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Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants

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Abstract

Amorphous nanoparticles of cefuroxime axetil (CFA), a poorly water-soluble drug, were produced by the controlled nanoprecipitation method without any surfactants at room temperature. The influence of the operation parameters, such as the types of solvent and anti-solvent, the stirring speed, the solvent/anti-solvent (S/AS) volume ratio, the drug concentration and the precipitation temperature, were experimentally investigated. The results indicated that increasing the stirring speed and the S/AS volume, decreasing the drug concentration and the temperature favored to decrease the particle size from 700 to 900 nm to ~300 nm. The XRD analyses confirmed that the as-prepared CFA was amorphous nanoparticles. Furthermore, the amorphous CFA nanoparticles exhibited significantly enhanced dissolution property when compared to the commercial spray-dried product. The results demonstrated that the controlled nanoprecipitation method is a direct and feasible technology which could be utilized for preparation of the poorly water-soluble pharmaceutical nanoparticles.

Keywords: Cefuroxime axetil; Poorly water-soluble drug; Controlled nanoprecipitation; Nanoparticles

1. Introduction

Cefuroxime axetil (CFA, cefuroxime 1-acetoxyethyl ester, molecular structure as shown in Fig. 1) is a cephalosporin antibiotic possessing a high activity against a wide spectrum of Gram-positive and Gram-negative microorganisms. Commonly, it exists polymorphous of crystalline and amorphous forms, of which the latter exhibits a higher bioavailability (Crisp and Clayton, 1985; Crisp et al., 1989; Sasinowska et al., 1995; Oszczapowicz et al., 1995). As a poorly water-soluble drug, CFA features a low solubility and dissolution rate in the gastrointestinal tract, which limits its effective absorption and bioavailability.

According to the Ostward–Freundlich and Noyes–Whitney equation, the saturation solubility and dissolution rate of a drug can be increased by reducing the particle size to increase the interfacial surface area (Mosharrafand and Nyström, 1995; Müller et al., 2001). Many approaches (Yu, 2001; Dateand and Patravale, 2004) have been attempted to produce submicron amorphous drug powders. The common way for reducing the particle size is to disrupt the previously formed larger particles by milling methods such as spiral jet milling (Midoux et al., 1999). In addition, some new communication techniques including the media milling technology (Liversidge et al., 2003) and the high-pressure homogenization (Krause and Müller, 2001) were also developed. These techniques, however, need high energy and show some disadvantages in practice such as electrostatic effects and broad particle size distributions. In the last decade, supercritical fluid-based technologies have been widely investigated to obtain submicron or nanosized drug particles, such as the supercritical anti-solvent precipitation (SAS) (Reverchon, 1999), the supercritical anti-solvent enhanced mass transfer method (SAS-EM) (Chattopadhyay and Gupta, 2001), the rapid expansion of supercritical solutions (RESS) (Charoenchaitrakool et al., 2000)

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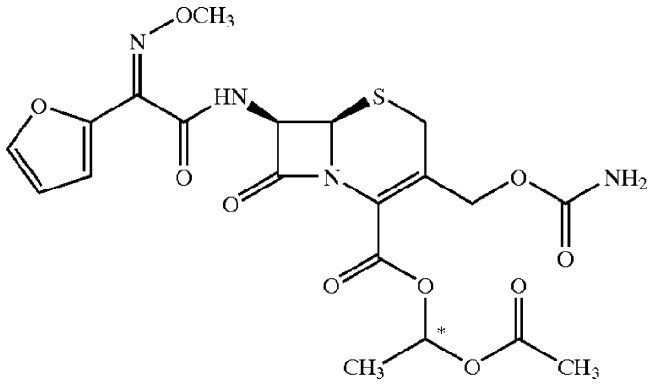

Fig. 1. Structure of Cefuroxime axetil.

and the rapid expansion of a supercritical solution into a liquid solvent (RESOLV) (Pathak et al., 2004). However, these methods are difficult to control and scale up.

Anti-solvent method is also utilized to prepare nanosized polymer or drug particles (Violanto and Fischer, 1989; Ruch and Matijević, 2000; Rasenackand and Müller, 2002; Kayser et al., 2003; Chen et al., 2004; Bilati et al., 2005). In this method, briefly, the polymer or the drug is first dissolved in the solvent and the formed solution is quickly poured to the anti-solvent. Precipitation occurs immediately by a rapid desolvation of the polymers or drug. Currently, aqueous solutions containing some surfactants are used as the anti-solvent. The stabilizers presented in the aqueous are absorbed on the formed drug particles to inhibit crystal growth. This technique is a rapid and direct process, which can be performed with ease. However, there might be some disadvantages such as the contamination of drug particles by surfactants absorbed on the surface of the drug particles.

