Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2024; Vol 23: Issue 1, https://carcinogenesis.com/

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* drug release profile, TEM, and stability evaluation of the optimized formulation. Canagliflozin-loaded NLCs were prepared using a high-speed homogenization method followed by ultrasonication. 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 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 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.*

How to Cite: Miss. Chandrawanshi Mayuri J., Nagoba Shivappa N., (2024) Formulation and Evaluation of Canagliflozin-Loaded Nanostructured Lipid Carrier, *Journal of Carcinogenesis, Vol.23, No.1*, 198-215

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, sustained 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 ethanol, (1000 µg/ml) respectively. Then 0.1ml of above solution was diluted to 10 ml ethanol to make 10 µg/ml solution.

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

Calibration Curve of Canagliflozin in Ethanol

Preparation of stock solution in Ethanol:

Standard stock solution was prepared by taking 50 mg in 50 ml of ethanol (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 the 1000 µg/ml stock solution, 10 ml was diluted to 100 ml standard solutions with concentrations of 2, 4, 6, 8, 10, and 12 ppm were prepared by diluting with 10 ml ethanol. Absorbance recorded at 292 nm & 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 of Active Pharmaceutical Ingredient (API). The X-ray diffraction (XRD) patterns 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]

- Solid lipid, liquid lipid, and canagliflozin were first weighed precisely and melted together at 70 °C.
- The molten lipid mixture was then introduced dropwise into the aqueous surfactant solution, which was kept at 70 °C.
- Homogenization at 15,000 rpm was carried out to obtain a pre-emulsion, followed by ultrasonication for 15 minutes at 70 °C to prevent any lipid crystallization.
- The formed oil-in-water emulsion was finally cooled gradually to room temperature with continuous stirring, allowing the lipids to recrystallize and form the NLCs.

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]

A Quality by Design (QbD) framework was adopted for formulation optimization. Response surface methodology was applied to examine the impact of lipid and surfactant concentrations on critical quality attributes such as drug content (Y1), entrapment efficiency (Y2), and drug release (Y3). The experimental design and optimization process were executed using Design-Expert® software. All the finalized independent variables and the response variables are described in Table below.

Table 01: Details of variables

Independent variable	Low value (-1)	Middle Value (0)	High value (+1)	Dependent variables	Constraints
Lipid conc (%)	2	3.5	5	Drug content (%)	Maximize
Surfactant conc (%)	1	1.25	1.5	Entrapment Efficiency (%)	Maximize
				Drug release (%)	Maximize

Table 02: DOE suggested batches for Canagliflozin

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

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 was determined by dissolving 1 mL of prepared NLCs in 10 mL of ethanol. The amount of Canagliflozin (%) in each formulation was determined spectrophotometrically by measuring the absorbance of the clear supernatant at max of 292 nm. Each experiment was performed in triplicate.

Entrapment efficiency (%)[24]

An entrapment efficiency study was 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 14000 rpm for 30 min to separate the lipid and aqueous phase. The supernatant was then diluted and analysed on spectrophotometer at 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 diffusion study**

In vitro drug diffusion studies of Canagliflozin-loaded nanostructured lipid carriers (NLCs) were conducted using the Franz diffusion with phosphate buffer (pH 6.8) as the release medium at 37 °C. A 2 mL volume of the NLC formulation was accurately measured 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 (0.5, 1, 2, 4, 6, 8, 10 and 12 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

Approximately 1000 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 400–4000 cm^{-1}.

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 on 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]

The optimized Nanostructured Lipid Carriers (NLCs) containing Canagliflozin were filled into glass vials, sealed with screw caps, and subjected to stability studies at 2-8 ° C for 6 month to ensure safety, efficacy & quality throughout their shelf life, in accordance with ICH guidelines [Q1A (R2)].

3. RESULT AND DISCUSSION

Spectrophotometric characterization of Canagliflozin in UV Spectroscopy.

