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Preparation of uniform prednisolone microcrystals by a controlled microprecipitation method

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Abstract

Prednisolone (PDL) microcrystals were successfully prepared by a controlled microprecipitation method. The characterization of PDL microcrystals by SEM and PSD indicated that the hexagonal and tetragonal PDL microcrystals with an average particle size of 1.60 and 1.46 μm could be prepared under a stirring speed of 10,000 rpm at 14 and 4 °C, respectively. The morphology and the particle size of PDL could be well controlled, from 1.60 to 6.12 μm for hexagonal microcrystals and 1.46 to 3.90 μm for tetragonal ones, by altering the operating parameters such as temperature, stirring speed and stabilizers. The XRD, TGA–DSC, FT-IR and physical stability studies demonstrated that the as-prepared hexagonal and tetragonal PDL microcrystals with the same pseudopolymorphic form were much more stable in water than the commercial micronized PDL with another crystal form. The dissolution tests showed that the hexagonal and tetragonal PDL microcrystals exhibited significantly enhanced dissolution property when compared to commercial micronized PDL.

Keywords: Prednisolone; Microcrystals; Microprecipitation; Anti-solvent; Morphology

1. Introduction

Prednisolone (PDL) is a synthetic adrenal corticosteroid at least existing two polymorphic forms and two pseudopolymorphic forms (Young and Eui, 2003), which has potent anti-inflammatory performances and is widely used in a variety of inflammatory conditions such as arthritis, colitis, asthma, bronchitis, certain skin rashes, and allergic or inflammatory conditions of the nose and eyes (Alessi et al., 1996; Eerikainen and Kauppinen, 2003; Mazurek and Szostak, 2006). As a poorly water-soluble drug, PDL exhibits a low solubility and dissolution rate in the gastrointestinal tract, which limits its effective absorption and bioavailability (Zhang et al., 2006).

According to the Noyes–Whitney equation, the dissolution rate could be increased by reducing the particle size at the micro- or nano-scale to increase the interfacial surface area (Douroumis and Fahr, 2006). Several approaches have been attempted to produce micronized PDL particles. Submicron suspension of PDL had been prepared by a high-pressure homogenization process, but dry submicron particles were hard to obtain (Kipp et al., 2005; Keck and Müller, 2006). Presently, PDL dry particles with a mean particle size smaller than 5 μm could be prepared by utilizing the supercritical fluid processing, whose particles showed better properties than the jet-milled products. However, the as-prepared PDL dry particles were unstable in water and had a wide size distribution (Steckel et al., 1997; Meure et al., 2004; Kalogiannis et al., 2005; Meziani et al., 2006; Park et al., 2006).

The existing technologies to prepare microcrystals can be divided into the so-called "top down" and the "bottom up" technologies. The top down technologies are the mechanical comminution of previously formed larger particles, including jet mills, pear-ball mills and high-pressure homogenizers. How-

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ever, these technologies do not represent the idea way for the production of microcrystals because drug substance properties and surface properties are altered in a mainly uncontrolled manner. The "bottom up" technologies start from the molecules which are dissolved and precipitate them by adding the solvent to a non-solvent, such as spray-drying, supercritical fluid (SCF) technology, spray-freezing into liquid process, evaporative precipitation into aqueous solution (EPAS) and liquid solvent change process (Chen et al., 2002; Rogers et al., 2002; Hu et al., 2004; Rasenack and Müller, 2004; Rasenack et al., 2004; Keck and Müller, 2006).

