ORIGINAL ARTICLE

Nano Lipid Carrier System Development for Dapagliflozin: Central Composite Design Approach

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Abstract

Original Research: The content involves original experimental work on the development of a nano lipid carrier system for Dapagliflozin using a central composite design approach. **Methodology:** It describes specific methodologies, such as the central composite design (CCD), and includes experimental data and results. **Objective Analysis:** The aim is to optimize a drug delivery system, which typically falls under the category of research articles in scientific journals.

Key words: Nano Lipid Carrier, Dapagliflozin, Central Composite Design (CCD) and Drug Delivery System

INTRODUCTION

In recent years, solid lipid nanoparticles (SLNs) have emerged as a hopeful drug delivery system for enhancing the therapeutic efficacy of poorly water-soluble drugs. Dapagliflozin, a sodium-glucose co-transporter 2 inhibitor widely used in treating type 2 diabetes mellitus, presents inherent challenges due to its poor aqueous solubility.[1,2] Encapsulating dapagliflozin within SLNs offers a compelling solution to address this limitation. By leveraging the encapsulation capabilities of SLNs, dapagliflozin's solubility can be significantly enhanced, thereby improving its dissolution rate and overall bioavailability.[3] In addition, SLNs allow controlled and sustained release of dapagliflozin, aligning well with its once-daily dosing regimen and ensuring prolonged therapeutic effects.[4]

Moreover, SLNs can be tailored for targeted delivery, potentially concentrating dapagliflozin at its site of action in the kidneys, thus maximizing its pharmacological effects while minimizing systemic side effects. Furthermore, the stability of dapagliflozin can be preserved through encapsulation within SLNs, safeguarding its potency during storage and transportation.[5] Overall, the formulation of dapagliflozin into SLNs represents a promising approach to enhance its therapeutic outcomes, offering improvements in solubility, bioavailability, sustained release, targeted delivery, and stability, thereby advancing the type 2 diabetes mellitus treatment.[6,7]

These SLNs are an innovative carrier system that offers an alternative to polymeric nanoparticles, liposomes, and oil-in-water emulsions.[8-10] These aqueous dispersions are stabilized by suitable surfactants and composed of lipids that remain solid at both room and body temperatures. Compared to polymeric nanoparticles, SLNs present distinct advantages, particularly in topical and oral drug delivery, where the same lipids can serve as the matrix material. In addition, the wide variety of surfactants and stabilizers used in conventional formulations can also be employed in SLNs, ensuring compliance with regulatory standards for excipients.[11,12]

Lipid nanoparticles have been widely researched for percutaneous drug delivery and offer notable advantages over other colloidal delivery systems. SLNs, in particular, stand out due to their biocompatibility, scalability, and capacity to modulate drug release, enhancing overall performance. These qualities make SLNs an attractive option for optimizing oral drug delivery.[13,14]

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The literature contains limited reports on using SLNs to bypass first-pass metabolism. However, notable studies have shown significant improvements in oral bioavailability when drugs are loaded into SLNs. For example, the oral bioavailability of all-trans retinoic acid in rats increased four- to five-fold when delivered through SLNs compared to a suspension.[15] Similarly, SLNs enhanced the oral bioavailability of cryptotanshinone. In addition, research has examined the pharmacokinetics and tissue distribution of clozapine-loaded SLNs following intraduodenal administration.[16]

In this study, dapagliflozin-loaded SLNs were prepared using stearic acid as the lipid, Tween 80 as the surfactant, and soya lecithin as the co-surfactant through a hot homogenization followed by the ultra-sonication method. The SLNs were characterized, and the optimized formulation was evaluated for its potential to improve the oral bioavailability of dapagliflozin.[17]

MATERIALS AND METHODS[9]

Materials

Dapagliflozin was generously provided as a gift sample by Aurobindo Labs, Hyderabad. Stearic acid and palmitic acid were procured from Sigma-Aldrich, Merck, whereas soya-lecithin was sourced from SRL Laboratory Pvt. Ltd. Poly sorbate 80 was obtained from LobaChemie Pvt. Ltd., and Poloxamer-188 was acquired from Himedia, Mumbai. Chloroform was purchased from Qualigens, India, and methanol from Rankem, India. The dialysis membrane utilized in the study was sourced from HiMedia, Mumbai. All other reagents used were of analytical grade.

Screening of lipids[18]

The solid lipids for formulating dapagliflozin were chosen based on the drug's maximum solubility. To determine the drug's solubility, it was tested in various solid lipids, including, palmitic acid, glyceryl monostearate, stearic acid, and soya lecithin. One gram of each solid lipid was individually placed in a glass beaker and heated on a magnetic stirrer to a temperature exceeding 10°C over the melting point of the lipid. The drug was then added gradually in specified amounts with small increments while maintaining constant stirring. The mixture was stirred continuously for 30 min after each addition to ensure the drug completely dissolved. The clarity and transparency of the mixture were monitored during this process. A loss of transparency indicated the drug's saturation point in the lipid.

Surfactant screening for size and stability assessment[19]

In selecting the most appropriate surfactant, our focus centered on its impact on the formulation's size and stability. We conducted screening with various surfactants featuring different hydrophilic-lipophilic balance (HLB) values. Polysorbate 80 HLB value was found 15.2, along with Poloxamer 188, sodium deoxycholate, and Span 80, which was selected for evaluation based on its HLB value. These surfactants were assessed for their impact on the size and stability of blank nanostructured lipid carriers (SLNs). The stability was determined by monitoring phase separation/creaming or floccule formation in the placebo formulation. This systematic approach allowed for a comparative assessment of surfactants based on their ability to maintain the desired particle size and stability of the SLN formulation.

Experiment design (Response surface methodology [RSM])[20]

RSM, specifically employing a three-level approach, was utilized for experimental design and formulation optimization of SLNs dapagliflozin intended for oral drug delivery. Design Expert® software (Version 13.0.5.0, State-Ease Inc., and India) was employed for this purpose. Based on previous literature and variables in the formulation, the most suitable design for analyzing quadratic response surfaces was found to be the central composite design (CCD) over the linear responses, two-factorial interactions, and polynomial models. The CCD, requiring only 14 runs with 4 replicated center points, facilitated process optimization. By utilizing a computer-generated non-linear polynomial model quadratic equation, the three-factor three-level design was elucidated. This systematic approach enabled the optimization of SLN formulation parameters, focusing on desired characteristics for oral drug delivery.[21]

$$\text{Polynomial equation } (Y) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_{12} + b_{22}X_{22} + b_{33}X_3 \quad (1)$$

In the developed model, Y represents the dependent variable, with b_0 representing the intercept and b_1 to b_{33} denoting the regression coefficients found from individual responses. The independent variables are represented by X_1 to X_3. The prefixed variables corresponded to coded levels in the CCD. Specifically, X_1 represents the percentage weight of stearic acid, X_2 represents soya lecithin, and X_3 represents polysorbate 80. Furthermore, terms such as X_i^2 (where i = 1, 2, or 3) depict the interaction of independent variables, whereas the quadratic terms are denoted by X_i^2. Table 1 defines the encoded values and levels of the independent and dependent variables. This structured approach facilitates the interpretation and manipulation of variables in the regression analysis, aiding in the optimization of the SLN formulation process.

