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FORMULATION AND EVALUATION OF EMPAGLIFLOZIN-LOADED ETHOSOMES

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Abstract:

The goal of personalised care plans for people with type 2 diabetes is to enhance their quality of life. The major objective of this study was to create a new vesicular drug delivery system (ethosomal gel) that incorporates empagliflozin for the treatment of diabetes. The gel evaluated vesicle size, PDI, Zeta Potential, % Entrapment efficiency, and vesicular shape.According to SEM examination, optimised ethosomal gel was found to have unilamellar vesicles with a size range of a few microns to a few nanometres. DSC and FT-IR studies revealed that empagliflozin was successfully integrated into ethosomes, indicating that it was in an amorphous state within ethosomes.

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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. 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.

Materials and Methods:

Famotidine was obtained as a gift sample from Hetero lab, Soya Lecithin, Phospholipid from Sisco Research Laboratories, Propylene glycol, Carbopol, Cholesterol from Ozone chemicals, Mumbai.

Preparation of Phosphate Buffer 7.4 pH

Standard stock preparation

Using a phosphate buffer with a pH of 7.4, 100 mg of atorvastatin was dissolved in 5 ml of methanol as a cosolvent in a 100 ml volumetric flask. The stock solution had a concentration of 1000 µg/ml and was entirely clear after being sonicated for 15 minutes and filtered.

Preparation of the working solution

By adding 7.4 pH phosphate buffer to 1 ml of the original sample in a 10-ml volumetric flask, the volume was increased to 10 ml with 7.4 pH phosphate buffer. The sample was then scanned spectrophotometrically between 200 and 400 nm against a blank of 7.4 M phosphate buffer.

Preparation of Ethosomes by Cold Method

Ethanol was added to a clean, dry round-bottom flask to dissolve empagliflozin. After being accurately measured, the soy-lecithin and cholesterol were added to the ethanol. The ethanol components were combined using a mechanical stirrer. Next, while stirring, the lipid combination stated above was added to propylene glycol. The filtered water should be kept at 30 degrees Celsius in a water bath. In a water bath, the ethanolic mixture was brought to 30 degrees Celsius. The aqueous phase was then progressively added to the lipid phase in a very thin stream as the mechanical stirrer continuously mixed at 700 revolutions per minute. Following the full inclusion of the aqueous phase, ethosomal suspension was formed when mixing was continued for five minutes. The ethosomal solution was sonicated for 0.5 to 3 minutes using a probe sonicator to reduce the size of the ethosomes. After that, the sonicated ethosomal solution was constantly shaken for half an hour to produce stable ethosomes. The mixture was stored between 4 and 8 degrees Celsius until it was ready for use.

Characterization

Determination of entrapment efficiency (%)

In order to separate the free drug, the mixture was spun in a cooling centrifuge at 16,000 rpm and 4 °C [39]. In the supernatants, 10 mL of distilled water were diluted for three minutes [28]. We were able to ascertain that the maximum concentration of empagliflozin was 224 nm by using a spectrophotometer (Jasco UV-Vis spectrophotometer, Jasco, Japan).

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

Zeta Potential & Vesicle Size Analysis

Dynamic light scattering (DLS) is used by the Malvern Zetasizer (Nano ZS, Malvern, UK) to determine vesicle size and ZP.

In Vitro Release Study

Ten milligrammes of Empagliflozin were present in one millilitre of each formulation, which was placed into a dialysis bag. For the release tests, we used 40 millilitres of Sorensen's phosphate buffer, which has a pH of 6.5. Before being immersed in the prepared release media, the dialysis bag was put in a dissolving apparatus set to 32±0.5 °C and 100 rpm. We collected 1-mL samples at 1, 2, 4, 8, 12, and 24 hours and replaced them with an equivalent volume of fresh medium. The sample concentrations were determined using spectrophotometric analysis at 224 nm [31,55].

