An impact of nanocrystals on dissolution rate of Lercanidipine: Supersaturation and crystallization by addition of solvent to antisolvent

Dolly Tulsibhai Gadhiya1*, Jayvadan K. Patel2 and Arji Arjanbhai Bagada1

Abstract

Background: Nanocrystals of any drug are pure solid drug particles with a mean diameter in nanometer range. Dissolution is a crucial factor for absorption of medicine in case of water-insoluble or poorly soluble drugs. The aim of this study was to develop nanocrystals of a hydrophobic drug, Lercanidipine, by addition of solvent to an antisolvent with high-speed homogenization to achieve dissolution and solubility enhancement. Addition of organic solvent to antisolvent results in genesis of nanosized particles due to fast nucleation process and rapid mixing. The nanosuspension was formulated using PVP K30 as a stabilizer. Further, nanosuspensions were lyophilized to convert into solid nanocrystals using mannitol as a cryoprotectant. The developed nanosuspensions were characterized for particle size, zeta potential, saturation solubility, and in vitro dissolution studies. Lyophilized solid nanocrystals were characterized for FTIR, SEM, XRD, and zeta potential ($\zeta$).

Results: Central composite design was executed to study influence of amount of stabilizer and solvent to antisolvent ratio (independent variables) on particle size and % drug release at 10 min (dependent variables). The particle size of the developed Lercanidipine nanosuspensions were observed in the range of 302.00 ± 10.58 to 484.33 ± 6.51 nm measured by Zetatrac. A considerable increase was found in the solubility and dissolution rate of the nanocrystals as compared to pure drug. The drug release from Lercanidipine nanosuspensions was increased up to 88.95% within 10 min as compared to pure Lercanidipine which was only 21.53%. The X-ray diffraction study of lyophilized nanocrystals showed sharp and distinct peaks due to increase in crystallinity of Lercanidipine. Particle morphology was studied by scanning electron microscopy revealed that nanoprecipitated particles with lyophilization in the presence of mannitol exhibited dendrite needle-like crystals.

Conclusion: The nanocrystal development by antisolvent precipitation procedure using methanol as solvent, water as antisolvent, and low amounts of PVP K30 as stabilizer is a very promising and effective method to increase the dissolution rate of Lercanidipine.

Keywords: Lercanidipine, SAS ratio, Nanocrystals, Central composite design, Dissolution enhancement, Nucleation process, Supersaturation

* Correspondence: dollygadhiya2012@gmail.com
1Department of Pharmaceutical Sciences, Saurashtra University, University Road, Rajkot, Gujarat 360 005, India
Full list of author information is available at the end of the article

© The Author(s). 2021 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.
Background
Crystal engineering has been the center of attention for many researchers to improve physicochemical properties of drug during the past few years. In crystal engineering, nanocrystals approach denotes advantages such as high drug loading capacity, enhanced stability, and less toxicity in comparison to other approaches. Nanocrystal is developed by two concepts: top-down or bottom-up techniques [1]. Top-down technology involves the mechanical grinding down of coarse drug powder to make it refined by employing media milling with zirconium beads and high-pressure homogenization [2]. Bottom-up technologies fabricate nanocrystals using precipitation method. Anti-solvent precipitation is simple, cost effective and promising approach which involves steps like dissolution, nucleation, solution diffusion, and particle growth. The patented techniques of nanocrystals are available in market instanced by Dis-socubes®, Nanopure®, Nanocrystlas®, Nanomill®, and Dynamill® [3]. Formulation of poorly soluble drugs is a difficult task in pharmaceutical field, especially for those compounds which are poorly soluble in an aqueous and organic medium [4]. The poor solubility of drugs in aqueous medium resulting in reduced oral bioavailability is for all time drawback in pharmaceutical dosage form. Solubility and dissolution rate are rate-limiting steps for bioavailability and absorption of drug. There are lots of techniques that have been used to augment dissolution rate or solubility of poorly water-soluble drugs.

Lercanidipine hydrochloride (LER) belongs to chemical class 1,4-dihydropyridine. It is used as an antihypertensive and antianginal agent. It acts by blocking the calcium channels of smooth muscles. This results in peripheral vasodilatation and reduction in blood pressure [5]. Lercanidipine hydrochloride is from BCS class II, which is practically insoluble in water and its lipophilicity is high. Because of high lipophilicity and low solubility, Lercanidipine hydrochloride shows only 10% of oral bioavailability [6].

Different techniques have been applied to encounter the issues associated with poorly water-soluble drugs. The implement of solid dispersion technique [7], liquisolid technique [8], co crystallization [9], and microcrystals preparation [10] are some examples which put an effort to improve solubility and dissolution of poorly soluble drugs. In the present study, the antisolvent precipitation approach has been applied to formulate Lercanidipine nanocrystals (LER NCs) with the aim to improve its solubility and dissolution properties which further improve its oral bioavailability.

This investigation aimed to prepare and study impact of nanocrystals on dissolution behavior of Lercanidipine by antisolvent nanoprecipitation method. Quality by design (QbD) is a proficient way to deal with design creation configuration and convey any drug item with predefined item specifications [11]. In the present study, central composite design (CCD) was used for optimization of formulation variable after preliminary study. It is one of the competent designs to study the effect of independent variables on properties of formulation with minimum experiment runs [12].

Methods
Materials
Lercanidipine was obtained as a gift sample from Alembic Pharmaceuticals, Gujarat, India. Methanol used as a solvent was of analytical grade obtained from Merck specialities Pvt. Ltd., Mumbai, India. Polyvinyl pyrrolidone K30 (PVP K30) was used as a polymer and mannitol was used as a cryoprotectant were supplied by S. D. Fine Chemical Ltd., Mumbai, India. All other chemicals like Poly vinyl alcohol (Himedia Laboratories (P) Ltd. Mumbai, India), Poloxamer 188 & 407 (BASF India Ltd., Mumbai, India), HPMC 3LV and 6LV (Yarrow Chemical Ltd, Mumbai, India) were obtained as a gift sample. Other solvents like acetonitrile, dimethyl formamide, and dimethyl sulfoxide were obtained from Merck specialities Pvt. Ltd., Mumbai, India. Distilled water was obtained from an Ultrapure water system (Milli-Q, Merck).

