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Formulation and Evaluation of Canagliflozin-Loaded Nanostructured Lipid Carrier

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ABSTRACT

Aim of this research work is to develop and evaluate Canagliflozin-loaded Nanostructured Lipid Carriers (NLCs) to improve the drug's solubility, oral bioavailability, and therapeutic efficacy in type 2 diabetes mellitus.

NLCs was formulated by using suitable solid and liquid lipids with surfactants, followed by characterization through particle size, zeta potential, entrapment efficiency, in vitro release profile, morphological analysis via TEM, and stability evaluation of the optimized formulation. Canagliflozin-loaded NLCs were prepared using a high-speed homogenization method followed by ultrasonication. Various formulations were developed by altering lipid and surfactant composition. The prepared NLCs were evaluated for particle size, zeta potential, drug content, entrapment efficiency, and in vitro drug release. TEM analysis was performed to study morphology. Stability studies were carried out under accelerated conditions in compliance with ICH guidelines.

FTIR study was conducted for identifying functional groups and drug-excipient compatibility study and found that they are compatible with each other. The in-vitro drug release profile confirmed improved release characteristics. The optimized formulation (CF3) exhibited favourable particle size of 305 nm, high drug content of 96.23 %, and high entrapment efficiency of 94.56%. TEM images confirmed smooth and spherical particle morphology, indicating successful NLC formation. Stability studies shown good physical stability under accelerated conditions and there were no significant changes in the physic-chemical properties of formulation. So it was concluded that, the Canagliflozin-loaded NLCs are potential and effective drug delivery system.

Keywords: *NLCs, Nanotechotechnology, antidiabetic, canagliflozin etc.*

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1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Among various therapeutic approaches, oral antidiabetic drugs remain a cornerstone for the management of type 2 diabetes. Canagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, has gained significant attention due to its ability to reduce renal glucose reabsorption, thereby promoting glycosuria and lowering blood glucose levels independently of insulin action.

However, like many oral antidiabetic agents, Canagliflozin suffers from limitations such as poor aqueous solubility, variable oral bioavailability, and potential gastrointestinal side effects. These challenges can compromise its therapeutic efficacy and patient compliance.

To overcome these issues, nanotechnology-based drug delivery systems have emerged as promising alternatives. Nanostructured Lipid Carriers (NLCs), which are second-generation lipid nanoparticles, offer several advantages including enhanced drug loading capacity, improved physical stability, controlled drug release, and increased bioavailability. By incorporating Canagliflozin into NLCs, it is possible to enhance its pharmacokinetic profile and therapeutic performance.

This study focuses on the formulation and evaluation of Canagliflozin-loaded NLCs. The aim is to develop a stable, efficient nano-formulation that can improve drug delivery and therapeutic outcomes. The NLCs are prepared using suitable

lipid and surfactant systems, characterized for physicochemical properties, and evaluated for stability, in vitro release, and surface morphology.

2. MATERIALS AND METHODS

Materials

Canagliflozin was purchased from Cipla Pvt Ltd., Pune, India. Whereas excipients available in research centre were obtained from: glycerol monostearate from molychem, Mumbai, castor oil from vikas Pharmaceuticals, stearic acid from cosmochem, pune, tween 20, 40,60 ,80 from oxford lab fine chem, oleic acid from labware chemicals.

Method [7,8,9,10]

Spectrophotometric characterization of Canagliflozin in UV Spectroscopy.

- **Scanning for λmax of Canagliflozin**

UV visible spectrophotometric analysis of canagliflozin was carried out by using UV spectrophotometer. Spectrum was scanned in range between 200-400 nm. The stock solution of the canagliflozin was prepared by dissolving 25 mg of drug in 25 ml of methanol, (1000 µg/ml) respectively. Then 0.1ml of above solution was diluted to 10 ml methanol to make 10 µg/ml solution.

- **Standard calibration curve of Canagliflozin by UV Spectroscopy.**

Calibration Curve of Canagliflozin in Methanol

Preparation of stock solution in Methanol:

Standard stock solution was prepared by taking 50 mg in 50 ml of Methanol (1000µg/ml). The stock solution was scanned in the range 400-200 nm by UV spectrophotometer; the solution showed maximum absorbance at 292 nm.

Preparation of dilutions for the standard curve:

From 1000µg/ml, prepare solutions of 2, 4, 6, 8, 10 and 12 ppm by diluting100 -500 µl stocks to 10 ml Methanol. Absorbance was taken at 292 nm using Methanol as a blank. The absorbance v/s concentration graph is plotted.

IR Spectroscopy [11, 12, 13]

FTIR study was conducted to analyse the chemical composition as well as molecular structure of a material. It is primarily used to identify functional groups present in a compound by detecting their characteristic vibrational frequencies. Study is conducted in relation to formulation to confirm the chemical compatibility and interactions between the active pharmaceutical ingredients (APIs) and excipients used in the formulation and identify incompatibilities that may occur during the formulation process. The spectra were noted for pure drug using FTIR. The scanning range was 400-4000 cm-1.

X-Ray Diffraction Studies [14, 15, 16]

This study was conducted to analyse physical properties and interactions between **active pharmaceutical ingredients (APIs)** with **excipients**. The X-ray diffraction (XRD) patterns of NLCs were determined using XRD diffractometry. Study was performed on a Siemens DIFFRAC plus 5000 powder diffractometer with Cu / 40 kV/ 30 mA. The tube voltage and amperage were set at 40 kV and 30 mA, respectively. Each sample was scanned between 10◦C and 90◦C in 2θ with a step size of 0.01◦C at 1 step/ scanning speed of 10.0000 deg/min.

Preparation of Canagliflozin loaded Nanostructured lipid carrier (NLC)

Canagliflozin loaded NLCs was prepared by using High-speed homogenization method followed by ultrasonication.[17,18,19]

1. Briefly, weighed Stearic acid and Triglyceride and the Canagliflozin were dissolved into 10 ml of mixed organic solvent of Methanol in a water bath at 55 °C.
2. The mixture was then added drop wise to Tween 80 in aqueous phase at 70ºC, a pre-emulsion was obtained by homogenization at 15000 rpm and ultrasonicate for 30 min at 70ºC.
3. Further, this pre-emulsion was ultra sonicated for 15 min to prevent the crystallization of lipids.
4. The o/w emulsion obtained was subsequently cooled down to room temperature with continuous stirring and the lipid was recrystallized to form Nanostructured lipid carrier (NLC).

