Formulation and Characterization of Dasatinib Loaded Solid Lipid Nanoparticles by Design of Experiment

Baji Hussain Khan¹, Sandhya P²*

¹Research Scholar, Career Point University, Kota, Rajasthan-325003, India; ²Research Supervisor, Career Point University, Kota, Rajasthan-325003, India.

ABSTRACT

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

Aims: The objective of the research is aimed at formulation of solid lipid nanoparticle (SLN) of dasatinib a tyrosine kinase inhibitor used in the treatment of chronic myelogenous leukemia.

Methodology: By investigating the relationship between design factors and experimental data using response surface methodology. A 33 Box-Behnken design was chosen using amount of glycerol monostearate (X1) the amount of poloxamer 407 (X2) and amount of tyloxapol (X3) level as independent factors. SLN prepared by hot emulsification / ultra-sonication method technique using glyceryl monostearate as the solid lipid, and poloxamer 407 as the surfactant. The dependent variables include particle size (Y1), entrapment efficiency(Y2) and drug release after 12 h(Y3). Properties of SLN such as the morphology, FT-IR, particle size, zeta potential, entrapment efficiency and drug release behavior were investigated.

Results: From the results DF10 was found to be optimized formulation, which shown that the nanoparticle designed are spherical in shape with a mean particle size of 112 nm with PI of 0.40 and zeta potential of −25.6 mV. The in vitro release studies showed that more than 98.78% of drug was released from optimized SLN after 12 h, which is higher when compared with marketed formulation. The release kinetics of the optimized formulation followed zero Intriorder drug release and best fitted the Korsemeyer-peppas model.

Conclusion: These results indicated that the dasatinib-loaded SLN formulation could potentially be exploited for the treatment of chronic myelogenous leukemia with controlled release manner.

Key Words: Dasatinib, Solid lipid nanoparticle, Chronic myelogenous leukemia, Box-Behnken design, Particle size, SEM

INTRODUCTION

Nanoparticulate drug delivery systems are considered for drug delivery because due to their possibility of modulating drug release, increased penetration, and prolonging residence time at site of action. Amongst these systems solid lipid nanoparticles (SLN) appear very promising. SLN are alternative to traditional colloidal carrier systems such as emulsion, liposome, and polymeric micro-and nanoparticles. They are highly biocompatibility, have low cytotoxicity, target-specific, scalability, prolonged drug release, and ease of production in industrial scale 1-5

A traditional formulation and optimization approach makes it extremely difficult (and inefficient) to achieve an optimized formulation that allows for rapid and complete dissolution. Hence attention is paid towards the formulation optimization of SLN dispersion systems by using factorial design. The nature of lipids and emulsifiers effect the quality of SLN dispersions and the nature of SLN is controlled by the relative number of excipients used in the formulation. Various experimental designs6,7 are applied for optimizing the formulation that involve less experiments and provide estimates of the relative significance of different variables. 8

The response surface methodology (RSM) is useful in simultaneously analyzing process variables when variable interactions are very complicated. RSM has been demonstrated in multiple studies to be useful for determining the optimal formulation in various drug delivery systems. This study used
the Box-Behnken design, a RSM design, since it requires fewer runs in a 3-factor experimental design than all other RSM designs, and is especially useful when extreme combinations of treatments need to be avoided or minimized.

As an oral tyrosine kinase inhibitor, dasatinib is approved for treating patients with chronic myelogenous leukemia (CML), whether they are BCR/ABL positive or negative. The major targets of Dasatinib are the BCR/ABL, SRC, Ephrins and GFR. Dasatinib is a Biopharmaceutics Classification System Class II drug having very low solubility and high permeability. In addition, the low aqueous solubility and poor dissolution of dasatinib result in poor bioavailability, therefore, limiting its therapeutic effectiveness. There is evidence that the absolute bioavailability of dasatinib is approximately 14 to 34% due to extensive first-pass effects in animals.