Preparation of SLNs loaded with dapagliflozin[22]

Dapagliflozin-loaded SLNs were prepared through a sequential process involving hot homogenization followed by ultra-sonication. Table 2 shows the formulation composition overview. Initially, the required amount of stearic acid as the

solid lipid and the emulsifier (soya lecithin) were dissolved in a 1:1 ratio of 10 mL mixture of methanol and chloroform. The organic solvents were completely removed using a rotary flash evaporator. The resulting lipid layer was melted by heating to approximately 5°C above the lipid's melting point.

The stabilizer (polysorbate 80) was dissolved in distilled water (1.5% w/v) to prepare an aqueous phase, heated to match the oil phase's temperature. Subsequently, the hot aqueous phase was added to the oil phase, and homogenization was conducted for 5–10 min at 12,000 rpm using a homogenizer (model T-18 D, IKA T18, Ultra-Turrax, Germany). The coarse oil-in-water emulsion obtained was subjected to sonication using a probe sonicator (Model No. HV-PRO-650) for 20 min. The dapagliflozin-loaded SLNs were obtained by allowing the hot nanoemulsion to cool to room temperature (RT). Subsequently, lyophilization was carried out until the nanoemulsion dried.

In this present work, dapagliflozin-loaded SLNs were fabricated using an RSM approach. The study design employed a randomized, non-block, central composite, model quadratic with 3 factors of non-dependent variables at three levels (−1, 0, and +1). The experimental data are summarized in Table 1.

SLNs characterization

Fourier transform infrared (FTIR) studies for drug-excipient interactions[23]

Briefly, FTIR spectroscopy analysis was conducted to investigate potential interactions and compatibility between the drug and lipids used in SLNs preparation. An FTIR spectrometer from Bruker, India, was utilized for the analysis. Dapagliflozin samples were blended with potassium bromide (KBr), and FTIR spectra were recorded for the pure drug, physical mixture of the drug and lipid, and surfactant mixture. Scanning was performed in transmission mode across a wavenumber range from 4000 to 400 cm^{-1}.

Differential scanning calorimeter (DSC) characterization[24]

DSC analysis was performed using the Mettler-Toledo DSC 821e instrument in Columbus, OH, USA. DSC scans were

Table 1: Dapagliflozin-loaded SLNs: Model variables and central composite design coded levels

Name of variables			
In dependent (%w/v)	Stearic acid (X1)	Soya Lecithin (X2)	Polysorbate 80 (X3)
Dependent (Responses)	Particle in size nm (Y1)	PDI (Y2)	Entrapment efficiency (%) (Y3)
Coded levels	Low	Centre	High
	−1	0	+1
A: Stearic acid (X1)	5	7.5	10
B: Soy Lecithin (X2)	2.5	3.75	5
C: Polysorbate 80 (X3)	1.5	1.75	2

Table 2: Experimental runs conducted employing central composite design

Run	**Independent variable (%w/v)**		
	Stearic acid (X1)	**Soya Lecithin (X2)**	**Polysorbate 80 (X3)**
1	7.5	3.75	1.75
2	5	5	2
3	7.5	5	1.75
4	10	5	1.5
5	10	3.75	1.75
6	7.5	3.75	1.75
7	7.5	2.5	1.75
8	7.5	3.75	1.75
9	7.5	3.75	2
10	5	2.5	1.5
11	7.5	3.75	1.5
12	5	3.75	1.75
13	7.5	3.75	1.75
14	10	2.5	2

conducted for all combinations of drug and lipid, employing a heating rate of 10°C/min over a temperature range of 50–250°C.

Measurement of particle size (nm), polydispersity index (PDI), and zeta potential (ZP) of SLN[25]

Size, PDI, and ZP of dapagliflozin-loaded SLNs were evaluated utilizing a Malvern Zetasizer (Nano ZS90, UK). Approximately 100 µL of the prepared SLN dispersion was diluted to 5 mL with double-distilled water before being subjected to analysis using the zeta sizer.

Determination of entrapment efficiency (EE) (%)[26]

To determine EE, free drug concentration in an aqueous medium was quantified using ultrafiltration with centrisart tubes (Sartorius, USA). After centrifugation, SLNs containing the drug remained in the outer chamber while the aqueous phase migrated to the recovery chamber. Dapagliflozin concentration in the aqueous phase was analyzed using high-performance liquid chromatography (HPLC).

In vitro drug release studies[27]

In vitro release studies were conducted using the dialysis method with a Himedia membrane (India) featuring a pore size of 2.4 nm and a molecular weight cutoff between 12,000 and 14,000. The membrane was pre-soaked overnight in double distilled water. Phosphate buffer pH 6.8 served as the release medium. The experimental setup included donor and receptor compartments. In the donor compartment, 1 mL of SLN dispersion was placed in a boiling tube tied to the dialysis membrane. The receptor compartment, a 250 mL beaker filled with 100 mL release medium, was maintained at 37 ± 0.5°C. Samples were withdrawn at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24-h intervals and replaced with an equal volume of release medium. Samples were diluted and analyzed at 224 nm using an ultraviolet-visible spectrophotometer (SL-150, ELICO, and India).

Drug release kinetics and mechanism analysis

To ensure the drug release kinetics and mechanisms of dapagliflozin-SLNs, we systematically evaluated the *in vitro* dissolution profiles for optimized formulation. These profiles underwent analysis using a range of appropriate models, including zero-order kinetics, first-order kinetics, Higuchi's plot, and the Korsmeyer–Peppas (K-P) model. The statistical tool DDSolver software facilitated this analysis.[28]

In this assessment, crucial parameters such as the adjusted regression values (r^2 adjusted), the range of the Akaike Information Criterion (AIC), and the Model selection criterion (MSC) values played a pivotal role.[29] These parameters generated distinct values that formed the basis of comparison. By analyzing these values, it became possible to determine the best-fitting models that elucidate the release order behavior for the optimized formulation. Insights into the kinetics and mechanisms governing drug release from the selected formulations are provided by this systematic approach.

Physical stability studies[30]

The optimized formulation of dapagliflozin-loaded SLNs was stored at RT (25°C, 60 ± 5% RH) and refrigerator temperature was maintained at (4°C) for 60 days. The average size (nm), ZP (-mV), PDI, and EE (%) were determined in triplicate.

Statistical analysis

All experiments were conducted in triplicate and reported values to represent the average of three measurements with standard deviation (±SD). Statistical analysis involved comparing various study groups using ANOVA for analysis of variance. Design Expert® (Version 13.0.5.0, State-Ease Inc., and India) and DDSolver 1.0 (Microsoft Corp., U.S.A.) were utilized for statistical analysis of different parameters. A significance level of $P < 0.05$ was considered.

RESULTS AND DISCUSSION

Lipid and surfactant screening

We concluded that stearic acid, when combined with soya lecithin and polysorbate 80, constitutes the highly favorable solid lipid for drug-loaded SNPs. This conclusion stems from its high solubility, biocompatibility, and stability. Soya lecithin plays a key role in stabilizing nanoparticles, facilitating the encapsulation of both drugs that have lipophilic and hydrophilic natures. In addition, polysorbate 80 enhances drug release kinetics and prevents aggregation, thereby ensuring efficient drug delivery.

