Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

# Formulation and *In-vivo* Evaluation of Dasatinib-loaded Solid Lipid Nanoparticles

**Baji H. Khan, Pamu Sandhya***

*Department of Pharmaceutics, Career Point University, Kota-325003, Rajasthan, India*

ARTICLE INFO

**Article history:**

Received: 02 October, 2022

Revised: 21 October, 2022

Accepted: 28 October, 2022

Published: 30 November, 2022

**Keywords:**

Box-behnken design, Dasatinib, *In-vivo* and *in-vitro* analysis, Solid lipid nanoparticle.

**DOI:**

10.25004/IJPSDR.2022.140621

ABSTRACT

The current research is aimed at formulation and *in-vivo* evaluation of dasatinib solid lipid nanoparticles (SLN) for enhanced solubility and bioavailability. A $3^3$ Box-Behnken design (BBD) was used to optimize the formulations in which amount of glycerol monosterate($X_1$) the amount of poloxamer 407 ($X_2$) and amount of tyloxopol ($X_3$) were chosen as independent variables while particle size, entrapment efficiency and drug release after 12 hours were chosen as dependent variables. About 17 SLN formulations of dasatinib were prepared using glyceryl monostearate, poloxamer 407, Tyloxopol and Tween 80 and optimised using desirability function. All the SLNs were evaluated for fourier transform infrared spectroscopy (FTIR), particle size, zeta potential, entrapment efficiency and drug release behavior were investigated. The *in-vivo* pharmacokinetic evaluation of opimised SLN was carried out in rats. Based on BBD and desirability function the formulation comprising of X1:10, X2:06 and X3:02 was chosen optimal. The model was proven to be validated since a fine agreement existed between the predicted and observed results. It can be seen that the experimental values were in very close agreement with the predicted values, indicating the success of the BBD combined with a desirability function for the evaluation and optimization of SLNs formulations. The formulation DF10 displayed 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 hours, which is higher when compared with marketed formulation. The release kinetics of the optimized formulation followed zero order drug release and best fitted the Korsemeyer-peppas model. At any time, point, the drug plasma concentrations in animals administrated with optimized SLN was higher than that of pure drug. $C_{max}$ of the dasatinib optimised SLN 158.26 ± 0.54 ng/mL was significant ($p < 0.05$) as compared to the pure drug suspension formulation 32.08 ± 0.21 ng/mL. $T_{max}$ of both optimised SLN formulation and pure drug was 1.0 ± 0.47 and 1.0 ± 0.04 hour, respectively $AUC_{0-\infty}$ infinity for dasatinib optimised SLN formulation was higher (702.23 ± 1.26 ng.h/mL) than the pure drug suspension 191.67 ± 1.27 ng.h/mL. Statistically, $AUC_{0-t}$ of the optimised SLN formulation was significantly higher ($p < 0.05$) as compared to pure drug suspension formulation. These results indicated that the dasatinib-loaded SLN formulation could potentially be exploited for the treatment of chronic myelogenous leukemia with controlled release manner.

## Introduction

The field of nanotechnology is rapidly developing, and solid lipid nanoparticles (SLN) have several potential applications in drug delivery, clinical medicine and research. The unique properties of lipid nanoparticles enable development of new therapeutics. As drugs can be incorporated into nanocarriers, a new prototype for drug delivery can be developed that could be used for secondary and tertiary drug targeting.[1-3] Due to their controlled and site-specific delivery capability, SLN have attracted much attention from researchers. An in-depth analysis of SLN is presented in this review, which discusses their benefits, limitations, and possible solutions. In addition to discussing SLN, nanostructured lipid carriers, and

***Corresponding Author:** Ms. Pamu Sandhya

**Address:** Department of Pharmaceutics, Career Point University, Kota-325003, Rajasthan, India

**Email** ✉: sandhya.mpharm2022@gmail.com

**Tel.:** +91-9849395823

**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

lipid drug conjugates, the different types of nanocarriers are also discussed. An overview of different methods for producing SLN that can be scaled up to large volumes is provided.[5]

SLNs combine the advantages and avoid the drawbacks of several colloidal carriers of its class. Potential disadvantages such as poor drug loading capacity, drug expulsion after polymeric transition during storage and relatively high water content of the dispersions (70–99.9%) have been observed. The drug loading capacity of conventional SLN is limited by the solubility of drug in the lipid melt, the structure of the lipid matrix and the polymeric state of the lipid matrix. If the lipid matrix consists of especially similar molecules (i.e, tristearin or tripalmitin), a perfect crystal with few imperfections is formed. Since incorporated drugs are located between fatty acid chains, between the lipid layers and also in crystal imperfections, a highly ordered crystal lattice cannot accommodate large amounts of drug. Therefore the use of more complex lipids is more sensible for higher drug loading.[6-9]

