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Research Article

Application of Box-Behnken Design in the Development of Nanostructured Lipid Carriers for an Anticancer Drug

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Abstract
The present study aimed to develop and optimize Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) to enhance the solubility, entrapment efficiency, and sustained drug release of the BCS Class IV anticancer drug, Ceritinib, using the Box-Behnken Design (BBD). Capmul concentration (A), Egg Lecithin concentration (B), and Sonication time (C) were selected as independent variables, while Particle Size (Y1), Entrapment Efficiency (Y2), and Drug Release (Y3) were response variables. 17 experimental formulations were prepared and evaluated, and the data was analyzed using a quadratic polynomial model with ANOVA validation. The optimized formulation was prepared using 10% Capmul, 3% Egg Lecithin, and 5 minutes of sonication time, which resulted in a particle size of 214.23 nm, entrapment efficiency of 94.25%, and drug release of 85% over 48 hours. FTIR and DSC studies confirmed the compatibility and stability of Ceritinib with excipients, while SEM images revealed spherical, uniform nanoparticles with smooth and porous surfaces. In vitro release studies showed biphasic drug release with an initial burst followed by sustained release, following Korsemeyer-Peppas release kinetics (R^2 = 0.995), suggesting a diffusion-controlled non-Fickian release mechanism. Stability studies over six months under refrigerated, long-term, and accelerated conditions demonstrated minimal changes in particle size, entrapment efficiency, and drug content, indicating good formulation stability. The study concluded that C-NSLCs are a promising delivery system for improving the bioavailability and therapeutic efficacy of Ceritinib.

Key Words: Bioavailability, BCS Class IV, Anti cancer Drugs, Box Behnken Design

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Introduction

Cancer is a complex and uncontrolled proliferation of abnormal cells, which can invade surrounding tissues and metastasize to distant organs, leading to life-threatening conditions. Effective chemotherapeutic drugs are needed to combat cancer, the world's largest cause of death (Siegel et al., 2023). Anticancer medications damage DNA, limit mitotic spindle formation, and disrupt tumor-growth signalling pathways (El-Najjar et al., 2020). Due to inadequate solubility and absorption, many anticancer medicines have inferior therapeutic results (Wang et al., 2021).Cancer cells are targeted by biochemical pathways in anticancer medicines. These medications

may impede cell proliferation, cause apoptosis, or disrupt DNA replication and repair. These pathways must be understood to produce successful cancer treatments.

Anticancer medicines or chemotherapy
These medicines impair rapidly dividing cancer cells' growth. Mechanistic classifications of drugs include:

Cytotoxins Making DNA Damaged
Cancer cells undergo apoptosis due to direct DNA damage from these medications. Examples include, Alkylating drugs like cyclophosphamide and cisplatin impair DNA replication.
Topoisomerase inhibitors etoposide and doxorubicin prevent DNA unwinding for replication and transcription (El-Najjar et al., 2020).

Blockers of mitosis
These medicines prevent cell division by inhibiting mitotic spindle formation. This includes: Vinca alkaloids like vincristine and vinblastine hinder microtubule assembly.
Paclitaxel and docetaxel stabilise microtubules, preventing depolymerisation and interrupting mitosis.

Targeted treatment
This therapy targets cancer-related molecular targets. Traditional medicines can impact malignant and healthy cells, unlike our technique. Targeted therapy improves efficacy and reduces negative effects by targeting pathways or mutations. Targeted treatment suppresses cancer-related proteins, receptors, and signalling pathways, unlike traditional chemotherapy, which affects both healthy and malignant cells. TKIs including lapatinib, cabozantinib, and erlotinib suppress cancer cell signalling enzymes. Trastuzumab and rituximab, monoclonal antibodies (mAbs), attach to cancer cell antigens to help the immune system kill them.

Hormonal therapy
This is used to treat hormone-sensitive cancers like breast and prostate. These drugs suppress hormones to stop tumour growth. In breast cancer, selective oestrogen receptor modulators (SERMs) like tamoxifen block receptors. Abiraterone suppresses testosterone production in prostate cancer.

Immunotherapy, checkpoint inhibitors
These drugs increase the immune system's ability to fight cancer. Immune cells may recognise and attack cancers with checkpoint inhibitors like pembrolizumab and nivolumab, which block the PD-1/PD-L1 and CTLA-4 pathways. A patient's T cells are genetically modified to target and kill cancer cells in CAR-T cell therapy.

Anticancer Drug Challenges
Anticancer medications face formulation and pharmacokinetic issues that reduce efficacy despite treatment advances.

Reduced solubility and permeability
Many anticancer drugs, notably BCS Class IV, have limited solubility and permeability, resulting in uneven absorption and low bioavailability (Wang et al., 2021). Lapatinib, Cabozantinib, and Paclitaxel require solubilising agents, which may increase toxicity (Rizvi & Saleh, 2022).

Quick Half-Life and Elimination
5-Fluorouracil (5-FU) has a short plasma half-life, necessitating several doses that increase systemic toxicity and decrease patient adherence (Chen et al., 2021).

High systemic toxicity
Both malignant and healthy cells are affected by chemotherapy, causing myelosuppression,
gastrointestinal damage, and neuropathy (Chaudhary et al., 2022).

Pharmacological resistance
Overexpression of efflux transporters (e.g., P glycoprotein), drug target mutations, and alternative survival pathways reduce therapeutic effectiveness in cancer cells (Bhatia et al., 2020).

Limitations of the BBB
Many anticancer drugs cannot cross the blood-brain barrier, limiting their effectiveness in treating brain tumours such glioblastoma (Rizvi & Saleh, 2022). Advanced drug delivery methods, such as Nanostructured Lipid Carriers (NLCs), may increase bioavailability, toxicity, and therapeutic results (Jain et al., 2023).
The BCS classifies medicines by solubility and permeability. BCS Class IV drugs have limited solubility and permeability, making oral bioavailability variable (Amidon et al., 2021). Lapatinib, Cabozantinib, and Ceritinib are examples of anticancer medicines with formulation issues (Rizvi & Saleh, 2022). Dissolution rate-limited absorption and poor permeability limit systemic uptake of these medicines (Singh et al., 2020). Therapeutic plasma concentrations sometimes require a greater dosage, increasing systemic toxicity and side effects (Kumar et al., 2023).
BCS Class IV anticancer medicines' limited oral bioavailability requires dosage escalation, which may exacerbate drug toxicity (Patel et al., 2022). This causes poor patient compliance and treatment failure (Chen et al., 2021). Drug delivery techniques should increase chemical solubility, permeability, and stability to improve bioavailability and therapeutic index (Mishra et al., 2019).
Ceritinib, a second-generation ALK inhibitor, is approved for ALK-positive non-small cell lung cancer. Selectively reducing ALK phosphorylation blocks downstream signalling pathways involved in cancer cell growth and survival. Sharma et al. (2021) Ceritinib, a BCS Class IV medication, has limited permeability and low water solubility, resulting in low and variable oral bioavailability. It is appropriate for formulation as Nanostructured Lipid Carriers (NLCs) to increase

