Development and Optimization of Solid Lipid Nanoparticle Formulation for Enhanced Solubility of Ceritinib Using Box–Behnken Design

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Abstract

Background: Ceritinib is an anaplastic lymphoma kinase (ALK) inhibitor that exhibits low water solubility and poor drug compressibility hence depressed bioavailability. Objective: The objective of the current research is to develop ceritinib-loaded solid lipid nanoparticles (SLNs) for enhancing bioavailability. Materials and Methods: Box–Behnken design (BBD) was employed to optimize variables in the formulation process of ceritinib-loaded SLNs containing three factors and evaluated at three levels. The independent variables include the ratio of drug to lipid (A), concentration of glyceryl monostearate (B), and Poloxamer-188 concentration (C), whereas dependent variables were particle size (Y1) and entrapment efficiency (Y2). The SLNs prepared by single emulsification and solvent evaporation method. Three optimized formulations of ceritinib SLN prepared using the BBD and subjected for physicochemical characterization. Results: The formulation F1 with mean particle size (167.9 nm), polydispersity index (0.645), zeta potential (−24.9 ± 1.48mV), and % entrapment efficiency (90.24%) is chosen for further investigation. The scanning electron microscopy study confirms a spherical shape. Conclusion: The in vitro studies indicate a maximum drug release of 95.12% in 360 min for F1 which is much higher that of control (30.12% in 360 min). No significant difference (P < 0.05) in physicochemical properties was observed even after 90 days of stability studies.

Key words: Box–Behnken design, ceritinib, lymphoma kinase inhibitor, solubility

INTRODUCTION

Solid lipid nanoparticles (SLNs) are substitute to emulsions, liposomes, microparticles, and other drug-carrying systems since the early 1990s. Initially, nanoparticles were designed to carry vaccines and anticancer drug with the strategy of enhancing drug targeting further coating of nanoparticles with hydrophilic substances to reduce the intake of these nanoformulations by reticuloendothelial system cells.\(^1\)\(^2\) The use of hydrophilic substances such as poloxamers, polyvinyl alcohol, and polyethylene glycol further minimizes the non-specific interaction of drug with other proteins.\(^3\) Ceritinib is a small molecule drug belonging to the second-generation selective ALK inhibitors with activity 20 times more than crizotinib. Ceritinib is chemically 5-chloro-N4-[2-[(1-methylethyl)sulfonyl]phenyl]-N2-[5-methyl-2-(1-methylethoxy)-4-(4-piperidinyl)phenyl][2,4-pyrimidine diamine used for the treatment of positive lung cancer. Ceritinib is BCS Class IV drug exhibiting very low solubility of 0.02 mg/ml at room temperature along with low permeability making the drug difficult to formulate.\(^4\) Furthermore, picking and sticking problems are encountered during the formulation process of ceritinib due to its physical characteristics, thus resulting in poor compression. Ceritinib sticky nature causes a high drug load to further negatively influence the manufacturing of tablets due to enhanced sticking/picking. Clinical effectiveness of ceritinib on administration requires its delivery in a dosage form which would result in high bioavailability in addition to decreased variability within or in between subjects.

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In the current study, the ceritinib was formulated as SLNs for enhancement of its oral bioavailability, solubility, and protein-binding capacity. The formulation and process variables of these SLNs are optimized by statistically experimental design methodology followed by Box-Behnken design (BBD). Different parameters were evaluated on the optimized SLN formulations such as particle size analysis, polydispersity index (PDI), zeta potential (ZP), entrapment efficiency, drug loading capacity, and scanning electron microscopy (SEM) analysis.

EXPERIMENTATION

Materials

Ceritinib was gifted from Caplin Point Laboratories Limited, Bengaluru, India. Glyceryl monostearate (GMS), glyceryl tripalmitate, glyceryl tristearate, Compritol 888 ATO (glyceryl behenate), glyceryl palmitostearate, and dialysis tubing (molecular weight cutoff 12–14 kDa) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tween® 80 was a product from SD Fine Chem Ltd. (Mumbai, India). Soy lecithin, Poloxamer-188, and PVA were gift samples from Dr. Reddy’s Laboratories Ltd., India.

Instruments

Chemical analysis conducted using Fourier transform infrared (FTIR) spectrophotometer (Shimadzu FTIR 8400S, Japan), thermal analysis by PerkinElmer differential scanning calorimeter (DSC)/7 DSC (PerkinElmer, CT-USA), and PXRD on Bruker D8 Advance X-ray diffractometer. The SEM (JOEL SEM, Model 6400F, Japan) used for studying the morphology of the formulations.