Physicochemical characterization

Employing FTIR analysis, potential interactions between drugs and SLN components were investigated. The study focused on peak intensity and peak shifting to assess compatibility among pure drug and excipient interactions in optimized dapagliflozin-SLNs. All the results are depicted in Figures 1 and 2.

Pure dapagliflozin displayed characteristic peaks was measured at 3352.08 cm^{-1} (OH stretching), 1625.27 cm^{-1} (aromatic C=C), and C-O ester stretching 1286.17 cm^{-1}. In addition, common peaks generated from dapagliflozin included a 1039.04 cm^{-1} and 1025 cm^{-1} recorded for C-Cl bond, a 3318.38 cm^{-1}peak for O-H, and a C-C bond noted at 1608.37 cm^{-1}.

In dapagliflozin-SLNs (optimized formulation) spectra, characteristic peaks for O-H stretching were found at

3354.02^{-1}, C=O stretching was 1285.37cm^{-1}, and aromatic C=C stretching 1595.27 cm^{-1} was identified. Furthermore, shifts in the peaks of lipid's C=O were recorded at 1607.28 cm^{-1} and dapagliflozin's aromatic C=C was found at 1625.47 cm^{-1}. Figure 2 illustrates the FTIR spectra of dapagliflozin (pure API), with a physical mixture. Notably, the optimized dapagliflozin formulation's spectra contained all functional group peaks observed in the pure (dapagliflozin') spectra, there with no additional peaks was detected. This indicates no interaction between the drug and excipients used in manufacturing dapagliflozin-loaded SLNs.

DSC thermograms were investigated for both the pure API form of dapagliflozin and the optimized formulation of dapagliflozin loaded with SLNs containing the drug with selected lipids, surfactants, and co-surfactants, as illustrated in Figures 3 and 4.

Figure 3 displays the DSC thermogram of the pure drug dapagliflozin, which exhibited an onset peak of endothermic at 190.09°C, indicating its melting point. This peak corresponds to the drug's crystalline nature and purity. In contrast, Figure 4 shows the DSC thermogram for the optimized formulation, which includes dapagliflozin, and all excipients. The thermogram reveals both wide and sharp endothermic peaks, with a significant one observed very close to 190.15°C.

From these observations, it can be resolved that the onset of the endothermic melting points of both with other additives is very similar. Consequently, these DSC studies did not show significant variations that could not be distinctly differentiated between the pure drug and its mixture in terms of thermal behavior. Hence, the DSC results indicate that there were no substantial interactions or modifications in the

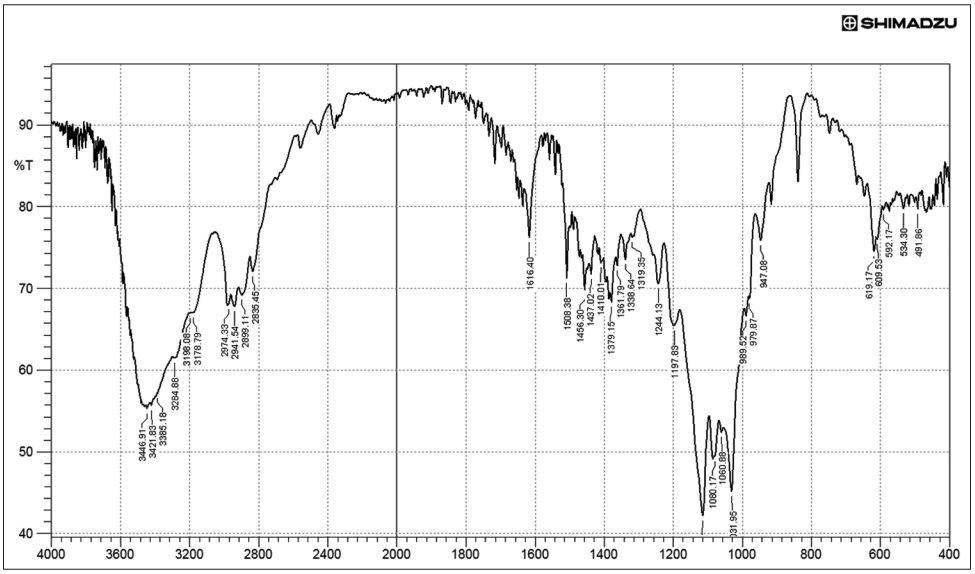

Figure 1: Fourier transform infrared spectra of pure dapagliflozin

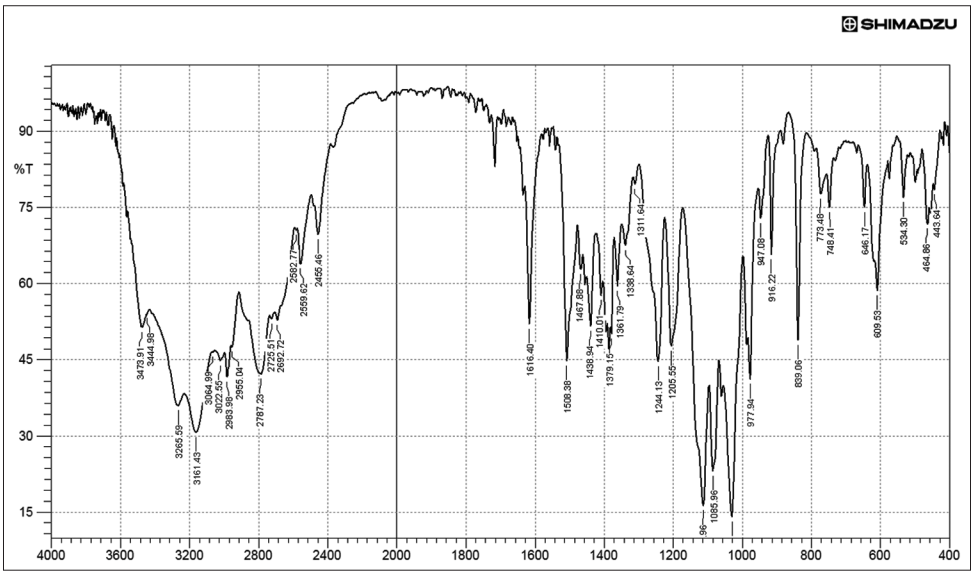

Figure 2: Fourier transform infrared spectra of dapagliflozin solid lipid nanoparticle's optimized formulation