- **Scanning for λmax of Canagliflozin**

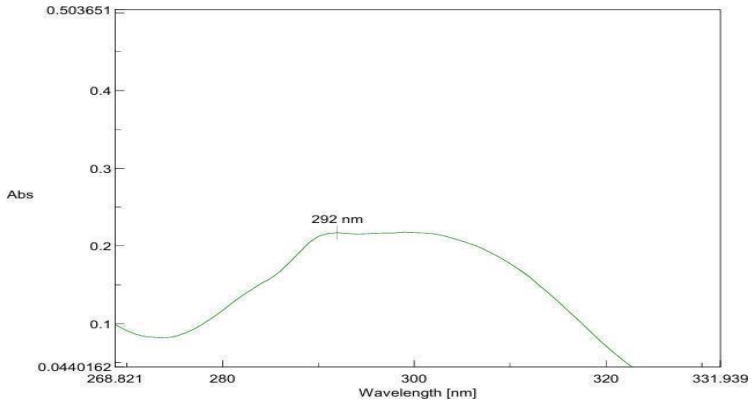

Figure 01: UV spectrum of Canagliflozin in ethanol (10µg/ml)

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 Ethanol

Concentration (µg/ml)	Absorbances
0	0
2	0.0364±0.013
4	0.0681±0.011
6	0.0983±0.019
8	0.1267±0.098
10	0.1558±0.017
12	0.1869±0.026

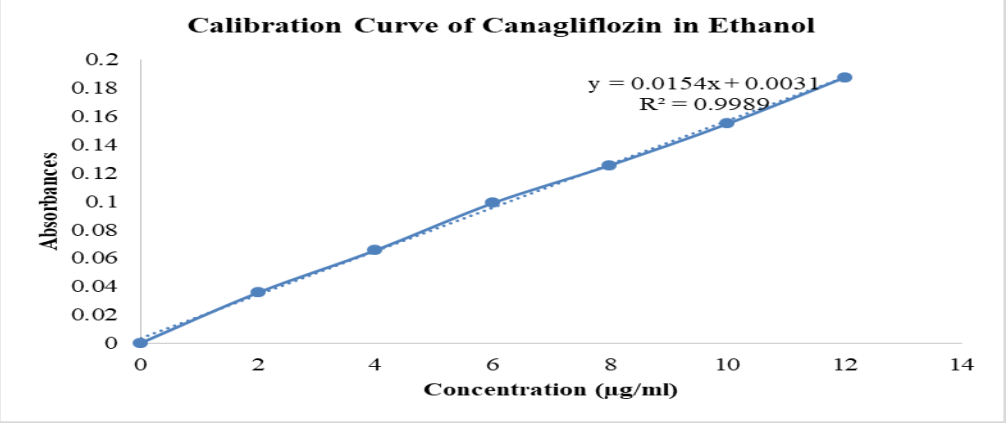

Figure 02: Calibration Curve of Canagliflozin in ethanol

IR Spectroscopy

FTIR of Canagliflozin

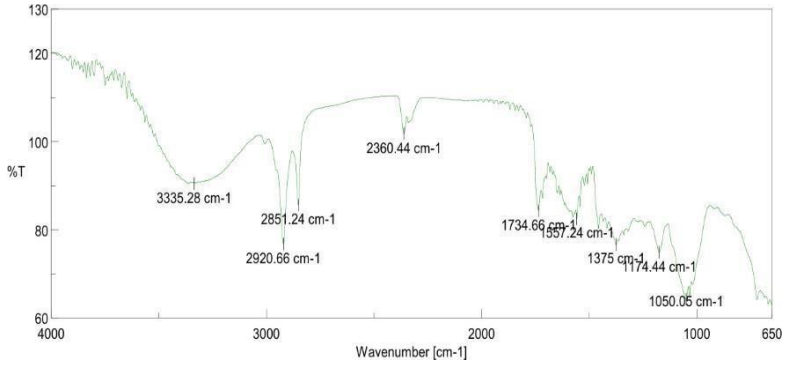

Figure 03: FTIR spectra of Canagliflozin

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.

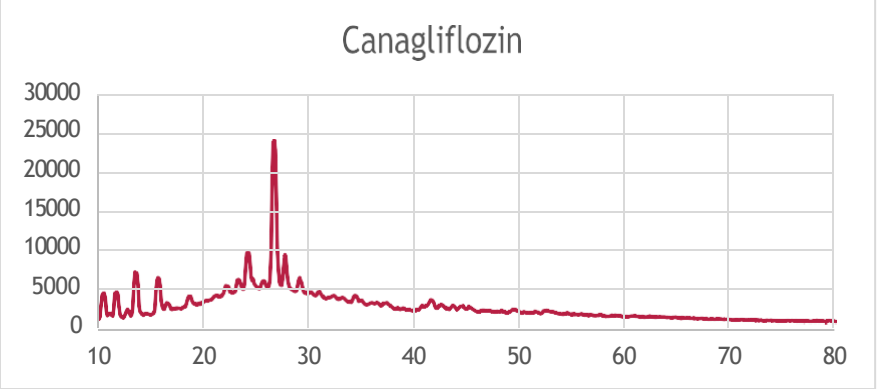

Figure 04: XRD graph of Canagliflozin pure drug

Evaluation of NLC

Drug Content (%)