Selection of the Liquid Lipid and Surfactant [20,21]

To determine the solubility of Canagliflozin in various liquid lipids surfactants and Co surfactants, an excessive amount of Canagliflozin (approximately 10 mg) was added to a 2.0 ml tube containing 2 ml of each vehicle. The mixtures were shaken for 24 h at 25°C to reach an equilibrium state using a vortex shaker at 50 rpm. The mixtures were then centrifuged at 1000

rpm for 15 min, and the supernatant was passed through a 0.20 µm syringe filter to remove the excess Canagliflozin.

Experimental design [22]

The response surface methodology (RSM) was employed to perform Quality by Design approach for constructing and investigating the polynomial models, using fewer experimental runs. Central composite Design comprising of 2-factors and 2- levels was employed to examine the quadratic response surfaces by assessing the effect of pre-defined independent variables on different response dependent variables Drug content, Entrapment efficiency (%) and Drug release (%), was coded as Y1, Y2 and Y3. Three independent variables namely Lipid conc (%), Surfactant conc (%) and Homogenization speed (C) were chosen. Each of the variables was varied at two different levels, known as high and low levels. All the finalized independent variables and the response variables are described in Table below.

Table 01: List of Independent and Dependent variables in Box–Behnken design

Independent variable	Low value (-1)	High value (+)	Dependent variables	Constraints
Lipid conc (%)	2	5	Drug content (%)	Minimize
Surfactant conc (%)	1	1.5	Entrapment Efficiency (%)	Maximize
			Drug release (%)	Minimize

Table 02: DOE suggested batches for Canagliflozin

Formulation code	Canagliflozin (mg)	Lipid Conc (%)	Solid lipid (gm)	Liquid lipid (gm)	Surfactant Tween 80 conc (%)	Methanol (ml)	Homogenization (Rpm)
CF1	100	3.5	0.735	0.315	1.5	10	15000
CF2	100	3.5	0.735	0.315	1.25	10	9000*
CF3	100	2	0.42	0.18	1.5	10	15000
CF 4	100	5	1.05	0.45	1	10	16000*
CF 5	100	5	1.05	0.45	1.5	10	15000
CF 6	100	3.5	0.735	0.315	1	10	12500
CF 7	100	2	0.42	0.18	1	10	12500
CF 8	100	5	1.05	0.45	1.25	10	10000
CF 9	100	3.5	0.735	0.315	1.25	10	12500

***Indicates noise**

Evaluation of NLCs

Drug content [23]

Drug content study was conducted to determine total amount of drug in formulation. **Amount of drug** in a formulation determined by dissolving 1 mL of prepared NLCs in 10 mL of Methanol. The amount of Canagliflozin (%) in each formulation was determined spectrophotometrically by measuring the absorbance of the clear supernatant at max of 292. Each experiment was performed in triplicate. Acetonitrile was used as blank for UV absorbance.

Entrapment efficiency (%)[24]

An **entrapment efficiency study** is conducted to determine how much of the **drug** is successfully **encapsulated** within a **drug delivery system.** A volume of 4 ml of each drug-loaded sample was centrifuged at rpm for 30 min to separate the lipid and aqueous phase. The supernatant was then diluted with Acetonitrile spectrophotometer and analyze 292 nm. The entrapment efficacy of NLC was calculated as follows:

$$EE(\%) = \frac{Wa - Ws}{Wa} \times 100$$

Where, Wa stands for the mass of Canagliflozin added to the formulation and

Ws is the analyzed weight of drug in supernatant.

In vitro drug release study

In vitro release studies of Canagliflozin-loaded nanostructured lipid carriers (NLCs) were conducted using phosphate buffer (pH 6.8) as the release medium at 37 °C. A 2 mL volume of the NLC formulation was accurately weighed and transferred into a pre-soaked dialysis membrane. The formulation was gently positioned to ensure direct contact with the membrane surface.

The dialysis membrane was then immersed in 100 mL of phosphate buffer (pH 6.8), serving as the receiving compartment. This setup was placed on a magnetic stirrer operating at 75 rpm and maintained at 37 °C throughout the experiment.

Samples of 5 mL were withdrawn from the receiving medium at predetermined time intervals (1, 2, 3, 4, 5, and 6 hours). The withdrawn samples were analysed using a UV-Visible spectrophotometer at 292 nm to determine the amount of Canagliflozin released. After each sampling, an equal volume (5 mL) of fresh phosphate buffer was added to the receiving compartment to maintain sink conditions.

Particle size and Zeta potential

A 100 µL sample of the NLC formulation was mixed with distilled water and subjected to sonication for 30 minutes. The particle size analysis was conducted at a temperature of 25 °C. The same procedure was followed for zeta potential measurement.

FTIR spectroscopy

The compatibility between the drug and excipients was evaluated using the Fourier Transform Infrared (FTIR) spectroscopy technique. The optimized formulation (CF3) was scanned over a wavenumber range of 500–4000 cm^{-1} using the diffuse reflectance scanning method.

Transmission Electron Microscopy (TEM) [25]

The surface morphology of the optimized batch CF3 was examined using Transmission Electron Microscopy (TEM). A few microliters of the diluted NLC suspension of Canagliflozin (batch CF3) were placed onto a 300-mesh copper grid coated with a carbon film and allowed to air-dry at room temperature. After complete drying, the sample was negatively stained with a 2% w/v phosphotungstic acid solution, and excess stain was removed using filter paper. The samples were then analyzed, and images were captured using Digital Micrograph and Soft Imaging Viewer software.

Stability study [26]

Accelerated stability study

The optimized Nanostructured Lipid Carriers (NLCs) containing Canagliflozin were filled into Class I glass vials, sealed with screw caps, and subjected to stability studies under both long-term and accelerated conditions, in accordance with ICH guidelines [Q1A (R2)].

3. RESULT AND DISCUSSION

Spectrophotometric characterization of Canagliflozin in UV Spectroscopy.

- **Scanning for λmax of Canagliflozin**

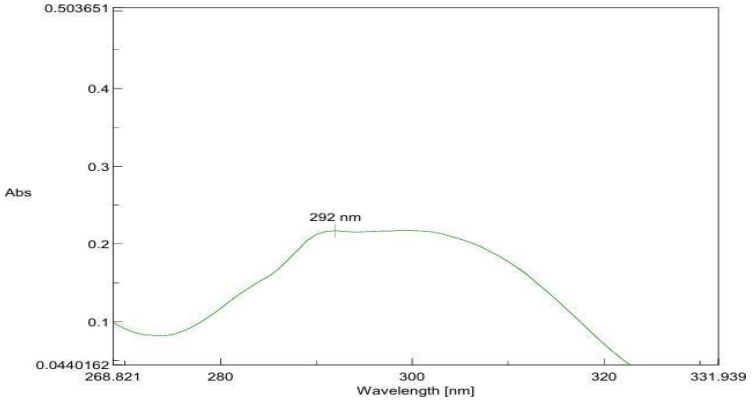

Figure 01: UV spectrum of Canagliflozin in methanol (10µg/ml)

Table 03: λmax of Canagliflozin

Drug	λmax	Reported λmax
Canagliflozin	292	290

The observed λmax values for Canagliflozin 292 nm are in close agreement with their reported values 290 nm, indicating the purity and proper identification of the compounds through UV-Visible spectrophotometry.