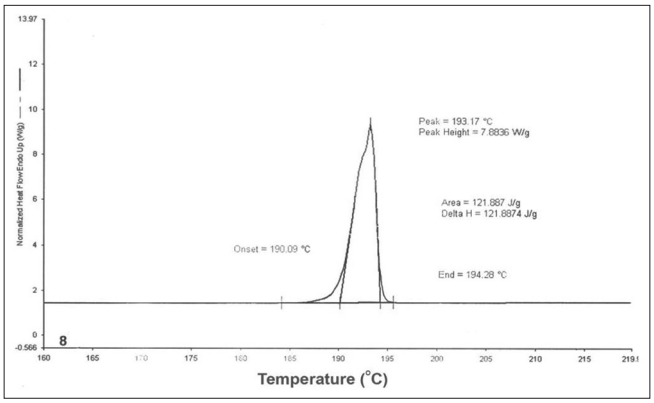

Figure 3: Differential scanning calorimeter thermograph of pure dapagliflozin

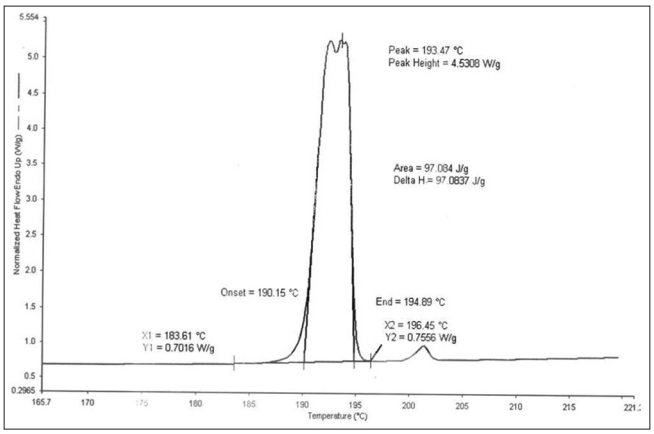

Figure 4: Differential scanning calorimeter thermogram of optimized dapagliflozin solid lipid nanoparticle optimized formulation

melting characteristics of dapagliflozin when combined with the selected components.

X-ray diffraction (XRD) crystallography

Powder-XRD analysis confirmed the molecular dispersion state of the drug using the established formulation method. It was additionally used to explore the polymorphic behavior and crystallinity of dapagliflozin. Figures 5 and 6 depict the diffraction patterns of the optimized SLNs compared to pure dapagliflozin. The pure XRD spectra exhibited multiple distinct, intense peaks recorded at diffraction angles (2 theta scale) against d-spacing values. The peaks were namely 12.5 (d-10.25), 13.5 (d-8.21), 20.2 (d-4.399), 21.9 (d-2.045), 21.58 (d-1.287), 28.9 (d-3.748), and 30.15 (d-1.541) and number of peaks were observed more.

In the case of dapagliflozin SLNs optimized formulation, the intensity of the XRD peaks pattern was obtained at 11.91 (d-8.21), 19.8 (d-2.41), 20.1 (d-1.258), and 17.54 (d-1.028); this would be significantly different from the pure API and also numbers of peaks were recorded at low with respect to the pure dapagliflozin. In addition to that, the model peaks disappeared as in the case of optimization formulation. The results are depicted in Figures 5 and 6.

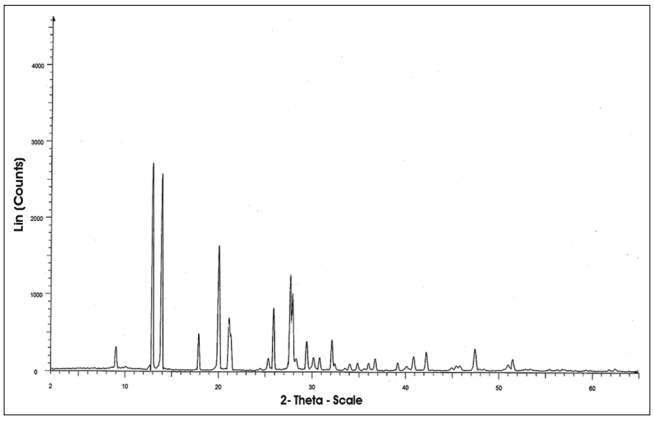

Figure 5: X-ray diffraction spectra of pure dapagliflozin

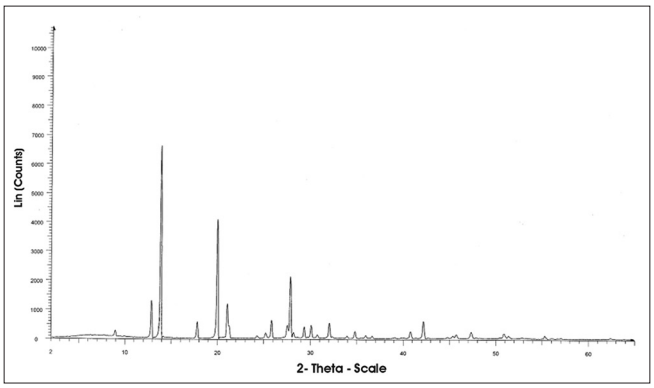

Figure 6: X-ray diffraction spectra of optimized dapagliflozin solid lipid nanoparticle

From the above observation, the optimized formulation of dapagliflozin resulted in a significant disruption of its crystalline nature, shifting it toward an amorphous stated due to several reasons as like, this transformation may enhance drug solubility and dissolution kinetics within the lipid matrix of SLNs, potentially improving the formulation's efficacy.

The optimized formulation of dapagliflozin shifted its crystalline nature significantly toward an amorphous state. This transformation could be attributed to various factors, such as the lipid matrix, potentially high in the amorphous phase, which played a role in disrupting dapagliflozin's crystalline structure or processing techniques such as high-pressure homogenization or solvent evaporation applied mechanical or thermal stress on dapagliflozin crystals and also, this study includes the interactions between dapagliflozin and lipid components of the formulation might have contributed to the disruption. Incorporating dapagliflozin into the matrices of lipids could have altered the packing arrangement of drug molecules, prompting the transition from crystalline to amorphous.

Overall, the disruption of dapagliflozin's crystalline nature and its shift toward an amorphous state in the optimized formulation suggest the successful incorporation of the drug into the lipid matrix of the SLNs. This transformation may

have implications for the formulation's drug release profile, bioavailability, and therapeutic efficacy.

Particle size (nm) PDI and ZP (mV) and EE (%)

All the experimental formulations[14] underwent comprehensive analysis to ascertain their particle size distribution, ZP, and PDI values, as outlined in Table 3. The particle size across all formulations ranged from 153 ± 0.23 nm to 179 ± 0.31 nm, while PDI varied from 0.241 ± 0.23 to 0.424 ± 0.31 and ZP spanned from −18.4 ± 0.14 to −23.6 ± 0.24. In addition, determining the EE (%) is crucial for characterizing SLNs, analyzed for each formulation using HPLC. Based on the results obtained, all formulations demonstrated excellent EE, ranging from 83.60 ± 0.13 to 91.78 ± 0.22. Notably, all the recorded values were fall within acceptable ranges. Based on these findings, it was confirmed that the selected independent variables significantly influenced the characteristics of the solid lipid nanoformulations. Consequently, the obtained results warranted further assessment to govern the optimized formulation through CCD.

Application of factorial design for optimization of dapagliflozin-SLNs formulations

The data were analyzed by fitting it into various models, resulting in actual, adjusted, and predicted regression coefficients. These coefficients were closely similar for each response variable. To select the best-fit model, we aimed to keep the difference between the adjusted r^2 value and the predicted r^2 value under 0.2.

The analysis revealed that a quadratic model was the most suitable representation for the responses. It showed that particle size, PDI, and EE of SLNs were influenced by the levels of lipid, surfactant, and co-surfactant. These factors interactions also played a significant role, as indicated by the quadratic model.

To delve deeper, we proposed further assessment through ANOVA, contour plots, and response plots to understand the relationship between the responses and variables. The selected best-fit models for the responses showed significance, with $P < 0.001$.

