RESEARCH ARTICLE

Design, Development and Characterization of a Niosomal Anticancer Drug Delivery System Formulation

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ABSTRACT

Objective: This study set out to create a niosomal formulation of ceritinib with the goal of delivering the medication specifically to the lungs while reducing side effects.

Method: Span 60, cholesterol and DCP were combined with the thin film hydration process to create niosomes. A central composite design was used to optimize the formulation, and the design of experiments was used to assess the impact of various variables on vesicle size and entrapment efficiency. The TEM and *in-vitro* drug release tests were used to analyse the optimized niosomes.

Results: 395.5 nm was the particle size, 75.28% entrapment efficiency, 0.321 polydispersity Index (PDI), and -28.0 mV zeta potential of the optimized niosomes. 68.03% of the medication was released after 48 hours. **Conclusion:** To address the issues with traditional oral ceritinib distribution, ceritinib-loaded niosomes were created in this study as a potential substitute.

Keywords: Ceritinib, Lung cancer, Niosomes, Central composite design.

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INTRODUCTION

Concerns about cancer in public health have grown dramatically over the last 50 years, particularly in relation to lung cancer. Of all lung cancer diagnoses, 15% are for small cell lung cancer (SCLC), while the remaining 85% are for non-small cell lung cancer (NSCLC). One of the main risk factors for lung cancer is smoking. Nonetheless, it is especially concerning that the number of nonsmoker instances of lung cancer is rising.[1-3]

Patients diagnosed with advanced-stage cancer often face lower survival rates and varied treatment outcomes. Similarly, many other cancers are frequently diagnosed only at stage four (IV).[4] Chemotherapy, surgery, and radiation therapy are among the lung cancer treatment options accessible. The treatment approach for lung cancer varies based on the stage and severity of the cancer cells. In cases of advanced-stage lung cancer, chemotherapy often serves as the primary treatment option. Typically, modern chemotherapy protocols involve administering medications intravenously (IV) into the patient's bloodstream.[5,6]

Niosomes have an amphiphilic nature, allowing them to encapsulate hydrophilic drugs in their core and entrap hydrophobic drugs within their vesicle bilayer, which is composed of non-ionic surfactants like Span and Tween, with or without cholesterol. Additionally, niosomes are very small, existing on the microscale or nanoscale.[7]

Non-ionic surfactants form films, while cholesterol serves as a stabilizer and rigidifier, preventing vesicle aggregation. Additionally, charge inducers create a surface charge on the niosomes, stabilizing the formulation by generating repulsive forces.[8]

Niosomes exhibit chemical stability, biodegradability, biocompatibility, low toxicity, improved solubility, and enhanced bioavailability. They have been used as carriers for various types of drugs, including synthetic, herbal, antigenic, and hormonal substances, as well as other bioactive compounds. Niosomes can be administered through oral, parenteral, topical, and pulmonary routes.[7,9,10] This study aimed to develop a niosomal formulation of ceritinib to target lung delivery specifically, thereby reducing the negative side effects commonly associated with ceritinib use.

MATERIALS AND METHODS

Materials

Materials: Ceritinib was generously provided by MSN Laboratory Pvt. Ltd., Hyderabad. Span 60 was a gift from

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Table 1: Formulation variables for central composite design with the experimental and coded values

S. No.	*Variable*	*Level*		
		Low	*Medium*	*High*
	Independent variables			
1	Concentration of surfactant (mg) (Span 60) (X1)	150	200	250
2	Concentration of cholesterol (mg) (X2)	50	100	150
3	Hydration time (min) (X3)	30	45	60
	Coded value	-1	0	+1
	Dependent variables			
1	R1= Vesicle size (nm) (Z-average value) R2=% Entrapment efficiency			

Mohini Organic Pvt. Ltd., Mumbai. Cholesterol was supplied by Fermenta Biotech Ltd., Mumbai, and diacetyl phosphate (DCP) was obtained from Sigma Chemical Co., USA. Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were procured from Merck. All analytical-grade organic solvents were procured from Loba Chemie Pvt. Ltd., Mumbai.