solubility, permeability, and controlled drug release for better therapeutic effects (Kumar et al., 2022). Ceritinib's therapeutic effectiveness is restricted by low gastrointestinal absorption, first-pass metabolism, and dose-dependent gastrointestinal toxicity. These limitations can be overcome using NLC-based formulations' enhanced bioavailability, less systemic toxicity, and targeted tumour tissue delivery. 2023 (Singh et al.)

Materials & Methods

Ceritinib was acquired as a complimentary sample from MSN Laboratories Pvt. Ltd., located in Hyderabad. Glyceryl monostearate, egg lecithin, poloxamer 188, and Capmul MCM were procured from S.D. Chemicals in Hyderabad. All chemicals and solvents utilised in the study are of analytical grade and are employed precisely as received.

Optimization by Box Behnken Design

The optimisation of Ceritinib-loaded Nanostructured Lipid Carriers (NLCs) was conducted utilising the Box-Behnken Design (BBD) within the framework of Response Surface Methodology (RSM) to assess the influence of formulation variables on essential quality attributes. The experimental design and statistical analysis were conducted utilising Design-Expert 13. (Trial Version)

Identification of Independent and Dependent Variables

The study examined three independent formulation variables and their impact on three dependent responses in order to attain an optimised formulation exhibiting desirable characteristics. The chosen independent variables and their respective levels were as follows: Capmul Concentration (% w/w) (A): 4 (-1), 10 (0), 16 (+1), Concentration of Egg Lecithin (% w/v) (B): 0.3 (-1), 1.65 (0), 3 (+1), Sonication Time (min) (C): 2 (-1), 5 (0), 8 (+1). The dependent variables identified for the purpose of optimisation were: Y_1: Particle size (nm) (Minimise), Y_2: Entrapment Efficiency (%) (Objective: Maximise), Y_3: Percentage of Drug Release (Optimisation Target: 80–90%). A total of 17 experimental trials were generated by BBD, including five centre points, to evaluate non-linear relationships and interaction effects among the independent variables. The responses derived from each experiment were subjected to analysis utilising a quadratic polynomial model.

Table 1. List of independent and dependent variables in Box Behnken Design (BBD)

Factors			Levels		
Variable	**Name**	**Units**	**Low**	**Middle**	**High**
A	Concentration of Capmul	% w/w	4	10	16
B	Concentration of Egg lecithin	% w/v	0.3	1.65	3
C	Sonication Time	Min	2	5	8
Responses			Goal		
Y1	Size of the Particle	nm	Minimize		
Y2	Percentage Entrapment Efficiency	%	Maximize		
Y3	Percentage Drug Release	%	Maximise		

Statistical Model Fitting and ANOVA

The responses derived from the experimental trials were subjected to statistical analysis through Analysis of Variance (ANOVA) to ascertain the significance of the model parameters. The equation can be expressed as follows:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$$

In this context, Y denotes the dependent variable, which may refer to particle size, entrapment efficacy, or drug release. The parameter β_0 signifies the intercept, while β_1 through β_3 represent the linear coefficients. The terms β_{12} to β_{23} indicate interaction effects, and β_{11} to β_{33} correspond to the quadratic terms. The statistical significance of the model parameters was assessed utilising p-values ($p < 0.05$) and F-values.

The adequacy of the model was assessed through the following methods:

R^2 (Coefficient of Determination) Modified R^2 Predicted Residual Error Sum of Squares (PRESS) Sufficient Precision Models exhibiting a non-significant lack of fit ($p > 0.05$) were deemed valid.

Response Surface Analysis and Contour Plots

To illustrate the impact of formulation variables on the responses, three-dimensional surface plots and contour plots were created. These diagrams helped identify interactions and trends between variables. The Pareto chart was employed to prioritise the impact of individual factors.

Optimisation Utilising the Desirability Function: Numerical optimisation was conducted utilising the desirability function approach to ascertain the optimal formulation. The criteria were established as follows:

Reduce the particle size (Y_1) to improve bioavailability, Optimise the Entrapment Efficiency (Y_2) to achieve an increased drug loading capacity, Optimise the drug release (Y_3) to achieve a range of 80% to 90% for sustained release The desirability function generated by the software yielded an optimal formulation, with a desirability value (D) approaching 1, signifying the most advantageous combination of factors.

Preparation of Ceritinib Nanostructured lipid carriers

The improved homogenisation procedure was used to create C-NSLCs, which was then followed by probe sonication. Egg lecithin was dissolved in 1:1 ratio of methanol and chloroform which was vapourised for 20 min to completely evaporate the solvent and resulted in

the creation of egg lecithin film. The combination mentioned above was supplemented with glyceryl monostearate and capmul. After then, the drug was included in this combination. The poloxamer solution was heated to the same temperature and then mixed in with that. To create a coarse emulsion, the liquid was heated continuously at 60 degrees Celsius and homogenised for 10 minutes at 2000 revolutions per minute (rpm). Keeping the temperature constant guarantees that the product will remain stable. After that, the mixture was sonicated with a probe for 15 minutes at 50°C. The mixture was then cooled to room temperature in order to convert the lipid into a solid. (Lohan.s.et.al., 2013)

Particle Size, Polydispersity Index, and Zeta Potential

The particle size and polydispersity index (PDI) of the formulations were assessed using dynamic light scattering using a Zetasizer (Malvern Panalytical Ltd., Malvern, UK) Nano ZS, following appropriate dilution (1:100) with distilled water. The technology identified variations in light intensity, attributed to the Brownian motion of lipid nanoparticles, which are utilised to determine particle size and distribution. The zeta potential of the optimised C-NSLCs was assessed using a zeta sizer. The particle size and zeta potential were assessed through sample dilution. (Nekkanti.v.et.al., 2016)

