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Micronization of atorvastatin calcium by antisolvent precipitation process

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ABSTRACT

Amorphous atorvastatin calcium (AC) ultrafine powder has been successfully prepared by antisolvent precipitation and spray drying process, in which hydroxypropyl methylcellulose (HPMC) was employed to control the particle size and morphology. The effects of experimental parameters, such as stirring time, drug concentration and drying methods, on particle size and morphology were investigated. The average particle size of AC obviously increased from 410 nm to 1200 nm as the stirring time changed from 30 s to 60 min. The enhancement of drug concentration favored to decrease the particle size from 410 nm to 240 nm. After spray drying process, ultrafine AC powder was obtained, which had good dispersibility and narrow particle size distribution of 1–3 μm. The as-prepared ultrafine AC was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, thermal gravimetric analysis (TG), differential scanning calorimetry (DSC), specific surface area and dissolution test. The XRD analyses indicated that the ultrafine AC was amorphous. In the dissolution tests, the amorphous AC ultrafine powder exhibited enhanced dissolution property when compared to the raw material.

1. Introduction

It is estimated that a significant proportion of drugs on the market are poorly soluble in water, and it is expected that this will be even more pronounced in the future (Lindfors et al., 2006). A substantial factor that prevents the development of such substances is the limited dissolution rate. Consequently the bioavailability is very often below the therapeutic level (Merisko-Liversidge et al., 2003; Douroumis and Fahr, 2006; Müller and Peters, 1998).

Several techniques are commonly used to improve dissolution and bioavailability of poorly water-soluble drugs, such as size reduction (Perrut et al., 2005), the use of surfactants (Raghavan et al., 2001a,b; Goddeeris et al., 2007), the formation of solid dispersions (Leuner and Dressman, 2000) and the transformation of crystalline drug to amorphous state (Goddeeris et al., 2008). Among various strategies to address the solubility issue, drug particle size reduction has emerged as an effective and versatile option (Pathak et al., 2004). In regard to the Noyes–Whitney and Ostwald–Freundlich equations, size reduction can offer increased dissolution and solubility characteristics (Hecq et al., 2006). Many approaches have been attempted to produce microparticles, including mechanical commutation (Krause and Müller, 2001), supercritical fluid technique (Tenorio et al., 2007) and antisolvent precipitation process (Chiou et al., 2007). The mechanical commutation methods need high-energy input and show some disadvantages in practice such as electrostatic effects and broad particle size distributions (Zhang et al., 2006). Supercritical fluid technique is believed to be attractive methods for the size reduction, providing particles with narrow size distribution. However, they also have the limitations of low yield and high equipment cost (Wang et al., 2007).

Antisolvent precipitation process is a promising technique to prepare ultrafine drug particles, which is based on the change of supersaturation caused by mixing the solution and the antisolvent (Zhong et al., 2005; Zhang et al., 2006). In this method, it requires two solvents that are miscible. Ideally, the drug must dissolve in the solvent, but not in the antisolvent. Precipitation occurs instantaneously by a rapid desolvation of the drug. The key to producing ultrafine particles by antisolvent precipitation is to create conditions that favor very rapid particle formation and little or no particle growth. The technique presents some advantages, in that it is a straightforward method, rapid and easy to perform. This technique has been successfully used to prepare several drugs, such as budesonide (Rasenack et al., 2003), danazol (Zhao et al., 2007), beclomethasone dipropionate (Wang et al., 2007) and prednisolone (Li et al., 2007). Recently, hydrophilic-based microparticles have became one of the most novel and advanced release systems and are an extremely important type of dosage form for oral drug delivery (Reverchon et al., 2008). For instance, aqueous solution containing hydrophilic stabilizer could be used as the antisolvent. HPMC is a

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semi-synthetic ether derivative of cellulose that is widely used in many fields (Reverchon et al., 2008). In particular, it is frequently used in the preparation and formulation of poorly water-soluble drugs because of its non-toxic nature, easy to manufacture and water-soluble property (Raghavan et al., 2001a,b, 2003; Won et al., 2005; Larsson et al., 2008). In addition, the stabilizer presented in the aqueous solution would absorb on the surface of the formed hydrophobic drug particles to inhibit particle growth and aggregation (Raghavan et al., 2003).

