Formulation & Optimization of Nilotinib Loaded Solid Lipid Nanoparticles Using Design of Experiment

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ABSTRACT

Introduction: SLN is alternative to traditional colloidal carrier systems such as emulsion, liposome, and polymeric micro-and nanoparticles. They are highly biocompatible, have low cytotoxicity, target specific, scalability, prolonged drug release, and ease of production in industrial scale.

Objective: Current study aims to formulate and characterize Nilotinib-loaded solid lipid nanoparticles (SLNs) carrier system.

Methods: About 17 SLN formulations were fabricated employing hot emulsification/ ultrasonication technique. A full factorial design (3³) was employed for formulation batches (i.e., 17 formulations) in which 3 factors namely lipid, surfactant and co-surfactant were tested at 3 levels of concentration. The effect of different levels of factors was evaluated at the particle size of resultant formulation, entrapment efficiency and % cumulative drug released. Kinetic model fitting for all nilotinib SLN formulations was done to interpret the release rate from the SLN. Optimized formulation was subjected for FTIR, SEM and stability studies.

Results: The mean particle size, PDI, zeta potential, entrapment efficiency (EE), content uniformity and in-vitro drug release profile of optimized nilotinib-loaded SLNs (NF12) were found to be 132.11 ± 3.47 nm, 0.128 ± 0.02 , -18.02 ± 2.17 mV, $84.12 \pm 2.66\%$, $98.96 \pm 1.23\%$ and $98.86 \pm 2.12\%$ respectively. The release kinetics suggest that drug release followed zero order and release was anomalous non-fickian diffusion super case II transport. The FTIR studies revealed no incompatibilities between the drug and excipient, and SEM images showed that the nanoparticles appeared to be more porous and spherical in shape. The formulation was shown to be stable over a period of six months by stability studies.

Conclusion: According to the results, the method of SLN preparation proposed in this study could be considered as the most suitable method for generating colloidal carriers loaded with nilotinib.

Key Words: Nilotinib, Solid-lipid nanoparticle, Box-Behnken design, Independent variables, Release order kinetics, Solubility

INTRODUCTION

Lipid-based formulations showed success in reducing the inherent limitations of slow and incomplete dissolution of poorly water-soluble drugs by facilitating the formation of solubilized phase and further, improving the absorption.¹ Solid lipid nanoparticles (SLNs) are emerging as alternative carriers to other colloidal drug systems, in controlled and targeted drug delivery. Submicron in size (50-1000nm) and made from biocompatible and biodegradable materials that can be combined with lipophilic and hydrophilic drugs. SLNs combine the advantage of different colloidal carriers, vesicular carriers and polymeric carriers² as physiologically acceptable systems and also in imparting the controlled re-

lease of drug from lipid matrix.³ Furthermore, these systems enhance the lymphatic transport of the lipophilic drugs, irrespective of the route of administration, and therefore increase the systemic availability of drug molecule.⁴

Nilotinib is a second-generation tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia (CML) caused by the breakpoint cluster region Abelson murine leukemia (BCR-ABL) oncogene. It acts by inhibiting the tyrosine kinase activity of the BCR-ABL protein. Nilotinib has poor aqueous solubility and low oral bioavailability.⁵

A pharmaceutical formulation approach with an improved solubility could intern enhance bioavailability. Using QbD, we will be able to deduce suitable SLN formulations as well

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as understand the fate of the nanoparticles as potential carriers for improving dissolution rate-limited absorption. The present research investigation was aimed to develop the Nilotinib loaded SLN formulation by using Box-Behnken design (BBD).⁶

MATERIAL AND METHODS

Nilotinib was purchased from Hetero drugs Ltd, Hyderabad. Dynasan 116, cremophor RH40, soy lecithin, tween 80, chloroform and methanol were purchased from Gattefosse, Mumbai. All the reagents used were of analytical grade. Marketed product-Tasigna 200mg capsule.