Solubility studies of Lercanidipine (solvent selection)
The solvent selection was carried out on the basis of their ability to dissolve a maximum drug. Various solvents like methanol, acetonitrile, dimethyl formamide, and dimethyl sulfoxide were screened on solubility basis using the “shake-flask method.” An excess quantity of Lercanidipine was added to 5 ml of each solvent in a test tube and sonicated for 5 min using Ultra sonicator (Remi instruments, India) in order to facilitate the mixing of drug with vehicle. Then test tubes containing mixtures are shaken for 3 days on Shaker Incubator (Tempo Instrument & equipment, India). Shaker temperature maintained at 37 °C. After that, each of the test tubes was centrifuged using ultracentrifuge at 10,000 rpm for 15 min. The supernatant of each sample was collected and filtered using a membrane filter (0.45 μm, Whatman). The samples were diluted in volumetric flask and estimated using UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against blank. The study was conducted in triplicate and their mean values were reported [13].

Preliminary screening of critical parameters and their ranges
To achieve nanosized particles, set of experiments were carried out by trying different stabilizer (PVA Cold, Poloxamer 188, Poloxamer 407, PVP K30, HPMC 3LV, HPMC 6LV), concentration of stabilizer (0.125, 0.25,
0.50, 0.75, 1.0% w/v), concentration of drug (10, 20, 30, 40 mg/ml), stirring speed (5000 and 10000 rpm), and solvent–anti solvent (SAS) ratio (1:10, 1:20, 1:30, 1:40) by keeping all parameters constant against any one parameter. The levels of parameters were selected on the basis of average particle size and polydispersity index (PDI) of the Lercanidipine nanosuspension.

Preparation of Lercanidipine nanocrystals

The LER nanosuspensions were prepared using SAS precipitation technique with high-speed homogenization [11, 14, 15]. A known quantity of LER was completely dissolved in solvent (methanol) which was completely water miscible having concentration 10 mg/ml and then sonicated (Jain Scientific, India) for 30 s. The solution was filtered through a 0.45 μm Whatman filter paper to remove impurities if any. Solution of PVP K30 (0.5% w/v) was prepared in water which act as an antisolvent. Then, drug solution was injected by syringe (needle size 0.55 × 25 mm of 24 gauges) into stabilizer solution which was placed on high-speed homogenizer (Omni PDH, Omni International, USA) with stirring speed 10,000 rpm for 10 min. Precipitation of drug particles occurred immediately upon addition of LER solution in stabilizer solution (antisolvent) and formed a suspension with a yellowish color appearance. The prepared suspension was then centrifuged at 10,000 rpm for 10 min under cool condition. A supernatant portion was removed and the solid was lyophilized by adding 0.5% w/v mannitol as cryoprotectant and stored until further studies.

Optimization using central composite design

A central composite design, a type of response surface methodology, was used to statistically optimize critical factors and to estimate main interaction and quadratic effects of factors on critical quality attribute of Lercanidipine loaded nanosuspension [16]. Based on the results obtained from preliminary studies, two critical parameters, polymer concentration (X1) and SAS ratio (X2), were taken as independent variables and their levels were suitably coded as per Table 1. Particle size and % cumulative drug release in 10 min (CDR10) were taken as the response variables. The design consists of total CCD1–CCD9 experimental runs which included 4 factorial points, 4 star points, and 1 center point as shown in Table 1; designed and analyzed by the statistical software Design Expert® 12 (Stat-Ease Inc., USA). The data obtained from experimental batches were employed to construct surface response plot. The batch size (5 ml), drug concentration (2% w/v), PVP K30 as stabilizer, and homogenizer speed (10,000 rpm) were kept constant in the experimental batches. All 9 batches of drug-loaded nanosuspension was formulated in triplicate in order to estimate reproducibility of the model. A second-order polynomial Eq. (1) was successfully used to evaluate the responses against independent variable. The polynomial equation with coded variables and coefficients can be written as below:

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2
\]

where Y is dependent variable(responses), \(\beta_0\) is arithmetic mean of nine runs, and \(\beta_1\) is the estimated coefficient for relative factor \((X_1, X_2,...)\). The main effects \((X_1, X_2)\) signify average result of altering one factor at a time from its lowest to highest value, whereas the interaction terms \((X_1X_2)\) indicates change in responses when two factors were simultaneously altered. The polynomial terms \((X_1^2, X_2^2)\) were added to investigate non-linearity of the model. Data obtained were further analyzed by Microsoft Excel® for regression analysis. Analysis of variance (ANOVA) study was used to assure nonsignificant difference. Contour and response surface plots were generated to study response variations against independent variables using Design Expert® 12 (Stat-Ease Inc., USA) software. Pareto chart were constructed using Minitab® statistical software. The percentage relative error of each response was calculated using the following equation in order to decide validity of the model.

\[
\text{%Relative error} = \frac{|\text{Predicted value–Actual value}|}{\text{Actual value}} \times 100
\]

Characterization of LER nanosuspension

Particle size and polydispersibility index

The particle size and size distribution was performed using Zetatrack (Microtrac Inc. USA). Each sample was analyzed in triplicate and results were reported. PDI value indicates heterogeneity of particle size in dispersion medium.

Zeta potential (ζ)

Zetatrack measures the particle size based on dynamic light scattering principle [17]. In a constant electric field, particle moves from cathode to anode which is internally fitted in sample holder, at a constant velocity. The charge and zeta potential of suspended particles were determined by velocity of particle in electric field. The charged particles were oscillate with high frequency in generated alternate current filed. This phenomenon result in a measurement of zeta potential of particles.

Saturation solubility studies

Excess amount of lyophilized nanosuspensions were added to distilled water and placed into 10 ml capped glass vial to avoid any changes due to evaporation and
subjected for solubility study. Then glass vial containing mixtures was shaken for 3 days on Shaker Incubator (Tempo Instrument & equipment, India). Shaker temperature maintained at 37 °C. After that, sample was centrifuged in ultracentrifuge and supernatant was collected and filter through 0.45-μm syringe filter. The filtered samples were analyzed using UV-Visible spectrophotometer at λ_max 236 nm after appropriate dilutions. Experiments were carried out in triplicate and mean and standard deviation was calculated [18].

**In vitro dissolution studies**
Dissolution studies were carried out in 900 ml 0.01 N HCl (pH 2.4) at 37 °C at 50 rpm (Electrolab Dissolution Tester TDT-06P, Mumbai, India) by USP type II Paddle method [12, 13, 17]. Twenty milligrams of pure LER and its equivalent formulations were added to dissolution medium and 5 ml of sample was withdrawn at time interval of 5, 10, 15, 30, 45, and 60 min. The volume of dissolution fluid adjusted to 900 ml by adding fresh 5 ml of dissolution medium after every sampling with pipette. The solutions were filtered with Whatman filter paper (0.22 μm) and read against blank.