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

Formulation code	Drug Content (%)
CF1	91.88±0.45
CF2	89.36±0.14
CF3	96.23±0.23
CF 4	92.49±0.66
CF 5	93.68±0.42
CF 6	88.91±0.49
CF 7	87.76 ±0.87
CF 8	85.17±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	102.35	5	20.45	13.82	0.0276	significant
A-Lipid Conc	2.34	1	2.34	1.58	0.2961	
B-Surfactant Conc	21.61	1	21.61	14.60	0.0314	
AB	7.02	1	7.02	4.73	0.1172	
A^2	2.47	1	2.47	1.66	0.2849	
B^2	68.85	1	68.85	46.53	0.0063	
Residual	4.45	3	1.47			
Cor Total	106.79	8				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 13.82 implies the model is significant. There is only a 2.76% 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.	1.21		**R^2**	0.9583
Mean	90.45		**Adjusted R^2**	0.8890
C.V. %	1.33		**Predicted R^2**	0.8889
			Adeq Precision	9.5739

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

Drug Content	=
+87.28	
-0.6265	A
+1.89	B
+1.32	AB
-1.11	A^2
+5.86	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	=
+235.34536	
-1.36036	Lipid Conc

-239.50667	Surfactant Conc
+3.53334	Lipid Conc * Surfactant Conc
-0.496295	Lipid Conc^2
+93.89334	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.

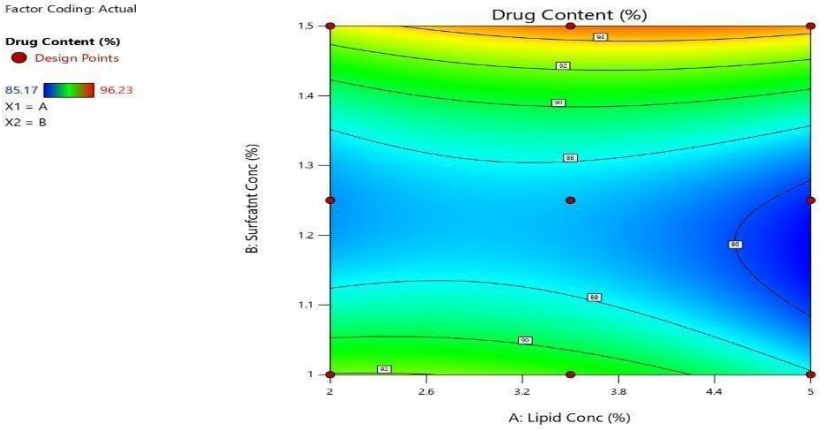

Figure 05: Counter plot for Response 1: Drug Content (CF1-CF9)

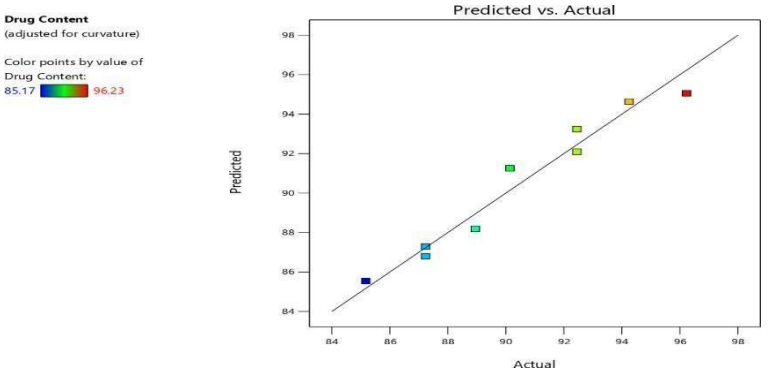

Figure 06: Predicted Vs Actual plot for Response 1: Drug Content (CF1-CF9)