Amorphous CFA can be prepared by spray drying techniques, roller drying, freeze drying or supercritical fluids (Crisp et al., 1991; Jun et al., 2005). The commercial amorphous CFA is currently prepared by spray drying the CFA solution dissolved in acetone. The spray-dried CFA particles, however, have a large size with a broad particle size distribution (PSD). Furthermore, the drug may be decomposed during the drying process because of the high temperature.

The objective of this study was to directly produce amorphous CFA nanoparticles by the controlled nanoprecipitation method. In this method, both solvent and anti-solvent were organic solvents. The pure amorphous CFA nanoparticles were successfully precipitated without any surfactants at room temperature. Furthermore, filtering and vacuum drying instead of lyophilization and spray drying, which usually used in liquid precipitation process, was used to obtain powder product. The controlled nanoprecipitation process was thus more straightforward and feasible.

In this study, the influence of the operation parameters, such as the types of solvent and anti-solvent, the stirring rate, the solvent/anti-solvent (S/AS) volume ratio, the drug concentration and the temperature were experimentally investigated. The CFA nanoparticles produced by the controlled nanoprecipitation method were characterized by scan electronic microscope (SEM), Fourier transform infrared spectrophotometry (FT-IR), powder X-ray diffraction (XRD) and dissolution testing. The data of commercial spray-dried CFA were also shown for comparison.

2. Materials and methods

2.1. Materials

Crystalline cefuroxime axetil (purity 98%) and spray-dried CFA were both obtained from North China Pharmaceutical Group Corporation (NCPC), China. Ethyl acetate, methylene chloride, chloroform, formic acid, isopropyl ether and acetone (A.R. grade) were supplied by Beijing Chemical agent Company. Water was purified to type I reagent grade by passing through a Barnstead (NANOpure II) filtration system.

2.2. Methods

2.2.1. Experiment

Crystalline CFA was dissolved in the solvent at definite concentration. The solution was filtrated through 0.45 μm pore size membranes to remove the possible particulate impurities. The CFA nanoparticles were then prepared by the controlled nanoprecipitation. Briefly, 5 mL CFA solution was quickly poured into the anti-solvent under magnetic stirring and the precipitation was formed immediately upon mixing. The effects of the process parameters, such as the types of solvent and anti-solvent, the stirring rate, the CFA concentration, the S/AS volume ratio and the reaction temperature, on the properties of the formed particles were investigated.

The freshly formed nanoparticles were then filtered and dried under vacuum at 50 °C for 12 h. The dried particles were analyzed by FT-IR, XRD, and dissolution testing measurements.

2.2.2. Particle size, morphology and particle size distribution

Particle size and morphology were examined by SEM (S250MK3, Cambridge, UK). Freshly formed nanoparticles suspensions were deposited on the glasses upon the evaporation of solvent. The glasses were then fixed on aluminum stubs using doubled-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 particle size distribution was determined by the IBAS I/II Image Analyzer System (Germany) via the obtained SEM photographs.

2.2.3. Fourier transform infrared spectrometry (FT-IR)

FT-IR spectra were recorded with a Nicolet 60-SXB spectrometer in the range 450–4000 cm^{-1} using a resolution of 4 cm^{-1} and 16 scans. Samples were diluted with KBr mixing powder, and pressed to obtain self-supporting disks.

2.2.4. X-ray diffraction (XRD)

The physical state of raw material and the formed particles were characterized by X-ray powder diffraction measurements. Phase identification was conducted using an X-ray diffractometer (Shimadzu XRD-6000) with Cu Kα radiation at scanning speed of 0.05°/min.

2.2.5. Differential scanning calorimetry (DSC)

The phase transition of various CFA were analyzed by differential scanning calorimeter (Pyris 1, Perkin-Elmer, USA) at a heating rate of 10 °C/min from 30 to 190 °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.

2.2.6. Dissolution testing

Dissolution testing for CFA sample was carried out using a dissolution apparatus (D-800LS, Tianjin, CN) following the USP Apparatus II (paddle) method. Paddle speed and bath temperature were set at 100 rpm and 37.0 ± 0.5 °C, respectively. Approximately 20 mg CA was placed into vessels containing 900 mL 0.1 M HCl solution. Five milliliters 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 (UV-3000, Shimadzu, Japan) at 278 nm.