- **Standard calibration curve of Canagliflozin by UV Spectroscopy.**

Table 04: Calibration Curve of Canagliflozin in Methanol

Concentration (µg/ml)	Absorbances
0	0
2	0.0356
4	0.0654
6	0.0987
8	0.1254
10	0.1547
12	0.1874

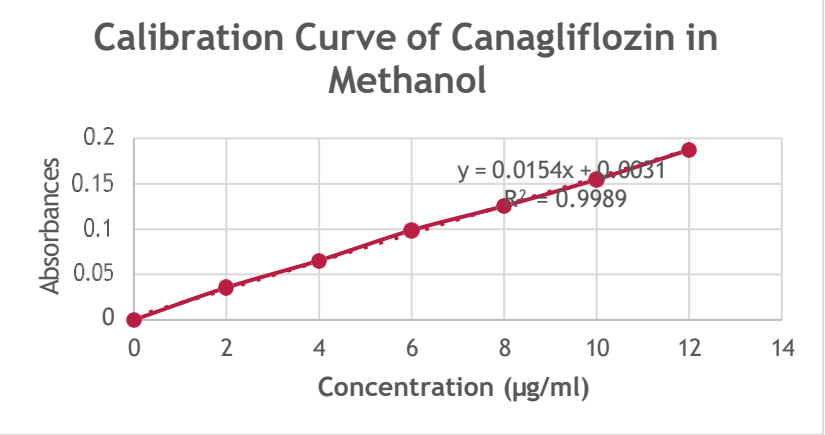

Figure 02: Calibration Curve of Canagliflozin in Methanol

IR Spectroscopy

FTIR of Canagliflozin

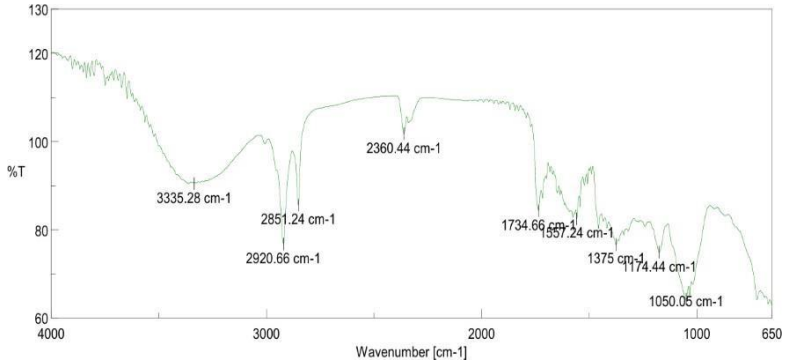

Figure 03: IR spectra of Canagliflozin

Table 05: Interpretation of Canagilflozin FTIR

Functional Group	Wavenumber (cm^{-1})	Observation
O–H stretch	3000–3500 (≈ 3335.28)	Hydroxyl groups in canagliflozin
Aromatic C–H stretch	≈ 2920.66	Aromatic C–H bonds
Aromatic C=C ring stretch	≈ 1557.24 or 1375	Aromatic ring vibrations
Alkyl C–H stretch (CH_3)	≈ 2851.24	Methyl group vibrations
Aryl fluoride C–F stretch	≈ 1174.44	Aryl fluoride moiety

X-Ray Diffraction Studies

The X-ray diffraction (XRD) pattern of Canagliflozin displays clear peaks at defined 2θ angles, confirming its crystalline structure. These sharp, well-defined peaks indicate that Canagliflozin is primarily in a crystalline state, which is essential for evaluating its purity, stability, and performance.

The 2θ values obtained correspond to the crystallographic planes, and its peak intensity indicates the degree of crystallinity, which affects drug solubility and bioavailability. The XRD analysis confirms that Canagliflozin is crystalline, which is important for its pharmaceutical properties.

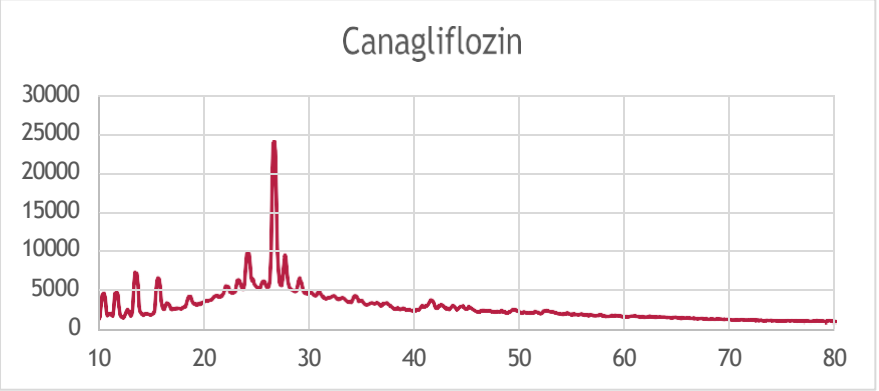

Figure 04: XRD graph of Canagliflozin pure drug

Evaluation of NLC

Drug Content (%)

Table 06: Drug Content (%) of Canagliflozin loaded NLCs

Formulation code	**Drug Content (%)**
CF1	92.38±0.45
CF2	82.31±0.14
CF3	96.38±0.23
CF 4	92.49±0.66
CF 5	93.98±0.42
CF 6	88.91±0.49
CF 7	87.96 ±0.87
CF 8	79.25±1.02
CF 9	90.84±0.63

*****Data were expressed as Mean ± SD, n=3

ANOVA for Quadratic model

Table 07: Summary of statics for Response 1: Drug Content (CF1-CF9)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	102.37	5	20.47	13.83	0.0277	significant
A-Lipid Conc	2.36	1	2.36	1.59	0.2962	
B-Surfactant Conc	21.62	1	21.62	14.61	0.0315	
AB	7.02	1	7.02	4.74	0.1175	
A^2	2.49	1	2.49	1.69	0.2850	
B^2	68.87	1	68.87	46.54	0.0064	
Residual	4.44	3	1.48			
Cor Total	106.81	8				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 13.83 implies the model is significant. There is only a 2.77% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 08: Summary of Fit Statistics for Response 1: Drug Content (CF1-CF9)