Preparation of Nilotinib SLN

Nilotinib-SLNs were prepared by a hot emulsification / ultrasonication method. Nilotinib (200 mg), Glycerol Mono Dynasan 116(%) was dissolved in a mixture of chloroform and methanol (1:1) (20mL). The solvent was completely removed using a rotary evaporator. The drug-embedded lipid layer was melted by heating at 75°C. An aqueous phase was formulated by dissolving the Cremophor RH 40 & Soy Lecithin in double-distilled water and adding to the molten lipid phase Coarse hot oil in water emulsion was obtained, which was then ultrasonicated using a probe sonicator. Finally, the obtained hot nanoemulsion was allowed to cool to room temperature to prepare Nilotinib-SLNs.⁷

Experimental Design

Box-Behnken Experiment Design (BBD)

Three-factor, three-level BBDs were employed to study and optimize the main effects, interaction effects, and quadratic effects of the formulation ingredients on the results of the SLN. The design is suitable for studying quadratic response surfaces and constructing second-order polynomial models (table 1). Accordingly, the following table shows 17 randomized experimental runs for the selected independent variables, including five replicates at the centre point (asterisk-marked) resulting from a three-factor, three-level BBD analysis and their results. BBD matrix was generated with Design Expert® software. (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), and the data obtained were analyzed by the same software and Composition of Nilotinib SLNs formulation by BBD is given in Table 2.

Optimization Using the Desirability Function

As part of the present study, all three responses were optimized simultaneously by a desirability function based on a numerical optimization method introduced by Derringer and Suich using the Design-Expert software (Version8.0, Stat-Ease Inc., Silicon Valley, CA, USA).⁸

Characterization of Nilotinib SLN

The particle size, PDI and zeta potential of SLN evaluated using photon correlation spectroscopy using a Zetasizer 3000HSA (Malvern, UK) as per the referred procedure.⁹

Drug Content

1 ml SLN mixed with 100 ml methanol and sonicated for 5 min in bath sonicator. The contents filtered and filtrate analyzed spectrophotometrically at 263nm.¹⁰

Entrapment Efficiency

Entrapment efficiency (EE%) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium as reported.¹¹ The amount of Nilotinib in the aqueous phase was estimated by HPLC method and the entrapment efficiency was calculated by the equation:

$$\%EE = \frac{\text{analysed weight of drug in SLN}}{\text{theoretical weight of drug loaded}} \times 100$$

In-vitro release Study

In-vitro release studies were performed in 0.1N HCl (pH 1.2) using modified franz diffusion cell and dialysis membrane having pore size 2.4 nm, molecular weight cut-off between 12,000-14,000 was used. Membrane was soaked in double distilled water for 12 h. the study carried out as per the referred procedure and samples withdrawn at 0.5, 1,2,3,4,6,8 and 12 h time points. The samples analyzed by UV-visible spectrophotometer at 263 nm.¹² The data obtained from drug release studies was fitted into various linear models and evaluated.¹³

Characterization of the optimized nilotinib SLN

Fourier transform infrared spectroscopy (FT-IR)

The FTIR of pure nilotinib and optimized formulations recorded using FTIR spectrometer Prestige-21 (Shimadzu-Japan). The scanning range adjusted between 400-4000 cm⁻¹ and resolution to 1cm⁻¹.¹⁴

SEM studies

The surface and shape morphology of optimized SLN analyzed using scanning electron microscopy by placing the formulations on metal stub and photographs were taken at proper magnification.¹⁵

Stability studies

The optimized SLN formulation was subjected to stability study at temperature of 40 °C ± 2 °C and relative humidity of 75±5% RH for a time period of 6 months in Humidity chamber (REMI, Mumbai). The specifications to be evaluated in stability study period include particle size, entrapment efficiency, *in vitro* drug released.^{16,17,18}

RESULTS AND DISCUSSION

Characterization of SLNs

The drug content for all was within 96.17 -98.96% and found to increase with increase in the concentration of surfactant. The highest drug content was observed for NF12 formulation. The EE of all formulation ranged between 66.45 - 84.12% with NF12 displaying the maximum value. The PS ranged between 132 - 212 nm. The nanoformulations exhibited -ve surface charge with the inclusion of Nilotinib which clearly suggested the orientation of nilotinib in the lipid matrix. The polydispersity index of all SLNs indicated narrow size distribution which reveals the higher stability of Nilotinib SLNs. The zeta potential of NF12 was not sufficient to keep the particles dispersing stably. However, the particle size did not change significantly within 30 days, which should contribute to the following point. (table 3).