Atorvastatin calcium ([R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, AC) is a synthetic lipid-lowering agent of great medicinal and commercial importance (Wade et al., 1997; Rádl et al., 2002). AC is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer (Kim et al., 2008a,b). AC is rapidly absorbed after oral administration. However, the fraction absorbed and the absolute bioavailability of AC are only approximately 30% and 12%, respectively (Kim et al., 2008a,b). Therefore, it is of great significance to enhance the dissolution rate and improve the low systemic availability of AC. Recently, Kim et al. reported that the solubility and bioavailability of crystalline AC could be improved by physical modification such as particle size reduction and generation of amorphous state (Kim et al., 2008a).

The objective of this work was to prepare amorphous AC ultrafine powder via antisolvent precipitation and spray drying process. In this study, HPMC was used as a hydrophilic polymer to control the particle morphology and inhibit particle growth. The effects of experimental parameters, such as stirring time, the concentration of AC methanol solution and the drying method, on particle size and morphology were investigated. The raw AC and ultrafine AC were characterized with scan electronic microscope (SEM), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), thermal gravimetric analysis (TG), differential scanning calorimetry (DSC), specific surface area and dissolution testing.

2. Materials and methods

2.1. Materials

The raw AC (Form I, purity is 99.4%) was purchased from Beijing Mediking Biopharm Co., Ltd. (Beijing, RPC) and used without further purification. Methanol, sodium hydroxide (NaOH) and potassium dihydrogen phosphate (KH_2PO_4) were analytical grade and obtained commercially from Beijing Chemical Reagents Company (Beijing, RPC). Hydroxypropylmethylcellulose (HPMC, ZW-5, 3–7 mPa s) was obtained from Zhejiang Joinway Pharmaceutical Co. Ltd. Glacial acetic acid and acetonitrile were HPLC grade and provided by FisherChemical (NJ, USA). Deionized water was prepared with Hitech-K Flow Water Purification System (Hitech Instruments Co. Ltd., Shanghai, China).

2.2. Preparation of ultrafine AC

The experimental process for the preparation of ultrafine AC was illustrated in Scheme 1. In a typical procedure, methanol and water were used as solvent and antisolvent of AC, respectively. Raw AC was dissolved in methanol and the solution was then filtrated through a membrane with pore size of 0.45 μm to remove the possible particulate impurities. 0.1 g HPMC was added into 100 mL water (0.1 wt%), which was used as initial antisolvent. At 20 °C, a certain concentration of AC methanol solution (5 mL) was poured into antisolvent (50 mL) with a vigorous stirring speed of 1000 rpm. Immediately, particles precipitated from the antisolvent and a milk-like suspension formed simultaneously. After stirring for 30 s, the suspension was processed with spray drying method to obtain ultrafine AC powder. Spray drying was carried out using laboratory scale spray dryer (SD-Basic, Labplant, UK) under the following conditions: inlet temperature, 120 °C; outlet temperature, 70–80 °C; spray flow rate, 20 mL/min; atomization air pressure, 0.65 MPa.

2.3. Characterization of product

2.3.1. Particle size analysis

The particle size of the suspension was analyzed using laser diffractometer (Malvern; ZETASIZER-3000HS). The sample was diluted with three times water (HPMC, 0.1 wt%) and then sonicated to create a homogenous suspension.

2.3.2. Scanning electron microscopy (SEM)

Particle morphology was observed using scanning electron microscopy (SEM) JSM-6360LV (JEOL Inc., Japan). The samples, an appropriate amount of AC powder or a glass slide with a small drop of the suspension, were fixed on an SEM stub using double-sided adhesive tape and coated with Au at 50 mA for 6 min through a sputter-coater (KYKY SBC-12, Beijing, China). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 10 kV.

2.3.3. X-ray diffraction studies (XRD)

X-ray diffraction analysis was employed to detect the crystallinity of AC, which was conducted using a XRD-6000 diffractometer (Shimadzu, Japan). The powder was placed in a glass sample holder. Cu Kα radiation radiation was generated at 30 mA and 40 kV. Samples were scanned from 5° to 50° with a step size of 0.05°.

