FORMULATION AND EVALUATION OF A TRANSFEROSOMAL BUCCAL FILM CONTAINING EMPAGLIFLOZIN – AN ANTIDIABETIC AGENT

ASHISH P. GORLE, JANHAVI WAGH and SWATI RAYSING

R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India
✉ *Corresponding author: A. P. Gorle, ashish.gorle@rcpatelpharmacy.co.in*

Received July 9, 2026

Diabetes mellitus is one of the most difficult chronic diseases around the world, and it is as important to discover ways to better deliver antidiabetic drugs as it is to discover them. Empagliflozin is a selective SGLT2 inhibitor that has a restricted therapeutic potential when administered as an oral tablet. To overcome this limitation, it was chosen in this study as a drug to deliver through a transferosomal buccal film. Lipid vesicles (Lipoid S100 and Tween 80) were prepared by the ethanol injection method and optimised using a Central Composite Design. The mean particle size, entrapment efficiency and the zeta potential of the optimized transferosomes were found to be 119 nm, 86% and -28.3 mV, respectively (Batch F4). They were then incorporated into a film prepared by the solvent casting technique using mucoadhesive materials, such as HPMC E5, PVP K30 and PEG 400. The results indicated that Batch B3 was the best batch having the drug content of 97.67%, moisture content of 4.46% and folding endurance of 127. The optimized formulation released 97.85% of drug *in vitro* in 60 minutes and had *ex-vivo* buccal permeation that was 18.93% higher than that of a plain film, having a zero-order release profile; also, it was found stable when kept at different temperatures. Most importantly, *in-vivo* pharmacokinetic evaluation in Sprague Dawley rats further confirmed enhanced systemic absorption, with the transferosomal film showing markedly higher bioavailability ($AUC_{0-\infty}$: 8.706 vs. 5.519 μg·h/mL) and C_{max} (0.93 vs. 0.56 μg/mL) relative to the plain buccal film.

Keywords: transferosomes, buccal film, SGLT2 inhibitor, Central Composite Design, *in vitro* release, *in vivo* pharmacokinetic evaluation, stability studies

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition, which involves a sustained increase in blood glucose levels because of a failure of the normal production of insulin or the action of insulin, or both. It is one of the biggest health problems of the 21st century, it was estimated that, in 2021, 589 million adults worldwide had diabetes, and by 2045, the figure is expected to reach 783 million.[1] India is known as the 'diabetes capital of the world' and has a disproportionately large burden of diabetes, having over 77 million diagnosed cases.[2] In addition to glucose dysregulation, diabetes brings about significant macrovascular complications (coronary artery disease, stroke and peripheral arterial disease) and microvascular complications (nephropathy, retinopathy and neuropathy) that have a significant impact on patient quality of life.[3]

There are four major types of *diabetes mellitus*:

•Type 1 *Diabetes mellitus* (T1DM): autoimmune disease-causing destruction of beta cells (accounts for 5-10% of diabetes; usually occurs in childhood or early adulthood; insulin must be taken lifelong);

•Type 2 *Diabetes mellitus* (T2DM) (>90%): insulin resistance in peripheral tissues and progressive failure of beta-cells, strongly associated with obesity, sedentary lifestyle, genetic susceptibility;

•Gestational *Diabetes mellitus* (GDM): glucose intolerance that occurs during pregnancy, which leads to higher risk of later life T2DM in mother and foetus;

•Other specific types: these include exocrine pancreatic diseases, drug/chemical induced diabetes and monogenic diabetes syndromes (MODY).[4]

The mechanism of the T2DM is polyetiological. The eight related defects are

referred to as DeFronzo's 'Ominous Octet': decreased insulin secretion from the beta-cells; increased glucose production in the liver; insulin resistance in the muscle; excessive lipolysis in fat; incretin deficiency or resistance; glucagon secretion from alpha-cells; increased reabsorption of glucose in the kidney via SGLT2 transporters; and central neurotransmitter dysfunction. This complexity requires a multi-targeted pharmacological approach to achieve good glycaemic control.[5]

The glomeruli of the kidneys filter about 180 g of glucose per day, which is almost entirely reabsorbed from the proximal convoluted tubule (PCT). This reabsorption is mediated by SGLT2 (covers ~90%) and SGLT1 (covers ~10%) in the S1/S2 segments of the PCT and S3 segment of the PCT respectively. It is logical and validated that SGLT2 is an interesting drug target in T2DM, because of its upregulation in hyperglycaemia. SGLT2 inhibitors (gliflozins) inhibit SGLT2 selectively, resulting in glucosuria of 60-90 g/day. The main benefits are: insulin independent mechanism, even when there is a great beta cell failure present; no danger of hypoglycaemia because of self-limiting glucose filtered load; small but constant weight loss (about 2–3 kg); blood pressure control through osmotic diuresis and natriuresis.[6]

Empagliflozin is a highly active, selective SGLT2 inhibitor that has been approved for the treatment of T2DM.[7] However, while effective clinically, the usual oral route of administration causes a lot of first pass metabolism in the liver and side effects in the gut, reducing the bioavailability and patient acceptance of the drug. These constraints have led to investigation of alternate delivery methods.[8]

Transferosomes are ultra-deformable, lipid-based vesicular carriers that have revolutionized transdermal drug delivery by overcoming the limitations posed by the *stratum corneum* barrier. Composed of phospholipids and edge activators, such as surfactants or bile salts, transferosomes exhibit exceptional elasticity, enabling them to squeeze through pores much smaller than their own diameter while maintaining structural integrity.

Transferosomes are composed of four key elements: (i) phospholipids, such as phosphatidylcholine, dipalmitylphosphatidylcholine, lipoid S100 and distearylphosphatidylcholine; (ii) an edge activator such as a surfactant or bile salt, typically ranging from 10 to 25% (*e.g*., sodium cholate, sodium deoxycholate, Tween® 80, Span® 80); (iii) ethanol in a lower percentage, usually below 10%; and (iv) water as a vehicle.[9] The phospholipids act as membrane formers, and edge activators act as flexibility enhancers. Edge activators or bile salts make the transferosome vesicles ultra-deformable. The edge activators also serve as penetration enhancers.[10]

The edge activator plays a key role as it provides a high radius of curvature that can destabilize the lipid bilayer, increasing the deformability of the membrane. This self-optimizing deformability allows transferosomes to dehydrate and deform to cross through small gaps, while they can recover their shape by a rehydration process. Compared to conventional vesicular systems like liposomes, niosomes, and ethosomes, transferosomes demonstrate superior permeation, high drug entrapment efficiency, biocompatibility, and the capability to encapsulate both hydrophilic and lipophilic drugs.[9]

Transferosomes via the buccal route

Transferosomes are lipid-based vesicles that have gained significant attention in the field of drug delivery due to their unique structure and properties. The buccal cavity, also known as the oral cavity, is a complex anatomical structure that presents a promising route for drug delivery, particularly for systemic drug absorption.

Future directions in transferosome research may involve stimuli-responsive systems, buccal and intranasal delivery, and microneedle arrays or a personalized medicine model. Recent literature specifically identifies buccal transferosomes as a breakthrough approach, particularly for drugs requiring direct systemic entry, while avoiding gastrointestinal degradation.[11]

The ultra-deformable nature of transferosomes allows them to permeate the buccal mucosa through both transcellular and paracellular pathways. Their nano-size range (typically 100–300 nm), combined with the flexibility conferred by edge activators, allows intimate contact with the mucosal epithelium, facilitating enhanced permeation. When formulated as mucoadhesive gels, patches, or films, transferosomes prolong residence time in the buccal cavity, further improving drug absorption.[12]

The buccal mucosal route avoids hepatic first-pass metabolism, increases the resistance of drugs to gastrointestinal enzyme degradation, increases

the vascularity of the mucosa for fast systemic absorption and increases ease of access.[12] The buccal films are thin and flexible, in polymeric dosage form, which can adhere to the mucosa and release the drug in a controlled manner. They are accurate for dosage, have high patient compliance (particularly paediatric and geriatric patients), have a low choking risk and can be taste masked.[14]

EXPERIMENTAL

Materials

The drug was obtained from AARTI Pharma Labs as a gift sample. Lipoid S100 was sourced from GmbH (Germany). Tween 80, HPMC E5 and PEG 400 were procured from Molychem. Beta-cyclodextrin and ethanol were obtained from Research Lab. PVP K 30 was from a Molecular Biology Kit supplier. Citric acid was from Loba Chem Pvt. Ltd. All chemicals were of analytical reagent grade.

Preformulation studies

The preformulation study is one of the most important prerequisite steps in the development of any drug delivery system. It gives the information needed to define the nature of the drug substance, provides a framework for the drug combination, with pharmaceutical excipients in the dosage form. Hence, preformulation studies on the obtained sample of drug are required.