In-vitro dissolution testing of Nilotinib SLNs

More than 85% of drug was dissolved from NF12 after 12 hrs. However, the original Nilotinib powder showed only approximately 88% dissolved after the same time period. The enhanced dissolution may be due to the decrease in crystallinity and the increase in solubility of the drug. (figure 1-3)

Kinetic analysis of Nilotinib SLN

The results show that the regression coefficient value closer to unity in case of zero order plot i.e., 0.986 indicates that the drug release follows a zero-order mechanism. Further the n value obtained from the Korsmeyer-Peppas plots i.e., 0.957 indicating non Fickian (anomalous) transport thus it projected that delivered its active ingredient by coupled diffusion and erosion. (figure 4,5)

Statistical analysis of the designed experiment

The range of particle size (Y1) for all batches was 132.11 ± 3.47 - 212.48 ± 4.14 nm. Similarly, the range for %EE (Y2) was $66.45 \pm 2.38\%$ - $84.12 \pm 2.66\%$ and the range for cumulative percentage of drug released in 12 h (Y3) was 79.65 ± 1.80 - $98.86 \pm 2.12\%$. All responses were fitted to a second quadratic model and the adequacy of this model was verified by ANOVA; tests provided by Design- Expert software.

Particle size (Y1)

The quadratic model generated for particle size revealed that the amount of Dynasan 116, amount of Cremophor RH40 and amount of Soy Lecithin have a significant influence on Y1. This may also explain the significant interaction between the amount of lipid and surfactant (figure 6A,6B).

Entrapment Efficiency (Y2)

The quadratic model generated revealed that the amount of

Dynasan 116 and amount of Cremophor RH40 have a significant influence on Y2. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The mathematical model generated for Entrapment Efficiency (%) (Y2) was found to be significant with F-value of 0.0143 implies the model is significant. (fig 7A,7B).

Cumulative percent drug released (Y3)

The quadratic model generated revealed that the amount of Dynasan 116, amount of Cremophor RH40 and amount of Soy Lecithin have a significant influence on Y3. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The mathematical model generated for percent drug released in 12 hrs (Y3) was found to be significant with F-value of 0.0316 implies the model is significant. (figure 8A,8B).

Optimization by desirability function

The responses were transformed into the desirability scale, respectively. Among them, Y1 and Y2 had to be minimized, while Y3 had to be maximized. For individual desirability function, $Y_{\max}$ and $Y_{\min}$ were taken as the highest objective function (D) was calculated by Equations for each response. The maximum function value was obtained at X1:06, X2:06 and X3:06. We prepared three batches of formulations under the optimum composition in order to verify the model's capacity to predict, and we examined three responses for each formulation.. (Table 4).

Stability studies

No significant change in particle size, entrapment efficiency, in-vitro release and drug content observed at $40^\circ\text{C} \pm 2^\circ\text{C}/75\%$ RH $\pm 5\%$ and at $25^\circ\text{C} \pm 2^\circ\text{C}/60\%$ RH $\pm 5\%$ (table 5)

Characterization of Nilotinib SLN

The FTIR spectrum of pure nilotinib (figure 9) showed a peak at 1741.78 cm^{-1} for C=O stretching, 3396.76 cm^{-1} for O-H stretching, 1741.78 cm^{-1} for C-H stretching, broad band at 1687.77 cm^{-1} for N-H resulting from salt formation between the quaternary nitrogen of nilotinib and -OH of hydrogen sulfate the bands for C-O stretching appeared at 1066.80 , 1155.40 and 1228.70 cm^{-1} . The FTIR spectra solid lipid nanoparticles of nilotinib optimized formulation (NF12) (Figure 10) showed similar prominent peaks pure drug and these results indicate the absence of any chemical interactions between the drug nilotinib and used excipients in the formulation.

SEM studies

The drug crystals seemed to be smooth-surfaced, round in shape. The drug surface in solid lipid nanoparticles of nilotinib optimized formulation seems to be more porous in nature. Drug crystals appeared to be incorporated into the particles of

the polymers looked like a matrix particle. The results could be attributed to dispersion of the drug in the molten mass of the polymer which lead to the sustained release of the drug. (figure 11A,11B)

Particle size and zeta potential

The particle size of the nanoparticles was found to be in the range of $132.11 \pm 3.47 - 212.48 \pm 4.14$ nm. The zeta potential values were ranging from -18 to -26mV and least values were observed for NF12 (figures 12 and 13).