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

Preparation of Ethosomal gel

The ethosomal gels of empagliflozin were created by spinning distilled water with 1–4% w/w Carbopol 940 P at room temperature. As a preservative, we used equal amounts of sodium propyl and sodium methyl hydroxybenzoate. Propylene glycol, a stabilising and humectant ingredient, was added to the preparation with preservatives. An Empagliflozin ethosomal preparation with a medication concentration of 10 mg is included in the gel formulation. After 15 minutes of mixing, the weight of the gel formulation was attuned to 100% w/w. After 15 minutes of sonication, the ethosomal gel's air bubbles disappeared, allowing the collapsable aluminium tube to be filled.

Table No 5: Preparation of Ethosomal gel

Ingredient	G1	G2	G3	G4	G5	G6	G7
Carbopol 940	0.5	1.0	1.5	2.0	2.5	3.0	4.0
Propylene Glycol	2.0	4.0	6.0	2.0	4.0	6.0	8.0
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Dist. water	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Characterization of Ethosomal gels

pH Determination

A pH meter was used to measure the pH of each batch of ethosomal gel

Viscosity determination

By the Brookfield Viscometer, the viscosity (cp) was calculated. Ethosomal gel (2 mL) was put into the sample cup. Viscosity was measured using a cone spindle (CPA-41Z) and HADV-II+ pro modelling.

Spreadability

The spreadability apparatus was made up of a male perspex cone that could be moved and was put on top of a female perspex cone that was fixed to a wooden board. Twenty grammes was the weight of the male moveable perspex cone. A 5 g sample of gel was held in a female perspex cone and then shaved to a smooth surface using a knife. After that, the male perspex (weight) was gradually brought closer to the female perspex cone over a distance of six centimetres, and the interval between each sample was noted.

$$\text{Spreadability} = \frac{\text{Weight of male perspex cone}}{\text{Time is taken to move towards female perspe}}$$

Drug content

Using a 10 ml volumetric flask, measure 100 mg of ethosomal gel. Methanol and phosphate buffer pH 7.4 (70:30 ratio) were combined to create a solvent mixture that was entirely filled into each sample's flask. Following the mixing and filtering of the solutions by means of a 0.45 µm membrane, 1 ml of the filtered solution was moved to a 10-ml volumetric flask, while the residual 9 ml was occupied with a solvent mixture. To analyse these solutions, a UVSpectrophotometer was utilised at λmax 224 nm.

In vitro drug release study

For the in vitro drug release investigation, Franz diffusion cells were employed. To investigate drug diffusion, the egg membrane was employed as a model for a semi-permeable membrane. An Empagliflozin vesicular gel formulation containing 100 mg was directed to each donor compartment. After filling receptor compartment with a 7.4 pH phosphate buffer solution, the temperature was kept at 32±0.5 °C. Throughout the experiment, a magnetic stirrer was used to continually mix the dissolving medium at 400 rpm. The same volume of new sample was used to replace the samples that were taken. The extracted sample was examined using a UV Spectrophotometer set to a maximum of 224 nm.

Evaluation

Fourier Transform Infrared Spectroscopy (FTIR)

The pellets were created by mixing 10 mg of soy lecithin and cholesterol with 100 mg of dry potassium bromide powder. The infrared spectra were recorded using FTIR following the placement of the thin pellet on the pellet plate.

Differential Scanning Calorimetry (DSC) Study

DSC was used to evaluate the thermal stability of sample by heating them in a nitrogen environment at a frequency of 20 °C/ min.

Stability study as per ICH

The product's stability with the principal pack was tested by storing batches at 5±30C (2o to 8o C), 25°C/60%RH, and 30°C/75%RH for three months (0, 1, 2, 3 Month). Samples were removed from the stability chambers at random intervals and subjected to colour, pH, viscosity, drug content, and drug diffusion analyses.