3. Results and discussion

3.1. Effect of the types of solvent and anti-solvent

CFA nanoparticles were prepared by the nanoprecipitation method in this study. The effect of the types of solvent and anti-solvent on the physical state of CFA was investigated. As shown in Table 1, the physical state of the formed particles was significantly influenced by the nature of anti-solvent. When organic solvent (isopropyl ether) was used as anti-solvent, amorphous CFA nanoparticles were prepared. However, with water as anti-solvent, the product liked gel and was crystalline form as amorphous CFA is unstable and easily transformed to its crystalline form in water. The solvents of drug had no influenced on the crystallinity of the CFA. Because CFA has higher solubility in acetone than that in ethyl acetate, methylene chloride or chloroform, acetone and isopropyl ether were chosen as solvent and anti-solvent in the following experiment.

3.2. Effect of the stirring rate

SEM images of CFA particles prepared at different stirring rates are shown in Fig. 2. It can be clearly seen from SEM images that the size of CFA particles decreased from ∼800 to ∼340 nm with the increasing of the stirring rate from 300 to 1200 rpm. This indicated the size of CFA particles is strongly dependent on the stirring rate.

The decreasing of the particle size can be explained by the intensification of the micromixing (i.e. mixing on the molecular level) between the multi-phases with the increasing of stirring rate. High micromixing efficiency enhanced the mass transfer and the rate of diffusion between the multiphase, which induced high homogenous supersaturation in short time and thus rapid nucleation to produce smaller drug particles. Simultaneously, the CFA particles had a narrower PSD due to the uniform supersaturation. Hence, higher stirring rate favored the formation of the smaller and more uniform drug particles.

3.3. Effect of the CFA concentration and the solvent volume ratio

A series of experiments were conducted to investigate the effect of the CFA concentration on the particle size. As shown in Fig. 3, the particle size decreased from ∼800 to ∼300 nm as the CFA concentration in the acetone phase decreased from 120 to 60 mg/mL.

This behavior might be explained by considering two factors: the number of nuclei formed in the solvent/anti-solvent interface and the influence of concentration on the viscosity (Galindo-Rodriguez et al., 2004). On one hand, the large number of nuclei formed in the interface of two phases lead to aggregate and thus formation of larger nanoparticles. Simultaneously, those nuclei decrease the diffusion from solvent to anti-solvent, which limited nanoparticles formation and decreased the yield (Stainmesse et al., 1992). On the other hand, the viscosity of drug solution increased with the increasing of concentration, which hindered the diffusion between solution and anti-solvent and thus resulted in nonuniform supersaturation. As a result, the drug particles had a large size and broad PDS. The same phenomenon was also observed by other researchers (Bilati et al., 2005).

Furthermore, the effect of the S/AS volume ratio was investigated. The result is shown in Fig. 4. As S/AS volume ratio was increased from 1:14 to 1:20 (it should be mentioned that when S/AS volume ratio was below about 1:13, the precipitation was viscous gel and result the product of crystalline form), the decrease in particle size was observed. At a volume ratio of 1:14, the particles had a mean size of about 700 nm with a board size distribution. As the volume ratio increased to 1:20, the mean particle size decreased to 400 nm.

Table 1
Effect of the solvent type on the crystallinity of final precipitate

No.[a]	Solvent (S)	Anti-solvent (AS)	CFA concentration (mg/mL)	Volume of anti-solvent (mL)	S/AS volume ratio	NP[b]
1	Ethyl acetate	Isopropyl ether	50	50	0.1	Amorphous
2	Methylene chloride	Isopropyl ether	60	50	0.1	Amorphous
3	Chloroform	Isopropyl ether	60	50	0.1	Amorphous
4	Acetone	Isopropyl ether	120	100	0.05	Amorphous
5	Acetone	Water	120	100	0.05	Crystalline
6	Formic acid	Water	220	100	0.05	Crystalline

[a] Drug concentration: 50 mg/mL; reaction temperature: 20 °C.
[b] By XRD.