2.3.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of samples were recorded with a Nicolet model 8700 spectrometer (Nicolet Instrument Corporation, USA) in the wavenumber range of 500–4000 cm^{-1} to evaluate the molecular states of raw AC and the as-prepared ultrafine AC. Samples were diluted with KBr mixing powder at 1% and pressed into self-supporting disks.

2.3.5. Thermal gravimetric analysis (TG)

Thermal gravimetric analysis was performed using a Thermogravimetrical Analyzer (TGS-2, PerkinElmer, USA). The experiment was performed with a heating rate of 10 °C/min using nitrogen flow (50 mL/min) and samples were weighed (approximately 4.5 mg) in open aluminum pans and the percentage weight loss of the samples was monitored from 25 °C to 250 °C.

2.3.6. Differential scanning calorimetry (DSC)

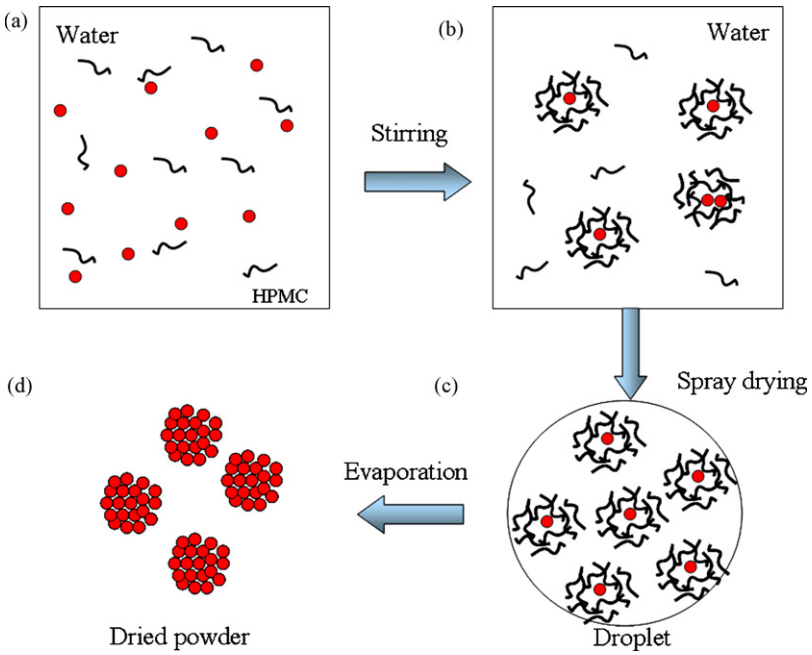
The phase transition of samples was analyzed by differential scanning calorimeter (Pyris 1, PerkinElmer, USA) at a heating rate of 10 °C/min. A dry nitrogen purge of 20 mL/min was employed in the process. Calibration of the instrument with respect to temperature and enthalpy was achieved using high purity standard of indium.

2.3.7. Specific surface area

The specific surface areas of raw AC and ultrafine AC powder were determined using N_2 adsorption method. The calculation was implemented by Surface Area Analyzer ASAP 2010-M (Micromeritics Instrument Corporation, USA) based on the BET equation. Before measuring, dry powder was loaded into a sample cell and degassed for at least 4 h.

2.3.8. HPLC instrumentation and conditions

The alliance HPLC system comprised of a Waters 2996 Photodiode Array Detector and a Waters 2695 Separations Module



Scheme 1. Experimental process to prepare ultrafine AC.

including an autosampling system, column oven, integrated solvent and sample management configuration (Waters Corporation, Miford, MA, USA). A Waters' Empower 2 Chromatography Data Software was used to record and evaluate the data collected during and following chromatographic analysis. The chromatographic separation was accomplished using a Waters Sunfire™ C18, reverse-phase column (150 mm × 4.6 mm i.d., 5 μm particle size) protected by a guard column (10 mm × 4.6 mm i.d.) which was packed with the