Results & Discussion

Table No 11: Calibration curve of Empagliflozin with Phosphate buffer pH 7.4

Concentration (µg/ml)	Absorbance
0.0	0
2.0	0.145
4.0	0.253
6.0	0.360
8.0	0.482
10.0	0.568

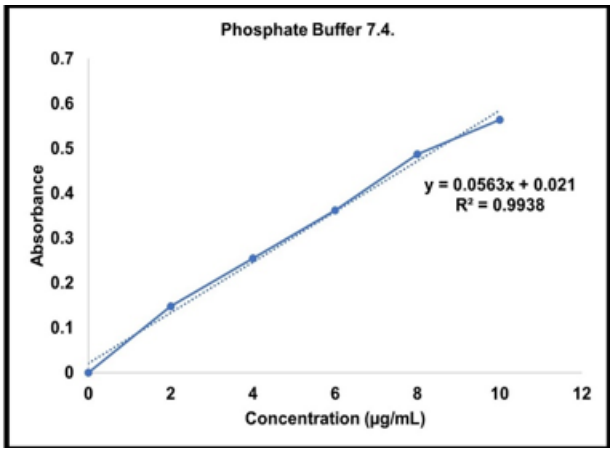

Figure 15: Standardisation studies of pH 7.4

Table 12: Ethosomes formulations.

Formulation	Drug (mg)	Cholesterol (mg)	phospholipon® 90G (mg)	Lecithin (mg)	Ethanol (ml)
F1	10	85	-	-	5
F2	10	80	-	-	5
F3	10	75	-	-	5
F4	10	-	85	-	5
F5	10	-	80	-	5
F6	10	-	75	-	5
F7	10	-	-	85	5
F8	10	-	-	80	5
F9	10	-	-	75	5

Characterization of ethosomes

PDI and ZP

In terms of PDI values, the prepared vesicle population was rather stable, ranging from 0.12 to 0.35. The range of ZP values for all ethosomal preparations was -21.03 to -39.82. The vesicles' charge changes from positive to negative when ethanol is added, which explains why ethosomal compositions have a negative ZP value.