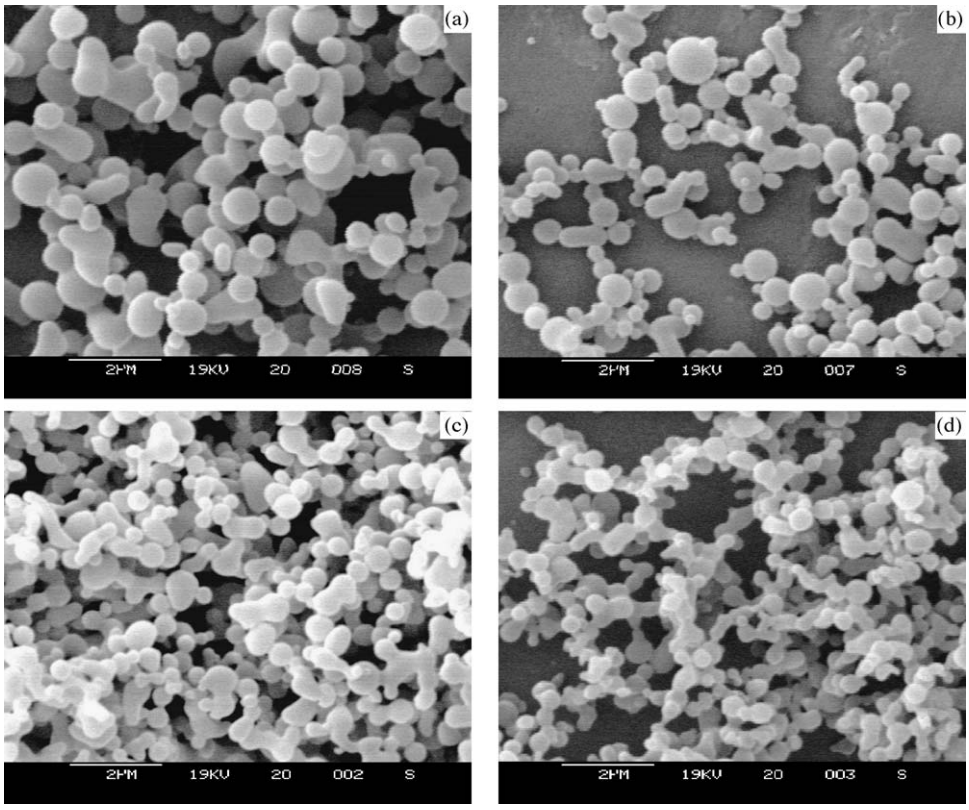

Fig. 2. SEM images showing the effect of stirring rate on particle size (CFA concentration: 8% (w/v); S/AS volume ratio: 1:20; *T*: 20 °C). (a) 300 rpm; (b) 600 rpm; (c) 900 rpm; (d) 1200 rpm.

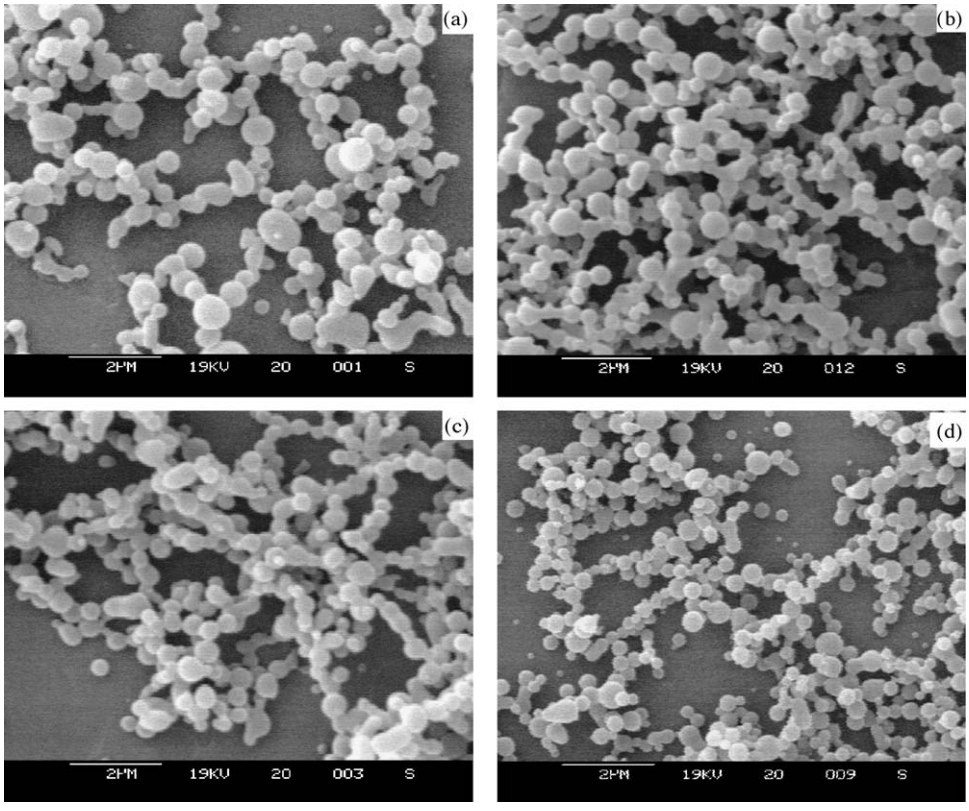

Fig. 3. SEM images showing the effect of CFA concentration on particle size (stirring rate: 1200 rpm; S/AS volume ratio: 1:20; *T*: 20 °C). (a) 12% (w/v); (b) 10% (w/v); (c) 8% (w/v); (d) 6% (w/v).