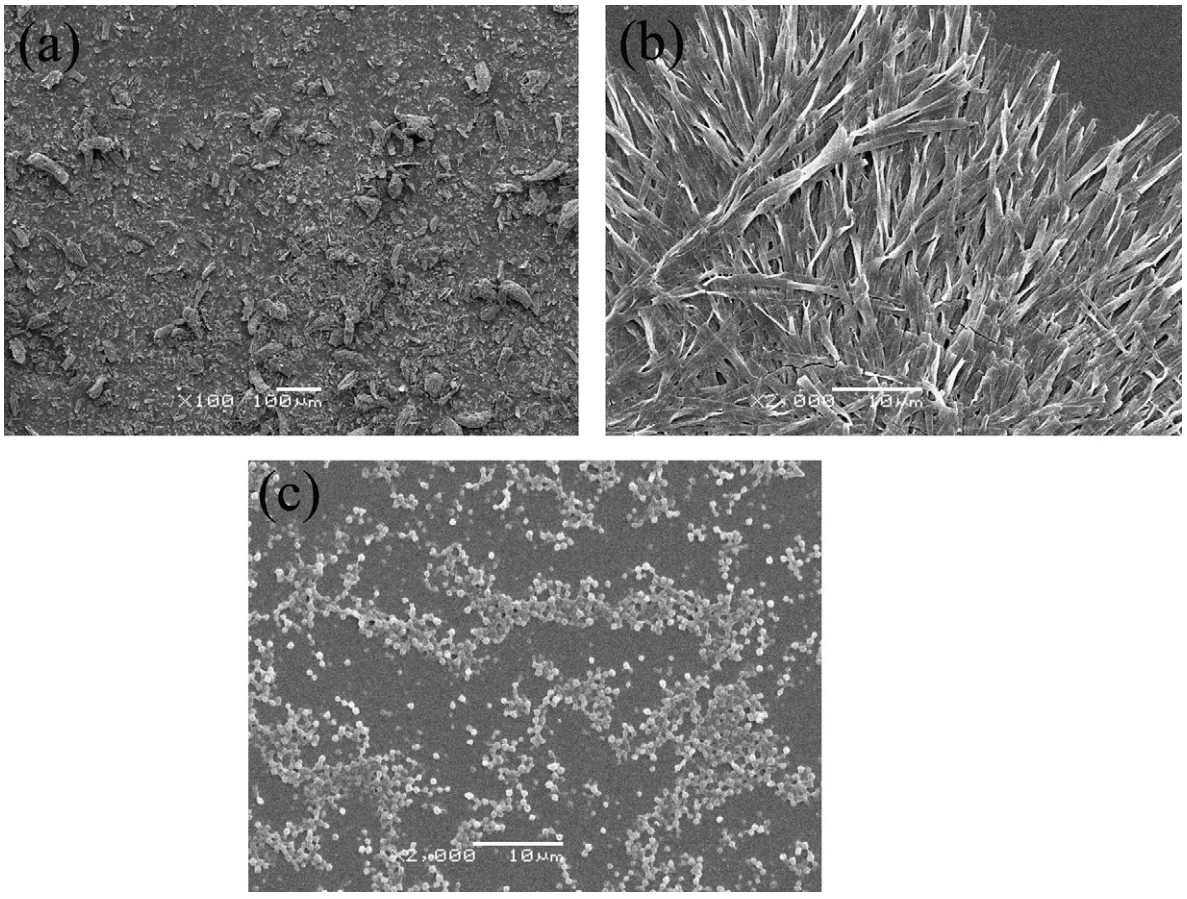


Fig. 1. SEM images of (a) raw AC and particles precipitated from (b) pure water; (c) 0.1 wt% HPMC aqueous solutions (AC methanol concentration: 20 mg/mL; stirring time: 30 min).

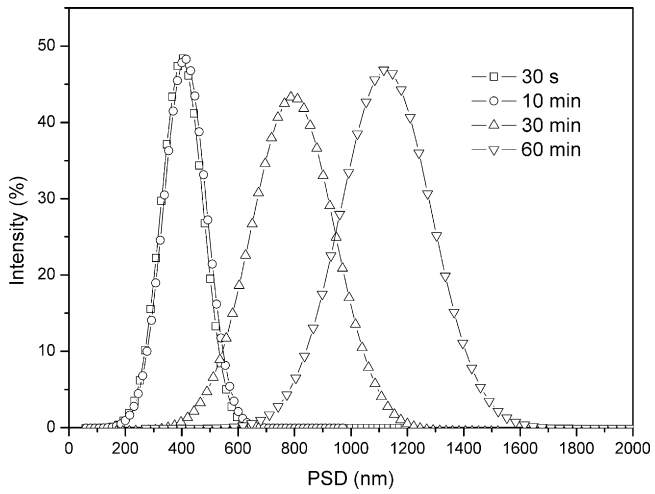


Fig. 2. Particle size distribution of AC particles prepared with different stirring time (AC methanol concentration: 20 mg/mL).

