



Surfactant Free Preparation of Celecoxib Microcrystals by a Controlled Precipitation Process

Sepideh Mardani¹, Maryam Maghsoodi^{1*}, Hamed Hamishehkar¹

¹Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Article Info

Article History:

Received: 8 October 2016

Accepted: 8 January 2017

ePublished: 30 September 2017

Keywords:

-Celecoxib
 -Microcrystals
 -Precipitation
 -Acetone
 -Surfactant free

ABSTRACT

Background: The antisolvent precipitation technique has evolved into an effective method to prepare microcrystals of drug. Although this method has advantages, such as cost effectiveness, the processing is significantly sensitive to stabilizer. The aim of present study was to prepare celecoxib (CLX) microcrystals via antisolvent precipitation technique without any surfactants.

Methods: Acetone was used as a solvent for the CLX and water was used as an antisolvent. During the precipitation process, several experimental parameters, such as the volume ratio of antisolvent to CLX solution, the concentration of CLX solution, the temperature and the stirring speed, were investigated.

Results: The results showed that external characteristics such as particle size and its distribution were strongly influenced by the process parameters, while the internal structures such as chemical composition and crystal structure were unaffected during the process. Higher volume ratio of antisolvent to CLX solution, lower temperature and more- intense stirring led to the smaller crystals. However, increasing the concentration of CLX solution resulted in aggregation of crystals and consequently formation of large crystals with more heterogeneous distribution. It was found that this effect could be prevented by using of ethanol as co-solvent with volume ratio to acetone 5:1. Under the optimum conditions, the yielded powder had a mean particle size of 7 μm and 70 % of the particles were distributed in the range of 0.3- 15 μm .

Conclusion: Results of this study offer a useful starting point for a conceptual framework to guide the preparation of microcrystals without using a stabilizer.

Introduction

The average particle size and the particle size distribution play key roles in pharmaceutical formulation. These factors may affect the bioavailability and the processability. A small average particle size with a narrow particle size distribution is desirable for poorly water-soluble drugs.

The performance of pulmonary drug delivery is influenced by both characteristics of the powder formulation and breathing patterns. Among the formulation factors, the size and size distribution of particles are the main factors that influence the efficiency of pulmonary drug delivery.

Many techniques have been used to obtain particles with optimal respirable size from DPI formulations, such as milling,^{1,2} supercritical fluids,³⁻⁵ spray drying,¹ precipitation,⁶ and so on. Milling is the most frequently used technique to reduce particle size of the active pharmaceutical ingredients for lung delivery. This technique is energy intensive and applies high pressure on the input crystals

which may result in the formation of adverse properties of obtained products such as generation of amorphous disorder or defects within the crystalline lattice and increasing possibility of chemical degradation.⁷⁻¹¹ This technique also shows some disadvantages in practice such as poor control of particle size, shape, and surface charge. Furthermore, the milling abrasion introduces contamination into the obtained product.¹²

Supercritical fluid techniques and spray drying may be interesting methods for producing particles with tight size distribution. However, supercritical fluid techniques have the limitations of using expensive high-pressure equipment and spray drying results in construction of amorphous product which is thermodynamically unstable. Amorphous particles have high affinity for moisture and consequently show tendency to agglomerate and recrystallize on storage.¹³

In contrast to the aforementioned techniques, precipitation is promising method for industrial production, because of its high yield, low cost,

*Corresponding Author: Maryam Maghsoodi, E-mail: maghsoodim@tbzmed.ac.ir

©2017 The Authors. This is an open access article and applies the Creative Commons Attribution (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

convenience in processing, as well as the ease of scale-up. Antisolvent precipitation is based on the change of the supersaturation caused by mixing the solution and the antisolvent. This process has often been performed in presence of a stabilizing additive for decreasing particle size and producing micronized crystals.¹⁴⁻¹⁶ However, no safety data on inhaled stabilizing agents are presented. Therefore, in the case of DPI administration stabilizers should be used with caution because they are usually unsafe to lungs.