Anti-solvent method is an effective way to prepare micro- or nano-sized drug particles. In this method, briefly, the drug is firstly dissolved in the solvent and the formed solution is quickly poured to the anti-solvent. Precipitation occurs immediately by a rapid desolvation of the drug. Currently, aqueous solutions containing some stabilizers are commonly used as the anti-solvent. When comparing different potential stabilizers, hydroxypropylmethylcellulose (HPMC) was especially shown to stabilize the small drug particles. Since HPMC containing methoxyl and hydroxypropyl groups can form hydrogen bonds between the drug molecule and the polymer, the stabilizer presented in the aqueous solution is absorbed on the surface of the formed hydrophobic drug particles to inhibit crystal growth (Raghavan et al., 2001, 2003). This technique is a rapid and direct process (Rasenack and Müller, 2002; Rasenack et al., 2003a,b), which can be performed with ease. Rasenack et al. (2003a,b) successfully prepared micro-sized drug particles such as beclomethasone-17,21-dipropionate (BDP) and betamethasone-17-valerate (BV) with the addition of HPMC by a solvent change process (namely anti-solvent method) (Raghavan et al., 2001), but they failed in the preparation of PDL.

The objective of this study was to directly prepare hexagonal and tetragonal PDL dry microcrystals by a controlled micro-precipitation method. In addition, the effect of the operation parameters, such as the types of solvent and anti-solvent, the temperature and the stirring speed were also explored. The corresponding physical stability and dissolution property of the as-prepared PDL microcrystals were characterized by scanning electronic microscope (SEM), Fourier transform infrared spectrophotometry (FT-IR), thermogravimetric analysis–differential scanning calorimeter (TGA–DSC), powder X-ray diffraction (XRD) and dissolution testing.

2. Materials and methods

2.1. Materials

The commercial micronized PDL was supplied by Henan Lihua Pharmaceutic Co. Ltd. (China). *N*-Methyl-2-pyrrolidinone (NMP), ethanol, methanol and acetone were analytical grade and purchased from Beijing Yili fine chemicals Co. Ltd. Hydoxypropylmethylcellulose (HPMC, 50–55 mPa s) was obtained from Shandong Ruitai Chemicals Co. Ltd. Deionized water was prepared by Hitech-K flow water purification system.

2.2. Methods

2.2.1. Experimental

PDL NMP solution (1.0 g/ml) as solvent and 0.2 wt% HPMC aqueous solution as initial anti-solvent were firstly prepared. The succeeding process included the following two steps. Step 1: 500 ml of 0.2 wt% HPMC aqueous solution was added into 1000 ml beaker as the initial anti-solvent under a FLUKO® FM300 homogenizer. Then, 5 ml of drug solution was quickly added into the anti-solvent under a stirring speed of 2500–10,000 rpm. Immediately, particles precipitated from the anti-solvent and a milk-like suspension simultaneously formed. Finally, the suspension was filtered, and the filter cake was washed several times to remove most of NMP and HPMC. Hence, 1000 ml filtrate (NMP: 0.5 vol.%; HPMC: 0.1 wt%; water: 99.5 vol.%; PDL: saturated) was obtained. Step 2: 500 ml of the as-obtained filtrate was subsequently employed as the improved anti-solvent for the repeat of step 1. The resulting filter cake could be dried in an oven at 60 °C for 24 h or re-dispersed in deionized water uniformly for spray-drying to achieve the PDL microcrystals.

2.2.2. Particle size, morphology and particle size distribution (PSD)

Particle size and morphology were examined by SEM (JEOL, JSM-6360LV, Japan). The dried PDL 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. The largest diameters of at least 500 particles were measured by Image-Pro Plus software (release 5.0, MediaCybernetics, USA) via the obtained SEM photographs to determine the mean (arithmetic mean) particle size and PSD.

2.2.3. FT-IR, TGA–DSC and XRD characterization

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.

The phase transition of various PDL were analyzed by differential scanning calorimeter (Pyris 1, Perkin-Elmer, USA) at a heating rate of 10 °C/min from 30 to 600 °C. A dry nitrogen purge of 25 ml/min was employed in the process. Calibration of the instrument with respect to temperature and enthalpy was achieved using high purity standard of indium.

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

2.2.4. BET surface area

The specific surface area was determined using the gas adsorption method. Calculation is based on the BET equation. Surface Area Analyzer ASAP 2010-M (Micromeritics

Instrument Corporation, USA) was used. Before measuring, the sample of PDL was degassed for 4 h.

