Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 301 (2005) 286–293
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation and characterization of uniform nanosized cephradine by combination of reactive precipitation and liquid anti-solvent precipitation under high gravity environment

Jie Zhong, Zhigang Shen, Yan Yang, Jianfeng Chen *,[1]

Key Laboratory for Nanomaterials, Ministry of Education, Research Center of the Ministry of Education for High Gravity Engineering and Technology, Beijing University of Chemical Technology, Beijing 100029, PR China

Received 3 February 2005; received in revised form 20 May 2005; accepted 5 June 2005
Available online 28 July 2005

Abstract

In this work, a novel direct method, which was combined with reactive precipitation and liquid anti-solvent precipitation under high gravity environment, had been developed to prepare nanosized cephradine with narrow particle size distribution. Compared with commercial crude cephradine, the prepared cephradine showed a significant decrease in particle size, a significant increase in the specific surface area and shorter dissolving time when used for injection. The characteristic particle size was between 200–400 nm. The specific surface area increased from 2.95 to 10.87 m^2/g after micronization. When the amount of L-arginin decreased from 0.25 to 0.18 g, the mixture of nanosized cephradine and L-arginine could still dissolve in 1 min. The X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) analysis indicated that the physical characteristics and molecular states remained unchanged after the recrystallization process. This method had potential application in industrial fields because of its low cost, efficient processing and the ease of scaling-up.

Keywords: Nanosized pharmaceuticals; Cephradine; Reactive precipitation; Liquid anti-solvent precipitation; Dissolving time

1. Introduction

Cephradine, a member of the first generation cephalosporins, is widely used in clinic for its activity against both Gram-positive and Gram-negative microorganisms. Cephradine is sparingly soluble in water and appears to be slowly released from the site of injection to give the level of antibiotic in plasma. A lot of newly developed pharmaceuticals have low solubility and dissolution rate just like cephradine, resulting in low bioavailability and/or erratic absorption, which have presented obstacles in marketing them for clinical use. Many techniques have been utilized

* Corresponding author. Tel.: +86 10 64446466; fax: +86 10 64434784.
E-mail address: chenjf@mail.buct.edu.cn (J. Chen).
[1] Present address: Beijing University of Chemical Technology, No. 15 Bei San Huan Dong Road, P.O. Box 35, Beijing 100029, PR China.

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.06.005

to improve the dissolution rate and bioavailability for poorly soluble pharmaceuticals in the development of pharmaceuticals formulation (Betageri and Makarla, 1995; Ward and Schultz, 1995; Shinji et al., 1999). One of the most effective methods is by reduction of their sizes to the nanometer range (Rawlings and Tindall, 1977; Hu et al., 2002; Rasenack and Müller, 2002).

Conventional techniques for the particle size reduction are mechanical techniques based on high shear or impaction including microfluidization, high-pressure homogenization and milling (Ripple, 1985; Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Müller et al., 2001). However, these techniques are inefficient due to high-energy input or pharmaceuticals denaturation while milling. Spray drying (Esclisa-Díaz et al., 1996; Elversson et al., 2003) and supercritical fluids techniques (Domingo et al., 1997; Reverchon and Porta, 1999; Chattopadhyay and Gupta, 2001) have received a particular emphasis on preparing pharmaceuticals with optimal size directly and non-occurrence of the pharmaceuticals denaturation, whereas these techniques have the limitation of low yields and high equipment expenditure.

In contrast with techniques hereinbefore, liquid precipitation technique, which includes reactive precipitation and liquid anti-solvent precipitation, has good prospect in industrial fields because of its low cost, convenience in processing, as well as the ease of scale-up (Violanto and Fischer, 1989; Ruch and Matijević, 2000; Cushing et al., 2004). However, the tendency of the pharmaceutical particles to grow and hard to inhibit the growth, has also restricted the wide use of this method. Hu et al. (2002) developed a spray freezing into liquid technology to produce micronized pharmaceutical ingredients to inhibit crystal growth of pharmaceutical particles. The cost will be increased due to the use of cryogen, such as liquid nitrogen. Rasenack and Müller (2002) prepared microsized pharmaceutical particles in the presence of stabilizing agents. The stabilizing agents can stop the molecular association and crystal growth by forming a protective layer around the nucleation. But the stabilizing agent has introduced a potential toxicity.

