ORIGINAL ARTICLE

Design, Evaluation, and Statistical Optimization of Nintedanib Solid Lipid Nanoparticles

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Abstract

Introduction: Nintedanib (NTD), a triple tyrosine kinase inhibitor targeting vascular endothelial growth factor receptor, fibroblast growth factor receptor, and platelet-derived growth factor receptor, is widely used in cancer therapy but is limited by poor aqueous solubility and variable bioavailability. Solid lipid nanoparticles (SLNs) offer a promising approach to enhance drug stability, entrapment, and sustained release. This study aimed to formulate and statistically optimize NTD-loaded SLNs (NTD-SLNs). **Materials and Methods:** NTD-SLNs were prepared using glyceryl monostearate as lipid, Tween 80 as surfactant, and Pluronic F-68 as stabilizer by the solvent injection method. Response surface methodology was employed to optimize three formulation variables: surfactant concentration, stabilizer concentration, and aqueous-to-organic phase ratio. The optimized formulation was characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency (EE), drug loading (DL), morphology scanning electron microscopy (SEM), and *in vitro* drug release using the dialysis membrane technique. **Results:** The optimized SLNs exhibited a particle size of 246.2 nm and a PDI of 0.272, closely matching predicted values, confirming model reliability. The formulation showed high EE (86.5 ± 3.33%), DL (18.5 ± 1.2%), and percentage yield (94.3%). SEM images confirmed spherical and uniform nanoparticles. *In vitro* release studies demonstrated sustained drug release, with 98.9% cumulative release over 24 h compared to rapid release of pure NTD within 4 h. **Conclusion:** The optimized NTD-SLNs demonstrated favorable physicochemical properties and sustained release behavior, indicating their potential to enhance therapeutic performance and controlled delivery of NTD.

Key words: Entrapment efficiency, nintedanib, particle size optimization, response surface methodology, solid lipid nanoparticles

INTRODUCTION

Cancer is a complicated illness that includes a wide range of problems that are marked by continuous and unending development. Cancer has plagued mankind for all of recorded history. As a formidable obstacle, cancer persists despite remarkable developments in cancer biology; it is, after all, the biggest killer on a global scale. There are more than 200 distinct cancer forms worldwide. Worldwide, 12.7 million instances of cancer were recorded in 2008, leading to 7.6 million fatalities; developing nations accounted for 64% of these fatalities. This disease's clinical variability and resistance to therapy are explained by its complexity at the genetic and phenotypic levels.[1-5]

Nintedanib (NTD) is a medication that inhibits three varieties of receptor tyrosine kinases, which are involved in the growth and repair of cells. The fibroblast growth factor receptor, vascular endothelial growth factor receptor, and platelet-derived growth factor receptor are among the receptors. The effects of NTD on cells can vary. During necrosis, the cells

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may lose their membrane structure and perish rapidly as a result of the cell's disintegration.

Alternatively, the cells may cease to divide and develop, resulting in a decrease in their survival rate. Another possibility is that the cells initiate a programmed process known as apoptosis, which is a controlled method of cell demise. When necrosis occurs, cells typically enlarge rapidly, lose their membrane structure, cease energy consumption, and discharge their internal contents into the surrounding area.[6-10]

Nanotechnology, an emerging area of study that brings together chemistry, engineering, biology, and medicine, offers several potential uses in cancer biology, including the identification of tumors at an early stage, the identification of biomarkers that indicate cancer's progression, and the creation of new therapies.[11-13] The unique properties of solid lipid nanoparticle (SLNs), which are colloidal carriers composed of lipids and remain solid at both room and body temperature, make them attractive for use in improving the performance of pharmaceuticals, neutraceuticals, and other materials. SLNs are small (50–500 nm), have a large surface area, and can contain a high drug loading (DL).[14-15] In addition, the interaction of phases at their interfaces adds to their appeal.

In the present study, SLNs of NTD were prepared and optimized by a statistical optimization technique.

Table 1: Solubility of lipids in ethanol

Lipids	5 mg	15 mg	50 mg	100 mg
Stearic acid	X	X	X	X
Cetyl alcohol				
Glyceryl monostearate				
Gelucire 50/13	X	X	X	X
Precirol	X	X	X	X
Dynasan 114	X	X	X	X

Tabe 2: Solubility of lipids in methanol

Lipids	5 mg	15 mg	50 mg	100 mg
Stearic acid	X	X	X	X
Cetyl alcohol	X	X	X	X
Glyceryl monostearate				
Gelucire 50/13	X	X	X	X
Precirol	**X**	**X**	**X**	**X**
Dynasan 114	X	X	X	X

Table 3: Solubility of lipids in acetone

Lipids	5 mg	15 mg	50 mg	100 mg
Stearic acid	X	X	X	X
Cetyl alcohol	X	X	X	X
Glyceryl monostearate	X	X	X	X
Gelucire 50/13	X	X	X	X
Precirol	**X**	**X**	**X**	**X**
Dynasan 114	X	X	X	X

MATERIALS AND METHODS

Materials

Nintedanib from Lupin Pharma Ltd, Glyceryl monostearate from Gattefosse, France, Tween 80 from Merck(Mumbai), Pluronic F68 from Merck (Mumbai).