Moreover, stabilizers may result in the change of solid state of drug crystals from crystalline to amorphous which is undesirable as mentioned above. Therefore, producing micronized crystals without stabilizers is an important effort for DPI use.

Celecoxib (CLX), a cyclooxygenase-2 (COX-2) inhibitor, was chosen as the model drug in present work. Cyclooxygenase-2 enzyme is believed to play an important role in the pathogenesis of lung tumors.¹⁷ COX-2 is over expressed in lung cancer with implications in inhibition of apoptosis and suppressing the immune response, stimulating angiogenesis, and increased invasiveness of lung cancer.¹⁸ Furthermore, CLX has been demonstrated to increase the response of malignant cells to anticancer agents.¹⁷ In this work, we explored the possibility of producing micronized crystals of CLX by precipitation method without using stabilizing agents. The effects of various parameters including antisolvent to CLX solution ratio, the concentration of CLX solution, the temperature and the stirring speed were investigated, and the obtained crystals of CLX were characterized.

Materials and Methods

Materials

In this research celecoxib (CLX) was supplied by Arastoo chemical company (Iran) and all solvents were purchased from Merck (Germany).

Experimental Procedure

Selection of the Solvent-Antisolvent System

CLX is practically insoluble in water,¹⁹⁻²⁰ therefore, in this work, water was selected as an antisolvent. The solvent (acetone, ethanol, ethyl acetate, and PEG400) can be selected in accordance with the supersaturation ratio (S):

$$S = \frac{C}{C_{mix}} \quad \text{Eq.(1)}$$

where, C and C_{mix} are the concentration of CLX in solvent and in the solvent-antisolvent mixture, respectively. CLX was added to 5 ml of solvent under stirring at 25 °C until the CLX did not dissolve and stirring was continued for 120 min. Samples passed through a 0.45 μm membrane filter (Millipore), and analyzed spectrophotometrically employing a UV detector (UV-160A, Shimadzu,

Japan) to determine the concentration of CLX in solvent. Using determination of the terminal value of S of the solvent-antisolvent system at 25 °C, the volume ratio of solvent to antisolvent (water) was 1/10 (v/v). The concentration in solvent-antisolvent mixture was also attained in accordance with the abovementioned manner. Thus, the terminal value of S could be calculated.

Precipitation Process

CLX solution was obtained by dissolving raw CLX in acetone at 25°C. The concentration of the solution was less than its solubility in acetone. CLX solution (5 mL) was immediately poured into water (at 25°C) placed in a cylindrical vessel (250 ml) in a certain ratio of antisolvent to solution under stirring using a propeller type stirrer with four blades.

After 15 min agitation (400 rpm) the CLX suspension was filtered under vacuum and the filtered paste was dried in an oven at 60°C for 3 h.

Characterization

The morphology of CLX powder was observed using a model LEO 1430 VP scanning electron microscopy (SEM) system (JEOL, Japan). A small amount of dried CLX powder was scattered on the copper stub and then coated with gold under an argon atmosphere and observed by scanning electron microscope.

The volume particle size was measured using a laser diffractometer (SALD-2101, SHIMADZU, Japan). Each sample was appropriately diluted with the water in the presence of dispersant (Tween 80, 0.01%, w/w). Concentration of samples was adjusted as much as obscuration was not less than 10%. Mean size was determined three times for each sample.

Infrared spectra were recorded using an FT-IR spectrophotometer (M-B-100, Bomem, Canada) utilizing potassium bromide discs. Samples were prepared by gently grinding the powder with KBr. The data region was 500–2000 cm⁻¹.

A Seimens (Model D5000, Germany) X-ray diffractometer was used at 40 kV, 30 mA and a scanning rate of 0.06° min⁻¹ over a range of 2–40 2θ, using CuK_{α1} radiation of wavelength 1.5405 Å.

DSC studies were carried out to determine polymorphic composition of prepared crystals.

Samples of the crystals (3–5 mg) were heated (range 25-250°C) at 10 °C/min in crimped aluminum pans under a nitrogen atmosphere. The melting point was automatically calculated using the software provided by Shimadzu (Japan).