To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid-based drug delivery systems such as solid lipid nanoparticles (SLNs) can be used.

The aim of this research was to evaluate the main and interaction effect of compositional variation and to optimize the dasatinib-loaded SLN formulation using the Box-Behnken design.

**MATERIAL AND METHODS**

**Materials used**
The drug dasatinib was obtained from Hetero drugs Ltd, Hyderabad. The excipients Glycerol Monostearate, Poloxamer 407, Tyloxopol were obtained from Rubicon research Pvt Ltd, Thane, Maharashtra. Tween 80 was purchased from SD Fine Ltd, Mumbai and solvents chloroform, methanol were purchased from Merck Specialties Pvt Ltd, Mumbai, India.

**Preparation of dasatinib loaded SLN**
The SLNs were prepared by a hot emulsification / ultrasonication method. Dasatinib (100 mg), glycerol monostearate (%), was dissolved in a mixture of chloroform and methanol (1:1) (20mL). The solvent was then completely removed using a rotary evaporator. The drug-embedded lipid layer was melted by heating at 75°C. An aqueous phase was then formulated by dissolving the surfactant and co-surfactant (%), such as poloxamer 407 and tyloxopol in double-distilled water and adding it to the molten lipid phase followed by homogenization for 3 minutes. Coarse hot oil in a 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 SLNs.

**Experimental Design**
A 3^3 BBD employed for optimizing the main, interaction, and quadratic effects of formulation components on characteristics of SNEDDS. Seventeen experiments run randomly for chosen independent variables, that include 5 repetitions at center (asterisk-marked) obtained from 3 factor, 3-level BBD and their subsequent responses noted are specified in table 1 and 2.

The BBD matrix obtained using Design Expert® software (Version 7.0, Stat-Ease Inc., Silicon Valley, CA, USA), the second-order quadratic equations are as:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 + \beta_3^2 X_3^2 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 + \beta_3^2 X_3^2 \]

\[ Y - \text{Level of the measured response} \]

\[ \beta_0 - \text{intercept} \]

\[ \beta_i - \beta_4 - \text{regression coefficient} \]

\[ X_1, X_2, X_3, \text{main effects} \]

\[ X_1 X_2, X_2 X_3, \text{and} X_1 X_3 - \text{interaction between the main effects} \]

\[ X_1^2, X_2^2 \text{and} X_3^2 - \text{quadratic terms of independent variables}\]

**EVALUATION OF SLNs**

**Determination of drug content and entrapment efficiency (EE)**

Determination of drug content and EE was done according to reported procedures in reference.

**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. During the experiments, the solution in receptor side was maintained at 37°C ± 0.5°C and stirred at 50 rpm with magnetic stirring bars for 2 hours. Then, the pH was increased to pH 6.8 for the remaining 10 hours. An aliquot of the sample (5 mL) was and analyzed by UV-visible spectrophotometer at 323 nm.

**Kinetic Model Fitting**

The drug release data was fitted into various linear models include Zero order, Higuchi, Hixon – Crowell, Quadratic and Polynomials, whereas the nonlinear models include First order, Weibull, KorsMeyer – Peppas, Logistic etc.

**Characterization of optimized SLN formulation**

Fourier transform infrared spectroscopy (FT-IR), SEM, particle size, zeta potential and stability studies were performed and reported according to reference procedures.

**RESULTS & DISCUSSION**

**Drug content**
The drug content uniformity of all formulations (DF1-DF17) ranges between 96 to 99% with DF10 displaying maximum of 99.42% drug uniformity.
In-vitro dissolution study of dasatinib SLNs
To understand the release mechanisms of dasatinib SLNs formulations, the drug release from the formulation DF10 was shown to be 98.78%, whereas marketed product was shown to be 89.61% after 12 h (figures 1-3). The enhanced dissolution may be due to the decrease in crystallinity and the increase in solubility of the drug. The increase in cumulative drug released is mainly attributed to rapid self-emulsification of the formulations due to instantaneous dispersion in the medium after dissolution of the capsule shell.