Methods

Screening of component

Lipid solubility in several water miscible solvents was investigated in a qualitative assay. To find the best lipid to use in making SLNs, solubility testing. Two milliliters of each solvent – Methanol, ethanol, N,N-dimethylformamide, and dimethyl sulfoxide – were measured into small vials with a capacity of 5 mL. An increasing quantity of lipids – stearic acid, cetyl alcohol, glyceryl monostearate, compritol ATO 888, precirol ATO 5, dynasan 114, and dynasan 118, gelucire

Table 4: Average particle size and % EE of SLNs by three different methods

Method	Average particle size (nm)	% EE
Glyceryl monostearate		
Melting dispersion	665.3±30.5	54.30±5.52
Self-emulsification solvent-evaporation	308.6±20.3	70.3±2.25
Injection method	121.1±30.2	88.91±6.46
Cetyl alcohol		
Melting dispersion	801.7±10.6	50.2±5.29
Self-emulsification solvent-evaporation	371.2±25.2	63.3±5.7
Injection method	187.5±13.6	86.3±3.3

Mean+standard deviation, *n*=3. EE: Entrapment efficiency, SLNs: Solid lipid nanoparticles

Table 5: Response surface regression evaluation of NTD-SLNs formulations showing 27 runs

RUN	Factor 1 (% of surfactant)	Factor 2 (% of stabilizer)	Factor 3 (Aq: oil)	Response 1 (Particle size nm)	Response 2 (PDI)
1	0.2	0.5	10	312	0.38
2	0.35	0.2	5.5	268	0.31
3	0.35	0.5	1	255	0.29
4	0.5	0.5	5.5	298	0.34
5	0.5	0.35	5.5	276	0.32
6	0.2	0.35	1	284	0.33
7	0.5	0.2	5.5	305	0.36
8	0.35	0.5	10	262	0.30
9	0.5	0.2	10	320	0.40
10	0.2	0.5	1	248	0.28
11	0.5	0.2	5.5	290	0.35
12	0.2	0.2	1	275	0.33
13	0.35	0.35	5.5	260	0.30
14	0.5	0.35	10	270	0.31
15	0.5	0.35	10	315	0.39
16	0.2	0.35	1	265	0.32
17	0.2	0.35	10	310	0.38
18	0.35	0.5	10	250	0.29
19	**0.35**	**0.35**	**5.5**	**245**	**0.27**
20	0.35	0.2	10	300	0.37
21	0.35	0.35	10	285	0.34
22	0.5	0.5	5.5	258	0.30
23	0.5	0.2	10	295	0.36
24	0.2	0.2	1	260	0.31
25	0.35	0.35	1	255	0.29
26	0.2	0.2	5.5	275	0.33
27	0.2	0.5	5.5	265	0.31

NTD-SLNs: Nintedanib-solid lipid nanoparticles, PDI: Polydispersity index, Bold values indicate the optimized formulation obtained based on desirability criteria

Table 6: Fit Summary - Response 1: Particle size

Source	Sequential *P* value	Lack of fit *P* value	Adjusted R^2	Predicted R^2	Significance
Linear	0.0200		0.2565	0.0317	
2FI	0.0859		0.3805	−0.1245	
Quadratic	**0.0265**		**0.5701**	**0.0637**	**Suggested**
Cubic	0.0635		0.7575	−0.0346	Aliased

Bold values represent the selected model based on significance ($p < 0.05$), model fit, and adequacy criteria; aliased models are excluded.

50/13 – were added to the solvent, with amounts ranging from 5 to 300 mL. The mixture was then placed in a sonicator and left to run for 10 s at a constant temperature of 25 ± 1°C. Based on visual inspection, the capacity of several solvents to dissolve the lipids was taken into account.[16-18]

Formulation of NTD SLNs

There are three distinct approaches for preparing SLNs: A combination of lipids for drug entrapment, an emulsifier, and a stabilizer for the emulsification and stability of the two phases – lipidic and aqueous. These were used in melting dispersion, solvent-emulsification solvent evaporation, and injection techniques.[19-21]

Optimization of SLNs

Optimization of method for NTD-SLN preparation

The method that produced the smallest particle size in

Table 7: ANOVA for Quadratic model - Response 1: Particle size

Source	Sum of squares	Degrees of freedom	Mean square	F-value	*P*-value	Significance
Model	9419.64	9	1046.63	4.83	0.0026	Significant
A-quantities of surfactant	128.00	1	128.00	0.5909	0.4526	
B-stabilizer concentration	2112.50	1	2112.50	9.75	0.0062	
C-C	2244.50	1	2244.50	10.36	0.0050	
AB	736.33	1	736.33	3.40	0.0827	
AC	1240.33	1	1240.33	5.73	0.0285	
BC	396.75	1	396.75	1.83	0.1937	
A^2	1849.19	1	1849.19	8.54	0.0095	
B^2	64.46	1	64.46	0.2976	0.5925	
C^2	647.57	1	647.57	2.99	0.1019	
Residual	3682.44	17	216.61			
Cor Total	13102.07	26				

Table 8: Fit statistics for Response 1

Standard deviation	14.72	R^2	0.7189
Mean	277.81	Adjusted R^2	0.5701
C.V. %	5.30	Predicted R^2	0.0637
		Adeq precision	7.4647