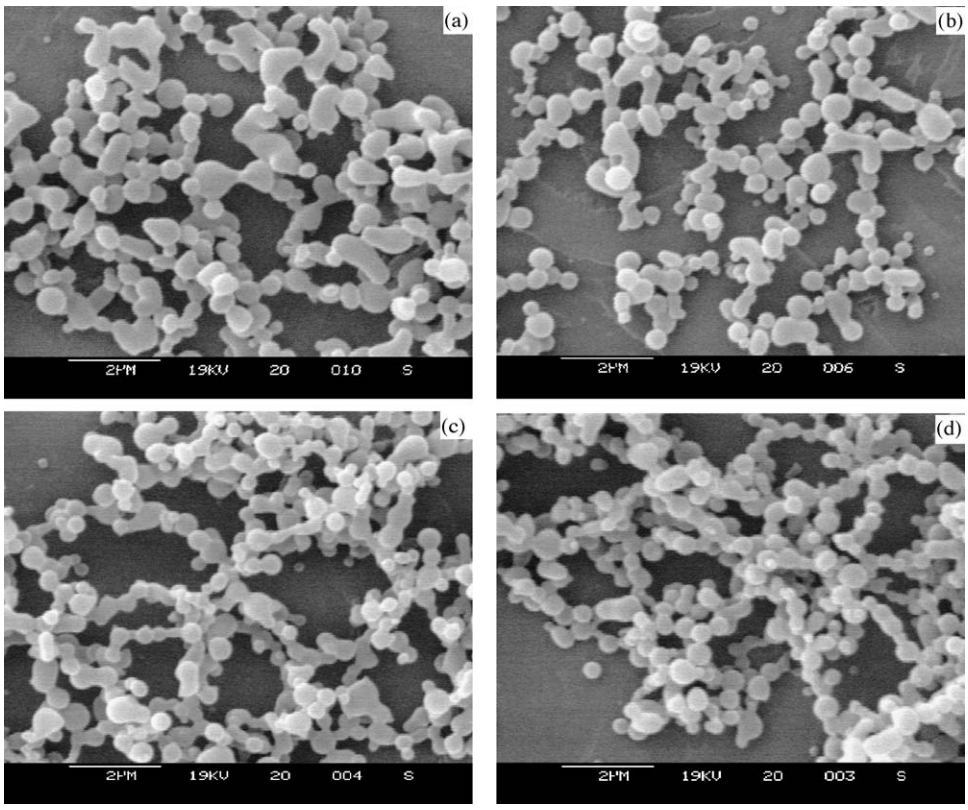

Fig. 4. SEM images showing the effect of S/AS volume ratio on particle size (CFA concentration: 8%; stirring rate: 1200 rpm; *T*: 20 °C). (a) 1:14; (b) 1:16; (c) 1:18; (d) 1:20.