In this study, a new method directly to prepare nanosized pharmaceutical particles is proposed. This method, which combines the reactive precipitation and anti-solvent precipitation, can overcome the disadvantages that each of these techniques known when used alone. It is suitable for pharmaceuticals, which are amphoteric like cephradine and insoluble in most aqueous and organic solvents but soluble in acidic or alkaline solutions in salt form. The pharmaceuticals are prepared by adding acid or alkali (original reactant) to the salt solution. In this study, a mixture of original reactant (triethylamine) and additional solvent (acetone), which is miscible with original reactant, was used without the introduction of foreign materials into the body. The effect of additional solvent is just as anti-solvent to generate a higher supersaturation and the solution to anti-solvent ratio is 3:1.

The conventional precipitation process carried out in stirred tanks or column reactors cannot guarantee the quality of the products and control the morphology, size and size distribution of the produced particles by reason of the poor micromixing. In this paper, a new technology called high gravity technology for the mass production of nanoparticles was used to prepare nanosized pharmaceutical powder. High gravity technology in the form of a rotating packed bed (RPB) on the earth has been used to intensify mass transfer and micromixing (Chen et al., 2000). The technique has successfully been used to produce nanomaterials including $CaCO_3$ (15–40 nm), $Al(OH)_3$ (1–10 nm) and $SrCO_3$ (40 nm). Apart from the success with inorganic nanomaterials, the application of this technique for nanosized benzoic acid and microsized cephradine as model drugs was also reported (Chen et al., 2004; Shen et al., 2004).

The aim of this paper is to study the feasibility of the micronization of organic pharmaceutical compounds, such as cephradine, using a combination of reactive precipitation and anti-solvent precipitation under high gravity environment.

2. Materials and methods

2.1. Materials and equipment

The cephradine hydrochloride solution was obtained by dissolving commercial crude cephradine in dilute hydrochloric acid, the pH value and concentration of the solution were about 2.5 and 0.2 g/ml, respectively. The commercial crude cephradine was supplied by BETA Inc., NCPC, China. Acetone (industrial grade), hydrochloride acid (industrial

grade) and triethylamine (industrial grade) were all supplied by BETA Inc., NCPC, China too.

The experimental set-up for the high gravity precipitation is shown schematically in Fig. 1. The key part of the rotating packed bed (Higee machine) is a packed rotator. More details about this equipment can be seen in our previous paper (Chen et al., 2000). The cephradine hydrochloride solution and the mixture of original reactant (triethylamine) and anti-solvent (acetone) were continuously pumped from their storage tanks into different slotted pipe distributors in the RPB simultaneously. After entering the RPB, the two fluids going through the packing were spread or split into very fine droplets, thread and thin film under the high gravity environment. This results in intense micromixing among the fluid elements, which greatly favors homogenous nucleation of the product in the reaction and precipitation process.

2.2. Preparation of nanosized cephradine

The two liquid streams, cephradine hydrochloride solution and the mixture of original reactant (triethylamine) and anti-solvent (acetone), entered the RPB to mix and react together to yield particles. The mixture flowed in the radial direction under centrifugal force, passed the packing and outside space between the rotator and shell, and then finally left the equipment through the liquid outlet for collection. After the reaction was finished, the cephradine suspension was filtered and the solid was then washed with acetone.

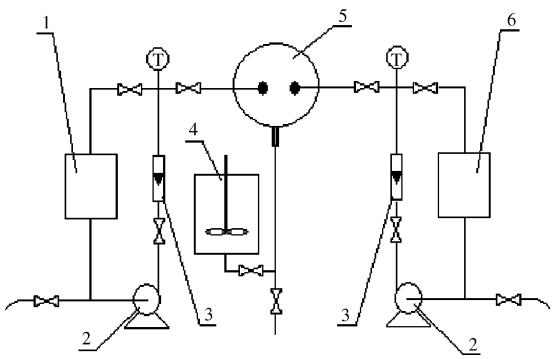

Fig. 1. Schematic representation of high gravity apparatus for cephradine particle precipitation. 1, Mixture of triethylamine and acetone tank; 2, pump; 3, flowmeter; 4, stirring tank; 5, high gravity reactor and 6 cephradine hydrochloride solution tank.