Entrapment efficiency

This was assessed by acidifying the formulation with 0.1 N HCl, as Ceritinib exhibits optimal solubility at acidic pH, leading to the aggregation and separation of lipid nanoparticles. These nanoparticles were then centrifuged at 10,000 rpm for 30 minutes using a high-speed centrifuge. The supernatant was discarded, and the pellets were reconstituted in 10 mL of methanol prior to analysis at 320 nm using a UV spectrophotometer. (Ke Z et.al., 2016)

Compatibility studies of drug & excipients:

FTIR is an effective method for detecting and confirming potential interactions between excipients and the drug. The FTIR spectrum of Ceritinib and the other excipients utilised was recorded using FTIR (FTIR-4800, Shimadzu). A 1 mg solid sample was compressed with 100 mg of KBr into a disc. For the liquid sample, droplets were placed onto a NaCl or KBr aperture plate and subsequently sandwiched between another aperture plate to create a thin liquid membrane. The sample was scanned for absorbance in the range of 4000 to 400 cm^{-1}. The acquired spectra were correlated with one another.(Potu AR et.al., 2012)

Thermal analysis data was obtained utilising a Differential Scanning Calorimeter (DSC 204 Fl.Phoenix) for both the pure drug sample and all excipients incorporated in the formulation.(Fang.CL et.al., 2013)

Scanning Electron Microscopy

The surface morphology of optimized C-NSLCs was studied using SEM (Model JSM-7800F, JEOL Ltd., Tokyo, Japan). The sample was dusted on double sided tape onto aluminium stub coated with gold by using cold sputter coater in SEM chamber of thickness 400A^{o} The graphs were recorded with voltage of 15Kv electron beam.(Shah.P et.al., 2017)

In vitro drug release studies

The dialysis membrane method was employed to perform in-vitro drug release studies on both the optimised formulation and the commercial medication. The dialysis membrane, with a molecular weight ranging from 3500 to 5000Da, was immersed in water overnight using 0.01M HCl as the dissolution medium. The donor compartment contains 150 mg of the optimised formulation, whereas the receptor compartment contains 100 mL of release media maintained at 37°C ± 0.5°C. Approximately 1 ml of the sample solution was collected from the receptor compartment at intervals ranging from 1 to 12 hours, diluted with dissolving buffer, and analysed using a UV spectrophotometer at 320 nm.(Mishra B.et.al., 2017)

Assessment of drug release kinetics

The dissolution data from the optimised formulation were analysed using various kinetic equations, including zero order, first order, Korsmeyer-Peppas, and Higuchi, to determine the mechanism and manner of drug release. The release data from the NSLCs were ascertained using a curve fitting method. Curve fitting entails the selection of a mathematical model (e.g., zero-order, first-order) that most accurately represents drug release behaviour. Model parameters are optimised through nonlinear regression to reduce the discrepancy between experimental data and model predictions. Goodness-of-fit metrics such as R-squared and RMSE evaluate the precision of the fitted curve. The visualisation of the fitted curve with the experimental data confirms the model's appropriateness. The interpretation of fitted parameters yields insights into the release mechanism and kinetics, facilitating the optimisation of formulations for controlled drug delivery systems.

Stability Studies

The stability study of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) aimed to evaluate the retention of particle size, which affects solubility, drug content and entrapment efficiency, which influences drug availability, over time, thereby ensuring product quality and bioavailability are maintained. The optimised C-NSLCs were stored under various temperature conditions in accordance with ICH guidelines, utilising a stability chamber (Model ICH-256, Memmert GmbH + Co. KG, Schwabach, Germany). The formulations underwent long-term stability testing at 25°C ± 2°C with 60% ± 5% relative humidity, refrigerated conditions at 4°C ± 2°C, and accelerated stability testing at 40°C ± 2°C with 75% ± 5% relative humidity over a period of six months.

Particle size and entrapment efficiency were periodically assessed to evaluate the formulation's stability.

Results & Discussion

The optimisation of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) through Box-Behnken Design (BBD) effectively delineated the relationship between formulation variables and critical quality attributes. The experimental data were analysed using a quadratic polynomial model, and its adequacy was validated through ANOVA analysis. The p-values (<0.05) demonstrate that the quadratic model is statistically significant for all responses, with no notable lack-of-fit.

Table 2: Composition & Evaluation parameters of Ceritinib Nanostructured Lipid Carriers (C-NSLCs)

Formulation Code	Capmul (% w/w)	Egg Lecithin (% w/v)	Sonication Time (min)	Particle Size (nm)		Entrapment Efficiency (%)		Drug Release (%)	
				OV	PV	OV	PV	OV	PV
CF1	4	0.3	5	218.12	216.25	58.12	59.13	91.62	91.75
CF2	10	1.65	5	200.02	200.00	85.01	85.00	85.02	85.00
CF3	16	0.3	5	320.03	320.00	72.23	69.13	72.01	71.63
CF4	10	3	2	192.12	193.75	90.32	86.00	83.21	81.88
CF5	10	3	8	190.32	188.75	88.12	85.75	84.01	84.13
CF6	10	0.3	2	260.67	261.25	65.00	67.25	78.03	77.88
CF7	4	1.65	8	200.45	201.25	63.23	62.38	92.12	91.50
CF8	16	3	5	289.23	287.50	78.24	81.38	74.13	74.63
CF9	16	1.65	8	291.34	293.75	75.00	73.88	76.21	75.25
CF10	10	1.65	5	200.04	200.00	85.01	85.00	85.01	85.00
CF11	4	0.3	5	250.12	252.50	55.34	51.62	90.23	89.38
CF12	10	1.65	5	200.00	200.00	85.04	85.00	85.01	85.00
CF13	4	3	5	170.02	170.00	70.43	72.88	94.32	94.38
CF14	10	0.3	8	228.23	236.25	67.34	71.00	79.12	80.13
CF15	16	1.65	2	310.01	308.75	73.32	73.63	70.23	70.50
CF16	10	1.65	5	200.00	200.00	85.09	85.00	85.21	85.00
CF17	10	1.65	5	200.01	200.00	85.11	85.00	85.13	85.00

OV – Observed Value, PV – Predicted Value

Effect on Particle Size

The particle size of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) was significantly affected by the formulation variables, specifically Capmul concentration (A), Lecithin concentration (B), and Sonication Time (C). The derived response surface equation for particle size is presented as follows:

Particle Size = 200 + 46.25A - 28.25B - 7.50C + 12.50AB + 0.00AC + 5.00BC + 46.25A² + 11.25B² + 8.75C²

A, B, and C denote the Capmul concentration (% w/w), Lecithin concentration (% w/v), and Sonication Time (min), respectively. The coefficients, both positive and negative, signify the direction and magnitude of each factor's effect on particle size. The interactive effects of these variables on particle size are illustrated in the 3D response surface plots and contour plots.