Std. Dev.	1.22		**R^2**	0.9584
Mean	90.46		**Adjusted R^2**	0.8891
C.V. %	1.34		**Predicted R^2**	0.8889
			Adeq Precision	9.5740

Table 09: Summary of Final Equation in Terms of Coded Factors for Response 1: Drug Content (CF1-CF9)

Drug Content	=
+87.29	
-0.6267	A
+1.90	B
+1.33	AB
-1.12	A^2
+5.87	B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 10: Summary of Final Equation in Terms of Actual Factors for Response 1: Drug Content (CF1-CF9)

Drug Content	=
+235.34537	
-1.36037	Lipid Conc

-239.50667	Surfactant Conc
+3.53333	Lipid Conc * Surfactant Conc
-0.496296	Lipid Conc^2
+93.89333	Surfactant Conc^2

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

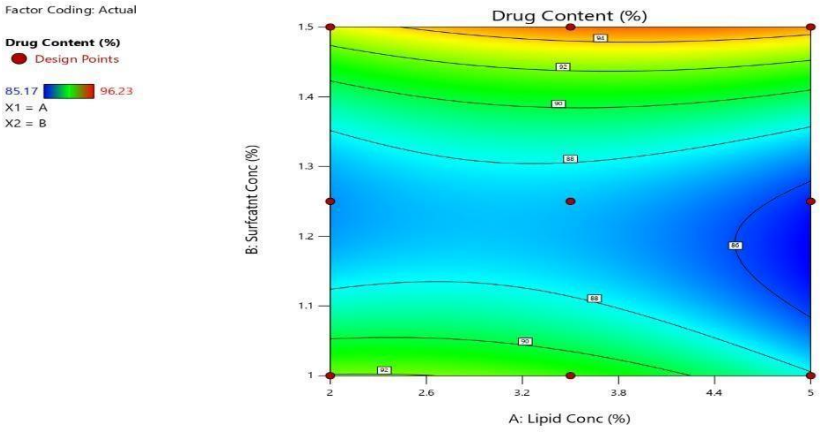

Figure 05: Counter plot for Response 1: Drug Content (CF1-CF9)

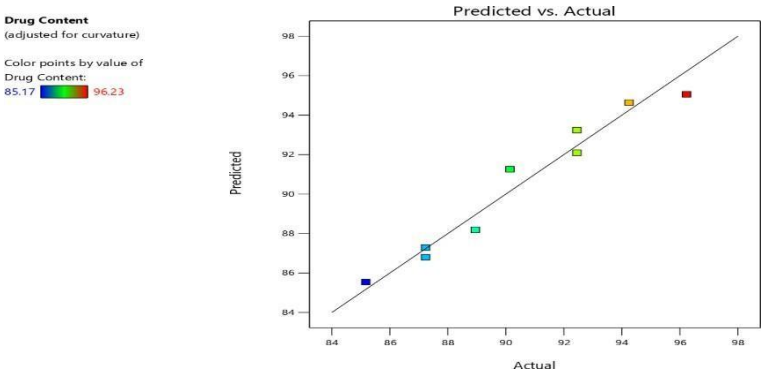

Figure 06: Predicted Vs Actual plot for Response 1: Drug Content (CF1-CF9)

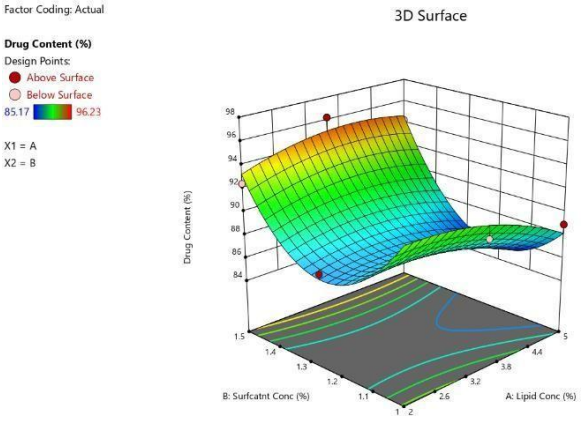

Figure 07: 3D Surface response plot for Response 1: Drug Content (CF1-CF9)

Entrapment efficiency

Table 11: Entrapment efficiency of Canagliflozin loaded NLCs

Formulation code	Entrapment efficiency (%)
CF1	89.46±1.27
CF2	80.41±1.44
CF3	95.63±1.39
CF 4	93.26±1.13
CF 5	90.24±1.08
CF 6	84.57±1.60
CF 7	85.26±1.36
CF 8	77.59±1.81
CF 9	92.15±0.98

*****Data were expressed as Mean + SD, n=3

ANOVA for Quadratic model

Table 12: Summary of statics for Response 2: Entrapment efficiency (CF1-CF9)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	139.21	5	27.84	12.32	0.0326	significant
A-Lipid Conc	0.0368	1	0.0368	0.0163	0.9065	
B-Surfactant Conc	61.31	1	61.31	27.13	0.0138	
AB	0.4096	1	0.4096	0.1812	0.6990	
A^2	10.17	1	10.17	4.50	0.1240	
B^2	67.28	1	67.28	29.77	0.0121	
Residual	6.78	3	2.26			
Cor Total	145.99	8				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 12.32 implies the model is significant. There is only a 3.26% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 13: Summary of Fit Statistics for Response 2: Entrapment efficiency (CF1-CF9)

Std. Dev.	1.50		**R^2**	0.9536
Mean	86.29		**Adjusted R^2**	0.8762
C.V. %	1.74		**Predicted R^2**	0.8760
			Adeq Precision	9.2305

Table 14: Summary of Final Equation in Terms of Coded Factors for Response 2: Entrapment efficiency (CF1-CF9)

Entrapment efficiency	=
+83.93	
+0.0783	A
+3.20	B
+0.3200	AB
-2.26	A^2
+5.80	B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 15: Summary of Final Equation in Terms of Actual Factors for Response 2: Entrapment efficiency (CF1-CF9)

Entrapment efficiency	=
+204.21667	
+6.00111	Lipid Conc
-222.20000	Surfactant Conc
+0.853333	Lipid Conc * Surfactant Conc
-1.00222	Lipid $Conc^2$
+92.80000	Surfactant $Conc^2$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