Statistical evaluation of data

All data reported in the present study were the mean ± standard deviation (SD). ANOVA was used to compare the mean values of the data

obtained and comparison between the two means was determined using the Tukey's test with statistical significance evaluated at $P < 0.05$.

Results and Discussion

In fact, selection of suitable solvent (S) and antisolvent (AS) is a key step to precipitate microcrystals. In this work, water was served as antisolvent. The supersaturation ratios of the acetone-water, ethanol-water, ethylacetate-water and PEG400-water systems were reported in Figure 1. The Acetone-water system was the best of the four systems, because it induced the highest degree of supersaturation, which usually resulted in small particles. Therefore, acetone was used as solvent to get microcrystals without addition of any other stabilizers. Acetone is a good concern from both low toxicity and easily removing point of view. Moreover, according to the ICH solvent toxicity, acetone belongs to class III with very low toxicity.

During the antisolvent precipitation process, several factors that influence particle construction should be considered, including the volume ratio of antisolvent to solution, the concentration of the CLX-acetone solution, the temperature, and the stirring speed. In this paper, the effect of these parameters on particle size of the CLX crystals was studied.

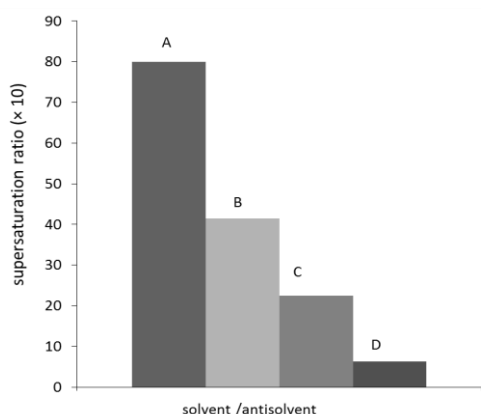


Figure 1. Supersaturation ratio in different solvent-antisolvent systems: (A) acetone-water, (B) PEG 400-water, (C) ethylacetate-water and (D) ethanol-water.

Effects of Experimental Parameters on the Particle Size

Effect of the Volume Ratio of Antisolvent to Solution

Different volume ratios of antisolvent to solution (5/1 and 20/1) were explored when the CLX solution concentration were 10 and 20 mg/ml. The mean particle size and the particle size distribution obtained at different volume ratios of antisolvent to solution (AS/S) is indicated in Table 1 and Figure 2. The crystal size is inversely proportional to the volume ratio of AS/S. With the increase of volume ratio, there is a significant ($p < 0.05$) decrease in

crystal size of CLX. In similar works it has also been shown that the particle size decreases as the solvent content is reduced by increasing the AS to S ratio.²¹⁻²⁴ The shift from a larger crystal size at lower volume ratio to a smaller crystal size at higher volume ratio can be elucidated as follows. Keeping the same solute content in the system, if the ratio of AS/S is increased, the degree of supersaturation ratio is raised. Crystallization consists of two main steps of nucleation and crystal growth. Compared to the crystal growth rate, the rate of nucleation is more dependent on supersaturation ratio, and the nucleation rate strongly influences the final crystal size distribution.²⁵⁻²⁶ A higher degree of supersaturation ratio leads to a high nucleation rate and as a result, larger number nuclei are formed. Therefore, there is a much greater probability that form new nuclei rather than each nucleus could grow. Therefore, higher supersaturation leads to smaller crystals due to formation of a larger number of nuclei. In contrast, at lower AS/S ratio, the acetone-water mixture is a better solvent for CLX, which results in a lower degree of supersaturation and consequently larger crystals, for the reason that fewer nuclei being formed.

