

Characterization of A Dual-Drug Liposomal Nanoformulation of Metformin Hydrochloride and Canagliflozin Hemihydrate for Sustained Oral Delivery.

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ABSTRACT

Objective: The purpose of this study was to design and optimize a dual-drug-loaded liposomal nanoformulation containing metformin hydrochloride and canagliflozin hemihydrate for sustained oral delivery utilizing a Quality-by-Design (QbD) approach. A Box-Behnken design was used to develop a robust formulation with ideal physicochemical properties.

Methods: Liposomes were created using the thin-film hydration technique using soya lecithin and cholesterol. The effects of lipid content, hydration duration, and sonication time on particle size, zeta potential, and entrapment efficiency were improved using a Box-Behnken design with three factors and three levels. The optimized formulation was thoroughly tested for physicochemical properties, shape, solid-state features, in vitro drug release, release kinetics, and enhanced stability.

Results: The Optimized liposomal nanoformulation had a particle size of 151.2 ± 2.5 nm, polydispersity index of 0.168 ± 0.012 , zeta potential of -34.8 ± 1.4 mV, and entrapment efficiencies of $98.4 \pm 0.5\%$ and $84.0 \pm 0.6\%$ for metformin hydrochloride and canagliflozin hemihydrate, respectively. FTIR verified the absence of substantial drug-excipient interactions, whereas DSC and XRD showed reduced drug crystallinity after encapsulation. SEM examination revealed uniformly distributed spherical vesicles with a smooth shape. The formulation demonstrated a biphasic release profile with sustained drug release over 24 hours, producing cumulative releases of $97.3 \pm 1.5\%$ for metformin hydrochloride and $94.1 \pm 1.4\%$ for canagliflozin hemihydrate. Drug release followed the Korsmeyer-Peppas model, showing that anomalous diffusion was the primary release mechanism. Enhanced stability experiments revealed excellent physicochemical stability over three months.

Conclusion: The QbD-driven optimization resulted in a stable dual-drug-loaded liposomal nanoformulation with high encapsulation efficiency, regulated drug release, and enhanced stability. These findings emphasize the potential of this nanocarrier as an oral combination delivery method for the treatment of type 2 diabetes mellitus, necessitating additional in vivo pharmacokinetic and pharmacodynamic studies.

KEYWORDS: Metformin hydrochloride; Canagliflozin hemihydrate; liposomes; Quality by Design; Box-Behnken design; prolonged oral medication administration; type 2 diabetes.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a fast expanding global health concern, accounting for 90-95% of all diabetes cases and imposing a significant clinical and socioeconomic burden around the world.[1,2] This metabolic disease is characterized by insulin resistance, pancreatic β -cell malfunction, and chronic hyperglycemia. Chronic blood glucose increase adds to severe microvascular and macrovascular consequences such as diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disease, all of which continue to be important causes of morbidity and mortality in people with diabetes.[3] Despite tremendous breakthroughs in pharmacotherapy, maintaining glucose control while reducing side effects and increasing patient compliance remains a key therapeutic problem.[4]

Metformin hydrochloride is the first-line oral antihyperglycemic medication for the treatment of type 2 diabetes because to its proven efficacy, excellent safety profile, low cost, and insulin-sensitizing characteristics. It predominantly inhibits hepatic gluconeogenesis, increases peripheral glucose utilization, and improves insulin sensitivity while causing minimal hypoglycemia.[5] However, metformin has significant pharmacokinetic constraints, such as low intestinal permeability, inadequate oral bioavailability, a short

elimination half-life, and gastrointestinal side effects that may impair patient compliance. These limitations underscore the need for new drug delivery methods capable of increasing absorption, Sustained drug release, and improving therapeutic efficacy.[6]

Canagliflozin hemihydrate, the first clinically authorized sodium-glucose cotransporter-2 (SGLT2) inhibitor, has emerged as a promising treatment option due to its insulin-independent mechanism of action. By specifically inhibiting SGLT2 in the proximal renal tubules, it lowers renal glucose reabsorption while increasing urine glucose excretion, reducing plasma glucose levels.[7] Beyond glucose control, canagliflozin has shown significant cardiovascular and renoprotective effects, prompting its incorporation into modern diabetes therapy. Nonetheless, as a Biopharmaceutics Classification System Class II medication, canagliflozin has low water solubility, limiting dissolution rate and oral bioavailability.[8] As a result, formulation techniques that improve its solubility while preserving prolonged systemic exposure are critical for maximizing therapeutic efficacy.[9]

Metformin hydrochloride and canagliflozin combination therapy has received widespread clinical approval due to the complimentary mechanisms by which both medicines regulate glucose homeostasis.[10] Metformin largely decreases hepatic glucose synthesis and improves insulin sensitivity, whereas canagliflozin reduces blood glucose by increasing urine glucose excretion. When used together, they provide better glycemic control, a lower risk of hypoglycemia, and extra benefits in body weight management, cardiovascular protection, and renal function than monotherapy.[11] However, the obvious physicochemical variations between these medicines present significant formulation issues. Metformin is extremely hydrophilic, but canagliflozin is lipophilic and less water soluble, making simultaneous integration into a single oral dose form problematic and frequently resulting in variations in dissolving behavior, release profile, and systemic exposure.[12]

Nanotechnology-based medication delivery systems have received a lot of interest as a viable way to overcome the constraints of traditional oral formulations.[13] Liposomes stand out among these systems because to their biocompatibility, biodegradability, low toxicity, and unique structural structure. Liposomes, which are made up of phospholipid bilayers surrounding an aqueous core, can encapsulate both hydrophilic and lipophilic medicines.[14] This dual-loading feature makes them ideal for co-delivering medicines with opposing physicochemical qualities. Furthermore, liposomal formulations can preserve encapsulated pharmaceuticals from premature degradation, enable regulated and prolonged release, improve intestinal absorption, and possibly increase oral bioavailability while minimizing dose-related side effects. These characteristics have established liposomes as adaptable nanocarriers for oral combination drug release.[15]

Quality by Design (QbD) has arisen as a systematic, scientifically supported, risk-focused approach to pharmaceutical formulation design. Unlike traditional trial-and-error methods, QbD focuses on established quality targets and the identification of critical quality attributes, critical material attributes, and critical process parameters, allowing for a better understanding of formulation and process variability.[16] Statistical experimental designs, such as the Box-Behnken design, enable efficient optimization by establishing links between formulation variables and product performance while minimizing the number of experimental runs. As a result, QbD has emerged as a valuable technique for designing.[17]

Although nanocarrier-mediated formulations for metformin and canagliflozin have been studied individually, creating a single liposomal carrier capable of efficiently co-encapsulating these physicochemically different drugs remains difficult. Furthermore, systematic optimization of such dual-drug liposomal systems utilizing a QbD framework, as well as thorough physicochemical characterization and release evaluation, has received

little attention. Addressing these issues is critical for the creation of stable, repeatable oral liposomal formulations for combination antidiabetic therapy.[18]

Accordingly, the present study aimed to develop and optimize a dual-drug-loaded liposomal nanoformulation containing metformin hydrochloride and canagliflozin hemihydrate using a Quality-by-Design-guided Box–Behnken experimental design. Liposomes were prepared by the thin-film hydration method, and the effects of critical formulation and process variables on key quality attributes were systematically optimized. The optimized formulation was comprehensively characterized with respect to physicochemical properties, morphology, encapsulation efficiency, solid-state characteristics, in vitro drug release, release kinetics, and stability to evaluate its potential as a sustained oral nanocarrier for combination therapy in patients with type 2 diabetes mellitus.[19]

MATERIALS AND METHODS

2.1 Materials

Metformin hydrochloride (MET) was procured from Yarrow Chem Products (Mumbai, India), while canagliflozin hemihydrate (CAN) was kindly provided by Exemed Pharma Pvt. Ltd. (Vapi, Gujarat, India). Soya lecithin (phosphatidylcholine) and cholesterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade), chloroform, ethanol, potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium bromide (spectroscopic grade), and other analytical-grade chemicals and reagents were obtained from Merck Life Science Pvt. Ltd. (Mumbai, India). Ultrapure water generated using a Milli-Q purification system (Millipore, Bedford, MA, USA) was used throughout the study. Dialysis membranes with a molecular weight cut-off (MWCO) of 12–14 kDa were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Unless otherwise specified, all chemicals and reagents were of analytical or HPLC grade and were used as received.