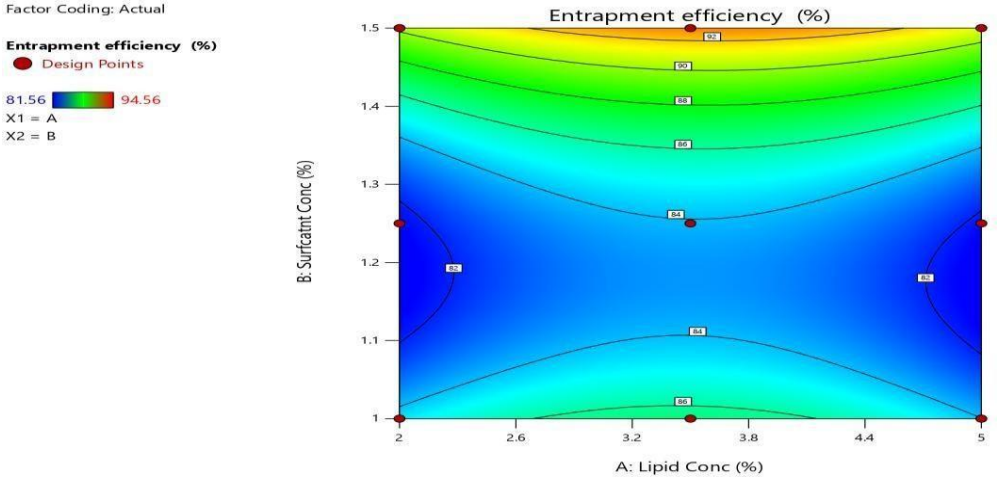

Figure 08 : Counter plot for Response 2: Entrapment efficiency (CF1-CF9)

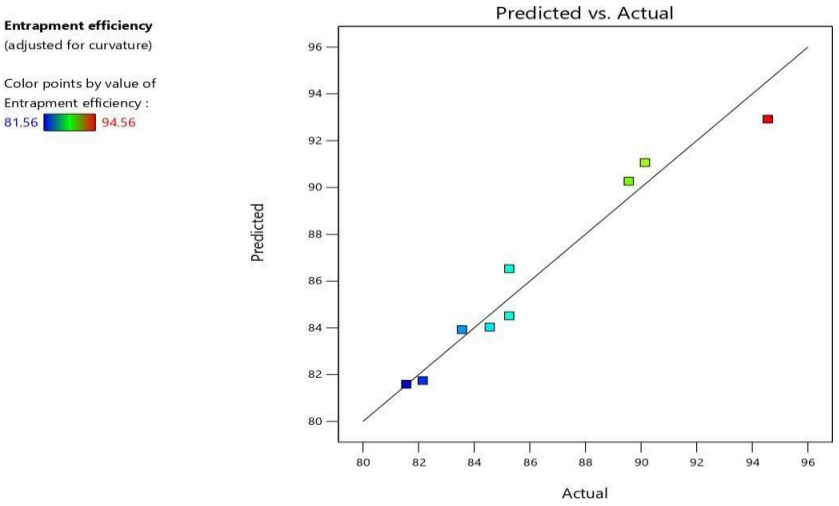

Figure 9: Predicted Vs Actual plot for Response 2: Entrapment efficiency (CF1-CF9)

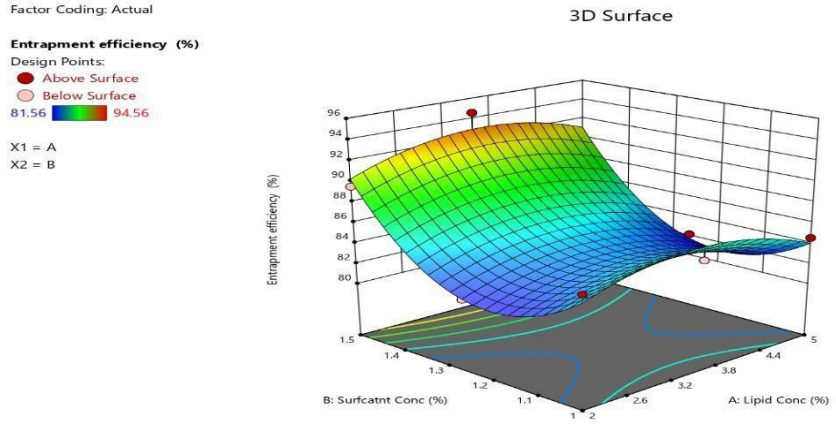

Figure 10: 3D Surface response plot for Response 2: Entrapment efficiency (CF1-CF9)

In Vitro drug diffusion study

Table 16: In Vitro drug diffusion (%) Study of Canagliflozin loaded NLCs (CF1-CF9)

Time (hr)	CF1 (%)	CF2 (%)	CF3 (%)	CF4 (%)	CF5 (%)	CF6 (%)	CF7 (%)	CF8 (%)	CF9 (%)
0	0	0	0	0	0	0	0	0	0
1	20.96±1.11	22.34±0.93	25.16±1.24	28.49±0.95	21.53±1.29	31.87±0.65	24.73±0.89	20.62±1.08	23.48±1.13
2	33.65±0.54	34.19±1.15	38.43±0.79	36.58±0.84	33.29±0.89	43.74±0.77	34.67±0.78	32.59±1.12	31.72±1.02
3	51.37±1.02	49.31±0.67	58.72±1.01	51.46±1.23	48.38±0.47	55.31±0.28	47.39±0.43	43.72±0.98	43.67±0.85
4	69.78±1.23	68.89±0.56	72.58±1.13	62.39±1.05	60.66±0.73	67.85±0.17	59.74±0.25	57.81±1.35	63.59±1.47
5	84.42±0.87	76.88±0.74	87.35±0.87	79.43±0.94	84.97±0.81	78.29±0.61	73.53±0.49	72.43±1.26	70.23±0.58

6	92.56± 0.97	85.74±1. 35	95.14±0. 73	89.97±1. 02	93.56±0. 57	87.35±0. 64	88.36±0. 98	80.37±1. 21	83.45±0. 82