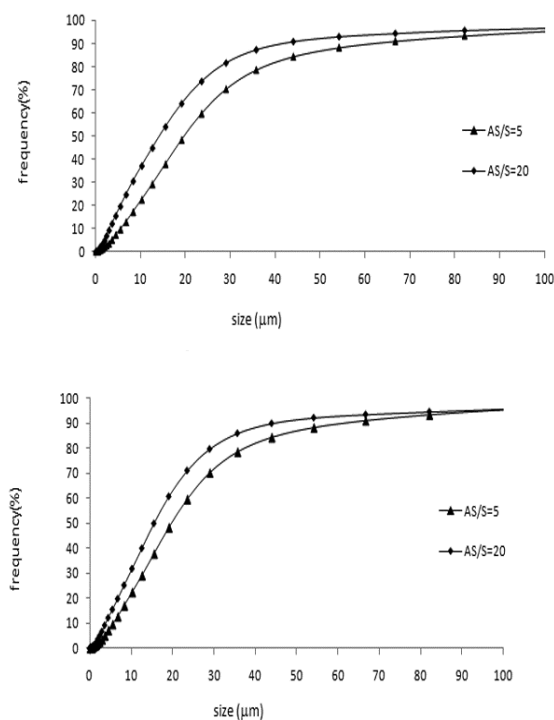


Figure 2. Particle size distribution of CLX obtained at different volume ratios of antisolvent to solution (A/S). Solution concentration 10 mg/ml (up) 20 mg/ml (down)

Effect of Concentration of the CLX-Acetone Solution

CLX solution concentration of 10 and 20 mg/ml were explored at different volume ratio of AS/S

(5/1 and 20/1). Table 1 and Figure S1 (supplementary file) show the mean particle size and the particle size distribution of CLX as a function of solution concentration. Higher concentrations of drug solution produces higher degree of supersaturation upon mixing with the AS, leading to an increasing in the nucleation rate.²⁷ As aforementioned increasing the nucleation rate stands for the creation of large number of nuclei and consequently formation of smaller crystals. Contrary to our expectation, it was found that at both AS/S ratios the particle sizes significantly ($p < 0.05$) increased when the concentration of the drug solution increased from 10 to 20 mg/ml. These results could be explained as follows. When the degree of supersaturation is increased by a higher drug concentration, the particle size is increased above a certain limit because of higher availability of solute molecules near the nascent surface favoring the particle growth.²⁸

These results can also be elucidated by considering the dependency of the micromixing between the multiphases on the solution concentration. Higher solute molecules in solution results in higher viscosity which in turn hinders the diffusion of nuclei between the solvent and the antisolvent phases.²⁹⁻³⁰ Existing of large number of nuclei and decreased their diffusion lead to increase the collision rate of nuclei with each other and consequently the particle aggregation.

Wide particle size distribution and presence of coarse particle ($>400\mu\text{m}$) in the samples prepared with CLX solution concentration of 20 mg/ml, as can be seen in Figure S1 (supplementary file), confirmed the particle aggregation in these samples. Similar results have been reported in literature of previous studies that an optimum drug concentration is needed to achieve the smallest particle size and use of drug concentration above this level results in larger particles.^{21,29}

Cosolvents system

It was concluded from above results that the viscosity for solution with the increasing of drug concentration should be concerned cautiously because of its negative effect on diffusion between S and AS and non-homogenous supersaturation. To avoid the negative effect of increasing drug

concentration, the cosolvents system was taken into consideration, which was mixture of acetone and ethanol. It should be noted that, at a temperature of 25 °C, the solubility of CLX in acetone is 800 mg/ml, whereas the corresponding value in ethanol is 63 mg/ml. Given the incorporated properties of both solvents, the volume ratio of acetone and ethanol was adjusted to balance the solvent solvency and solution viscosity. In contrary to obtained results of using acetone alone, when the mixture of ethanol and acetone was used at 5:1 V/V ratio, with the increase of drug concentration, from 10 mg/ml to 50mg/ml the average particle sized did not rise. However, a conversion appeared and the particle size increased when ethanol increased from 5:1. Therefore, it could be suppose that the ratio of 5:1 should be appropriate for the co-solvents system.