Particle size (Y1)

We evaluated the impact of lipid, surfactant, and co-surfactant on particle size. Statistical analysis, ANOVA, was conducted to understand this relationship. The model quadratic provided the best fit with a $P < 0.001$, and a lack of fit non-significant test indicated that the model accurately represents the data. The ANOVA results revealed a significant influence of the selected variables, with high sum square values for the model and linear responses, indicating a strong overall factor influence. Interaction terms (AB, AC, and BC) also showed significant effects and high mean square values confirmed the substantial impact of the preferred variables on particle size. The polynomial equation for particle size response is shown below.

$$\text{Particle size} = +12.00\,A + 1.00\,B - 1.50\,C - 0.7500\,AB - 2.25\,AC + 1.25\,BC + 8.05\,A^2 + 0.0455\,B^2 - 0.4545C^2 \quad (2)$$

Stearic acid exhibited larger coefficient values of 12.00 for A and 8.05 for A^2 in the polynomial equation, indicating its substantial impression on particle size in SLNs. The coefficient sign positive suggests that increasing stearic acid concentration leads to proliferation in particle size.

Table 3: Particle size, PDI, zeta potential, and entrapment efficiency (%) of dapagliflozin SLN's formulations

Runs	Particle size (nm)	PDI	zeta potential (mV)	Entrapment efficiency (%)
1	158 ± 0.01	0.247 ± 0.31	−19.3 ± 0.21	86.62 ± 0.11
2	156 ± 0.05	0.320 ± 0.22	−20.4 ± 0.23	83.60 ± 0.13
3	158 ± 0.25	0.256 ± 0.14	−18.4 ± 0.14	87.76 ± 0.24
4	179 ± 0.31	0.410 ± 0.35	−21.6 ± 0.13	91.78 ± 0.22
5	177 ± 0.14	0.302 ± 0.25	−22.5 ± 0.17	91.24 ± 0.28
6	155 ± 0.21	0.250 ± 0.21	−23.6 ± 0.24	87.56 ± 0.24
7	156 ± 0.33	0.291 ± 0.14	−19.5 ± 0.24	86.74 ± 0.16
8	156 ± 0.02	0.241 ± 0.23	−23.2 ± 0.33	88.78 ± 0.33
9	155 ± 0.15	0.285 ± 0.15	−19.6 ± 0.17	87.04 ± 0.35
10	151 ± 0.17	0.410 ± 0.21	−20.5 ± 0.26	83.27 ± 0.41
11	158 ± 0.21	0.267 ± 0.16	−19.7 ± 0.24	87.66 ± 0.26
12	153 ± 0.23	0.390 ± 0.21	−21.7 ± 0.08	83.50 ± 0.38
13	157 ± 0.24	0.266 ± 0.18	−22.5 ± 0.16	87.09 ± 0.36
14	171 ± 0.23	0.424 ± 0.31	−20.9 ± 0.44	90.32 ± 0.37

±SD values $n = 3$

This phenomenon can be attributed to stearic acid's larger molecular size compared to other lipid components commonly used in SLNs. Its long hydrocarbon chains enable molecules to pack densely together. Consequently, higher concentrations of stearic acid result in more of these larger molecules being integrated into the nanoparticles, leading to increased packing density and larger particle sizes overall. This efficient packing facilitates swift particle size increase, as depicted in Figures 7 and 8.

The contour plot and 3D surface plot demonstrate that smaller particle sizes for dapagliflozin SLN lipid nanoparticles are achieved when lipid concentrations are from 5% to 10%, surfactant concentrations range from 2.5% to 5%, and co-surfactant concentrations range from 1.5% to 10%. These plots vividly illustrate the complex interactions between lipid-surfactant, lipid-co-surfactant, and surfactant-co-surfactant, offering valuable insights for optimizing formulations to achieve desired smaller particle sizes.

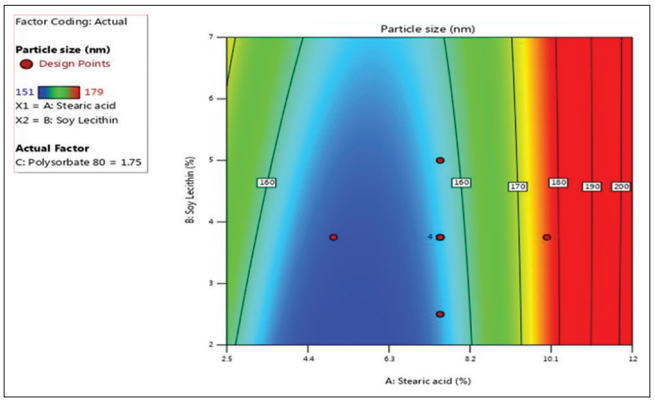

Figure 7: 2D contour plot of independent variables influencing dapagliflozin solid lipid nanoparticle particle size

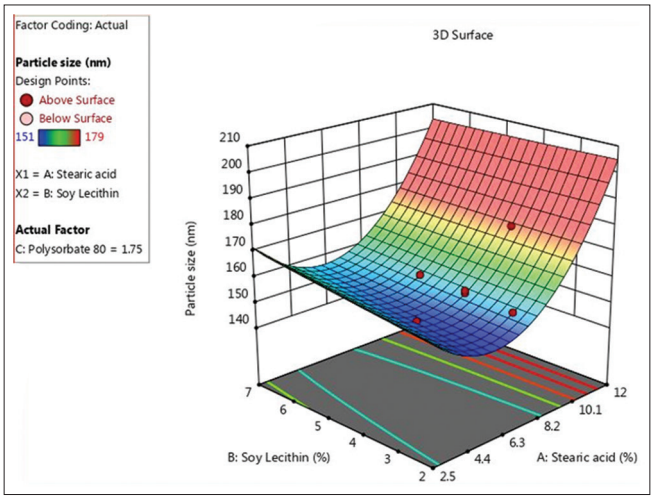

Figure 8: 3D response surface methodology plot of independent variables influencing dapagliflozin solid lipid nanoparticle particle size

PDI Y2

The analysis systematically evaluated the impact of independent variables lipid, surfactant, and co-surfactant on the PDI, followed by a rigorous statistical examination to extract key insights. ANOVA was performed on the PDI response across multiple experimental runs, yielding significant findings. The quadratic model emerged as the most suitable fit for the data, supported by a highly significant $P < 0.001$. A non-significant lack of fit test is preferred, indicating the model accurately represents the relationship between independent and dependent variables, facilitating reliable predictions and valid inferences.

ANOVA revealed significant influences of the overall factor (quadratic response) and interaction terms, particularly for A and BC, shown by the high sum of squares (SS) values. Elevated mean square values highlighted the strong impact of independent variables on the dependent variable. Specifically, surfactant and co-surfactant interactions significantly affected the PDI, with high F-values for A (44.99) and BC interaction (75.91).

$$PDI = -0.0440\,A - 0.0175\,B + 0.0090\,C + 0.0280\,AB + 0.0085\,AC - 0.0700\,BC + 0.0941\,A^2 + 0.0216\,B^2 + 0.0241C^2 \quad (3)$$

From the above polynomial equations, the components of A and BC were shown significant negative sign effects on the dependable response of PD. This negative coefficient sign suggests that increasing the concentrations of stearic acid and the surfactant to co-surfactant ratio leads to a decrease in the PDI of the fabricated SLNs.