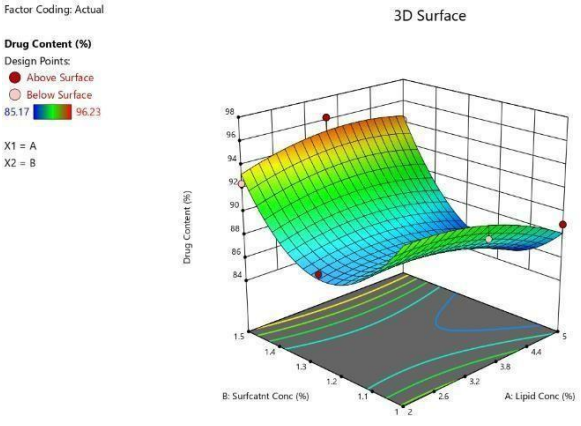

Figure 07: 3D Surface response plot for Response 1: Drug Content (CF1-CF9)

Entrapment efficiency

Table 11: Entrapment efficiency of Canagliflozin loaded NLCs

Formulation code	Entrapment efficiency (%)
CF1	89.46±1.27
CF2	82.41±1.44
CF3	94.56±1.39
CF 4	91.89±1.13
CF 5	90.24±1.08
CF 6	84.57±1.60
CF 7	85.26±1.36
CF 8	81.56±1.81
CF 9	91.15±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	139.19	5	27.83	12.31	0.0325	significant
A-Lipid Conc	0.0366	1	0.0368	0.0162	0.9064	
B-Surfactant Conc	61.29	1	61.29	27.12	0.0137	
AB	0.4095	1	0.4095	0.1811	0.6989	
A^2	10.16	1	10.16	4.49	0.1239	
B^2	67.27	1	67.27	29.76	0.0120	
Residual	6.76	3	2.25			
Cor Total	145.98	8				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 12.31 implies the model is significant. There is only a 3.25% 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.	1.49		**R^2**	0.9535
Mean	86.28		**Adjusted R^2**	0.8761
C.V. %	1.73		**Predicted R^2**	0.8759
			Adeq Precision	9.2304

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

Entrapment efficiency	=
+83.92	
+0.0782	A
+3.19	B
+0.3199	AB
-2.25	A^2
+5.79	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	=
+204.21665	
+6.00110	Lipid Conc
-222.20000	Surfactant Conc
+0.853332	Lipid Conc * Surfactant Conc
-1.00221	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.

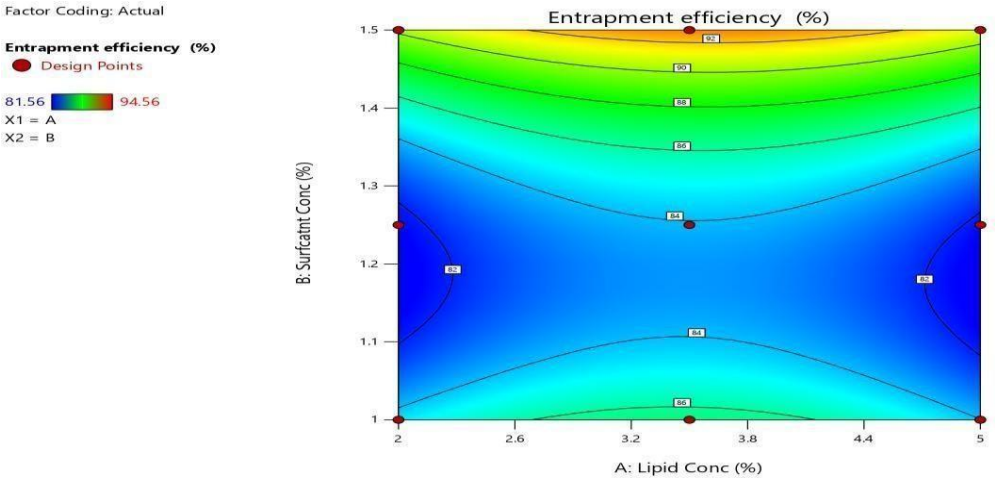

Figure 08 : Counter plot for Response 2: Entrapment efficiency (CF1-CF9)