**Percentage drug content estimation**
The drug content was determined by dissolving equivalent (~20 mg) quantity of LER nanosuspension in methanol. The solution was stirred sufficiently to dissolve the drug and centrifuged at 5000 rpm for 15 min. The supernatant was collected, diluted, and measured at 236 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against blank. The drug content was performed in triplicate and mean of results were reported in Table 2.

**Lyophilization (conversion to solid state)**
An optimized Lercanidipine nanosuspension was lyophilized to convert it into solid state to enhance its stability to a longer period of time in dry powder state. The properties of drug remain intact because lyophilization includes removal of water content without adding excess heat to the product. Cryoprotectant; Mannitol (1% w/v) was used for lyophilization of Lercanidipine nanosuspension. Mannitol and freshly prepared nanosuspension was mixed in a glass vial. The sample was shaken on sonicator bath until the cryoprotectant was dissolved completely. The prepared sample was lyophilized subsequently using a lyophilizer. Mannitol offers great flow ability to dried powder. Dried powder can be used for further solid state characterization after easy reconstitution.

**Solid state characterization of lyophilized Lercanidipine nanocrystals**

**Fourier transform infrared spectroscopy**
LER, PVP K30, physical mixture LER and PVP K30 (1:1), and lyophilized nanocrystals were compared using Fourier transform infrared spectroscopy (CARY 630, Agilent Technologies) which precisely measures the amount of light absorbed by the sample. This absorbance creates a unique spectral fingerprint that is used to identify the molecular structure of the sample with the help of unique Michelson interferometer. The sample was mounted on sample area and spectra obtained were compared for compatibility study of excipient and drug.

---

**Table 1** Layout of central composite design batches (*average ± SD, n = 3*)

| Run  | $X_1$ (Conc of PVP, %w/v) | $X_2$ (SAS Ratio) | $Y_1$ (Particle size, nm)* | $Y_2$ (%CDR10)* |
|------|--------------------------|-------------------|---------------------------|-----------------|
| CCD1 | 0                        | 0                 | 311.00 ± 4.00             | 87.39 ± 3.89    |
| CCD2 | +1.41                    | 0                 | 352.67 ± 3.79             | 79.71 ± 4.84    |
| CCD3 | −1.41                    | 0                 | 414.67 ± 6.03             | 67.65 ± 5.95    |
| CCD4 | +1                       | +1                | 324.67 ± 6.51             | 84.98 ± 4.62    |
| CCD5 | 0                        | +1.41             | 302.00 ± 10.58            | 88.95 ± 4.99    |
| CCD6 | −1                       | −1                | 464.33 ± 5.51             | 58.41 ± 5.07    |
| CCD7 | 0                        | −1.41             | 484.33 ± 6.51             | 56.29 ± 4.79    |
| CCD8 | +1                       | −1                | 383.67 ± 6.51             | 72.97 ± 4.60    |
| CCD9 | −1                       | +1                | 341.33 ± 4.16             | 83.59 ± 4.56    |
| LER NCs | 0.56               | 27                | 308.18 ± 10.56            | 87.50 ± 1.17    |

| Independent variables | Levels |
|-----------------------|--------|
| $X_1$ = Concentration of PVP | $a(-1.41)$ | $-1$ | 0 | $+1$ | $a(+1.41)$ |
| $X_2$ = SAS ratio | 0.15 | 0.25 | 0.5 | 0.75 | 0.85 |

---
Fourier transform infrared (FTIR) spectra were recorded in the range of 4000–400 cm\(^{-1}\).

**Powder X-ray diffraction**

Powder X-ray diffraction (PXRD) study was conducted using (PANalytical, Netherlands model: PW 3040/60 X’pert PRO) diffractometer to study crystalline nature of pure untreated Lercanidipine API and lyophilized LER-NCs. Samples were characterized by X-Ray diffraction method at room temperature using Cu K\(\alpha\) X-ray radiation source over 2\(^\theta\) range from 0° to 80° with a step size of 0.02° and scan rate of 0.04/s.

**Scanning electron microscopy**

The morphological characteristic of the optimized LER nanosuspension and pure Lercanidipine was evaluated by scanning electron microscope (Zeiss EVO-18, Germany). Prior to the measurement, the sample was diluted with distilled water (1:100) and agitated gently to assure proper dispersion of the suspension then mounted on the scanning electron microscopy (SEM) sample stab. The interaction between electrons and atoms present in sample produces various signals that contain information about surface morphology.

**Stability studies of nanocrystals**

Stability studies of optimized batch were carried out at 40 °C/75% RH for a period of 6 months. The sample was periodically analyzed for particle size, PDI, zeta potential, and CDR10. Each sample was analyzed in triplicate.

**Evaluation parameters for lyophilized drug nanocrystals**

Particle size, size distribution, zeta potential, saturation solubility, percentage drug content and in vitro drug release of lyophilized nanocrystals were carried out as mentioned earlier under section characterization of LER nanosuspensions.

**Results**

**Solubility studies of LER (solvent selection)**

For selection of solvent, solubility studies of LER was done using “shake-flask method.” Lercanidipine showed solubility in solvents like methanol (131.15 ± 1.31 mg/ml), acetonitrile (29.67 ± 0.36 mg/ml), dimethyl formamide (120.12 ± 0.19 mg/ml), and dimethyl sulfoxide (77.72 ± 1.67 mg/ml). The results of solubility study indicated that LER showed highest solubility in methanol and was selected as the solvent phase for further study and water having solubility 0.0060 ± 0.003 mg/ml was selected as an antisolvent.

**Effect of stabilizer**

An appropriate stabilizer must be added to settle the nanosuspension, control the aggregation of drug particles, and Ostwald aging by giving steric prevention at the drug–solvent interface during particle precipitation [19, 20]. The different formulations were prepared by using varying type of stabilizer at a constant concentration of stabilizer. The prepared nanosuspensions were evaluated for particle size, PDI, and zeta potential. Effect of different stabilizer on particle size and zeta potential was depicted in Figs. 1 and 2.

