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

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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 carriers ² as physiologically acceptable systems and also in imparting the controlled release 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

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 ± 3.47nm, 0.128 ± 0.02, -18.02 ± 2.17mV, 84.12±2.66%, 98.96±1.23% and 98.86±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
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).  

**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.  

**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. (Version 7.0, 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 (Version 8.0, Stat-Ease Inc., Silicon Valley, CA, USA).  

**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.

**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.

**Entrapment Efficiency**

Entrapment efficiency (EE%) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium as reported.  

\[
\text{EE} = \frac{\text{analyzed weight of drug in SLN}}{\text{theoretical weight of drug loaded}} \times 100
\]

**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, 12 h time points. The samples analyzed by UV-visible spectrophotometer at 263 nm.  

**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.

**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.
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±2.38% – 84.12±2.66% and the range for cumulative percentage of drug released in 12 h (Y3) was 79.65±1.80 – 98.86±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 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°C±2°C/75% RH±5% and at 25°C±2°C/60% RH±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±3.47 – 212.48±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 ± 3.47nm, 0.128 ± 0.02, -18.02 ± 2.17mV, 84.12±2.66%, 98.96±1.23% and 98.86±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.

**ACKNOWLEDGEMENT**

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

**Conflict of Interest**

No conflict of interest

**Author Contribution**

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

**Financial Support**

Self-financed

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Table 1: List of dependent and independent variables in BBD

| Independent variables | Variable | Name | Units | Low (-1) | Middle (0) | High (+1) |
|-----------------------|----------|------|-------|----------|------------|----------|
| A                     | A        | Amount of Dynasan 116 | %     | 4        | 6          | 8        |
| B                     | B        | Amount of Cremophor RH40 | %     | 2        | 4          | 6        |
| C                     | C        | Amount of Soy Lecithin | %     | 2        | 4          | 6        |

Dependent variable

| Y1 | Particle size | nm | Minimize |
| Y2 | Entrapment Efficiency | % | Minimize |
| Y3 | 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               | 6                 | 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   | 96.77±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± 0.02         |
| 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 variable | Nominal values % | Predicted values | Batch | Particle size (Y1) (nm) | Entrapment Efficiency (% Y2) | %CDR (Y3) | Percent drug release in 12 hrs (Y3) |
|----------------------|------------------|------------------|-------|-------------------------|-----------------------------|------------|-----------------------------------|
| Amount of Dynasan 116 (A) | 06               | 133.36           | 86.19 | 98.38                   |
| Amount of Cremophor RH4 0 (B) | 06               | 131.88           | 85.45 | 98.11                   |
| Amount of Soy Lecithin (C) | 06               | 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      | 83.7±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 (NF 13-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.
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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).