Dasatinib (Sprycel®) is approved in 2006 for IM-resistant CML treatment, represent a frontline therapy for patients.[10] Currently, 350 clinical trials (63 in recruitment) regarding dasatinib are focused on chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), hodgkin and non-hodgkin lymphoma, neck, head, breast, non-small cell lung cancer (NSCLC), melanoma, mesothelioma, ovarian, colorectal, glioblastoma and central nervous system (CNS) tumors. Dasatinib is an orally bioavailable synthetic small molecule-inhibitor of SRC-family protein-tyrosine kinases The aim of the present work is to formulate dasatinib nanoparticle in order to enhance the drug solubility and bioavailability by reducing the particle size using double emulsion solvent evaporation technique method.

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 (BBD).

## Materials and Methods

### Materials Used

The drug dasatinib was obtained from Hetero drugs Ltd, Hyderabad. The excipients glycerol monosterate, poloxamer407, tyloxopol were obtained from Rubicon research Pvt Ltd, Thane, Maharashtra. Tween 80 was purchased from SD Fine Ltd, Mumbaiand solvents chloroform, methanol were purchased from Merck Specialties Pvt Ltd, Mumbai, India.

### Preparation of Dasatinib-loaded SLN[11-13]

The SLNs were prepared by a hot emulsification/ultrasonication method using dasatinib(100 mg), glycerolmonosterate in a mixture of chloroform and methanol (1:1) (20 mL) as per the referred procedure. (Table 1).

### Experimental Design

A $3^3$ BBD employed for optimizing the main, interaction, and quadratic effects of formulation components on characteristics of self-nanoemulsifying drug delivery systems (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.[14]

The BBD matrix obtained using Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), the second-order quadratic equations are as:

$$Y = \beta_1 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2$$

Y-Level of the measured response

$\beta_0$–intercept

$\beta_1$ to $\beta_9$-regression coefficient

$X_1$, $X_2$, and $X_3$-main effects

$X_1X_2$, $X_2X_3$, and $X_1X_3$-interaction between the main effects $X_1^2$, $X_2^2$ and $X_3^2$-quadratic terms of independent variables.[14-16]

### Optimization Using the Desirability Functions

To optimize multiple responses, they should be highly correlated with each other. It is unlikely that the values desirable to optimize the effect of one response will have same effect on the second response, thus a conflict can occur between them. Hence, the most favorable compromising zone must be sought for each of the responses without any bias. In the present study, all three responses were simultaneously optimized by a desirability function that uses the numerical optimization method introduced by derringer and suich in the design-expert software (Version8.0, Stat-Ease Inc., Silicon valley, CA, USA). Recently, the desirability function approach was reported in several articles for the optimization of multiple responses.

### 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.[17,18]

#### *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°Cand 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.[19]

#### *Kinetic Model Fitting*

The drug release data was fitted into various linear models include zero order, Higuchi, Hixon-Crowell, Quadratic and

**Table 1:** Composition of dasatinib SLNs formulation

| *S. No* | *Dasatinib (mg)* | *Glycerol monosterate(%)* | *Poloxamer407 (%)* | *Tyloxopol (%)* | *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* | | |
|---|---|---|---|---|---|
| *Variable* | *Name* | *Units* | *Low (-1)* | *Middle (0)* | *High (+1)* |
| A | Amount of glycerol monosterate | % | 6 | 8 | 10 |
| B | Amount of poloxamer407 | % | 2 | 4 | 6 |
| C | Amount of tyloxopol | % | 1 | 2 | 3 |
| *Dependent variable* | | | *Goal* | | |
| Y1 | Particle size | nm | Minimize | | |
| Y2 | Entrapment efficiency | % | Minimize | | |
| Y3 | Drug release after 12 hours | % | Maximize | | |

polynomials, whereas the nonlinear models include first order, Weibull, KorsMeyer-Peppas, logistic etc.[20,21]

### *Characterization of Optimized SLN Formulation*

Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), particle size, zeta potential and stability studies were performed and reported according to reference procedures.[22]

### *Stability Studies*

Among all batches of dasatinib-loaded SLNs were subjected to immutability studies in accordance with guidelines of intracerebral brain hemorrhage (ICH) stability protocol. The test specifications include 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.

## Pharmacokinetic Studies of Dasatinib

### *Animal Preparation[23]*

Healthy wistar rats were (Weighing 180–200 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (temperature 25°C, relative humidity 45% and 12 hours alternate light and dark cycle) with 100% fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee (IAEC NO: 1477/PO/RE/S/11/CPCSEA-53/A).