Table 2: Compositions of the checkpoint baches

Factor	*Name*	*I*	*II*	*III*
A	Span 60	170.00	150.00	250.00
B	Cholesterol	150.00	50.00	150.00
C	Hydration time	45.00	30.00	60.00

Preparation of the Ceritinib Niosomal Formulation:

Using the thin film hydration method, niosomes were created. Accurate measurements were made of the drug (50 mg), surfactant (200 mg), cholesterol (100 mg), and DCP (10 mg). Chloroform (10 mL) was used to dissolve every ingredient, including the drug, in a 25 mL round-bottom flask. This flask formed a thin film on its wall after being exposed to low pressure at 60°C for one hour while attached to a rotating evaporator. After that, the niosomal solution was hydrated for an additional hour at 60°C using PBS (10 mL, pH 7.4). After the suspension was hydrated, a 10-minute sonication was used to create a unilamellar niosome dispersion. The finished product was kept for further investigation at 4 ± 2°C.[11]

Central Composite Design (CCD)

To enhance the niosomal formulation, three formulation variables were pinpointed following preliminary trials. Their impacts on vesicle size and the percentage of entrapment efficiency were evaluated through a 3-level central composite design (CCD). with $\alpha = \pm 1$. This design predicts interactions between significant and nonsignificant variables, if any. Table 1 illustrates the evaluation of three factors at three levels each, leading to experimental trials with seventeen possible combinations.

Checkpoint Batches

Checkpoint batches were prepared and evaluated for their responses, as shown in Table 2.

Characterization of the niosomal formulation

Vesicle size, polydispersity index (PDI) and zeta potential

The particle size, polydispersity Index (PDI), and zeta potential of the vesicles were analyzed using a Zetasizer HAS 3000 from Malvern Instruments. Vesicle size was determined by appropriately diluting the vesicular suspension with PBS (pH 7.4) to avoid multiple scattering events. Each measurement was conducted in triplicate for every study to ensure precision and consistency.[12]

Percent entrapment efficiency

Ceritinib-loaded niosomes (2 ml) were isolated by centrifugation at 20,000 rpm for 60 min using a cooling centrifuge at 4°C (REMI C-24). The obtained precipitate was diluted with phosphate buffer (pH 7.4) to obtain a niosomal dispersion. Subsequently, 0.1 mL of the niosome dispersion was taken, and 0.2 mL of isopropyl alcohol (IPA) was added to disrupt the niosomes. To prepare assay samples, the drug samples were diluted to 5 mL with methanol. The ceritinib content was estimated by measuring the absorbance at λ_{max} 304 nm with a UV spectrophotometer (Shimadzu Corporation, Model: 1800 UV/visible, Japan).[13]

$$\%\text{Entrapment Efficiency} = (\text{Amount of entrapped drug}) / (\text{Total amount of drug}) \times 100\%$$

Estimation of drug content percentage

In a 10 mL volumetric flask (VF), a niosomal suspension containing 10 mg of ceritinib was taken. The volume was adjusted with PBS (pH 7.4), and the vesicles were disrupted by sonication for 40 minutes. Subsequently, 1 ml of this solution was transferred to a 50 ml volumetric flask (VF), and the volume was adjusted with buffer. The solution was further sonicated to ensure complete dispersion. The concentration of ceritinib in the solution was determined using a UV spectrophotometer (UV-1800, Shimadzu Corporation) by measuring the absorbance at 307.5 nm.[14]

Transmission electron microscopy (TEM)

Using TEM, the morphological properties of the niosome formulation was investigated. On carbon-coated copper grids, a drop of the diluted niosomal sample was applied, and any extra liquid was wiped off using filter paper. After allowing the samples to air dry, 2% (w/v) phosphotungstic acid was added to them to increase contrast. A Philips Tecnai 20 microscope running at an 80 kV accelerating voltage was used to take TEM pictures.[15]