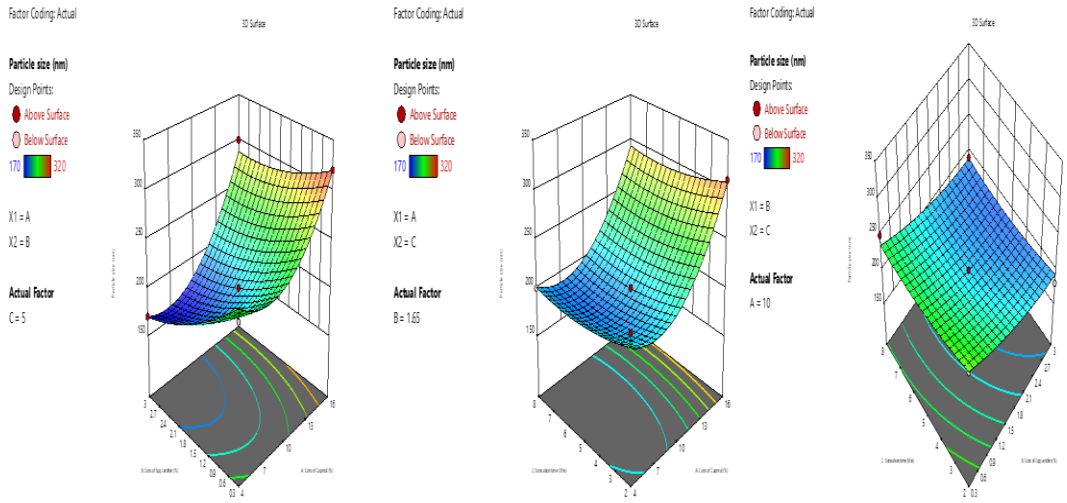

Fig. 1 Response Surface Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Particle Size

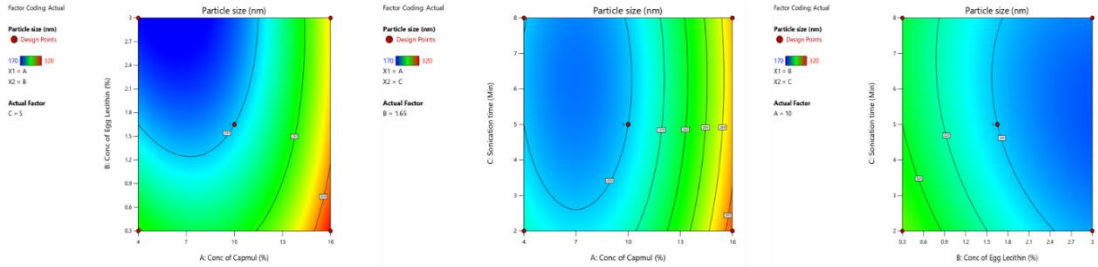

Fig.2. Contour Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Particle Size

The positive coefficient (+46.25A) indicates that an increase in Capmul concentration significantly enhances particle size. The presence of higher lipid content elevates the viscosity of the formulation, leading to the formation of larger lipid droplets during the emulsification process. The response surface plots demonstrate that elevated Capmul concentrations (16% w/w) correspond to increased particle sizes (~320 nm), while reduced Capmul concentrations (4% w/w) yield smaller sizes (~170 nm).

The negative coefficient (-28.25B) signifies that an increase in Lecithin concentration leads to a decrease in particle size. Lecithin functions as a stabiliser by improving the dispersion of lipid nanoparticles, minimising coalescence, and inhibiting aggregation, which results in smaller and more uniform particles. The response surface plots indicate that formulations with higher Lecithin concentrations (3% w/v) result in smaller particle sizes (~170 nm), whereas lower Lecithin levels (0.3% w/v) correspond to larger sizes (~320 nm).

The negative coefficient (-7.50C) indicates that an increase in sonication time leads to a reduction in particle size. Sonication disrupts larger lipid aggregates, enhancing emulsification and decreasing droplet size. The response surface plots indicate that extended sonication (8 min) results in a reduction of particle size, while shorter sonication (2 min) produces comparatively larger particles. The impact of sonication is less pronounced than that of Capmul and Lecithin concentrations, as evidenced by the smaller coefficient magnitude.

The study demonstrated that Capmul concentration (A) is the primary factor influencing particle size, with higher lipid content resulting in increased particle size. The concentration of lecithin (B) exhibits a significant inverse relationship, leading to a reduction in particle size as a result of its emulsifying and stabilising characteristics. Sonication duration (C) influences size reduction, yet its effect is relatively minor in comparison to the composition of the lipid phase. The response surface plots and regression equation indicate that an optimal combination of Capmul (10% w/w), Lecithin (3% w/v), and Sonication Time (5–6 min) yields the smallest and most stable NLCs, approximately 200 nm in size.

Effect on Percentage entrapment efficiency

The entrapment efficiency (%) of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) was significantly affected by the formulation variables, as illustrated by the quadratic equation:

Entrapment efficiency = 85 + 6.50A + 8.38B + 0.8750C – 2.25AB - 0.75AC – 1.0BC – 13.25A² -3.00B² -4.50C².

The positive coefficients for A and B suggest that higher concentrations of Capmul and Lecithin improve entrapment efficiency, with Lecithin (B) exhibiting the most significant impact (+8.38), likely attributed to its function in stabilising the drug within the lipid matrix. The slight positive impact of Sonication Time (C, +0.8750) indicates that a moderate duration of sonication enhances drug encapsulation, whereas extended sonication may result in decreased EE% due to disruption of the lipid matrix. The negative interaction term (AB, -2.25) indicates that increased levels of Capmul and Lecithin are associated with a slight decrease in EE%, likely due to the impact of excessive lipid content on drug incorporation. The negative quadratic terms (A², B², and C²) suggest a non-linear response, wherein elevated levels of any variable result in decreased entrapment efficiency, potentially attributable to phase separation, drug expulsion, or unstable lipid dispersion. The 3D response surface plots corroborate these trends, indicating that optimal EE% (approximately 90%) is attained at moderate Capmul levels (around 10% w/w), elevated Lecithin concentrations (approximately 3% w/v), and regulated sonication duration (approximately 5 minutes).