same Sunfire™ C18 material. The mobile phase was consisted of a 55:45 (v/v) mixture of acetonitrile: 0.5% (v/v) glacial acetic acid in water. The column was maintained at 30 °C and equilibrated for 60 min with the analytical mobile phase before injection. The injection volume was 20 μL, and the mobile phase was pumped isocratically at a flow rate of 1.0 mL/min. The effluent was monitored at 245.5 nm.

2.3.9. Dissolution study

Dissolution study was carried out following the USP Apparatus 2 (paddle) method (D-800LS, Tianjin, CN). The paddle speed and bath temperature were set at 75 rpm and 37.0 ± 0.5 °C, respectively. Phosphate buffer (pH 6.8) was employed as the dissolution medium. Approximately, 30 mg powder was added into vessels containing 900 mL of the dissolution medium. The samples (2 mL) were withdraw each time at specific intervals and filtered using a 0.45 μm filter. The filtrate was diluted with methanol and the concentrations of AC were measured by HPLC system. Each sample was analyzed in triplicate.

3. Results and discussion

3.1. The effect of HPMC

Fig. 1 presents the SEM images of raw AC and the particles obtained without and with HPMC as surfactant. The raw AC had an irregular shape and broad particle size distribution ranged from 2 μm to 100 μm. In comparison, the particles precipitated from pure water and 0.1 wt% HPMC aqueous solution exhibited regular morphology. When pure water is used as antisolvent, most of the particles appeared as fibrous flake with a width of 2–3 μm and a length up to several micrometers (Fig. 1b). However, the particle size was not well controlled. When 0.1 wt% HPMC aqueous solution is used as antisolvent, well-dispersed spherical AC particles with an average diameter of about 800 nm are obtained (Fig. 1c). The above results indicate that HPMC plays a key role in controlling the morphology and size of AC particles. Since HPMC