### *Study Design[24]*

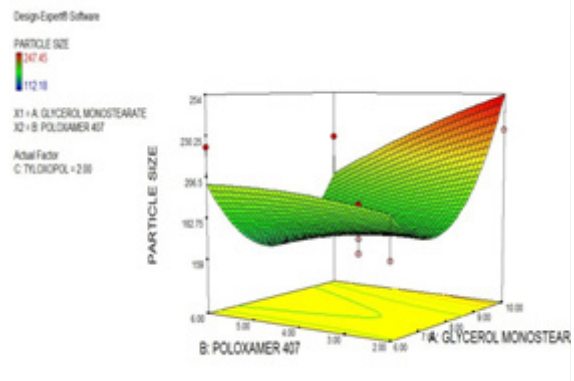
Rats were divided in to two groups at random. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group

**Table 3:** Optimized values obtained by the constraints applies on Y1, Y2 and Y3

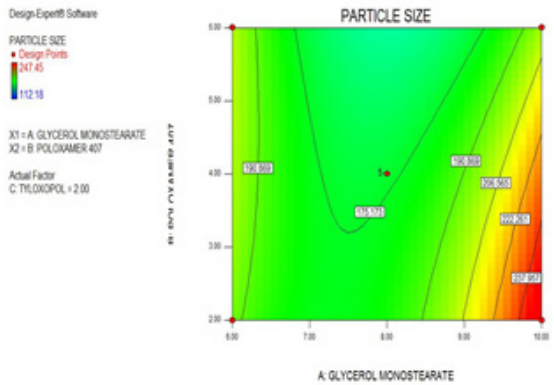
| *Independent variable* | *Nominal values %* | *Predicted values* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Particle size (Y1) (nm)* | *Entrapment Efficiency (%) (Y2)* | *%CDR (Y3)* | *Batch* | *Particle size (Y1) (nm)* | *Entrapment Efficiency (%) (Y2)* | *Percent drug release in 12hrs (Y3)* |
| Amount of Glycerol Monostearate (A) | 10 | 112 | 96.41 | 98.78 | 1 | 113 | 96.28 | 98.47 |
| Amount of Poloxamer407 (B) | 06 | | | | 2 | 112 | 96.09 | 98.24 |
| Amount of Tyloxopol (C) | 02 | | | | 3 | 114 | 96.54 | 98.18 |

**Table 4 :** Physico-chemical parameters of Dasatinib SLNs

| *S. No* | *#Content uniformity (%)* | *%Entrapment efficiency* | *Mean particle size (nm)* | *Zeta potential (mV)* |
|---|---|---|---|---|
| DF1 | 98.45 ± 1.26 | 88.96 ± 1.46 | 247.45 ± 4.23 | 25.25 ± 6.62 |
| DF2 | 97.33 ± 2.89 | 86.42 ± 1.57 | 188.63 ± 4.95 | 19.66 ± 5.61 |
| DF3 | 97.12 ± 2.43 | 79.89 ± 1.65 | 212.19 ± 8.91 | 25.41 ± 2.34 |
| DF4 | 96.18 ± 1.52 | 81.83 ± 2.36 | 175.54 ± 4.29 | 19.56 ± 4.18 |
| DF5 | 97.51 ± 2.16 | 87.77 ± 1.19 | 221.17 ± 3.53 | 26.47 ± 2.27 |
| DF6 | 98.73 ± 2.33 | 88.53 ± 1.54 | 224.60 ± 4.47 | 20.31 ± 2.29 |
| DF7 | 96.41 ± 2.67 | 83.67 ± 2.88 | 169.28 ± 5.12 | 21.85 ± 6.62 |
| DF8 | 96.55 ± 2.89 | 80.83 ± 1.72 | 210.04 ± 7.66 | 19.03 ± 5.61 |
| DF9 | 98.11 ± 2.53 | 84.79 ± 1.57 | 159.32 ± 4.23 | 25.77 ± 2.64 |
| DF10 | 99.42 ± 2.12 | 96.41 ± 2.35 | 112.18 ± 3.53 | 16.74 ± 2.25 |
| DF11 | 97.88 ± 2.65 | 79.54 ± 1.23 | 232.36 ± 2.52 | 27.95 ± 1.69 |
| DF12 | 96.23 ± 1.62 | 85.27 ± 2.91 | 195.78 ± 3.65 | 23.26 ± 2.14 |
| DF13 | 98.56 ± 1.89 | 80.32 ± .2.84 | 173.30 ± 6.24 | 17.82 ± 6.62 |
| DF14 | 97.01 ± 2.39 | 88.29 ± 1.70 | 223.92 ± 8.46 | 28.63 ± 6.11 |
| DF15 | 96.78 ± 2.17 | 86.27 ± 2.67 | 168.46 ± 4.23 | 22.78 ± 5.58 |
| DF16 | 97.45 ± 2.56 | 83.63 ± 1.42 | 222.37 ± 3.53 | 18.35 ± 4.48 |
| DF17 | 97.26 ± 2.13 | 82.82 ± 2.18 | 176.28 ± 4.37 | 27.12 ± 3.15 |




