H stretch of alcohol. The presence of alkyl and aromatic C-H stretches, as well as the C-C stretch of alkynes, were recognized by two additional strong peaks at 2923 and 2396 cm^{-1}, respectively. C=O and C=Cstretch were found to correspond to the other prominent peak at 1708 cm^{-1}. The C-N stretch was detected at 1532 cm^{-1} in the IR spectra, while the C-N

and P=O stretches were detected at 1194 cm-1. Aromatic C-O stretch was also detected, with peaks at 1047cm-1. There was zero evidence of drug-excipient interaction in the infrared range.

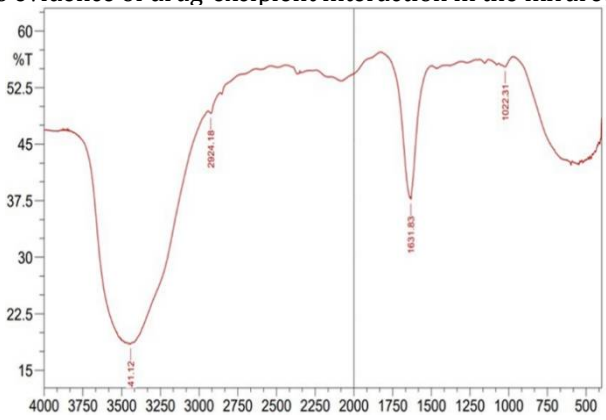

Fig. 7: FTIR spectrum of ethosomal suspension

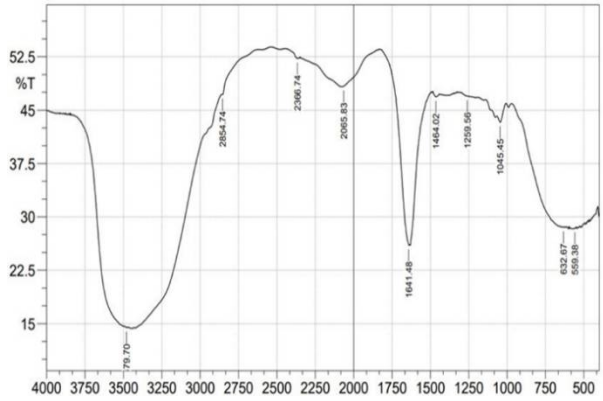

Fig. 8: FTIR spectrum of ethosomal gel

Differential Scanning Calorimetry (DSC)

On the melting curve, the dapagliflozin has an onset temperature of 80.14 °C, a peak temperature of 74.9 °C, and an endset temperature of 83.19 degrees Celsius (DHm = -19.4 J/gm).

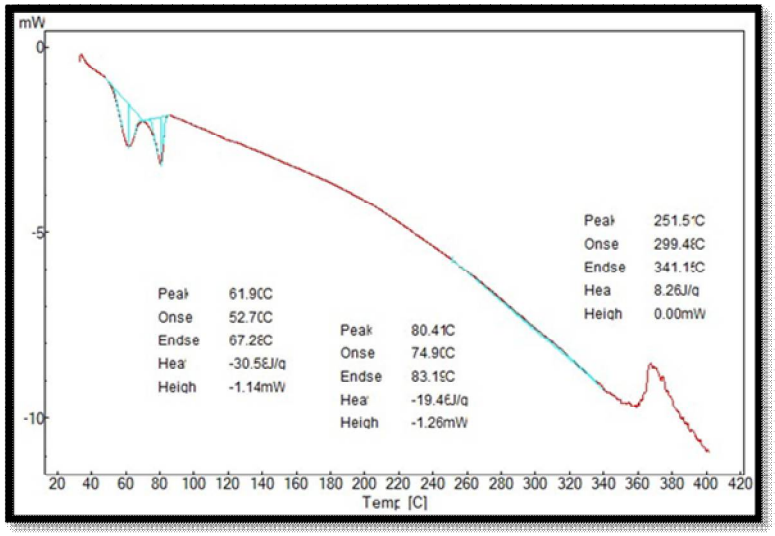

Fig. 9: DSC thermogram of Pure Drug

At temperatures between 148 and 155 °C, cholesterol displayed a significant exothermic peak. Soy lecithin caused a wide curve with a jump line from 37.60 to 160.38 °C in a physical combination of drug-excipients. Solubilization and dilution of the medication in molten lipid mass resulted in an endothermic

peak between 36.11 and 150.93 °C during the formulation of dapagliflozin ethosomes and ethosomal gel, during which no change or interaction was detected.

