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Original Research Article

Formulation and evaluation of Dapagliflozin -Loaded Ethosomes as Transdermal Drug Delivery Carriers: Statistical Design

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Abstract:

Diabetes that includes risk factors for noncommunicable diseases like hypertension are only one example of the various situations in which chronic disease is connected with other conditions. Due to the challenges in overcoming the adverse effects of a complicated therapeutic treatment regimen, the treatments available for such chronic comorbid disorders are restricted and tough. 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. Different parameters, such as in-vitro skin permeation, skin irritation, in-vivo antidiabetic, and antihypertensive activities, were used to characterise the developed formulations. Dapagliflozin is a potent, oral, reversible, highly selective, and competitive inhibitor of human SGLT2 used to treat type 2 diabetes. The ethosomes that contain dapagliflozin are the focus of our current study. By incorporating this medication inside lipid nanocarriers, we were able to generate ethosomes, where the vesicular size and lipid used for formulation controlled the sustained release of medicines. The purpose of this study is to create Dapagliflozin-loaded ethosomes for the management of diabetes, and then to statistically optimise and characterise them. 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 factors. Following incorporation of the optimised ethosomes into Carbopol® 940 gel, their rheological behaviour, in-vitro release, and ex-vivo skin permeation studies were characterised. When compared to drug solutions, in vitro and ex vivo permeation studies yielded more positive results. All things considered, the ethosomal vesicles showed promise as a carrier, allowing for improved topical administration of Dapagliflozin.

Keywords: Dapagliflozin, Diabetes, SGLT2, in-vitro release, and ex-vivo, phosphatidylcholine, topical administration, 3^3-level factorial design.

Introduction

For drugs to be administered in the conventional manner, they must be packed in a suitable form, such as tablets for oral use or a liquid for intravenous injection. It has been shown that these dosage formulations have major downsides, including the need for decreased potency, toxicity, and unpleasant side effects despite larger dosages[1]. Modern medicine administration technologies have been developed or are in the process of development to solve the limitations of conventional drug delivery methods in order to meet the needs of the healthcare industry. This method may be broken down into two subsets: controlled drug delivery and drug distribution[2].

Although classifying patients with diabetes is essential because it affects how they are treated, it is a difficult task because many patients, especially younger adults, do not easily fit into a single class[3]. The American Diabetes Association (ADA) still uses and endorses the traditional categorization of diabetes into types 1, 2, and others, including gestational diabetes mellitus (GDM), as recommended in 1997. "Type 1 and type 2 diabetes are the same disorder of insulin resistance set against different genetic backgrounds," as Wilkin put it in his accelerator theory. Obesity and, by extension, insulin resistance, are hypothesized to be at the heart of the difference between the two kinds, with quicker progression correlating to a more predisposed genotype and an earlier onset of symptoms[4].

When applied to healthy skin, transdermal medications release their contents into the systemic circulation at a controlled rate via the skin and into the body. The transdermal drug delivery system (TDDS) has emerged as a significant component of emerging drug delivery systems. Distribution through the transdermal method is intriguing since it is simple and safe[5].

As the definition puts it, "ethosomes" are liposomes that are dissolved in ethanol. Ethosomes are recognized as non-invasive carriers that transport drugs deep into the skin and/or the body's circulatory system. The goal of these pliable, mobile vesicles is to boost delivery of their respective active components. The vesicles' function in facilitating communication between cells and particles has been known for quite some time. The vesicles will then act as a dose control and release mechanism over a lengthy period of time, protecting the drug from immune reactions and other removal processes while releasing the correct amount of medication[6,7]. One of the most significant advances in vesicle research was the identification of a new kind of derivative, the vesicle ethosome. Ethosomes are a little modification of the conventional liposome used as a drug carrier[8]. The components of ethosomes include phospholipids, water, and the alcoholic compounds ethanol and isopropyl alcohol. Ethosomes are fluid vesicles made of ethanol, water, and (more abundant) phospholipids. The ethosomes' sizes will be on the order of tens of nanometers (nm) to microns, and they may quickly penetrate the skin's layers[9]. Scan differential calorimetry was developed to measure the temperature of lipid transport in ethosomal systems. It is important to highlight that the temperatures of lipid transformation in ethosomes and conventional liposomes

revealed lower values in ethosomes for the lipids, indicating that they are more fluid[10].

The primary objective of this research was to create a new vesicular drug delivery system (ethosomal topical gel) containing Dapagliflozin for the treatment of diabetes.

Materials & Methods:

Identification and Characterization of Dapagliflozin

For the purpose of medication authentication, many factors were considered. Physical characteristics, melting point, and FTIR analysis confirmed the authenticity of the drug sample.The obtained sample was compared to standards using FTIR spectroscopy, UV spectroscopy, XRD, and DSC. The medication was then characterized by its organoleptic qualities, solubility, melting point, and partition coefficient.

Physical Appearance

Color and odor were used to describe the excipients' visual characteristics.

Determination of Melting Point

Using a capillary technique and melting point equipment (CL-725-6, Chemiline Technologies), we were able to measure the melting point of cholesterol and soy-lecithin powder. The melting point was estimated by placing the powder in a glass capillary and sealing off one end with a flame. Powder in a capillary was melted by heating it slowly in a heater containing silicon oil. The capillary was dipped in liquid paraffin within a thiele's tube. The melting point of the excipient was recorded as the temperature at which it began to melt[11].

Solubility

A saturated solution is one that has reached a state of equilibrium between the undissolved solute and the dissolved solute. The solubility of a material was defined as the amount required to form an immersed solution at a constant temperature and pressure. Powdered cholesterol and soy-lecithin were tested for their solubility in various solvents by adding 0.1 mg to each of 10 ml volumetric flasks filled with mineral oil, fatty acid, water, ethanol, chloroform, vegetable oil, and methanol. To reach dissolving equilibrium, the samples were sonicated for 30 minutes to 1 hour in a shaker. The clarity and solubility of the solutions were tested[12].

Analytical Characterization

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. 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[13].

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[14].

Drug-excipient compatibility study

The FTIR, DSC, and UV Spectrophotometer were used to investigate whether or not the medicine and excipients were compatible with one another.Lists the drug-to-excipients ratio used in the formulation. Dapagliflozin, soy-lecithin, cholesterol, the drug/excipient physical combination, and the formulated ethosomes were all subjected to FTIR and DSC analysis. Dapagliflozin drug and Dapagliflozin-excipients physical mixtures were also kept in the stability chamber (Thermo Lab Scientific Equipment's, 90/90/130 liters) for one month at 25°C ± 2°C/60% ± 5% RH, 30°C ± 2°C/75% RH ± 5% RH and 40±2ºC/75±5% RH to check

the degradation through analyzing the drug content by using UV Spectrophotometer and compared the difference of initial and 1 month exposed samples of different conditions[15].

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. A mechanical stirrer was used to combine the ethanol components. The afore mentioned lipid combination was then added to propylene glycol while being stirred. Put the filtered water in a water bath and keep it at 30 degrees Celsius. The ethanolic mixture was heated in a water bath to 30 degrees Celsius. 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 700 rotations per minute. 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 degrees Celsius until it could be used[16].

Box–Behnken Experimental Design

To produce ethosomes carrying Dapagliflozin with the highest EE%, smallest vesicle size, maximum ZP, and largest DR%, we used a Box-Behnken (BB) three-level three-factor design, as shown in Table 3. Design-Expert software (Version 12.0.3.0, Stat-Ease Inc., Minneapolis, MN, USA) was used in the development and assessment of the experimental plan[17].

The quantities of Lecithin (X1), cholesterol (X2), and ethanol and isopropyl alcohol (X3) (all in the range of 0% to 1% by weight) were varied throughout 17 separate tests. Conversely, we selected EE% (Y1), VS (Y2), ZP (Y3), and DR% (Y4) as our dependent variables.