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




**Contour plot showing the influence of amount of glycerol monostearate and amount of Poloxamer 407 on particle size fixed level of C**

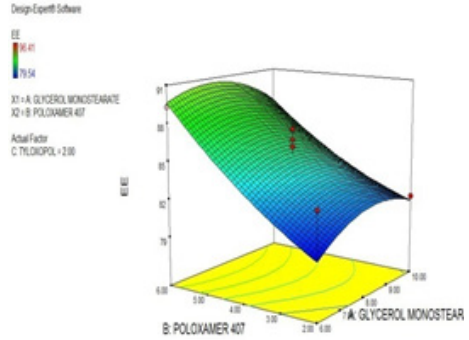
**Fig. 1:** Response surface and counter plots for particle size.

was administered with pure dasatinib (as such) made suspension with 0.5% methocel and second group was administered prepared dasatinib optimised SLN diluted in 0.5% methocel by oral route at a dose of 0.25 mg.

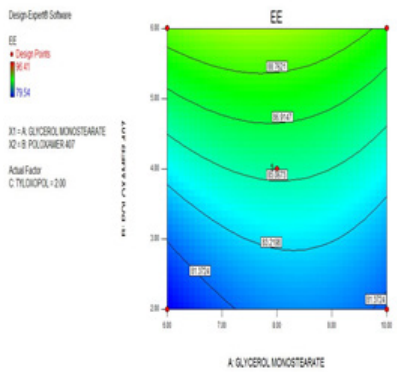
**Table 5:** Stability studies of optimized formulation

| *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)

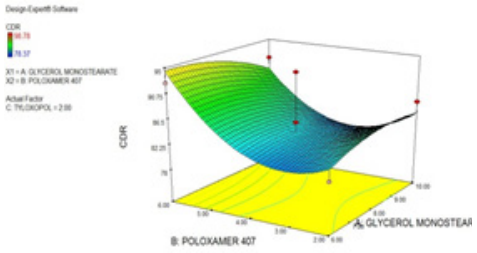



**Response 3D surface plot showing the influence of amount of Glycerol Monostearate and amount of Poloxamer 407 on Entrapment Efficiency (%) fixed level of C.**

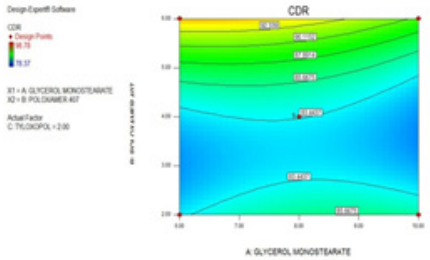



**Contour plot showing the influence of amount of amount of Glycerol Monostearate and amount of Poloxamer 407 on Entrapment Efficiency (%) fixed level of C.**

**Fig. 2:** Response surface and counter plots for Entrapment efficiency.




**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.**




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

**Fig . 3:** Response surface and counter plots for Cumulative % drug release

Then approximately 250 µL of blood samples were collected from retro-orbital plexus into heparinized 1.5 mL centrifuge tube at 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 hours post-dosing. All blood samples were centrifuged at 10,000 rpm (4°C) for 10 minutes, and the plasma samples were collected and stored at -20°C until analysis

## Determination of Dasatinib in Rat Plasma by high-pressure liquid chromatography (HPLC) Method[25,26]

### *Instrumentation*

The high-pressure liquid chromatographic (HPLC) system utilized was an agilent HPLC 1260 series with the GI311C quaternary pump, thermo scientific $C_{18}$ column (5 µm particle size X 4.6 × 250 mm) (made in the USA) and a diode array detector G1315D was utilized. Ezchrome elite software was used for chromatography data acquisition, processing, and control of HPLC chromatograph. Digital pH meter (systronics model-802), an electronic balance (Shimadzu TX223L), a sonicator (spectral lab, model UCB 40) and UV-visible spectrophotometer (systronics model-2203) were used in this study. Lapatinib was used as internal standard (IS), and separation took place at a flow rate of 0.2 mL/min. The column temperature was maintained at 20°C and the temperature of the auto sampler was maintained 4°C. The complete chromatographic run time of each sample was 10 minutes, which separated