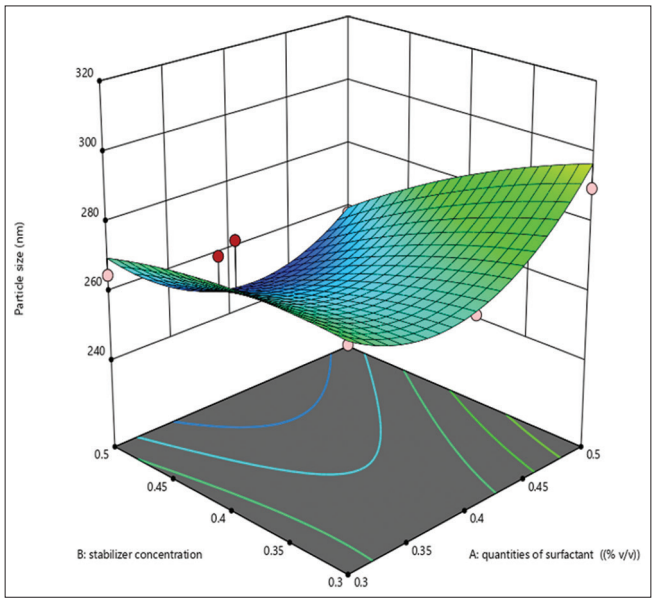

Figure 1: 3D response surface curve showing the effect of surfactant concentration and stabilizer concentration on average particle size

nano-range and the highest entrapment efficiency (EE) was selected for preparation of NTD-SLNs.

Optimization of formulation variables

The formulation factors were optimized with Design Expert software. Three independent formulation factors were examined in the study: Surfactant concentration (A), stabilizer concentration (B), aqueous to organic phase ratio

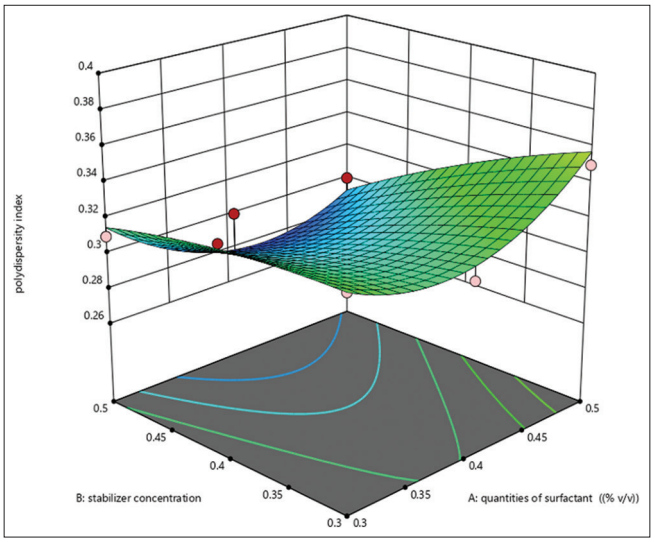

Figure 2: 3D response surface curve showing the effect of surfactant: stabilizer ratio and stabilizer concentration on particle size distribution

(C). The examined dependent variables were particle size (X) and polydispersity index (PDI) (Y). The design comprised 32 experimental sites (Table 5). The data were assessed to conform to the polynomial equation.[22-25] The individual and interaction impacts of these formulation parameters were examined by producing SLNs at varying amounts of all variables. All parameters were evaluated at two levels: Surfactant concentration (0.3% and 0.5% v/v), stabilizer concentration (0.3% and 0.5% w/v), aqueous to organic phase ratio (10:1 and 25:1 v/v).

Characterization of NTD SLNs

EE and DL

EE of NTD-SLNs was determined by quantifying the free drug content in the supernatant obtained after centrifugation of SLN suspension at high speed (16000 rpm for 30 min at

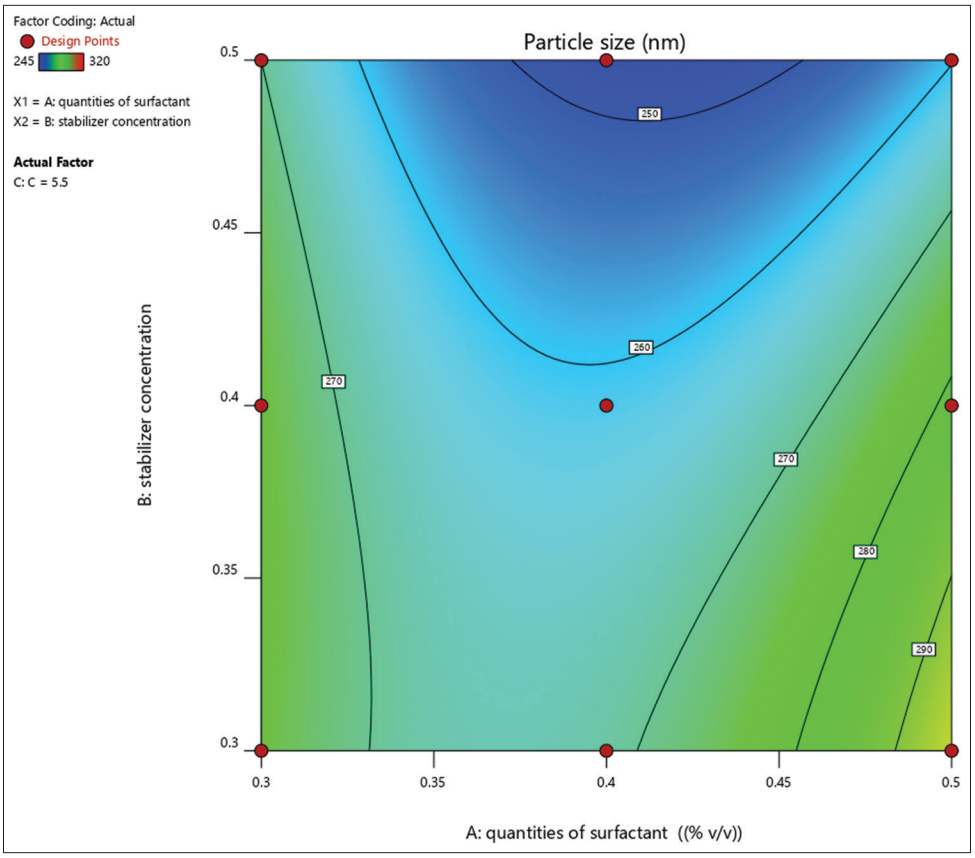

Figure 3: 2D contour plots indicating different interactions between the variables and particle size