Formulations of ethosomes were made with a concentration of 2-4 w/w% lecithin. Most ethosomal formulations had a concentration of ethosomes between 20 and 40 w/w%. In recent studies, ethosomes were prepared with a cholesterol content of 0% to 1% w/w. The most preferable formula was selected for further analysis, and this process was repeated[18,19].

Characterization of the Dapagliflozin - Loaded Ethosomes

Determination of entrapment efficiency (%)

The percentage of Dapagliflozin contained inside vesicles (EE%) is reported for each formulation. 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). Dilutions of 10 mL of distilled water (3 minutes) were made in the supernatants. Using a spectrophotometer (Jasco UV-Vis spectrophotometer, Jasco, Japan), we were able to determine that the max of Dapagliflozin was 224 nm; this value was derived from a standard calibration curve. The EE% may be determined by taking the initial quantity of Dapagliflozin and subtracting the amount found in the supernatant, and then dividing that number by the original amount[20]:

EE%=Total amount of drug−Free drug/ Total amount of drug ×100

Vesicle Size Analysis

The Malvern Zetasizer (Nano ZS, Malvern, UK) uses dynamic light scattering (DLS) to measure vesicle size. To increase scattering intensity and get rid of numerous scattering phenomena, all formulations were diluted with distilled water and shaken before testing. After transferring the samples to glass cuvettes, the particle size was determined. The results are shown as the

mean SD from three independent tests performed on each formulation[21].

Zeta Potential Analysis

We used an 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[22].

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 (Sorensen's phosphate buffer). 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, we took 1-mL samples and replaced them with the same volume of fresh medium. Spectrophotometric analysis at 224 nm was used to calculate the sample concentrations[23,24].

$$DR\% = \frac{The\ amount\ of\ drug\ released\ at\ time\ t}{The\ initial\ amount\ of\ entrapped\ drug} X100$$

Optimization and Experimental Model Validation

Statistically valid polynomial equations are selected by Design-Expert® software. After factoring in the degree and sign of the derived coefficients, these equations are used to illustrate inferences about each answer. There is "synergism" when the sign is positive and "antagonism" when it is negative[25].

The relationship between each element and the final answer is graphically shown in three dimensions using the Design-Expert® program. The optimal value of each answer to an independent variable is calculated using the desirability index (Di) in the optimization procedure. Di = 0 indicates a bad formula, whereas Di = 1 indicates a good one [31]. The purpose of this research was to identify the best recipe for optimizing EE percentage (Y1), vesicle size (Y2), zeta potential (Y3), and DR percentage (Y4). If the deviation of the data from the best formula falls within the prediction interval of the confirmation node, then the model is approved. Each of the three improved formulas was made three times to ensure accuracy. Formulas 9, 13, and 16 were evaluated for the permeation investigation, all of which had varied release patterns (highest-lowest and medium values)[26,27].

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 Sorensen phosphate buffer (pH = 6.8) was utilized. 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 detection range ranged from 1-20 µg/mL, while the LOQ was 0.84 µg[28-30].

In terms of penetration, animal skin may be a better model than human skin. 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). The predicted resistance may be

affected by a number of variables, such as the kind of instrument used, the applied frequency, the resulting current, the ionic strength of the solution, and the sample's surface area of skin. In the study, 1 kΩ was chosen as the reference value[31].

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), we may determine the steady-state flux, denoted by jss. By dividing jss 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)[32].

Kp = jss/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. We mixed equal parts sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate as a preservative. Propylene glycol, which serves as both humectant and a stabilizing agent, 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 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. Gel formulation was mixed for 15 minutes, the weight adjusted to 100% w/w. After 15 minutes of sonication, the air bubbles in the ethosomal gel were gone, and the tube of collapsible aluminum could be filled. The ethosomal gel formulations were made using the tabled recipe[33,34].

Table 1: Formulation of Ethosomal gel

Ingredient	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.S100	Q.S100	Q.S100	Q.S100	Q.S100	Q.S100	Q.S100

Characterization of Ethosomal gels
Physical appearance

Before, during, and after storage, the produced batches were inspected visually at predetermined intervals and under varying stability circumstances. Aesthetic properties such as color, homogeneity, rheology, consistency, and phase separation in storage were evaluated in gel compositions[35].

pH Determination

Each batch of ethosomal gel had its pH evaluated using a meter. Before each measurement, a pH meter (Orion star A211, Thermo Scientific, Singapore) was checked against solutions of known pH 4, 7, and 10. Triple-checked values were used[35].

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. 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. Modeling HADV-II+ pro on a cone spindle (CPA-41Z) were used messuring viscosity. That spindle had a 3° cone angle and a 2.4 cm cone radius. Using the vernier ring, we attached the cone spindle to the sample cup and set the gap between the two in accordance with established practice. After affixing the sample cup to the viscometer, the system was let around 15 minutes to reach thermodynamic equilibrium. A shear rate of 2.00 N sec^{-1} was applied. Spindle speeds of 10, 20, 30, 40, and 50 rpm were used in the estimate. All measurements of viscosity were taken in triplicate at room temperature[36].

Spread ability

The gel's capacity to spread to the damaged area of skin necessitated a test of its 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. 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[36]:

Weight of male perspex cone X Length moved on fixed female perspex cone

Spreadability = (g.cm/sec) Time is taken to move towards female perspex cone

Drug content

Ethsomal gel's drug content was measured using an ultraviolet (UV) spectrophotometer. 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. Values are checked three times to ensure accuracy[37].

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. 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. The membrane was then inserted into the modified Franz diffusion cells between the donor and receptor compartments. 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 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[38].

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). There are two distinct regions inside a cell: the donor region and the receptor region. 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. 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. After that, we sandwiched several modified Franz diffusion cells between the skin and their donor and receptor compartments. 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. Each formulation was tested over the course of six experiments (n=6). Slope of the line connecting the steady-state values of the cumulative quantity of medication over time was used to calculate the flux. Using the equation Flux=KpC0, where Kp is the permeability coefficient and C0 is the starting concentration of medication in the gel applied to the donor compartment, we were able to determine the flux rate through the gel[38].

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 Month), 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[39].

Results & Discussion:

Identification and characterization of Dapagliflozin

Dapagliflozin was successfully identified and characterized physicochemically, yielding the following findings:

FTIR (Fourier Transform Infra-Red Spectroscopy):

A drug's molecular structure and fingerprint may be determined via FTIR, a kind of vibrational spectroscopy. Pure Dapagliflozin 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 Dapagliflozin at 3367.10 cm1 (OH stretching), 1613.16 cm1 (C=C, aromatic), and 1246.70 cm1 (C-O ester stretching). Peaks for the C-Cl bond at 1018 cm1, the O-H elastic response at 3375 cm1, and the C-C bond at 1614 cm1 were all produced by Dapagliflozin in all of the compounds.

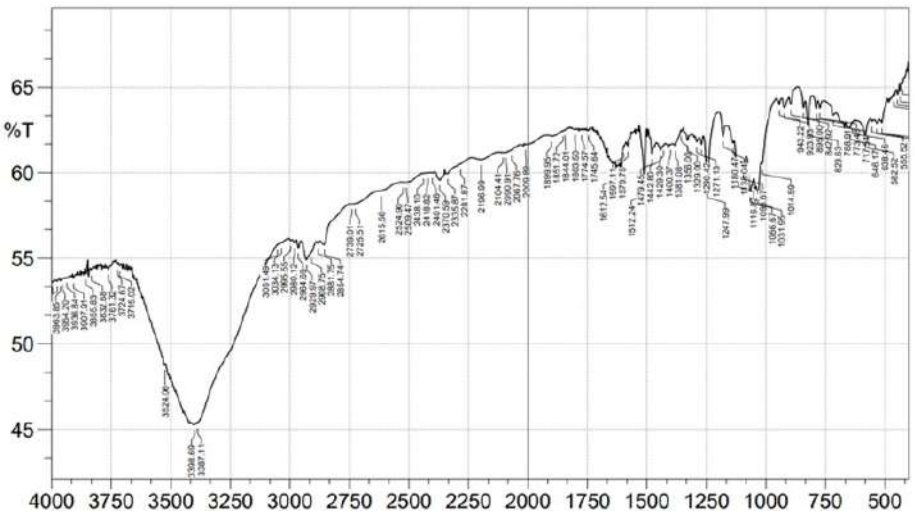

Figure 1: FTIR spectral studies of Pure Drug (Dapagliflozin)

Differential scanning calorimetry

The thermodynamic compatibility of Dapagliflozin with various polymers was studied by determining their crystalline melting temperature and glass transition temperature, since this is one of the most practical approaches for evaluating drug and excipient mix compatibility. The melting endotherm is shown by the DSC curve's departure from and subsequent return to the baseline. The melting point, or onset temperature, is found by extrapolating the base line from the point where the tangent intersects the greatest slope on the main side of the peak. At a heating rate of 2^0C min^{-1}, the endotherm of melting Dapagliflozin is shown in the figure. On the melting curve, the sample has an onset temperature of 80.14 degrees Celsius, a peak temperature of 74.90 degrees Celsius, and an endset temperature of 83.19 degrees Celsius (DHm = -19.4 J/gm).