**Optimization of nanocrystals**

Based on preliminary trials, a total of 9 formulations, with two critical parameters, concentration of polymer and SAS ratio among all studied parameter, were selected to study their effect on response at five level each (0, −1, +1, −\(\alpha\), +\(\alpha\)), and were prepared as per the experimental design shown in Table 1. The actual value and coded value for both factors summarized in

| Batch | PDI    | Saturation Solubility(\(\mu\)g/ml) | Zeta potential(mv) | % Drug content |
|-------|--------|----------------------------------|--------------------|----------------|
| CCD1  | 0.13 ± 0.05 | 88.95 ± 2.01 | 27.70 ± 2.78 | 81.27 ± 1.64 |
| CCD2  | 0.21 ± 0.03 | 89.53 ± 3.14 | 26.91 ± 1.73 | 80.47 ± 2.15 |
| CCD3  | 0.26 ± 0.01 | 85.62 ± 2.12 | 25.06 ± 2.39 | 86.22 ± 2.04 |
| CCD4  | 0.19 ± 0.01 | 85.38 ± 2.89 | 30.38 ± 1.15 | 94.56 ± 2.20 |
| CCD5  | 0.18 ± 0.01 | 88.08 ± 2.56 | 27.29 ± 1.32 | 92.51 ± 1.98 |
| CCD6  | 0.26 ± 0.03 | 84.44 ± 2.96 | 29.59 ± 1.37 | 91.71 ± 2.40 |
| CCD7  | 0.19 ± 0.01 | 89.91 ± 2.58 | 25.66 ± 2.54 | 93.07 ± 1.98 |
| CCD8  | 0.12 ± 0.02 | 91.79 ± 2.62 | 27.18 ± 1.34 | 93.14 ± 2.40 |
| CCD9  | 0.15 ± 0.01 | 80.59 ± 2.17 | 27.89 ± 1.32 | 90.65 ± 1.91 |
| LER NCs | 0.20 ± 0.34 | 87.78 ± 3.27 | 28.69 ± 1.56 | 93.15 ± 2.24 |
| LER   | 3.35 ± 0.33 | 6.03 ± 0.65  | 3.51 ± 0.62  | –              |
Table 1. The prepared batches were analyzed and further characterized for responses like particle size (nm) and CDR10. The results obtained were reported in Table 1. For all the 9 batches, particle size ($Y_1$) and CDR10 ($Y_2$) demonstrated wide variations from 302.00 ± 10.58 to 484.33 ± 6.51 nm and 56.29 ± 4.79 to 88.95 ± 4.99 (%) respectively indicating good influence of independent variables ($X_1$ and $X_2$) on the selected responses ($Y_1$ and $Y_2$). The data obtained from experimental runs were subjected to regression analysis. For the selected responses ($Y_1$ and $Y_2$), coefficients $\beta_1$, $\beta_2$, $\beta_{11}$, and $\beta_{22}$ were found to be
significant \((p < 0.05)\) while coefficient \(\beta_{12}\) was found to be insignificant \((p > 0.05)\) as per Table 3. Hence, this insignificant term was removed from their full model in order to develop a reduced one. The removal of insignificant terms was further justified by ANOVA test (Table 4). The high values of correlation coefficients \((R^2)\) for particle size \((Y_1)\) and saturation solubility \((Y_2)\) illustrate fitness of adopted model with predicted value. The critical \(F\) values for \(Y_1\) and \(Y_2\) were found to be 10.13 \((df = 1, 3)\). For both responses, \(Y_1\) and \(Y_2\), calculated \(F\) values was found 2.98 and 6.68 were less than their respective critical values which suggested that nonsignificant differences among the full and reduced model. The Pareto chart as shown in Fig. 3A, B revealed that polymer concentration and SAS ratio had standardized effect at 95% confidence interval on mean particle size and CDR10. The fitted polynomial Eq. (2–3) relating the responses particle size \((Y_1)\) and CDR10 \((Y_2)\) were the transformed in actual value as below. The significant \(F\) ratio and \(R^2\) in Table 4 suggested that there was a good linearity between the predicted and the observed values as a result of this \%relative errors were also less as reported in Table 5 [17].

\[
PS (Y_1) = 311.0 - 23.13 PVP K30 - 54.98 \frac{S}{AS} + 33.9 PVP K30*S/PVP K30 + 38.6 \frac{S}{AS}* \frac{S}{AS} + 16.00 PVP K30*S \frac{S}{AS}
\]  

\[
CDR10(Y2) = 87.39 + 4.125 PVP K30 + 10.424 \frac{S}{AS} - 6.40 PVP K30 \left(3\right)
\]

**Influence of concentration of PVP K30 on particle size**

Stabilizer concentration plays an important role in stability of nanosuspension by offering their affinity on surface of drug particles. The relationship between the dependent and independent variables was further enlightened using response surface plot and polynomial equation [18]. In the polynomial Eq. 2, negative value of coefficient for PVP K30 concentration suggested that the increase in the concentration of polymer decreased the mean particle size [17]. The lowest particle size of 302.00 ± 10.58 nm was observed with batch CCD5.

| Table 3 Statistical analysis of particle size and CDR10 |
|----------------|----------------|
| Independent factors | \(P\) value |
| \(X_1 = (conc of PVP, %w/v)\) | \(X_2 = (S/AS ratio)\) |
| Particle size \((Y_1)\) | CDR10 \((Y_2)\) |
| \(X_1\) | 0.034 | 0.016 |
| \(X_2\) | 0.003 | 0.001 |
| \(X_{11}\) | 0.046 | 0.020 |
| \(X_{22}\) | 0.033 | 0.016 |
| \(X_{12}\) | 0.166 | 0.070 |