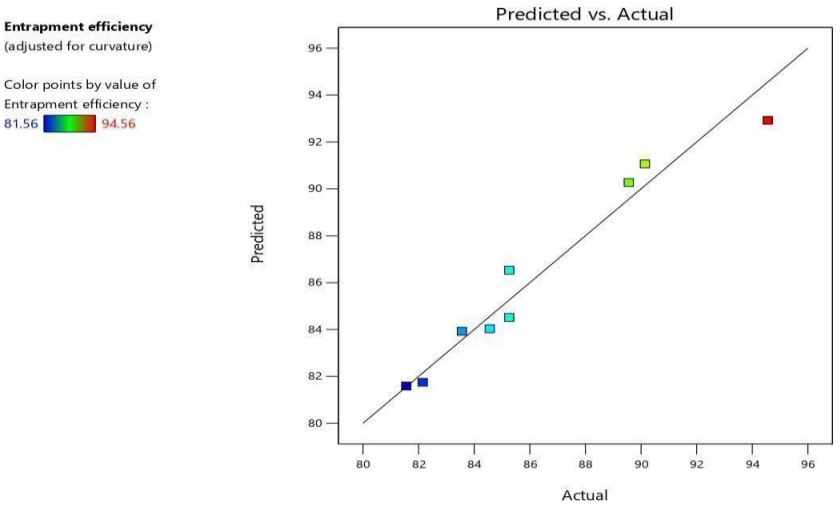

Figure 9: Predicted Vs Actual plot for Response 2: Entrapment efficiency (CF1-CF9)

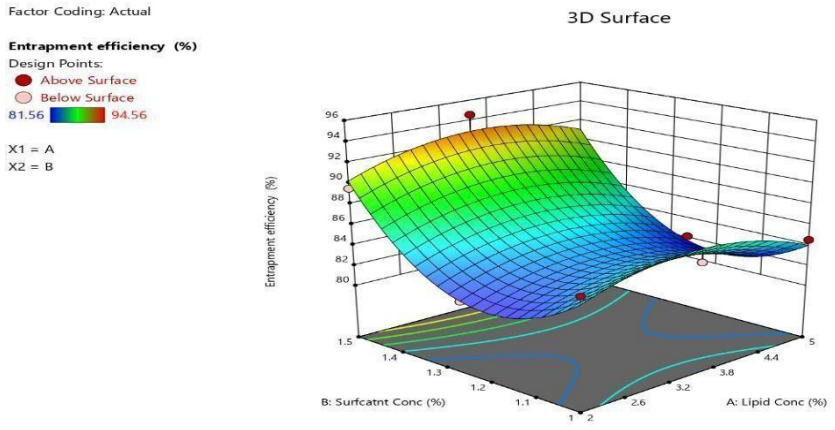

Figure 10: 3D Surface response plot for Response 2: Entrapment efficiency (CF1-CF9)

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
0.5	21.49±0.95	14.48±1.13	28.16±1.24	23.96±1.11	25.53±1.29	16.74±0.93	15.63±0.89	12.62±1.08	17.87±0.65
1	29.58±0.84	23.72±1.02	37.43±0.79	31.65±0.54	32.29±0.89	24.19±1.15	22.67±0.78	20.59±1.12	26.74±0.77
2	37.46±1.23	31.67±0.85	49.72±1.01	41.53±1.02	43.38±0.47	33.31±0.67	32.59±0.43	29.72±0.98	35.31±0.28
4	48.39±1.05	39.59±1.47	58.58±1.13	50.78±1.23	51.66±0.73	41.89±0.56	40.74±0.25	37.81±1.35	43.85±0.17
6	60.92±0.84	47.64±0.67	67.89±1.07	62.59±0.36	63.42±1.12	56.23±0.59	53.87±1.13	44.79±1.21	57.67±0.85
8	72.53±0.94	53.23±0.58	79.35±0.87	74.42±0.87	76.97±0.81	68.88±0.74	64.84±0.49	50.43±1.26	70.29±0.61

10	80.96±1.13	66.69±1.15	87.35±0.62	82.35±1.14	85.54±0.38	77.94±0.16	74.65±1.18	63.68±1.09	79.38±1.16
12	89.96±1.02	84.45±0.82	95.14±0.73	90.47±0.97	91.56±0.57	87.35±1.35	85.36±0.98	83.55±1.21	87.74±0.64