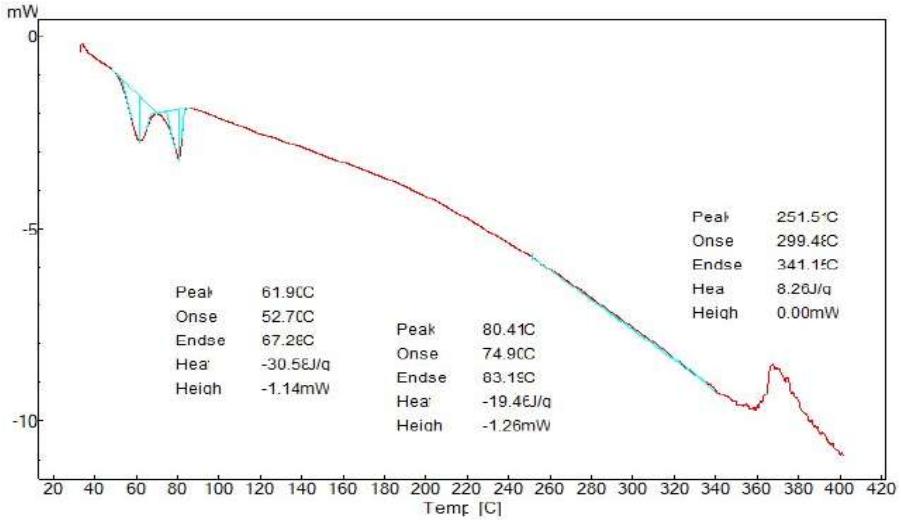

Figure 2: DSC thermogram of Pure Drug

Powder X-ray diffraction (P-XRD) analysis

The graphic displays the XRD spectrum of Dapagliflozin . Dapagliflozin XRD spectra are consistent with the reference data provided by Savi Pharma (India). The two peaked at the same 21° frequency. The drug's crystalline nature is shown by the sharp peak in the XRD spectra. Using an XRD pattern, the molecular structure of Dapagliflozin powder was analyzed. This graphic clearly shows that there were 10 peaks between the 10th and 30th positions.

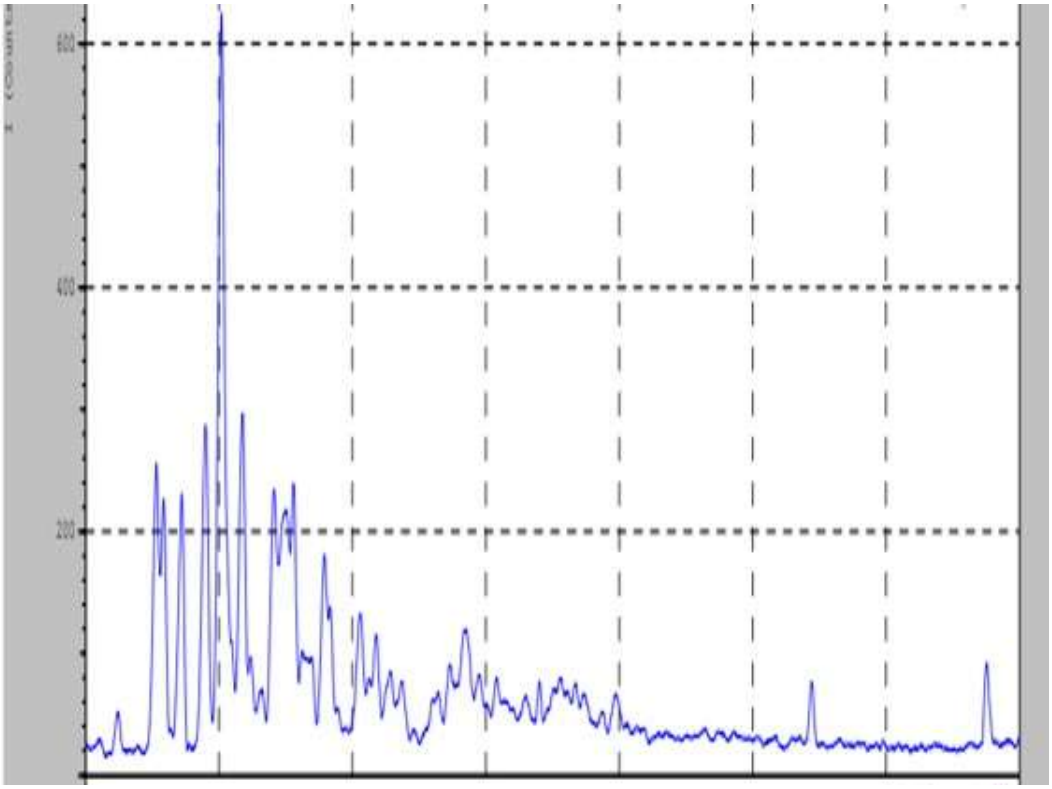

Figure 3: Powdered X ray diffraction studies of Dapagliflozin

The purity and authenticity of the sample of Dapagliflozin acquired were validated by the aforementioned tests. Dapagliflozin is somewhat crystalline, as seen by a large, sharp peak in XRD spectra and a DSC thermogram.

Preformulation studies of Dapagliflozin

The nature of the medication has been confirmed via preformulation experiments. All the ranges in the preformulation trials are quite close to those seen with regular Dapagliflozin

Melting Point

Capillary fusion was used to determine the drug's melting point. The drug's melting point was measured and found to be within the typical range. In the table below, you'll find the results. The melting point of Dapagliflozin was determined to be 74.09±1.59 ^{0}C using the glass capillary technique and melting point equipment, and this value was found to be consistent with the melting point of Dapagliflozin as described in the scientific literature (74 - 78°C).

Table 6: Melting Point of Dapagliflozin

Apparatus	Observed value	Reference Value
Melting point apparatus	74.09±1.59 ^{0}C	**74 - 78°C**

Solubility studies

Using the equilibrium solubility technique, the solubility of Dapagliflozin in a variety of solvents was investigated using a cyclone mixer (REMI CM 101, India). Solubility tests of the drugs in the solvents and buffer media listed below. Dapagliflozin is very little soluble in water (0.173±0.23 mg/mL), however it dissolves much more easily in methanol (25.36±0.12 mg/mL) and poly ethylene glycols (>25 mg/mL) at 25 degrees Celsius. The medication is almost insoluble in water but soluble in methanol, according to the solubility research. Dapagliflozin has a solubility of 0.8 mg/mL in water, according to published reports.