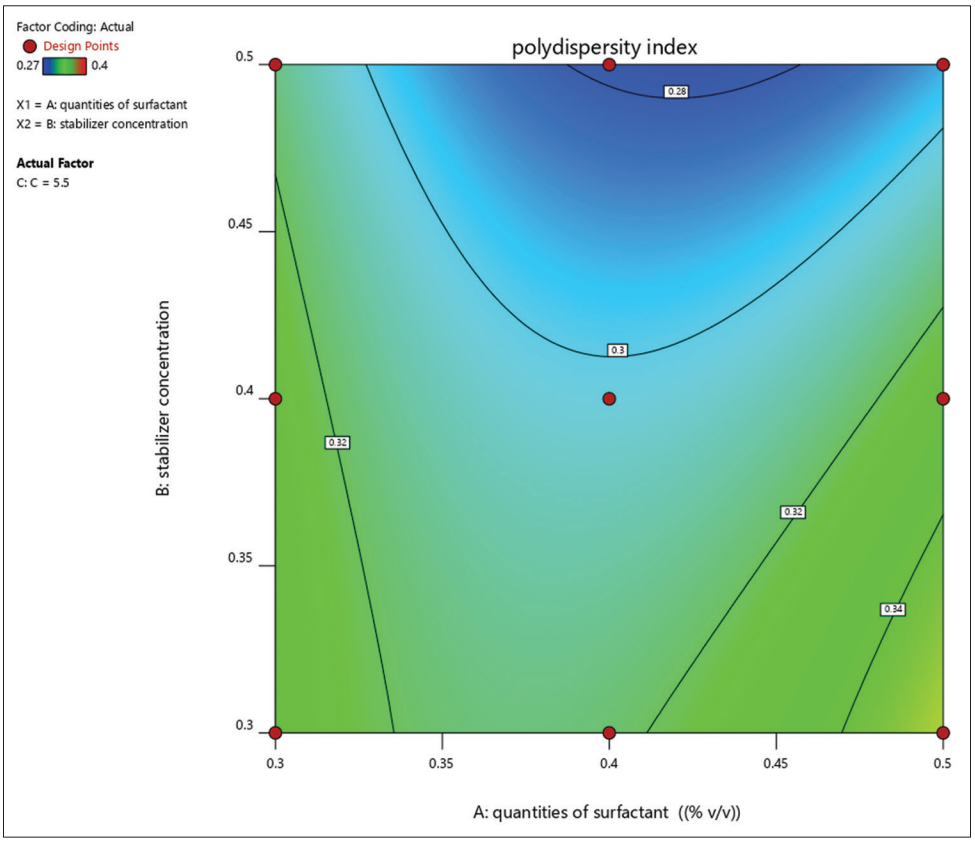

Figure 4: 2D contour plots indicating different interactions between the variables and polydispersity index

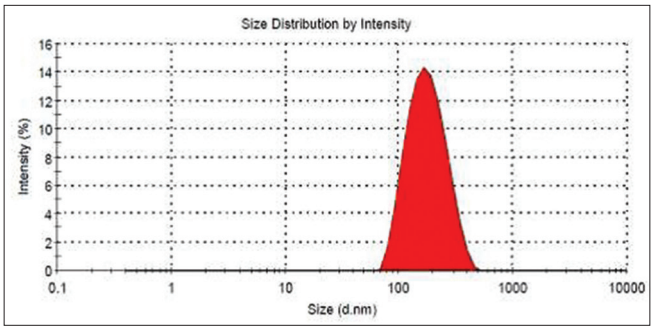

Figure 5: Statistical representation of particle size distribution of adenosine loaded-solid lipid nanoparticles

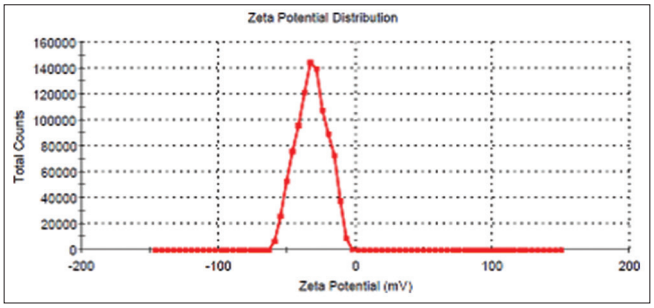

Figure 6: Statistical representation of zeta potential of solid lipid nanoparticles prepared by GM