Preliminary studies

Preliminary studies carried out to check the influence of dependent variables of SLNs (drug-to-lipid ratio [A], concentration of GMS [B], and Poloxamer-188 concentration [C]) on variables that were dependent (particle size [Y1] and entrapment efficiency [Y2]). The time and speed of homogenization, stirring speed, stirring time, and time of sonication were fixed during the study. Analysis of the three independent variables at low (−1), medium (0), and high (+1) levels was done. Stat-Ease Design-Expert® software V8.0.1 was employed to study the variables response. Conformational experiments carried out to verify the validity of experimental statistics.

Design of experiments (DOEs)

The BBD was used to optimize the formulation variables of ceritinib-loaded SLNs containing three factors and evaluated at three levels. The ratio of drug to lipid (A), concentration of GMS (B), and concentration of cosurfactant (Poloxamer-188, C) as independent variables, while particle size (Y1) and entrapment efficiency (Y2) were selected as dependent responses. Design of experiments (DOEs) The BBD was used to optimize the formulation variables of ceritinib-loaded SLNs containing three factors and evaluated at three levels. The ratio of drug to lipid (A), concentration of GMS (B), and concentration of cosurfactant (Poloxamer-188, C) as independent variables, while particle size (Y1) and entrapment efficiency (Y2) were selected as dependent responses [Table 1]. The experimentation designed using DOE software (Stat-Ease Design-Expert® software V8.0.1) by employing one-way analysis of variance (ANOVA) at 0.05 levels. F test was employed for the evaluation of each parameter with the use of multiple linear regression analysis in the generation of quadratic model for each response parameter of the form given below:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \]

| Independent variables | Name                      | Units            | Low    | Middle | High   |
|------------------------|---------------------------|------------------|--------|--------|--------|
| A                      | Drug-to-lipid ratio       | -                | 1:10   | 1:15   | 1:20   |
| B                      | Concentration of glyceryl monostearate | mg     | 50     | 75     | 100    |
| C                      | Concentration of Poloxamer-188 | mg     | 20     | 30     | 40     |

**Table 1: List of dependent and independent variables in Box–Behnken design**

| Dependent variable    | Goal          |
|-----------------------|---------------|
| Y1                    | Minimize      |
| Y2                    | Maximize      |
Pure error sum of square analysis was carried out by designing 17 formulations using the software with five center points. Among the linear, two-factor interaction and quadratic model experiments were performed for choosing the best model. Statistical significance as considered when obtained $P < 0.05$.

**Preparation of ceritinib SLN formulations**

Single emulsification and solvent evaporation method were employed for the preparation of SLNs of ceritinib. Ceritinib (100 mg), GMS, and soy lecithin taken into 10 ml volumetric flask and dissolved in 3 ml chloroform. The contents then added to 10 ml of 1.5% w/v of Poloxamer-188 solution. The dispersion was homogenized at 10,000 rpm for 6 min and sonicated for 15 min followed by stirring for 3 h at 1000 rpm. The obtained dispersion was again subjected to centrifugation for 45 min at 15,000 rpm. Nanoparticles were subjected to further purification after the obtained pellets were subjected to 3–4 times washing with Milli-Q.[9]

**Characterization of ceritinib-loaded SLNs**

Ceritinib SLNs formulation that was optimized was subjected to the evaluation of various physicochemical parameters such as size of the particle, entrapment efficiency, and percentage drug loading. Comparison of response values observed to the values predicted was performed.

**Measurement of ZP, PDI, and particle size**

ZP and size and PDI of the formulated SLNs were analyzed by Nano ZS90 Zetasizer (Malvern, Worcestershire, UK) by dilution of 100 ml formulation to about 5 ml by the use of distilled obtain 50–200 Kcps.[10-13]

**Entrapment efficiency and drug loading determination**

Ten milliliters of ceritinib-loaded SLN formulation centrifuged for 20 min at 10,000 rpm (Remi Instruments Pvt. Ltd., India). The lipid portion isolated followed by ultraviolet (UV) spectroscopic determination of drug amount in supernatant (Shimadzu 1800, Japan) at $\lambda_{\text{max}}$ 320 nm.