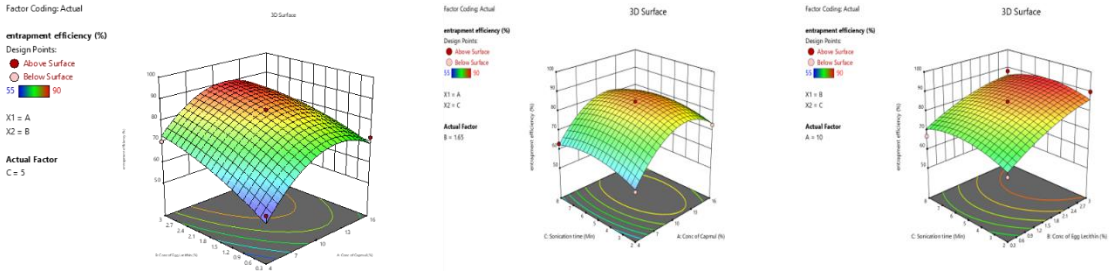

Fig.3. Response Surface Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Entrapment Efficiency

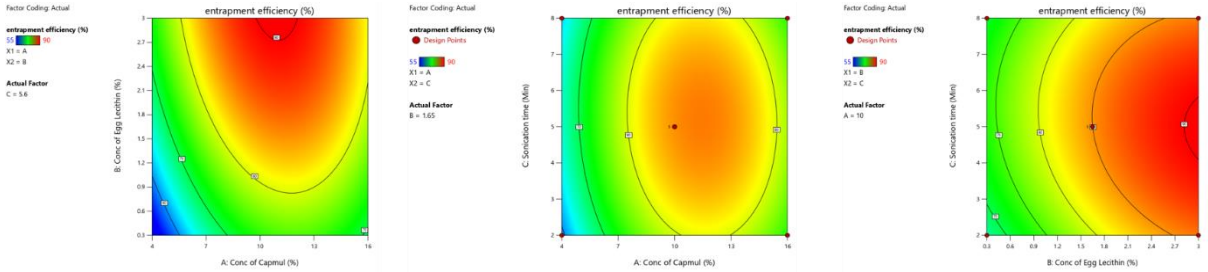

Fig.4. Contour Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Entrapment Efficiency

Effect on Percentage Drug Release

The drug release percentage of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) was significantly affected by the formulation variables, as indicated by the quadratic equation,

Drug Release = 85 – 9.38A + 2.00B + 1.13C – 0.50AB + 1.25AC + 0.0BC – 0.6A^2 – 1.87B^2 – 2.13C^2

The negative coefficient for Capmul (-9.38A) suggests that increased Capmul concentrations lead to a notable reduction in drug release, probably due to the development of a denser lipid matrix that hinders drug diffusion. The positive coefficient for Lecithin (+2.00B) indicates that an increase in Lecithin concentration enhances drug release, likely by improving drug dispersion in the aqueous phase. The marginal positive effect of Sonication Time (+1.13C) suggests that extended sonication enhances drug release rates, presumably through a reduction in particle size and an increase in surface area. The interaction terms (AB, AC) exhibit negligible effects, suggesting weak synergistic interactions among the variables. The negative quadratic terms (A^2, B^2, C^2) indicate a non-linear response, implying that high levels of any variable result in reduced drug release, potentially due to phase separation or lipid crystallisation. The 3D response surface plots confirm these results, indicating that optimised drug release (approximately 85%) is attained with moderate Capmul (10% w/w), elevated Lecithin (3% w/v), and regulated sonication (around 5 minutes). The findings highlight the importance of maintaining a balanced lipid composition and regulating processing parameters to attain a sustained drug release profile in NLC formulations.

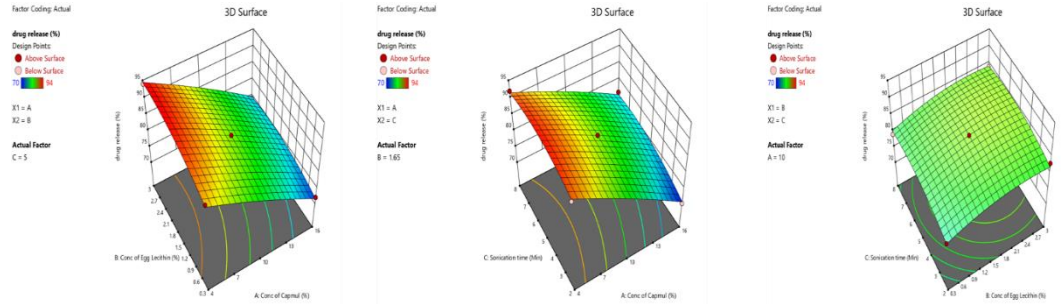

Fig.5. Response Surface Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Percentage Drug Release

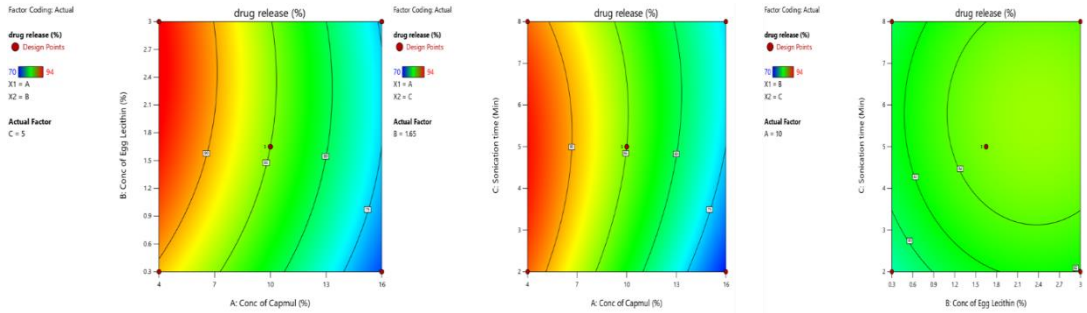

Fig.6. Contour Plots Showing the Effect of Capmul, Lecithin, and Sonication Time on Percentage Drug Release

Table 3: Experimental Results

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response 1	0.9704	0.9325	0.9272	1.27	0.54
Response 2	0.9562	0.90	0.80	1.48	0.78
Response 3	0.9934	0.9849	0.8945	0.86	1.05

Particle Size, PDI & Zeta Potential

The analysis of particle size distribution, polydispersity index (PDI), and zeta potential of the optimised Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) was conducted to evaluate their stability and homogeneity. The average particle size measured 214.23 nm, suggesting appropriateness for improved bioavailability and cellular absorption. A PDI value of 0.31 indicates a moderately uniform particle distribution, which contributes to minimal aggregation and enhanced colloidal stability. Nanoparticles in the 100–300 nm range are beneficial for targeted drug delivery because of the enhanced permeability and retention (EPR) effect. A zeta potential of +38.20 mV signifies substantial electrostatic repulsion, which diminishes the likelihood of particle aggregation and fosters a stable dispersion system. Zeta potential values exceeding ±30 mV typically signify favourable physical stability, advantageous for extended storage periods. The presence of a positive surface charge may facilitate cellular uptake through interactions with negatively charged biological membranes. The physicochemical properties of the formulation facilitate prolonged circulation, controlled drug release, and effective delivery. The optimised C-NSLCs demonstrate a favourable equilibrium of stability, uniformity, and biopharmaceutical performance, contributing to enhanced therapeutic efficacy.