Table 7: solubility determination in different mediums

S. No	Medium	Solubility determination Concentration (mg/mL)
1	Methanol	28.36±0.12
2	Dichloromethane	22.34±3.45
3	PEG 200	15.36±3.21
4	PEG 400	18.39±2.46
5	PEG 600	12.84±1.27
6	Ethanol	5.39±1.25
7	Chloroform	16.43±0.83
8	0.1 N HCl pH 1.2	18.37±0.07
9	Buffer pH 6.8	2.35±0.023
10	Buffer pH 7.4	1.26±0.053
11	Water	In soluble

Ultraviolet (UV) spectrum

The development of an analytical method for determining a drug's efficacy is essential for preformulation research. C-glycosyl Dapagliflozin is a beta-D-glucose derivative with a 4-chloro-3-(4-ethoxybenzyl)phenyl group in lieu of the anomeric hydroxy group. Propanediol monohydrate is used to help persons with type 2 diabetes better regulate their blood sugar levels when combined with a healthy diet and regular exercise. UV absorption spectra of Dapagliflozin in methanol and buffers containing 1% sodium lauryl sulphate (SLS) are shown in figure. Maximum absorption (max) of Dapagliflozin in methanol with SLS 1% buffer was found to occur at 235.5 nm.

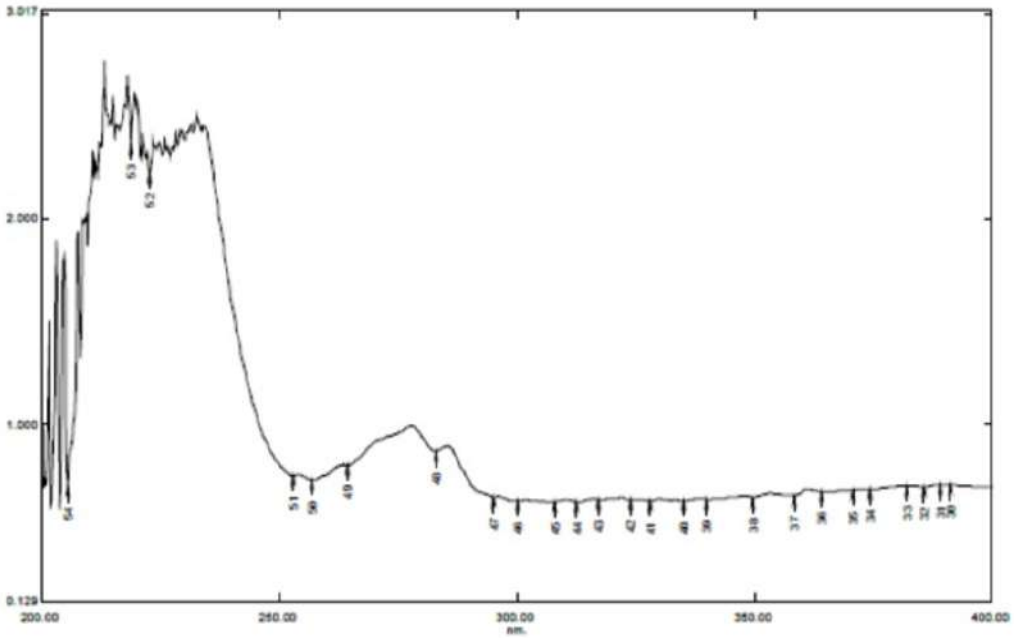

Figure 4: Determination of λmax by UV Visible spectrophotometer

Partition coefficient determination

Predicted values of log P for Dapagliflozin range from 2.11±0.06 to 2.52±0.02, indicating that it is hydrophobic and explaining its affinity for lipid membranes, interactions with hydrophobic domains of proteins, and translocation through the blood-brain barrier, among other experimental results. Due to the afore

mentioned spectrum features, Dapagliflozin may be quickly identified and its concentration accurately determined. Dapagliflozin has a log P value of 2.13±0.03 in octanol: water. The calculated log P value, ~3.4, agreed with the previously reported value, ~3.4. If the drug's log P value is more than 1, it is hydrophobic.

Experimental design for the preparation and optimization of Dapagliflozin ethosomes

Touitou et al., 2000, devised a conventional cold approach for preparing the Dapagliflozin -loaded ethosomes. It's the most preferable method for making tiny ethosomes. 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. After analyzing the results of our early tests and a comprehensive literature search, we settled on the independent variables and their respective values. Responses for % EE, vesicle size, zetapotential, and %CDR are reported in Table 2 for a total of 17 formulations created using the factorial design. Linear regression, two-factor interaction (2FI), and quadratic models were used inside the Design-Expert® program to fit the collected data (Talluri et al., 2017). 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. Decisions were made using the polynomial equation by factoring in the magnitudes of the coefficients as well as the positive and negative mathematical signs associated with them. Coefficient values closer to one indicate more important model variables, whereas those farther from one indicate less significant model components. In a polynomial equation, a positive sign implies that a rise in the level of one variable results in an increase in the linked response, whereas a negative sign suggests the opposite. 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. Design-Expert®so ftware was used to construct 2D-contour plots, 3D-response surface plots, and perturbation graphs, all of which helped to illustrate and clarify the connections between the explanatory and analytic variables. In addition, the linear correlation graphs between the observed and forecasted data proved the reliability of the model. Goodness of fit is indicated by high values of R2 (>0.9000) between observed and predicted values for all answers (Chaudhary et al., 2011). 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.

Table 2: 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	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

Fitting of data to the model

Design-Expert® software was used to concurrently match the observed responses for all 17 formulations to the different mathematical models to determine the best suited model. Table 3 displays the mean values, standard deviations, multiple correlation coefficients, adjusted multiple correlation coefficients, projected multiple correlation coefficients, coefficients of variation, and predicted residual sums of squares (PRESSs) for all models. The quadratic model was shown to have the greatest fit for all four answers (Y1, Y2, Y3, and Y4), as measured by high R2, adjusted R2, and anticipated R2s values and low SD, CV, and PRESS values. If the model is a good match for the data, the PRESS statistic will show it. The preferred model will have a low PRESS relative to the alternatives.

Effect of independent variables on % entrapment efficiency (Y1)

The entrapment effectiveness of nanovesicular formulations is the percentage of the whole medication contained inside them. Calculating the entrapment efficiency is crucial since it establishes the system's drug storage and eventual delivery capabilities. Table 2 displays the outcomes of the entrapment effectiveness of the Dapagliflozin ethosomes formulations. 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

where X1 is the phospholipid, X2 is the cholesterol, and X3 is the ethanol, and Y1 is the entrapment efficiency in percent. 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. This suggests that a higher phospholipid content raises the ethosomes' % EE, whereas a higher cholesterol content reduces it. Cholesterol's impact on Dapagliflozin ethosome EE was more pronounced than phospholipid's, as shown by the larger X2 coefficient value. With an F-value of 80.54,

the model may be considered statistically significant. The likelihood of seeing an F-value this high as a result of random chance is less than 0.01%. Model terms are statistically significant when their p-values are less than 0.0500. In this scenario, B, C, AC, A2, and C2 are all crucial components of the model. With an F-value of 8.52, the Lack of Fit is statistically significant. The likelihood of seeing a Lack of Fit F-value as great as this one being the result of random chance is just 3.28 percent. The difference between the Predicted R2 of 0.8657 and the Adjusted R2 of 0.9781 is less than 0.2, indicating that the two values are consistent with one another. The signal-to-noise ratio is evaluated using Adeq Precision. It's preferable to have a ratio higher than 4. Your signal-to-noise ratio of 28.439 is good.

Figure depicts the influence of independent factors on the % EE as evidenced by 3D-response surface graphs and their associated 2D- contour plots. The figures demonstrate that the entrapment effectiveness of Dapagliflozin in the ethosomes improved when the phospholipid concentration was raised from 2% to 4%. It has been shown that the EE of lipophilic medicines is much greater than that of hydrophilic medications when encapsulated in ethosomes (Abdel Messih et al., 2017). Since hydrophobic drugs are better able to interact with the vesicle membrane hydrophobically, increasing the phospholipid concentration in the vesicle improves drug EE (Faisal et al., 2018). The availability of the lipid phase to drug molecules and their subsequent accommodation in the lipid bilayers may also be enhanced by an increase in phospholipid concentration (Aggarwal et al., 2013). However, 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 (Ahad et al., 2014). Dapagliflozin is soluble in ethanol because it has a lipophilic molecular structure. Increasing the ethanol concentration would therefore promote medication solubilization and trapping inside the vesicle's hydro-ethanolic core and lipid bilayers. Ethanol at high concentrations renders the vesicles leaky because ethanol partially solubilizes the phospholipid bilayer in ethanol (Ahad et al., 2014). This resulted in a decrease in % EE when the ethanol concentration was increased up to 50%. Figure 2C's perturbation graph demonstrates that the percentage of ethanol was the most critical factor influencing the drug's EE, whereas factor A's (the phospholipid concentration) slope was very shallow.