**Table 6:** Pharmacokinetic Parameters of dasatinib optimised solid lipid nanoparticles formulation and pure drug

| *Pharmacokinetic parameters* | *Dasatinib pure drug* | *Dasatinib optimised solid lipid nanoparticle formulation* |
|---|---|---|
| $C_{max}$ (ng/mL) | 32.08 ± 0.21 | 158.26 ± 0.54 |
| $AUC_{0-t}$ (ng.h/mL) | 150.27 ± 1.58 | 582.21 ± 0.41 |
| $AUC_{0-\infty}$ (ng.h/mL) | 191.67 ± 1.27 | 702.23 ± 1.26 |
| $T_{max}$ (h) | 1.0 ± 0.04 | 1.0 ± 0.47 |
| $t_{1/2}$ (h) | 1.25 ± 0.08 | 1.02 ± 0.03 |

**Table 7:** Statistical analysis of PK parameters of Dasatinib.

| *PK parameters* | *Dasatinib pure drug vs. Dasatinib optimized solid lipid nanoparticle formulation* | |
|---|---|---|
| | Significant difference between means (P < 0.05) | P |
| $C_{max}$ | Yes | <0.0001 |
| $T_{max}$ | Yes | <0.0001 |
| $AUC_{(0-t)}$ | Yes | <0.0001 |
| $AUC_{0-\infty}$ | Yes | <0.0001 |
| $t_{½}$ | Yes | <0.0001 |

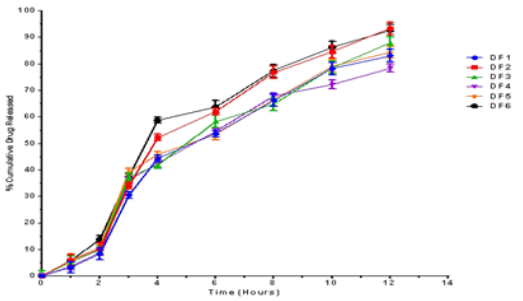



**Fig. 4:** *In-vitro* drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF1-DF6.

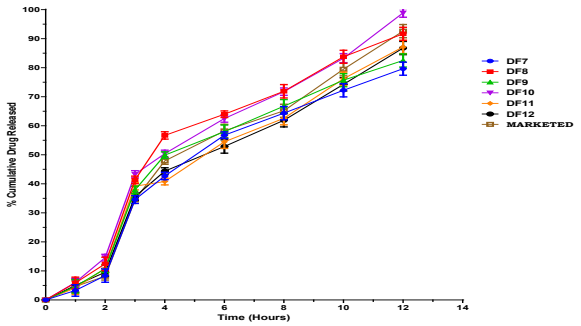



**Fig. 5:** *In-vitro* drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF7-DF12.

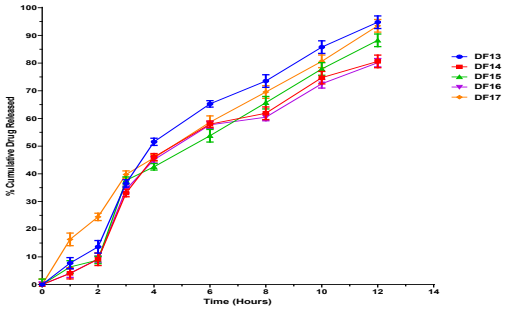



**Fig. 6:** *In-vitro* drug released profile of prepared dasatinib loaded solid lipid nanoparticles DF13-DF17.

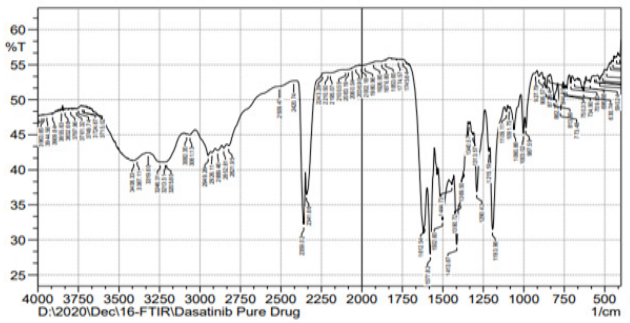



**Fig. 7:** FTIR of pure drug.

dasatinib and lapatinib from each other with retention times 2.5 and 5.4 minutes, respectively. Samples were analysed at wavelength of 322 nm.