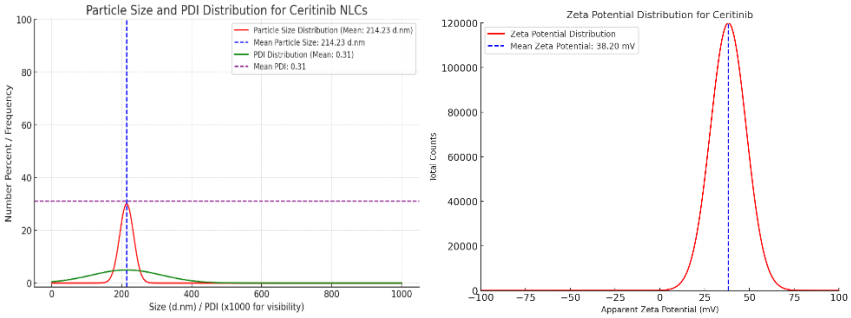

Fig. 7. Particle size, PDI & Zeta potential of the optimised C-NSLCs

Percentage Entrapment Efficiency

The entrapment efficiency (%) of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) demonstrated notable variation across the experimental runs, as illustrated in the column chart. The values varied between 55% and 90%, with the maximum entrapment efficiency noted in formulations that included moderate Capmul (10% w/w) and high Lecithin (3% w/v). The elevation of Lecithin concentration enhanced entrapment efficiency, presumably attributable to its emulsifying characteristics that augment drug retention within the lipid matrix. In contrast, formulations containing low Lecithin (0.3% w/v) and high Capmul (16% w/w) exhibited decreased entrapment efficiency, likely attributable to phase separation and insufficient lipid stabilisation. The findings highlight the significance of lipid composition and processing conditions in optimising NLC formulations to improve therapeutic efficacy.

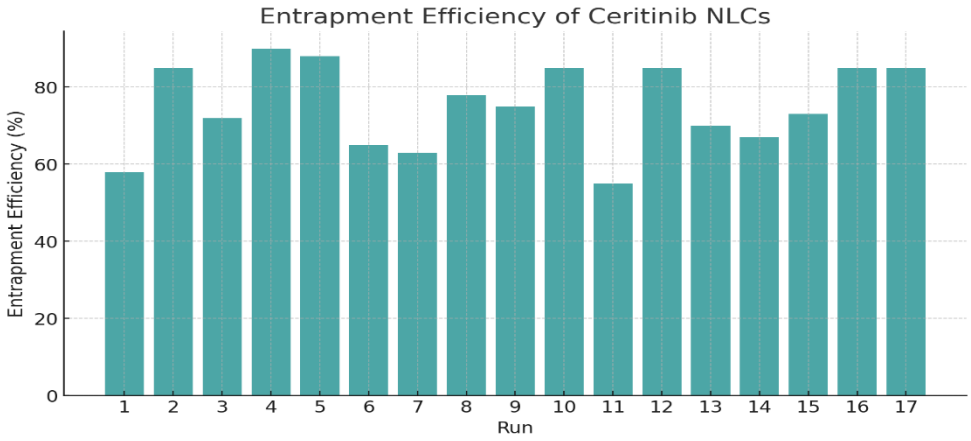

Fig 8. Percentage entrapment efficiency of all the formulations of C-NSLCs

Drug Excipient compatibility studies by FTIR

A compatibility study using FTIR was performed to assess potential chemical interactions between Ceritinib and its excipients. The FTIR spectra of the pure drug and excipients were analysed in comparison to the spectrum of the physical mixture, as illustrated in the FTIR overlay. The characteristic peaks of Ceritinib, including N-H stretching (~3400 cm^{-1}), C=O stretching (~1625 cm^{-1}), and C-F stretching (~1250 cm^{-1}), were retained in the physical mixture without significant shifts, indicating the absence of chemical interactions between the drug and excipients. The characteristic peaks of Egg Lecithin (P=O at ~1230 cm^{-1}), Poloxamer 100 (C-O-C at ~1100 cm^{-1}), Capmul (C=O at ~1745 cm^{-1}), and

Glyceryl Monostearate (C-H at ~2925 cm^{-1}) were preserved in the physical mixture, indicating their compatibility with Ceritinib. No major peak disappearance, shifting, or new peak formation was observed, which confirms that no strong molecular interactions or degradation occurred between the drug and excipients. The findings indicate that the chosen excipients exhibit both physical and chemical compatibility with Ceritinib, thereby ensuring the stability of the nanostructured lipid carrier (NLC) formulation.

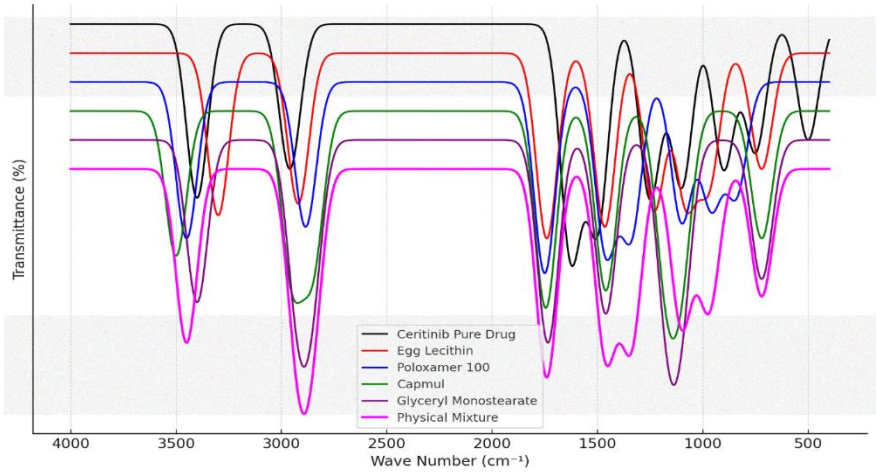

Fig 9. FTIR spectra of pure drug, excipients used and combination of drug and excipients