***Data were expressed as mean ± SD, n=3

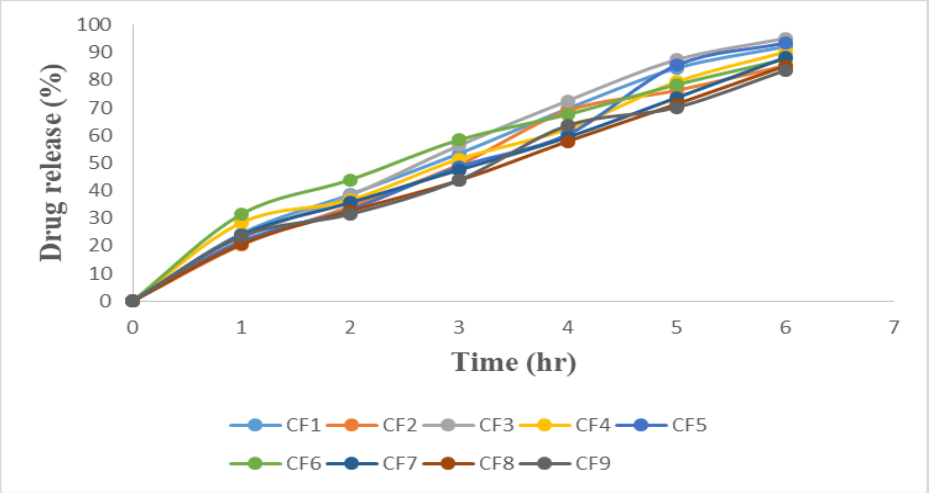

Figure 11: In Vitro release Study of Canagliflozin (CF1-CF9)

ANOVA for quadratic model

Table 17: Summary of statics for Response 3: Drug release (CF1-CF9)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	126.82	5	25.36	22.44	0.0139	significant
A-Lipid Conc	1.41	1	1.41	1.25	0.3452	
B-Surfactant Conc	36.85	1	36.85	32.60	0.0107	
AB	3.90	1	3.90	3.45	0.1602	
A^2	1.41	1	1.41	1.24	0.3461	
B^2	83.25	1	83.25	73.65	0.0033	
Residual	3.39	3	1.13			
Cor Total	130.21	8				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 22.44 implies the model is significant. There is only a 1.39% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 18: Summary of Fit Statistics for Response 3: Drug release (CF1-CF9)

Std. Dev.	1.06		**R^2**	0.9740
Mean	88.96		**Adjusted R^2**	0.9306
C.V. %	1.20		**Predicted R^2**	0.9304
			Adeq Precision	11.8114

Table 19: Summary of Final Equation in Terms of Coded Factors for Response 3: Drug release (CF1-CF9)

Drug release	=
+85.22	
-0.4850	A
+2.48	B
+0.9875	AB
-0.8383	A^2
+6.45	B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 20: Summary of Final Equation in Terms of Actual Factors for Response 3: Drug release (CF1-CF9)

Drug release	=
+242.20713	
-1.00685	Lipid Conc
-257.37000	Surfactant Conc
+2.63333	Lipid Conc * Surfactant Conc
-0.372593	Lipid $Conc^2$
+103.22667	Surfactant $Conc^2$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

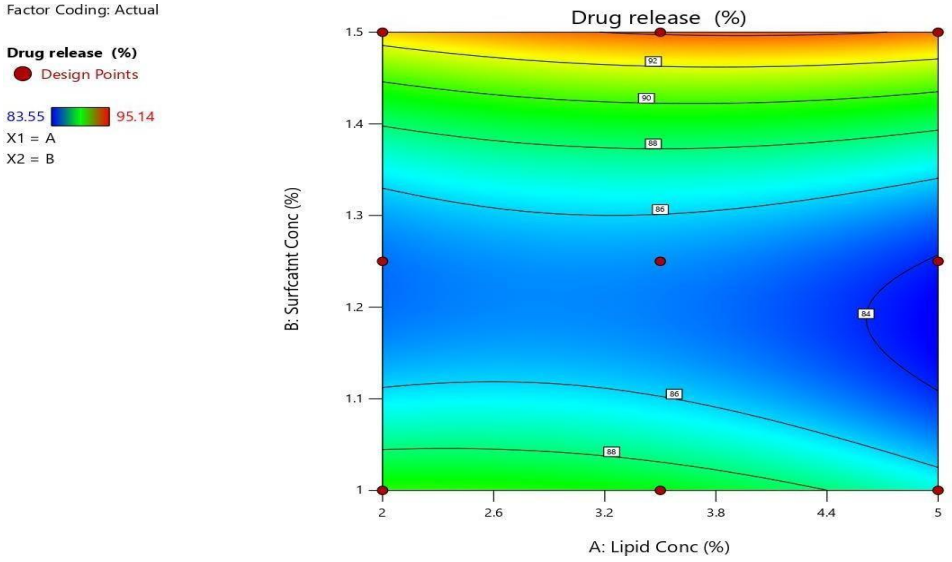

Figure 12: Counter plot for Response 3: Drug release (CF1-CF9)

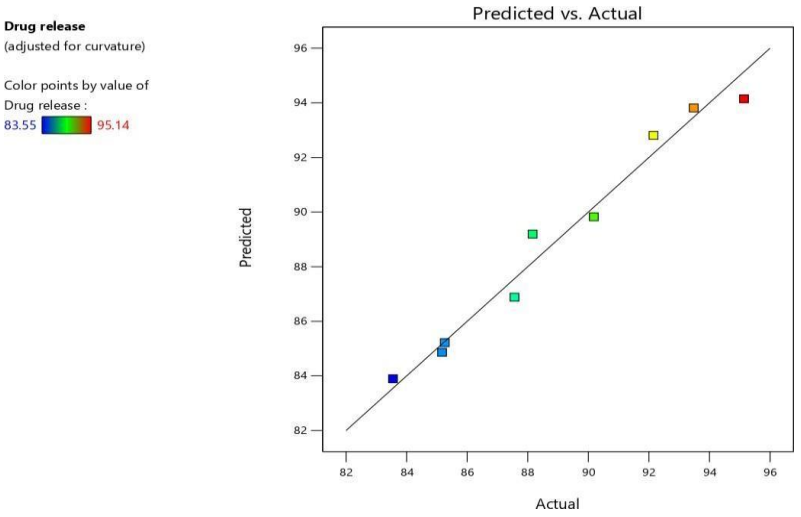

Figure 13: Predicted Vs Actual plot for Response 3: Drug release (CF1-CF9)