Effect of Temperature

The effect of temperature on the particle size is revealed in Table 1 and Figure S2 (supplementary file). The mean size of CLX crystals decreased from 17.75 μm to 9.96 μm in length and narrowed the particle size distribution as the temperature decreases from 25 °C to 4 °C. Temperature influences various parameters of the precipitation process which can account for the phenomenon of size decrease. The equilibrium solubility of drug becomes lower with the temperature reduce, which results in higher degree of supersaturation. It is well known that the rate of nucleation increases with increasing of supersaturation leading to formation of larger numbers of nuclei and less growth of individual crystals. Moreover, the crystal growth rate decreases with reductions in the temperature.³¹ As a result, the drug particles are small at low temperature. The reduction of solubility of drug decreases the Ostwald ripening phenomenon resulting in a reduced aggregation of the particles and consequently formation of crystals with more homogenous particle size distribution. As far as the dependency of particle size on temperature is concerned in previous studies, a decrease in the antisolvent temperature generally reduces the particle size and narrows the particle size distribution.^{22-24,32}

Table 1. Mean particle size of prepared samples.

Samples	Drug solution concentration (mg/ml)	Antisolvent/solvent ratio	Stirring (rpm)	Temperature (°C)	Mean particle size \pm SD
F1	10	5	1000	25	17.75 \pm 0.42
F2	10	20	1000	25	14.71 \pm 0.38
F3	20	5	1000	25	20.01 \pm 0.46
F4	20	20	1000	25	15.86 \pm 0.45
F5	10	5	1200	25	9.06 \pm 0.45
F6	10	5	1000	4	9.96 \pm 0.48

Effect of Stirring Speed

Table 1 and Figure S2 (supplementary file) shows that stirring speed has a significant effect ($p < 0.05$) on the mean particle size and size distribution. Large particles with a mean size of $17.75 \mu\text{m}$ are obtained at a stirring speed of 1000 rpm. When the stirring speed is 1200 rpm, produced particles are smaller, with a mean size of $9.06 \mu\text{m}$, compared to that obtained at 1000 rpm. Furthermore, at higher stirring speed, the particle size distribution grows to be narrower. These results can be interpreted by considering the micromixing between the solution and AS. Higher stirring speed decreases the mass transfer resistance and increases diffusion rate between the solution and AS, which results in rapid high homogenous supersaturation and, therefore, fast nucleation to generate particles with smaller size and narrower size distribution.

Characterizations of the CLX Crystals Obtained under the Optimal Condition

To further elucidate the effect of the precipitation process on the particle formation, other characterizations are also used. The CLX crystals are those prepared under the optimum condition, and the process parameters are as follows: AS/S ratio, 20/1 (v/v); concentration of CLX-acetone solution, 50 mg/mL; temperature, $4 \text{ }^\circ\text{C}$ and stirring speed, 1200 rpm.

Morphology

The SEM images of the untreated and CLX crystals prepared under the optimum condition are shown in

Figure 3. It is obvious that the external shape of the untreated CLX particles is an irregular tabular. It should be noted that the magnification of the images of the processed particles are different. Obviously, the particle size of prepared CLX is significantly smaller and more uniform than that of untreated CLX. It is found that the crystals prepared under various conditions exhibit the same morphology (Figures not shown).

FT-IR, XRD and DSC Analysis

The molecular structure and the crystalline of the untreated and optimized CLX were studied by means of XRD, FT-IR and DSC. The resultant profiles are shown in Figure S3, S4 and 4 (supplementary file).

XRD patterns indicated that the micronized CLX are crystallized and keep the same crystalline peaks between 10° and 40° as untreated CLX. Therefore, it can be declared that the crystal structure of the CLX was not changed after the precipitation process. XRD patterns show that the peaks of the micronized CLX are broader than those of the untreated CLX. This may be attributed to smaller particle size of the micronized CLX compared to untreated sample,³³ which was confirmed with the SEM photomicrographs.

FTIR spectra between untreated and microsized CLX are identical, which demonstrated that the whole preparation process did not influence the chemistry composition of CLX.