| Table 4 Result of analysis of variance |
|----------------|----------------|
| Particle size \((Y_1)\) | \(df\) | \(SS\) | \(MS\) | \(F\) ratio | \(P\) value | \(R\) square |
| Full model | Regression | 5 | 34141.62 | 6828.32 | 19.88 | 0.016 | 0.9707 |
| | Residual | 3 | 1030.33 | 343.44 | | | |
| | Total | 8 | 35171.95 | | | | |
| Reduced model | Regression | 4 | 33117.62 | 8279.41 | 16.12 | 0.009 | 0.9416 |
| | Residual | 4 | 2054.33 | 513.58 | | | |
| | Total | 8 | 35171.95 | | | | |
| CDR10 \((Y_2)\) | \(df\) | \(SS\) | \(MS\) | \(F\) Ratio | \(P\) Value | \(R\) Square |
| Full model | Regression | 5 | 1200.76 | 240.15 | 37.08 | 0.006 | 0.9841 |
| | Residual | 3 | 19.42 | 6.47 | | | |
| | Total | 8 | 1220.19 | | | | |
| Reduced Model | Regression | 4 | 1157.40 | 289.35 | 18.43 | 0.007 | 0.9485 |
| | Residual | 4 | 62.78 | 15.69 | | | |
| | Total | 8 | 1220.19 | | | | |
Fig. 3 Pareto chart of the standardized effects on a particle size and b CDR10
Influence of solvent to antisolvent volume ratio on particle size
The effect of the SAS volume ratio on particle size reduction was investigated. As shown in Eq. (2), the coefficient for SAS ratio has negative sign indicating that as SAS ratio increased from 1:5 to 1:35, the decrease in the mean particle size was observed. It was further confirmed by Fig. 4A; the volume of antisolvent increased, and the particle size decreased. It could be explained by two theories; (i) when drug solution was injected into antisolvent, drug concentration reduced rapidly with an increased part of antisolvent which leads to proliferative precipitation of the drug into nanoparticles [21]; (ii) larger volume of antisolvent (water) headed to fastened nucleation rate and generates smaller nuclei, at the same time the growth will rise. Further excess amount of antisolvent would increase diffusion distance for growing nuclei and it becomes the limiting step for nuclei to growth [22]. Consequently, high supersaturation condition results in genesis of small particles due to the development of large number of nuclei [23]. Figure 3A revealed that solvent to antisolvent ratio had maximum standardized effect at 95% confidence interval, on mean particle size. With these results and discussion, it was concluded that increasing the solvent to antisolvent volume ratio would decrease the mean particle size.

Influence of independent variable \( X_1 \) and \( X_2 \) on CDR10
The experimental studies suggested higher drug release for the nanosuspension as compared to the bulk drug. The increase in surface area as well as the reduction in particle size may account for fast drug release [24]. This could be also explained by solvent diffusion process and sink condition between drug particle and solvent; this will be discussed extensively in the same paragraph. Figure 4B shows the response surface plot characterizing increase in the CDR10 with increase in the SAS ratio. It was due to the decrease in the particle size at these levels. This phenomenon was clearly understood by Noyes and Whitney’s equation. Dissolution rate is increased as a result of particle size reduction and increased surface area of drug particles [25]. Figure 3B also revealed that solvent to antisolvent ratio had maximum standardized effect at 95% confidence interval, on % cumulative drug release. The positive signs in polynomial Eq. (3) suggested that increase in polymer concentration leads to increased drug release. Previously, it was reported that concentration of PVP as such did not show any profound influence on dissolution rate of Lercanidipine; therefore, the increase in drug release in the presence of PVP could be ruled out. Hence, drug release increased as a result of size reduction of particle and increased surface area.

Characterization of LER nanosuspension

**Particle size, particle size distribution, and zeta potential**
Particle size, PDI, and zeta potential (\( \zeta \)) value of the prepared drug-loaded nanosuspensions were reported in Tables 1 and 2. Particle size ranges from 302.00 ± 10.58 to 484.33 ± 6.51 nm. As shown in Table 1, the lowest mean particle size of batch CCD5 was found to be 302 ± 10.58 nm. PDI values of the formulation ranges from 0.12 ± 0.02 to 0.26 ± 0.01. Particle size distribution of batch CCD5 demonstrated in Fig. 5 showed narrow distribution. Zeta potential values of CCD1 to CCD9 as depicted range from 25.06 ± 2.39 to 30.38 ± 1.15 mV. The zeta potential (\( \zeta \)) values in the range of 25 mV to 30 mV in either charge are considered a stable formulation [26].

**In vitro dissolution studies**
Figure 6 represents a comparison of the dissolution profiles of bulk LER and prepared nanosuspension in 0.01N HCl pH 2.4. Drug nanocrystals exhibited noteworthy enhancement in dissolution rate compared to pure drug. (CCD5 > CCD1 > CCD4 > CCD9 > CCD2 > CCD8 > CCD3 > CCD7 > CCD6 > pure LER). According to Noyes-Whitney equation, the dissolution rate is inversely proportional to particle size [27]. Dissolution rate will be improved as a result of particle size reduction. The particle size could be a significant factor in increasing and diminishing the dissolution velocity of drug. The dissolution rate of drug nanocrystals might have increased due to increase in

---

**Table 5** Actual vs. predicted experiment value (*relative error*)

| Batch code | Particle size (nm) | %CDR10 | Actual | Predicted | %RE  | Actual | Predicted | %RE  |
|------------|--------------------|---------|--------|-----------|------|--------|-----------|------|
| CCD1       | 311.00             | 311.56  | 0.18   | 87.39     | 87.34| 0.06   |           |      |
| CCD2       | 352.67             | 346.01  | 1.92   | 79.71     | 80.43| 0.79   |           |      |
| CCD3       | 414.67             | 411.41  | 0.79   | 67.94     | 68.76| 1.19   |           |      |
| CCD4       | 324.67             | 321.35  | 1.03   | 84.98     | 85.32| 0.40   |           |      |
| CCD5       | 302.00             | 310.45  | 2.72   | 88.95     | 88.28| 0.76   |           |      |
| CCD6       | 464.33             | 477.56  | 2.77   | 58.41     | 56.23| 3.88   |           |      |
| CCD7       | 484.33             | 465.96  | 3.94   | 56.29     | 58.80| 4.27   |           |      |
| CCD8       | 383.67             | 399.36  | 3.93   | 72.97     | 71.06| 2.69   |           |      |
| CCD9       | 341.33             | 335.60  | 1.71   | 83.59     | 83.66| 0.08   |           |      |
| Optimized batch | 311.33         | 290.42  | 7.20   | 93.35     | 91.36| 2.20   |           |      |
surface area as a result of size reduction of drug particles [25].

**Saturation solubility studies**

The results of saturation solubility studies of pure LER and LER nanosuspensions and optimized batch of LER nanosuspensions are depicted in Table 2. The saturation solubility of LER nanosuspensions were enhanced as compared to pure LER. This might be due to decrease in the particle size thereby increasing the surface area due to nanoparticles formation. Saturation solubility of pure drug was 6.03 μg/ml while
prepared nanosuspensions showed up to 91.79 μg/ml of solubility.