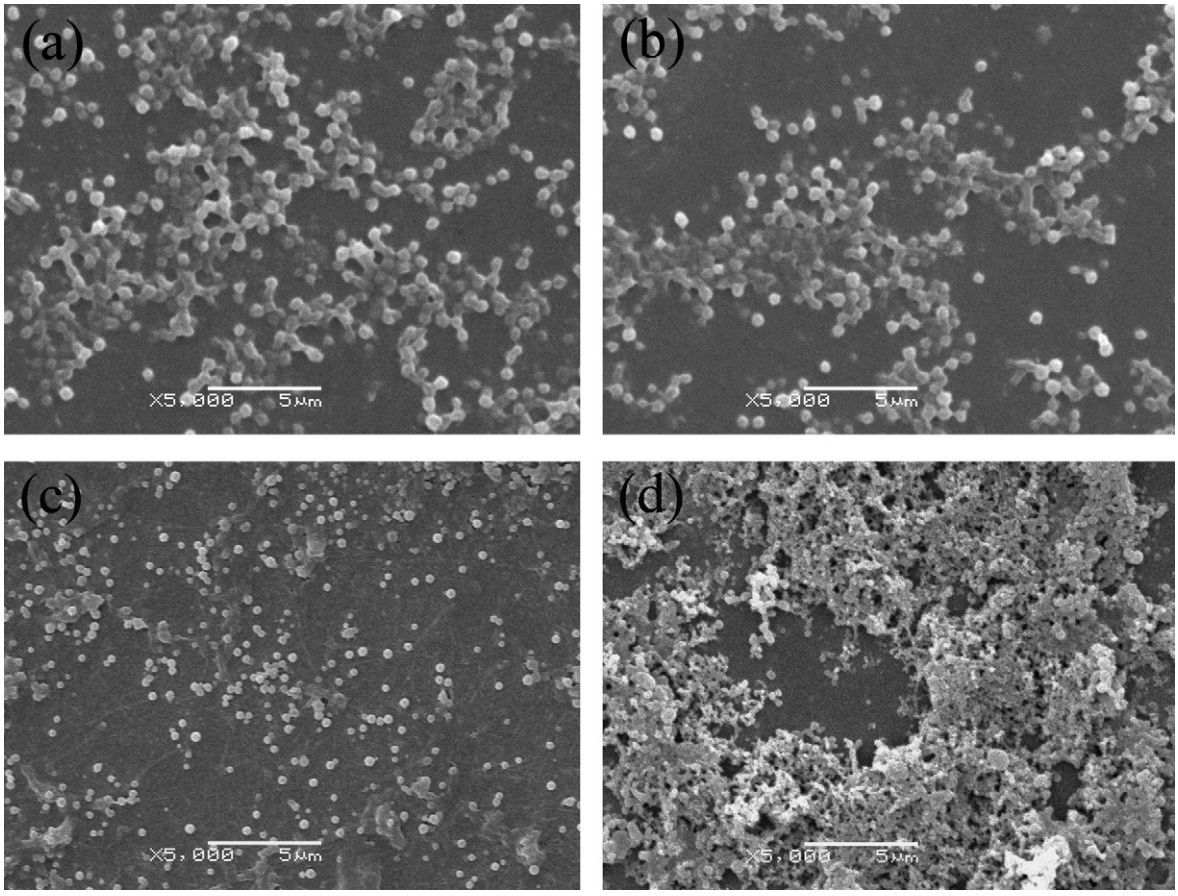


Fig. 3. SEM images of AC microparitcles prepared with different AC concentrations: (a) 20 mg/mL; (b) 40 mg/mL; (c) 60 mg/mL; (d) 80 mg/mL (stirring time: 30 s).

contains methoxyl and hydroxypropyl groups, which can form hydrogen bonds between the drug molecule and polymer, the stabilizer presented in the aqueous solution is absorbed on the surface of the formed hydrophobic drug particles to inhibit particle growth (Raghavan et al., 2003; Rasenack et al., 2004; Li et al., 2007).

3.2. The effect of stirring time

Stirring time is usually an important factor to influence the particle size in the antisolvent precipitation process. The relationship between the particle size distribution and stirring time is revealed in Fig. 2. It is evident that there is a notable increase in particle size during the stirring process. When the stirring time is 30 s, the average particle size is about 410 nm. Increasing the stirring time to 10 min, the particle size does not change obviously (420 nm). However, when the stirring time increases to 30 min, the particle size is dramatically increased to 800 nm. With the stirring time prolonged to 60 min, the average AC particle size is further increased to 1200 nm. It can be concluded that the shorter the stirring time, the smaller the particle size. The phenomenon of particle size increasing with stirring time can be explained by particle growth. When AC methanol solution is added into aqueous solution, small particles are formed immediately. During the stirring process, the formed small particles would collide and aggregate together, which may cause particle growth to larger ones. Moreover, it can be found that the AC particles own a narrow size distribution under shorter stirring time. Therefore, stirring for 30 s is believed as an optimum condition to obtain small particles.

3.3. The effect of drug concentrations

Fig. 3 shows SEM images of particles prepared with AC concentrations of 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL, respectively. As shown in Fig. 3a–c, the particles appear to have a spherical shape and exhibit good dispersibility. The average particle sizes under three different concentrations are 410 nm (20 mg/mL), 380 nm (40 mg/mL) and 240 nm (60 mg/mL). The particle size is slightly decreased with the increase of concentration, which could be explained as follows. Precipitation includes the main steps of nucleation and crystal growth. Compared to the crystal growth rate, the nucleation rate is more dependent on supersaturation, and the rate of nucleation rate greatly affects the final particle size (Dirksen and Ring, 1991; Wang et al., 2007). A higher drug concentration would create a higher supersaturation level and nucleation rate, resulting in small particle size (Chen et al., 2004). However, if the concentration was further increased to 80 mg/mL, the resultant particles tended to agglomerate together during the course of the precipitation, and the final product exhibited a poor distribution in both size and shape. This phenomenon might be interpreted by considering two factors: the number of nuclei formed in the solvent and antisolvent interface, and the influence of concentration on the viscosity. On one hand, at higher concentration, a large number of nuclei formed in the interface of two phases. Simultaneously, these nuclei decrease the diffusion from solvent to antisolvent, which would lead to particle aggregation. One the other hand, the viscosity of drug solution increased with the increasing of concentration, thereby hindering the diffusion between solution and aqueous solution (Zhang et al., 2006). Consequently, the particles show irregular shape and tend to aggregate at AC concentration of 80 mg/mL (Fig. 3d).