2.2.5. Physical stability studies

Dry powder (4 mg) of each PDL sample was re-dispersed uniformly in 10 ml deionized water. The bottles of resulted suspensions were placed on a table under room conditions, samples were collected at different time for SEM characterization.

2.2.6. UV and drug content analyses

UV–vis spectrophotometer (Shimadzu, UV-2501, Japan) was used for the UV analyses (Spireas and Sadu, 1998; Wittaya-areekul et al., 2006) of all PDL samples in deionized water at 248 nm in triplicate. The calibration curve is $Y = 0.00519 + 0.04012C$; C: 2–18 μg/ml; $R^2 = 0.99984$. Drug contents of the as-prepared products were determined according to the calibration curve and the mean value of the three measured drug contents was adopted.

2.2.7. Dissolution testing

Dissolution testing for PDL sample was carried out using a dissolution apparatus (D-800LS, Tianjin, China) following the USP 29 Apparatus II (paddle) method. Paddle speed and bath temperature were set at 50 rpm and 37.0 ± 0.5 °C, respectively. Approximately 20 mg PDL was placed into vessels containing 900 ml degassed deionized water. Five milliliters of the sample was withdrawn at specific intervals. These samples were filtered using a 0.22 μm filter. The concentration of samples was analyzed in an ultraviolet spectrophotometer (Shimadzu, UV-2501, Japan) at 248 nm. Each sample was analyzed in triplicate.

3. Results and discussion

3.1. The choice of solvent and anti-solvent

As a comparison, the SEM image of the commercial micronized PDL was given in Fig. 1A. The commercial