**Solid state characterization of nanocrystals**

*Fourier transform infrared spectroscopy*

The overlay Fourier transform infrared (FTIR) spectra were showed in Fig. 7. An untreated LER showed the strong characteristic peaks at 1680 cm\(^{-1}\) due to the stretching of C=O group, 1500 cm\(^{-1}\) due to the stretching of N-O group, 3180 cm\(^{-1}\) due to the stretching of O-H group, and 1440 cm\(^{-1}\) due to the bending of O-H group. The presence of these major peaks in treated LER sample ruled out any changes in chemical structure of LER. A sharp peak at 1680 cm\(^{-1}\) shown in untreated LER was broadened and shifted to lower wavenumber at 1650 cm\(^{-1}\) in LER NCs with PVP K30, suggesting a difference in the molecular environment of the ketone group.

*Powder X-ray diffraction studies*

The effect of antisolvent precipitation and lyophilization on crystallinity of LER-NCs against pure LER has been studied by XRD studies. The spectra of pure LER showed halo pattern while precipitated lyophilized nanocrystals sample showed sharp and narrow distinct peaks (Fig. 8) which suggested highly crystalline nature of LER in formulation. The XRD patterns in the case of nanocrystals indicate some new peaks as compared to pure
drug due to presence of mannitol and PVP K30 on the surface of drug particles [28].

**Scanning electron microscopy**

SEM images of pure drug powder and lyophilized LER nanocrystals samples were illustrated in Fig. 9. As depicted in Fig. 9A, the pure LER showed cubic-like shaped crystals; after antisolvent crystallization, there was a considerable change in shape of drug. The precipitated particles in the presence of PVP K30 as shown in Fig. 9B exhibited dendritic needle-like crystals.

**Accelerated stability studies**

For accelerated stability studies, optimized LER formulation evaluated at specific time intervals (0, 1, 2, 3, and 6 months) at 40 °C/75% RH. The results of evaluated parameters like particle size, PDI, zeta potential, saturation solubility, and CDR10 of optimized LER formulation are depicted in Table 6. The results of stability studies showed that nanocrystals were
stabled as there was no significant change in parameters after evaluation. This might be due to PVP K30 which offers good stability to nanocrystals against the aggregation.

**Discussion**

**Super saturation ratio and selection of solvent antisolvent**

Based on preliminary study, among all organic solvent, methanol showed highest solubility for LER and was selected as the solvent. Water was selected as an antisolvent phase in our precipitation process because of high miscibility with methanol. The solvent methanol can be chosen according to the supersaturation ratio \( S \):

\[
S = \frac{C}{C_{eq}}
\]

where \( C \) is concentration of Lercanidipine in the solvent and \( C_{eq} \) is the equilibrium concentration of LER in the solvent-antisolvent mixture at a certain temperature.

In present investigation, nanocrystals were prepared by bottom up technique; so far, it is also known as antisolvent precipitation. The drug solution was prepared in a solvent (methanol), which is added to solvent-miscible anti-solvent generally water with the help of syringe. Precipitation consists of three process; supersaturation, nucleation, and subsequent growth of particles. The solubility of Lercanidipine in solvent–antisolvent mixture was lowered as compared to original solution (methanol), as a result of this environment was set for supersaturation. Supersaturation is prerequisite for the nucleation process to occur [23].

**Effect of concentration of stabilizer**

A competent particle size reduction was seen with all the stabilizers but in the case of PVP K30, reduction of particle size was highest. Hence, it was decided to make use of PVP K30 as a stabilizer to prepare Lercanidipine nanoparticles. PVP K-30 is a linear nonionic polymer which is soluble in both water and organic solvents which meet one of the criteria for solvent antisolvent precipitation [29]. The concentration of PVP K30 affects the particle size during crystallization of drug in antisolvent medium. It was observed that the particle size initially decreased and then increased with increasing PVP concentration (Fig. 4A). This effect was very likely due to the adhesive nature of PVP K30 and Ostwald ripening at higher polymer concentration. Similar behavior has been reported previously [2].

**Experimental factors affecting particle size**

During the precipitation process, several factors influence the particle size were previously described in result section, including solvent to antisolvent volume ratio, concentration of LER in methanol, stirring speed, and the stirring time. In this investigation, the size of particle in nanometer was used as the characteristic parameter.

Effect of antisolvent to solvent ratios was studied by varying it from 5:1 to 35:1. The particle size is inversely

---

**Table 6** Accelerated stability studies data at 40 °C/75% RH

| Time (months) | Particle size (nm) | PDI | Zeta potential (mV) | Saturation solubility (µg/ml) | %CDR10 |
|--------------|--------------------|-----|---------------------|-----------------------------|--------|
| 0            | 308.18 ± 1.56      | 0.20 ± 0.4 | 26.69 ± 1.56       | 87.78 ± 3.27               | 87.50 ± 1.17 |
| 1            | 311.33 ± 9.02      | 0.18 ± 0.06 | 25.47 ± 1.24       | 85.36 ± 0.86               | 85.33 ± 0.90 |
| 3            | 303.67 ± 8.02      | 0.26 ± 0.07 | 24.68 ± 0.96       | 84.20 ± 0.83               | 86.41 ± 1.11 |
| 6            | 314.33 ± 5.51      | 0.29 ± 0.04 | 27.39 ± 1.17       | 87.75 ± 0.44               | 85.34 ± 1.75 |

---

![SEM Images](image.png)

**Fig. 9** SEM Images of the a Lercanidipine and b Lercanidipine nanocrystals
proportional to the quantity of antisolvent (water). As shown in Fig. 10A, the study was revealed that as volume of antisolvent increased the particle size decreased. It could be justified by higher supersaturation in the interface of two liquid phases leading to a quick nucleation and produces smaller nuclei. It was previously reported that high supersaturation condition results in small particles due to the formation of large number of nuclei [23]. When the LER drug solution is added into a miscible non-solvent (water) under agitation, it leads to a sudden high supersaturation, resulting in rapid nucleation and precipitation.

Different concentrations of the LER in methanol were studied with constant volume ratio of solvent to antisolvent (1/20). According to Fig. 10B, it was revealed that higher drug concentration creates a high degree of supersaturation, resulting into reduction of particle size. However, after 40 mg/ml, any further increase in concentration dose not influence the particle size. It was concluded that methanol can uptake up to 40 mg/ml of drug (drug loading). For the present study, 20 mg/ml drug concentration was selected.