The powder was then dried at 40 °C under vacuum condition.

2.3. Product characterization

2.3.1. The morphology and particle size studies

The morphology of the cephradine particles was determined by scanning electron microscopy (SEM). The dried cephradine powder was fixed on aluminium stubs using double-sided adhesive tape and coated with Au at 50 mA for 30 s using a Pelco Model 3 sputter-coater under an Ar atmosphere. A Cambridge S250MK3 scanning electron microscope (Cambridge Instruments Inc., UK) at an accelerating voltage of 10 kV with a secondary electron detector was used to obtain digital images of the samples. The particle size and particle size distribution were determined by the IBAS I/II Image Analyzer System (Germany) via the obtained SEM photographs.

2.3.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded with a Bruker IFS66 spectrometer in the range 400–4000 cm^{-1} using a resolution of 2 cm^{-1} and 32 scans. Samples were diluted with KBr mixing powder at 1% and pressed to obtain self-supporting disks.

2.3.3. X-ray diffraction studies (XRD)

X-ray diffraction analysis was performed using XRD-6000 (SHIMADZU Inc., Japan) to detect any changes in the physical characteristics and crystallinity of the nanosized cephradine. The measuring unit consists of a rotating anode in transmission technique and with the following specifications: Cu $K\alpha_1$ radiation generated at 30 mA and 40 kV. The scanning speed is 10°/min from 5° to 55° with a step size of 0.02°.

2.3.4. BET surface area

The specific surface area of commercial crude cephradine and nanosized cephradine was determined using the gas adsorption method. Calculation is based on the BET equation. Surface Area Analyzer ASAP 2010-M (Micromeritics Instrument Corporation, America) was used. Before measuring, the sample of cephradine was degassed for 4 h.

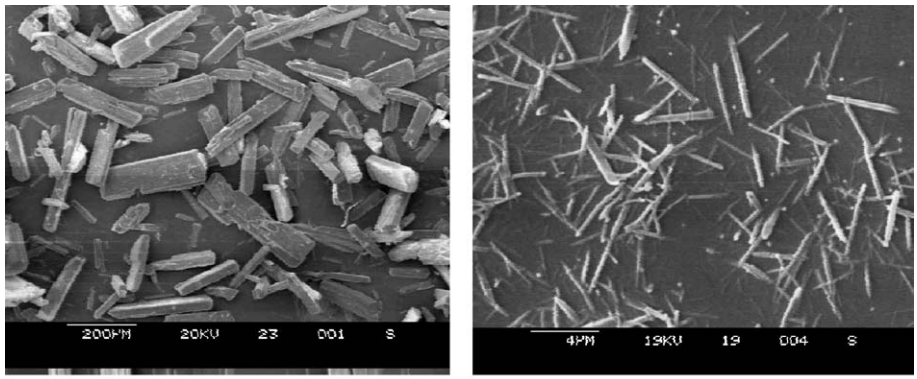

Fig. 2. SEM photographs of commercial crude cephradine and nanosized cephradine.

2.3.5. Dissolving time of cephradine for injection

Cephradine for injection medicament was a dry powder mixture of cephradine and L-arginine as solubilizer. The L-arginine to cephradine ratio was ranged from about 1:2 to 1:2.9 in weight ratio, wherein the weight of cephradine was 0.5 g. Deionized water (5 ml) was added to the mixture and shaken gently till clear solution was observed. This indicated complete dissolution and rendered the mixture suitable as an injectable formulation. The time taken for complete dissolution was recorded. Three readings were taken and the mean value was obtained.