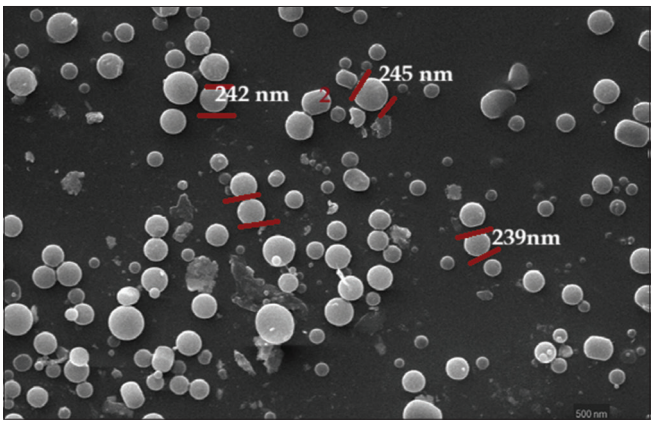

Figure 7: Scanning electron microscopy photograph of nintedanib solid lipid nanoparticles

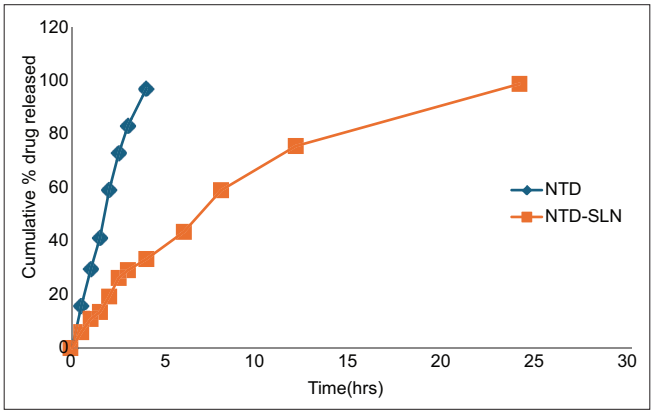

Figure 8: *In vitro* release profile of nintedanib from solid lipid nanoparticles

30°C) using Remi cooling centrifuge (Mumbai, India). The EE of NTD-SLNs was determined, as the ratio between the actual and theoretical loadings.[26-28] The drug loaded in the SLNs could be calculated with the total amount of NTD, subtracting the amount of free drug in NTD-SLNs dispersion, and the drug EE and DL could be achieved by the following equation:

$$EE\ (\%) = W_s/W_{Total} \times 100$$

$$DL\ (\%) = W_s/W_{Lipid} \times 100$$

Where, *Ws* - Amount of NTD loaded in the SLNs; W_{Total} - Total NTD amount in NTD-SLNs dispersion; W_{Lipid} - Weight of the vehicle.

DL (%) was calculated as drug entrapped in the SLN versus the total amount of the lipid added during preparation.

Yield of SLNs

To find the yield, which is the amount of SLN that was recovered throughout the preparation procedure, 10 mL of the suspension was dried in an oven at 30°C until a consistent weight was achieved. The ratio of the quantity of lipids in the suspension to the theoretical amount is the way it is stated as a percentage.

Particle size distribution

Photon correlation spectroscopy was used with a Zetasizer 1000 HS to estimate the average particle size and PDI of the NTD-SLN dispersions. To make sure the light scattering intensity was within the range of the instrument's sensitivity, the SLN solution was diluted with double-distilled water and filtered through a 0.45 µm membrane filter. All measurements were taken at 25°C with a scattering angle of 90°. The z-average diameter and PDI of the particles were calculated after triple measurements of each.

Zeta potential determination

The electrophoretic mobility and zeta potential of the lipid carriers were measured by Zetasizer 1000 HS. Before the measurements, the NTD-SLNs samples were diluted with double-distilled water (1:5, v/v). Each sample was analysed in triplicate.

Scanning electron microscopy (SEM)

The morphology of the particles was observed by SEM. Samples were analysed in the form of aqueous dispersion, using Quanta 200 environmental SEM. Analysis was performed at 25 ± 2°C.

In vitro dissolution study

An *in vitro* dissolution study of NTD-SLNs was performed using dialysis membrane. Within 24 h of preparation, studies for NTD and NTD-SLNs were performed. A dialysis tube

Table 9: Fit Summary - Response 1: Polydispersibility Index

Source	Sequential *P* value	Lack of fit *P* value	Adjusted R^2	Predicted R^2	Significance
Linear	**0.0044**		**0.3529**	**0.1628**	Suggested
2FI	0.1457		0.4279	−0.0200	
Quadratic	**0.0292**		**0.5981**	**0.1117**	Suggested
Cubic	0.0509		0.7852	0.0459	Aliased

Bold values represent significant models ($p < 0.05$) with acceptable fit criteria; aliased models are excluded from consideration.

Table 10: ANOVA for Quadratic model - Response 1: Polydispersibility Index

Source	Sum of squares	Degrees of freedom	Mean square	F-value	*P*-value	Significance
Model	0.0242	9	0.0027	5.30	0.0016	Significant
A-quantities of surfactant	0.0000	1	0.0000	0.0438	0.8367	
B-stabilizer concentration	0.0072	1	0.0072	14.20	0.0015	
C-C	0.0068	1	0.0068	13.42	0.0019	
AB	0.0019	1	0.0019	3.70	0.0714	
AC	0.0016	1	0.0016	3.22	0.0905	
BC	0.0008	1	0.0008	1.64	0.2171	
A^2	0.0039	1	0.0039	7.73	0.0128	
B^2	0.0001	1	0.0001	0.2337	0.6350	
C^2	0.0018	1	0.0018	3.51	0.0783	
Residual	0.0086	17	0.0005			
Cor total	0.0328	26				