Kinetic analysis
From the above results it is apparent that the regression coefficient value of DF10 closer to unity in case of zero order plot i.e., 0.975 indicates that the drug release follows a zero-order mechanism. Further the n value obtained from the Korsmeyer-Peppas plots i.e., 0.953 indicating non Fickian (anomalous) transport thus it projected that delivered its active ingredient by coupled diffusion and erosion (table 3).

Design of experiments
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 (table 4).

Particle size
The particle size of the nanoparticles was found to be in the range of 112-247 nm. The quadratic model generated revealed that the amount of glycerol monostearate, amount of poloxamer 407 and amount of tyloxopol have a significant influence on the Particle size. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The factorial equation for particle size (Y1) was found to be significant with F-value of 0.0175 implies the model is significant (figure 4A and 4B).

Entrapment efficiency (%)
The Entrapment Efficiency (%) of the SLNs was found to be in the range of 79.54% to 96.41%. The quadratic model generated revealed that the amount of Glycerol Monostearate and amount of Poloxamer 407 have a significant influence on the Entrapment Efficiency (%). The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The factorial equation for Entrapment Efficiency (%) showed a good correlation coefficient (0.9997) (figure 5A and 5B).

Cumulative percent drug released
The cumulative percent drug release in 12 hrs from the SLNs was found to be in the range of 78.66 – 98.78%. The quadratic model generated revealed that the amount of Glycerol Monostearate, amount of Poloxamer 407 and amount of Tyloxopol have a significant influence on the particle size. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The factorial equation for percent drug release showed a good correlation coefficient (0.9994) (figure 6A and 6B).

Optimization by desirability function
An optimization process was undertaken with desirability function to optimize the three responses simultaneously. The results are shown in Table 5.

CHARACTERIZATION OF SLN

Fourier-transform infrared spectroscopy (FTIR)
The characteristic peaks of the drug in IR spectrum of Dasatinib and optimized formulation were identified by absorption peaks at 3068 cm\(^{-1}\) (secondary amine N-H stretch), 3086 cm\(^{-1}\) (C-H aromatic ring), 985 cm\(^{-1}\) (C-H bending), 1192 cm\(^{-1}\) (C-N stretch), 1444 cm\(^{-1}\) (C=O stretch) and 1290 cm\(^{-1}\) (C=C) stretch, aromatic ring. There were no particular interactions between drug and excipients and drug remain intact in the formulation. (figure 7, 8)

Droplet size and zeta potential
The mean globule size of selected SLN formulation DF10 was 112 nm with low polydispersity index 0.40 and zeta potential of -25.6 mV which is indicated the ability of the present technology to produce nanoemulsion that offers larger interfacial surface area required for drug absorption. (figure 9).

The optimized SLN showed high absolute zeta potential value of -17.7 mV. The emulsion stability is directly related to the magnitude of the surface charge. (figure 10)

SEM studies
The SEM data indicates spherical and uniform particles of optimized formulation DF10, that are slightly porous with rougher surfaces. The roughness of surface is due to quick moisture loss from wet mass possessing higher liquid content due to porous surface. (figures 11A and 11B)

Stability studies
The formulation DF10 is found stable for 6 months with no significant variations in the values of particle size, entrapment efficiency, drug release profile and drug content values (table 6).

CONCLUSION
Optimization of an SLN formulation is a complex process, which requires one to consider a large number of variables and their interactions with each other. The present study
conclusively demonstrates that the optimal formulations of dasatinib SLN contain 10% (w/v) of glycerol monostearate, 6% (w/v) poloxamer 407, and 2% tyloxapol using the Box-Behnken design. The derived polynomial equations and response surface plots aid in predicting the values of selected independent variables for preparation of optimum formulations with desired properties. From the results DF10 was found to be optimized formulation, which shown that the nanoparticle designed are spherical in shape with a mean particle size of 112 nm with PI of 0.40 and zeta potential of −25.6 mV. The in vitro release studies showed that more than 98.78% of drug was released from optimized SLN after 12 h, which is higher when compared with marketed formulation. The release kinetics of the optimized formulation followed zero order drug release and best fitted the Korsmeyer-peppas model. These results indicated that the dasatinib-loaded SLN formulation could potentially be exploited with controlled drug release.