**FTIR spectroscopy and DSC characterization**

FTIR spectroscopy (FTIR-8400S Spectrophotometer, Shimadzu, Japan) study conducted to check the compatibility of drug with excipients. The samples prepared using KBr disk and scanned over range of 4000–400 cm$^{-1}$. The DSC thermogram recorded over PerkinElmer DSC/7 DSC (PerkinElmer, CT-USA).

**SEM**

Scanning electron microscope (SEM, Hitachi, Tokyo, Japan) was used for the study of particle surface morphology. The SLNs post freeze-drying was observed at 15,000 volts after dilution with water at 1:100 ratio and subsequent air drying.

**In vitro drug release evaluation**

**Drug release kinetic evaluation**

Elucidation of mechanism and mode of drug release was performed by fitting the dissolution data into different kinetic model equations such as zero-order, first-order, Korsmeyer–Peppas, and Higuchi’s model. The release data from the nanoformulation were determined by curve fitting method.

**Stability studies**

Evaluation of the stability of nanoparticles suspension of ceritinib was performed for 60 days by division of six samples contained in screw-capped glass vials into two groups followed by storage at 25°C and 4°C. At predefined intervals till 2 months, the drug leakage from samples and average particle size were evaluated.[14]

**RESULTS AND DISCUSSION**

**Optimization of formulation variables**

Determination of ceritinib solubility was performed in five varied lipids, glyceryl palmitostearate, GMS, glyceryl tripalmitate, glyceryl distearate, and glyceryl behenate. GMS, in which drug exhibited maximum solubility, was chosen as lipid part for experimentation carried further.[15-17] Most relevant surfactant was selected by the preparation of nanoparticles using lipid GMS and evaluating for size of particles, entrapment efficiency, and PDI. The specified three parameters were found to be best with Poloxamer-188 formulated suspensions that also have generally recognized as safe status and thus were chosen as relevant surfactant.[14,18]
Sodium taurocholate, soy lecithin, and glyceryl monostearate were tested as cosurfactants using fixed amount of surfactant (Poloxamer-188), by titration method with water. Nanoparticles with soy lecithin had decreased particle size with increased entrapment efficiency.

Effect of solvent volume on particle size and entrapment efficiency was observed. Experimentation of the same resulted in selection of 3 ml solvent volume which resulted in maximum entrapment efficiency.

**Experimental design optimization and response surface approach**

In total, 17 formulations by emulsification-solvent evaporation method were prepared and characterized for particle size and PDI. A significant effect of the ratio of drug to lipid (A), GMS concentration (B), and Polaxomer-188 concentration (C) on particle size (Y1) and entrapment efficiency (Y2) of formulated SLNs was observed. Thus, the study of these three variables A, B, and C at three varied levels low (−1), medium (0), and high (+1) was done by fixing speed of homogenization (10,000 rpm), homogenization time (6 min), time of sonication (5 min), and stirring speed (1000 rpm). Table 1 lists factors that are independent and variables that are dependent. Table 2 depicts obtained responses for dependent variable by conducting experiments as per design. These preliminary studies gave a conclusion that the ratio of drug to lipid (1:10–1:20), glyceryl monostearate concentration (50–100 mg), and Poloxamer-188 concentration (20–40 mg) are variables in formulation.

**DOE**

On the basis of BBD, designing of about 17 experiments was done, as shown in Table 2. Regression coefficients, regression equation, and ANOVA were obtained by analyzing the data by Stat-Ease Design-Expert® software V8.0.1. Table 2 shows multiple linear regressions analysis generated mathematical relationships of specified variables. These equations are a representation quantifiable effect of drug-to-lipid ratio (A), glyceryl monostearate concentration (B), and concentration of Poloxamer-188 (C) on particle size (Y1) and entrapment efficiency (Y2). Interaction terms and quadratic relationship are represented by one factor and higher factor order coefficients. Synergistic and antagonistic effects are indicated by positive and negative signs, respectively. Data fitting to quadratic model was done using a backward elimination process. Both the polynomial equations were significant statistically ($P \leq 0.01$), as determined using ANOVA, as per the provisions of Design-Expert software.