2.2 Experimental Design and Optimization

A Quality by Design (QbD)-based approach was adopted to systematically develop and optimize the dual-drug-loaded liposomal formulation. Response Surface Methodology (RSM) employing a three-factor, three-level Box–Behnken design (BBD) was used to investigate the influence of critical formulation and process variables on the physicochemical characteristics of the liposomal system. Based on preliminary studies and risk assessment, lipid concentration (X_1), hydration time (X_2), and sonication time (X_3) were selected as the independent variables, while particle size (Y_1), zeta potential (Y_2), and entrapment efficiency (Y_3) were considered the critical quality attributes (CQAs).[20]

The experimental design comprised 17 runs, including 12 factorial edge points and 5 center points, generated using Design-Expert® software (Version 13.0, Stat-Ease Inc., Minneapolis, MN, USA). Experimental data were fitted to second-order polynomial models to establish the relationship between the independent variables and the measured responses. Model adequacy and statistical significance were evaluated by analysis of variance (ANOVA) based on regression coefficients, F-values, p-values, coefficient of determination (R^2), adjusted R^2 , predicted R^2 , coefficient of variation (CV), lack-of-fit, and adequate precision.[21]

The effects of the formulation variables and their interactions were visualized using three-dimensional response surface plots and two-dimensional contour plots. Numerical optimization was performed using the desirability function approach to obtain an optimized formulation with minimum particle size, maximum absolute zeta potential, and maximum entrapment efficiency. The optimized formulation predicted by the model was prepared experimentally, and the observed responses were compared with the predicted values to validate the reliability and predictive capability of the optimization model.[22]

Table 1: Independent variables and response variables used in the Box-Behnken experimental design.

Factor	Independent Variable	Low (−1)	Medium (0)	High (+1)
X ₁	Lipid concentration (mg)	50	100	150
X ₂	Hydration time (min)	30	60	90
X ₃	Sonication time (min)	30	60	90
Response	Critical Quality Attribute		Optimization Goal	
Y ₁	Particle size (nm)		Minimize	
Y ₂	Zeta potential (mV)		Maximize absolute value	
Y ₃	Entrapment efficiency (%)		Maximize	

2.3 Preformulation Studies

Preformulation studies were carried out to evaluate the physicochemical characteristics of metformin hydrochloride (MET) and canagliflozin hemihydrate (CAN) prior to formulation development. These studies included organoleptic characterization, equilibrium solubility determination, melting point analysis, and Fourier transform infrared (FTIR) spectroscopy to assess the suitability and compatibility of both drugs for incorporation into the liposomal nanoformulation.[23]

2.3.1 Organoleptic Characterization

The organoleptic properties of MET and CAN were evaluated by visual inspection for colour, appearance, physical state, texture, odour, and the presence of any visible particulate matter. The observed properties were compared to the manufacturer's standards to ensure the identity and physical integrity of the pharmaceutical drug compounds.[24]

2.3.2 Solubility Determination

The equilibrium solubility of MET and CAN was investigated using distilled water, methanol, ethanol, chloroform, acetone, and phosphate buffer (pH 7.4). To achieve equilibrium, surplus drugs were added to 10 mL of each solvent in sealed glass vials and swirled continuously for 24 hours at 25 ± 2 °C. The suspensions were filtered through a 0.45 µm membrane filter, diluted, and examined with a UV-Visible spectrophotometer (Shimadzu UV-1900, Shimadzu Corporation, Kyoto, Japan). The solubility profile was used to determine liposomes as an appropriate carrier system for co-encapsulating hydrophilic and lipophilic medicines. [25]

2.3.3 Melting Point Determination

The melting points of MET and CAN were calculated using the open capillary method on a digital melting point instrument (Toshniwal Instruments, India). Drug samples were placed in capillary tubes and heated at a controlled rate of around 2 °C/min. The melting onset and completion temperatures were documented, and all data were taken three times.[26]

2.3.4 Fourier Transform Infrared (FTIR) Spectroscopy

The drug-excipient interaction was determined using a PerkinElmer Spectrum Two FTIR spectrophotometer (PerkinElmer Inc., Waltham, MA, USA) and the potassium bromide (KBr) pellet method. The FTIR spectra of pure medicines, individual excipients, physical mixes, and the optimized liposomal formulation were acquired over the spectral range of 4000-400 cm^{−1} with a resolution of 4 cm^{−1} using 32 scans per sample. The spectra were evaluated for typical absorption bands, such as peak shifts, disappearance, broadening, or the emergence of new peaks, to determine any potential physicochemical interactions among drug molecules and formulation ingredients.[27]

2.4 Preparation of Liposomes

The thin-film hydration approach was used to create dual-drug liposomes containing metformin hydrochloride (MET) and canagliflozin hemihydrate (CAN). Soya lecithin and cholesterol were utilized as membrane-

forming lipids, with a soya lecithin-to-cholesterol molar ratio of 7:3. The lipids were precisely weighed, dissolved in chloroform:methanol (2:1 v/v) in a round-bottom flask. The organic solvent was evaporated under decreased pressure using a rotary vacuum evaporator (Heidolph Instruments GmbH, Schwabach, Germany) at 40 ± 2 °C and 60 rpm, resulting in a thin, homogenous lipid coating on the flask wall. The film was then vacuum-dried to ensure that any remaining solvent was completely removed.[28]

The dried lipid film was hydrated with phosphate buffer (pH 7.4) containing 50 mg MET and 5 mg CAN. The buffer had been heated to 60 °C before being gradually added with continuous vortex mixing to ensure consistent hydration and vesicle formation. The hydration procedure was carried out at the parameters described in the experimental design.[29]

The multilamellar vesicle (MLV) dispersion was reduced to nanosized liposomes by probe sonication using a Vibra-Cell™ ultrasonic processor (Sonics & Materials Inc., Newtown, CT, USA). The processor was operated at 40% amplitude with a pulse sequence of 3 seconds on and 2 seconds off. The sonication time was changed using the Box-Behnken design matrix. Liposomal compositions were transferred to amber glass jars and stored at 4 ± 2 °C for subsequent analysis.[30]

Table 2: Physicochemical Characterization of Optimized Liposomes

Parameter	Instrument/Method	Purpose
Particle size	Dynamic Light Scattering	Vesicle size determination
Polydispersity Index	Dynamic Light Scattering	Uniformity of particle distribution
Zeta potential	Electrophoretic Light Scattering	Colloidal stability
Entrapment efficiency	Ultracentrifugation + Quantification	Drug encapsulation efficiency
Drug loading	Mathematical calculation	Drug incorporation capacity
FTIR	KBr Pellet Method	Drug-excipient compatibility
DSC	Differential Scanning Calorimetry	Thermal behavior
XRD	Powder X-ray Diffraction	Crystallinity
SEM	Scanning Electron Microscopy	Surface morphology
In vitro release	Dialysis membrane diffusion	Drug release profile
Stability	ICH Q1A(R2)	Long-term physicochemical stability

2.5 Characterization of Liposomal Formulations

2.5.1 Particle Size, Polydispersity Index, and Zeta Potential

The mean particle size, polydispersity index (PDI), and zeta potential of the liposomal formulations were measured with a Zetasizer Nano ZS (Malvern Panalytical Ltd., Malvern, UK). To eliminate numerous scattering effects, materials were diluted with filtered deionized water before being analyzed. DLS was used to quantify particle size and PDI, whereas electrophoretic light scattering was used to assess zeta potential using the Smoluchowski approximation. All measurements were taken at 25 ± 0.5 °C. All measurements were taken in triplicate, and the findings are shown as mean $\pm$ standard deviation.[31,32]

2.5.2 Entrapment Efficiency and Drug Loading

Ultracentrifugation was used to separate the untrapped and liposome-associated drugs and estimate entrapment efficiency (EE). The liposomal dispersion was centrifuged under cold conditions, and the supernatant containing the free medication was collected and evaluated using the validated simultaneous UV spectrophotometric method detailed in Section 2.X. The EE was computed using the following equation:

$$DL (\%) = \frac{\text{Entrapped drug}}{\text{Total weight of lipid + drug}} \times 100$$