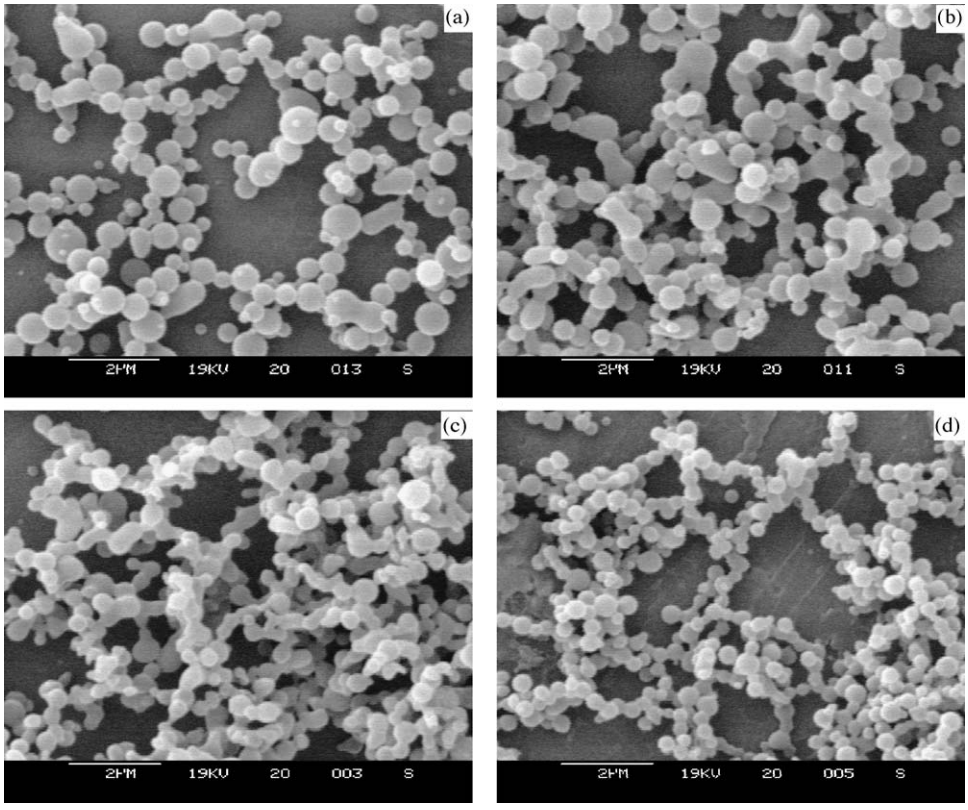

Fig. 5. SEM images showing the effect of temperature on particle size (CFA concentration: 8% (w/v); stirring rate: 1200 rpm; S/AS: 1:20). (a) 40 °C; (b) 30 °C; (c) 20 °C; (d) 10 °C.

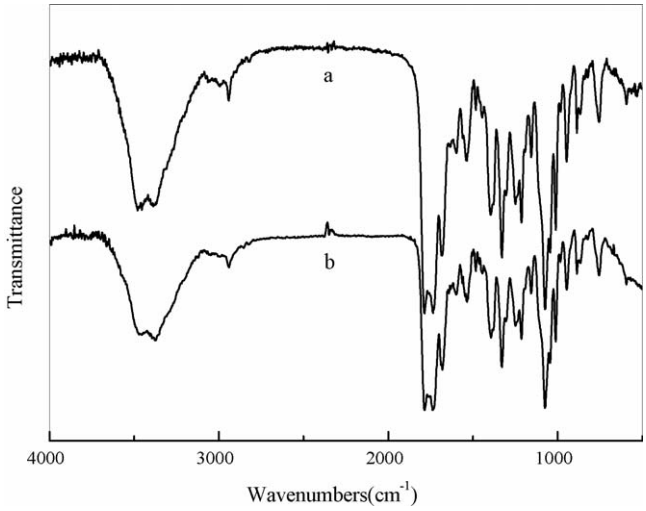

Fig. 6. FT-IR spectrums of various CFA. (a) nanosized CFA (CFA concentration: 8%; stirring rate: 1200; S/AS: 1:20; *T*: 20 °C); (b) raw CFA.

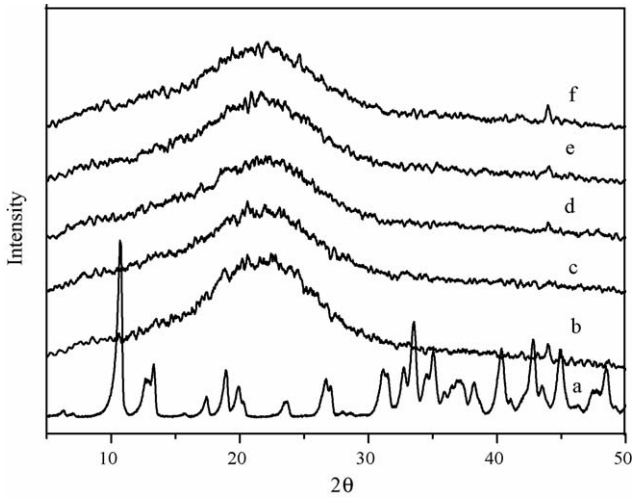

Fig. 7. X-ray diffraction patterns of various CFA. (a) Raw CFA; nanosized CFA (CFA concentration (w/v), stirring rate, *T*, S/AS); (b) 8%, 1200 rpm, 20 °C, 1:20; (c) 12%, 1200 rpm, 20 °C, 1:20; (d) 8%, 300 rpm, 20 °C, 1:20; (e) 8%, 1200 rpm, 40 °C, 1:20; (f) 8%, 1200 rpm, 20 °C, 1:14.

3.4. Effects of the preparation temperature

The effect of temperature, ranging from 10 to 40 °C at a 10 °C interval, was also studied. It can be seen from Fig. 5 that the particle size decreased from about 800 to 300 nm with the decreasing of temperature from 40 to 10 °C. This indicated the lower the precipitation temperature, the smaller the particles. The follow reasons may be responsible for this. Firstly, at low temperature, the drug solubility decreased and the metastability zone became narrow, thus, it was easily to reach high supersaturation when solution was infused into anti-solvent. Secondly, the nucleating process was a process of free energy decrease and heat release, thus, it favors to form the high nucleation rate at low temperature. Thirdly, a lower temperature can inhibit the particles growth (Cushing et al., 2004). Therefore, the particles with smaller size were formed as a result of the high nucleation rate and low growth rate at lower temperature.