3. Results and discussion

3.1. Particles size and morphology

The SEM photographs of the commercial crude cephradine and nanosized cephradine were shown in Fig. 2. The particle size distributions were shown in Fig. 3. The width of the as-prepared cephradine is 200–400 nm with uniform size distribution and the mean particle size is about 300 nm. The width of the commercial crude cephradine is about 20–70 μm with non-uniform size distribution and irregular morphology and the mean particle size is about 37 μm. The

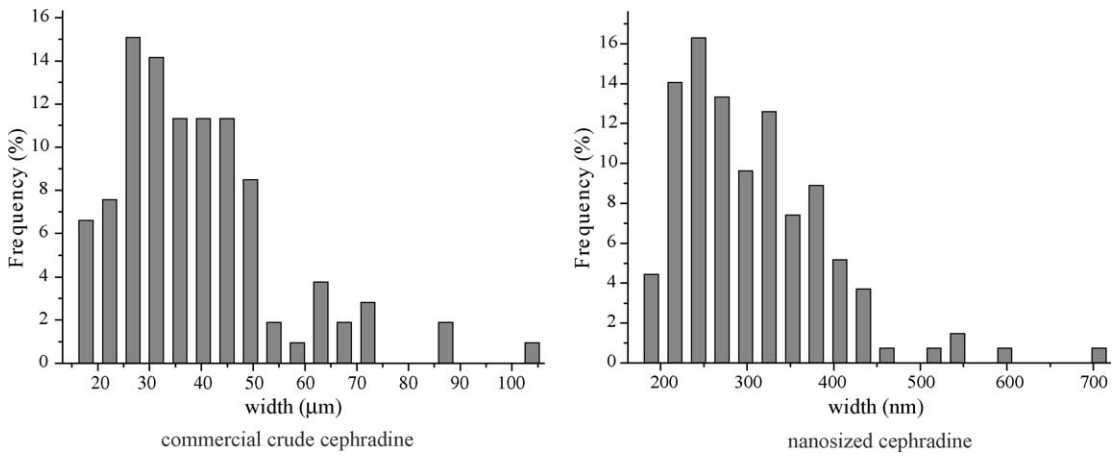

Fig. 3. The particle size distribution of commercial crude cephradine and nanosized cephradine.

width decreased more than 100 times after recrystallization.

Chen and Shao (2003) studied the rules of obtaining nanoparticles with narrow size distribution and controlled morphology by reactive precipitation method. Besides the well-micromixing and well-macromixing are expected, another factor is the separation of the reaction and nucleation zones (RNZ) from the crystal growth zone (CGZ). In this study, when the mixture of the solvents commixed with the cephradine hydrochloride solution, the formulated cephradine particles dispersed into acetone quickly to inhibit the growth of the particles. In addition, the existence of a large volume of acetone can also achieve higher supersaturation. The magnitude of micromixing and mass transfer rate in an RPB are much larger than these in a conventional stirring tank reactor, which are very helpful for generating higher supersaturated concentrations and more uniform spatial concentration of the product in the precipitation process, and thus obtaining the nanosized cephradine particles. At the same time, the particle size distribution is narrower than that of cephradine prepared in conventional stirred tank reactor. The width of the as-prepared cephradine is 200–400 nm with uniform size distribution and the mean particle size is about 300 nm. The conventional technique of crystallization for cephradine is reactive precipitation in stirred tank reactor. The cephradine size obtained by this technique was about 37 μm with irregular morphology and broad particle size distribution due to poor micromixing and macromixing. Shen et al. (2004) prepared microsized cephradine by reactive precipitation using high gravity reactive precipitator instead of stirred tank reactor. The cephradine particle size obtained by this method was reduced to 4 μm due to higher degree of supersaturation generated by RPB than that by the traditional stirring tank reactor. Because of the slow nucleation rate, fast growth rate and slow separation of the RNZ and CGZ, the size of the cephradine particles was reduced by 10 times only, hence the size is in the micrometer range.

3.2. FT-IR studies

FT-IR analysis was performed to evaluate the molecular states of commercial crude cephradine and nanosized cephradine according to the information on vibrations in the powder composition. FT-IR spectra of unprocessed and processed cephradine were reported in Fig. 4. From the spectra, it can be concluded that there are no changes in composition induced by the micronization process.