In-vitro drug release study

To find out the drug release profile, the dialysis method was employed. Initially, a cellulose dialysis tube (dimensions: 28 x 46 x 17 mm; HiMedia) was activated by soaking it in double distilled water for three to four hours. Next, the dialysis tubing was filled with the niosomal suspension, securely tied, and immersed in PBS (pH 7.4, 50 mL) at 37°C. The buffer solution was stirred using a magnetic stirrer at 100 rpm. At predetermined time intervals (2 to 48 hours), samples were withdrawn and analyzed spectrophotometrically at 307.5 nm (UV-1800, Shimadzu Corporation) to quantify the ceritinib content and assess its release kinetics.[16]

RESULTS

Data Analysis and Optimization Study

Based on the preliminary trials, prior knowledge, and current understanding of the product, three independent factors were identified and selected for the DoE study. For optimization, three-factor response surface methodology was used with a face-centered composite design (3 levels). The results of the response for all 17 formulations are depicted in Table 3.

Contour Plot

The impact of factors was further explained using the contour and 3D plot (Figures 1 and 2) for the Particle Size. Both graphs show the positive impact of the factors over response (R1-Particle Size).

3D Plot

The impact of factors was further explained using Contour and the 3D plot (Figures 3 and 4) for the %Entrapment Efficiency which shows there is a curvilinear relationship between independent variables and response for the response (R2-% Entrapment Efficiency).

Table 3: Three-level central composite design baches and measured responses

Batch	*Factor 1*	*Factor 2*	*Factor 2*	*Vesicle size (nm)*	*% Entrapment efficiency ± SD*	*Polydispersity index*	*Zeta potential (mV)*
	X1	*X2*	*X3*	*R1*	*R2*		
F1	-1	+1	-1	333.5	61.57 ± 1.72	0.511	-19.0
F2	0	0	0	401.6	74.47 ± 1.22	0.315	-32.2
F3	0	0	0	389.3	73.24 ± 2.17	0.322	-18.5
F4	+1	-1	-1	567.1	67.19 ± 1.82	0.215	-17.9
F5	-1	-1	-1	266.9	63.68 ± 2.41	0.203	-43.7
F6	-1	-1	+1	288.4	68.97 ± 1.97	0.209	-48.5
F7	-1	+1	+1	445.2	67.98 ± 1.85	0.515	-39.8
F8	+1	+1	-1	578.9	79.34 ± 1.11	0.592	-52.6
F9	0	0	0	395.5	75.28 ± 2.61	0.321	-28.0
F10	+1	+1	+1	617.8	80.28 ± 1.25	0.537	-28.5
F11	+1	-1	+1	587.4	76.19 ± 1.75	0.213	-21.4
F12	0	-1	0.0	398.3	75.91 ± 2.12	0.422	-20.3
F13	0	0	+1	590.4	78.98 ± 1.19	0.337	-21.9
F14	-1	0	0	425.8	76.21 ± 1.74	0.327	-35.3
F15	0	+1	0	397.8	65.31 ± 1.39	0.576	-20.2
F16	0	0	-1	358.9	68.89 ± 1.77	0.358	-38.7
F17	+1	0	0	326.8	69.21 ± 1.26	0.379	-29.7

Mean ± SD, n = 3; SD = standard deviation

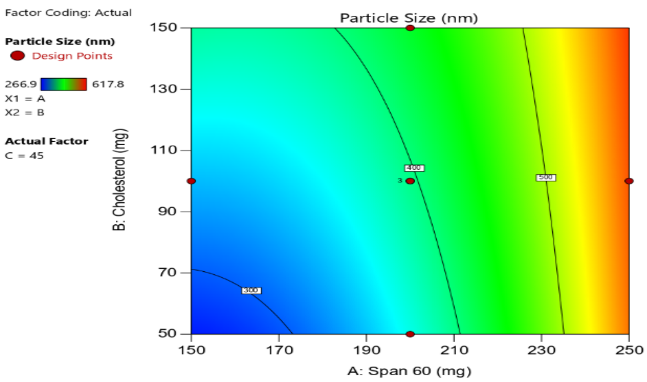

Figure 1: Contour plot showing the impacts of Span 60 and cholesterol on particle size (hydration time of 45 min)