3.5. FT-IR study

The molecular states of the raw and nanosized CFAs were studied by means of FT-IR. Fig. 6 shows the FT-IR spectrum of the CFAs in the range of 500–4000 cm^{-1}. The spectrums of the CFAs are characterized by the NH, NH_2 complex (3480–3210 cm^{-1}), β-lactam (1782 cm^{-1}), acetate (1760 cm^{-1}), 4-ester group (1720 cm^{-1}) and 7-amido (1676 and 1534 cm^{-1}). The close agreement between the FT-IR spectra of the raw and nanosized CFAs suggested that there were no changes in the CFA molecular structure caused by the nanoprecipitation process.

3.6. XRD and DSC analysis

XRD was performed to investigate the effect of the process parameters on the crystallinity of CFA in the controlled nanoprecipitation process. The XRD patterns are shown in Fig. 6. As seen from Fig. 7, the pattern of the raw CFA exhibited some intense crystalline peaks between 10° and 50° of 2θ, which proved that the raw CFA was crystalline form. However, instead of those intense crystalline peaks, only one broad and diffuse maxima peak was detected in the patterns of the nanosized CFA, which indicated that the nanosized CFA was amorphous form.

In order to further confirm the physical state, DSC was preformed to analyze the various CFA samples. The results were shown in Fig. 8. There are two endothermic bands around 125 and 181 °C with the DSC scan of raw material. It indicates that the raw material was polymorphs. However, only one endothermic band around 80 °C with lower enthalpy occurred in the DSC scans of nanosized CFA and spray-dried CFA. It proved that the nanosized and spray-dried CFA were substantially amorphous form.

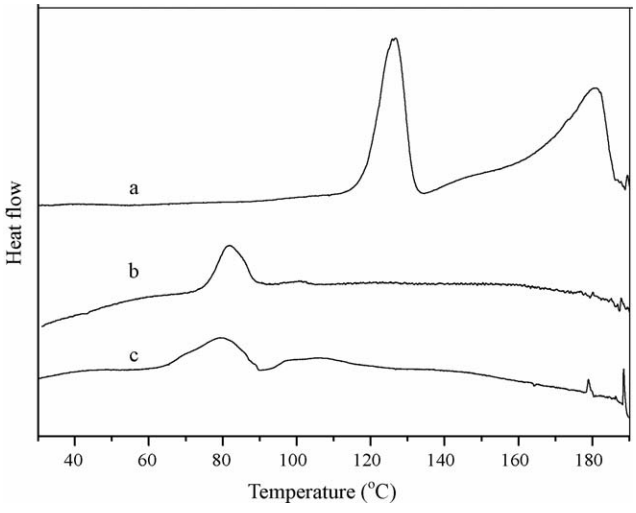

Fig. 8. DSC of various CFA. (a) Raw CFA; (b) spray-dried CFA; (c) nanosized CFA (CFA concentration: 8% (w/v), stirring rate: 1200 rpm, *T*: 10 °C, S/AS: 1:20).