Drug loading (DL) was measured as the percentage of entrapped drug divided by the total weight of lipids and drug in the liposomal nanoformulation.[33]

2.5.3 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to study the thermal behavior of pure medicines, excipients, physical mixtures, and improved liposomal formulations. Samples were enclosed in aluminum pans and scanned throughout a temperature range of 30-300 °C with a heating rate of 10 °C/min in a nitrogen atmosphere.[34]

2.5.4 Powder X-ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) was used to assess the crystalline properties of both the pure medicines and the improved formulation. Diffraction patterns were analyzed to determine changes in crystallinity after liposomal encapsulation.[35]

2.5.5 Scanning Electron Microscopy (SEM)

The surface morphology of the optimized liposomal formulation was investigated using scanning electron microscopy (SEM). Samples were dried, mounted on aluminum stubs with double-sided carbon tape, sputter-coated with a thin layer of gold, and evaluated under appropriate accelerating voltage and magnification to assess particle morphology and surface properties.[36]

2.5.6 In Vitro Drug Release Study

The in vitro release profiles of MET and CAN from the improved liposomal formulation were assessed using the dialysis membrane diffusion technique. Dialysis membranes (MWCO: 12-14 kDa) were soaked in distilled water for 12 hours before use. An aliquot of the liposomal formulation equivalent to the required medication dose was put into the dialysis bag, which was immersed in 250 mL of phosphate buffer (pH 7.4) and kept at 37 ± 0.5 °C with continuous stirring at 100 rpm.[37]

To maintain sink conditions, aliquots of the release medium were taken at regular intervals and promptly replaced with an equal volume of fresh prewarmed dissolving medium. The collected samples were filtered and examined with a validated simultaneous UV spectrophotometric technique. All experiments were done in triplicate.[38]

2.5.7 Drug Release Kinetics

The in vitro drug release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models to elucidate the release mechanism. The model providing the highest coefficient of determination (R^2) was considered the best fit.[39]

2.5.8 Stability Study

The stability of the optimized liposomal formulation was evaluated under accelerated storage conditions in accordance with ICH guideline **Q1A(R2)**. Samples were stored at 40 ± 2 °C/ $75 \pm 5\%$ RH for **3 months** and analyzed at **0, 1, and 3 months**. The formulations were evaluated for changes in particle size, PDI, zeta potential, entrapment efficiency, and drug content to assess their physicochemical stability during storage.[40]

RESULTS AND DISCUSSION

3.1 Preformulation Studies

Preformulation studies were conducted to characterize the physicochemical properties of metformin hydrochloride and canagliflozin hemihydrate before liposome development. These studies provided the basis for formulation design by identifying properties that influence encapsulation, stability, and release. Because the two drugs differ markedly in solubility and lipophilicity, preformulation assessment was essential for selecting a carrier system capable of co-encapsulating both compounds.

3.1.1 Organoleptic Characterization

The organoleptic properties of both active pharmaceutical ingredients were assessed to confirm their physical identity and quality before formulation. The observed characteristics were compatible with the corresponding pharmacopoeial requirements and certificates of analysis provided by the manufacturers. Both APIs appeared exactly as expected, with no discoloration, aggregation, moisture uptake, or apparent particulate contamination. Metformin hydrochloride flowed freely, whereas canagliflozin hemihydrate was non-hygroscopic, indicating appropriate processing qualities. The absence of aberrant organoleptic alterations demonstrated that both medicines maintained acceptable pharmaceutical quality and were suitable for future formulation development. Although preliminary, organoleptic examination is nonetheless a vital quality check since changes in color, odor, or appearance might indicate degradation, contamination, or poor storage.

Table 3: Organoleptic properties of metformin hydrochloride and canagliflozin hemihydrate.

Parameter	Metformin HCl	Canagliflozin Hemihydrate	Pharmaceutical Significance
Color	White	White	Confirms identity and purity
Physical appearance	Crystalline powder	Fine powder	Suitable for formulation
Texture	Fine, free-flowing	Fine, non-hygroscopic	Good handling characteristics
Odour	Odourless	Odourless	Indicates absence of degradation

3.1.2 Solubility Studies

The equilibrium solubility of both drug molecules was evaluated in solvents with varying polarities in order to understand their physicochemical behavior and guide formulation design. The results are shown in Table 4.

Table 4: Solubility profiles of metformin hydrochloride and canagliflozin hemihydrate in various solvents.

Solvent	Metformin HCl	Canagliflozin Hemihydrate	Interpretation
Distilled water	Highly soluble	Practically insoluble	Contrasting aqueous solubility
Methanol	Highly soluble	Soluble	Suitable analytical solvent
Ethanol	Slightly soluble	Soluble	Moderate organic solubility
Chloroform	Practically insoluble	Soluble	Confirms lipophilic behavior of CAN
Phosphate buffer (pH 7.4)	Highly soluble	Sparingly soluble	Relevant for release studies
Acetone	Slightly soluble	Soluble	Limited applicability

The investigation revealed an obvious difference between the two drug molecules. Metformin hydrochloride had great aqueous solubility, consistent with its BCS Class III profile, but canagliflozin hemihydrate had poor aqueous solubility but higher solubility in organic solvents, consistent with BCS Class II behavior. These findings were consistent with the known physicochemical features of both medications, highlighting the necessity for a formulation that can incorporate molecules with varying solubility profiles.

Liposomes were chosen as the delivery system as they may entrap both hydrophilic and lipophilic drug molecules in separate sections of the vesicle. Canagliflozin partitions into the phospholipid bilayer, whereas

metformin hydrochloride is likely to dwell in the aqueous core. This dual division provides a rational foundation for co-delivery and may increase release timing and overall formulation efficacy.

3.1.3 Melting Point Determination

Melting point determination was used to authenticate the drug's identification and purity, as well as to evaluate their thermal behavior prior to formulation. Both APIs had melting points similar to the indicated pharmacopoeial values, indicating high purity and verifying chemical identity.

Table 5: Melting point determination for the given APIs.

Drug	Observed Melting Point (°C)	Reported Value (°C)	Interpretation
Metformin hydrochloride	222-225	220-226	Within pharmacopoeial limits
Canagliflozin hemihydrate	144-146	143-147	Within reported range

There was no considerable decrease or increase of the melting range, indicating the absence of major impurities or polymorphism alterations. The higher melting point of metformin hydrochloride reflects its highly crystalline character and strong intermolecular interactions, whereas canagliflozin hemihydrate has a lower melting point due to its molecular structure and hydrated crystalline state. These thermal properties were considered throughout formulation, notably during hydration, when the processing temperature was kept substantially below the melting points of both medications to avoid degradation. The findings also offered a good baseline for future DSC investigation.

3.1.4 Fourier Transform Infrared Analysis

FTIR spectroscopy was used to investigate potential drug-excipient interactions. We examined the distinctive peaks of pure metformin hydrochloride, canagliflozin hemihydrate, phospholipids, cholesterol, the physical mixture, and the optimized liposomal nanoformulation were compared.

Table 6: Fourier Transform Infrared (FTIR) Analysis

Drug/Sample	Key Peaks (cm ⁻¹)	Functional Group Assignment	Observation in Liposomal Formulation
Metformin HCl	3350-3150	N-H stretch (biguanide)	Retained; slight broadening
Metformin HCl	1630	C=N stretch	Retained without shift
Metformin HCl	1070	C-N stretch	Retained
Canagliflozin	3380	O-H stretch (hydroxyl)	Retained; slight shift
Canagliflozin	1600-1450	Aromatic C=C stretch	Retained
Canagliflozin	830	C-F stretch (p-fluorophenyl)	Retained confirming structural integrity
Soya Lecithin	2920, 2850	Alkyl C-H stretch	Dominant peaks in formulation
Liposomal Formulation	All key drug peaks present	No new peaks observed	No chemical interaction confirmed

Metformin hydrochloride showed characteristic N-H stretching vibrations in the 3350–3150 cm^{-1} region, C=N stretching near 1630 cm^{-1} , and C-N stretching around 1070 cm^{-1} . Canagliflozin hemihydrate exhibited O-H stretching near 3380 cm^{-1} , aromatic C=C stretching in the 1600–1450 cm^{-1} region, and C-F stretching near 830 cm^{-1} . The lipid components showed their expected aliphatic C-H stretching bands between 2850 and 2920 cm^{-1} .