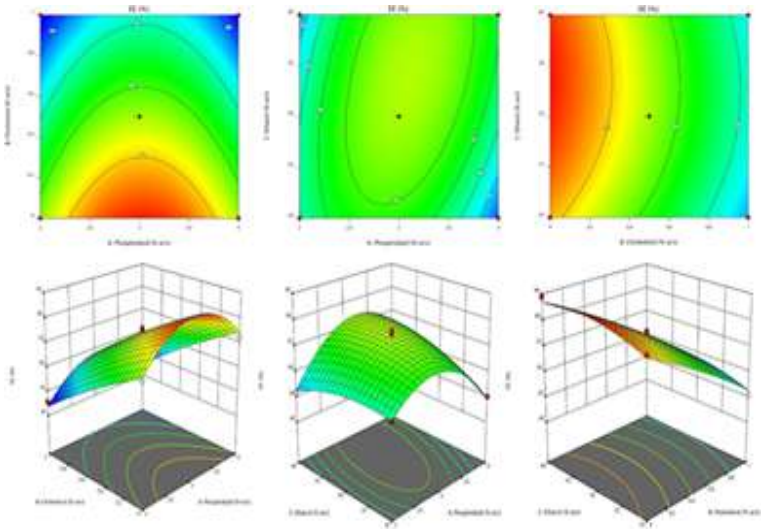

Figure 6: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on ethosomal Entrapment Efficiency (Y1)

Effect of independent variables on vesicle size (Y2)

Vesicle size is crucial for transdermal or topical medication administration. This is because only very small vesicles, those with a diameter of 300 nm or less (Zhai et al., 2015), can deliver their encapsulated medication to the dermal layers of the skin. Furthermore, vesicle uptake into cells and physical stability are both influenced by particle size (Jain et al., 2015). Table 2 displays the vesicle sizes of the various Dapagliflozin ethosome compositions. F4 vesicles were the smallest (103.462.17nm), 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

where Y1 represents the vesicle size, X1 represents the phospholipid percentage, X2 represents the cholesterol percentage, and X3 represents the ethanol %. The equation demonstrates a positive relationship between phospholipid and cholesterol concentrations and vesicle size, and a negative relationship between ethanol concentration and vesicle size. Increasing the phospholipid and choleterol percentages results in larger ethosome vesicles, whereas increasing the ethanol % results in smaller vesicles. The large X2 figure indicates that the proportion of cholesterol has a more noticeable influence on ethosome vesicle size than that of ethanol. The model is statistically significant, since the F-value for it is 67.97. The likelihood of seeing an F-value this high as a result of random chance is less than 0.01%. Model terms are statistically significant when their p-values are less than 0.0500. Important words in this model include AB, CA, BC, AA, and C2. A lack of significance between the Lack of Fit and the pure error is shown by an F-value of 1.54. The likelihood of seeing a Lack of Fit F-value as great as this one being the result of random chance is 33.53 percent. The 0.8949 predicted R2 and the 0.9741 adjusted R2 are quite close to one another (the difference is less than 0.2). The signal-to-noise ratio is evaluated using Adeq Precision. It's preferable to have a ratio higher than 4. You have an acceptable signal with a ratio of 22.602.

3D- response surface graphs (Figure 1A) and 2D contour plots (Figure 1B) depict the impact of varying the independent factors on the 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 (Wilson et al., 2017). It's possible that the formation of a phase with inter diffusing hydrocarbon chains is to blame for the drastic loss in phospholipid bilayer thickness seen when large amounts of ethanol were present in ethosomes. Ethanol provides a net negative charge to the ethosomal systems and lends some steric stability to the lipid vesicles, both of which may contribute to a reduction in the vesicle size of the ethosomes (Chen et al., 2010). These findings correlate well with those of Ahad et al. (2013), who reported a similar trend in their description of the shrinking vesicle size of valsartan-loaded nanoethosomes in response to increasing concentrations of ethanol. The vesicle size was found to be significantly affected by the phospholipid percentage, as seen by the steep slope of factor A (% phospholipid) and the minor bend of factor B (percentage ethanol) in the perturbation graph (Figure 1C)

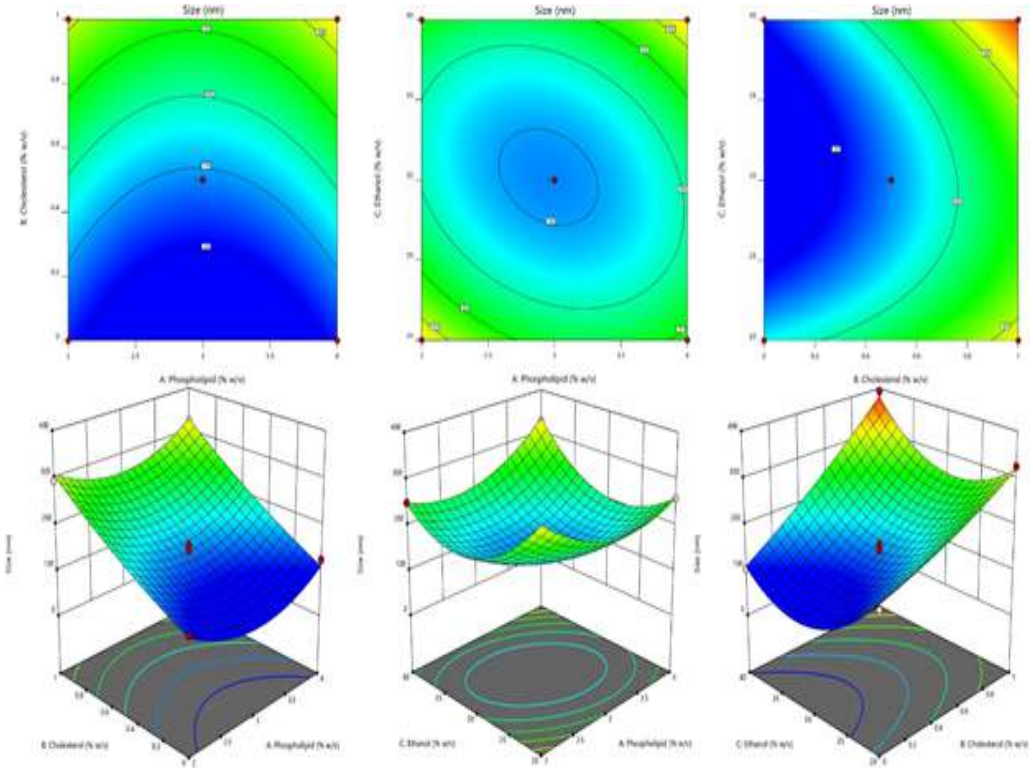

Figure 7: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on Vesicle size (Y2)

Efects of Experimental Variables on ZP (Y3)

If you want to know how resistant a vesicular dispersion is to particle aggregation, growth, and sedimentation, the best method is to calculate its zeta potential (53). Zeta potential values for Dapagliflozin -loaded ethosomes varied from -21.03±1.03 (F10) to -39.82±3.02mV (F12), as reported in Table. The electric repulsion between vesicles is strong enough at these zeta potential levels to avoid aggregation and preserve the physical stability of the dispersion [17, 18]. 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 [7, 53, 54]. Ethmosomal vesicles may be transported more easily through the intestinal membrane because of the negative charge's destabilizing effect on membranes [53]. The model terms X1, X1X2, and X1X3 had a significant influence (P≤0.05) on zeta potential (Y3), but X2, X3, and X2X3 did not (P > 0.05) according to the results of an ANOVA on the quadratic model.