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

REFERENCES

1. Badawi A, El-Laithy H, El Qidra R, El Mofty H, El dally M. Chitosan based nanocarriers for indomethacin ocular delivery. Arch Pharm Res 2008;31(8):1040–1049.
2. De Jong W, Born P. Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine 2008;3(2):133–149.
3. Üner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. Int J Nanomedicine. 2007;2(3):289–300.
4. Freitas C, Müller R. Correlation between long-term stability of solid lipid nanoparticles (SLN (TM)) and crystallinity of the lipid phase. Eur J Pharm Biopharm 1999;47(2):125–132.
5. Müller R, Rühl D, Runge S, Schulze-Forster K, Mehnert W. Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm Res 1997;14(4):458–462.
6. Huang Z, Hua S, Yang Y, Fang J. Development and evaluation of lipid nanoparticles for camptothecin delivery: a comparison of solid lipid nanoparticles, nanostructured lipid carriers, and lipid emulsion. Acta Pharmacologica Sinica 2008;29(9):1094–1102.
7. Müller R, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. Eur J Pharm Biopharm 2000;50(1):161–177.
8. Chang J, Huang Y, Hou S, Wang R, Wu P, Tsai Y. Formulation optimization of meloxicam sodium gel using response surface methodology. Int J Pharm 2007;338(1–2):48–54.
9. Liu C, Wu C, Fang J. Characterization and formulation optimization of solid lipid nanoparticles in vitamin K1 delivery. Drug Dev Ind Pharm 2010;36(7):751–761.
10. Arafath AA. Enhancement of Oral Bioavailability via Solid Lipid Nanoparticles of Anticancer Drug Dasatinib - An in Vitro Cytotoxicity and Pharmacokinetic Study. Asian J of Pharmac Clin Res 2019;12(6):143-5.
11. ArchanaNerella, BasavaRaju D, Aruna Devi M, Formulation, Optimization and In-vitro Characterization of Letrozole Load ed Solid Lipid Nanoparticles, Int J of Pharm Sci and Drug Res 2014; 6(3): 183-188.
12. Mehnert W, Mader K. Solid lipid nanoparticles: Production, characterization and applications. Adv. Drug Deliv Rev 2001;47:165-174.
13. Huang Z, Hua S, Yang Y, Fang J. Development and evaluation of lipid nanoparticles for camptothecin delivery: a comparison of solid lipid nanoparticles, nanostructured lipid carriers, and lipid emulsion. Acta Pharmacologica Sinica 2008;29(9):1094–1102.
14. Chang J, Huang Y, Hou S, Wang R, Wu P, Tsai Y. Formulation optimization of meloxicam sodium gel using response surface methodology, Int J Pharm 2007; 338(1–2):48–54.
15. Myers R, Montgomery D, Anderson-Cook C. Response surface methodology: process and product optimization using designed experiments. New York: John Wiley & Sons 2009; 73(1):25–33.
16. Rahman Z, Zidan A, Habib M, Khan M. Understanding the quality of protein loaded PLGA nanoparticles variability by Plackett-Burman design. Int J Pharm 2010; 389(1–2):186–194.
17. Schwarz C, Mehnert W, Luckw J, Müller R. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J Control Release 1994;50(1):83–96.
18. Yang C, Zhao X, Hu H. Preparation, Optimization and Characteristic of Huperzine a Loaded Nanostructured Lipid Carriers. Chem Pharm Bull. 2010; 58(5):656–661.
19. Kovacevic AB, Muller RH, Savic S. Solid lipid nanoparticles (SLN) stabilized with polyhydroxy surfactants: preparation, characterization and physical stability investigation. Colloid Surf A 2014; 444:15–25.
20. Chidambaram N, Porter W, Flood K, Qiu. Formulation and characterization of new layered diffusion matrices for zero-order sustained release. J Control Rel 1998;52: 149–158.
21. Bayomi MA. Geometric approach for zero-order release of drugs dispersed in an inert matrix. Pharm Res 1994;11: 914-916.
22. Panner S R, Kulkarni PK, Dixit M. Self Emulsifying Formula tion, Platform For Solubility Enhancement: A Review. IJCCR 2011;3(1):28-38.
23. Kumar DC, Madhab DK, Kumar PS. Lipid-Based Solid Dispersions of Azilsartan Medoxomil with Improved Oral Bioavailability: In Vitro and In Vivo Evaluation. Int J Cur Res Rev 2020;12 (19): 134-139.
Table 1: Composition of dasatinib SLNs formulation