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

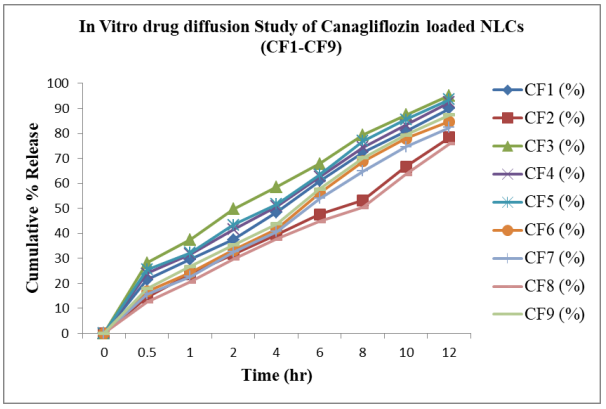

Figure 11: *In Vitro* drug diffusion Study of Canagliflozin (CF1-CF9)

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	126.79	5	25.35	22.43	0.0138	significant
A-Lipid Conc	1.39	1	1.39	1.24	0.3449	
B-Surfactant Conc	36.84	1	36.84	32.59	0.0106	
AB	3.89	1	3.89	3.44	0.1601	
A^2	1.40	1	1.40	1.23	0.3459	
B^2	83.24	1	83.24	73.64	0.0032	
Residual	3.38	3	1.12			
Cor Total	130.19	8				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 22.43 implies the model is significant. There is only a 1.38% 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.	1.05		**R^2**	0.9739
Mean	88.95		**Adjusted R^2**	0.9305
C.V. %	1.19		**Predicted R^2**	0.9303
			Adeq Precision	11.8113

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

Drug release	=
+85.23	
-0.4849	A
+2.47	B
+0.9874	AB
-0.8382	A^2
+6.44	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	=
+242.20710	
-1.00679	Lipid Conc
-257.37000	Surfactant Conc
+2.63332	Lipid Conc * Surfactant Conc
-0.372592	Lipid $Conc^2$
+103.22665	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.

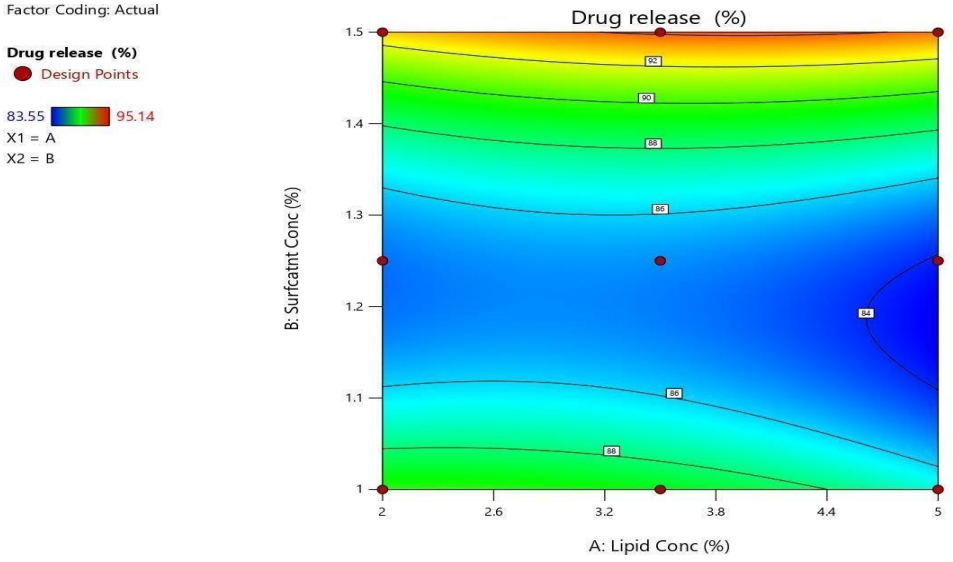

Figure 12: Counter plot for Response 3: Drug release (CF1-CF9)