In the Optimized liposomal nanoformulation, both medicines' distinctive peaks remained visible, with only slight broadening caused by weak hydrogen-bonding interactions inside the phospholipid matrix. There were no significant peak changes, disappearances of typical bands, or new absorption peaks identified. These findings suggest that the formulation procedure did not result in any major chemical interactions between the medicines and excipients. As a result, the interactions required in encapsulation were primarily physical rather than chemical, which contributed to the liposome system's compatibility and stability.

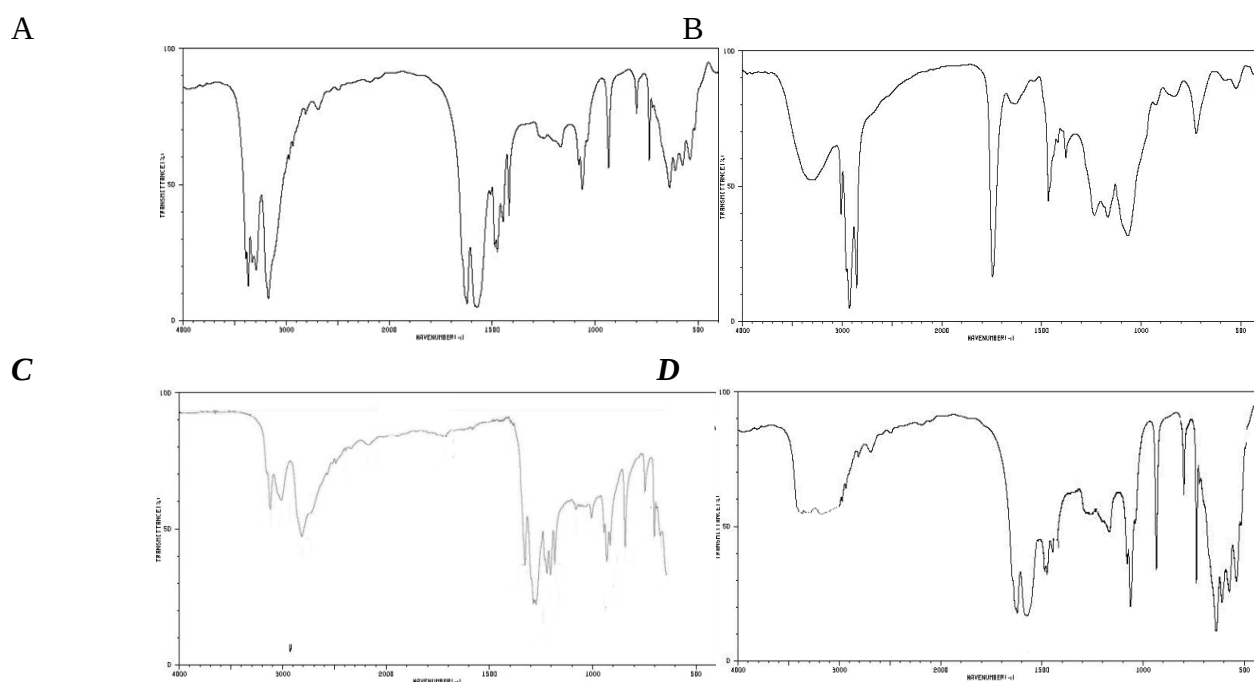


Fig. 1: FTIR spectra of (A) Metformin HCl, (B) Canagliflozin, (C) Lecithin, and (D) Physical mixture

3.2 Optimization of Dual Drug-Loaded Liposomal Nanoformulation Using Box– Behnken Design

The dual-drug-loaded liposomal nanoformulation was optimized using a Quality by Design (QbD)-based optimization technique that included a three-factor, three-level Box-Behnken design (BBD). The independent variables were lipid content (X_1), hydration time (X_2), and sonication duration (X_3). The critical quality attributes (CQAs) were particle size (Y_1), zeta potential (Y_2), and entrapment efficiency (Y_3). The experimental design and statistical analysis were carried out with Design-Expert® software (Version 13.0, Stat-Ease Inc., Minneapolis, MN, USA).

3.2.1 Experimental Design Matrix

The Box-Behnken design produced 17 experimental runs, each with 12 edge points and 5 center points, allowing for the examination of the selected variables' linear, interaction, and quadratic effects. The experimental design employed an efficient optimization method while decreasing the number of experimental trials. Table 7 shows the design matrix and the experimentally observed responses. The significant variation in particle size, zeta potential, and entrapment efficiency among formulations confirmed that the selected formulation and process variables had a significant effect on the liposomal system's physicochemical characteristics, validating the design space chosen for response surface optimization.

Table 7: Experimental design matrix generated using the Box– Behnken design.

Run	Lipid (mg)	Hydration (min)	Sonication (min)	Particle Size (nm)	Zeta Pot. (mV)	EE (%)
1	50	30	60	182.0	-26.5	71.5
2	50	90	60	182.5	-26.2	72.0
3	150	30	60	180.8	-27.0	71.2
4	150	90	60	183.2	-25.8	72.5
5	50	60	30	178.5	-27.2	73.0
6	50	60	90	179.6	-26.8	73.5
7	150	60	30	176.8	-28.5	72.2
8	150	60	90	180.5	-26.5	73.8
9	100	30	30	172.5	-29.0	75.5
10	100	30	90	174.0	-28.2	76.8
11	100	90	30	171.8	-29.5	75.8
12	100	90	90	175.5	-27.8	77.2
13	100	60	60	150.0	-35.5	83.5
14	100	60	60	151.2	-34.8	84.0
15	100	60	60	149.5	-35.2	83.0
16	100	60	60	150.8	-34.9	83.8
17	100	60	60	150.3	-35.1	83.6

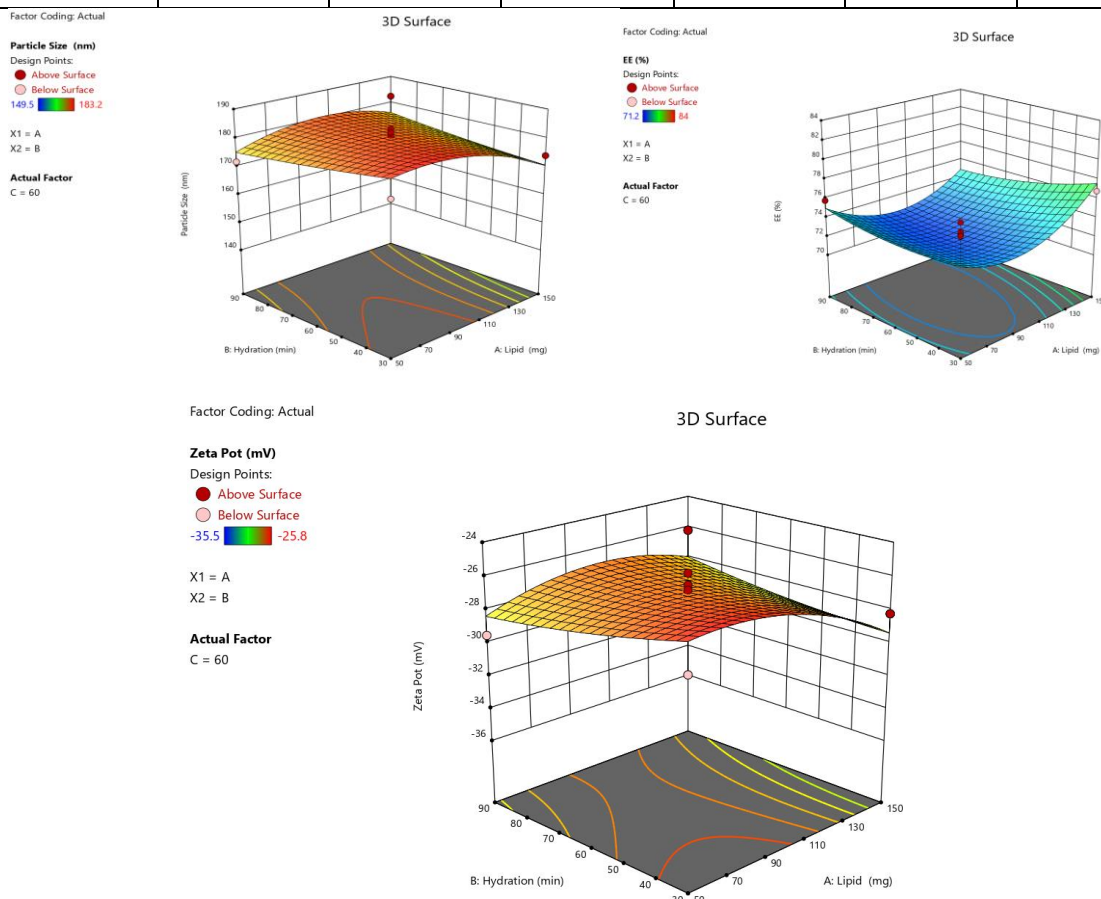


Fig. 2: Experimental responses obtained for the prepared liposomal formulations.