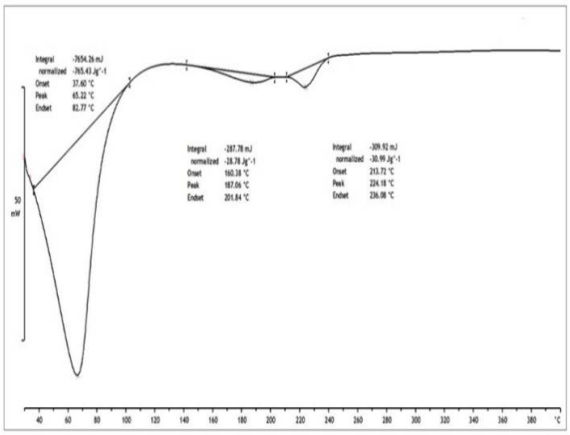

Fig. 10: DSC of ethosomal suspension

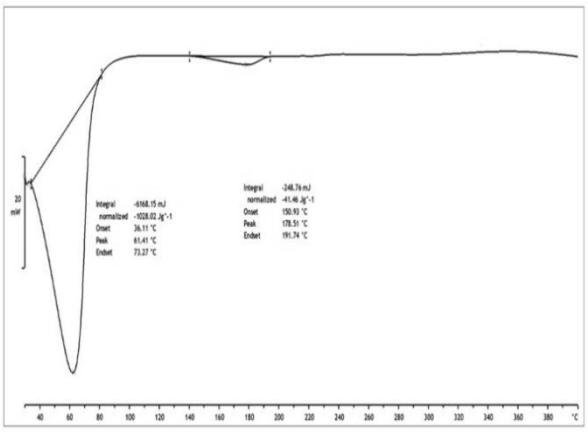

Fig. 11: DSC of ethosomal formulation

In vitro drug release test (IVRT)

Less drug was released at the final time point of 12 hours in an in vitro drug release investigation of several formulations of drug solution, ethosomal suspension, and ethosomal gel formulation. Maximum drug release was seen at the 6-hour mark for both the ethosomal solution and gel formulations; hence, these formulations were chosen for the subsequent in vitro drug permeation investigation (IVPT).

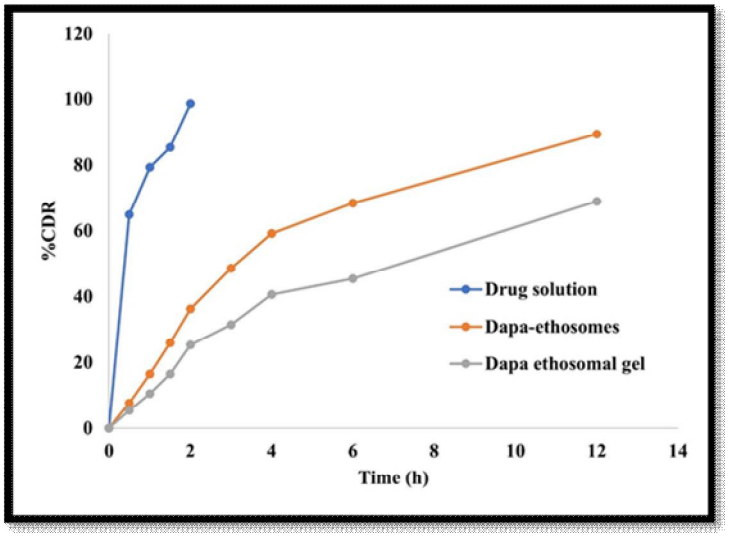

Fig.12: invitro drug release studies comparison between the optimized ethosomal gel and ethosomal suspension