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

Individually, X1 had a detrimental impact on zeta potential (P0.05). Reasons for this include an increase in cholesterol concentration, which leads to an increase in electrical conductivity [56, 57], and an increase in lipid concentration, which has been reported to introduce a negative charge onto the surface of the vesicles due to the uneven polarity distribution of the lipid (X1) hydroxyl group [55, 56]. The significant negative X1X3 interaction (P0.05) indicated the antagonistic combined efect of X1 and X3, while the negative coeffcient of X1X2 interaction (P0.05) indicated the antagonistic effect of increasing X2 from 1 to 17 with the negative effect of increasing X1 from 2 to 4%w/v on the absolute value of zeta potential.

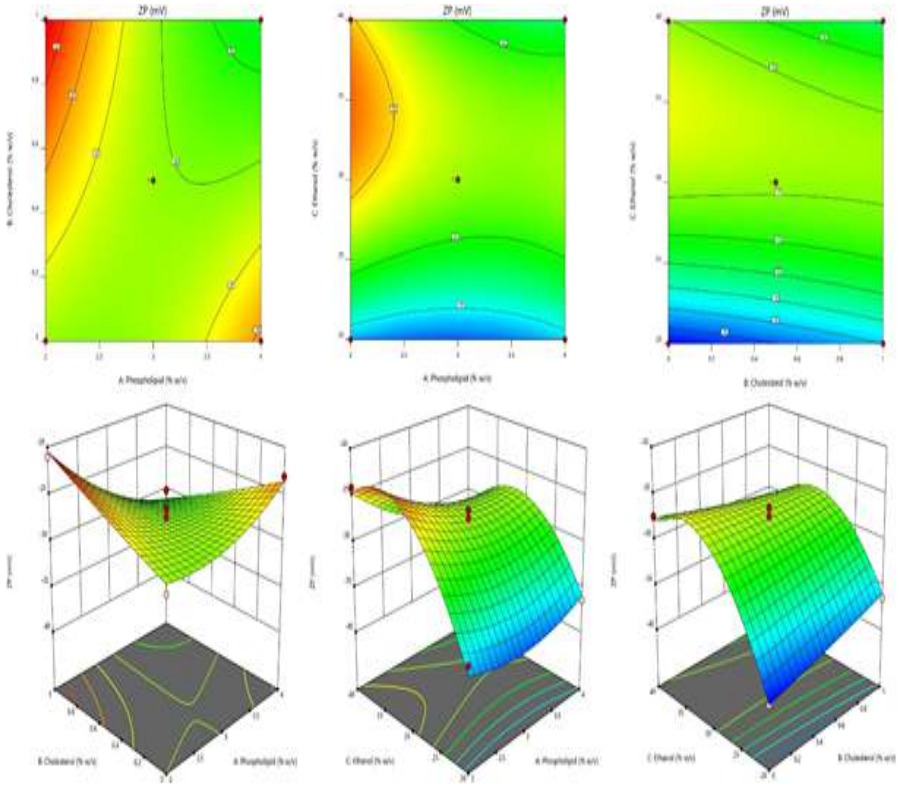

Figure 8: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on Zeta potential (Y3)

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 ethosoma [41]. An F-value of 26.61 for the model indicates that it is statistically significant. The likelihood of seeing an F-value this high as a result of random chance is less than 0.01%. Model terms are statistically significant when their p-values are less than 0.0500. Relevant model words here are A, C, AB, AC, BC, A2, C2. Compared to the pure error, the Lack of Fit F-value of 1.20 is not statistically significant. The likelihood of seeing a Lack of Fit F-value this high owing to random chance is 41.57 percent. The 0.7611 predicted R2 and the 0.9351 adjusted R2 are not too far off from one another. The signal-to-noise ratio is evaluated using Adeq Precision. It's preferable to have a ratio higher than 4. Your signal-to-noise ratio of 19.084 is good.

Effect on In Vitro Drug Release

Figure depicts the in vitro drug release profile of Dpagliflozin-loaded ethosomes and free drug. As can be shown in Figure, 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). Dapagliflozin 's rapid absorption after administration of an ethosomal formulation may be due to the drug's location in the vesicles' outer membrane [32]. The effect of varying formulation factors on in vitro drug release from formed ethosomes, including lipid content and cholesterol concentration, was also studied. Drug release efficiency from ethosomes was clearly diminished when lipid contents increased. Possible explanations for this include (a) the

phospholipid's amphiphilic capabilities and (b) the positive impact of increasing lipid content on the vesicle size. However, the in vitro drug release pattern from the prepared ethosomal formulations was not significantly affected by cholesterol concentration. Dapagliflozin release from various ethosomal formulations was shown to follow the Higuchi kinetics, elucidating a diffusion-controlled mechanism [33], whereas free Dapagliflozin suspension followed first-order kinetics.

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

With an F-value of 71.45, the model is statistically significant. The likelihood of seeing an F-value this high as a result of random chance is less than 0.01%. Model terms are statistically significant when their p-values are less than 0.0500. In this scenario, important model terms were B, C, AC, BC, B2, and C2. With an F-value of 7.10, the Lack of Fit is statistically significant. A Lack of Fit F-value as high as this one is very unlikely to be random. The 0.8523 predicted R2 and the 0.9754 adjusted R2 are quite close to one another (the difference is less than 0.2). The signal-to-noise ratio is evaluated using Adeq Precision. It's preferable to have a ratio higher than 4. Your signal-to-noise ratio of 34.664 is rather good.

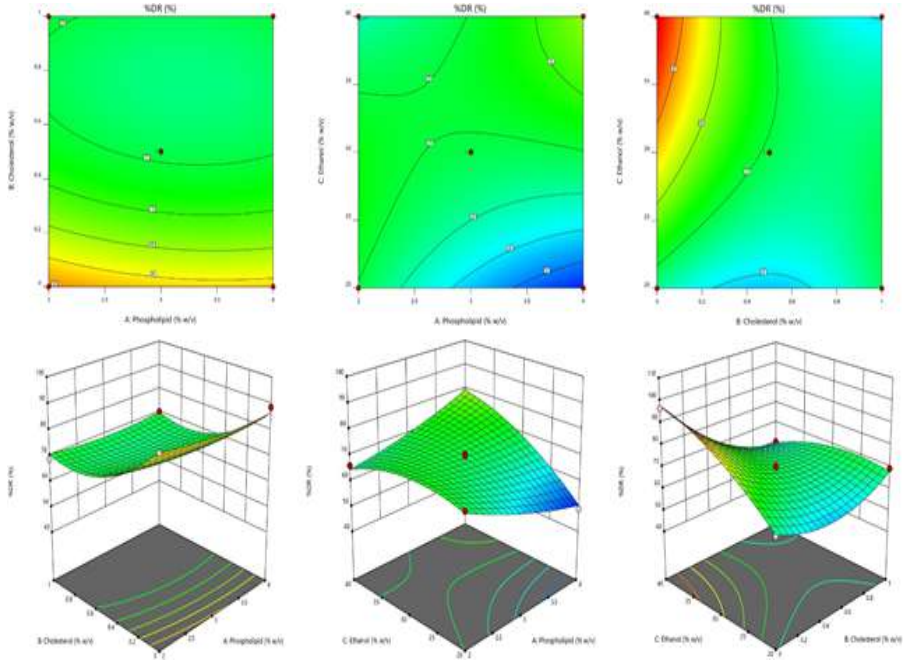

Figure 9: 2D contour graph, 3D response surface plot demonstrating the influence of independent variables (X1), (X2) and (X3) on %CDR (Y1)

Characterization of ethosomes

PDI and ZP

Dapagliflozin ethosomes' PDI and ZP values are shown in Table. In Figure, we see the optimized ethosomes' vesicle size distribution as well as their zeta potential (ZP). The particle size distribution index (PDI) is a metric for assessing the consistency of the nanovesicles. High PDI values imply a vesicle population that is very diverse, whereas low PDI values describe a homogeneous population (Mbah et al., 2014). The prepared vesicle population was quite consistent, with PDI values between 0.12 and 0.35. ZP is associated with the charge on the nanovesicle surface and may affect the formulation's physical stability as well as the vesicle skin contacts. Long-term stability of vesicular formulations is improved by a high ZP value (positive or negative), since this reduces the likelihood

of aggregation amongst vesicles with the same charge owing to electrostatic repulsion. All of the ethosomal preparations had ZP values between -21.031.03 and -39.823.02 mV. The addition of ethanol causes a change from a positive to a negative charge on the vesicles, which accounts for the negative ZP value seen in ethosomal formulations (Touitou et al., 2000). Ethanol molecules in ethosomes were found both in the aqueous core and the lipid bilayer membrane. Negative ZP may result via hydrogen bonding between hydroxyl groups in the ethanol molecules or between ethanol and water molecules (Zhai et al. 2005; Mbah et al. When more ethanol is added, more negative charge is added to the vesicles.