3.2.2 Effect of Formulation Variables on Particle Size

Figure 3 shows how lipid concentration, hydration duration, and sonication time affect particle size. Among the variables tested, lipid concentration had the greatest effect on vesicle size. Increasing lipid content resulted

in larger vesicles due to increased phospholipid bilayer formation and dispersion viscosity, reducing the effectiveness of vesicle size reduction during sonication. Hydration time facilitated lipid film expansion and vesicle production; however, extended hydration increased vesicle fusion, resulting in a gradual rise in particle size. In contrast, increasing sonication time resulted in a considerable reduction in particle size by converting multilamellar vesicles into smaller unilamellar vesicles by ultrasound cavitation. However, excessive sonication can jeopardize membrane integrity due to localized heat effects. Response surface analysis revealed a significant correlation between lipid concentration and sonication time, implying that formulations with higher lipid concentrations required more ultrasonic energy to produce nanosized vesicles. The optimized formulation had a narrow particle size distribution in the nanoscale range, indicating that vesicle size may be effectively controlled by optimizing formulation and process variables simultaneously.

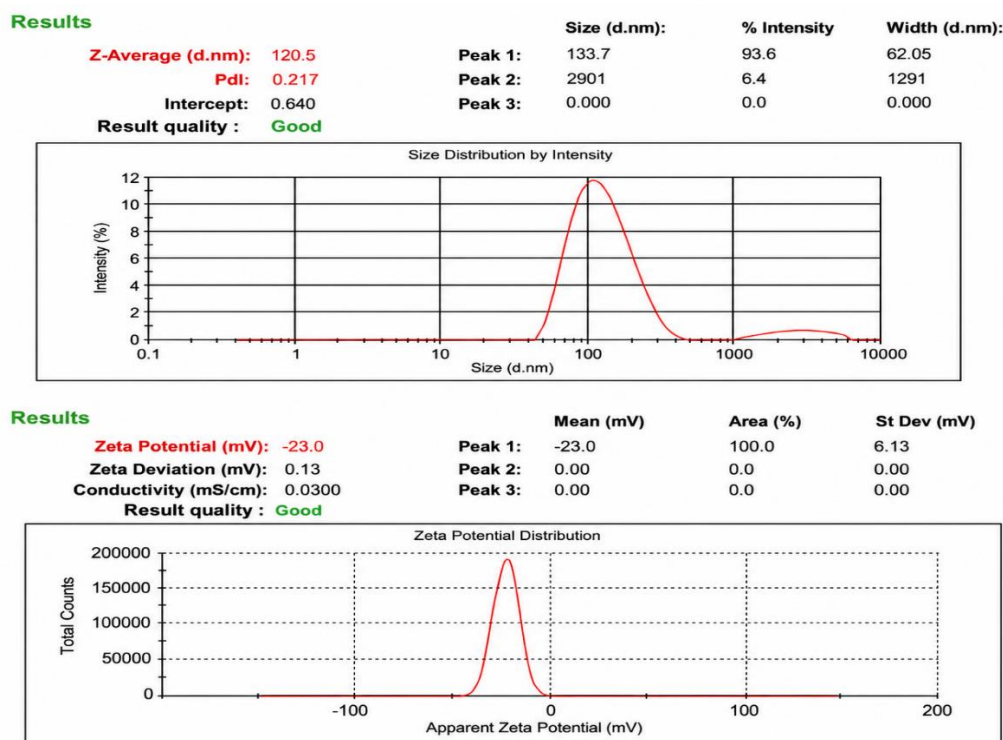


Fig. 3: Particle size and Zeta Potential Optimized Liposomal Formulation

3.2.3 Effect of Formulation Variables on Zeta Potential

The response surface plots (Figure 3) showed that lipid concentration, hydration duration, and sonication time all had an effect on the liposomal formulations' zeta potential. Lipid concentration influenced phospholipid packing and membrane organization, which influenced the electrical double layer surrounding the vesicles. Adequate hydration facilitated homogenous bilayer development and a more consistent surface charge, whereas insufficient hydration resulted in increased zeta potential variability. Moderate sonication enhanced vesicle homogeneity and colloidal stability; however, prolonged sonication may disrupt membrane organization, resulting in modest surface charge shifts. The optimized formulation had a comparatively high absolute zeta potential, indicating good electrostatic stability and a lower tendency for vesicle fusion during storage.

3.2.4 Effect of Formulation Variables on Entrapment Efficiency

The entrapment efficiency was significantly impacted by all three formulation variables (Figure 3). Lipid concentration had the largest favorable effect, as higher phospholipid content increased the liposomal bilayer's ability to encapsulate both hydrophilic metformin hydrochloride and lipophilic canagliflozin hemihydrate. Hydration duration also influenced drug encapsulation by encouraging complete bilayer hydration and vesicle formation; conversely, excessive hydration increased drug diffusion into the external medium, resulting in

decreased encapsulation. Sonication enhanced formulation homogeneity for moderate periods, while longer sonication increased membrane permeability, resulting in partial leakage of the encapsulated medicines. Response surface analysis indicated that optimizing lipid concentration, hydration duration, and sonication time concurrently was required to enhance drug entrapment while preserving optimal particle size and colloidal stability.

3.2.5 Numerical Optimization and Model Validation

The desirability function in Design-Expert® software was used for numerical optimization to produce a formulation with the smallest particle size, highest absolute zeta potential, and highest entrapment efficiency. The software's optimized formulation was tested experimentally, and the observed responses were quite close to the predicted values. The reduced prediction error demonstrated the accuracy and predictive capability of the constructed polynomial models. These findings confirmed the robustness of the optimization method and the feasibility of the proposed design space for developing the dual-drug-loaded liposomal nanoformulation. The improved formulation was then chosen for extensive physicochemical analysis, in vitro drug release, and stability evaluations.

3.3 Physicochemical Characterization of the Optimized Liposomal Nanoformulation

The optimized dual-drug-loaded liposomal nanoformulation developed utilizing the Box-Behnken design was evaluated to ensure its physicochemical qualities and appropriateness as an oral nanocarrier. The formulation was tested for particle size distribution, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM).

Table 8: Physicochemical Characterization of the Optimized Liposomal Nanoformulation

Parameter	Observed Value	Interpretation
Particle Size (nm)	151.2 ± 2.5 nm	Nanosized vesicles
Polydispersity Index (PDI)	0.168 ± 0.012	Uniform size distribution
Zeta Potential	-34.8 ± 1.4 mV	Good colloidal stability
Entrapment Efficiency (MET)	$98.4 \pm 0.5\%$	Efficient drug encapsulation
Entrapment Efficiency (CAN)	$84.0 \pm 0.6\%$	Efficient drug encapsulation
Drug Content (MET)	$99.1 \pm 0.3\%$	High drug incorporation
Drug Content (CAN)	$98.3 \pm 0.4\%$	High drug incorporation

3.3.1 Particle Size, Polydispersity Index, and Zeta Potential

The Optimized liposomal nanoformulation had an average particle size of 151.2 ± 2.5 nm, a PDI of 0.168 ± 0.012 , and a zeta potential of -34.8 ± 1.4 mV (Table 12). The nanoscale particle size and limited size distribution demonstrate that homogeneous liposomal vesicles were successfully prepared. A PDI less than 0.20 indicates excellent formulation consistency with minimal aggregation, yet the comparatively strong negative zeta potential offers adequate electrostatic repulsion to maintain colloidal stability during storage. The optimal lipid concentration, hydration conditions, and sonication time all led to the creation of uniformly distributed nanosized vesicles. Liposomes of this size range have been shown to have increased physical stability, better contact with the intestinal epithelium, and more predictable drug release behavior, making them ideal for oral drug delivery applications. Previous studies have shown that phospholipid-based liposomal formulations with particle sizes less than 200 nm and zeta potential values larger than ± 30 mV have high colloidal stability.