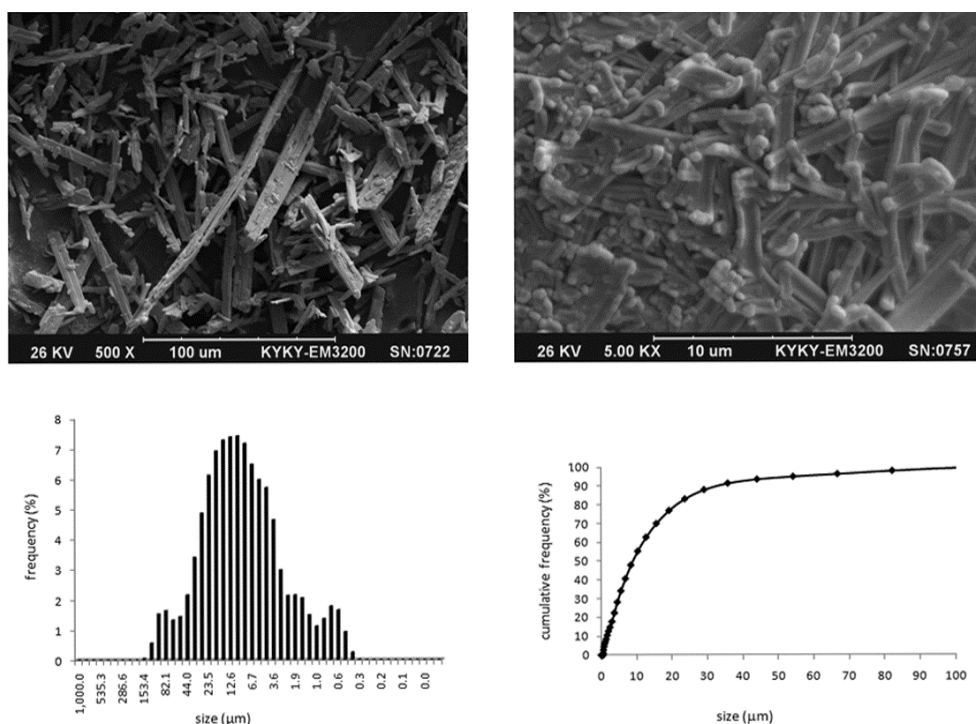


Figure 3. SEM photographs of CLX: (up) untreated CLX, (down) micronized CLX obtained under optimum condition and particle size distribution of micronized CLX obtained under optimum condition.

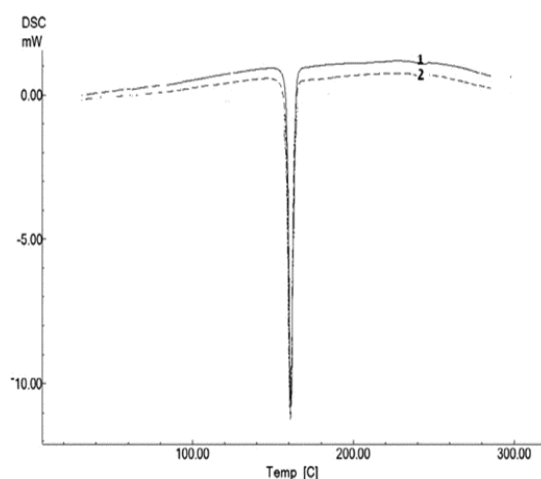


Figure 4. DSC scans of (1): micronized CLX obtained under optimum condition and (2): untreated CLX.

DSC analyses were also carried out to screen the differences in solid state of CLX in the samples. DSC thermograms (Figure 4) showed that there was no significant difference between melting points of untreated CLX (160 °C) and the micronized sample confirming the XRD, that both samples had the same crystalline structure.

Conclusion

In this paper, we proposed a liquid antisolvent precipitation pathway without any stabilizer to prepare micronized CLX particles. In the precipitation of CLX, water is used as the antisolvent and acetone as the solvent, as superior supersaturation could be achieved. There are several factors that influence the process, such as the volume ratio of antisolvent to solution, the concentration of CLX-acetone solution, the temperature, and the stirring speed. The particle size of CLX decreases as the volume ratio of antisolvent to solvent and the stirring speed increase. Furthermore, decreasing the temperature can also result in the smaller particles. However, increasing the concentration of CLX-acetone solution leads to increasing the particle size. In this work, we found out that this effect could be compensated by using ethanol and acetone as co solvents with optimum volume ratio of ethanol: acetone (5:1). By adjusting the process parameters without any surfactant, the particle size of CLX particles can be well-controlled with the same chemical and crystal structure as the untreated CLX.