| F. No | Dasatinib (mg) | Glycerol Monostearate (%) | Poloxamer 407 (%) | Tyloxapol (%) | Tween 80 (ml) | Chloroform: Methanol (1:1) | Distilled Water (mL) |
|-------|----------------|---------------------------|-------------------|---------------|---------------|-----------------------------|---------------------|
| DF1   | 100            | 6                         | 2                 | 2             | 0.5           | 20                          | Q. S                |
| DF2   | 100            | 10                        | 2                 | 2             | 0.5           | 20                          | Q. S                |
| DF3   | 100            | 6                         | 6                 | 2             | 0.5           | 20                          | Q. S                |
| DF4   | 100            | 8                         | 6                 | 1             | 0.5           | 20                          | Q. S                |
| DF5   | 100            | 6                         | 4                 | 1             | 0.5           | 20                          | Q. S                |
| DF6   | 100            | 10                        | 4                 | 1             | 0.5           | 20                          | Q. S                |
| DF7   | 100            | 6                         | 4                 | 3             | 0.5           | 20                          | Q. S                |
| DF8   | 100            | 10                        | 4                 | 3             | 0.5           | 20                          | Q. S                |
| DF9   | 100            | 8                         | 2                 | 1             | 0.5           | 20                          | Q. S                |
| DF10  | 100            | 10                        | 6                 | 2             | 0.5           | 20                          | Q. S                |
| DF11  | 100            | 8                         | 2                 | 3             | 0.5           | 20                          | Q. S                |
| DF12  | 100            | 8                         | 6                 | 3             | 0.5           | 20                          | Q. S                |
| DF13  | 100            | 10                        | 4                 | 2             | 0.5           | 20                          | Q. S                |
| DF14  | 100            | 8                         | 4                 | 1             | 0.5           | 20                          | Q. S                |
| DF15  | 100            | 8                         | 4                 | 6             | 0.5           | 20                          | Q. S                |
| DF16  | 100            | 6                         | 4                 | 2             | 0.5           | 20                          | Q. S                |
| DF17  | 100            | 8                         | 4                 | 2             | 0.5           | 20                          | Q. S                |

Table 2: List of dependent and independent variables in in Box-Behnken design

| Independent variables | Levels | Middle (o) | High (+1) |
|-----------------------|--------|------------|-----------|
| Variable               | Units  | Low (-1)   |           |
| A                     | %      | 6          | 8         | 10        |
| B                     | %      | 2          | 4         | 6         |
| C                     | %      | 1          | 2         | 3         |

| Dependent variable      | Goal   |
|-------------------------|--------|
| Y1                      | Minimize |
| Y2                      | Minimize |
| Y3                      | Maximize |