The drug confirmation test was carried out by UV spectroscopy, melting point, attenuated total reflectance infrared spectroscopy (ATRIR), thin layer chromatography (TLC), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies.[15]

UV spectroscopy

A double beam UV spectrophotometer was used to determine the lambda maximum of the drug. For this purpose, 10 mg of empagliflozin was dissolved in a 10 mL of methanol to prepare a 1000 µg/mL solution of the drug, marked as a standard solution. From this standard solution, 1 mL was taken in a beaker and diluted to 100 mL with the solvent (methanol) to prepare a solution containing 10 µg/mL of active drug. The final solution was evaluated by a UV scan between the wavelengths of 200 and 400 nm to determine the λ_{max} of drug.[16]

Attenuated total reflectance infrared spectroscopy

The IR spectrum of the drug was measured in the solid state. Pure empagliflozin powder was scanned using an ATRIR spectrometer-430 (Shimadzu 8400S, Japan).[17]

Differential scanning calorimetry

The melting point of empagliflozin was determined by using DSC (Mettler DSC1 star system, Mettler-Toledo, Switzerland). The pure drug was sealed in a perforated aluminium pan and heated at a constant rate of 10 °C/min with nitrogen purging at 50 mL/min over the temperature range of 30-300 °C.[18]

X-ray diffraction study

The X-ray pattern of empagliflozin was obtained using a diffractometer with a rotating objective X-ray tube (TrO) and a goniometer at wide angle. The X-ray tube was operated with a voltage of 50 kV and a current of 150 mA. The swing range (2) was 5° to 60°, with a scanning step of 0.02° at a rate of 4° per minute, in 0.1° increments.[19]

Standard calibration curve of the drug

For spectrophotometric analysis, the standard calibration curve of the drug was drawn by serial dilutions of the drug in methanol. Concentrations of 1, 2, 4, 6, up to 12 µg/mL were prepared starting from the standard solution (10 µg/mL) accordingly. The absorbance of every dilution was measured at 221 nm wavelength using a UV spectrophotometer, and a graph was plotted by taking concentrations at the X-axis and the corresponding absorbance on the Y-axis.[20]

Thin layer chromatography

Thin layer chromatography was performed to analyse the drug using a suitable solvent system. Pre-coated silica gel TLC plates were used as the stationary phase. The mobile phase consisted of a mixture of toluene: ethyl acetate: methanol (4:4:2 v/v/v). The solvent system was prepared and poured into a developing chamber, which was then allowed to saturate for about 15–20 minutes. A small amount of the sample solution was applied as a spot on the TLC plate using a capillary tube, approximately 1–2 cm above the base line. The plate was then placed carefully in the chamber, ensuring that the spot remained above the level of the mobile phase. The plate was allowed to develop until the solvent front travelled an appropriate distance. It was then removed, and the solvent front was immediately marked. The plate was dried and visualized under UV light.[21]

$$\text{Retention factor (Rf)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}} \quad (1)$$

Determination of partition coefficient

The partition coefficient was determined by dissolving 20 mg of the drug in separating funnels containing n-Octanol and PBS (pH 6.8). The separating funnels were shaken on a mechanical shaker for 24 hours. Two phases were separated and the aqueous phase was filtered through Whatman filter paper and the amount of the drug in aqueous phase was determined spectrophotometrically at 241 nm.[22]

Drug-excipient compatibility study

Drug excipient compatibility is the most important aspect as it helps to maximize the stability of a final drug

product because any physical or chemical interaction between a drug and excipient might alter the drug's properties, such as efficacy, toxicity and shelf-life. The compatibility between the physical mixture of the drug and excipients was determined by ATR-FTIR spectroscopy. Empagliflozin and excipients were firstly ground and the sample was placed on the surface of the crystal for ATR-FTIR analysis. Their physical mixture and the final formulation were also analysed.[23]

Formulation of empagliflozin transferosomes

Empagliflozin-loaded transferosomes were prepared by the ethanol injection method. Empagliflozin, soya phosphatidylcholine (S100), and the edge activator were dissolved in ethanol to form the organic phase, which was injected dropwise into phosphate buffer (pH 6.8) under continuous magnetic stirring (800–1000 rpm). The resulting vesicular dispersion was stirred for 45 min to ensure complete ethanol evaporation and vesicle stabilization, followed by probe sonication for 4 min to reduce vesicle size. The formulation was then ultracentrifuged (15,000 rpm, 30 min, 4 °C) to separate the unentrapped drug. Finally, the transferosomal dispersion was freeze-dried in the presence of a cryoprotectant to obtain stable, free-flowing empagliflozin-loaded transferosomes for further characterization and incorporation into buccal films.[24]

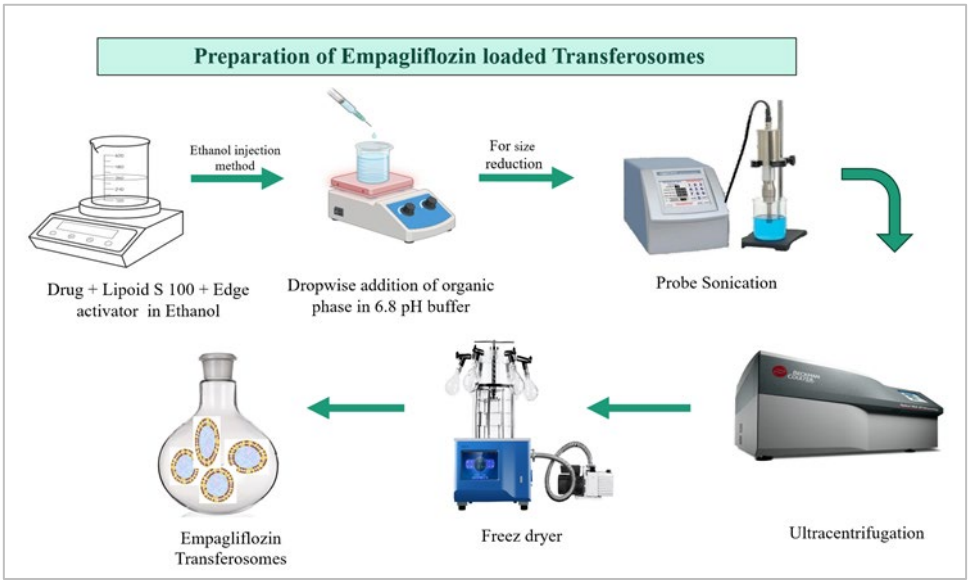


Figure 1: Preparation of transferosomes using ethanol injection

Optimization of empagliflozin transferosomes

Empagliflozin-loaded transferosomes were optimized using a Central Composite Design (CCD), with lipid concentration (100–400 mg) and surfactant concentration (20–40% w/w) selected as the independent variables based on preliminary studies.[25]

Characterization of optimized empagliflozin transferosomes

Vesicle size and polydispersity index (PDI)

The average vesicle size and PDI of the optimized transferosomal formulation were determined using Dynamic Light Scattering (DLS). Vesicle size influences drug permeation through biological membranes, while PDI indicates the homogeneity of the vesicle population. A PDI value below 0.3 suggests a uniform size distribution.[26]

Zeta potential (ZP)

A Zetasizer was used to measure the zeta potential (ZP) of the transferosomes. The stability of the colloidal system in terms of particle size was evaluated based on zeta potential values and was established based on the dynamic light scattering technique.[26]

Entrapment efficiency (EE)

Entrapment efficiency was determined by separating the unentrapped drug through centrifugation and quantifying the drug content using UV-Visible spectrophotometry. High entrapment efficiency indicates effective incorporation of empagliflozin within the transferosomal vesicles. % EE was calculated by using following equation:[27]

% Entrapment efficiency

$$= \frac{\text{Total amount of drug - Amount of drug in supernatant}}{\text{Total amount of drug}} \times 100 \qquad (2)$$

Fourier transform infrared spectroscopy (FTIR)

FTIR studies were conducted to investigate possible interactions between empagliflozin and formulation

excipients. The retention of characteristic peaks of the drug in the formulation confirms compatibility.[28]

X-ray diffraction study (XRD)

XRD studies were carried out to assess the crystalline nature of the drug before and after encapsulation. A reduction in peak intensity indicates conversion of the drug from a crystalline to an amorphous state within the vesicles.[29]

Differential scanning calorimetry (DSC)

DSC analysis was performed to evaluate the thermal behaviours of the drug and the formulation. The disappearance or reduction in intensity of the characteristic melting peak of empagliflozin suggests successful entrapment within the lipid bilayer.[30]

Scanning electron microscopy (SEM)

The morphology of the formulation was analysed using scanning electron microscopy (SEM) (Zeiss Gemini SEM 500, Germany). The samples were placed on an aluminium stub and coated with a thin layer of gold-palladium using an Auto Fine Coater, achieving a coating thickness of 400 Å. Images were captured from randomly selected areas and examined using a scanning electron microscope operated at an acceleration voltage of 15 kV.[29]

Stability studies

The stability study of the optimized transferosomal formulation was performed as per ICH Q1A(R2) guidelines. The formulation was stored under specified conditions (4 °C ± 3 °C, and 25 °C ± 2 °C / 60% RH ± 5% RH) for an accelerated stability study. Samples were withdrawn initially, and after the 1st, 2nd, and 3rd month, and evaluated for particle size, PDI and zeta potential using the Zetasizer (Malvern Instruments, UK).[31]

Formulation of Emp transferosomal buccal film

Preparation of casting solution

Buccal films were prepared by the solvent casting method using HPMC and PVP K-30 as film-forming polymers, and PEG 400 as a plasticizer. A 5% polymeric solution was prepared by dissolving HPMC and PVP K-30 in distilled water under continuous magnetic stirring until complete swelling was achieved. PEG 400 was then added, followed by 1% Tween 80 solution and mixed uniformly. Transferosomal powder equivalent to 138 mg of empagliflozin and β-cyclodextrin (β-CD) as a permeation enhancer were incorporated into the polymeric solution and stirred for 45 min to obtain a homogeneous dispersion.