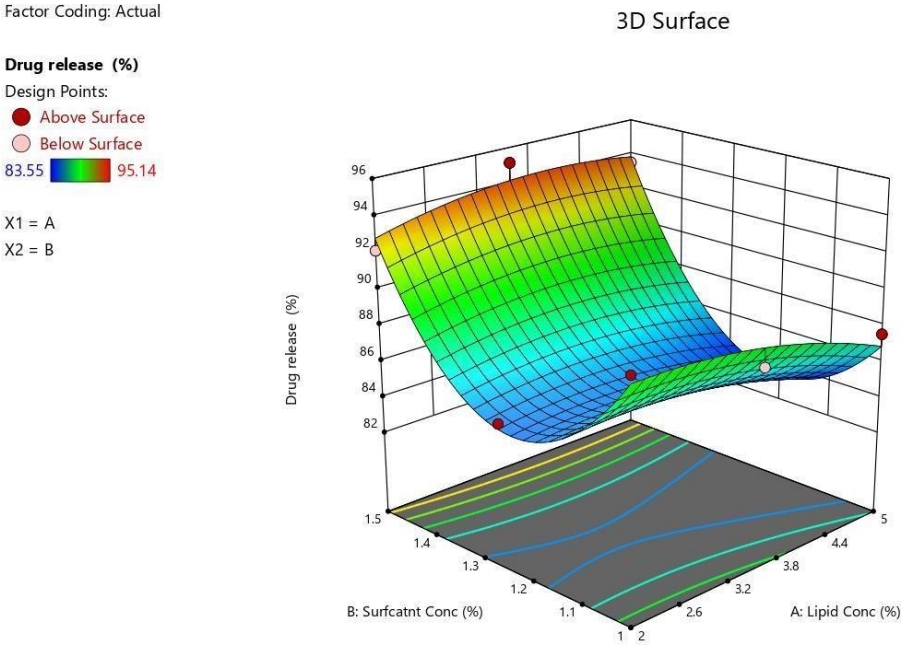

Figure 14: 3D Surface response plot for Response 3: Drug release (CF1-CF9)

Particle size analysis and Zeta potential

Table 21: Particle size analysis of Canagliflozin (CF1-CF9) NLC formulation

Optimized Batch	Particle size (nm)	Zeta potential (mV)	PDI
CF3	305.0	-38.5	0.219

Figure 15: Particle size and PDI of CF3

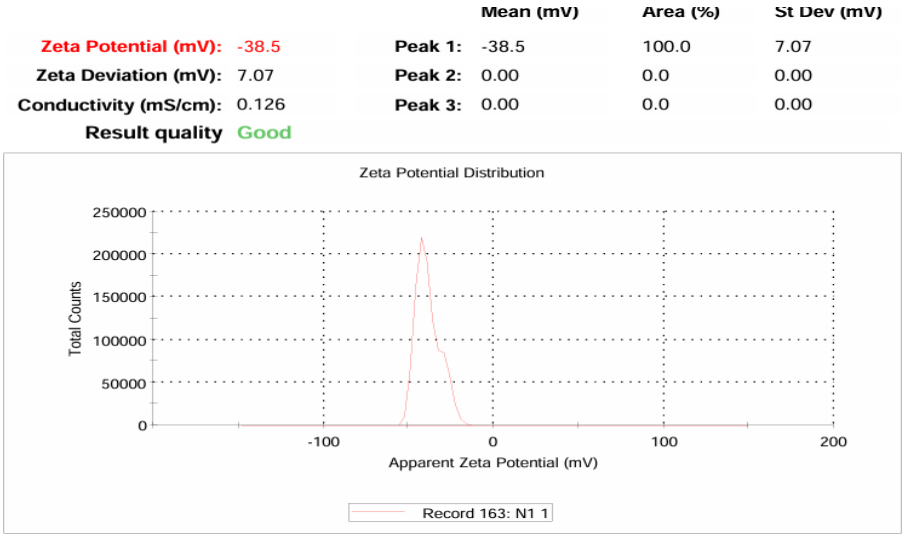

Figure 16: Zeta Potential of CF3

FTIR of Canagliflozin NLC physical mixture

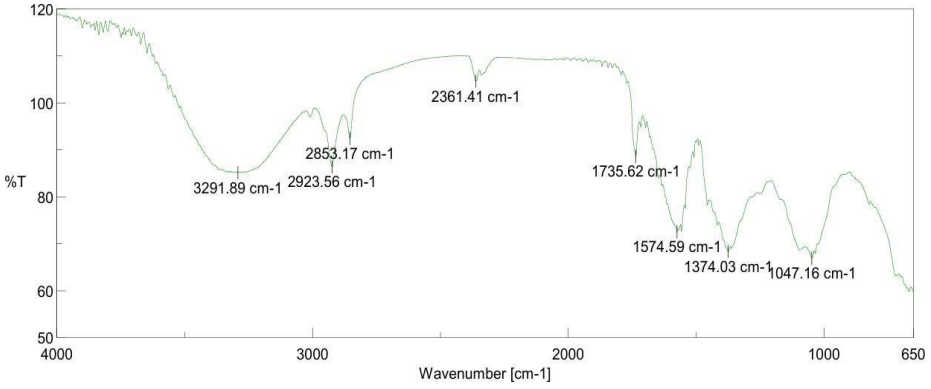

Figure 17: IR spectra of Canagliflozin NLC Physical mixture

Analyzing the FTIR spectra of a physical mixture of Canagliflozin Nanostructured Lipid Carriers (NLC) is a crucial step in assessing potential interactions between the drug and the carrier components. The FTIR spectra show that there is no any incompatibility of Canagliflozin with the excipients.

The groups showing for the physical mixture corresponding to the vibration frequencies of pure Canagliflozin drug.

Table 22 : Table 05: Interpretation of Canagilflozin and Excipients FTIR

Functional Group	Wavenumber (cm^{-1})	Observation
O–H Stretch	3000–3500 (≈3291.89)	Broad band indicating hydroxyl groups present in Canagliflozin
Aromatic C–H Stretch	≈2923.56	Stretching vibrations of aromatic C–H bonds
Alkyl C–H Stretch (–CH_3)	≈2853.17	Stretching vibrations due to methyl groups
Aromatic C=C Ring Stretch	≈1574.59, 1374.03	Stretching vibrations within aromatic rings
Aryl Fluoride C–F Stretch	≈1047.16	Stretching vibration of C–F bond in the aryl fluoride moiety

Transmission Electron Microscopy (TEM) (JEOL, 2200FS)

TEM was used to determine the morphology of optimized formulation CF3 NLC. The morphology can be seen as curved spherical particle. The image of TEM is shown in figure below.

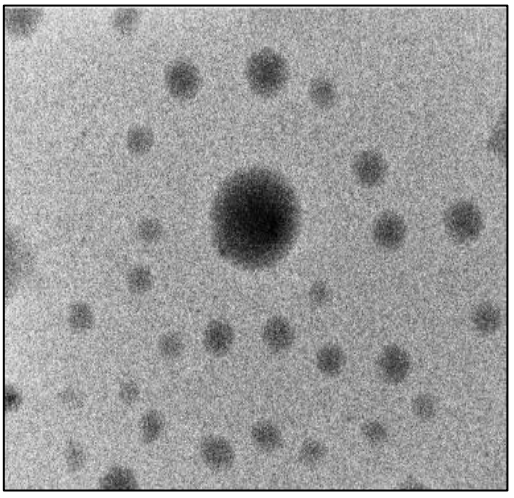

Figure 18: TEM of optimized batch (CF3)

Stability Study

The stability studies of Canagliflozin optimized NLC formulation indicate that there were no significant changes in the physico-chemical properties of the formulation. There were no significant changes in physico-chemical characteristics.