In general, surfactants and co-surfactants play crucial roles in reducing the PDI of nanoparticles through several mechanisms. They form a protective layer around nanoparticles, preventing aggregation and ensuring uniformity in particle size distribution, thereby lowering PDI. In addition, by reducing the surface tension of the formulation medium, they enhance nanoparticle dispersion, minimizing the tendency for particles to aggregate and form larger sizes. Moreover, these agents influence the kinetics of particle growth, regulating the formation of nanoparticles to maintain a more homogeneous size distribution and minimize the occurrence of larger particles, thus further reducing PDI. Furthermore, their interaction with lipid components affects the structure and stability of nanoparticles. Optimizing their composition can lead to a more uniform particle size distribution and lower PDI. The results are depicted in Figures 9 and 10.

EE (%) Y3

The study systematically assessed the influence of independent variables – Lipid, surfactant, and co-surfactant – on EE (%), followed by comprehensive statistical analysis to uncover key findings. ANOVA results were conducted

on the EE (%) response from multiple experimental runs, revealing significant insights. The resulting ANOVA results summarizing the influence of lipid, surfactant, and co-surfactant on EE are presented below.

Statistical analysis identified the quadratic model as the best fit ($P < 0.001$), confirming its accuracy in representing the data. ANOVA showed substantial influences from the overall factor (linear response) and interaction terms, with factor A being particularly significant (SS = 87.94, F = 317.73). Elevated mean square values further emphasized the strong impact on the dependent variable.

Based on the observations, it was concluded that factor A significantly influences EE, while factors B and C do not. In addition, no significant interaction effects (AB, BC, and AC) were observed on EE. The relationship between stearic acid concentration and EE in nanoparticles can be attributed to several key factors. Higher stearic acid levels increase its ability to encapsulate the active ingredient, leading to improved EE. Optimal stearic acid concentrations also promote the formation of smaller nanoparticles, this provides increased surface area for encapsulation. Furthermore, stearic acid stabilizes and forms nanoparticles, ensuring efficient encapsulation and its interactions with other formulation components also contribute to its role. Overall, higher stearic acid concentrations generally result in better EE due to its vital roles in nanoparticle formation and encapsulation.

$$EE\ (\%)\ Y3 = +3.83\ A + 0.468\ B - 0.2917C \quad (4)$$

From the above polynomial equations, the components of A were shown a significantly positive co-efficient sign effect on EE. This positive coefficient recommended, that as the amount of stearic acid increases, the entrapment of the core drug into the nanoparticle will increase. The results are depicted in Figures 11 and 12.

Selection of optimized batch as a function of desirability of all response variables

The selection of the best and most optimized formulation relied on the desirability response, a key function in Design Expert software. This approach played a crucial role in identifying the most reliable formulation. The desirability graph in the software combined various responses from dependent variables, thus facilitating the creation of an ideal formulation with the desired physicochemical properties.

For optimization purposes, the desirability is scaled from 0 to 1. It aligned the values of individual response variables and was applied to evaluate each solution from 1 to 21 based on considerations of the mentioned constraints depicted in Table 4, and to meet the objective of the present study, the desirability value close to zero indicated unfavorable and unacceptable conditions for the responses, rendering a formulation undesirable. Conversely, as the value approached one, the formulation became highly preferable and desirable.

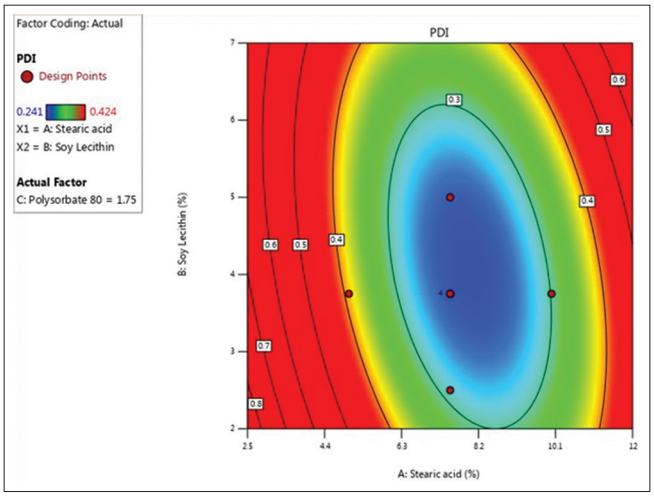

Figure 9: 2D contour plot of independent variables influencing dapagliflozin solid lipid nanoparticle polydispersity index

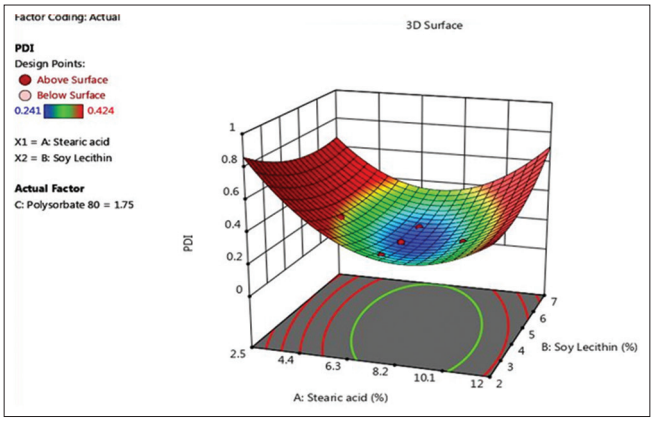

Figure 10: 3D response surface methodology plot of independent variables influencing dapagliflozin solid lipid nanoparticle polydispersity index

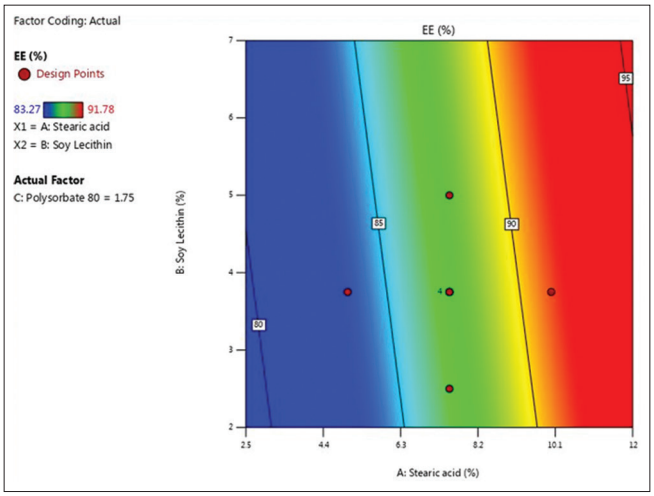

Figure 11: 2D contour plot of independent variables influencing dapagliflozin solid lipid nanoparticle entrapment efficiency (%)

In light of the results [Figure 13], the desirability of all solutions[20] was compared graphically using numerical optimization with software. This approach offered potential solutions for optimizing the dapagliflozin-loaded SLN batch. The information was illustrated through desirability and overlay plots, showcased in Figures 14 and 15. Notably, from the desirability plot, it was evident that the percentage weight composition of steric acid 9.81, soya lecithin 2.50, and polysorbate (80) 1.50 stood out as the most desirable formulation, given its highest desirability value of 0.934, highlighted by a yellow circle and composition of each i variables of present study is depicted in Figure 15.