Differential Scanning Colorimetry

The DSC analysis was conducted to evaluate the thermal stability and compatibility of Ceritinib, along with the excipients Glyceryl Monostearate, Egg Lecithin, Poloxamer 188, and Capmul, as well as their physical mixture. The thermogram of pure Ceritinib exhibited a distinct endothermic peak, thereby confirming its crystalline nature. The excipients displayed either broad or minor transitions, reflecting their amorphous or semi-crystalline properties. The physical mixture exhibited a slightly shifted yet preserved endothermic peak, indicating no notable interactions or incompatibility between the drug and excipients. The identification of all characteristic peaks in the mixture verifies the stability and compatibility of Ceritinib, indicating that the chosen excipients are appropriate for the formulation of Nanostructured Lipid Carriers (NLCs).

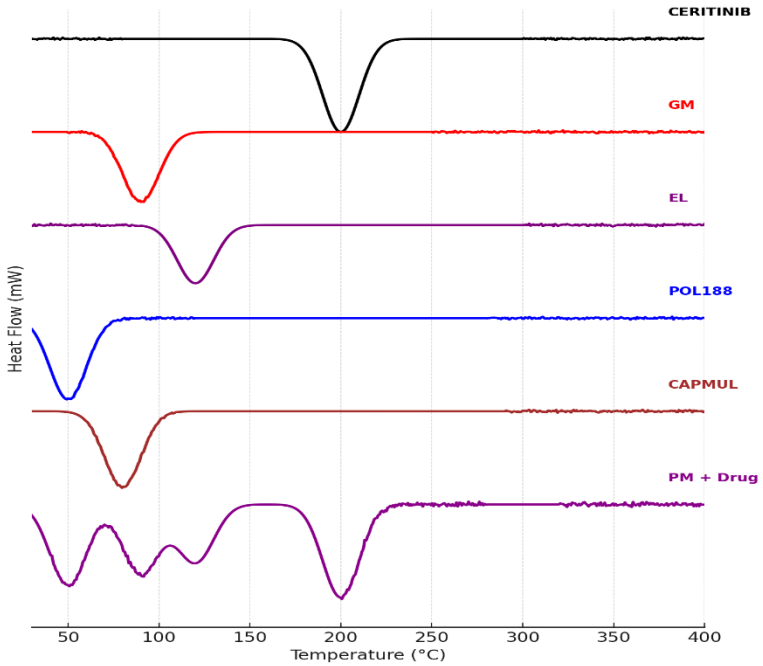

Fig 10. DSC graphs of pure drug, excipients used and combination of drug and excipients

SEM Analysis

The SEM analysis of optimised Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) demonstrated spherical particles with smooth and porous surfaces, indicative of effective lipid encapsulation. The left image displays uniformly distributed particles with minor surface pores, likely resulting from lipid crystallisation or solvent evaporation, while the right image illustrates a densely packed nanostructure, affirming the nanoscale nature of the formulation. The

lack of significant aggregation implies good stability, and the uniform particle morphology reflects efficient emulsification and homogenisation. These findings confirm that the C-NSLCs exhibit advantageous characteristics for improved drug delivery and bioavailability.

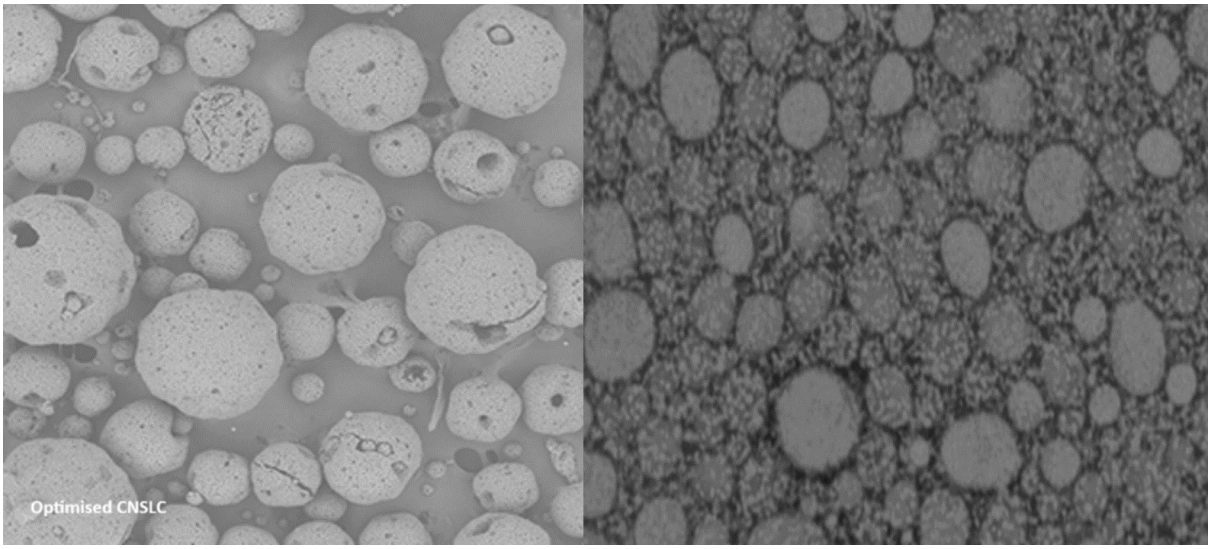

Fig 11. SEM pictures of optimised C-NSLCs

Drug Release Studies

The drug release studies of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) were performed over a 48-hour period to assess the release profiles of various formulations. The release data demonstrated a biphasic pattern, characterised by an initial burst release occurring within the first 2 to 4 hours, succeeded by a sustained release phase lasting up to 48 hours. The formulations exhibited diverse drug release percentages, varying from 70% to 94%, contingent upon the lipid and stabiliser composition. The release profiles adhered to diffusion-controlled mechanisms, as indicated by the Higuchi model, thereby ensuring extended drug availability. Lipid carriers significantly regulated drug release, minimising the risk of dose dumping and enhancing bioavailability. The optimised formulations demonstrated sustained and controlled release, rendering them appropriate for improved therapeutic efficacy in targeted drug delivery applications.