Table 11: Fit statistics

Standard deviation	0.0225	R^2	0.7372
Mean	0.3281	Adjusted R^2	0.5981
C.V. %	6.86	Predicted R^2	0.1117
		Adeq precision	8.2087

holding 1 ml of the dispersion was sealed and then placed into a vial with 10 mL of acetate buffer (pH 4.5) containing 0.5% tween 80. Using a shaker set at 37 ± 1°C and 50 strokes/min, the samples were horizontally shaken. At 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h intervals, a 2 mL sample of the medium was removed, and a portion of the medium was replaced with new medium. Using a ultraviolet (UV) spectrophotometer set at 390 nm, the drug content of the samples was examined. A comparison was made between the drug suspension and the SLN formulation in terms of NTD release.[29-30]

RESULTS AND DISCUSSION

Screening of component for preparation of NTD-SLNs

Selection of lipids

SLNs possess a solid lipid core matrix that can solubilize lipophilic molecules. The lipid core is stabilized by surfactants (emulsifiers). The ability of the solvent to dissolve the lipid was considered when the content appeared transparent on visual observation.

The solubility of lipids (stearic acid, cetyl alcohol, glyceryl monostearate, Gelucire 50/13, Precirol, Dynasan 114) was observed visually in three selected solvents in Methanol (Table 1), Acetone (Table 2), and Ethanol (Table 3).

It was observed that glyceryl monostearate was highly soluble in methanol and ethanol (solubility increased by changing the temperature). Dynasan 118, Gelucire 50/13, Stearic acid, and Precirol are insoluble in all the selected solvents. Cetyl alcohol was completely soluble in ethanol.

The selected lipids, Cetyl alcohol and Glyceryl monostearate, were entirely soluble in ethanol; hence, ethanol was chosen as a common solvent as it effectively and fully dissolved both lipids.

Cellulose acetate (CA) is challenging to handle; however, it possesses an oily texture and lacks flavor or odor, rendering it highly advantageous as a pharmaceutical excipient.

Glyceryl monostearate is widely used in food and cosmetics for its excellent emulsifying, thickening, and moisturizing properties, effectively blending oil and water phases.

As per the Food and Drug Administration, CA is considered an inactive ingredient. Therefore, Glyceryl monostearate was selected for further studies.

Selection of surfactant and stabilizer

After selecting the lipid, the next procedure was picking the surfactant/emulsifier. SLNs have two phases: An organic phase containing lipids and an aqueous phase. An emulsifier is essential for this composition, which comprises two immiscible phases. The surfactant is a crucial factor in emulsion formation and stability, with its effectiveness dependent on its structure and the properties of the two phases involved.

These agents may be classified according to the chemical group of the hydrophilic head into ionic, which can be further split into anionic and cationic, non-ionic, and zwitterionic.

Tween 80 (or polysorbate 80), a hydrophilic non-ionic surfactant, is the most prevalent surfactant and has demonstrated the ability to boost the solubility of substances, resulting in improved absorption of therapeutic candidates. Tween 80 is a prevalent excipient in several human dosage formulations. Pluronic F-68 was chosen as the stabilizer following compatibility assessments.

Table 12: Final equation in terms of actual factors

Particle size	=
+321.20439	
−940.18519	Quantities of surfactant
+537.50000	Stabilizer concentration
+10.98628	C
−783.33333	Quantities of surfactant*stabilizer concentration
−22.59259	Quantities of surfactant*C
−12.77778	Stabilizer concentration*C
+1755.55556	Quantities of surfactant^2
−327.77778	Stabilizer concentration^2
+0.513032	C^2
polydispersity index	=
+0.420110	
−1.39074	Quantities of surfactant
+0.757407	Stabilizer concentration
+0.012743	C
−1.25000	Quantities of surfactant*stabilizer concentration
−0.025926	Quantities of surfactant*C
−0.018519	Stabilizer concentration*C
+2.55556	Quantities of surfactant^2
−0.444444	Stabilizer concentration^2
+0.000850	C^2

*indicates statistically significant model terms ($p < 0.05$), ns indicates non-significant terms ($p > 0.05$).

Formulation of SLNs by different methods

SLNs were synthesized using three distinct methodologies: Melting dispersion, solvent-emulsification evaporation, and injection techniques. The technique was refined based on entrapment effectiveness and particle size. The preparation of SLNs using other procedures entails significant factors, including hazardous solvents, elevated temperatures, high emulsifier concentrations, and substantial pressures.

The particle size of SLNs produced by the injection approach was the smallest compared to those formed by the other two procedures. The particle size ranged from 125 to 200 nm, and the entrapment effectiveness of the generated SLNs was between 80% and 90%. The entrapment effectiveness of SLNs was superior using this technique compared to the other two techniques. Consequently, the injection approach was employed thereafter.