### *Pharmacokinetic Analysis*[27]

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration ($C_{max}$), time to attain $C_{max}$ i.e., $T_{max}$ and $t_{½}$ values, area under plasma concentration–time curve from zero to the last sampling time ($AUC_{0-t}$), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$).

## Results And Discussion

A full factorial design ($3^3$) was employed for formulation batches (*i.e.,* 17 formulations)in which 3 factors namely lipid, surfactant and co-surfactant (glycerol monosterate, Poloxamer 407 and Tyloxopol) Tween 80 were tested at3 levels of their concentration *i.e.,* low, medium and high.

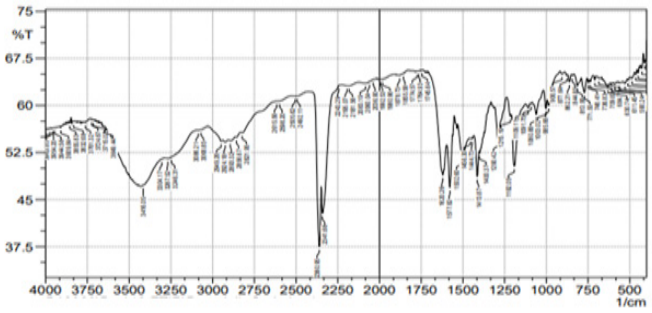



**Fig. 8:** FTIR of optimized formulation.

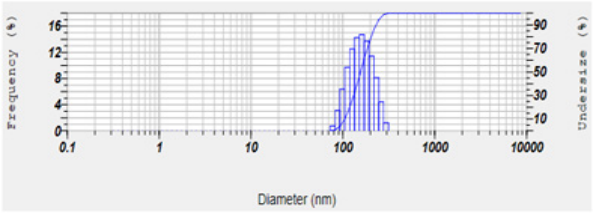



**Fig. 9:** Particle size of optimized SLN.

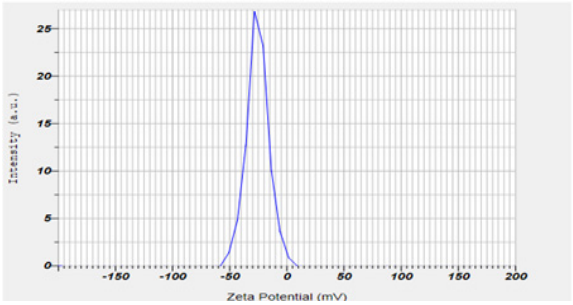
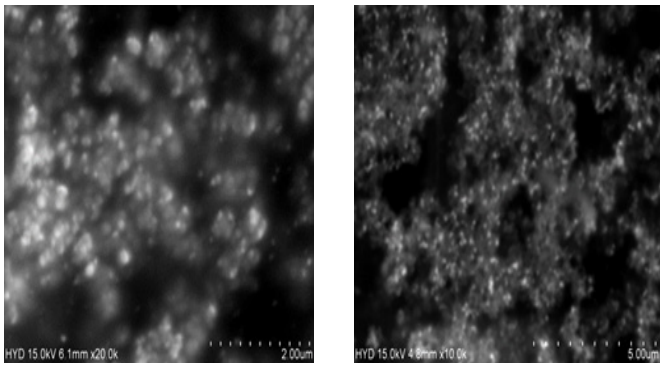



**Fig. 10:** Zeta potential of optimized formulation.



A B

**Fig. 11A & 11 B:** SEM images of optimized formulation.

The effect of different levels of factors was evaluated at the particle size of resultant formulation, entrapment efficiency and %cumulative drug released (*i.e.,* response). The composition of all formulation batches are given in Table 2.

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.