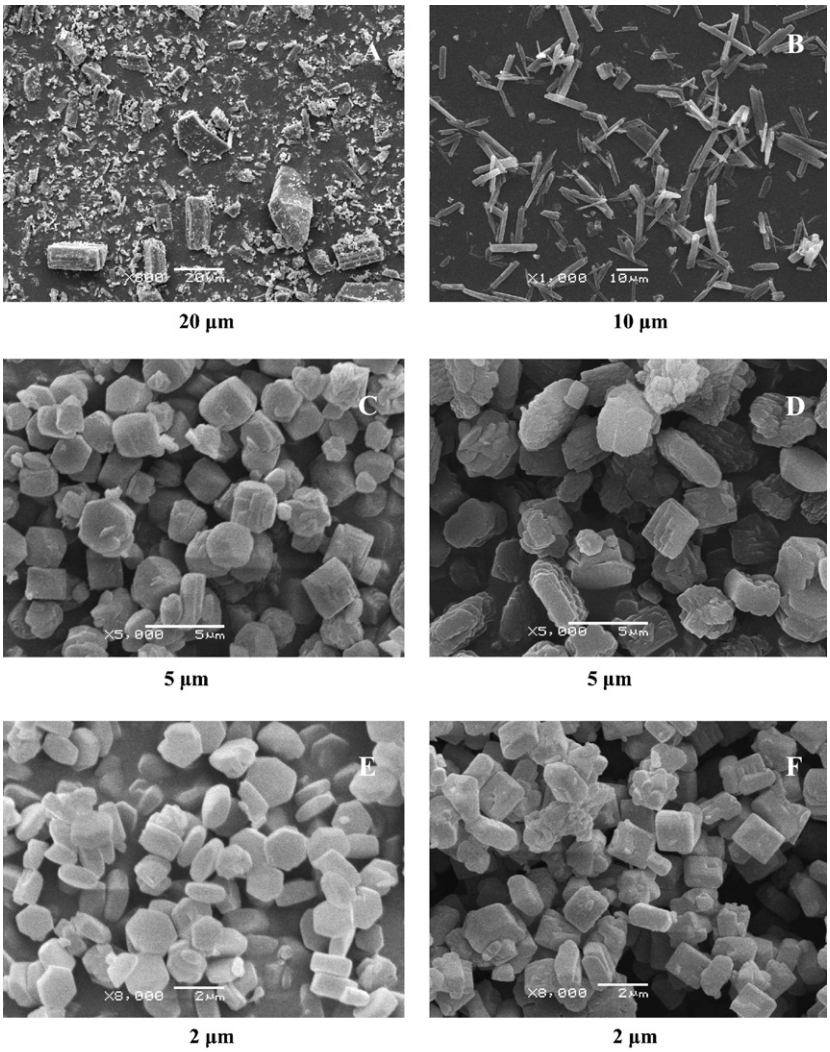

Fig. 1. SEM images of (A) commercial micronized PDL and particles precipitated from (B) pure water (T: 14 °C); (C) 0.2 wt% HPMC aqueous solution (T: 14 °C); (D) 0.2 wt% HPMC aqueous solution (T: 4 °C); (E) hexagonal PDL microcrystals precipitated from the filtrate at 14 °C; (F) tetragonal PDL microcrystals precipitated from the filtrate at 4 °C (PDL concentration: 1.0 g/ml; S/AS ratio: 1:100; stirring speed: 10,000 rpm).

micronized PDL had an irregular shape and a wide particle size distribution of about 40 μm. As a very slightly water-soluble drug, PDL is freely soluble in NMP while has a low solubility in other organic solvents such as ethanol, methanol and acetone. Therefore, pure water and NMP were firstly adopted as the anti-solvent and solvent, respectively. As shown in Fig. 1B, most of the particles appeared rods with a length of about 5–20 μm and a width of 2–3 μm, which were not well controlled. When 0.2 wt% HPMC aqueous solution acted as the anti-solvent, uniform particles with a mean diameter of about 2.5 μm could hence be obtained at 4 and 14 °C (Fig. 1C and D). Subsequently, step 2 was carried out by introducing the aforementioned filtrate as the novel anti-solvent to increase the yield ratio. The corresponding SEM images of the as-prepared samples obtained at the above-mentioned same two temperatures were also illustrated in Fig. 1E and F. As a result, PDL microcrystals with a smaller size (about 1.6 μm), narrower PSD and both interesting morphologies (hexagonal at 14 °C and tetragonal at 4 °C) were successfully prepared, indicating that the morphologies could be well controlled by the HPMC contained in the filtrate and the temperature. Obviously, the two microcrystals have significantly smaller and more uniform particle sizes than that of the commercial micronized drug. The possible reason was that when the filtrate saturated with PDL was quickly mixed with PDL NMP solution with a high concentration; high homogenous supersaturation degree could be generated in a very short time, thereby leading to rapid nucleation and formation of smaller microcrystals. According to the mechanism, Rasenack's failure with PDL was probably because the PDL ethanol solution and HPMC aqueous solution could not provide enough high supersaturation degree in an enough short time.

3.2. Effect of the stirring speed

SEM images of PDL microcrystals prepared at different stirring speeds were depicted in Fig. 2, respectively. It could be clearly found that typical hexagonal and tetragonal PDL particles with different particle sizes, uniform size distribution and