3.4. The effect of drying methods

Fig. 4 showed SEM images of powders obtained from oven drying and spray drying method. The cake dried in the oven at 60 °C was fractured into aggregates and could not be completely pulverized. Therefore, in this study, spray drying was chosen as the drying method. As shown in Fig. 4b, the spray dried powder had good dispersibility and narrow particle size distribution of 1–3 μm. Moreover, it could be clearly found that the resultant ultrafine AC was composed of some smaller particles, which kept the similar morphology and particle size with those of particles in the suspension (Fig. 3b). The possible formation mechanism of the obtained granular powder could be illustrated in Scheme 1. When the suspension passes the atomizer, the solution is sprayed as numerous small droplets containing many AC particles. With the evaporation of liquid, the particles included in the droplet would adhere together to form granular powder (Reinhard, 2008).

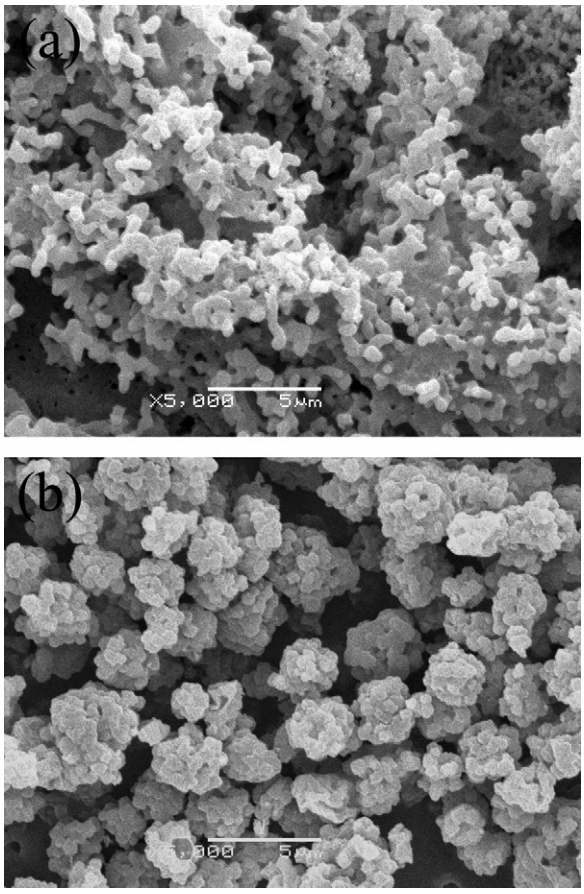


Fig. 4. SEM images of AC powders obtained from (a) normal oven drying and (b) spray drying (AC methanol concentration: 40 mg/mL).

3.5. X-ray diffraction studies

X-ray diffraction was performed to determine the physical state of raw AC and ultrafine AC. The corresponding patterns were displayed in Fig. 5. The raw AC was highly crystallized and exhibited intense crystalline peaks between 5° and 50°. However, a broad and diffuse maxima peak at 20° was detected in the pattern of ultrafine AC, demonstrating that the as-prepared ultrafine AC existed in the amorphous nature. It has been known that transforming the physical state of the drug to the amorphous would lead to a high-energy state and high disorder, resulting in enhanced dissolution rate and bioavailability (Won et al., 2005; Pathak et al., 2004).

3.6. FT-IR spectroscopy

Typical FT-IR spectra of raw AC, ultrafine AC and HPMC were compared in Fig. 6. It can be seen that the FT-IR spectrum of