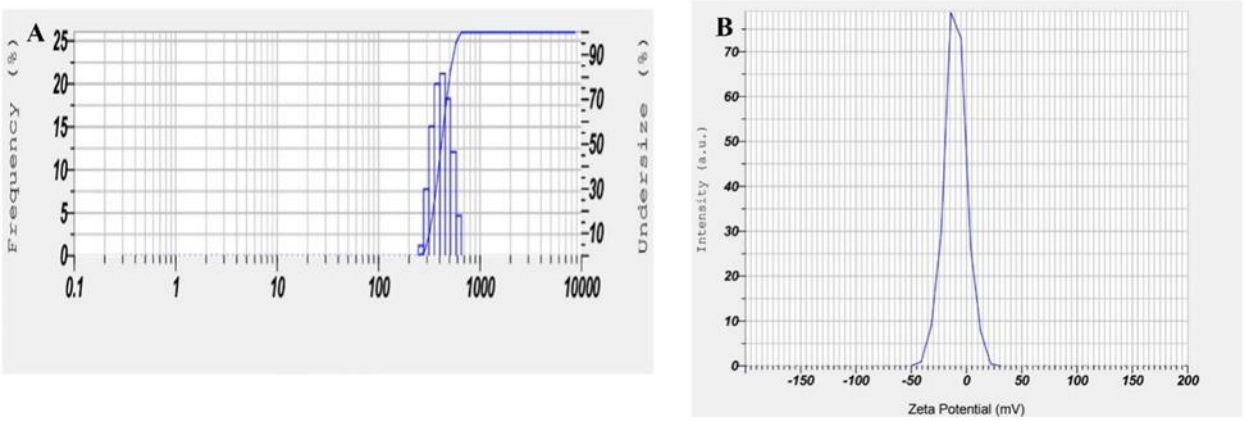

Figure 20: Particle size distribution and zeta potential of improved preparation

Table No 25: Characterization of gel formulations

Formulation	pH	Viscosity	Drug content	Spreadability
G.1	6.02±0.11	15683±1.15	95.64±0.05	11.46±0.54
G.2	6.36±0.21	16831±1.52	97.82±0.01	15.26±0.83
G.3	6.83±0.13	16680±1.18	95.76±0.03	10.74±0.79
G.4	6.73±0.05	14938±1.21	97.36±0.05	12.35±0.31
G.5	6.53±0.17	15863±1.59	96.27±0.02	13.49±0.59
G.6	6.45±0.04	16179±1.42	96.60±0.03	11.37±0.46
G.7	6.49±0.01	15578±1.23	96.17±0.01	12.80±0.62

Vesicle shape and morphology

The optimised ethosomal gel is found to be unilamellar vesicles with sizes ranging from a few nanometres to a few microns, according to SEM examination. The uneven shapes are caused by the presence of ethanol, which provides the bilayer membrane a great deal of flexibility.

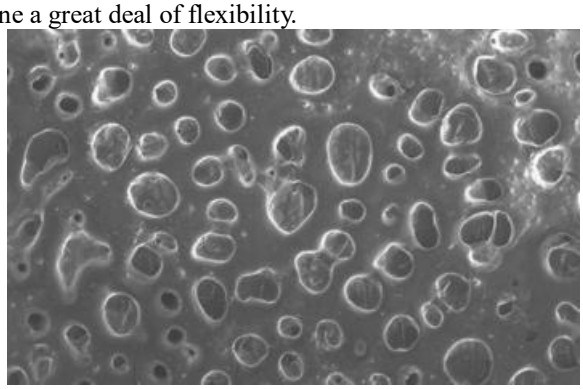

Figure 21: SEM images of Optimized formulation

FTIR:

A type of vibrational spectroscopy called FTIR can be used to identify the chemical structure and fingerprint of a medicine. O-H, C=C, aromatic C-O, O-H, C=O, and C=C groups were the characteristic bands seen in pure empagliflozin. The FTIR spectra of pure empagliflozin showed absorption peaks at 1246.70 cm-1 (C-O ester stretching), 336.10 cm-1 (OH stretching), and 1613.16 cm-1 (C=C, aromatic).