Figure 10C shows that stirring speed has a considerable effect on particle size. As stirring speed increased from 5000 to 15,000 rpm, there was a drastic change in particle size. The relationship between particle size and stirring speed explained by intense micromixing between the multiphases with higher stirring speed will lead to particle size reduction. At lower rpm, particle size reduction was less due to insufficient energy in term of mixing speed. Further stirring at higher rpm reduces the mass-transfer resistance and enhances the rate of diffusion between the multiphase system, which leads to high, consistent and vigorous supersaturation in a short period of time and as a result of this rapid nucleation occur to produce smaller drug particles. Therefore, it was concluded that higher stirring speed generates smaller particles. For the present study, 10,000 rpm stirring speed was selected.

The stirring time also affects the particle size of drug, as shown in Fig. 10D. When the stirring time is 5 min, the particles are larger because period of agitation was not sufficient to decrease particle size up to desired level.

Fig. 10 Effect of a Antisolvent volume ratio, b Conc. of drug, c stirring time, and d stirring speed on particle size
As the stirring process continues for more time, the particles are broken up, because high energy is introduced into the system thus there was a notable decrease in particle size [24]. For present study, 15 min stirring time was selected.

**Effect of particle size on drug release and saturation solubility**

In the present study, drug release of nanosuspension compared to the bulk drug was increased. The increased dissolution is dependent on particle size reduction. The size diminution of particle leads to an increased surface area and thus according to the Noyes–Whitney equation (Eq. 5), the dissolution rate is increased [30]. Furthermore, saturation solubility will be increased as particle size is reduced. As a result of this, concentration gradient (Cₛ–Cₜ) also increased which enhance dissolution velocity according to Noyes–Whitney equation:

\[
\frac{dx}{dt} = \frac{DA}{hD} (Cₛ–Cₜ)
\] (5)

where \(\frac{dx}{dt}\) is the dissolution velocity, \(D\) is the diffusion coefficient, \(A\) is the surface area, \(hD\) is the diffusional distance, \(Cₛ\) is the saturation solubility and \(Cₜ\) is the concentration around the particles. Further, the increase in PVP concentration beyond the level (30% w/v) leads to decreased dissolution rate of Lercanidine. It could be explained by diffusion of solvent toward the antisolvent will be drop off due to high viscosity of the solution, which in turn increases the mean particle size and so drug release also decreased [1]. The drug release from Lercanidine nanosuspensions was up to 88.95% within 10 min while the pure LER dissolved only 21.53% during the same period of time. A considerable enhancement in saturation solubility of LER-NCs was observed due to the wetting phenomena of stabilizer and increased in specific surface area of drug particles due to size reduction [18].

In statistical evaluation part, regression analysis, ANOVA, \(p\) value, correlation co-efficient \(R^2\), predicted vs. actual value and polynomial equation coefficients were analyzed. As shown in Table 5, predicted and actual value of particle size and CDR10 showed very strong and close agreement between results. It was suggested that second-order quadratic model was employed successfully. The significant \(F\) ratio and \(R^2\) suggested (Table 4) that there was a good linearity between the predicted and the observed values. The \((p < 0.05)\) in Table 3 indicates that concentration of PVP K30 and volume ratio of S/AS had significant effect on particle size and drug release. The dissolution profile of lyophilized nanocrystals and nanosuspension (Fig. 11) indicated that dissolution of Lercanidine was increased significantly after precipitation and lyophilization.

The XRD interpretation of final LER-NCs formulation shows sharp and intense peaks as compared to the plain drug suggested that crystallinity of drug increased in nanocrystals. Some new peaks are also observed which may be due to the presence of mannitol and polymer on the surface of nanocrystals [32].

The SEM image of pure Lercanidine showed cubic-like shaped crystals; after antisolvent crystallization, there was a considerable change in shape of drug. The
precipitated lyophilized nanocrystals exhibited dendrite needle-like crystals.

**Conclusion**

In the present study, nanocrystals were prepared to enhance the dissolution properties of poorly water-soluble drug Lercanidipine. The antisolvent precipitation procedure using methanol as solvent, water as antisolvent, and low amounts of PVP K30 as stabilizer is a very promising and effective method to increase the dissolution rate of Lercanidipine. The method is simple and cost effective and uses safe materials. The physicochemically stabilized Lercanidipine nanocrystals obtained in this method were remarkably showed higher dissolution rate and saturation solubility as compared to pure Lercanidipine.

**Abbreviations**

SAS: Solvent antisolvent; PXRD: Powder X-ray diffraction; SEM: Scanning electron microscopy; LER NCs: Lercanidipine nanocrystals; FTIR: Fourier transform infrared; CDR10: % Cumulative drug release at 10 min; QbD: Quality by design; CCD: Central composite design; PDI: Polydispersity index; ANOVA: Analysis of variance; df: Degree of freedom; SD: Standard deviation

**Acknowledgment**

The authors are thankful to Alembic Pharmaceuticals, Gujarat, India for providing gift sample of Lercanidipine hydrochloride and Department of Pharmaceutical Sciences, Saurashtra University, Gujarat, India for providing all facility and support to conduct research.

**Authors’ contributions**

DG designed, conduct, and collect data of the whole experiments. AB and DG analyzed the data and interpreted the results. DG was a major contributor in writing the manuscript. JP supervised DG. All authors gave their individual comments and review and final approval of the version to be submitted.

**Funding**

Not applicable.

**Availability of data and materials**

All data and material are available upon request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1. Department of Pharmaceutical Sciences, Saurashtra University, University Road, Rajkot, Gujarat 360 005, India.
2. Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Mehsana, Gujarat 384315, India.