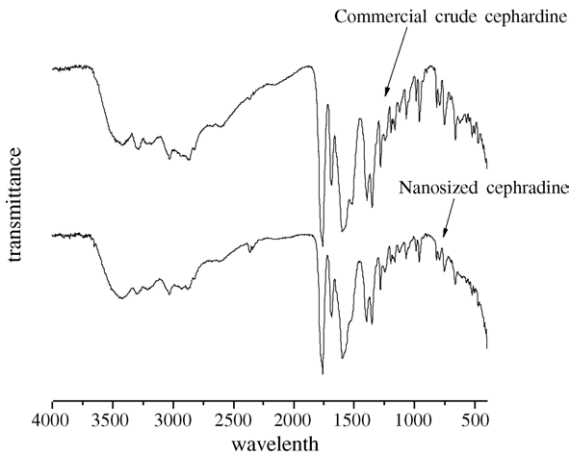

Fig. 4. FT-IR spectra of commercial crude cephradine and nanosized cephradine.

3.3. XRD studies

Fig. 5 shows the XRD patterns of the nanosized cephradine and commercial crude cephradine. It is clear that the peak positions of the nanosized sample is the same as that of commercial crude cephradine, which indicated that micronization would not affect the physical characteristics of cephradine. But the full width at half maximum (FWHM) values of the peaks of the nanosized cephradine were broader than those of

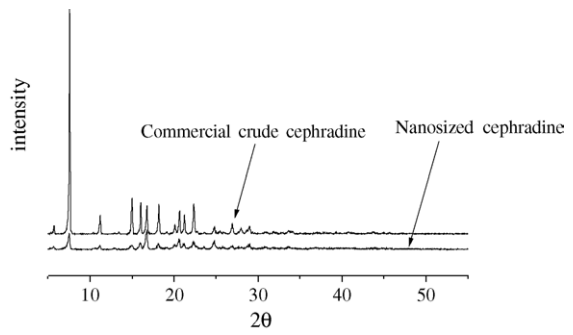

Fig. 5. XRD patterns of commercial crude cephradine and nanosized cephradine.

Table 1
Comparison of the *d*-value, FWHM and intensity of the commercial crude cephradine and the nanosized cephradine

Commercial crude cephradine				Nanosized cephradine			
2θ (°)	*d* (Å)	FWHM (°)	Intensity (counts)	2θ (°)	*d* (Å)	FWHM (°)	Intensity (counts)
7.6969	11.47687	0.1200	2890	7.6189	11.59419	0.25020	193
15.0551	5.88002	0.15580	479	14.9815	5.90874	0.36300	51
16.0986	5.50116	0.11370	394	16.0350	5.52283	0.25150	80
16.8286	5.26413	0.17400	379	16.7840	5.27802	0.27140	242
18.2424	4.85922	0.14240	378	18.1812	4.83544	0.26670	64
20.7062	4.28626	0.14270	297	20.6255	4.30284	0.26250	121
22.4028	3.96534	0.16820	402	22.3748	3.97024	0.29940	81

commercial crude cephradine (see Table 1). The average crystallite sizes could be evaluated by means of the Scherrer's equation (Torrado et al., 1998):

$$d_{\mathrm{XRD}} = \frac{K\lambda}{\beta \cos\theta}$$

where β is the difference in FWHM between broadened and standard maxima. According to the equation, larger FWHM values indicate smaller particle sizes. This was consonant with the SEM photographs.

The XRD patterns also shows that the peaks of commercial crude cephradine have higher intensities than those of nanosized cephradine, indicating higher crystallinity in the former case. The pharmaceuticals that have lower crystallinity often resulted in higher solubility and bioavailability (Sarkari et al., 2002). The loss in crystallinity in nanosized cephradine is expected to enhance its solubility and bioavailability.