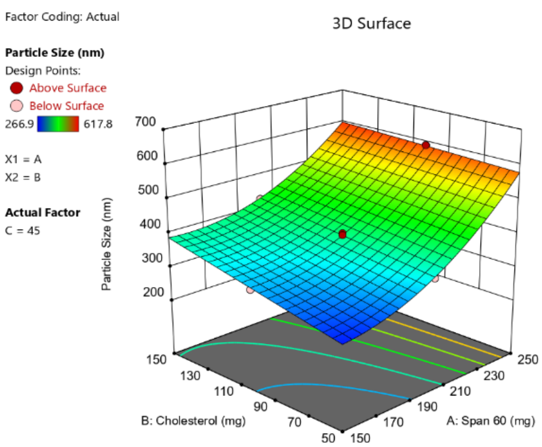

Figure 2: 3D surface plot showing the impacts of Span 60 and cholesterol on particle size (hydration time of 45 min)

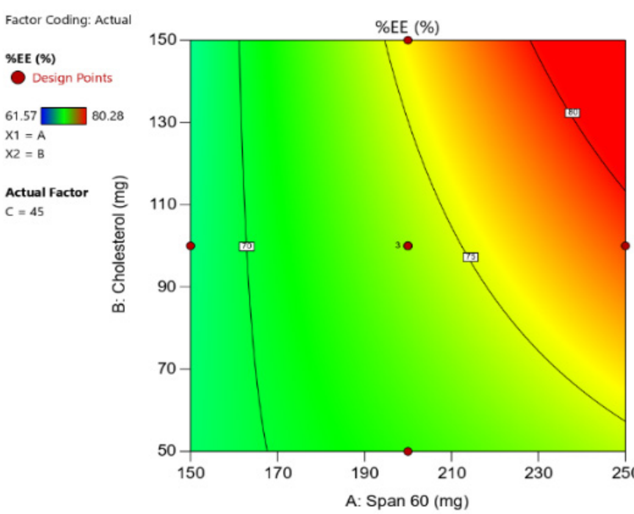

Figure 3: Contour plot displaying the effects of Span 60 and cholesterol on the percentage of encapsulation efficiency at a hydration time of 45 minutes

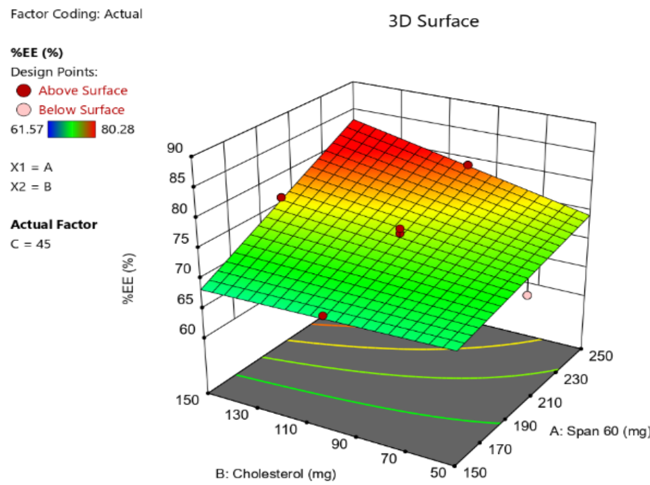

Figure 4: 3D surface plot showing the impacts of Span 60 and cholesterol on % EE (hydration time of 45 min)

Table 4: Effects of the independent variables on the responses

Name	*Goal*	*Lower limit*	*Upper limit*
A: Span 60	in range	150	250
B: Cholesterol	in range	50	150
C: Hydration time	in range	30	60
Particle size	in range	200	400
%EE	in range	NLT 70%	