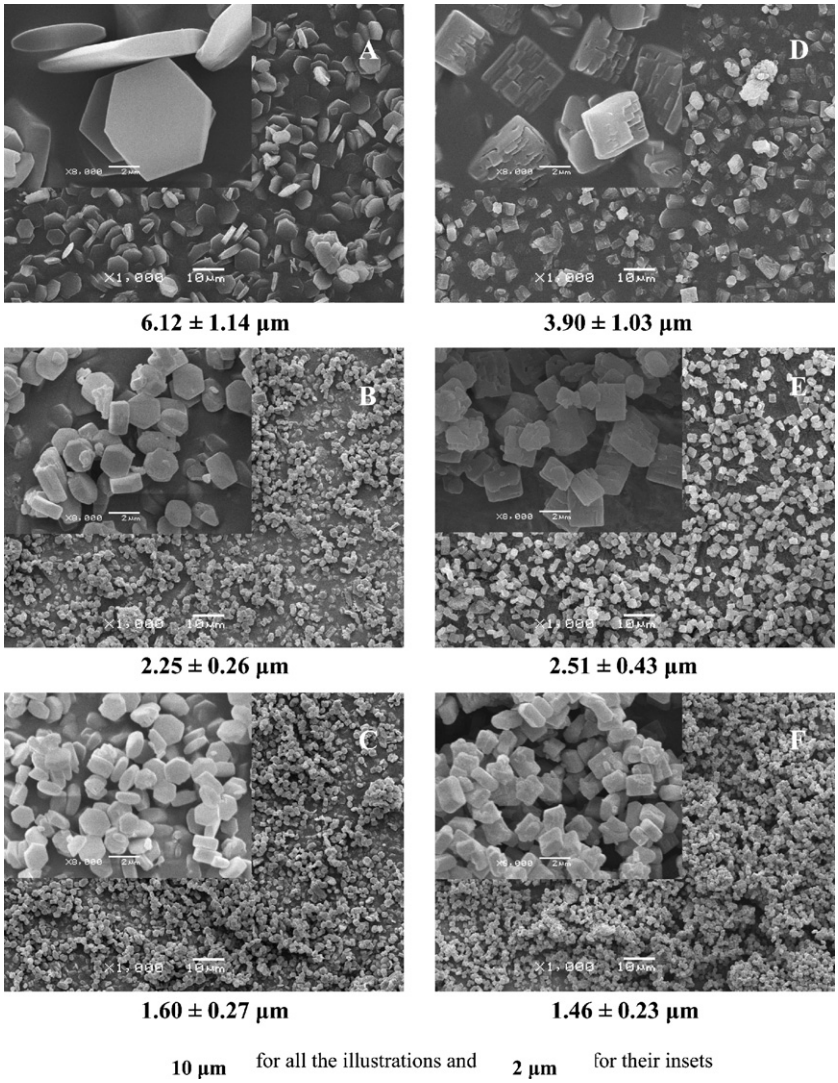

Fig. 2. SEM images of PDL hexagonal microcrystals (*T*: 14 °C for A–C) and tetragonal microcrystals (*T*: 4 °C for D–F) precipitated from the filtrate with different stirring speeds (PDL concentration: 1.0 g/ml; S/AS ratio: 1:100) (A and D) 2500 rpm; (B and E) 5000 rpm; (C and F) 10,000 rpm.

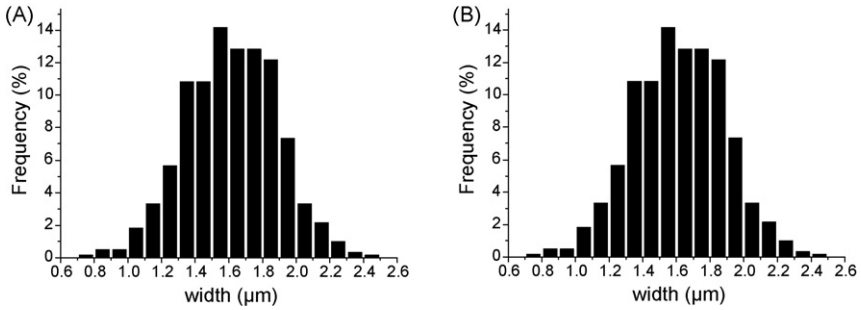

Fig. 3. PSD of PDL microcrystals (A) hexagonal microcrystals (see Fig. 1E) precipitated from the filtrate at 14 °C (mean particle size 1.60 ± 0.27 μm); (B) tetragonal microcrystals (see Fig. 1F) precipitated from the filtrate at 4 °C (mean particle size 1.46 ± 0.23 μm).