Conflict of interests

The authors claim that there is no conflict of interest.

Supplementary materials

Supplementary file contains Figure S1-S4.

References

1. Rasenack N, Steckel H, Müller BW. Micronization of anti-inflammatory drugs for pulmonary delivery by a controlled crystallization process. *J Pharm Sci.* 2003;92(1):35-44. doi:10.1002/jps.10274
2. Horn D, Rieger J. Organic nanoparticles in the aqueous phase—theory, experiment, and use. *Angew Chem Int Ed.* 2001;40(23):4330-61. doi:10.1002/1521-3773(20011203)40:23<4330::aid-anie4330>3.0.co;2-w
3. Steckel H, Thies J, Müller BW. Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide. *Int J Pharm.* 1997;152(1):99-110. doi:10.1016/s0378-5173(97)00071-9
4. Domingo C, Berends E, van Rosmalen GM. Precipitation of ultrafine organic crystals from the rapid expansion of supercritical solutions over a capillary and a frit nozzle. *J Supercrit Fluids.* 1997;10(1):39-55. doi:10.1016/s0896-8446(97)00011-9
5. Chattopadhyay P, Gupta RB. Production of griseofulvin nanoparticles using supercritical CO₂ antisolvent with enhanced mass transfer. *Int J Pharm.* 2001;228(1):19-31. doi:10.1016/s0378-5173(01)00803-1
6. Zhong J, Shen Z, Yang Y, Chen J. Preparation and characterization of uniform nanosized cephadrine by combination of reactive precipitation and liquid anti-solvent precipitation under high gravity environment. *Int J Pharm.* 2005;301(1-2):286-93. doi:10.1016/j.ijpharm.2005.06.005
7. Willart JF, Descamps M. Solid state amorphization of pharmaceuticals. *Mol Pharm.* 2008;5(6): 905-20. doi:10.1021/mp800092t
8. Ticehurst MD, Basford PA, Dallman CI, Lukas TM, Marshall PV, Nichols G, et al. Characterisation of the influence of micronisation on the crystallinity and physical stability of revatropate hydrobromide. *Int J Pharm.* 2000;193(2):247-59. doi:10.1016/s0378-5173(99)00347-6
9. Briggner LE, Buckton G, Bystrom K, Darcy P. The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. *Int J Pharm.* 1994;105(2):125-35. doi:10.1016/0378-5173(94)90458-8
10. Brodka-Pfeiffer K, Langguth P, Graß P, Häusler H. Influence of mechanical activation on the physical stability of salbutamol sulphate. *Eur J Pharm Biopharm.* 2003;56(3):393-400. doi:10.1016/s0939-6411(03)00134-6
11. Begat P, Young PM, Edge S, Kaerger JS, Price R. The effect of mechanical processing on surface stability of pharmaceutical powders: Visualization by atomic force microscopy. *J*