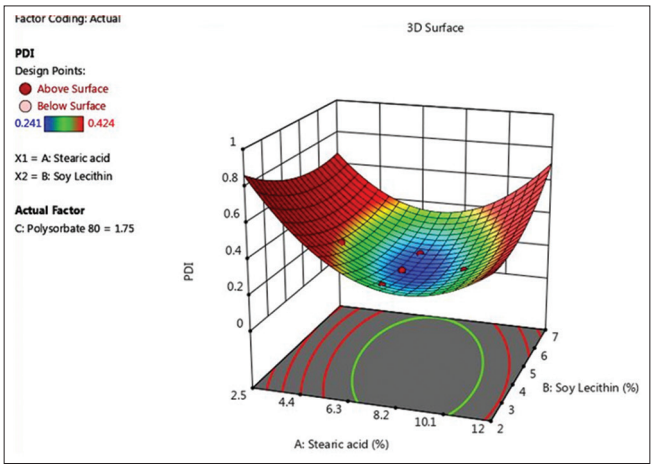

Figure 12: 3D response surface methodology plot of independent variables influencing dapagliflozin solid lipid nanoparticle entrapment efficiency (%)

In vitro drug release studies

This present work aimed to develop a formulation capable of sustaining the release of dapagliflozin over a 24-h period. The optimized dapagliflozin-loaded SLNs were evaluated under specified conditions using 6.8 phosphate buffer as the medium for dissolution. This release study demonstrated that the formulated SLNs successfully achieved this objective, exhibiting a remarkable drug release of 97.29 ± 0.25% within the designated timeframe. Such an extended-release profile holds significant clinical value, particularly for drugs necessitating once-daily dosing regimens. This prolonged-release feature not only enhances patient convenience but also ensures the maintenance of therapeutic efficacy throughout the treatment duration.

Initially, during the first 4 h, the release rate is relatively slow, ranging from 9.34 ± 0.28 to 25.31 ± 0.34 percent, as dapagliflozin molecules diffuse through the lipid matrix. This gradual release is attributed to the sustained-release properties of stearic acid, which moderates the drug release, keeping it at a moderate level.

However, as time progresses, particularly beyond 6 h, there is a notable increase in the release rate, reaching 47.25 ± 0.52 percent. This acceleration in release is indicative of the onset of lipid matrix erosion or degradation. The gradual breakdown of the lipid matrix allows for a more rapid and sustained release of dapagliflozin over extended periods, contributing to the observed sustained release profile.

Between the 6 and 12-h time intervals, the percentage of drug release escalated from 47.25 ± 0.52 to 76.11 ± 0.14.

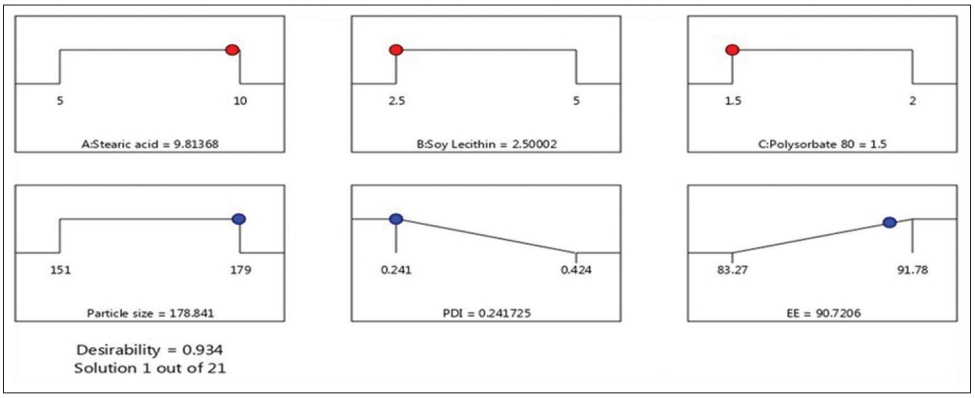

Figure 13: Independent variable components with the highest desirability

Table 4: Constraints and importance of independent variables

Name of the components	Goal	Limit (Low-Upper)		Importance
A: Stearic acid	In range	5	10	+++
B: Soy Lecithin	In range	2.5	5	+++
C: Polysorbate 80	In range	1.5	2	+++
Particle size	In range	151	179	+++++
PDI	Minimize	0.241	0.424	+++++
Entrapment efficiency (%)	Maximize	83.27	91.78	+++++

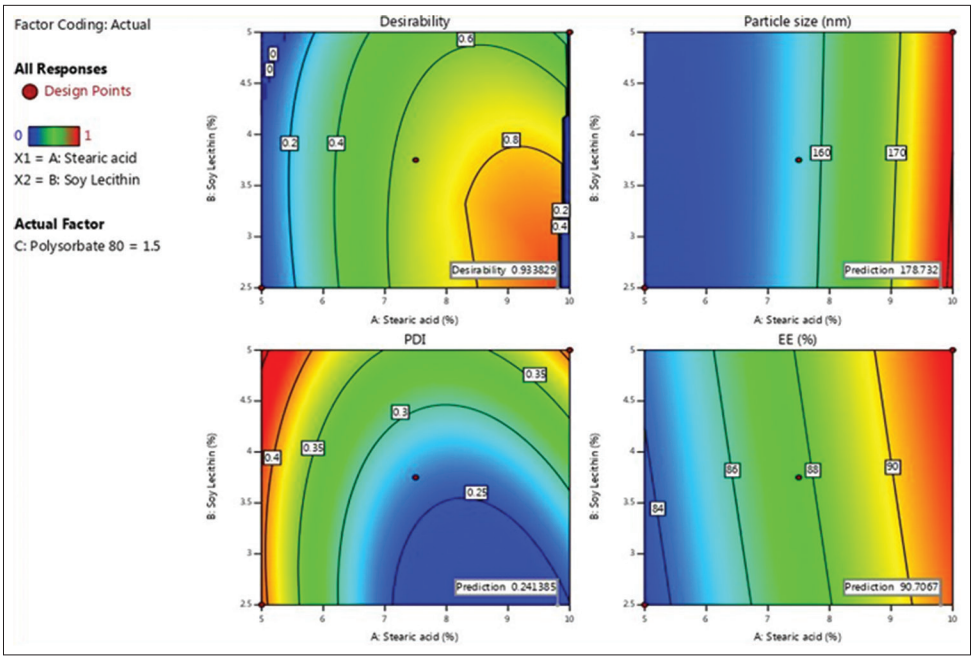

Figure 14: Desirability plot for particle size, polydispersity index, and entrapment efficiency (%)

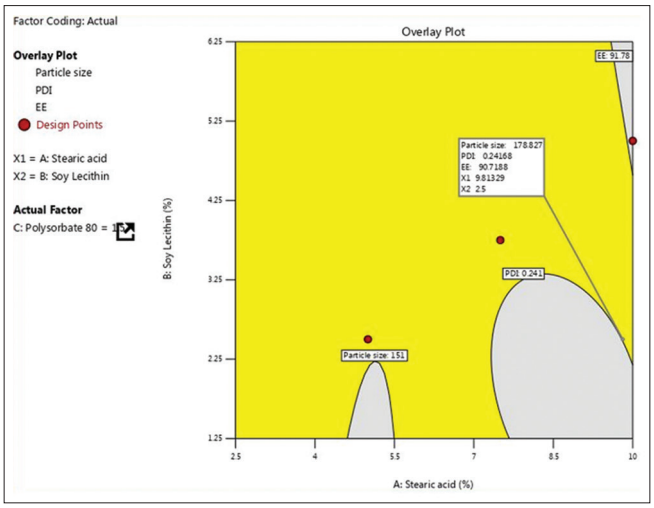

Figure 15: Overlay plot for optimized formulation

During this period, the erosion or degradation of stearic acid intensified, leading to a consistent rise in drug release. As the lipid matrix continued to break down, more drug molecules became exposed to the dissolution medium, further contributing to the increasing drug release.