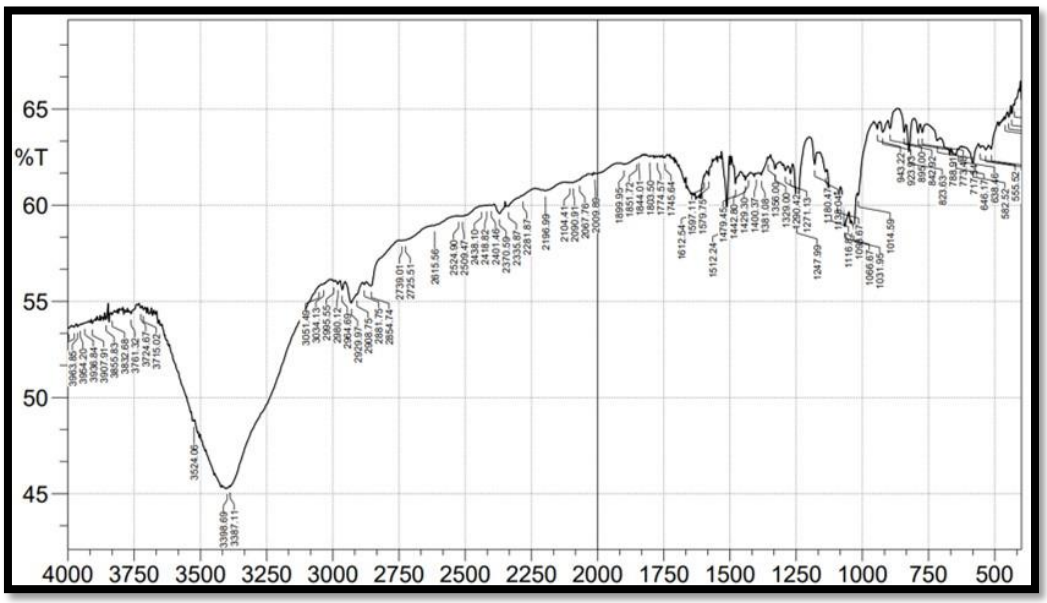

Figure 11: FTIR spectral studies of Empagliflozin

Differential scanning calorimetry

The sample has a temperature of 80.14 ℃ at the beginning of the melting curve, 74.90 degrees Celsius at the peak, and 83.19 ℃ at the end.

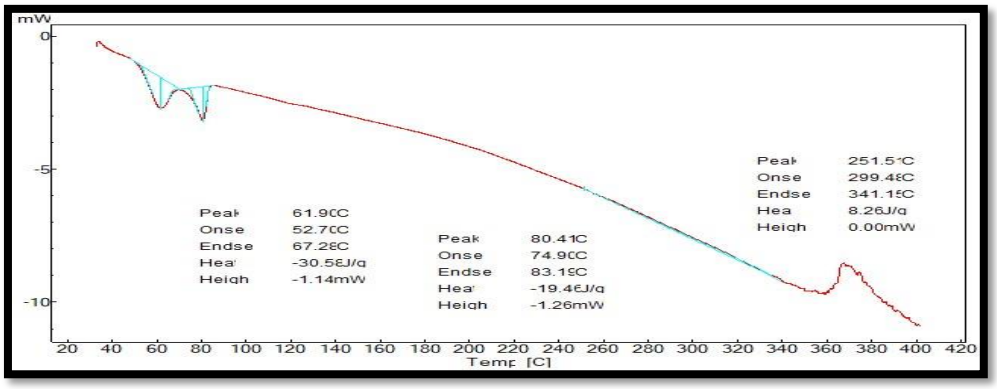

Figure 12: DSC thermogram of Pure Drug

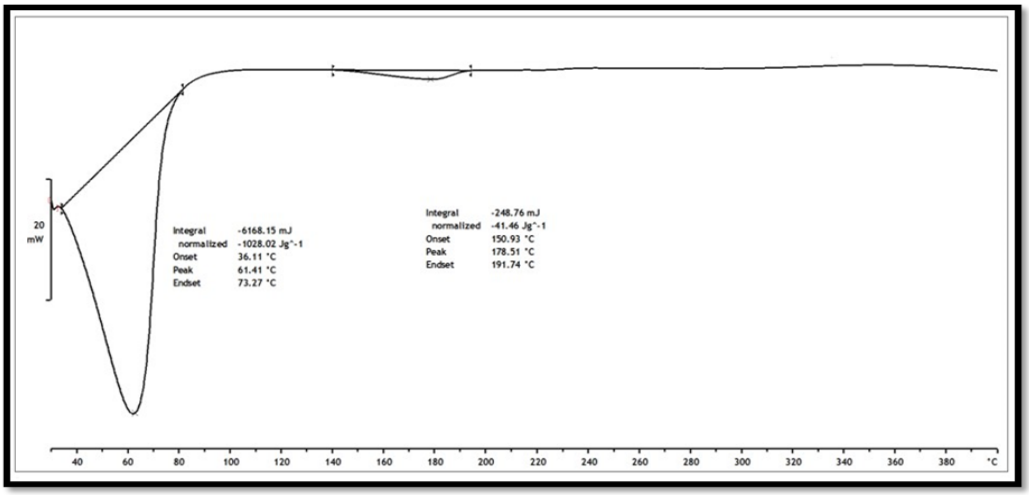

Figure 29: DSC thermogram of optimized ethosomal formulation

During the formulation of Empagliflozin ethosomal gel, the drug was dissolved and diluted in melted lipid form, resulting in an endothermic peak amid 36.11 and 150.93 °C. During this time, no change or interaction was observed.