3.3.2 Entrapment Efficiency and Drug Loading

The optimized liposomal nanoformulation showed $98.4 \pm 0.5\%$ entrapment efficiency for metformin hydrochloride (MET) and $84.0 \pm 0.6\%$ for canagliflozin hemihydrate (CAN). Drug loading was $99.1 \pm 0.3\%$ for MET and $98.3 \pm 0.4\%$ for CAN, indicating effective incorporation of both medicines into liposomal vesicles.

The great encapsulation efficiency can be related to liposomes' amphiphilic architecture, which allows for the simultaneous inclusion of hydrophilic MET into the aqueous core and lipophilic CAN into the phospholipid bilayer. Furthermore, optimizing the lipid composition and processing parameters reduced drug leakage during vesicle formation and size reduction. Efficient drug loading reveals that the designed lipid matrix can handle both active medicinal ingredients while maintaining vesicle integrity. Similar findings have been reported for dual-drug liposomal systems, where adjusted phospholipid composition increased encapsulation efficiency and formulation durability.

3.3.3 Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy was used to determine the compatibility of the medications and formulation excipients (Figure 4). The improved formulation retained the typical absorption bands of MET and CAN, with only moderate peak broadening and wavenumber alterations. No typical peaks disappeared or new absorption bands appeared, indicating that no chemical interactions or degradation occurred during formulation.

The observed spectral alterations are attributed to weak intermolecular interactions, such as hydrogen bonding and van der Waals interactions, caused by the medicines' physical encapsulation within the phospholipid bilayer. These data validate the compatibility of the formulation components and show that both medicines' molecular integrity was maintained throughout the liposome designing procedure.

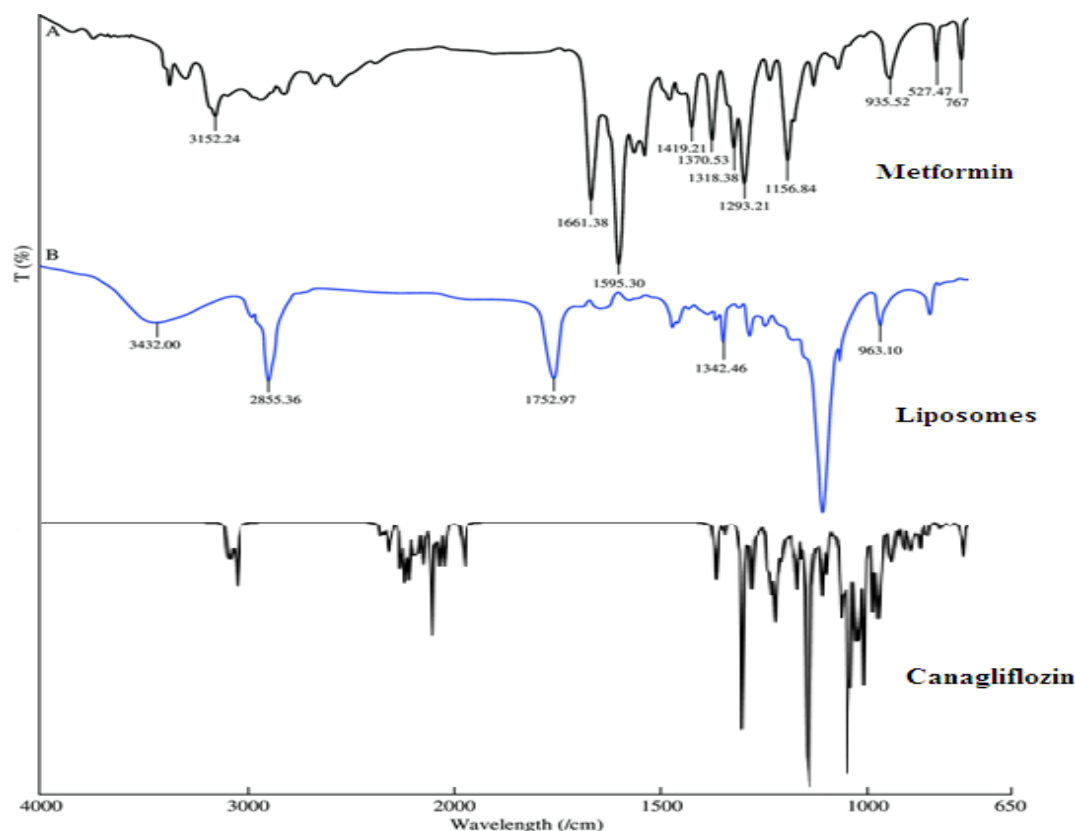


Fig. 4: FTIR spectra of metformin hydrochloride, canagliflozin hemihydrate, and the optimized liposomal nanoformulation.

3.3.4 Differential Scanning Calorimetry (DSC)

Figure 5 shows the DSC thermograms for the pure medicines and optimized formulation. Pure MET and CAN showed distinctive endothermic melting peaks due to their crystalline structure. In contrast, the improved liposomal formulation showed a significant reduction in peak intensity and broadening, showing that both medicines were successfully incorporated into the phospholipid matrix.

The decrease in crystallinity shows that the medicines are partially dispersed within the lipid bilayer, which may contribute to enhanced dissolution and long-term release. There were no more heat events identified, indicating that drug-excipient incompatibility did not occur during formulation. These observations are consistent with the FTIR results and confirm the effective encapsulation of both medicines within the liposomal nanocarrier system.

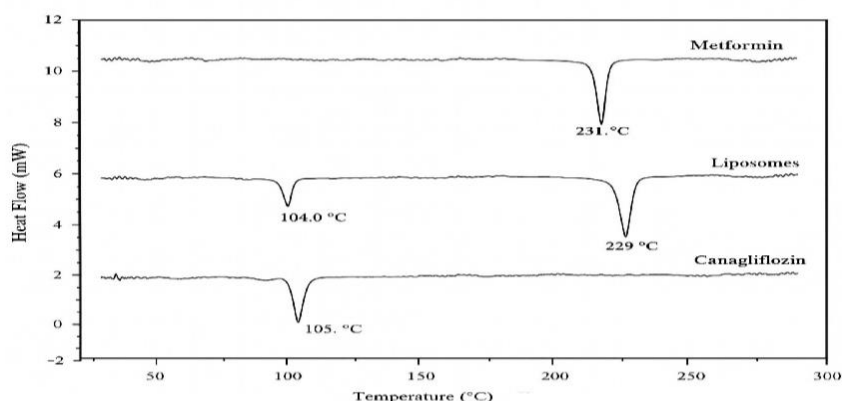


Fig. 5: DSC thermograms of metformin hydrochloride, canagliflozin hemihydrate, and the optimized liposomal nanoformulation.

3.3.5 Powder X-ray Diffraction (PXRD)

Figure 6 shows the PXRD patterns of the pure medicines and their improved formulations. Both MET and CAN showed distinctive strong diffraction peaks, indicating their crystalline structure. Following encapsulation, the improved formulation showed a significant decrease in peak intensity, followed by larger diffraction patterns, demonstrating a partial loss of crystallinity following inclusion into the lipid matrix. The reduction in crystallinity indicates that a considerable fraction of the drug molecules were in a molecularly dispersed or partially amorphous state within the liposomes, which could lead to improved dissolving properties and controlled release. No new diffraction peaks were observed, showing that liposome production did not cause undesired solid-state interactions or the development of new crystalline phases.