The resulting dispersion was poured into glycerine-lubricated Petri dishes and dried at room temperature under an inverted funnel to ensure controlled solvent evaporation. After drying, the films were carefully peeled off and stored for further evaluation. Plain empagliflozin buccal films were prepared using the same procedure for comparative studies.[32,33]

Evaluations of empagliflozin transferosomal buccal film

Physical appearance

The appearance of a buccal film is an important physical characteristic that reflects its quality, acceptability, and suitability for mucosal drug delivery**.** All the prepared patches were visually inspected for colour, clarity, entrapment of any air bubble, flexibility and smoothness.[34]

Thickness

The thickness of the empagliflozin transferosomal buccal film was determined using a digital vernier calliper. The thickness of patches was measured at five different places and the average values were reported.[35]

Weight variation

Weight uniformity testing was carried out by individually weighing several film samples to confirm consistency across a batch. Minimal variation in weight indicates that the drug and excipients have been evenly distributed during preparation, which is essential for achieving reproducible dosing.[26]

Folding endurance

Folding endurance was determined by repeatedly folding a small strip of film (approximately 2 x 2 cm) at the same place until it broke. The number of times patches could be folded at the same place, without breaking, gave the value of folding endurance and it was recorded.[33]

Moisture content

The prepared transferosomal buccal films were weighed individually and kept in desiccators containing fused calcium chloride at room temperature for the duration of 24 hours. After 24 hours, the films were re-weighed,[8] and the percentage moisture content was determined by the given formula:

$$\%\ moisture\ content\ = initial\ weight - final\ weight\ /\ initial\ weight\ \times 100 \quad (3)$$

Drug content

A 2 × 2 cm² buccal film was dissolved in 100 mL phosphate buffer (pH 6.8) and stirred until complete dissolution. The solution was filtered, suitably diluted, and analysed using a UV-Visible spectrophotometer at 221 nm. The drug content was determined from the calibration curve of empagliflozin and expressed as percentage drug content.[36]

$$\%Drug\ Content\ = \frac{Actual\ Drug\ Content}{TheoreticalDrug\ Content} \times 100 \quad (4)$$

Swelling index

The initial weight of the film was determined using a digital balance (W0). Then, the films were allowed to

swell on the surface of a Petri plate and kept in an incubator maintained at 37 °C. The weight of the swollen film was determined (Wt) at predetermined time intervals of 5 min. The percentage of swelling (% S) was calculated using the following equation:[37]

$$\% S = (Wt - Wo)/Wo \times 100 \quad (5)$$

where Wt is the weight of swollen patch after time t, W0 is the initial weight of patch at t=0.

Surface pH

The compatibility in the pH of the oral preparation, especially for buccal drug delivery, is of great concern. It directly influences patient compliance, so the pH of the prepared films should be comparable to that of the buccal cavity. The film was introduced into 5 mL of DW for 15 min and then pH was determined using a pH meter.[38]

Mucoadhesive strength

Mucoadhesive strength was measured using a Texture Analyzer (Brookfield Engineering Laboratories, Inc., Ametek Brookfield, USA). A 2 × 2 cm² buccal film was brought into contact with freshly excised goat buccal mucosa for a fixed time, and the maximum detachment force required to separate the film from the mucosal surface was recorded as the mucoadhesive strength.[39] The corresponding formula was used to compute the mucoadhesive strength:

$$Mucoadhesive\ strength\ (dyne/cm^2) = M \times G \times 100/A \quad (6)$$

where M is the weight in grams required for detachment, G is the acceleration due to gravity, A is the area of buccal mucosa exposed (cm²)

Tensile strength

The tensile strength of the buccal film was determined using a Texture Analyzer (Brookfield Engineering Laboratories, Inc., Ametek Brookfield, USA). Film specimens of appropriate dimensions were cut and fixed between two grips of the instrument. The initial distance between the grips was maintained at a predetermined length. The upper grip was moved at a constant speed until the film broke. The maximum force required to break the film was recorded automatically by the instrument.[32] Tensile strength was calculated by dividing the breaking force by the cross-sectional area of the film:

$$Tensile\ Strength\ (N/mm^2) = Breaking\ force\ (N)/ Cross\ sectional\ area\ of\ sample\ (mm^2) \quad (7)$$

In-vitro *disintegration time*

In-vitro disintegration time (DT) was observed to determine the disintegration of the film in the oral cavity. It is defined as the time required by the film to disintegrate when it comes in contact with saliva. To evaluate DT, the films were cut to a size of 2 x 2 cm^2 and placed on a Petri plate having 10 mL of distilled water. The time was noted on an electronic time counter as disintegration time when the film started to break from the edge or in the centre.[40]

In-vitro *drug release study*

In-vitro drug release studies were performed using a Franz diffusion cell. The studies were performed to determine the release of the drug from the empagliflozin transferosomal buccal film. The receptor compartment of the diffusion cell was filled with phosphate buffer (pH 6.8). The whole assembly was placed on the magnetic stirrer and continuously stirred. The samples (2.0 mL aliquots) were withdrawn at 0, 2, 5, 10, 15, 20, 30 min, up to 60 min, and the same quantity was replaced with phosphate buffer, and analysed for drug content spectrophotometrically at 241 nm using a UV spectrophotometer (Shimadzu 1900, Japan). The same study was performed by incorporating plain empagliflozin into the buccal film prepared by the abovesaid method, and the results were compared.[41]

To determine the mechanism of drug release from the film, the findings of the *in-vitro* dissolution study were fitted into the following kinetic equations:

a) Zero order drug release: cumulative % drug release vs time;
b) First order drug release: log cumulative % drug retained vs time;
c) Higuchi's classical diffusion equation: cumulative % drug release vs square root of time;
d) Hixson-Crowell cube root law: cube root of cumulative % drug retained vs time;
e) Korsmeyer-Peppas model: cumulative % drug release vs log time.

Ex-vivo *drug release study*

An *ex-vivo* study of drug penetration was conducted on the plain drug film and the transferosomal film. A fresh buccal membrane of goat was obtained from the slaughterhouse. It was stored in 0.9% (w/v) saline until further use. The buccal membrane was fixed between the donor and recipient sections. A buccal film containing transferosomes of empagliflozin containing 10 mg of the drug was placed in the donor chamber. The medium of the receiver chamber was 10 mL of phosphate buffer of pH 6.8. The diffusion chamber was at 37 ± 0.5 °C and stirred at 50 rpm using a magnetic stirrer. At predetermined time intervals of 0, 2, 5, 10, 15, 20, 30 min, up to 60 min, 2 mL of the receiver medium was sampled and then replaced with 2 mL of fresh phosphate buffer, pH 6.8, to maintain the condition. Aliquots were filtered through a 0.45 µm pore filter and the total amount released was measured UV spectrophotometrically at 241 nm. A curve of percent drug release versus time was plotted. The same procedure was applied in the plain film diffusion study of the drug and the permeation study of the film containing the drug and the formulation was compared.[41]

***In-vivo* pharmacokinetic study**

Two types of buccal film formulation, namely the plain drug mucoadhesive film and the transferosomes loaded mucoadhesive film, were compared in an *in-vivo* pharmacokinetic study for their absorption and bioavailability of empagliflozin. The study was approved by the IAEC of R. C. Patel Institute of Pharmaceutical Education and Research, and was conducted as per the guidelines of CPCSEA (Registration No.: IAEC/RCPIPER/2025-26/54) during March 2026.

12 healthy male Sprague Dawley rats (220–250 g, 10–12 weeks old) were acclimatized under standard laboratory conditions and then randomly split into two groups (n = 6). The plain drug buccal film was given to group 1, and the buccal film with drug loaded transferosomes to group 2. The rats were dosed with 0.5 × 0.5 cm buccal film of 1 mg of empagliflozin per rat per day. Anaesthetics were given to rats before administration, to ensure appropriate placement of the films in the buccal cavity. Blood samples were taken at certain times up to 12 h after dosing from the tail vein.[42,43]

Empagliflozin concentration in the plasma was determined by protein precipitation with acetonitrile, followed by HPLC/LC-MS/MS analysis, which was validated. The plasma was centrifuged, stored at −20 °C, and subsequently analysed for empagliflozin concentration. Non-compartment analysis was used to calculate the following pharmacokinetic parameters: C_{max}, T_{max}, AUC, elimination half-life, elimination rate constant, and relative bioavailability. The results were presented as mean ± SD, and statistical analysis used SPSS version 25 with significance level < 0.05.