Table 3: Release kinetics of optimized formulation of Dasatinib SLNs (DF10) and marketed formulation

| Formulation | Correlation Coefficient ($r^2$) | Diffusional Exponent (n) | Inference               |
|-------------|---------------------------------|--------------------------|-------------------------|
| Marked      | Zero Order                      | First Order              | Higuchi Equation        | Korsmeyer -Peppas       | Korsmeyer -Peppas       | Zero-order & Super case II transport |
|             | 0.938                           | 0.854                    | 0.883                   | 0.929                   | 1.064                   |                                          |
| DF 10       | 0.975                           | 0.881                    | 0.914                   | 0.953                   | 1.241                   | Zero-order & Super case II transport    |
Table 4: Regression Equations of the fitted models

| Response                          | Equation                                                                 |
|-----------------------------------|--------------------------------------------------------------------------|
| Particle Size (Y1)                | $96.84X_1-9.32X_2-13.37X_3-28X_1^2+ 65X_2X_3 +45X_2^2-21X_3X_3 +53X_3^3$ |
| Entrapment Efficiency (Y2)        | $72.53+13.57X_1+1.65X_2+1.89X_3+0.54X_1^2-2.74X_2X_3-0.95X_2^2-2.50X_3X_3-3.44X_3^3$ |
| % Cumulative drug released (Y3)   | $79.75-4.52X_1+16.77X_2+14.39X_3+1.82X_1^2-13.91X_2X_3+4.14X_2^2-24.15X_3X_3+3.53X_3^3$ |

Table 5: Optimized values obtained by the constraints applies on Y1, Y2 and Y3

| Independent variable              | Nominal values | Predicted values | %CDR (%) | Batch | % Particle size (Y1) | % Entrapment Efficiency (Y2) | % Percent drug release in 12hrs (Y3) |
|-----------------------------------|----------------|-----------------|----------|-------|---------------------|----------------------------|----------------------------------|
| Amount of Glycerol Monostearate (A) | 10             | 112             | 96.41    | 1     | 113                 | 96.28                       | 98.47                            |
| Amount of Poloxamer 407 (B)       | 06             |                 |          | 2     | 112                 | 96.09                       | 98.24                            |
| Amount of Tyloxopol (C)           | 02             |                 |          | 3     | 114                 | 96.54                       | 98.18                            |

Table 6: Stability studies of optimized formulation

| Retest Time for Optimized formulation (DF10) | Particle Size (nm) | Entrapment Efficiency (%) | In-vitro drug release profile (%) | Drug content (%) |
|---------------------------------------------|--------------------|---------------------------|-----------------------------------|------------------|
| 0 days                                      | 112                | 96.41±1.17                | 98.78±1.06                        | 99.42±2.3        |
| 30 days                                     | 112                | 96.41±1.09                | 98.78±1.23                        | 99.42±2.9        |
| 60 days                                     | 112                | 96.39±1.15                | 98.72±1.15                        | 99.36±2.7        |
| 120 days                                    | 112                | 96.36±1.11                | 97.92±1.20                        | 99.24±1.4        |
| 180 days                                    | 112                | 96.27±1.05                | 97.68±1.13                        | 99.13±1.6        |

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

Figure 1: In-vitro drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF1-DF6.

Figure 2: In-vitro drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF7-DF12.
**Figure 3**: In-vitro drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF13-DF17.

**Figure 4A**: Response 3D surface plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on particle size fixed level of C.

**Figure 4B**: Contour plot showing the influence of amount of glycerol monostearate and amount of Poloxamer 407 on particle size fixed level of C.

**Figure 5A**: Response 3D surface plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on Entrapment Efficiency (%) fixed level of C.

**Figure 5B**: Contour plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on Entrapment Efficiency (%) fixed level of C.

**Figure 6A**: Response 3D surface plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on Cumulative % Drug Released fixed level of C.
Khan et al: Formulation and characterization of dasatinib loaded solid lipid nanoparticles by design of experiment

Figure 6B: Contour plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on Cumulative % Drug Released fixed level of C.

Figure 7: FTIR of pure drug.

Figure 8: FTIR of optimized formulation.

Figure 9: Particle size of optimized SLN.

Figure 10: Zeta potential of optimized formulation.

Figure 11A & 11B: SEM images of optimized formulation.