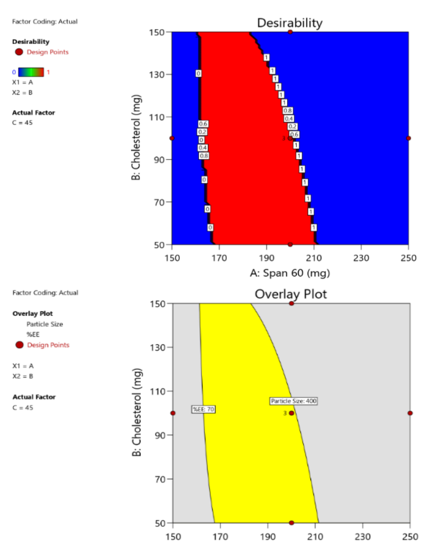

Figure 5: Desirability plot and overlay plot

Optimal Formula

After assessing the impact of independent variables on the responses, the optimal levels of these variables were investigated to achieve desired outcomes as shown in Table 4. Using a Software Design Expert, a desirability analysis was conducted to pinpoint the optimal formulation region. Table

Table 5: Result of checkpoint batches

Batch	*Analysis*	*Predicted mean*	*Observed mean ± SD*	*95% PI low*	*95% PI high*
I	Particle size	388.72	384.12 ± 7.0	358.89	418.54
	%EE	71.32	69.54 ± 3.2	67.48	75.15
II	Particle size	270.57	274.14 ± 5.6	233.70	307.44
	%EE	62.73	63.58 ± 2.2	58.45	67.01
III	Particle size	631.81 640.1 ± 4.2		594.94	668.68
	%EE	83.39	82.12 ± 2.1	79.11	87.66

Mean ± SD, n = 3; SD = standard deviation

5 displays the desirability values alongside upper and lower limits for all formulation variables and responses. Additionally, Figure 5 presents desirability and overlay plots for all variables, illustrating the optimal conditions for maximizing desired responses in the niosomal formulation of ceritinib.

The red area indicates higher values of desirability. Furthermore, an overlay plot was generated to identify the optimum region for the preparation of the formulation. Figure 5 shows the overlay plot within the yellow region, which gives the specific criteria for the optimum batch.

Checkpoint Batch

The results show that the measured response (observed) values were as expected. Predicted response values were found in the range of the 95% prediction interval (PI) of the observed value. Hence, it was concluded that the obtained mathematical models are valid for the response prediction.

Characterization of the optimized batch (F9)

From the results and statistical data, batch F9 was found to be the optimized batch, and was further evaluated in terms of the following parameters.

Vesicle size, PDI and zeta potential:

The vesicle size, PDI and zeta potential of the optimized batch were found to be 395.5 nm, 0.321 and -28 mV, respectively, as shown in Figures 6 and 7. The obtained values of all these parameters indicate that the optimized batch of niosomes gives a homogeneous dispersion without aggregation.

Entrapment Efficiency

The % entrapment efficiency of the optimized batch was found to be 75.28%.

Drug Content

The prepared niosomal formulation F9 was evaluated for drug content, which was found to be 95.67%.

Morphological Study

The surface morphology of the prepared niosomes was investigated using TEM. TEM images depicting the niosome formulation from optimized batch F9 are displayed in Figure 8. The niosomes appeared spherical in shape with minimal aggregation observed. The particle size was measured to be less than 500 nm