ATR IR studies

ATR-FTIR analysis of the optimized buccal film was performed using an ATR-FTIR spectrophotometer (Perkin Elmer, USA). Samples were directly placed on the ATR crystal and scanned over the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were analysed for any interaction or shifting of characteristic peaks between the drug and the excipients.[44]



Figure 2: Application of buccal film

XRD study

XRD analysis was performed using an X-ray diffractometer (Bruker) with CuKα radiation (λ = 1.54 Å) at 40 kV and 40 mA. Samples were scanned over a 2θ range of 5°–50° at a scanning rate of 2°/min. The diffractograms of the film were compared to assess the crystalline or amorphous nature of the drug within the film matrix.[44]

DSC analysis

DSC analysis was carried out using a DSC instrument from Mettler Toledo Instruments. The optimized film was crimped in aluminium pans and heated at a rate of 10 °C/min over a temperature range of 25 °C to 300 °C under nitrogen atmosphere. The thermograms were analysed for melting point, glass transition temperature, and any drug-excipient interactions.[45]

Stability studies

An optimized empagliflozin transferosomal buccal film (B3) was selected for stability studies. The optimized patches were placed at different temperatures, *i.e.*, 4±2 ℃ (refrigerator), 27 ±2 ℃ (room temperature) and 45±2 ℃ (stability chamber) for a period of three months. Physical appearance, moisture content, and drug content were examined each month.[46]

RESULTS AND DISCUSSION

Preformulation studies: drug confirmation and validation

Drug test identification was done based on organoleptic characteristics, such as colour, smell, appearance and further confirmation by melting point determination, infrared spectroscopy, UV spectrophotometry, which confirmed that the drug

is empagliflozin. Drug validation was also performed by XRD and DSC.

UV spectroscopy

The UV spectrum of empagliflozin was taken in the range of 200-400 nm by dissolving 10 mg of the drug in 10 mL of methanol. It showed maximum absorption at wavelength 221 nm in methanol and 241 nm in phosphate buffer pH 6.8, respectively, which was matched with reported values in the literature, and hence, the drug was confirmed.

Melting point

The melting point of the drug was determined by the capillary tube method. It was found to be in the range of 150-153 ℃. The determined melting point was matched with reported values in the literature. The melting point was also confirmed with DSC, which indicated the melting point with a sharp peak at 152.43 ℃.

DSC of empagliflozin

The DSC thermogram of pure empagliflozin showed a sharp endothermic melting peak at 150.35 °C, with an onset at 148.55 °C and an endset at 152.43 °C. The enthalpy of fusion was 86.55 J g^{-1}. The sharp melting peak confirms the crystalline nature and purity of empagliflozin, with no evidence of polymorphic transitions or decomposition prior to melting. This thermal behaviour is consistent with reported literature values for empagliflozin.

ATR-IR spectroscopy of empagliflozin

The ATR-IR spectrum of empagliflozin was recorded in the solid state. The FTIR spectrum showed characteristic absorption bands at 3423–3248 cm^{-1} (O–H stretching), 2928–2867 cm^{-1} (C–H stretching), 1670 cm^{-1} (aromatic C=C stretching), 1610–1490 cm^{-1} (aromatic skeletal vibrations), and 1275–1005 cm^{-1} (C–O–C/C–O stretching). These characteristic peaks confirm the chemical identity of empagliflozin and indicate the absence of any structural degradation. The ATR-IR spectrum of pure empagliflozin shows the functional group ranges typical of empagliflozin, similar to those of previously published literature reports, thus indicating that the drug was pure.

X-ray diffraction of empagliflozin

The PXRD pattern of pure empagliflozin showed several sharp and intense diffraction peaks, confirming its highly crystalline nature. The presence of distinct characteristic peaks indicates a well-ordered crystal structure, while the absence of a broad halo suggests that the drug exists predominantly in a crystalline form. These results are consistent with the reported crystalline characteristics of pure empagliflozin.

Standard calibration curve in methanol

The solution was scanned at 400 nm to 200 nm; an absorbance maximum was observed at 221 nm. The standard calibration curve of empagliflozin was plotted in methanol. Plotting the absorbance vs. concentration graph revealed that it was linear between 2 and 12 µg/mL. The graph followed Beer's and Lambert's laws, displaying an excellent concentration-dependent behaviour, with an R2 value of 0.9999.

Thin layer chromatography

The identification of empagliflozin was done by using thin layer chromatography, and the Rf value was calculated as 0.56. The recorded Rf value of empagliflozin matched the one reported in the literature, *i.e*., 0.59.

Determination of partition coefficient

A log P value of 1.9 indicates that the drug is moderately lipophilic (moderately soluble in lipids compared to water). This suggests that the drug has a balanced affinity for both aqueous and lipid environments.

Drug-excipient compatibility study

The FTIR spectra of pure empagliflozin, excipients, physical mixture, and the optimized transferosomes were compared to evaluate drug–excipient compatibility. The characteristic peaks of empagliflozin, including O–H stretching, C–H stretching, C=O stretching, and C–O stretching vibrations, were retained in the physical mixture and formulated film with only slight shifts in peak positions and intensity. The slight reduction in peak intensity and broadening observed in the optimized formulation are commonly associated with successful incorporation of the drug into the phospholipid matrix rather than chemical modification. Such spectral changes indicate molecular dispersion of empagliflozin within the lipid bilayer of Lipoid S100, while the preservation of the major functional group peaks confirms that the drug maintained its chemical integrity. Therefore, FTIR analysis supports the successful

encapsulation of empagliflozin in the lipid carrier, without any significant drug-excipient incompatibility.

Formulation and optimization of Emp transferosomes

Analysis of effect of factors on %EE

The quadratic model was found to be statistically significant ($F = 26.86$, $p < 0.0002$), indicating that the observed response was not due to random variation. Model terms A (phospholipid concentration), AB (interaction), A^2, and B^2 significantly influenced entrapment efficiency ($p < 0.05$). The lack-of-fit was non-significant ($p = 0.1394$), confirming a good fit of the model to the experimental data. The model also demonstrated good predictive ability, with a Predicted R^2 (0.7268) in reasonable agreement with the Adjusted R^2 (0.9151) and an Adeq. Precision of 15.926, indicating an adequate signal for design space navigation. The entrapment efficiency of the formulations ranged from 66.00% to 87.49%. The polynomial equation obtained for entrapment efficiency was:

$$Y_1 = 84.00 + 6.23A - 0.545B + 4.41AB - 2.05A^2 - 6.81B^2 \quad (8)$$

The results indicated that increasing the phospholipid concentration (X_1) significantly improved entrapment efficiency, whereas the effect of edge activator concentration (X_2) was comparatively less pronounced. The positive interaction between the two variables further contributed to enhanced drug entrapment, while the negative quadratic terms suggested that excessively high concentrations of either factor reduced the entrapment efficiency.

Table 1
Experimental runs, factors and measured responses of EMP transferosomes

Formulation code	Factor		Response		
	Conc. of lipid (mg)	Conc. of surfactatnt (mg)	Entrapment effciency	Particle size	Zeta potential
F1	100	20	77	190.4	-14.4
F2	37.868	30	69	221.6	-19
F3	250	44.1421	70	125.1	-17.5
F4	**250**	**30**	**86**	**119**	**-28.3**
F5	400	20	80	110.7	-18
F6	250	15.8579	70	120	-20.5
F7	100	40	66	117.95	-18.5
F8	400	40	86.64	234.8	-23.1
F9	462.132	30	87.499	256	-17.7

Analysis of effect of factors on mean particle size (MPS)

The quadratic model for particle size (Y_2) was found to be statistically significant ($F = 46.64$, $p < 0.0001$), indicating that the model adequately described the experimental data. The model terms A (phospholipid concentration), AB (interaction), and A^2 significantly influenced particle size ($p < 0.05$), whereas the lack-of-fit was non-significant ($p = 0.1023$), confirming the suitability of the model. The Predicted R^2 (0.8323) was in good agreement with the Adjusted R^2 (0.9500), and the Adeq. Precision value of 16.413 indicated an adequate signal for design space navigation. The particle size of the formulations ranged from 110.7 to 256 nm. The polynomial equation obtained for particle size was:

$$Y_2 = 106.56 + 10.72A + 7.36B + 49.14AB + 61.82A^2 + 3.69B^2 \quad (9)$$

The results demonstrated that phospholipid concentration (X_1) had a significant positive effect on particle size, with increasing phospholipid levels leading to the formation of larger vesicles. In contrast, increasing the edge activator concentration (X_2) reduced particle size due to enhanced membrane fluidity and vesicle flexibility. The significant interaction (AB) and quadratic (A^2) terms further indicate that an optimum combination of phospholipid and edge activator is essential for obtaining transferosomes with the desired nanoscale particle size.