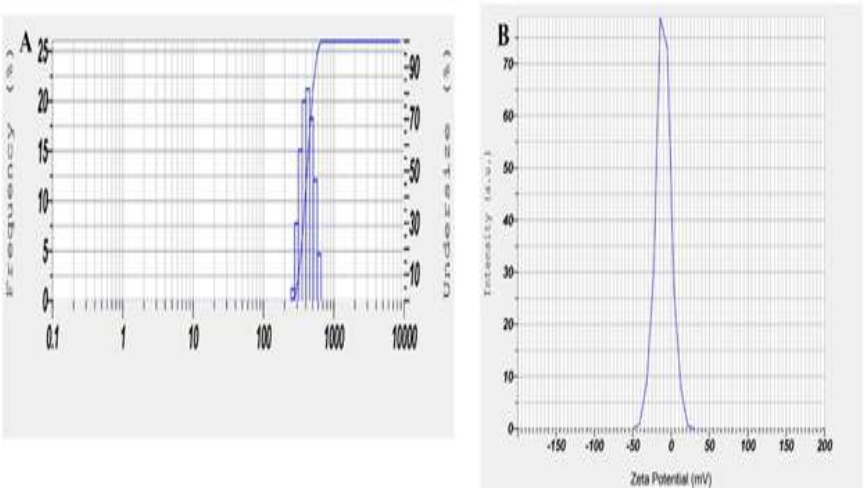

Figure 10: Particle size distribution and zeta potentail of optimized formulation

Evaluation of Ethosomal gel

Physical description

Physical testing revealed that the Dapagliflozin ethosomal gel is odorless and clear, with a color range from off-white to pale yellow.

pH determination

The pH of ethosomal gels was measured and compared; the findings are reported in Table.

Viscosity

The gels' thickness was measured using a plate and cone Brookfield Viscometer. The results of the HADV-II+ pro model run on the cone spindle CPA-41Z are listed below.

Spreadability

The findings of characterizing the spreadability of the ethosomal gel are listed in Table.

Drug content

UV spectrophotometer was used to characterize the drug content analysis of vesicular gel formulation to determine the quantity (%) of drug contained in the formulation. Table details the data acquired about the drug content in ethosomal gel.

Table 25: 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

SEM analysis confirmed the vesicular properties by evaluating the optimized nanovesicular formulation for shape and lamellarity. Images obtained using scanning electron microscopy (SEM) revealed optimized ethosomes and ethosomal gel to be unilamellar vesicles, ranging in size from a few nanometers to a few microns (Figure 3C). The presence of ethanol, which gives the bilayer membrane considerable flexibility (Faisal et al., 2018), gives the irregular forms. Sizes shown in scanning electron microscopy photomicrographs are often smaller than those determined using dynamic light scattering. Dehydrating and immobilizing the nanovesicles on a solid substrate is necessary for scanning electron microscopy imaging. Hydrophilic surfaces may contract and vesicle structures may become aberrant as a result of this dehydration stage. The outer hydrophilic surface of ethosomes is also notoriously difficult to stain (Zhai et al., 2015). Nanovesicles in their hydrated condition showed a higher hydrodynamic volume as a result of solvent effects, as measured by dynamic light scattering (DLS). To wit: (Ma et al. 2018, Abdel Messih et al.

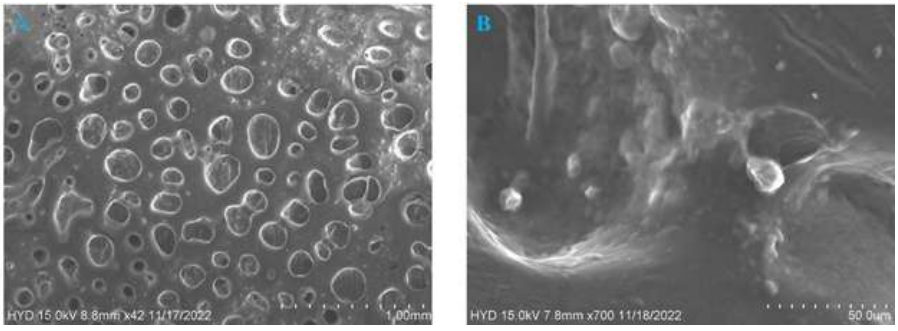

Figure 11: SEM images of Optimized formulations of Ethosomes and ethosomal gel

FTIR:

The alkyl C-H stretch, identified at 2922.74 cm-1, the C=O stretch, identified at 1707.74 cm-1, and the O-C-C stretch in ester, identified at 1044.78 cm-1, were all verified in phospholipid. C-N stretch and P=O stretch vibrations were found to be combined at 1188.45 cm-1, producing the other significant peak. The existence of C=C and P-O stretch was also verified by spectral peaks at 1546.32 cm-1 and 865.17 cm-1. Therefore, IR spectroscopy was used to identify Soy Lecithin as a pure compound.

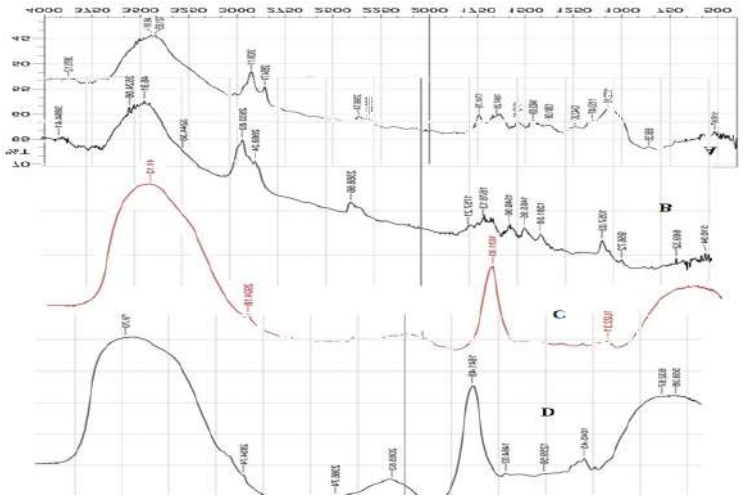

Figure 12: FTIR spectrum of A. Phospholipid, B. Cholesterol, C. Optimized ethosomal gel formulation and D. Optimized ethosomal gel formulation

The phenolic O-H stretch was clearly visible as a strong peak in the IR spectra of cholesterol, at 3618.81 cm-1. The presence of alkyl and aromatic C-H stretch was also recognized by a second strong signal at 2923.95 cm-1. It was determined that the other two prominent peaks, at 1545.61 and 1462.92 cm-1, originated from aromatic C=C stretch. Methyl C-H rock peaked at 1368.87 cm-1, long-chain methyl C-H rock peaked at 798.55 cm-1, and aromatic C-O stretch peaked at 1047.4 cm-1. The powder compound's purity was established using the IR spectrum.

Absorption of infrared photons caused vibrations of chemical bonds, and the resulting spectrum of distinctive bands provided a fingerprint that could be utilized for identification and characterisation of the sample using Fourier transform infrared spectroscopy (FTIR). Both ethosomes and ethosomal gel, which are used as medication excipients, revealed a high peak at 3743 cm1 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.