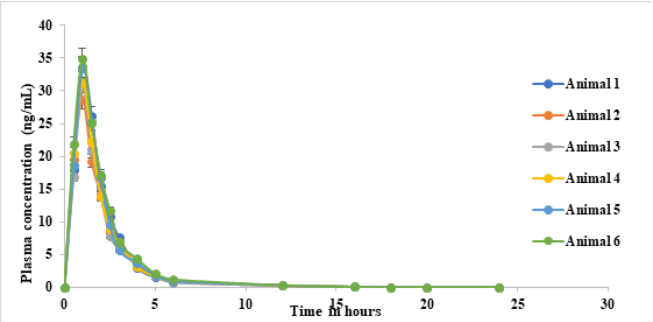



**Fig. 12A:** Plasma concentration-time profile of dasatinib pure drug in rat plasma.

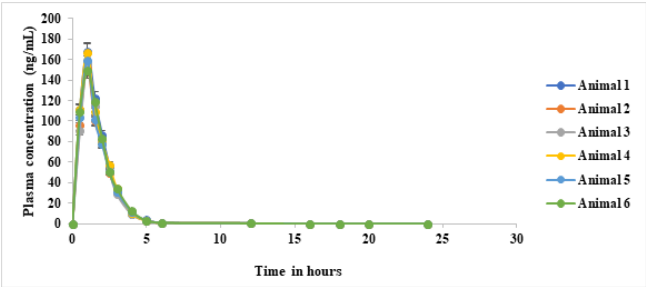



**Fig. 12B:** Plasma concentration-time profile of dasatinib optimized solid lipid nanoparticles in rat plasma.

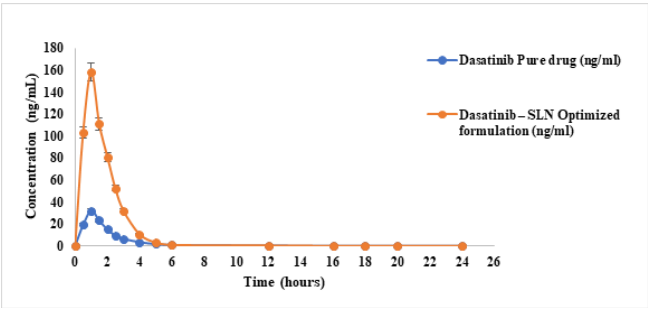



**Fig. 13:** Plasma concentration profiles of dasatinib optimised solid lipid nanoparticles and pure drug.

## Particle Size

**Y1=**$96+84X1-19X2-13X3-28X^2_1 + 65X_1X_3+45\ X^2_2-21\ X_2X_3 +53\ X^2_3$

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. The mathematical model generated for particle size (Y1) was found to be significant with f-value of 0.0175 implies the model is significant (Fig. 1 A and 1B).

## Entrapment Efficiency (%)

**Y2=**$72.53+13.37X1+6.15X2+1.89X3+0.54X^2_1 -2.74X_1X_3-09.55\ X^2_2 -2.50\ X_2X_3 -3.44\ X^2_3$

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) (Fig. 2).

## Cumulative %Drug Released

$$Y3 = 79.75 - 4.52X1 + 16.77X2 - 14.39X3 + 1.82X^2_1 - 13.91X_1X_3 + 4.14X^2_2 - 24.15X_2X_3 + 3.53X^2_3$$

The cumulative %drug release in 12 hours 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) (Fig. 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 3.

## Evaluation of SLN Formulations

The drug content uniformity of all formulations (DF1-DF17) ranges between 96 to 99% with DF10 displaying maximum of 99.42% drug uniformity. The entrapment efficiency ranged between 79–96% while the particle size and zeta potential values range between 112–247 nm and 16–27 mV, respectively.

## *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 hours (Fig. 4-6).

## 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 nonFickian (anomalous) transport thus it projected that delivered its active ingredient by coupled diffusion and erosion

## Characterization of SLN

### *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. (Figs 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 andzeta 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 (Fig. 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 (Fig. 10) (Table 4).

### *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 (Fig. 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 5).

### *Pharmacokinetic Data of Dasatinib*

Dasatinib concentrations in plasma following oral administration of pure drug and optimized dasatinib SLN formulation administered oral route are shown in plasma concentration-time curves are shown in Fig. 12A and 12B. Fig. 13 indicates plasma concentration–time curve recorded post single oral dose of dasatinib optimized SLN formulation in comparison to dasatinib pure drug suspension. At any time, point, the drug plasma concentrations in animals administrated with optimized solid lipid nanoparticles was higher than that of pure drug. $C_{max}$ of the dasatinib optimised SLN 158.26 ± 0.54 ng/mL was significant ($p < 0.05$) as compared to the pure drug suspension formulation 32.08 ± 0.21 ng/mL. $T_{max}$ of both optimised SLN formulation and pure drug was 1.0 ± 0.47 and 1.0 ± 0.04 hours, respectively. $AUC_{0-\infty}$ infinity for dasatinib optimised SLN formulation was higher (702.23 ± 1.26 ng.h/mL) than the pure drug suspension 191.67 ± 1.27 ng.h/mL. Statistically, $AUC_{0-t}$ of the optimised SLN formulation was significantly higher ($p < 0.05$) as compared to pure drug suspension formulation. (Table 6). The pharmacokinetic data was subjected to statistical analysis to test the significant differences between the pharmacokinetic parameters of two formulations. The results are shown in Table 7. The data indicated that there was significant difference in $C_{max}$, $T_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$,

between dasatinib pure drug and dasatinib optimised SLN formulation.