3.4. Dissolving time of cephradine for injection

Cephradine has been formulated for parenteral application either as aqueous suspensions, or by preparing water-soluble derivatives of the parent compound for its limited water solubility. The usage of solids in suspension severely limits the mode of parenteral administration. Furthermore, preparation of pharmaceutically acceptable solid derivatives is frequently accompanied by significant yield losses. Some suitable solid additives, such as L-arginine as solubilizer, are mixed with cephradine to obtain cephradine compositions for injection. However, the L-arginine has side effects to kidney, it is expected to diminish the amount of L-arginine blended with cephradine.

As appreciated from Fig. 6, we can see that the dissolving time of nanosized cephradine for injection has significantly decreased compared with commercial crude cephradine. The dissolving time is about 0.5 and 3 min, respectively, when the amount of L-arginine was 0.25 g. When the amount of L-arginin decreased, the dissolving time of the mixture of nanosized cephradine with L-arginine did not show any significant changes but that of commercial crude cephradine increased from 3 to 9 min. When the amount of L-arginin decreased from 0.25 to 0.18 g, the mixture of nanosized cephradine with L-arginine can still dissolve in 1 min. The decrease in dissolving time can be explained by the Noyes–Whitney equation, which states that dissolution rate depended on surface area. The specific surface area of nanosized cephradine is 10.87 m^2/g, compared with that of commercial crude cephradine, which is 2.95 m^2/g. Decreased amount of

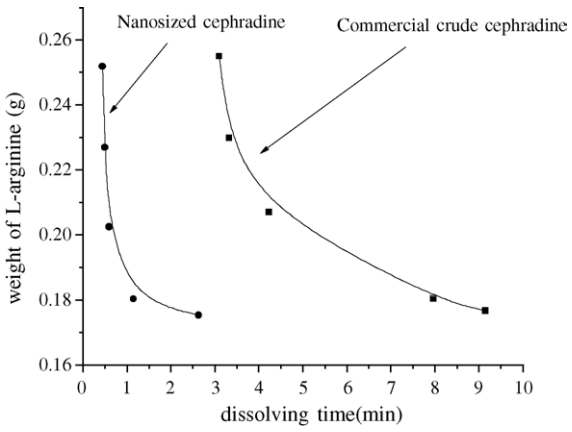

Fig. 6. Dissolving time of cephradine for injection.

L-arginine in nanosized cephradine for injection will result in lower cost of the cephradine for injection and debase the side effect of L-arginine.

4. Conclusions

In this study, nanosized particles with a narrow particle size distribution of cephradine were prepared by using a combination of reactive precipitation and liquid anti-solvent precipitation under high gravity environment. The as-prepared cephradine showed significant decrease in particle size with uniform particle size distribution because of higher degree of supersaturation generated by RPB and the anti-solvent acetone. The width of as-prepared cephradine was about 200–400 nm and the mean particle size was about 300 nm. The specific surface area increased from 2.95 to 10.87 m^2/g after micronization. There were no change of the physical characteristics and molecular states after processing according to the XRD and FT-IR analysis. The nanosized cephradine for injection also showed a shorter dissolving time than that of commercial crude cephradine. When the amount of L-arginin decreased from 0.25 to 0.18 g, the mixture of nanosized cephradine with L-arginine could still dissolve in 1 min. The reduction in the amount of L-arginine would result in lower cost of the cephradine for injection and debase the side effect of L-arginine. This method might be applied in industrial fields because of its low cost as well as efficient processing and the ease of scale-up.

Acknowledgements

The authors kindly acknowledge the financial support from the “863” Plan of China (Grant No. 2004AA218042) and NSF of China (No. 20325621, 20236020). The authors also wish to thank BETA Inc. NCPC, China for supplying the cephradine, acetone, hydrochloride acid and triethylamine.

References

Betageri, G.V., Makarla, K.R., 1995. Enhancement of dissolution of glyburide by solid dispersion and lyophilization techniques. Int. J. Pharm. 126, 155–160.

Chattopadhyay, P., Gupta, R.B., 2001. Production of griseofulvin nanoparticles using supercritical CO_2 anti-solvent with enhanced mass transfer. Int. J. Pharm. 228, 19–31.

Chen, J.F., Wang, Y.H., Guo, F., Wang, X.M., Zheng, Ch., 2000. Synthesis of nanoparticles with novel technology: high gravity reactive precipitation. Ind. Eng. Chem. Res. 39, 948–954.