CONCLUSION

In the present paper, the SLNs containing nilotinib were prepared and the influence of independent variables on the particle size, PDI and zeta potential was evaluated by BBD. In the following step, the formulation parameters were statistically optimized. Out of all formulations the mean particle size, PDI, zeta potential, entrapment efficiency (EE), content uniformity and in-vitro drug release profile of optimized nilotinib-loaded SLNs (NF12) were found to be 132.11 ± 3.47 nm, 0.128 ± 0.02 , -18.02 ± 2.17 mV, $84.12 \pm 2.66\%$, $98.96 \pm 1.23\%$ and $98.86 \pm 2.12\%$ respectively. The nanoparticles exhibited a prolonged release of nilotinib. The nilotinib SLN formulation proved to be a potential alternative for sustained drug release with enhanced solubility.

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Conflict of Interest

No conflict of interest

Author Contribution

All the two authors contributed equally towards the data collection, data analysis and compilation.

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Table 1: List of dependent and independent variables in BBD

Independent variables			Levels		
Variable	Name	Units	Low (-1)	Middle (0)	High (+1)
A	Amount of Dynasan 116	%	4	6	8
B	Amount of Cremophor RH40	%	2	4	6
C	Amount of Soy Lecithin	%	2	4	6
Dependent variable			Goal		
Y ₁	Particle size	nm	Minimize		
Y ₂	Entrapment Efficiency	%	Minimize		
Y ₃	Drug release after 12 h	%	Maximize		

Table 2: Composition of Nilotinib SLNs formulation by BBD

F. No	Nilotinib (mg)	Dynasan 116 (%)	Cremophor RH40 (%)	Soy Lecithin (%)	Tween 80 (ml)	Chloroform : Methanol (1:1)	Distilled Water (mL)
NF1	200	4	2	4	0.5	20	Q.S
NF2	200	8	2	4	0.5	20	Q.S
NF3	200	4	6	4	0.5	20	Q.S
NF4	200	6	6	2	0.5	20	Q.S
NF5	200	4	4	2	0.5	20	Q.S
NF6	200	8	4	2	0.5	20	Q.S
NF7	200	4	4	6	0.5	20	Q.S
NF8	200	8	4	6	0.5	20	Q.S
NF9	200	6	2	2	0.5	20	Q.S
NF10	200	8	6	4	0.5	20	Q.S
NF11	200	6	2	6	0.5	20	Q.S
NF12	200	6	6	6	0.5	20	Q.S
NF13	200	8	4	2	0.5	20	Q.S
NF14	200	6	4	2	0.5	20	Q.S
NF15	200	6	4	6	0.5	20	Q.S
NF16	200	4	4	4	0.5	20	Q.S
NF17	200	6	4	6	0.5	20	Q.S

Table 3: Physico-chemical parameters of Nilotinib SLNs

F. No	#Content uniformity (%)	% Entrapment Efficiency	Mean particle size (nm)	Zeta potential (-mV)	Polydispersity Index
NF1	98.53±1.69	74.92±1.47	212.48 ± 4.14	24.28 ± 6.13	0.164± 0.07
NF2	96.17±2.55	76.43±1.51	175.69 ± 4.18	18.62 ± 5.23	0.279 ± 0.01
NF3	97.05±2.45	78.80±1.62	202.14 ± 5.45	22.49 ± 2.45	0.183 ± 0.06
NF4	97.11±1.57	69.84±2.35	163.57 ± 4.67	18.53 ± 4.12	0.198± 0.03
NF5	98.59±2.14	67.72±1.17	212.19 ± 3.35	20.43 ± 2.46	0.236 ± 0.05
NF6	97.70±2.34	80.51±1.59	221.63 ± 4.57	22.38 ± 2.13	0.214 ± 0.09
NF7	97.46±2.13	73.68±2.83	155.27 ± 5.66	18.86 ± 6.47	0.269 ± 0.03
NF8	96.57±2.82	80.85±1.76	198.14 ± 7.79	20.45 ± 5.23	0.197± 0.007
NF9	98.18±2.52	75.72±1.58	161.85 ± 4.61	23.12 ± 2.89	0.232 ± 0.09
NF10	97.45±2.16	66.45±2.38	195.3 ± 3.07	19.75 ± 2.31	0.251 ± 0.01
NF11	96.84±2.62	78.57±1.22	213.37 ± 2.69	21.56 ± 1.43	0.188 ± 0.05
NF12	98.96±1.23	84.12±2.66	132.11 ± 3.47	18.02 ± 2.17	0.128 ± 0.02