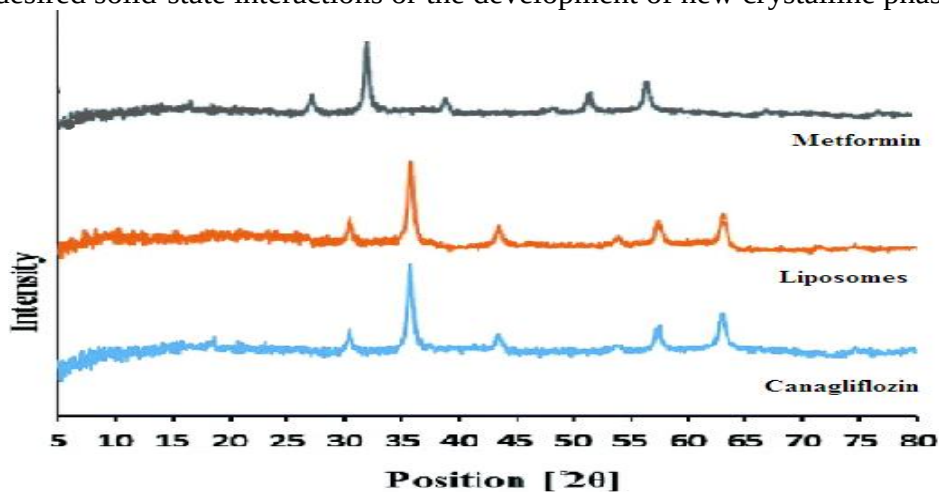


Fig. 6: PXRD patterns of metformin hydrochloride, canagliflozin hemihydrate, and the optimized liposomal nanoformulation.

3.3.6 Scanning Electron Microscopy (SEM)

SEM imaging was used to investigate the shape of the optimized liposomal formulation (Figure 7). The micrographs showed distinct spherical vesicles with smooth surfaces and consistent shape. The particles were effectively disseminated and displayed low aggregation, indicating that the thin-film hydration approach produced stable liposomes.

The observed morphology matched the dynamic light scattering data, indicating the creation of homogeneous nanosized vesicles. The absence of vesicle collapse or surface abnormalities confirms the improved formulation's structural stability and potential for long-term oral drug administration.

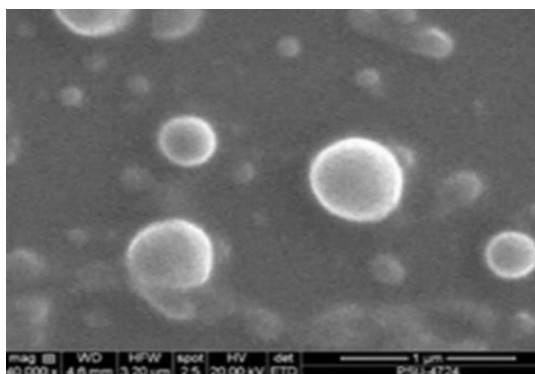


Fig. 7: SEM micrographs of the optimized dual-drug-loaded liposomal nanoformulation.

3.4 In Vitro Drug Release

In vitro release patterns of metformin hydrochloride (MET) and canagliflozin hemihydrate (CAN) from the improved liposomal formulation were examined using the dialysis membrane diffusion method in phosphate buffer (pH 7.4) at 37 ± 0.5 °C. The cumulative medication release profiles are shown in Figure 8, and the associated release data is presented in Table 9.

Table 9: Cumulative in vitro drug release profile of the optimized liposomal formulation.

Time (h)	Metformin HCl (% Cumulative Release)	Canagliflozin Hemihydrate (% Cumulative Release)
0	0.0 ± 0.0	0.0 ± 0.0
1	8.4 ± 0.6	7.1 ± 0.5
2	18.2 ± 0.9	15.5 ± 0.8
4	32.5 ± 1.1	28.7 ± 1.0
6	47.5 ± 1.3	43.4 ± 1.2
8	62.4 ± 1.4	58.2 ± 1.3
12	81.6 ± 1.3	77.5 ± 1.2
24	97.3 ± 1.5	94.1 ± 1.4

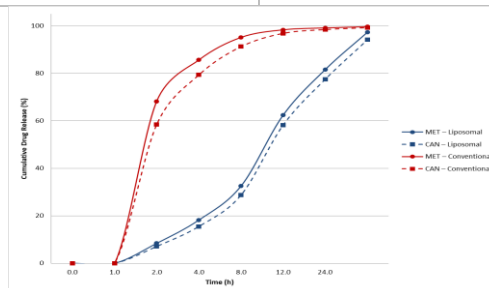


Fig. 8: In vitro drug release profiles of Metformin HCl (MET) and Canagliflozin (CAN) from optimized liposomal

The optimized liposomal nanoformulation demonstrated a distinctive biphasic release pattern, with an initial burst release followed by a sustained release phase lasting 24 hours. The trial concluded with approximately $97.3 \pm 1.5\%$ of MET and $94.1 \pm 1.4\%$ of CAN released. The initial burst release was attributed to drug molecules adsorbed or weakly linked with the liposomal surface, while the subsequent sustained release was caused by the slow diffusion of encapsulated drug molecules across the phospholipid bilayer.

The phospholipid membrane's barrier qualities and cholesterol's membrane-stabilizing impact, which lowered drug diffusion and minimized premature drug leakage, are responsible for the prolonged release behaviour. Furthermore, the dual-compartment structure of liposomes allowed for the simultaneous encapsulation of hydrophilic MET within the aqueous core and lipophilic CAN within the lipid bilayer, resulting in regulated release despite their conflicting physicochemical qualities.

Compared to the similar conventional formulations, the improved liposomal formulation had a much slower and more regulated drug release profile, indicating the efficacy of liposomal encapsulation in extending drug release. Such continuous release is predicted to reduce changes in plasma drug concentration, lower dose frequency, and increase therapeutic efficacy and patient compliance.

3.5 Drug Release Kinetics

To further understand the process of drug release, cumulative release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models. Table 9 displays the regression coefficients (R^2), whereas Figure 9 shows the kinetic graphs.

Table 10: Drug release kinetic parameters of the optimized liposomal formulation.

Kinetic Model	Mathematical Equation	R^2 (Metformin HCl)	R^2 (Canagliflozin Hemihydrate)	Interpretation
Zero-order	$Q_t = Q_0 + k_0t$	0.991	0.988	Constant release
First-order	$\log Q_t = \log Q_0 - k_1t/2.303$	0.942	0.938	Concentration-dependent release
Higuchi	$Q_t = kHt^{1/2}$	0.983	0.979	Diffusion-controlled release
Korsmeyer-Peppas	$M_t/M_\infty = ktn$	0.995	0.993	Mechanistic interpretation

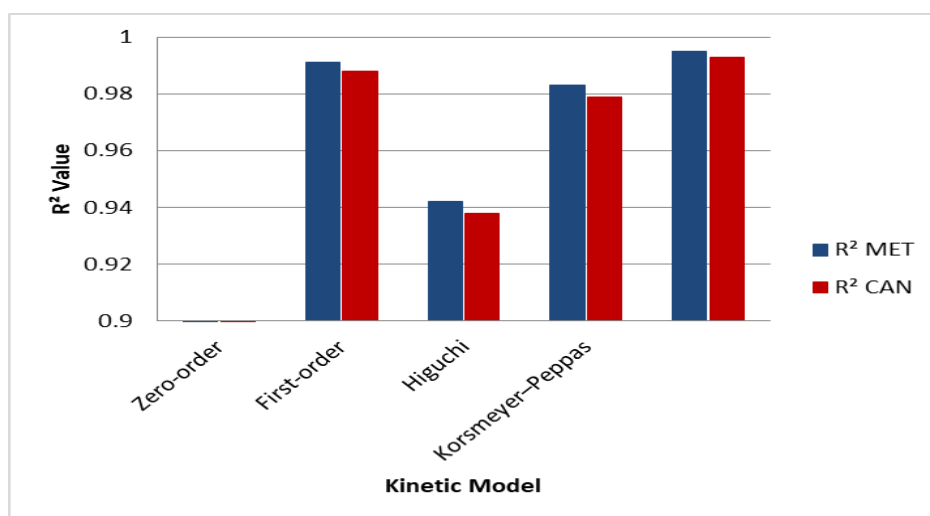


Fig. 9: Comparison of R^2 values for kinetic model fitting of MET and CAN release from optimized liposomal formulation.

The Korsmeyer-Peppas model had the greatest correlation coefficients ($R^2 = 0.995$ for MET and 0.993 for CAN), indicating a good fit for the released data. The release exponent (n) showed an unusual (non-Fickian) transport mechanism, indicating that drug release was controlled by a mix of diffusion across the phospholipid bilayer and progressive relaxation of the lipid matrix.