- Pharm Sci. 2003;92(3):611-20. doi:10.1002/jps.10320
12. Waltersson JO, Lundgren P. The effect of mechanical comminution on drug stability. *Acta Pharm Suec.* 1985;22(5):291-300.
13. Buckton G, Darcy P, Greenleaf D, Holbrook P. The use of isothermal microcalorimetry in the study of changes in crystallinity of spray-dried salbutamol sulphate. *Int J Pharm.* 1995;116(1):113-8. doi:10.1016/0378-5173(94)00322-v
14. Ruch F, Matijević E. Preparation of micrometer size budesonide particles by precipitation. *J Colloid Interface Sci.* 2000;229(1):207-11. doi:10.1006/jcis.2000.7012
15. Minyi Z, Jianfeng C, Xiaolin L. Preliminary Research on Recrystallization Method to Produce Micronized Ibuprofen Particles. *Chem Ind Eng Prog.* 2003;22(5):524-8.
16. Jing M, Yuhong W, Jianfeng C, Jimmy W. Preparation of ultrafine particles of salbutamol sulfate by anti-solvent precipitation. *J Beijing Univ Chem Technol Natur Sci.* 2003;30(6):6-9.
17. Hida T, Kozaki KI, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, et al. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res.* 2000;6(5):2006-11.
18. Shishodia S, Koul D, Aggarwal BB. Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF- κ B activation through inhibition of activation of I κ B α kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis. *J Immunol.* 2004;173(3):2011-22. doi:10.4049/jimmunol.173.3.2011
19. Chawla G, Gupta P, Thilagavathi R, Chakraborti AK, Bansal AK. Characterization of solid-state forms of celecoxib. *Eur J Pharm Sci.* 2003;20(3):305-17. doi:10.1016/s0928-0987(03)00201-x
20. Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B, et al. Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. *J Pharmacol Exp Ther.* 2001;297(2): 638-45.
21. Dalvi SV, Dave RN. Controlling particle size of a poorly water-soluble drug using ultrasound and stabilizers in antisolvent precipitation. *Ind Eng Chem Res.* 2009;48(16):7581-93. doi:10.1021/ie900248f
22. Wang Z, Chen JF, Le Y, Shen ZG, Yun J. Preparation of ultrafine beclomethasone dipropionate drug powder by antisolvent precipitation. *Ind Eng Chem Res.* 2007;46(14):4839-45. doi:10.1021/ie0615537
23. Zhang ZB, Shen ZG, Wang JX, Zhao H, Chen JF, Yun J. Nanonization of megestrol acetate by liquid precipitation. *Ind Eng Chem Res.* 2009; 48(18):8493-9. doi:10.1021/ie900944y
24. Zhao H, Wang JX, Wang QA, Chen JF, Yun J. Controlled liquid antisolvent precipitation of hydrophobic pharmaceutical nanoparticles in a microchannel reactor. *Ind Eng Chem Res.* 2007;46(24):8229-35. doi:10.1021/ie070498e
25. Dirksen J, Ring T. Fundamentals of crystallization: kinetic effects on particle size distributions and morphology. *Chem Eng Sci.* 1991;46(10):2389-427. doi:10.1016/0009-2509(91)80035-w
26. Schwarzer HC, Schwertfirm F, Manhart M, Schmid HJ, Peukret W. Predictive simulation of nanoparticle precipitation based on the population balance equation. *Chem Eng Sci.* 2006;61(1):167-81. doi:10.1016/j.ces.2004.11.064
27. Zhang X, Xia Q, Gu N. Preparation of all-trans retinoic acid nanosuspensions using a modified precipitation method. *Drug Dev Ind Pharm.* 2006;32(7):857-63. doi:10.1080/03639040500534184
28. Dalvi SV, Dave RN. Controlling particle size of a poorly water-soluble drug using ultrasound and stabilizers in antisolvent precipitation. *Ind Eng Chem Res.* 2009;48(16):7581-93. doi:10.1021/ie900248f
29. Zhang HX, Wang JX, Zhang ZB, Le Y, Shen ZG, Chen JF. Micronization of atorvastatin calcium by antisolvent precipitation process. *Int J Pharm.* 2009;374(1-2):106-13. doi:10.1016/j.ijpharm.2009.02.015
30. Zhang JY, Shen ZG, Zhong J, Hu TT, Chen JF, Ma ZQ, et al. Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants. *Int J Pharm.* 2006;323(1-2):153-60. doi:10.1016/j.ijpharm.2006.05.048
31. Chen JF, Zhou MY, Shao L, Wang YY, Yun J, Chew NYK, et al. Feasibility of preparing nanodrugs by high-gravity reactive precipitation. *Int J Pharm.* 2004;269(1):267-74. doi:10.1016/j.ijpharm.2003.09.044
32. Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, et al. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. *Eur J Pharm Sci.* 2010;40(4):325-34. doi:10.1016/j.ejps.2010.04.006
33. Torrado G, Fraile S, Torrado S, Torrado S. Process-induced crystallite size and dissolution changes elucidated by a variety of analytical methods. *Int J Pharm.* 1998;166(1):55-63. doi:10.1016/s0378-5173(98)00021-0