**Effects on particle size (Y1)**

The regression equations for the responses effect of particle size (Y1) are

$$162.08 + 112.37 A + 13.31 B + 12.47 C + 46.31 BC + 81.14 A^2 + 41.50 B^2 + 43.70C^2$$

Table 2 shows the nanoparticles particle size that ranged from 160.12 to 412.56 nm. The 3D response surface plots and

| Run | Factor A | Factor B | Factor C | Response Y1 | Response Y2 |
|-----|----------|----------|----------|-------------|-------------|
| 1   | 1:15     | 100      | 20       | 188.32      | 89.13       |
| 2   | 1:20     | 75       | 20       | 402.42      | 90.76       |
| 3   | 1:15     | 75       | 30       | 161.82      | 91.23       |
| 4   | 1:15     | 50       | 40       | 213.62      | 78.12       |
| 5   | 1:20     | 100      | 30       | 412.56      | 89.82       |
| 6   | 1:10     | 50       | 30       | 160.12      | 69.36       |
| 7   | 1:15     | 100      | 40       | 333.72      | 67.46       |
| 8   | 1:20     | 75       | 40       | 394.24      | 90.36       |
| 9   | 1:15     | 75       | 30       | 162.24      | 90.86       |
| 10  | 1:20     | 50       | 30       | 383.56      | 93.82       |
| 11  | 1:15     | 75       | 30       | 162.06      | 89.76       |
| 12  | 1:10     | 75       | 20       | 174.32      | 74.32       |
| 13  | 1:10     | 75       | 40       | 176.72      | 79.62       |
| 14  | 1:15     | 75       | 30       | 162.96      | 90.32       |
| 15  | 1:15     | 50       | 20       | 253.46      | 71.72       |
| 16  | 1:15     | 75       | 30       | 165.32      | 91.36       |
| 17  | 1:10     | 100      | 30       | 182.62      | 88.93       |
corresponding contour plots demonstrated the relationship between the variable that is dependent and independent in nature. B and C interactive effect on size of the particle (Y1) at fixed level A is demonstrated in Figure 1. An increase in Y1 values from 160.12 nm to 182.62 nm and from 383.56 nm to 412.56 nm is seen at low (drug-to-lipid ratio) and high levels of A.

**Effect on entrapment efficiency (Y2)**

The regression equation for the effect of entrapment efficiency (Y2) is

\[ 90.96 + 6.57 A + 2.79 B - 1.30 C - 5.89 AB - 7.02 BC - 6.03 B^2 - 7.75 C^2 \]

Table 2 depicts entrapment efficiency of nanoparticles that ranged between 67.46% and 93.82%.

Figure 2a shows that 3D response plots of Y2 response and interactive variables interactive effects on Y2 by keeping constant variable and varying the other two simultaneously in a particular range are depicted in Figure 2b. Extent and nature of interaction between different factors is revealed by shapes of response plots. A and B interaction on entrapment efficiency at C level fixed is depicted in Figure 2a; similarly, Figure 2b shows the B and C interaction at fixed level A. An increase in Y2 from 69.36% to 88.93% and from 89.82% to 93.82% was observed at low and high levels of A, respectively. Similarly, an increase in Y2 from 71.72% to 93.82% and from 67.46% to 89.82% was observed at high and low levels of B, respectively. On the other hand, a reduction in Y2 value from 90.76% to 71.72% at low levels of C and 90.36% to 67.46% at high levels of C was observed.

**Optimization experiments**

Table 3 depicts observed and predicted values of various parameters measured for optimized formulations. The obtained values of Y1 and Y2 were closer to predicted values. This stands as a demonstration of optimization procedure reliability in prediction of different parameters studied during the ceritinib nanoparticles formulation. The obtained three batches of formulation were subjected to further characterization.

Subjection of all optimized formulations that were prepared to particle size and distribution, entrapment efficiency, and ZP analysis was done, of which size distribution and ZP curve of optimized formulation are illustrated in Figures 3 and 4, respectively. An average of 167.9 ± 12.9–172.4 ± 9.9 nm as mean particle size and 89.46–90.24% as entrapment efficiency was observed for all formulations [Table 3]. A broad range of size distribution was indicated by PDI that ranged from 0.586 to 0.645. Inclusion of ceritinib in SLNs lipid matrix was indicated by the negative surface charge of prepared formulations that are a key regulating factor for particle stability. This ZP ranged between −0.6 ± 5.48 mV and −24.9 ± 2.89 mV for the prepared formulations [Table 4].