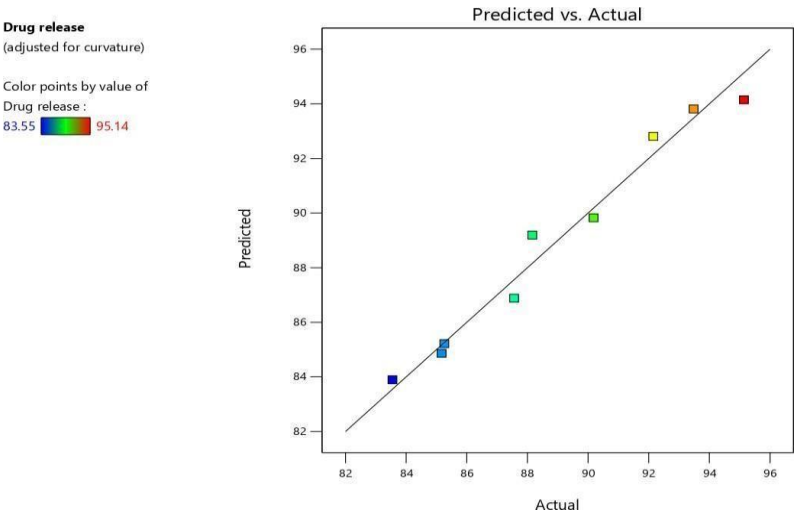

Figure 13: Predicted Vs Actual plot for Response 3: Drug release (CF1-CF9)

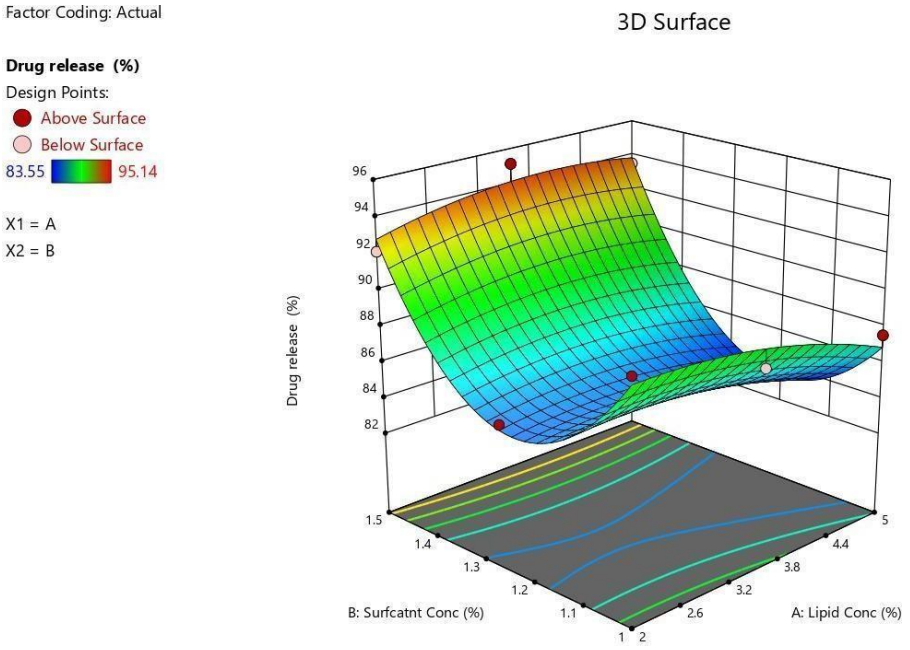

Figure 14: 3D Surface response plot for Response 3: Drug release (CF1-CF9)