Table 13: Factors

Factor	Name	Level	Low level	High level	Standard deviation	Coding
A	Quantities of surfactant	0.3044	0.3000	0.5000	0.0000	Actual
B	Stabilizer concentration	0.3024	0.3000	0.5000	0.0000	Actual
C	C	5.5	1.0000	10.00	0.0000	Actual

Table 14: Point prediction

	Two-sided					Confidence=95%		Population=99%	
Solution 1 of 100 response	Predicted mean	Predicted median	Observed	Standard deviation	SE mean	95% CI low for mean	95% CI high for mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Particle size	246.293	263.293		14.7178	8.39153	245.589	280.998	195.615	330.971
Polydispersity index	0.272047	0.312047		0.0225197	0.0128399	0.284957	0.339137	0.208493	0.415601

CI: Confidence interval, TI: Tolerance interval

Table 15: EE, drug loading,% yield and assay of NTD-SLNs prepared (mean±SD, *n*=3)

Formulation	% EE	Drug loading	% Yield
NTD-SLNs	86.5±3.33	18.50±1.2	94.3

EE: Entrapment efficiency, NTD-SLNs: Nintedanib-solid lipid nanoparticles

Optimization of SLNs

Optimization method for SLN preparation

The approach was chosen based on particle size and entrapment effectiveness. The mean particle size of the SLN formulation produced by three distinct processes is presented in the table 4. The EE of SLNs was assessed by measuring the concentration of unbound drug in the supernatant following high-speed spinning of the SLN solution at 16,000 rpm for 30 min at 30°C, utilizing a Remi cooling centrifuge (Mumbai, India). The mean percentage of encapsulation efficiency of SLNs is presented in the table 4. The minimal particle size of SLNs was achieved by the solvent injection approach, namely, 120–160 nm with glyceryl monostearate and 180–220 nm with cetyl alcohol.

The entrapment effectiveness of SLNs was greater for both lipids, specifically 85–95% for glyceryl monostearate and 80–90% for cetyl alcohol. The injection approach was chosen for the manufacturing of SLN due to its production of the smallest particles with the maximum entrapment effectiveness compared to the other two procedures.

Optimization of formulation variables

The formulation factors were optimized via the response surface methodology (RSM). The primary objective of RSM is to employ a series of structured tests to get an optimal answer. The response can be shown visually, either in three-dimensional space or as contour plots that facilitate the visualization of the response surface's form. Contours are curves representing continuous response, with all other variables held constant. Three independent variables were selected for the study to improve the formulation parameters of the SLN preparation. The optimized SLN formulation was chosen based on its reduced particle size, low PDI, and favorable attractiveness.

This approach assessed three parameters, each at two levels, with experimental trials conducted across all 27 potential combinations. The quantities of surfactant (A), stabilizer concentration (B), and aqueous to organic phase ratio (C) were designated as independent variables. The particle size (X) and the percentage production yield polydispersity index (Y) were the dependent variables (Table 5). Different concentrations (0.2% and 0.5%) of stabilizer (Polyvinyl Alcohol) and surfactant (tween 80) at different ratios (1:1 and 1:10, v/v) were used for formulating SLN formulations.

The relationship between independent and dependent variables was graphically represented by 3D response surface graphs (Figures 1 and 2) and 2D contour plots (Figures 3 and 4) generated by the model. Different shapes of the contour plots indicated different interactions between the variables.

The size distribution was also analysed for the PDI. The influence of formulation factors on particle-size distribution corroborated the aforementioned evidence on particle size. The formulation of SLNs with a surfactant and stabilizer concentration of 0.5% w/v GM and 0.5% (v/v) Tween 80 at a 1:10 (v/v) ratio resulted in low polydispersity indices, indicating uniform particle size distribution.

Fit summary

The quadratic model was suggested for particle size as it demonstrated a significant sequential (P = 0.0265) and a non-significant lack of fit (P = 0.5701), indicating an adequate fit of the model to the experimental data (Table 6). The presence of curvature suggests that particle size is influenced not only by the linear effects of formulation variables but also by their quadratic and interaction effects. Higher-order cubic models were aliased and therefore not considered.

Analysis of variance for quadratic model

Factor coding is coded.

Sum of squares is Type III – Partial.

Table 7 shows the Model F-value of 4.83 implies the model is significant. There is only a 0.26% chance that an F-value this large could occur due to noise.

$P < 0.0500$ indicates model terms are significant. In this case, B, C, AC, and A^2 are significant model terms. Values >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 8 shows the predicted R^2 of 0.0637 is not as close to the Adjusted R^2 of 0.5701 as one might normally expect; that is, the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal-to-noise ratio. A ratio >4 is desirable. Your ratio of 7.465 indicates an adequate signal. This model can be used to navigate the design space.

Fit summary

Table 9.

ANOVA for quadratic model

Table 10 shows the Model F-value of 5.30 implies the model is significant. There is only a 0.16% chance that an F-value this large could occur due to noise.

P <0.0500 indicates model terms are significant. In this case, B, C, and A^2 are significant model terms. Values >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.

Fit statistics

Table 11 shows the Predicted R^2 of 0.1117 is not as close to the Adjusted R^2 of 0.5981 as one might normally expect; that is, the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal-to-noise ratio. A ratio >4 is desirable. Your ratio of 8.209 indicates an adequate signal. This model can be used to navigate the design space.

Through multiple regression analysis on the experimental data, predicted response (Particle size and PDI) for the preparation of SLNs could be expressed by the following second-order polynomial equation in term of coded values:

The fitted equations that relates the responses particle size and PDI to the transformed factors are shown as above table 12.

The main effects represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when five factors are simultaneously changed. The polynomial terms are included to investigate non-linearity. Three-dimensional surface (3D) plots were drawn to illustrate the main and interactive effects of the independent variables on particle size and particle size distribution in terms of PDI. The optimum values of the selected variables were obtained from the software and also from the response surface plots.

Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables to obtain the desired response. An optimized formulation was developed by setting the constraints on the dependent and independent variables. The formulation developed was evaluated for the responses, and the experimental values obtained were compared with those predicted by the mathematical models generated.