It was observed that the optimized Canagliflozin NLC formulation is stable at accelerated stability conditions.

4. CONCLUSION

The present study successfully formulated and evaluated Nanostructured Lipid Carriers (NLCs) containing Canagliflozin with the objective of enhancing its solubility, bioavailability, and therapeutic efficacy. The NLCs were prepared using suitable solid and liquid lipids along with appropriate surfactants via the e.g., High speed homogenization method.

Physicochemical characterization revealed that the optimized formulation exhibited desirable particle size i.e. 305.0 nm, and satisfactory zeta potential, indicating good stability. Drug entrapment efficiency i.e. 94.56% was found to be high, suggesting effective incorporation of Canagliflozin into the lipid matrix.

In vitro drug release studies demonstrated a sustained release profile, indicating the potential of NLCs to provide controlled delivery of Canagliflozin over an extended period. Additionally, the formulation showed improved dissolution behaviour

compared to pure drug, which could contribute to better oral bioavailability.

Overall, the findings suggest that NLCs batch i.e. CF3 is optimised & a promising nanocarrier system for the effective delivery of Canagliflozin, potentially overcoming its limitations related to poor solubility and low bioavailability.

REFERENCES

[1] Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Research in pharmaceutical sciences. 2018 Aug;13(4):288.

[2] Pignatello, R., & Musumeci, T. Lipid nanoparticles as novel drug delivery systems: Basic concepts and current applications. Drug Discovery Today, 2010, 15(5–6), 219–230.

[3] Fang CL, A Al-Suwayeh S, Fang JY. Nanostructured lipid carriers (NLCs) for drug delivery and targeting. Recent patents on nanotechnology. 2013 Jan 1;7(1):41-55.

[4] Patil, S., Mahadik, K., & Paradkar, A. Polymeric nanoparticles for targeted oral drug delivery: An overview. International Journal of Drug Delivery, 2011, 3, 97–109.

[5] Qureshi, M. J., & Mallikarjun, C. Canagliflozin: A new era in SGLT-2 inhibitors. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 2015, 9(2), 126–130.

[6] Chauhan, D. S., & Chauhan, N. S. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. Asian Journal of Pharmaceutical and Clinical Research, 2020, 13(4), 13–20.

[7] Baka E, Comer JE, Takács-Novák K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. Journal of pharmaceutical and biomedical analysis. 2008 Jan 22;46(2):335-41.

[8] Shah, R., Eldridge, D., Palombo, E., & Harding, I. Lipid nanoparticles: Production, characterization and stability. SpringerPlus, 2014, 3, 1–9.

[9] Shah, R., & Iyer, A. K. Lipid-based drug delivery systems for oral delivery of poorly water-soluble drugs. Journal of Biomedical Nanotechnology, 2016, 12(1), 28–42.

[10] Naga Phani, P.J.V.V. et al. Enhancing the solubility of canagliflozin using SMEDDS: improved bioavailability. J. Chem. Health Risks, 2023,13(6): 361–374.

[11] Sajjad, A. et al. Design and optimization of canagliflozin nanocarriers for enhanced antidiabetic activity. ACS Omega, 2020, 5(28): 17510–17518.

[12] Reddy KA, Karpagam S. Preparation and Characterization of Drug-Loaded Phthalic Anhydride Based Hyperbranched Polyesteramide Microspheres. Pharmaceutical Chemistry Journal. 2017 Mar, 50(12):857-64.

[13] Zhang X, et al. Characterization of insulin-loaded NLC and its hypoglycemic effect on diabetic KK Ay mice. Drug Dev Ind Pharm, 2017, 43(12), 1908–1916.

[14] Muller, R. H., Radtke, M., & Wissing, S. A. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Advanced Drug Delivery Reviews, 2002, 54, S131–S155.

[15] Kwon, M. J., Yun, K. S., & Lee, J. Y. Preparation and evaluation of canagliflozin-loaded lipid nanocarriers for oral delivery. Pharmaceutics, 2020, 12(6), 521.

[16] Mehnert, W., & Mäder, K. Solid lipid nanoparticles: Production, characterization and applications. Advanced Drug Delivery Reviews, 2001. 47(2–3), 165–196.

[17] Unnisa A, et al. Nanostructured lipid carriers to enhance the bioavailability and solubility of ranolazine: optimization and pharmacological evaluations. Pharmaceuticals, 2023, 16(8), 1151.

[18] Haque, S. & Pal, K. Formulation and evaluation of nanostructured lipid carriers for oral delivery of teneligliptin: a novel anti-diabetic agent. J. Drug Deliv. Sci. Technol., 2020, 56: 101521.

[19] Han T, et al. Selenium-coated nanostructured lipid carriers of berberine for type 2 diabetes: formulation, characterization and enhanced anti-hyperglycemic effect. Nutrients, 2021, 13(2), 563.

[20] Jenning V, Thünemann AF, Gohla SH. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. International Journal of Pharmaceutics. 2000 Apr 20;199(2):167-77.

[21] Veseli A, Žakelj S, Kristl A. A review of methods for solubility determination in biopharmaceutical drug characterization. Drug development and industrial pharmacy. 2019 Nov 2;45(11):1717-24.

[22] Chen, M-L. et al. Nanoparticles in medicine: targeting, optimization, and clinical applications. Pharmacol. Rev., 2019, 71(4): 584–640.

[23] Vallianou, N. G., Evangelopoulos, A., Kazazis, C., & Vallianou, A. Canagliflozin and cardiovascular outcomes in type 2 diabetes. World Journal of Diabetes, 2020. 11(10), 444–450.

[24] Beloqui, A., Solinís, M. Á., Rodríguez-Gascón, A., Almeida, A. J., & Préat, V. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. Nanomedicine: Nanotechnology, Biology and Medicine, 2016 12(1), 143–161.

[25] Pattnaik, G. et al. Nanostructured lipid carriers for delivering bioactives – a review. Curr. Pharm. Des., 2021, 27(30): 3190–3202.

[26] Makoni PA, WaKasongo K, Walker RB. Short term stability testing of efavirenz-loaded solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) dispersions. Pharmaceutics. 2019 Aug;11(8):397.