Particle size analysis and Zeta potential

Table 21: Particle size analysis of Canagliflozin (CF3) NLC formulation

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

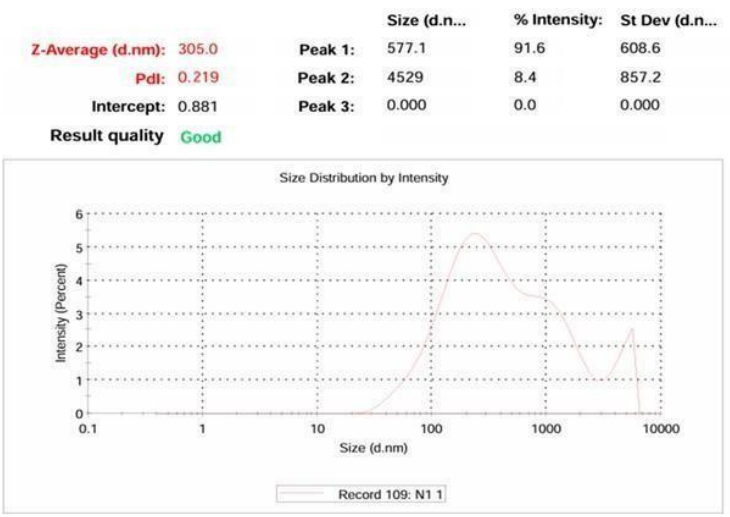

Figure 15: Particle size and PDI of CF3

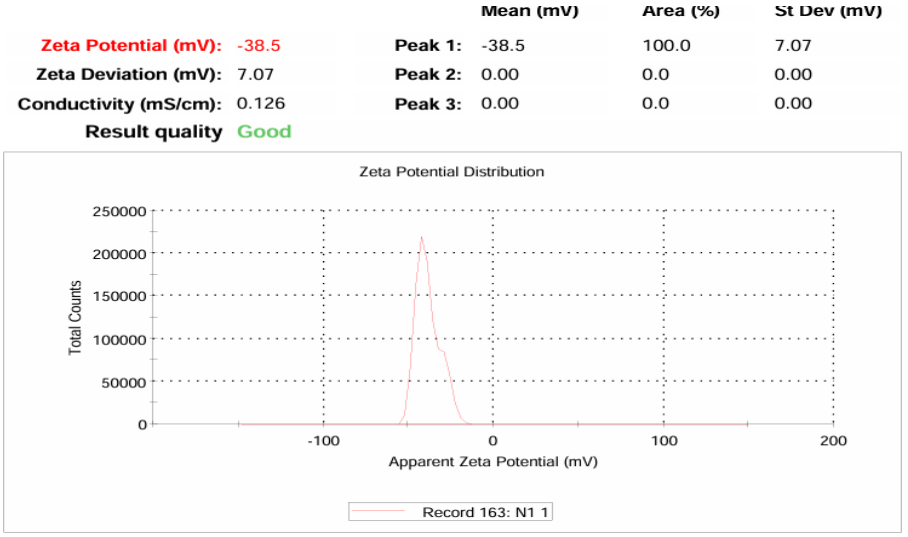

Figure 16: Zeta Potential of CF3

FTIR of Canagliflozin physical mixture

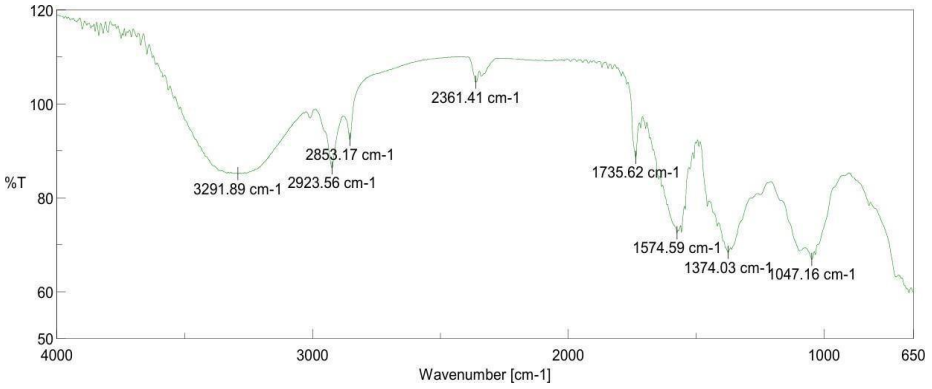

Figure 17: FTIR spectra of Canagliflozin Physical mixture

Analyzing the FTIR spectra of a physical mixture of Canagliflozin 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)

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.

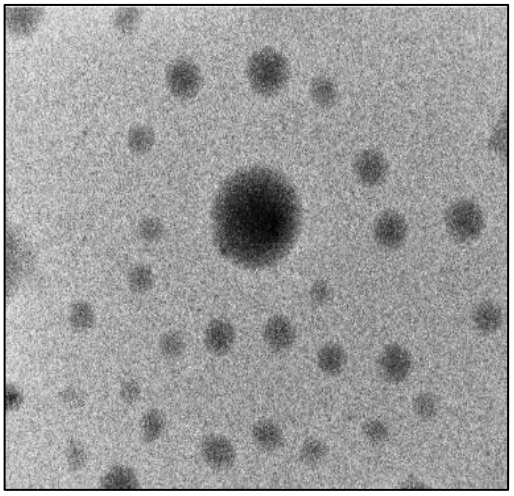

Figure 18: TEM of optimized batch (CF3)

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.

It was observed that the optimized Canagliflozin NLC formulation is stable at 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 by 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 diffusion studies demonstrated a sustained release profile, indicating the potential of NLCs to provide delivery of Canagliflozin over an extended period. Additionally, the formulation showed improved dissolution behavior,

which could contribute to better oral bioavailability.

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

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