Analysis of effect of factors on zeta potential

The quadratic model for zeta potential (Y_3) was statistically significant ($F = 4.32$, $p = 0.0412$), indicating that the selected model adequately explained the experimental response. Among the model terms, only the quadratic effects (A^2 and B^2)

were significant ($p < 0.05$), while the lack-of-fit was non-significant ($p = 0.1108$), confirming that the model fitted the experimental data well. Although the Predicted R^2 was negative, indicating limited predictive capability, the Adeq. Precision value of 4.599 exceeded the desirable value of 4, suggesting an adequate signal for design space navigation. The zeta potential of the formulations ranged from −28.3 to −14.4 mV. The polynomial equation for zeta potential was:

$$Y_3 = -25.26 - 0.7952A - 0.6197B - 0.2500AB + 3.50A^2 + 3.17B^2 \quad (10)$$

The results indicated that increasing the concentrations of phospholipid (X_1) and edge activator (X_2) initially produced more negative zeta potential values, which favored the electrostatic stability of the transferosomal dispersion. However, the positive quadratic terms (A^2 and B^2) suggested that further increases in these variables shifted the zeta potential toward less negative values, indicating the existence of an optimum concentration range for achieving maximum formulation stability.

Selection of optimized batch

Optimization of Emp loaded transferosomal formulation was achieved by fine tuning key parameters, notably the phospholipid and the edge activator. These factors played a crucial role in maximizing drug encapsulation efficiency and ensuring uniform distribution.

9 runs were produced by Central Composite Design (Design Expert 13.0.5). F4 was selected as the optimized batch, with the desirability value 0.908, with MPS 119 nm, %EE 86% and ZP -28.3. The optimized batch was subjected for further characterizations and was incorporated into the buccal film.

Characterization of optimized empagliflozin transferosomes (F4)

Mean particle size (MPS)

The MPS of optimized empagliflozin transferosomes (F4) was found to be 119 nm, which is ideal for transferosomes to cross the buccal membrane.

Zeta potential

The zeta potential of optimized empagliflozin transferosomes (F4), as shown in Table 2, was found to be -28.3, which is within the ideal range of -30 to +30. Thus, the formulation can be assumed as stable, and the transferosomes will be assumed to possess good shelf-life.

Entrapment efficiency (%EE)

%EE of the optimized empagliflozin transferosomes (F4) was found to be 86%. It showed that the method used to prepare EMP transferosomes was effective, as well as reproducible.

ATR-IR studies

The observed peaks in transferosomes indicate the presence of phospholipids, surfactants, and the drug encapsulated within the vesicular system. These characteristic peaks confirm the structural integrity and successful incorporation of empagliflozin (EMP) into the transferosomal formulation. In the ATR-IR spectrum of EMP transferosomes, the peak observed at 2925.71 cm^{-1} represented secondary amine and aliphatic C–H stretching vibrations, appearing as weak to moderate absorption bands. The broad tongue-shaped peak obtained at 3282.38 cm^{-1} indicated the presence of the hydroxyl (-OH) group, confirming hydrogen bonding interactions within the formulation. The characteristic aryl alkyl ether stretching band appeared at 1044.92 cm^{-1}, which showed strong C–O stretching vibrations in the fingerprint region. The absorption peak observed at 1457.33 cm^{-1} corresponded to C=C stretching vibrations, indicating the presence of aromatic groups in the structure of empagliflozin. The peak at 1731.97 cm^{-1} represented carbonyl (C=O) stretching, mainly attributed to phospholipid ester groups present in the transferosomal vesicles.

The ATR-IR spectra of pure EMP and optimized EMP transferosomes were compared. All the characteristic peaks of EMP were retained in the transferosomal formulation within their standard functional group ranges. No significant shifting or disappearance of characteristic peaks was observed in the optimized transferosome spectrum. These findings confirmed that no chemical interaction occurred between EMP and the excipients used in the formulation, indicating that EMP remained stable and retained its original chemical structure within the optimized transferosomal system.

XRD studies

The X-ray diffraction (XRD) pattern of lyophilized transferosomes exhibited several diffraction peaks with moderate to high intensities over the 2θ range of 5°–60°, indicating the presence of both crystalline and amorphous characteristics in the formulation. The

diffractogram showed prominent peaks mainly around 12°–14°, 19°–25°, 31°–36°, and 38°–42° 2θ regions, with the highest intensity peak observed near 25° 2θ. Compared with the pure drug, the peaks in the lyophilized transferosomal formulation appeared broadened and reduced in intensity, suggesting a decrease in crystallinity due to encapsulation of the drug within the vesicular system. The presence of broad halos along with reduced sharpness of peaks indicated partial conversion of the drug from crystalline to amorphous form during formulation and lyophilization. Hence, the XRD study confirmed successful incorporation of the drug into the transferosomal matrix, with reduced crystallinity and enhanced amorphous nature.

DSC studies

Differential scanning calorimetry (DSC) is a crucial analytical technique used to study the thermal properties of transferosome formulations. The DSC spectrum typically shows endothermic and exothermic peaks. Endothermic peaks indicate melting points of the lipids used in the formulation, while exothermic peaks can indicate crystallization or other thermal transitions. The type of lipids used in the transferosome formulation significantly affects the DSC profile. For instance, the presence of certain phospholipids can lead to distinct melting temperatures, which can be observed as sharp peaks in the DSC spectrum.

SEM studies

Scanning electron microscopy (SEM) studies confirmed the formation of spherical and uniformly distributed transferosomal vesicles, with smooth surface morphology. SEM was also used to measure the apparent size of dried particles. The sample preparation and drying procedure may induce partial aggregation, resulting in a larger apparent particle size. The vesicle size was found to be in the nanometric range (approximately 180–200 nm), indicating successful preparation of transferosomes. The SEM results suggest good structural integrity and support the potential application of the developed transferosomal formulation for efficient transdermal drug delivery.

Stability studies

The data regarding particle size and zeta potential, acquired for formulation F4 initially and over a three month period, at one month intervals, were tabulated in Table 2.

Table 2
Stability study of transferosomes (F4)

Sr No	Month	Particle size (nm)	Zeta potential (mV)
1	Initial	119	-28.3
2	1st Month	121.2	-27.8
3	2nd Month	129.2	-27.1
4	3rd Month	135.9	-25.4

Formulation and evaluation of optimized Emp transferosomal buccal film

Physical appearance, thickness and weight

The formulated film was found to be whitish, clear, smooth, uniform, flexible, and free from entrapped air bubbles. The thickness of the prepared film varied between 0.17 to 0.27 mm. The thickness of the optimized batch was found to be 0.23 mm.

The weight variation study showed that the films possessed good uniformity, with an average weight of 0.067 ± 0.003 g (mean ± SD, n = 3). The low standard deviation indicates uniform distribution of the formulation components and consistency in the film casting process.

Folding endurance

The prepared patches showed folding endurance ranging from 98 to 127 times, which was satisfactory for a buccal film. Sample batches B3, B4 and B5 have a greater concentration of hydrophilic polymer, which resulted in an increase in the folding endurance of 127 and 118, respectively. However, B1, and B2 have a lesser concentration of hydrophilic polymer, which resulted in a decrease in folding endurance. The increased concentration of the hydrophilic polymer in the formulation greatly affected the folding endurance, as it provided better elasticity.

Moisture content

The % moisture content in the prepared patches was found between 4.46% and 7.45%. The concentration of hydrophobic polymer was increased in B1, B2, and B3, which resulted in a decrease in moisture content. B4 and B5 had greater concentration of hydrophilic polymer, which resulted in an increase in moisture content. The high ratios of hydrophilic polymer resulted in increased % moisture content. Small moisture content in the formulation helps the patches to remain stable and prevents them from being completely dried and brittle.

Table 3
Experimental runs, factors and measured responses of EMP transferosomal buccal film

Batch	HPMC E-5 (mg)	PVP K 30 (mg)	PEG-400 (mL)	Drug content (%)	Moisture content (%)	Thickness (cm)	Folding endurance
B1	150	20	0.25	91.24 ± 0.62	6.82 ± 0.18	0.017 ± 0.001	102 ± 3
B2	175	25	1	93.58 ± 0.74	7.45 ± 0.22	0.019 ± 0.001	98 ± 4
B3	**200**	**30**	**0.5**	**97.67 ± 0.45**	**4.46 ± 0.14**	**0.023 ± 0.001**	**127 ± 3**
B4	225	35	0.3	95.12 ± 0.58	5.34 ± 0.16	0.025 ± 0.001	127 ± 3
B5	250	30	0.6	94.36 ± 0.66	5.91 ± 0.20	0.027 ± 0.002	8 ± 4

Drug content

The % drug content of all the batches was found to be 91.24% to 97.67%. The result showed that the method employed to prepare the film allowed producing films with almost uniform drug distribution.

Selection of optimized EMP transferosomal buccal film

Varying concentrations of HPMC did not show a significant effect on drug content and film thickness. Small moisture content in the formulation helps the film to remain stable and prevents it from becoming completely dry and brittle. The folding endurance was satisfactory in all the batches. Still, batch B3 was found to contain the highest % drug content (97.67%) and the lowest moisture content (4.46%), among all the batches, along with satisfactory thickness (0.23 mm) and folding endurance (127). Thus, batch B3 was selected as an optimized batch to prepare the EMP transferosomal buccal film, which was subjected to further characterisation, as well as *in-vitro* and *in-vivo* drug release studies.