In vitro drug release

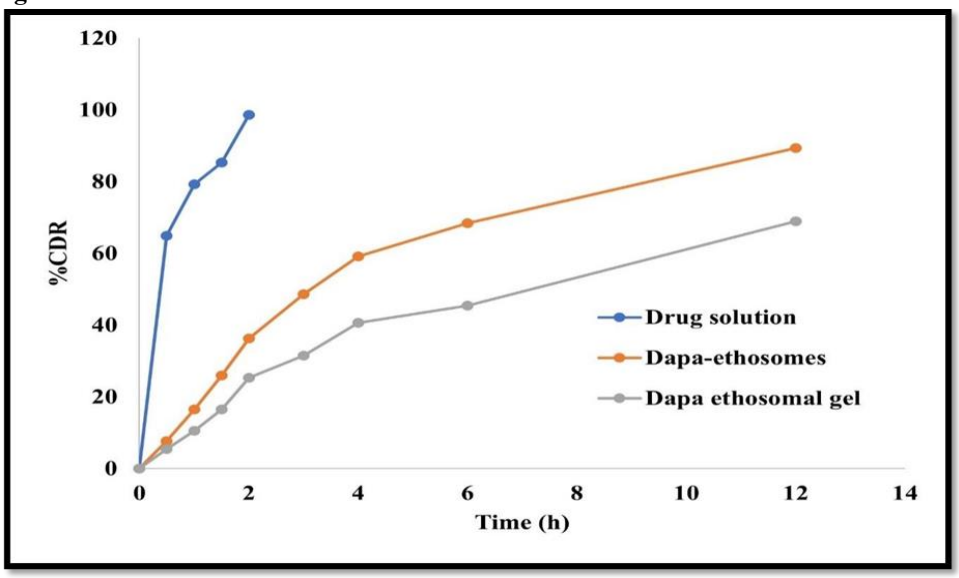

Figure 30: comparison invitro drug release studies

An analogous drug release profile was seen in vitro, quantified by a number. Several drug solution, ethosomal suspension, and ethosomal gel formulations were tested for drug release in vitro; at the final time point of 12 hours, less drug was released. At six hours, the ethosomal solution and gel formulations both showed their maximum drug release.

Stability Studies

One factor contributing to the decrease in entrapment efficiency is the potential for drug leakage from the ethosomes at higher temperatures. Consequently, ethosomes decreased the drug rention time as temperatures rose. The ethosomes' characterisation is altered by the accelerated stability studies.

Table No 26: Accelerated stability studies of Improved preparation

Months	Temperature (°C)	%EE	% CDR	Vesicle Size (nm)
I	Refrigeration (4±2 °C)	91.34±0.05	95.47±0.17	110.53±2.17
II		88.73±0.03	94.53±0.11	106.83±2.36
III		86.43±0.01	92.30±0.15	110.47±2.41
I	Room (25±2 °C)	89.43±0.01	95.49±0.16	105.38±2.1
II		86.73±0.05	93.26±0.17	112.51±2.3
III		80.26±0.06	91.15±0.14	120.63±1.7

SUMMARY & CONCLUSION:

Type 2 diabetes is a metabolic disease that can be prevented by making dietary changes, exercising frequently, and controlling one's weight. Public education is the key to controlling this new pandemic. There is currently no cure for the illness, despite improvements in our knowledge of its biology and the creation of encouraging new therapies. Individualised care programs that enhance their quality of life are what people with type 2 diabetes want. In the current study, we created a novel transdermal vesicular delivery system for empagliflozin ethosomes. The amorphous state of empagliflozin in ethosomes was demonstrated by DSC and FT-IR tests, indicating that it was successfully absorbed into ethosomes.

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