**Received:** 1 October 2020 **Accepted:** 26 May 2021

**Published online:** 29 June 2021

**References**

1. Sadeghi F, Ashofteh B, Homayounic A, Abbaspoura M, Nokhodchid A, Garekanif HA (2016) Antisolvent precipitation technique: a very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidone for solubility and dissolution enhancement. Colloids Surf B Biointerfaces 147:258–264. https://doi.org/10.1016/j.colsurfb.2016.08.004
2. Che E, Zheng X, Sun C, Chang D, Jiang T, Wang S (2012) Drug nanocrystals: a state of the art formulation strategy for preparing the poorly water-soluble drugs. Asian J of Pharm Sci 7(2):85–95
3. Liversidge EM, Liversidge GG, Cooper BR (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 18(2):113–120. https://doi.org/10.1016/S0928-0987(02)00251-8
4. Jain S, Patel N, Lin S (2013) Solubility and dissolution enhancement strategies: current understanding and recent trends. Drug Dev Ind Pharm 41(6):875–887. https://doi.org/10.3109/03639045.2013.871027
5. Shaikh FJ, Patel VB (2015) Enhancement of dissolution of Lercanidipine hydrochloride using solid dispersion technique. Res J of Recent Sci 4:299–207
6. Shaikh FJ, Patel MB, Surti NI, Patel VB (2017) Preparation and characterization of Lercanidipine hydrochloride inclusion complex with β-cyclodextrin and effect of complexation on solubility and dissolution. Res J Pharm Tech 10(4):951–958
7. Pandey S, Chandekar E, Wattamwar A, Pandit S, Joshee H, Patil A (2012) Enhancement of wettability and in vitro dissolution properties of Lercanidipine hydrochloride by solid dispersion technique. Thai J Pharm Sci 36:108–116
8. Asija R, Bhattacharya M, Asija S, Yadav A, Shah I (2014) Enhancement of solubility and dissolution of Lercanidipine by liquid dispersion technique. J Chem Pharm Res 6(6):2680–2686
9. Asija R, Mangukuia D, Asija S, Patel J, Patel C, Patel P (2013) Solubility enhancement of Lercanidipine hydrochloride by cocrystallisation. J Biomed Pharm Res 2(3):17–25
10. Mohan KV, Manjula BP (2013) Formulation and evaluation of Lercanidipine microcrystals for its solubility enhancement. Int J Pharm Res Dev Sci 5(5):37–47
11. Xiong S, Liu W, Zhou Y, Youcheng M, Liu Y, Chen X (2020) Enhancement of oral bioavailability and anti-Parkinsonian efficacy of reseratol through a nanocrystal formulation. Asian J of Pharm Sci 15(4):518–528. https://doi.org/10.1016/j.ajps.2019.04.003
12. Bagada A, Vadala KR, Raval MK, Gadhiya D (2019) To study the significance of processing variables using quality by design for optimization of nanoparticulate system of Cilnidipine. Int J Pharm Sci Drug Res 11(6):318–324
13. Parmar K, Patel J, Sheth N (2015) Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. Asian J Pharm Sci 10(5):396–404. https://doi.org/10.1016/j.ajps.2015.04.006
14. Thorat AA, Dalvi SV (2012) Liquid antisolvent precipitation and stabilization of nanoparticles of poorly water soluble drugs in aqueous suspensions: Recent developments and future perspective. Chem Eng J 181–182:31–34. https://doi.org/10.1016/j.cej.2011.12.044
15. Taneja S, Shilpi S, Khatr K (2016) Formulation and optimization of efavirenz nanosuspensions using the precipitation-ultrasonication technique for solubility enhancement. Antif Cells Nanomed Biotechnol 44:978–984
16. Patel M, Savant K (2017) A quality by design concept on lipid based nanof ormulation containing antipsychotic drug screening design and optimization using response surface methodology. Nanomed Nanotechnol 8(3):11–11
17. Shah SR, Parikh RH, Chavda JR, Sheth NR (2013) Application of Plackett–Burman screening design for preparing gelbenclamide nanoparticles for dissolution enhancement. Powder Technol 235:405–411. https://doi.org/10.1016/j.powtec.2012.10.055
18. Parmar R, Patel J, Sheth N (2015) Formulation and optimization of Embelin nanosuspensions using central composite design for dissolution enhancement. J Drug Del Sci Techno 29:1–7. https://doi.org/10.1016/j.jddst.2015.05.011
19. Verma S, Kumara S, Gokhale R, Burgess D (2010) Physical stability of nanosuspensions: investigation of the role of stabilizers on Ostwald ripening. Int J of Pharmaceutics 406(1–2):145–152
20. Maximino FP, de Paula LM, Figueiredo VF, de Andrade IM, Talvani A, Sabarretoec LC (2011) Benzimidazole microcrystal preparation by solvent change precipitation and in vivo evaluation in the treatment of Chagas disease. Eur J Pharm Biopharm 78:377–384. https://doi.org/10.1016/j.ejpb.2011.03.003
21. Lonare AA, Patel SR (2013) Antisolvent crystallization of poorly water soluble drugs. Int J Pharm 434:297–304. https://doi.org/10.1016/j.ijpharm.2012.10.055
22. Kakran M, Sahoo NG, Tan IL, Li L (2012) Preparation of nanoparticles of poorly-water-soluble antioxidant curcumin by antisolvent precipitation methods. J Nanopart Res 14(7):571–1–11
23. Wang Z, Chen JF, Le Y, Shen ZG, Yun J (2007) Preparation of ultrafine beclomethasone dipropionate drug powder by antisolvent precipitation. Ind Eng Chem Res 46(14):4839–4845. https://doi.org/10.1021/ie0615537
24. Rasenack N, Müller BW (2002) Dissolution rate enhancement by in situ micronization of Poorly Water-Soluble Drugs. Pharm Res 19(12):1894–1900. https://doi.org/10.1023/A:1021410002871
25. Chu KR, Lee E, Jeong SH, Park ES (2012) Effect of particle size on the dissolution behaviors of poorly water-soluble drugs. Arch Pharm Res 35(7):1187–1195. https://doi.org/10.1007/s12272-012-0709-3
26. Shakeel F, Haq N, El-Badry M, Alanazi FK, Alsanaa IA (2013) Ultra fine super self-nanoemulsifying drug delivery system (SNEDDS) enhanced solubility and dissolution of indomethacin. J Mol Liq 180:89–94. https://doi.org/10.1016/j.molliq.2013.01.008
27. Mosharraf M, Nyström C (1995) The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. Int J Pharm 122(1-2):35–47. https://doi.org/10.1016/0378-5173(95)00033-F
28. Sheskey PJ, Cook WG, Cable CG (2017) Handbook of Pharmaceutical Excipients, 8th edn. Pharmaceutical Press, London
29. Kk S, Patel MH, Patel K (2015) Cefdinir nanosuspension for improved oral bioavailability by media milling technique: formulation, characterization and in vitro–in vivo evaluations. Drug Dev Ind Pharm 42:758–768
30. Homayouni A, Sadeghi F, Varchosaz J, Garekani HA, Nokhodchi A (2014) Comparing various techniques to produce micro/nanoparticles for enhancing the dissolution of celecoxib containing PVP. Eur J Pharm Biopharm 88(1):261–274. https://doi.org/10.1016/j.ejpb.2014.05.022

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.