## 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% tyloxopolusing the BBD. 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 hours, which is higher when compared with marketed formulation. The release kinetics of the optimized formulation followed zero order drug release and best fitted the Korsemeyer-peppas model. These results indicated that the dasatinib-loaded SLN formulation could potentially be exploited with controlled drug release.

The indication of the enhanced oral bioavailability is evidenced from the AUC of the formulated SLNs and pure drug suspension. From the AUC parameter, about 3.50-fold enhancement in the bioavailability was observed in the SLN compared with pure drug suspension, which is statistically significant at a level of $p < 0.05$.

## 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, Borm P. Drug delivery and nanoparticles: applications and hazards. Int J Nanomed. 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 Nanomed. 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. Mohamed Yasir Arafath A, Jaykar. Enhancement Of Oral Bioavailability Via Solid Lipid Nanoparticles Of Anticancer Drug Dasatinib - An In-vitro Cytotoxicity And Pharmacokinetic Study. Asian J Pharm Clin Res. 2019; 6: 143-145.
7. Zhang L, Wang S, Zhang M, Sun J. Nanocarriers for oral drug delivery. J Drug Target. 2013;21:515-27.
8. Mehnert W, Mader K. Solid lipid nanoparticles production, characterization and applications. Adv Drug Deliv Rev. 2012;64:83-101.
9. Liu C, Wu C, Fang J. Characterization and formulation optimization of solid lipid nanoparticles in vitamin K1 delivery. Drug DevInd Pharm. 2010;36(7):751–761.
10. Arafath AA, MY. Enhancement of Oral Bioavailability via Solid Lipid Nanoparticles of Anticancer Drug Dasatinib - An In-vitro Cytotoxicity and Pharmacokinetic Study. Asian J of Pharma and Clinical Res. 2019;12(6): 143-5.
11. ArchanaNerella, Basava Raju D, Aruna Devi M. Formulation, Optimization and In-vitro Characterization of Letrozole Loaded 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 ofprotein loaded PLGA nanoparticles variability by Plackett-Burman design. Int J Pharm. 2010; 389(1–2):186–194.
17. Schwarz C, Mehnert W, Lucks J, Müller R. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J Cont. Release. 1994;30(1):83–96.
18. Yang C, Zhao X, Hu H, et al. Preparation, Optimization and Characteristic of Huperzinea Loaded Nanostructured Lipid Carriers. Chem Pharm Bull. 2010; 58(5):656–661.
19. Kovacevic AB, Muller RH, Savic SD.. Solid lipid nanoparticles (SLN) stabilized with polyhydroxy surfactants: preparation, characterization and physical stability investigation. Colloid Surf A. 2014;444:15–25.
20. Chidambaram NW, Porter K. Flood, Qiu Y. Formulation and characterization of new layered diffusion matrices for zero-order sustained release. J Cont Release. 1998, 52: 149-158.
21. Bayomi MA. Geometric approach for zero-order release of drugs dispersed in an inert matrix. Pharm Res. 11; 1994: 914-916.
22. Zhang Z, Gao F, Bu H, Xiao J.Solid lipid nanoparticles loading dasatinib cilexetil enhance oral bioavailability: In-vitro characteristics and absorption mechanism in rats. Nanomedicine. 2012; 8:740–747.
23. Zhang Z, Gao F, Bu H, Xiao J. Solid lipid nanoparticles loading Dasatinib cilexetil enhances oral bioavailability: In-vitro characteristics and absorption mechanism in rats. Nanomedicine 2012; 8:740–747
24. Narendar Dudhipala & Kishan Veerabrahma. Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: characterization, pharmacokinetic and pharmacodynamic evaluation. Drug Del. 2016; 23: 395-404.
25. Levêque, D, Becker G, Bilger K. Clinical Pharmacokinetics and Pharmacodynamics of Dasatinib. Clin Pharmacokinet. 2020; 59, 849–856.
26. Luo FR., Yang Z, Camuso A, Smykla R., McGlinchey K, Fager K. Dasatinib (BMS-354825) Pharmacokinetics and Pharmacodynamic Biomarkers in Animal Models Predict Optimal Clinical Exposure. Clinical Cancer Res. 2006;12(23):7180–7186.
27. Dudhipala N, Veerabrahma K. Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: Characterization, pharmacokinetic and pharmacodynamic evaluation. Drug Deliv. 2016;23:395-404. 12.


HOW TO CITE THIS ARTICLE: Khan BH, Sandhya P. Formulation and *In-vivo* Evaluation of Dasatinib-loaded Solid Lipid Nanoparticles. Int. J. Pharm. Sci. Drug Res. 2022;14(6):824-832. **DOI:** 10.25004/IJPSDR.2022.140621