From 12 h to 24 h, the drug release increased from 76.11 ± 0.54 to 97.29 ± 0.48. This gradual progression release of drugs in specified time intervals is influenced by several factors. These factors include decreased surface area, increased lipid matrix density, diffusion resistance, limited solubility of dapagliflozin, and potential polymerization or cross-linking of lipid matrix components. Together, these factors contribute to the sustained and controlled release of dapagliflozin over the extended release period, ultimately achieving the desired 24-h release profile.

In conclusion, the developed dapagliflozin SLN formulation shows promise for continuous dapagliflozin release, supported by robust *in vitro* release profiles and favorable physicochemical characteristics. This formulation holds the potential for enhancing patient compliance and therapeutic outcomes in conditions requiring prolonged drug delivery. All results are summarized in Figure 16.

Release order kinetics

The release orders for the dapagliflozin-SLN's optimized formulation were assessed using the DDSolver model software. This software served as a powerful tool for deciphering the intricate release patterns. By utilizing a range of mathematical models, the study yielded insightful results are shown in Table 5.

From the zero-order model, the optimized formulation exhibited a notable r^2 value of 0.995, indicating a robust correlation between the model and the observed data. Correspondingly, the AIC value was calculated as 28.32, while the MSC value reached 5.254. In the context of first-order studies, the model-optimized formulation resulted in a higher r^2 value of 0.976, confirming the model's strong fit. The recorded AIC value was 41.55, and the MSC value stood at 3.124.

Shifting focus to the Higuchi Model, the selected formulation demonstrated an r^2 value of 0.978, signifying the model's excellent alignment with the experimental data. The AIC value was determined as 35.24, while the MSC value reached 3.925. Notably, the inclusion of Hixson-Crowell release order analyses revealed that for formulation F1, the r^2 value

Table 5: Summary data on release kinetics of optimized dapagliflozin-SLNs from DDSolver software

Release order parameters	Zero-order (k0)	First order (k1)	Higuchi model (kH)	Hixon-Crowell (kHC)	Korsmeyer-Peppas (kKP)	*n*
Adjusted r^2	0.995	0.976	0.978	0.986	0.988	0.692
r^2	0.995	0.976	0.978	0.986	0.988	
AIC	28.32	41.55	35.24	32.49	34.25	
MSC	5.254	3.124	3.925	4.121	4.824	

Table 6: Stability studies of optimized dapagliflozin-loaded SLNs under varied storage conditions

	At room tempatrure (27°C)				At refrigerator (4°C)			
Time period	Particle size in (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Particle size in nm	PDI	Zeta potential (mV)	Entrapment efficiency (%)
Initial	178.85 ± 0.12	0.241 ± 0.14	−23.2 ± 0.22	90.72 ± 0.13	178.85 ± 0.15	0.24 ± 0.32	−22.17 ± 0.25	90.72 ± 0.14
Istmonth	192.37 ± 0.41	0.324 ± 0.47	21.07 ± 0.34	86.47 ± 0.28	190.38 ± 0.19	0.317 ± 0.37	−20.87 ± 0.14	88.17 ± 0.17
IInd month	210.38 ± 0.28	0.398 ± 0.29	20.68 ± 0.37	84.14 ± 0.31	205.37 ± 0.23	0.388 ± 0.13	−19.26 ± 0.25	86.39 ± 0.29

was 0.986, the AIC value was 32.49, and the MSC value was 4.121.

Extending the investigation, the Korsmeyer–Peppas equation provided deeper insights into the optimized formulation. The model suggested an r^2 value of 0.988, in strong agreement with the data. The calculated AIC value was 34.25, while the MSC value was 4.824. Notably, the exponent release (*n*) value stood at 0.692, indicating a non-Fickian diffusion-type mechanism governing the release.

Where, r^2 is regression co-efficient, AIC and MSC, etc.

Physical stability studies of optimized formulation during storage

Dapagliflozin-loaded SLNs were stored for 60 days at both RT (25°C/60 ± 5% RH) and refrigerated temperature (4°C). The PDI, ZP, average size, and EE (%) were measured.

Stability studies were performed for the optimized formulation, exhibiting improved size, PDI, ZP, and EE. Triplicate samples were analyzed, and the results are summarized in Table 6. The statistical t-test was applied, revealing a significant change only in the size of SLNs, with no notable differences observed in EE, PDI, and ZP throughout the storage period. Consequently, the optimized SLN preparation remained stable for a 2-month period at both RT and 4°C.

The statistical analysis of the data was conducted using an unpaired t-test in Excel (version 2013), with significance set at a $P = 0.05$. A significant difference was observed in the size of the SLNs ($P = 0.79$). However, no significant differences were found in terms of PDI, ZP, and EE during storage on the 1st, 30th, and 60th days.

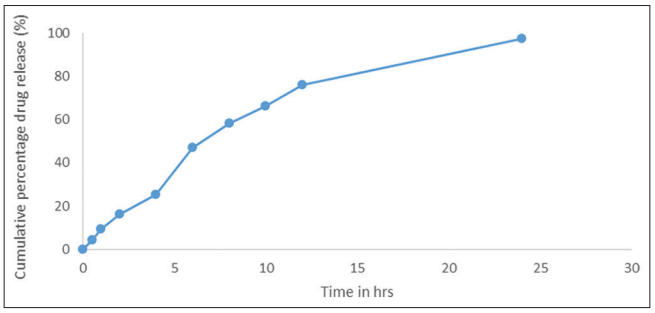

Figure 16: *In vitro* release profile of dapagliflozin-solid lipid nanoparticle's optimized formulation

CONCLUSION

The study successfully designed and optimized dapagliflozin-loaded SLNs for potential anti-diabetic therapy. The SLNs were prepared through high-pressure homogenization followed by ultra-sonication, with formulation optimization that could be done with CCD type and model RSM. The aim was to develop a cost-effective, biodegradable, and stable nanocarrier with enhanced drug entrapment and prolonged release over 24 h. The optimized formulation exhibited a particle size of 178.85 ± 0.12 nm, within the optimal range for oral delivery. In addition, it demonstrated high EE (90.72 ± 0.13%) and an extended drug release profile, with an initial burst followed by prolonged release (97.29 ± 0.25%). The release kinetics followed a zero-order equation, suggesting a non-Fickian diffusion-type mechanism with an exponent diffusional coefficient (*n*) value of 0.692. These results underscore the potential of SLNs as an effective delivery system for dapagliflozin, offering improved treatment outcomes for anti-diabetic actions. Further, *in vivo* studies and clinical trials are warranted to validate these promising *in vitro* findings and establish the clinical efficacy and safety of dapagliflozin-loaded SLNs.

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