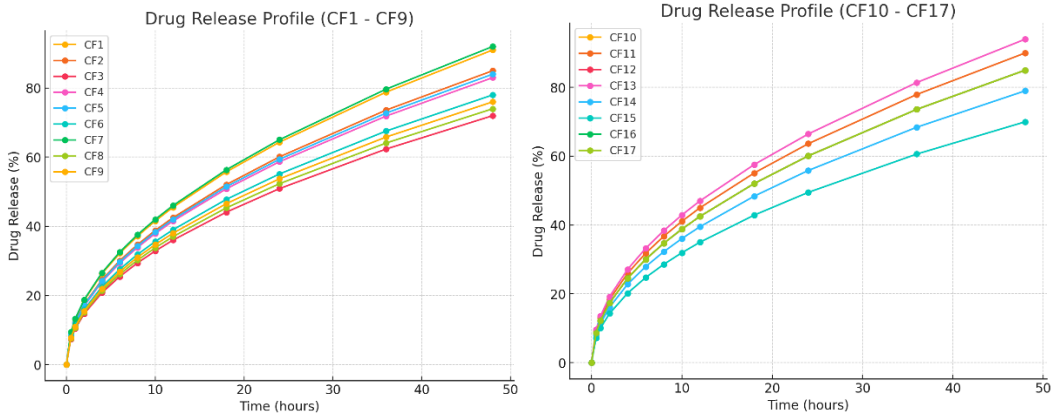

Fig.12 Drug release of all the formulations of C-NSLCs

Release Kinetics

Table 4. Ceritinib Release mechanism from optimized NSLCs

Release kinetic model	Equation	K	R^2
Zero Order	$C_t = C_o - K_t$	0.003	0.725
First Order	$\ln C_t = \ln C_o - K_t$	0.598	0.718
Korsemeyer Peppas	$\mathbf{M_t/M_\infty = kt^n}$	**0.045**	**0.995**
Hixon Crowel	$\sqrt[3]{W_0} = \sqrt[3]{W_i} + K_{Hc}t$	0.005	0.61
Higuchi	$Q = Kt^{0.5}$	0.039	0.625

The release kinetics of optimised formulation were examined through various mathematical models to identify the optimal mechanism for drug release behaviour. The Korsemeyer-Peppas model demonstrated the highest R^2 value (0.995), indicating adherence to a non-Fickian diffusion mechanism, which suggests a combination of diffusion and polymer relaxation-controlled release. The zero-order ($R^2 = 0.725$) and first-

order (R^2 = 0.718) models exhibited moderate correlation, signifying a gradual and sustained drug release rather than a solely concentration-dependent process. The Higuchi model (R^2 = 0.625) indicated that diffusion significantly influenced drug release, while the Hixon-Crowell model (R^2 = 0.610) suggested a minimal role for erosion-based kinetics. Overall, the findings affirm that optimised formulation demonstrates a controlled and sustained release profile, primarily regulated by diffusion and swelling mechanisms, indicating its potential as a formulation for extended drug release.

Stability Studies

The stability studies of Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) were performed under refrigerated (4 ± 2°C), long-term (25 ± 2°C / 60 ± 5% RH), and accelerated (40 ± 2°C / 75 ± 5% RH) conditions for six months to assess their physical and chemical stability. The particle size (PS) exhibited stability with negligible variations under all conditions, reflecting the formulation's structural integrity. Entrapment efficiency (EE) exhibited a marginal decline over time, particularly under accelerated conditions, indicating minor drug leakage attributed to temperature-induced stress. The drug content remained stable under refrigerated and long-term conditions, exhibiting only a minor decrease (≤1.5%), thereby confirming the formulation's chemical stability. At accelerated conditions, a gradual reduction in drug content was observed, reaching 95.49% at six months, suggesting potential degradation at elevated temperatures. The formulation demonstrated stability under refrigerated and long-term conditions, indicating suitability for storage. However, the minor reduction in drug content and encapsulation efficiency under accelerated conditions highlights the necessity for optimised packaging or controlled temperature storage to extend shelf life.

Table 5: Stability Studies

Time (Months)	0	1	3	6
Condition	**4 ± 2°C (Refrigerated Conditions)**			
PS (nm)	213.2 ± 1.3	213.2 ± 1.3	212.8 ± 1.2	216.82± 0.32
EE (%)	94.15 ± 1.32	94.17 ± 1.02	93.82 ± 0.62	93.12 ± 0.42
Drug Content (%)	99.42 ± 1.34	99.38 ± 1.01	99.31 ± 1.22	99.28 ± 1.04
Condition	**25 ± 2°C / 60 ± 5% RH (Long term Stability)**			
PS (nm)	213.2 ± 1.3	213.2 ± 1.3	213.8 ± 1.2	215.2 ± 0.5
EE (%)	94.25 ± 1.32	94.27 ± 1.02	93.92 ± 0.62	93.12 ± 0.42
Drug Content (%)	98.92 ± 1.2	98.76 ± 1.87	98.32 ± 1.23	97.98 ± 0.23
Condition	**40 ± 2°C / 75 ± 5% RH (Accelerated Stability)**			
PS (nm)	214.2 ± 1.3	212.2 ± 1.2	210.8 ± 0.8	210.2 ± 0.12
EE (%)	94.25 ± 1.32	93.16 ± 1.12	93.01 ± 0.2	92.82 ± 0.29
Drug Content (%)	98.42 ± 1.12	97.26 ± 1.76	96.32 ± 0.85	95.49 ± 1.10

Conclusion

The present study successfully developed and optimized Ceritinib-loaded Nanostructured Lipid Carriers (C-NSLCs) using the Box-Behnken Design (BBD) to overcome the solubility and bioavailability limitations of Ceritinib, a BCS Class IV anticancer drug. The optimized formulation exhibited spherical nanoparticles with a particle size of 214.23 nm, high entrapment efficiency of 94.25%, and sustained drug release of 85% over 48 hours. Drug-excipient compatibility was confirmed by FTIR and DSC analysis, and SEM images revealed uniform particles with smooth surfaces, suggesting successful lipid entrapment. Release kinetics followed the Korsemeyer-Peppas model, indicating a diffusion-controlled non-Fickian release mechanism, ensuring sustained drug delivery. Stability studies conducted over six months under refrigerated, long-term, and accelerated conditions demonstrated minimal changes in particle size, entrapment efficiency, and drug content, indicating good formulation stability. Overall, C-NSLCs proved to be a promising and stable delivery system for Ceritinib, with enhanced solubility, improved entrapment efficiency, and sustained drug release, offering potential benefits in cancer therapy.

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