good dispersion had been achieved. Moreover, the mean particle size of hexagonal PDL microcrystals markedly decreased from 6.12 ± 1.14 to 1.60 ± 0.27 μm while tetragonal ones from 3.90 ± 1.03 to 1.46 ± 0.23 μm with the increasing of the stirring speed from 2500 to 10,000 rpm (Fig. 2). So it could be concluded that the size of PDL microcrystals could be well controlled by the stirring speed. The obvious decrease of the particle size can be explained owing to the intensification of the micromixing (i.e. mixing on the molecular level) between the multi-phases with the rise of stirring speed. High micromixing efficiency enhanced the mass transfer and the rate of diffusion between the multiphases, resulting in high homogenous supersaturation in a very short time and thus rapid nucleation to produce smaller drug particles. The narrow PSD and the small size were hence attained. According to Fig. 1E and F, the corresponding PSD of PDL microcrystals at the optimum condition were measured, as shown in Fig. 3.

3.3. FT-IR, TGA–DSC and XRD studies

The molecular structures of the commercial micronized PDL and the two microcrystals were investigated by means of FT-IR. Fig. 4A shows the FT-IR spectra of three samples in the range of 500–4000 cm^{-1}. FT-IR spectra of hexagonal and tetragonal PDL microcrystals show some differences from that of commercial micronized PDL in the range of 3000–3500 cm^{-1} and no obvious difference in the fingerprint pattern, which could be ascribed to the formation of pseudopolymorph (hydrate).

The TGA–DSC curves (Fig. 4B) show two endothermic peaks at 102.47 and 244.97 °C and one exothermic peak at 144.97 °C. The weight loss of about 4.64% at the endothermic peak of 102.47 °C could be attributed to the removal of one molecule of H_2O. Therefore, it could be concluded that the hexagonal and tetragonal microcrystals were PDL monohydrate, which was similar to the PDL monohydrate in Young's article (Young and Eui, 2003).

The XRD patterns were performed to study the effect of the microprecipitation on the crystallinity of PDL, as illustrated in Fig. 4C. Commercial micronized drug exhibited an orthorhombic system according to database of JCPDS-ICDD 2004 (International Centre for Diffraction Data). In contrast, the hexagonal and tetragonal microcrystals had the same intense crystalline peaks belonging to monoclinic (P21/*(11)), demon-