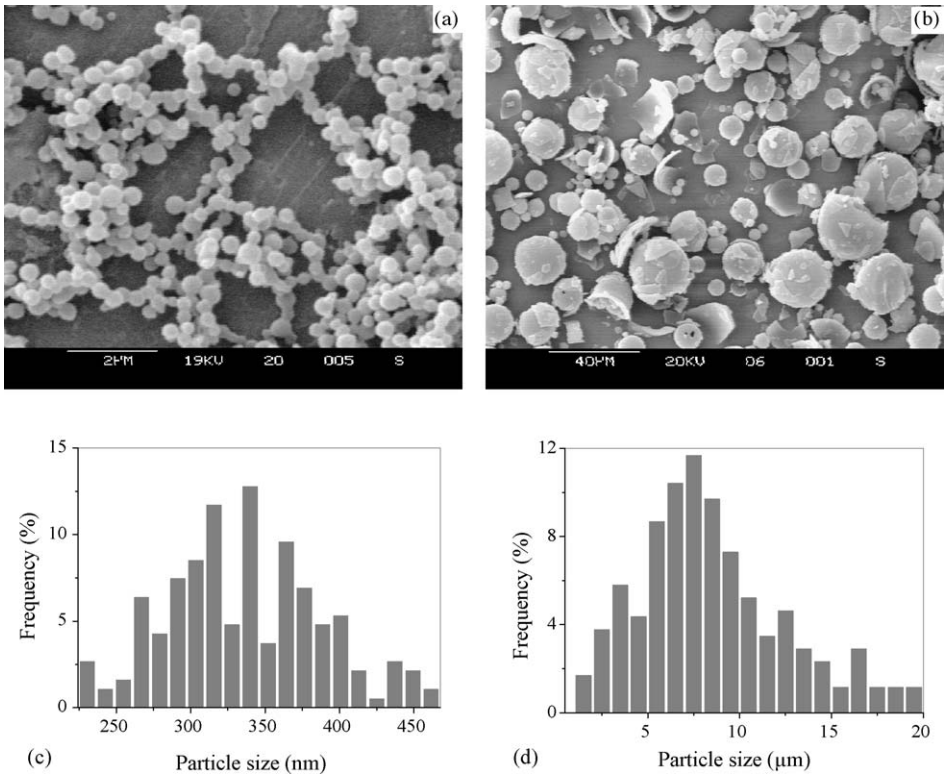

Fig. 9. SEM and PSD of nanosized and spray-dried CFA particles. (a and c) nanosized CFA (CFA concentration: 8% (w/v), stirring rate: 1200 rpm, *T*: 10 °C, S/AS: 1:20); (b and d) spray-dried CFA.

3.7. The particle size distribution (PDS)

The SEM images and the particles size distribution of the commercial spray-dried and the typical nanosized CFA particles are shown in Fig. 9. It can be seen that the spray-dried CFA particles are hollow spheres with the mean particle size of 10 μm and the PDS is broad from about 2 to 25 μm. While the mean particle size of the particles obtained via the controlled nanoprecipitation method was only about 300 nm with a narrow PSD. It is evident that the particle size of the nanosized CFA particles is significantly smaller and more uniform than that of the spray-dried CFA particles, which should be more beneficial to hence bioavailability.

3.8. The dissolution testing

The dissolution profiles of the commercial CFA and the nanosized CFA are shown in Fig. 10. Ninety percent of the nanosized CFA was dissolved in 60 min and complete dissolution occurred within 150 min; however, the commercial spray-dried CFA did not achieve complete dissolution (only about 63%) during the 150 min testing period. The amorphous CFA nanoparticles dissolved significantly faster than that of the commercial spray-dried CFA. It is due to that the particle size of the nanosized CFA was sharply induced to greatly increase the specific surface area, which offers significant potential for enhancing the dissolution of CFA. Thus, the nanoprecipitation method is an effective approach for decreasing particle size to enhance the solubility of water poorly solubility drug.

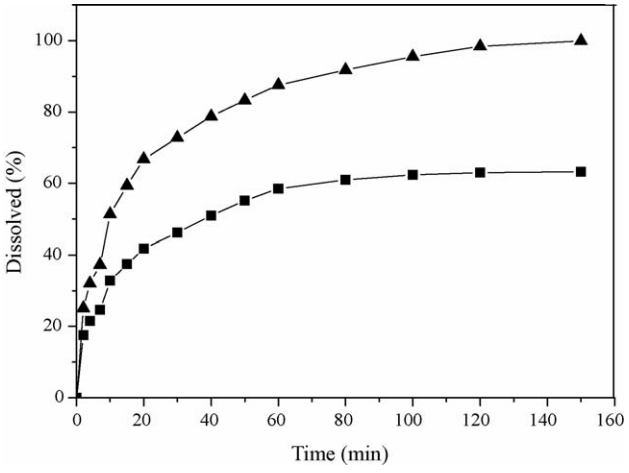

Fig. 10. Dissolution profiles: (■) spray-dried CFA; (▲) nanosized CFA (CFA concentration: 8% (w/v), stirring rate: 1200 rpm, *T*: 10 °C, S/AS: 1:20).

4. Conclusion

A controlled nanoprecipitation method was developed to prepare amorphous CFA nanoparticles without the addition of any chemical inhibitors or surfactants. In this process, the particle size and PSD of CFA can be controlled, from 300 to 800 nm, by adjusting the operation parameters, such as the drug concentration, the stirring rate, the temperature and the A/AS volume ratio. The drug particle size decreases with the increasing of the stirring rate and the S/AS volume ratio. In addition, lowering the drug concentration and the temperature can also result in the

smaller particles. The CFA nanoparticles produced via the controlled nanoprecipitation are amorphous with a narrow PSD. The dissolution of nanosized CFA is significantly enhanced compare with the spray-dried CFA. In conclusion, the controlled nanoprecipitation method offers a direct process to obtain drug nanoparticles of controllable size, amenable for continuous and consistent production.

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