The data clearly indicated that the values of particle size and PDI are strongly dependent on the selected independent variables. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative). The high values of correlation coefficient for particle size and PDI indicate a good fit, that is, good agreement between the dependent and independent variables. The equations may be used to obtain estimates of the response, as a small error of variance was noticed in the replicates.

The results obtained from the predicted model were used to create a contour plot for particle size, and the PDI is shown in Figures 2 and 4.

Factors

Table 13.

Point prediction

The software generated the optimum concentrations of surfactant (0.3% v/v Tween 80) and stabilizer (0.3% w/v GM) in the ratio of 10: 1 v/v. The aqueous: Organic phase ratio was optimized as 10:1 v/v, and the drug: Lipid ratio was optimized as 1: 10 w/w, which was very near to the predicted values. The software predicted the particle size and PDI as 245 nm and 0.272, respectively. Comparing with the values predicted by Design Expert, the results showed that the actual values of particle size (246.2 nm) and PDI (0.272) were very close to the predicted results. This indicated that the optimization achieved in the present study was reliable (Table 14).

Characterization of NTD SLNs

EE and DL

The concentration of NTD was measured using a UV spectrophotometer at 390 nm. The calibration plot for NTD in the SLN vehicle was established within a concentration range of 1.0–20.0 µg/mL, with a regression coefficient of r^2 = 0.999. The average EE and average DL of NTD-SLNs are presented in the table 15.

EE refers to the effectiveness of the preparation procedure in integrating the medication into the carrier system. An essential criterion for evaluating the appropriateness of a drug carrier system is its loading capacity. The loading capacity is often represented as a percentage in relation to the lipid phase (matrix lipid: Medication). The quantity of drug encapsulated also affects the efficacy of the drug delivery system, since it impacts the pace and magnitude of drug release from the system. Both DL and EE are contingent upon

the physicochemical qualities and the interactions among the drug, carrier matrix, and the surrounding media.

Yield: The yield denotes the quantity of SLNs obtained from the preparation procedure, expressed as the percentage ratio of the lipid content in the suspension to the theoretical amount. The yield of AD-SLNs is presented in the table 15.

Particle size distribution

This study employed photon correlation spectroscopy for particle size determination. Results from photon correlation spectroscopy are conventionally reported on an intensity basis, utilizing the Z-average and the PDI. The assessment of particle size is a crucial factor in optimizing the SLN formulation. The mean particle size of an improved NTD-SLN formulation was below 300 nm (Figure 5). The mean particle size of SLNs and the PDI are presented in the table 5.

A minimal ζ potential of ±30 mV is regarded as the standard for achieving a physically stable system. Charged particles (high zeta potential) are less prone to aggregation due to electrostatic repulsion (Figure 6).

The figure 7 presents SEM images of SLNs, providing insights into particle shape. The particles were round, smooth, and homogeneous in shape, with a mean diameter of approximately 230–250 nm for GM.

In vitro dissolution study

An *in vitro* release investigation for NTD and NTD-SLNs was conducted on SLN suspensions within 24 h of production using the dialysis membrane technique. The research was conducted in an acetate buffer at pH 4.5, comprising 0.5% Tween 80. Figure 8 clearly illustrates that the maximum release of 97.2% was achieved for normal NTD in 4 h, but the release of NTD SLNs was 33.3% in the same duration, suggesting that a portion of NTD was integrated at the surface of the SLNs. Consequently, NTD-SLNs exhibited a sustained-release characteristic, with a cumulative drug release percentage of around 98.9% over 24 h. The SLNs may diminish NTD release in the latter stage, likely because the solubilized/dispersed AD can only be released gradually from the lipid matrix by dissolution and diffusion. SLN formulations demonstrated superior outcomes relative to the usual NTD suspension due to their nanoscale dimensions and low polydispersity indices. The outcome was consistent with prior documented investigations demonstrating a sustained/controlled release pattern of drug-loaded SLNs.

CONCLUSION

The present study successfully developed and statistically optimized NTD -loaded SLN using glyceryl monostearate as the lipid, Tween 80 as the surfactant, and Pluronic F-68 as the stabilizer. Among the different preparation techniques evaluated, the solvent injection method was found to be superior, producing nanoparticles with a smaller particle size and higher EE compared to melting dispersion and solvent-emulsification methods. RSM effectively optimized the formulation variables, demonstrating that surfactant concentration, stabilizer concentration, and aqueous-to-organic phase ratio significantly influenced particle size and PDI. The optimized formulation showed a particle size of approximately 246 nm and a PDI of 0.272, closely matching the predicted values, thereby confirming the reliability and validity of the statistical model. The optimized NTD-SLNs exhibited high EE (86.5%), satisfactory DL (18.5%), and good percentage yield (94.3%). Morphological analysis confirmed spherical and homogeneous nanoparticles. The zeta potential values indicated acceptable physical stability of the formulation. *In vitro* release studies demonstrated a sustained drug release profile over 24 h compared to the rapid release observed with pure NTD suspension, indicating the potential of SLNs to provide controlled drug delivery.

Overall, the developed NTD-SLNs represent a promising nanocarrier system for improving the therapeutic performance of NTD by enhancing drug encapsulation, stability, and sustained release behavior. This optimized formulation may contribute to improved clinical efficacy and reduced dosing frequency in cancer therapy. Further *in vivo* pharmacokinetic and therapeutic evaluation studies are warranted to confirm its clinical applicability.

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