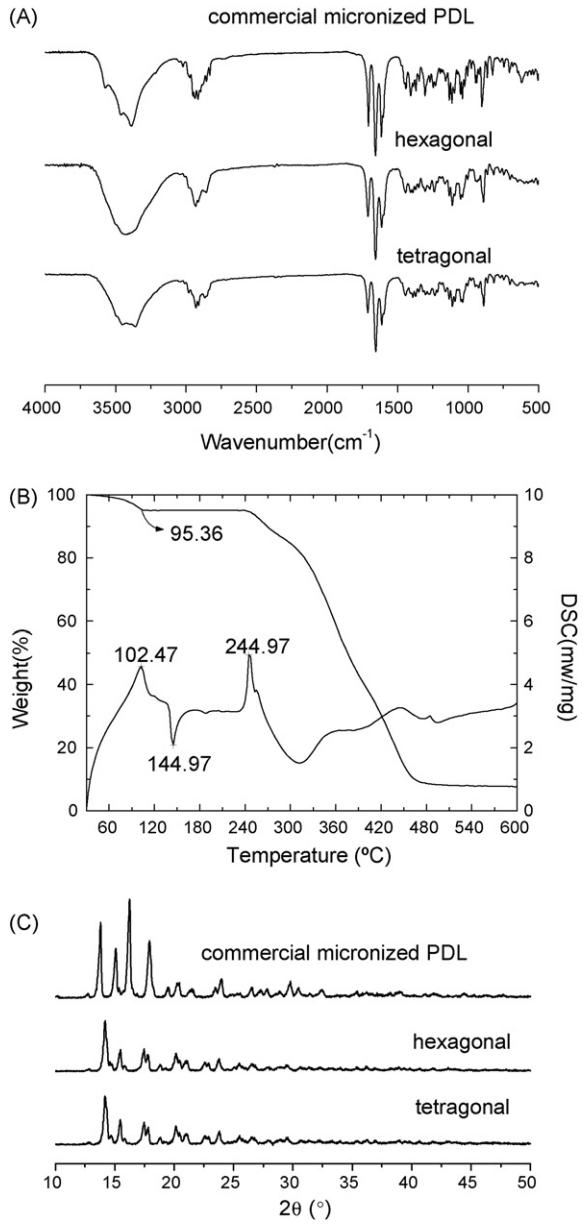

Fig. 4. (A) FT-IR spectra of commercial micronized PDL, hexagonal and tetragonal PDL microcrystals; (B) TGA–DSC curves of tetragonal (hexagonal) PDL microcrystals; (C) XRD patterns of commercial micronized PDL, hexagonal and tetragonal microcrystals.

strating that the hexagonal and tetragonal PDL microcrystals have a different crystalline form from commercial micronized PDL.

3.4. Physical stability studies

Polymorphism is a widespread phenomena observed for most of steroids. The stability of particles in the solid-state forms and/or in suspensions is of considerable importance (Beckmann et al., 2001). Stability studies indicated that the dry powder of commercial micronized PDL, hexagonal and tetragonal microcrystals could be stably held in plastic bags at room temperature for more than 3 months without any change. However, when uniformly re-dispersed in deionized water without any surfactant, most of commercial micronized drug particles (see Fig. 1A) changed into needle-like crystals (no crystal form transition and just different crystal habit) after 8 h (see Fig. 5A), while the hexagonal and tetragonal microcrystals maintained stable under the same condition for over 30 days. As PDL was slightly dissolved in water, hollow parts emerged in the hexagonal and tetragonal microcrystals. At the same time, some particles disrupted into smaller pieces, as shown in Fig. 5B and C.