**Drug-excipient compatibility study by FTIR spectroscopy**

FTIR analysis has been carried out to test the lipid (GMS) and drug interaction. Drug purity is confirmed by the presence of
Table 3: Predicted and observed values for constraints applies on Y1 and Y2

| Independent variable                  | Nominal values | Predicted values | Observed values |
|---------------------------------------|----------------|-----------------|-----------------|
|                                       |                | Y1              | Y2              | Batch | Y1       | Y2       |
| Drug-lipid ratio (A)                  | 1:12.5         | 160.12          | 91.35           | 1     | 167.9    | 90.24    |
| Conc. of glyceryl monostearate (B)    | 82.17          |                 |                 | 2     | 172.4    | 89.46    |
| Conc. of Poloxamer-188 (C)            | 27.69          |                 |                 | 3     | 169.6    | 89.72    |

Table 4: The mean particle size, PDI, zeta potential, entrapment efficiency, and % drug loading of optimized formulations

| Batch | MPS±SD (nm) | PDI | ZP±SD (mV) | % EE±SD      |
|-------|-------------|-----|------------|--------------|
| 1     | 167.9±12.9  | 0.645 | −24.9±1.48 | 90.24±0.28   |
| 2     | 172.4±9.9   | 0.629 | −6.0±3.22  | 89.46±0.17   |
| 3     | 169.6±3.8   | 0.586 | −7.2±3.89  | 89.72±0.42   |

n=3 (P<0.05). PDI: Polydispersity index

Figure 3: Polydispersity index of ceritinib nanoparticles (F1-F3)

major peaks at 3416 cm⁻¹, 3310 cm⁻¹, 2798 cm⁻¹, 1065 cm⁻¹, 1022 cm⁻¹, 1009 cm⁻¹, 942 cm⁻¹, 885 cm⁻¹, 863 cm⁻¹, 769 cm⁻¹, and 678 cm⁻¹, as illustrated in Figure 5, in FTIR analysis of pure drug. The presence of peaks at same wavenumbers was
Figure 4: Zeta potential of ceritinib nanoparticles of F1-F3

also found in the spectra of physical mixture of ceritinib, GMS, Poloxamer-188, soy lecithin, and physical mixture, as shown in Figure 6. In addition, the spectra also showed few additional peaks that might be characteristic of lipid functional groups.

Differential scanning calorimetry

Figure 7 illustrates thermal profile of the drug by DSC. The melting point of the drug is indicated by a sharp endothermic peak with high symmetricity at 179.1°C in the profile of pure drug. Two peaks endothermic in nature present at 65°C and 178°C are lipid and drug peaks, respectively. A possibility of two processes of degradation of ceritinib can be indicated by asymmetry in the drug’s characteristic peak along with a near to vertical line after peak maximum.

Surface morphology analysis

The SEM data indicated a spherical shape for ceritinib nanoparticles with uniform and relatively narrow particle
Figure 5: Fourier transform infrared of ceritinib drug

Figure 6: Fourier transform infrared spectrum of ceritinib physical mixture

Figure 7: Differential scanning calorimeter thermogram of ceritinib, glyceryl monostearate, soy lecithin, Poloxamer-188, and physical mixture
Table 5: Particle size and entrapment efficiency of ceritinib nanoparticles after 90 days of storage at refrigerated and room temperature

| Temperature (°C) | Particle size (nm) | Entrapment efficiency (%) | Release data (% CDR) |
|------------------|--------------------|---------------------------|----------------------|
|                  | 0 month 3 months   | 0 month 3 months          | 0 month 3 months     |
| 4±1°C            | 167±9±12.9         | 90.24±0.28                | 24.13±0.52           |
| 25±2°C           | 172±12±9.13        | 90.24±0.28                | 31.18±0.82           |
| n=3 (P<0.05)     | 167±9±12.9         | 90.24±0.28                | 39.12±1.15           |

Figure 8: Scanning electron microscopy images of ceritinib nanoparticles

Figure 9: *In vitro* release of ceritinib from nanoparticles

distribution [Figure 8]. Spherical particles could be uptake taken easily when compared with disfigured ones. Therefore, it is speculated that the mean particle size that was obtained using the laser diffraction method belongs to the agglomerated nanoparticles.

**In vitro drug release study**

Figure 9 illustrates SLN formulated with ceritinib dissolution profile which was about 94.91–95.12% at 6 h end, whereas 30.12% release was found in case of pure drug suspension which can be due to hydrophobicity of the drug that results in decreased solubility which is enhanced in case of nanoparticles formulation.