Chen, J.F., Shao, L., 2003. Mass production of nanoparticles by high gravity reactive precipitation technology with low cost. Chin. Particuol. 1, 64–69.

Chen, J.F., Zhou, M.Y., Shao, L., Wang, Y.H., Yun, J., Chew, N.Y.K., Chan, H.K., 2004. Feasibility of preparing nanodrugs by high gravity reactive precipitation. Int. J. Pharm. 269, 267–274.

Cushing, B.L., Kolesnichenko, V.L., O’Coonor, C.J., 2004. Recent advances in the liquid-phase syntheses of inorganic nanoparticles. Chem. Rev. 104, 3893–3946.

Domingo, C., Berends, E., van Rosmalen, G.M., 1997. Precipitation of ultrafine organic crystals from the rapid expansion of supercritical solutions over a capillary and a frit nozzle. J. Supercrit. Fluids 10, 39–55.

Elversson, J., Millqvist-Fureby, A., Alderborn, G., Elofsson, U., 2003. Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. J. Pharm. Sci. 92, 900–910.

Esclisa-Díaz, M.T., Guimaraens-Méndez, M., Pérez-Marcos, M.B., Vila-Jato, J.L., Torres-Labandeira, J.J., 1996. Characterization and in vitro dissolution behaviour of ketoconazole/β-and 2-hydroxypropyl-β-cycoldextrin inclusion compounds. Int. J. Pharm. 143, 203–210.

Hu, J.H., Rogers, T.L., Brown, J., Young, T., Johnston, K.P., Williams III, R.O., 2002. Improvement of dissolution rates of poorly water soluble APIs using novel spray freezing into liquid technology. Pharm. Res. 19, 1278–1284.

Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int. J. Pharm. 125, 309–313.

Liversidge, G.G., Cundy, K.C., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int. J. Pharm. 125, 91–97.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulation in therapy rationale for development and what we can expect for the future. Adv. Drug. Deliv. Rev. 47, 3–19.

Rasenack, N., Müller, B.W., 2002. Dissolution rate enhancement by in situ micronization of poorly water-soluble drugs. Pharm. Res. 19, 1894–1900.

Rawlings, E.A., Tindall, B., 1977. Bentley’s Textbook of Pharmaceutics, eighth ed. Baillière Tindall, London, pp. 1–17.

Reverchon, E., Porta, G.D., 1999. Production of antibiotic micro-and nanoparticles by supercritical anti-solvent precipitation. Powder Technol. 106, 23–29.

Ripple, E.G., 1985. Powders. In: Gennaro, A.R. (Ed.), Remington’s Pharmaceutical Sciences, 17th ed. Mack Publishing Company, Easton Pennsylvania, pp. 1585–1594.

Ruch, F., Matijević, E., 2000. Preparation of micrometer size Budesonide particles by precipitation. J. Colloid Interface Sci. 229, 207–211.

Sarkari, M., Brown, J., Chen, X.X., Swinnea, S., Williams III, R.O., Johnston, K.P., 2002. Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int. J. Pharm. 243, 17–31.

Shinji, S., Rika, S., Norio, S., Hiroshi, K., Mitsuru, A., Masahiro, H., 1999. Mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal tract. Int. J. Pharm. 177, 161–172.

Shen, Zh.G., Chen, J.F., Zhong, J., Hu, W.G., Yun, J., 2004. Properties of cephradine produced by high gravity technology. Chin. J. Pharm. 39, 36–39.

Torrado, G., Fraile, S., Torrado, S., Torrado, S., 1998. Process-induced crystallite size and dissolution changes elucidated by a variety of analytical methods. Int. J. Pharm. 166, 55–63.

Violanto, M.R., Fischer, H.W., 1989. Method for making uniformly sized particles from water-insoluble organic compounds. US Patent No. 4,826,689.

Ward, G.H., Schultz, R.K., 1995. Process-induced crystallinity changes in albuterol sulfate and its sulfate and its effect on powder physical stability. Pharm. Res. 12, 773–779.