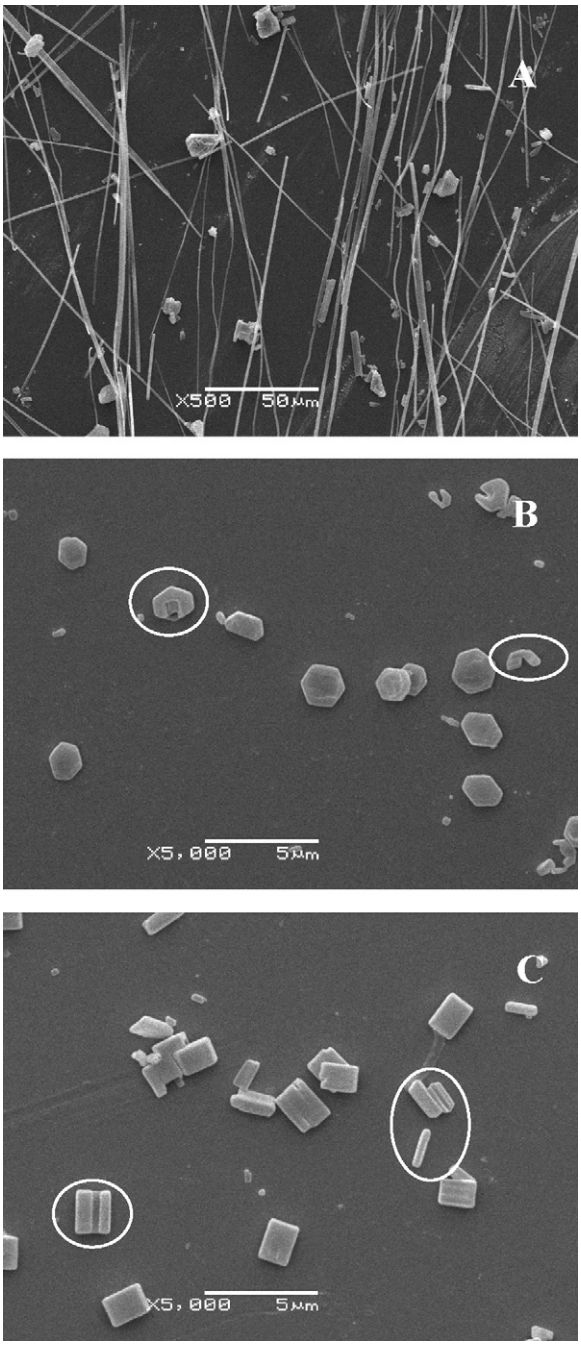

Fig. 5. SEM images of PDL particles re-dispersed in deionized water (0.4 mg/ml) (A) commercial micronized PDL (see Fig. 1A) for 8 h; (B) hexagonal microcrystals (see Fig. 1E) for 30 days; (C) tetragonal microcrystals (see Fig. 1F) for 30 days.

3.5. The drug content analyses

Since the microcrystals were obtained by using the filtrate containing about 0.1 wt% HPMC as the anti-solvent, some HPMC might be remained in the PDL microcrystals. Therefore, the related drug contents were examined by UV because of no chromophore of HPMC. The results showed that drug contents for both hexagonal and tetragonal microcrystals reached more than 98% ($98.78 \pm 0.37\%$ and $98.89 \pm 0.65\%$, respectively), which proved that most of the HPMC had been removed after the filter cake was washed several times.

3.6. The dissolution testing

The in vitro release profiles of the commercial micronized PDL, hexagonal and tetragonal PDL microcrystals were compared in Fig. 6. The hexagonal and tetragonal microcrystals displayed obviously higher dissolution rates than the commercial micronized drug, about 15% increase for the first 15 min. The increase of the dissolution rates of the products could be mainly attributed to the much better uniformity, the great reduction of the particle size, the enhancement of BET surface area (increased from 3.73 m^2/g for the commercial micronized drug to 5.41 and 4.89 m^2/g for the hexagonal and tetragonal microcrystals) and the improvement of particle dispersion. Thus, the microprecipitation method is an effective way for decreasing

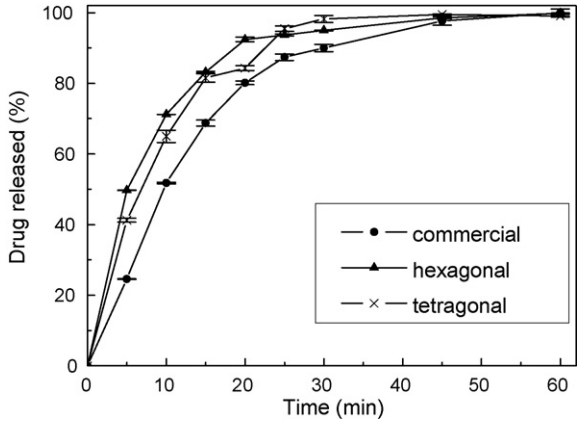

Fig. 6. The dissolution profiles of commercial micronized PDL, hexagonal and tetragonal microcrystals.

the particle size to enhance the dissolution rate of poorly water-soluble drugs.

4. Conclusions

In this study, a controlled microprecipitation method using the filtrate as the improved anti-solvent was developed to prepare PDL microcrystals. In this process, the two morphologies, hexagonal and tetragonal ones of the as-prepared PDL microcrystals could be well controlled by the HPMC contained in the filtrate and the temperature. The particle size and PSD of PDL can be controlled, from 1.60 to 6.12 μm for hexagonal microcrystals and 1.46 to 3.90 μm for tetragonal ones by adjusting the stirring speed. The PDL microcrystals produced by the controlled microprecipitation possessed the same pseudopolymorph (hydrate) with different crystal habit. The physical stability study indicated that the resulting hexagonal and tetragonal PDL microcrystals owned much better physical stability than commercial micronized drug when re-dispersed in pure water. In addition, both of the products exhibited a markedly fast dissolution process when compared to the commercial micronized drug. Therefore, using the filtrate saturated with drug as anti-solvent in a microprecipitation process, which resulted in high supersaturation and rapid nucleation, provided a potentially effective and economical way to prepare micro or nanodrugs for commercial procedure.

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