Swelling index

The swelling index of the optimized formulation was found to be 6.82 ± 0.67, indicating satisfactory swelling behaviour and hydration capacity of the film formulation.

Surface pH

The surface pH of optimized buccal film (B3) was found to be 7.2, which is close to the physiological pH of the buccal cavity.

Mucoadhesive strength

The mucoadhesive properties of the prepared transferosomal buccal film (TFA) were evaluated using a Brookfield Texture Analyzer. The force-time profile demonstrated good adhesion characteristics with distinct peaks during probe attachment and detachment, indicating effective interaction between the film and mucosal surface. The graph showed a sharp peak corresponding to maximum detachment force, followed by a rapid decline, confirming acceptable mucoadhesive behaviour.

The optimized transferosomal buccal film exhibited a mucoadhesive force of 12 g (0.118 N), indicating good adhesion to the buccal mucosa. This value falls within the commonly reported acceptable range (10–20 g) for mucoadhesive buccal films, suggesting adequate residence time, without causing excessive discomfort during removal. The favourable mucoadhesive behaviour can be attributed to the hydrophilic polymers in the film matrix, which form hydrogen bonds with mucin and promote intimate contact with the buccal mucosal surface.

Tensile strength

The tensile strength of the optimized buccal film was determined using a Texture Analyzer. The instrument recorded a maximum breaking load of 249 g (2.44 N) at the point of film rupture. Based on the film dimensions (width = 10 mm and thickness = 0.23 mm), the cross-sectional area was calculated to be 2.3 mm². Accordingly, the tensile strength of the film was 1.06 N/mm², indicating

adequate mechanical strength to withstand handling and application without tearing.

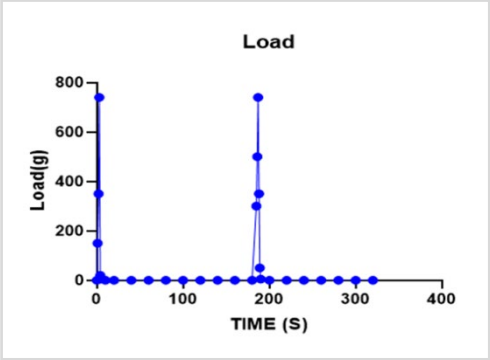


Figure 3: Mucoadhesive strength of buccal film

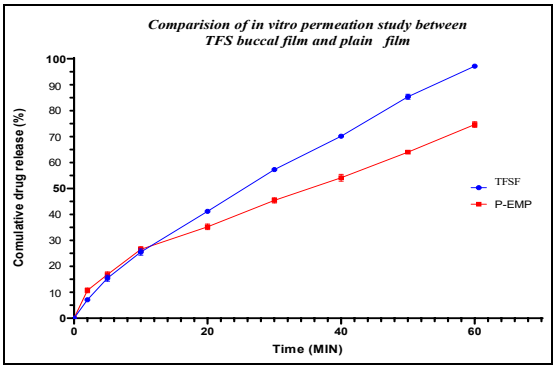


Figure 4: Comparison of *in-vitro* drug permeation of transferosomal EMP buccal film and plain EMP buccal film

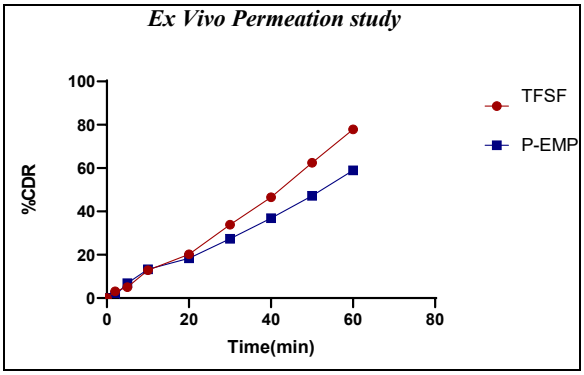


Figure 5: Comparison of *ex-vivo* diffusion study between transferosomal buccal film and plain EMP buccal film

In-vitro disintegration time

The disintegration time of the optimized mucoadhesive buccal film was found to be 3.2 ± 0.4 minutes, indicating rapid disintegration, along with adequate mucoadhesive properties, which are suitable for immediate drug release and enhanced patient compliance.

In-vitro drug release study

The *in-vitro* release profile is an important tool that predicts how a drug will behave *in vivo*. Release studies are required for predicting the rate of reproducibility and the duration of drug action. The drug release studies were performed for 60 min. The cumulative percentage of the drug released (%CDR) in 60 min for the plain EMP buccal film was found to be 74.64%, while the EMP loaded transferosomal buccal film released up to 97.85% of the drug. Thus, from the *in-vitro* drug release profile, it can be concluded that the % drug release increased due to the incorporation of EMP into transferosomes, instead of using plain EMP. Hence, transferosomes are more promising than the plain buccal film for EMP release system.

The *in-vitro* release data of all formulations was analysed by various kinetic models: the zero-order, first-order, Higuchi model, Hixson-Crowell cube root law, and Korsmeyer-Peppas model, and the R^2 value was determined for each of them. The highest correlation coefficient (R^2) value, which was derived for the respective kinetic model, indicated the pattern of drug release. The R^2 value of the zero-order model is nearer to 1, *i.e.* 0.9886, than the R^2 values of the other kinetic models. Thus, the drug release is considered to follow zero-order kinetics, which states that the drug release rate from the formulation is independent of the concentration of the drug. It shows ideal behaviour for a dosage form and leads to minimum fluctuations in drug plasma levels.

***Ex-vivo* drug release study**

The *ex-vivo* diffusion study was performed to compare the drug permeation of empagliflozin transferosomal buccal film (TFS film) and plain buccal film across the buccal membrane over a period of 60 minutes. The cumulative drug release (%CDR) was calculated at time intervals of 2, 5, 10, 20, 30, 40, 50, and 60 minutes. The results showed that the TFS film exhibited significantly higher drug permeation compared to the plain film throughout the study period. At the end of 60 minutes, the TFS film showed a %CDR of 77.85%, whereas the plain film showed only 58.92%, indicating an enhancement of approximately 18.93% in drug permeation. This superior permeation of the transferosomal formulation can be attributed to the vesicular carrier system of transferosomes, which enhances membrane fluidity and facilitates drug transport across the buccal mucosa due to their deformable and elastic nature.

HPLC analysis of plasma sample

Plasma samples were appropriately diluted prior to HPLC analysis, and the resulting concentrations of the diluted samples were within the validated calibration range. The linear regression data for the calibration curve shown in Figure 7 exhibited a good linear relationship over the concentration range of 2-12 ng/mL, with a correlation coefficient R2 = 0.9991.

***In-vivo* pharmacokinetic study**

The plasma concentration–time profile showed that the transferosome-loaded buccal film exhibited improved pharmacokinetic performance, compared to the plain buccal film containing empagliflozin.

The transferosomal formulation showed a higher C_{max} (0.93 µg/mL) compared to the plain film (0.56 µg/mL), indicating enhanced buccal absorption and improved bioavailability. Both formulations exhibited a T_{max} of 2 h, confirming rapid drug release and immediate release characteristics. Higher AUC and AUMC values observed for the transferosomal film further indicated greater systemic exposure and improved drug absorption, while similar MRT values suggested that the enhancement was mainly due to improved permeation rather than prolonged drug retention.

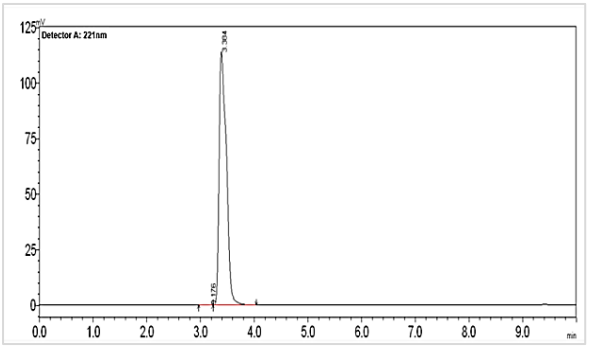


Figure 6: HPLC chromatogram of empagliflozin (3.3 min) in plasma

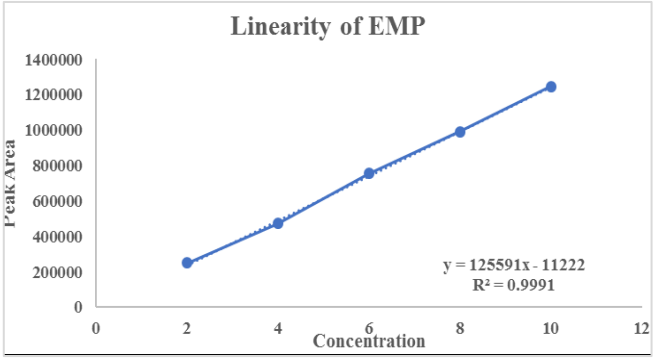


Figure 7: Standard calibration curve of EMP in plasma

Table 4
Comparative pharmacokinetic parameters of plain buccal film and transferosomal film containing EMP