In Vitro Skin Permeation

The steady-state flux was 1.93 µg/cm^2/h, the cumulative permeation percentage was 89%, and the quantity of drug penetrated was 1054±18.1 µg/cm^2 in the drug solution. The steady-state flow of dapagliflozin was 4.26 g/cm2/h, however the cumulative penetration was 97.6% when the best formulation of ethosomal suspension was used. The steady-state flow was 2.95 µg/cm2/h, and the percentage of cumulative permeation was 78%; moreover, optimized ethosomal gel exhibited a permeated quantity of dapagliflozin equivalent to 1348±21.7 µg/cm2. The observed electrostatic repulsion resulted in a trans epidermal resistance of more than 30±1.5 kΩ.

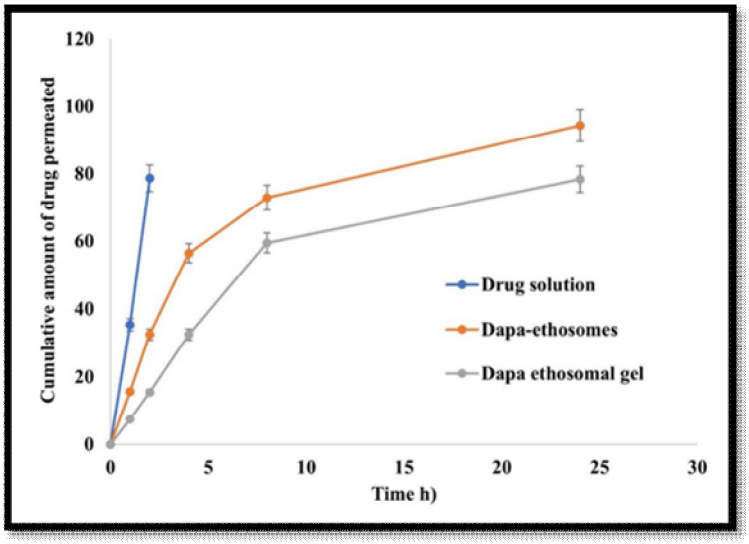

Fig. 13: Ex-vivo permeation studies of optimized ethosomal suspension and gel

Stability Studies

Drug retention capacity, entrapment efficiency, size, and CDR were all shown to be higher with ethosomal formulation (F4) held at 4°C compared to 25±2°C. Possible drug leaking from the ethosomes at higher temperature contributes to the decline in entrapment efficiency. As a result, rising temperatures caused ethosomes to reduce the drug rention time The acclerated stability studies changes the characterization of ethosomes.

Table 4: Acclerated stability studies of Optimized formulation

Months	Temperature (°C)	%EE	% CDR	Vesicle Size (nm)	Zeta value(mv)
1st Month	Refrigeration temperature	88.89±0.01	96.49±1.37	103.46±2.17	-27.41±1.26
2nd Month	(4±2^{0}c)	86.43±2.13	95.38±0.1	105.83±3.8	-28.94±1.15
3rd Month		85.24±0.58	93.42±0.4	112.47±2.4	-30.91±1.43
1st Month	Room temperature	88.89±0.01	96.49±1.37	103.46±2.17	-27.41±1.26
2nd Month	(25±2^{0}c)	84.56±5.21	94.26±0.2	110.28±2.5	-33.15±0.38
3rd Month		79.26±6.28	90.15±0.1	123.49±1.4	-35.41±0.26

CONCLUSION

People with type 2 DM want individualized care plans that improve their quality of life. We developed a new ransdermal vesicular delivery method for dapagliflozin ethosomes in the current investigation. Box behnken design using-response surface methods was used to improve the cold-prepared dapagliflozin ethosomes. Dapagliflozin ethosomes were tuned and characterized for EE, VS, ZP, PDI, and shape using scanning electron microscopy. Dapagliflozin 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 those-encapsulated dapagliflozin 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 dapagliflozin.

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