**Release order kinetics**

Drug release mechanism and order were found by fitting the *in vitro* release data of optimized formulation (F1) in different kinetic equations such as first-order, zero-order, Korsmeyer–Peppas, and Higuchi plots. Rapid initial drug release was indicated in the first-order plot whose regression coefficient was close to unity (dose-dependent kinetics). Zero-order plot gave a clear indication of decreased linearity. The use of mathematical models such as Korsmeyer–Peppas and Higuchi plots to get information of mechanism of release was also made. Value of n equal to 0.83847 in Korsmeyer–Peppas plots indicated non-Fickian (anomalous) diffusion with drug release by erosion and coupled diffusion.
Stability study

Stability tests indicate changes in drug substance or product’s quality with time brought about by varied environmental factors such as light, temperature, and humidity. Insignificant difference in particle size and entrapment efficiency ($P < 0.05$) for formulation optimized when stored at 20–25°C and refrigerated conditions was noted, as indicated in Table 5.

CONCLUSION

The present research demonstrated the use of a three-factor, three-level BBD, for optimizing variables of formulation for ceritinib SLNs nanoparticles preparation. The polynomial equation that was derived in conjunction with response surface plots helps in prediction of selected independent variables values that, in turn, aid in preparation of optimum formulations having desired properties. The independent variables include ratio of drug to lipid (A), concentration of GMS (B), and concentration of cosurfactant (Poloxamer-188, C), while particle size (Y1) and entrapment efficiency (Y2) were designated as dependent variables. The SLNs prepared by single emulsification-solvent evaporation method were evaluated for least particle size with highest entrapment efficiency. Observed response value of the optimized formulation was comparable with predicted value. The optimized batches were further investigated by FTIR, DSC, and SEM studies that indicated spherical morphology with no significant interaction of drug with the excipients. Significant enhancement of dissolution was observed with the nanoformulation of ceritinib indicating better therapeutically effects than the conventional oral formulations.

REFERENCES

1. Rane SS, Anderson BD. What determines drug solubility in lipid vehicles: Is it predictable? Adv Drug Deliv Rev 2008;60:638-56.
2. Bargoni A, Cavalli R, Zara GP, Fundarò A, Caputo O, Gasco MR. Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II--tissue distribution. Pharmacol Res 2001;43:497-502.
3. De Rossi E, Ainsa JA, Riccardi G. Role of mycobacterial efflux transporters in drug resistance: An unresolved question. FEMS Microbiol Rev 2006;30:36-52.
4. Chakraborty S, Shukla D, Mishra B, Singh S. Lipid-an emerging platform for oral delivery of drugs with poor bioavailability. Eur J Pharm Biopharm 2009;73:1-5.
5. Nagar M, Panwar KS, Chopra VS, Bala I, Trivedi P. Quality by design: A systematic approach to pharmaceutical development. Der Pharm Lett 2012;2:111-30.
6. Kakodka SS, Gajare P. Pharmaceutical quality-by-design (QbD): Basic principles. Int J Res Methodol 2015;1:1-25.
7. Roy RA. Primer on the Taguchi Method. New York: Van Nostrand Reinhold; 1990.
8. Myers RH, Montgomery DC. Response Surface Methodology: Product and Process Optimization Using Designed Experiments. 2nd ed. New York: John Wiley and Sons; 2002.
9. Pooja D, Kulhari H, Tunki L, Chinde S, Kuncha M, Grover P, et al. Nanomedicines for targeted delivery of etoposide to non-small cell lung cancer using transferrin functionalized nanoparticles. RSC Adv 2015;5:49122-31.
10. Rajendra C, Harish P, Jain AK, Himes HH, Saraogi GK. Preparation and evaluation of the self emulsifying drug delivery system containing atorvastatin HMG-COA inhibitor. Int J Pharm Pharm Sci 2011;3:147-52.
11. Nekkanti V, Wang Z, Betageri GV. Pharmacokinetic evaluation of improved oral bioavailability of valsartan: Proliposomes versus self-nanoemulsifying drug delivery system. AAPS PharmSciTech 2016;17:851-62.
12. Ruan G, Feng SS. Preparation and characterization of polylactide-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. Biomaterials 2003;24:5037-44.
13. Ke Z, Hou X, Jia XB. Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. Drug Des Devel Ther 2016;10:2049-60.
14. Lalit KT, Mohan LK. Stability study and in vivo evaluation of lornoxicam loaded ethyl cellulose microspheres. Int J Pharm Sci Drug Res 2014;6:26-30.
15. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci 2006;29:278-87.
16. Bondi ML, Di Gesù R, Craparo EF. Lipid nanoparticles for drug targeting to the brain. Methods Enzymol 2012;508:229-51.
17. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release 2004;95:627-38.
18. Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies. Adv Drug Deliv Rev 2008;60:625-37.

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