Sr. No	Parameter	Plain film	Transferosomal film
1	T_{max} (h)	2	2
2	C_{max} (µg/mL)	0.56	0.93
3	AUC_{0-8} (µg·h/mL)	3.212	5.352
4	$AUC_{0-\infty}$ (µg·h/mL)	5.519	8.706
5	$AUMC_{0-8}$ (µg·h^2/mL)	11.554	19.0472
6	MRT (h)	3.6	3.56

Characterisation of optimized EMP transferosomal buccal film by ATR-IR, XRD and DSC

In the ATR-IR spectrum of the EMP transferosomal buccal film (Fig. 8), the broad peak at 3435.96 cm^{-1} confirmed the presence of the hydroxyl (-OH) group. The peaks at 3006.61 cm^{-1} and 2893.89 cm^{-1} represented C–H stretching vibrations of aliphatic chains. The peak at 1733.37 cm^{-1} indicated carbonyl (C=O) stretching. The absorption band at 1453.73 cm^{-1} corresponded to aromatic C=C stretching, while the peaks at 1243.75 cm^{-1} and 1091.35 cm^{-1} represented C–O and C–O–C stretching vibrations. The peak at 718.73 cm^{-1} indicated alkyl chain rocking vibrations. The ATR-IR spectra of pure EMP and transferosomal buccal film showed no significant peak shifting, indicating absence of chemical interaction between EMP and film-forming excipients.

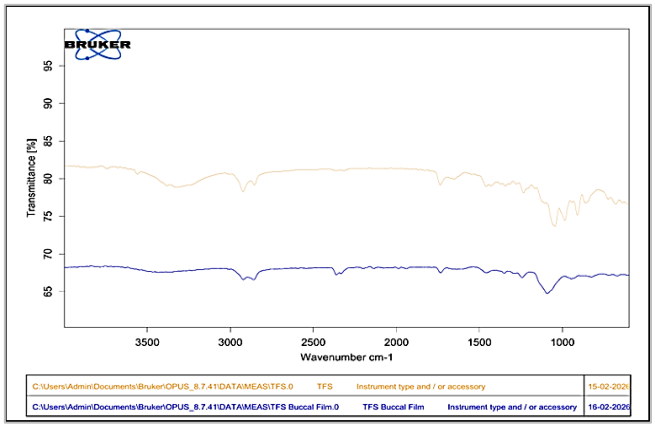


Figure 8: ATR overlay of transferosomal buccal film (TFS)

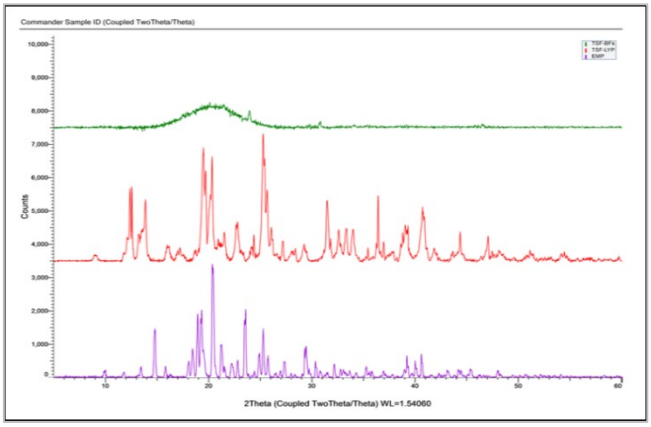


Figure 9: XRD overlay of transferosomal buccal film (TFS)

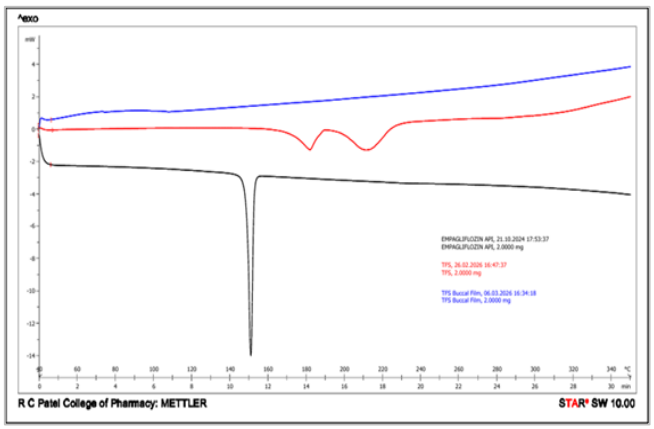


Figure 10: DSC overlay of transferosomal buccal film (TFS)

This confirmed that EMP remained stable and retained its original chemical structure within the buccal film formulation.

The XRD pattern of the transferosomal buccal film containing empagliflozin showed a broad diffuse peak, with reduced intensity and absence of sharp characteristic crystalline peaks of the pure drug. This indicates a decrease in crystallinity and suggests that empagliflozin was present in an amorphous or molecularly dispersed form within the polymeric matrix. The broad halo pattern confirms successful incorporation of the drug into the transferosomal film, without formation of any new crystalline impurities. Overall, the XRD study indicates good compatibility between the drug and excipients and supports the enhanced amorphous nature of the formulation.

The DSC thermogram of the transferosomal buccal film containing empagliflozin showed broad endothermic peaks around 74.20 °C and 108.98 °C. The reduction and broadening of the characteristic peak of empagliflozin indicate that the drug was successfully incorporated within the polymeric transferosomal matrix and may exist in a partially amorphous or molecularly dispersed state. The absence of any sharp crystalline peak of the pure drug suggests good compatibility between the drug and excipients, without significant chemical interaction. Overall, the DSC study confirms the stable incorporation of empagliflozin into the transferosomal buccal film formulation.

Stability studies

The optimized formulation (B3) was evaluated during storage at different temperatures, *i.e*., at 4±2 ℃ (refrigerator), 27±2 ℃ (room temperature), and 45±2 ℃ (stability chamber) for a period of three months. There were no insignificant physical changes in appearance. The moisture content was found to decrease with an increase in temperature. No significant changes were observed in drug content at 4±2 ℃. From the overall stability studies, it can be concluded that the formulated transferosomal buccal film was stable at all the three temperature conditions.

Table 5
Stability studies of the optimized transferosomal buccal film

Month	Temperature	Physical appearance	Moisture content	Drug content
1	4 ℃	whitish, smooth	4.46%	97.64%
	27±2 ℃	whitish, smooth	3.41%	95.61%
	45 ℃	whitish, smooth	3.08%	94.01%
2	4 ℃	whitish, smooth	3.52%	93.64%
	27±2 ℃	whitish, smooth	3.05%	93.58%
	45 ℃	whitish, smooth	2.47%	92.43%
3	4 ℃	whitish, smooth	4.25%	93.79%
	27±2 ℃	whitish, smooth	3.12%	92.88%
	45 ℃	whitish, smooth	2.47%	91.69%

CONCLUSION

The present study successfully developed and optimized an empagliflozin-loaded transferosomal buccal film using the Central Composite Design. The optimized transferosomal formulation exhibited desirable nanoscale characteristics, high drug entrapment, and good physicochemical stability, while its incorporation into a mucoadhesive buccal film produced a formulation with satisfactory mechanical strength, mucoadhesion, and drug content uniformity.

The transferosomal buccal film demonstrated superior *in-vitro* drug release, enhanced *ex-vivo* buccal permeation, and significantly improved *in-vivo* bioavailability compared with the conventional buccal film. Physicochemical characterization confirmed successful drug encapsulation, compatibility between the drug and excipients, and partial amorphization of empagliflozin, which contributed to the improved drug release and permeation characteristics. Furthermore, the optimized formulation remained stable under the evaluated storage conditions, indicating its suitability for pharmaceutical application.

Overall, the findings suggest that the transferosomal buccal film is a promising non-invasive drug delivery platform for empagliflozin, offering improved systemic absorption by overcoming hepatic first-pass metabolism and

enhancing buccal permeation. This strategy has the potential to improve therapeutic performance and patient compliance in the management of type 2 *Diabetes mellitus*. Further long-term pharmacodynamic and clinical studies are necessary to establish its clinical efficacy, safety, and translational potential.

***ACKNOWLEDGEMENTS*:** The authors would like to thank the R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, for providing the facility to perform the research work.