Differential Scanning Calorimetry (DSC)

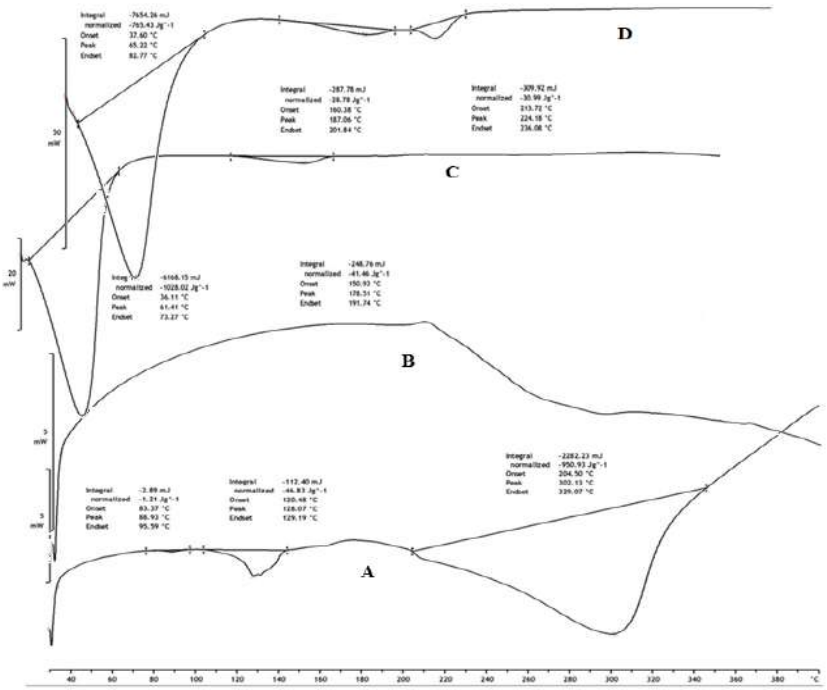

Figure 13: DSC thermogram of A. Cholesterol, B. Phospholipid, C. Optimized ethosomal suspension and D. Optimized ethosomal formulation

Both phospholipid and cholesterol, two crucial excipients, displayed an endothermic peak in a DSC analysis. At 83.37°C, 120.48°C, and 204.50°C, phospholipids showed exothermic peaks. When heated, bound water is released, which causes a disruption in the DSC thermogram and, perhaps, crystallization. Cholesterol's DSC thermogram exhibited a very clear exothermic peak at 148 to 155 °C, and no change or interaction occurred in that range. Figure13 depict the DSC

thermograms of the two excipients, respectively.

Scaning the DSC thermograms of the medication, excipients, and formulation from 5 to 300 °C revealed exothermic peaks in all cases, as well as a folding transition and a melting point close to the jump line. Between 61 and 74 degrees Celsius was discovered to be Dapagliflozin 's melting point. Exothermic peaks in soy-lecithin were recorded at 160 °C, 180 °C, and 190 °C. When heated, bound water is released, which causes a disruption in the DSC thermogram and, perhaps, crystallization. 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.

In vitro drug release test

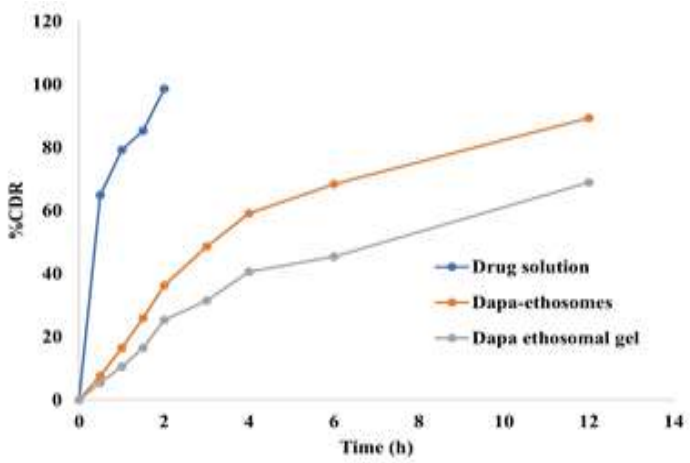

Figure 14: invitro drug release studies comparision between the optimized ethosomal gel and ethosomal suspension

By using the Franz diffusion cell (Teledyne Hanson Research, 6 cells), we were able to examine the in vitro drug release of a pure drug solution, an ethosomal suspension, and an ethosomal gel. The UV-Spectrophotometer was used to evaluate samples taken at half-hour, one- hour, two-hour, four-hour, and six-hour intervals. There was a comparable drug release profile seen in vitro. numerically; in terms of a number. Less drug was released at the final time point of 12 hours in an in vitro drug release investigation of several forulations 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).

In vitro Studies to Evaluate Skin Permeation

The steady-state flux was 1.93 µg/cm2/h, the cumulative permeation percentage was 89%, and the quantity of drug penetrated was 1054±18.1 µg/cm2 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. Finally, the ethosomal gel with the

highest ethanol concentration, the lowest lecithin concentration, and the moderate cholesterol concentration showed the best permeability at interval times with significance ($p \leq 0.05$). The observed electrostatic repulsion resulted in a trans epidermal resistance of more than 30±1.5 kΩ. It was a positive sign for the health of the skin.

In our formulations, the drug penetration rate rose as the concentration of phospholipid dropped. The medication permeation rate also increased when cholesterol concentration decreased. The stiffness of the ethosomal vesicle bilayer was also observed to increase with increasing lecithin and cholesterol concentrations in a prior research. Ethanol improved drug diffusion over the membrane by interacting with the polar head group of SC lipid molecules, which resulted in a decrease in the melting point of SC lipids and an increase in the fluidity and permeability of the lipid bilayer. Ethanol, vesicles, and SC lipid molecules worked together to increase the drug's permeability through the vesicles to its target. Carbopol is able to maintain the necessary pH and avoid skin irritation since it contains an anionic polymer and the best buffering capacity features. The optimal viscosity and bio-adhesion properties are achieved by combining carbopol with ethosomes.

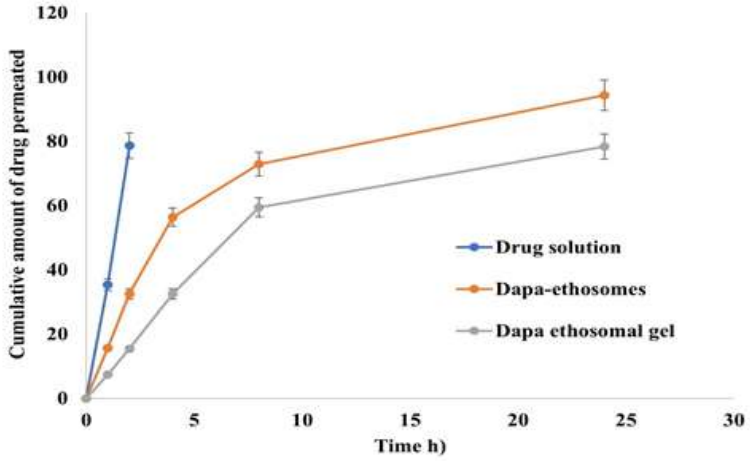

Figure 15: Ex-vivo permeation studies of optimized ethosomal suspension and gel

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 26: Acclerated stability studies of Optimized formulation

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

Conclusion:

Without reliable early detection technologies, diabetes mellitus will continue to spread like an epidemic. Preventing type 2 diabetes, a metabolic condition, requires adjusting one's diet, exercising regularly, and keeping one's weight in check. The key to managing this new pandemic is public education. Despite advances in our understanding of the disease's biology and the development of promising new treatments, no solution is yet in sight. 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 ethosome-encapsulated Dapagliflozin exhibited significantly higher cumulative penetration (Qn) and in vitro permeation compared to that of 25% pure drig 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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