Table 3: (Continued)

F. No	#Content uniformity (%)	% Entrapment Efficiency	Mean particle size (nm)	Zeta potential (-mV)	Polydispersity Index
NF13	97.59±1.80	77.33±2.85	181.35 ± 2.23	19.87 ± 6.32	0.233 ± 0.06
NF14	97.45±2.36	69.27±1.72	215.97 ± 3.15	26.61 ± 6.19	0.264 ± 0.08
NF15	97.71±2.18	73.28±2.60	168.46 ± 4.23	21.75 ± 5.36	0.154 ± 0.07
NF16	96.40±2.58	68.63±1.45	213.38 ± 3.15	19.39 ± 4.15	0.289 ± 0.01
NF17	97.21±2.17	72.85±2.17	181.27 ± 4.35	26.17 ± 3.43	0.216± 0.05

Table 4: Optimized values obtained by the constraints applies on Y₁, Y₂ and Y₃

Independent vari- able	Nominal values %	Predicted values						
		Particle size (Y1) (nm)	Entrapment Efficiency (%) (Y2)	%CDR (Y3)	Batch	Particle size (Y1) (nm)	Entrapment Efficiency (%) (Y2)	Percent drug release in 12 hrs (Y3)
Amount of Dynasan 116 (A)	06				1	133.36	86.19	98.38
Amount of Cremo- phor RH40 (B)	06	132.11	84.12	98.86	2	131.88	85.45	98.11
Amount of Soy Lecithin (C)	06				3	132.12	85.74	98.58

Table 5: Stability studies of optimized formulation

Retest Time for Optimized formulation (NF12)	Particle Size (nm)	Entrapment Efficiency (%)	In-vitro drug release profile (%)	Drug content (%)
0 days	132.11 ± 3.87	84.12±2.66	98.86±2.12	98.96±1.23
30 days	132.15 ± 2.56	84.08±1.89	98.53±2.47	98.67±1.15
60 days	132.28 ± 3.85	84.02±1.23	98.44±2.89	98.31±1.84
120 days	133.55 ± 2.74	83.85±1.77	98.36±1.88	97.96±1.61
180 days	133.43 ± 1.84	83.71±1.65	98.15±1.45	97.88±1.19

Values are expressed in mean± SD:(n=3)

Figure 1: In vitro dissolution data of Nilotinib SLN (NF1-NF6).

Figure 2: In vitro dissolution data of Nilotinib SLN (NF7-NF12).

Figure 3: In vitro dissolution data of Nilotinib SLN (NF13-NF17).

Figure 4: Zero order plot of NF12.

Figure 5: Korsmeyer-Peppas plot of NF12.

Figure 6A: Response 3D surface plot showing the influence of amount of Dynasan 116 and amount of Cremophor RH40 on particle size fixed level of C.

Figure 6B: Contour plot showing the influence of amount of Dynasan 116 and amount of Cremophor RH40 on particle size fixed level of C.

Figure 7A: Response 3D surface plot showing the influence of amount of Dynasan 116 and amount of cremophor rh40 on entrapment efficiency (%) fixed level of C.

Figure 7B: Contour plot showing the influence of amount of Dynasan 116 and amount of Cremophor RH40 on EE (%) fixed level of C

Figure 8A: Response 3D surface plot showing the influence of amount of Dynasan 116 and amount of cremophor RH40 on cumulative % drug released fixed level of C.

Figure 8B: Contour plot showing the influence of amount of Dynasan 116 and amount of cremophor RH40 on cumulative % drug released fixed level of C.

Figure 9A: SEM images of solid lipid nanoparticles of Nilotinib (NF12).

Figure 9B: SEM image of Nilotinib SLN (NF12).

Figure 10: Particle size of the optimized formulation solid lipid nanoparticles of Nilotinib (NF12).

Figure 11: Zeta potential for the optimized formulation solid lipid nanoparticles of Nilotinib (NF12).