These data demonstrate that the improved liposomal formulation delivers regulated and prolonged drug release via diffusion-controlled transport, indicating its applicability as an oral nanocarrier option for combination therapy.

3.6 Accelerated Stability Study

The improved liposomal formulation was tested for accelerated stability using ICH guideline Q1A(R2) and stored at 40 ± 2 °C/ $75 \pm 5\%$ RH for three months. At predefined intervals, samples were tested for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and drug content. Table 11 summarizes the stability statistics, whereas Figure 10 shows the equivalent graphical depiction.

Table 11: Stability profile of the optimized liposomal formulation under accelerated storage conditions.

Parameter	Initial	1 Month	3 Months	Observation
Particle size (nm)	151.2 ± 2.5	153.6 ± 2.7	155.9 ± 3.0	No significant change
PDI	0.168 ± 0.012	0.174 ± 0.013	0.180 ± 0.015	Maintained uniformity
Zeta potential (mV)	-34.8 ± 1.4	-34.2 ± 1.5	-33.9 ± 1.6	Stable surface charge
Entrapment efficiency (%) – MET	98.4 ± 0.5	97.9 ± 0.6	97.5 ± 0.7	Minimal drug leakage
Entrapment efficiency (%) – CAN	84.0 ± 0.6	83.4 ± 0.7	82.9 ± 0.8	stable during storage
Drug content (MET) (%)	99.1 ± 0.3	98.4 ± 0.4	97.8 ± 0.5	Acceptable stability
Drug Content (CAN) (%)	98.3 ± 0.4	98.3 ± 0.4	98.3 ± 0.4	Acceptable Stability

Throughout the experiment, the formulation showed only slight variations in particle size, PDI, zeta potential, entrapment efficiency, and drug content, all of which were within acceptable ranges. The minor increase in particle size seen after storage was attributable to limited vesicle aggregation, although the change was not significant enough to compromise the formulation's physicochemical stability. Similarly, relatively minor losses in entrapment efficiency and drug content were found, indicating low drug leakage and high retention of both MET and CAN within liposomal vesicles.

The constant zeta potential and low PDI confirmed colloidal stability during the storage duration. The remarkable stability can be due to the adjusted phospholipid content and cholesterol's membrane-stabilizing impact, which combined to retain vesicle integrity and reduce structural modifications during preservation. Overall, the Optimized nanoformulation showed excellent physicochemical stability under accelerated environments, validating the robustness of the Quality by Design-based optimization technique and indicating its promise for future pharmaceutical research and development.

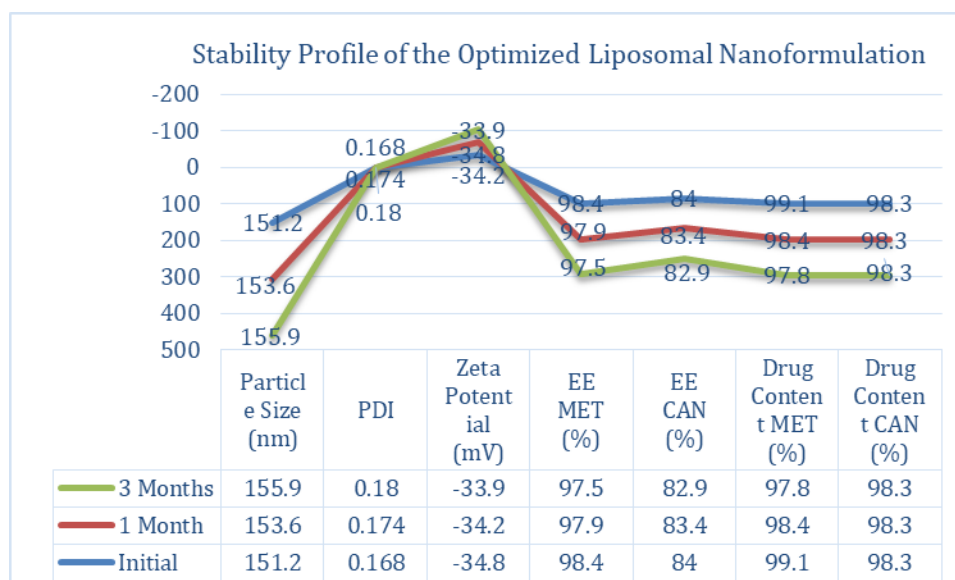


Fig. 10: Stability profile of the optimized liposomal formulation during accelerated storage.

CONCLUSION

The present research successfully design and developed a dual drug loaded liposomal nanoformulation a dual drug-loaded liposomal nanoformulation containing metformin hydrochloride (MET) and canagliflozin hemihydrate (CAN) using a systematic Quality by Design (QbD) approach.

The Box-Behnken design Using a systematic Quality by Design (QbD) approach, the authors effectively designed and optimized a dual drug-loaded liposomal nanoformulation combining metformin hydrochloride (MET) and canagliflozin hemihydrate (CAN). The Box-Behnken design enables the discovery and optimization of essential formulation and process factors, resulting in a strong formulation with desirable physicochemical properties.

The developed liposomal formulation produced nanosized vesicles with a limited particle size distribution, excellent colloidal stability, high entrapment efficiency, and effective drug loading. FTIR, DSC, PXRD, and SEM investigations indicated effective drug encapsulation inside the phospholipid matrix, with no signs of severe drug-excipient incompatibility or structural instability. The formulation showed a regulated biphasic release profile with sustained drug release for 24 hours, whereas kinetic studies revealed that drug release was mostly dominated by anomalous diffusion. Furthermore, accelerated stability testing demonstrated that the improved formulation maintained its physicochemical features and drug encapsulation efficiency under ICH-recommended storage settings, indicating good formulation stability.

A noteworthy finding of this study is the effective co-encapsulation of the hydrophilic medication metformin hydrochloride and the lipophilic drug canagliflozin hemihydrate in a single liposomal carrier. This demonstrates liposomes' potential to simultaneously deliver therapeutically complementary medicines with diverse physicochemical features, indicating a promising technique for combination therapy in type 2 diabetes mellitus.

Overall, the outcomes show that the improved liposomal nanoformulation provides a stable and efficient platform for long-term oral administration of MET and CAN. The combination of QbD principles and lipid-based nanotechnology creates a stable and scalable formulation technique that could aid future pharmaceutical development. Additional in vivo pharmacokinetic, pharmacodynamic, and long-term safety investigations are required to demonstrate the therapeutic potential and clinical application of the proposed liposomal nanoformulation.

Abbreviation Full Form

ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
BBD	Box– Behnken Design
BCS	Biopharmaceutics Classification System
CAN	Canagliflozin Hemihydrate
CMA	Critical Material Attribute
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
CV	Coefficient of Variation
DLS	Dynamic Light Scattering
DL	Drug Loading
DSC	Differential Scanning Calorimetry
EE	Entrapment Efficiency
FTIR	Fourier Transform Infrared Spectroscopy
HCl	Hydrochloride
HPLC	High-Performance Liquid Chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
MET	Metformin Hydrochloride
MLV	Multilamellar Vesicle
MWCO	Molecular Weight Cut-Off
NLC	Nanostructured Lipid Carrier
PBS	Phosphate Buffered Saline
PDI	Polydispersity Index
PXRD	Powder X-ray Diffraction
QbD	Quality by Design
RH	Relative Humidity
R ²	Coefficient of Determination
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
SGLT2	Sodium– Glucose Cotransporter-2
T2DM	Type 2 Diabetes Mellitus
UV	Ultraviolet
WHO	World Health Organization
XRD	X-ray Diffraction

DECLARATION OF THE USE OF ARTIFICIAL INTELLIGENCE

The authors exclusively utilized quillbot for language refining and grammar correction when working on this project. After utilizing this tool, the authors carefully reviewed and modified the content as needed, and they accept full responsibility for the manuscript's integrity, accuracy, and originality.

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The authors contributed equally to this article. First authors contributed to data gathering, analysis, and manuscript drafting. The second author contributed to the core idea, study design, and critical revisions.

Conflict of Interests

The Authors declares no conflict of Interest regarding this research work.

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