REFERENCES

[1] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova *et al*., *Diabetes Res. Clin. Pract*., **183**, 109119 (2022), https://doi.org/10.1016/j.diabres.2021.109119

[2] R. M. Anjana, R. Unnikrishnan, M. Deepa, R. Pradeepa, N. Tandon *et al*., *The Lancet Diabetes Endocrinol*., **11**, 474 (2023), https://doi.org/10.1016/S2213-8587(23)00119-5

[3] M. Zakir, N. Ahuja, M. A. Surksha, R. Sachdev, Y. Kalariya *et al*., *Cureus*, **15**, e45835 (2023), https://doi.org/10.7759/cureus.45835

[4] American Diabetes Association, *Diabetes Care*, **47**, S20 (2024), https://doi.org/10.2337/dc24-S002

[5] U. Galicia-Garcia, A. Benito-Vicente, S. Jebari, A. Larrea-Sebal, H. Siddiqi *et al*., *Int. J. Mol. Sci*., **21**, 6275 (2020), https://doi.org/10.3390/ijms21176275

[6] J. I. Fonseca-Correa and R. Correa-Rotter, *Front. Med*., **8**, 777861 (2021), https://doi.org/10.3389/fmed.2021.777861

[7] Y. Tang, Y. Tian, Y. Wang, X. Mei, L. Luo *et al*., *Front. Bioeng. Biotechnol*., **13**, 1638034 (2025), https://doi.org/10.3389/fbioe.2025.1638034

[8] A. Salawi, N. Tabassum, M. Zaman, M. A. Khan, W. Siddique *et al*., *ACS Omega*, **10**, 23492 (2025), https://doi.org/10.1021/acsomega.5c02082

[9] R. Fernández-García, A. Lalatsa, L. Statts, F. Bolás-Fernández, M. P. Ballesteros *et al*., *Int. J. Pharm*., **573**, 118817 (2020), https://doi.org/10.1016/j.ijpharm.2019.118817

[10] D. Mohapatra and T. W. Corson, *Nanomedicine (Lond)*, **20**, 2189 (2025), https://doi.org/10.1080/17435889.2025.2510197

[11] J. Medhi, C. Thalluri, M. Vasam and S. P. N. Bukke, *BioMed. Eng. OnLine*, **25**, 1 (2026), https://doi.org/10.1186/s12938-025-01490-6

[12] E. A. Nieves, M. C. Cotto and F. Márquez, *Pharmaceuticals*, **19**, 956 (2026), https://doi.org/10.3390/ph19060956

[13] M. Z. Ünükür Sevim, M. D. Arpa, K. Kolci, H. Sipahi and N. Üstündağ Okur, *Pharmaceutics*, **18**, 245 (2026), https://doi.org/10.3390/pharmaceutics18020245

[14] P. Chandrasekhar and R. Kaliyaperumal, *Recent Pat. Drug Deliv. Formul*., **18**, 262 (2024), https://doi.org/10.2174/0126673878312336240802113811

[15] G. Verma and M. K. Mishra, *Int. J. Pharm. Res. Rev*., **5**, 12 (2016)

[16] R. Grempler, L. Thomas, M. Eckhardt, F. Himmelsbach, A. Sauer *et al*., *Diabetes Obes. Metab*., **14**, 83 (2012), https://doi.org/10.1111/j.1463-1326.2011.01517.x

[17] S. D. Raysing and A. P. Gorle, *Colloid Polym. Sci*., **303**, 1501 (2025), https://doi.org/10.1007/s00396-025-05429-0

[18] F. Alemohammad, S. Abdollahi, Z. Raoufi and E. Moghimipour, *J. Polym. Environ*., **33**, 3407 (2025), https://doi.org/10.1007/s10924-025-03604-6

[19] M. Rizk, A. K. Attia, H. Y. Mohamed and M. S. Elshahed, *Chem. Res. J*., **5**, 6 (2020)

[20] A. Javed, R. M. Sarfraz, A. Mahmood, Z. Ahmad, M. R. Akram *et al*., *Naunyn-Schmiedeberg's Arch. Pharmacol*., **398**, 6739 (2025), https://doi.org/10.1007/s00210-024-03708-1

[21] S. Sahu, R. K. Pandey, S. S Shukla and B. Gidwani, *J. Appl. Spectrosc*., **92**, 405 (2024), https://doi.org/10.1007/s10812-024-01734-8

[22] M. Gundle, K. Danao, R. Shivhare, V. Rokde, A. Warokar *et al*., *J. Med. Pharm. Allied Sci*., **10**, 3206 (2021), https://doi.org/10.22270/jmpas.V10I4.1367

[23] A. Gumieniczek, A. Berecka-Rycerz, M. Dul and A. Pryjda, *Molecules*, **30**, 115 (2024), https://doi.org/10.3390/molecules30010115

[24] A. Dwivedi and G. Darwhekar, *Int. J. Pharm. Sci. Res*., **11**, 32 (2019)

[25] G. F. Balata, M. M. Faisal, H. A. Elghamry and S. A. Sabry, *J. Drug Deliv. Sci. Technol*., **60**, 101921 (2020), https://doi.org/10.1016/j.jddst.2020.101921

[26] S. Bhattacharyya, K. T. Lakshmanan and A. Muthukumar, *Turk. J. Pharm. Sci*., **21**, 303 (2024), https://doi.org/10.4274/tjps.galenos.2023.46735

[27] S. K. Prajapati, A. Jain and M. Bajpai, *RSC Adv*., **15**, 7799 (2025), https://doi.org/10.1039/d5ra00387c

[28] T. Khan, B. Nabi, S. Rehman, M. Akhtar, J. Ali *et al*., *Nano*, **16**, 2150149 (2021), https://doi.org/10.1142/S1793292021501496

[29] A. K. Chettupalli, A. Unnisa, H. Peddapalli, R. K. Jadi, K. Anusha *et al*., *Intelligent Pharmacy*, **3**, 193 (2025), https://doi.org/10.1016/j.ipha.2024.12.004

[30] N. E. Eleraky, M. EI-Badry, M. M. Omar, W. M. EI-Koussi, N. G. Mohamed *et al*., *Int. J. Nanomed*., **5831** (2023), https://doi.org/10.2147/IJN.S423251

[31] B. Tayade, V. Gharge, B. Jadhav, A. Patil, C. Parde *et al*., *Asian J. Pharm. Anal*., **16**, 41 (2026), https://doi.org/10.52711/2231-5675.2026.00007

[32] S. S. Haju and S. Yadav, *Int. J. Pharm. Pharm. Sci*., **13**, 34 (2021), https://dx.doi.org/10.22159/ijpps.2021v13i9.42641

[33] P. Chandrasekhar and R. Kaliyaperumal, *J. Appl. Pharm. Res.*, **14**, 61 (2026), https://doi.org/10.69857/joapr.v14i2.1523
[34] N. Islam, M. Irfan, A. F. Zahoor, M. S. Iqbal, H. K. Syed *et al.*, *Pharmaceutics*, **13**, 1315 (2021), https://doi.org/10.3390/pharmaceutics13081315
[35] R. Suma, P. V. Karwa and V. Kusum Devi, *Int. J. App. Pharm.*, **15**, 188 (2023), https://doi.org/10.22159/ijap.2023v15i6.48780
[36] S. Salehi and S. Boddohi, *Prog. Biomater.*, **6**, 175 (2017), https://doi.org/10.1007/s40204-017-0077-7
[37] D. A. Gaber, A. I. Alburaykan, L. M. Alruthea, N. S. Aldohan, R. F. Alharbi *et al.*, *Drug Des. Devel. Ther.*, **13**, 16 (2022), https://doi.org/10.2147/DDDT.S394523
[38] W. Siddique, R. M. Sarfraz, M. Zaman, M. H. Butt, Z. Hayat *et al.*, *Lat. Am. J. Pharm.*, **40**, 3002 (2021)
[39] A. B. Nair, J. Shah, S. Jacob, B. E. AL-Dhubiab, V. Patel *et al.*, *Pharmaceutics*, **13**, 728 (2021), https://doi.org/10.3390/pharmaceutics13050728
[40] I. Abbas, N. A. Rajab and A. A. Hussein, *Iraqi J. Pharm. Sci.*, **28**, 83 (2019), https://doi.org/10.31351/vol28iss2pp83-94
[41] W. Siddique, M. Zaman, A. M. Sarfraz, M. H. Butt, A. Ur Rehmanet *et al.*, *Polymers*, **14**, 3981 (2022), https://doi.org/10.3390/polym14193981
[42] I. Setiawan, A. Subarnas and Y. W. Wardhana, *Int. J. Curr. Pharm. Rev. Res.*, **14**, 60 (2022)
[43] O. Y. Mady, M. M. A. Abulmeaty, A. A. Donia, A. A. Al-Khureif, A. A. Al-Shoubki *et al.*, *Membranes*, **11**, 659 (2021), https://doi.org/10.3390/membranes11090659
[44] S. Abdella, F. Afinjuomo, Y. Song, R. Upton and S. Garg, *Pharmaceutics*, **14**, 542 (2022), https://doi.org/10.3390/pharmaceutics14030542
[45] A. Hussain, Z. Ahmad, A. Mahmood, S. Shchinar, M. I. Khan *et al.*, *BioNanoScienc.*, **14**, 2391 (2024), https://doi.org/10.1007/s12668-024-01511-z
[46] K. Pamlenyi, G. Regdon, D. Nemes, F. Fenyvesi, I. Bacskay *et al.*, *Pharmaceutics*, **14**, 1633 (2022), https://doi.org/10.3390/pharmaceutics14081633