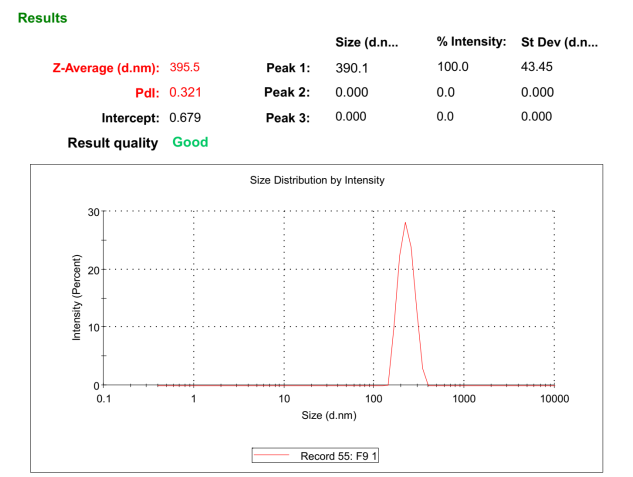

Figure 6: Particle size and PDI of the optimized niosomal formulation

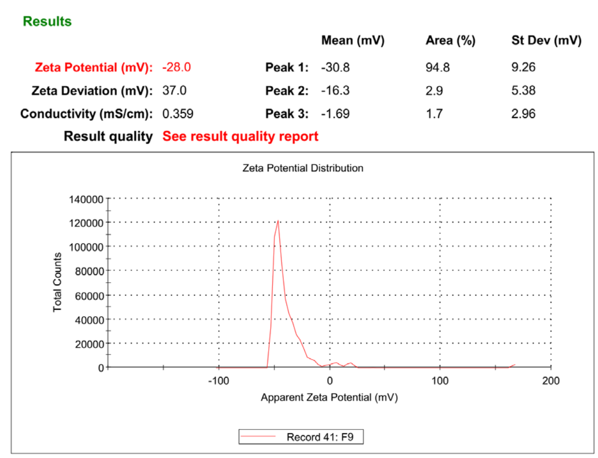

Figure 7: Zeta potential of the optimized niosomal formulation

In-vitro Drug Release

In the *in-vitro* drug release experiments, the optimized niosomal formulation (F9) exhibited a controlled release profile, achieving a release of 68.03% over 48 hours. This controlled release profile was compared to that of the pure drug (PD) in Figure 9, where "NS" denotes the niosomal formulation (F9) and "PD" denotes the pure drug.

The comparison in Figure 9 illustrates how the niosomal formulation (F9) provides a sustained release of ceritinib

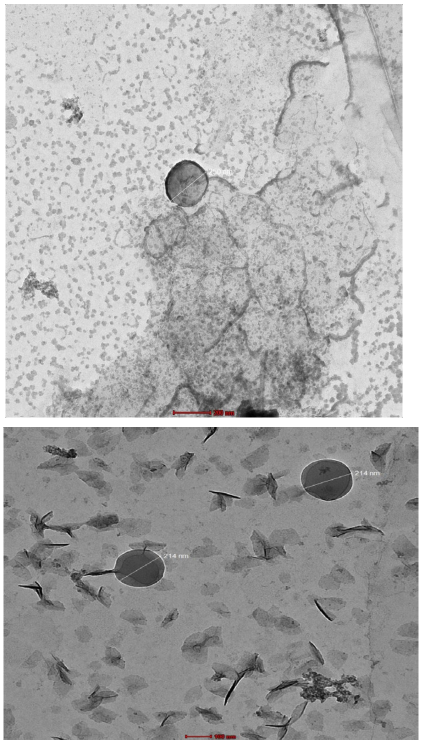

Figure 8: TEM images of drug-loaded niosomes

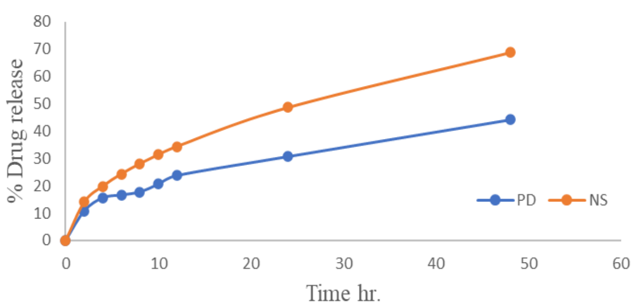

Figure 9: *In-vitro* percent drug release from the optimized niosomal formulation F9 and pure drug

compared to the rapid release observed with the pure drug formulation. This controlled release characteristic of the niosomal formulation is crucial for optimizing therapeutic outcomes and minimizing potential side effects associated with ceritinib administration.

CONCLUSION

The study's findings demonstrated that the thin-film hydration approach was effectively used to manufacture the niosomal formulation. The drug's longer release from the optimized formulation may have lessened its harmful effects. Niosomes have a higher entrapment efficiency and are more convenient for inhalation due to their smaller size as compared to pure medication.

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