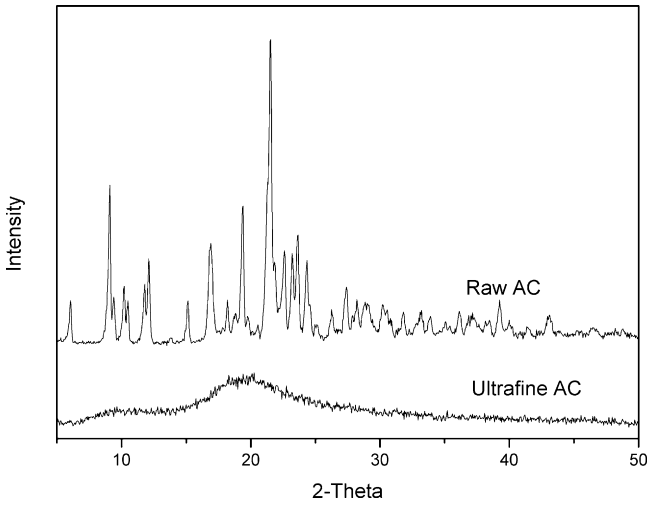


Fig. 5. XRD patterns of raw AC and ultrafine AC.

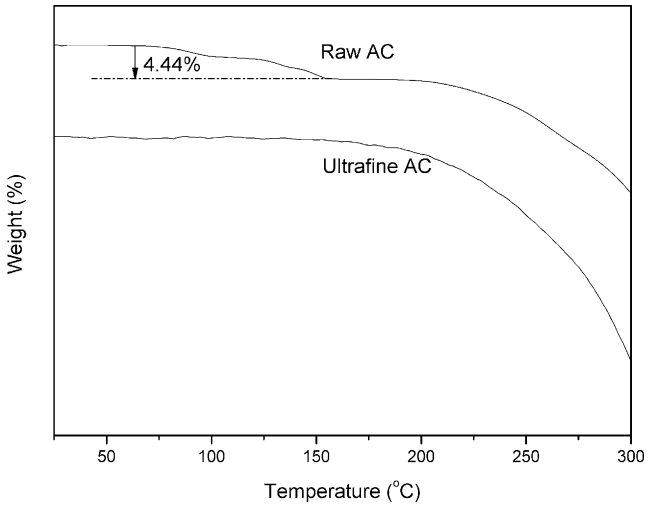


Fig. 7. TG curves of raw AC and ultrafine AC.

raw AC is different with that of ultrafine AC in two absorption bands (3700–3000 cm^{-1} and 1130–960 cm^{-1}). The absorption in 3666 cm^{-1} is assigned to the free O–H stretching, which was observed in the spectrum of raw AC, while not in the ultrafine AC. The peak at 3666 cm^{-1} probably belongs to the trihydrate functionality of raw AC (crystalline form). Moreover, three peaks at about 3364 (N–H stretching), 3261 (asymmetric O–H stretching) and 3056 cm^{-1} (symmetric O–H stretching) were found in the FT-IR spectra of raw AC, while broad bands with a shoulder at about 3407 cm^{-1}, 3320 cm^{-1} and 3058 cm^{-1} were observed for ultrafine AC, which could be ascribed to the amorphous nature of ultrafine AC (Kim et al., 2008b). In addition, the FT-IR spectrum of ultrafine AC is different with the raw AC in the range of 1130–960 cm^{-1} (C–O stretch). Since ultrafine AC is obtained by directly spray drying the suspension, some HPMC might be remained in dried powder. The HPLC study showed that drug content of ultrafine AC prepared with AC concentration of 40 mg/mL was approximately 81.49% (81.49 ± 0.63%). Therefore, the stronger absorbance of ultrafine AC in the range of 1130–960 cm^{-1} could be attributed to the existence of HPMC, because HPMC has strong absorption in this band (Weerd et al., 2004), as shown in Fig. 6.

3.7. TG analysis

TG curves corresponding to the thermal weight losses of raw AC and the ultrafine AC were shown in Fig. 7. Upon heating, the raw AC suffers pronounced weight loss step occurs in the region of 75–150 °C, which can be ascribed to the loss of three molecular water of crystallization. The total weight loss is about 4.44%, which is in agreement with theoretical value of a trihydrate (about 4.46%). However, weight loss was not observed in the ultrafine AC. From the TG results, it can be concluded that raw AC was transformed to anhydrous form by antisolvent precipitation and spray drying process.

3.8. DSC analysis

In order to further confirm the physical state, DSC was performed to analyze the raw AC and ultrafine AC, and the results were illustrated in Fig. 8. The DSC curve of raw AC showed two endothermic peaks, a broad endotherm in the region of 75–125 °C and a sharp endotherm at 158.8 °C. The broad endotherm ranging from 75 °C to 125 °C (with an enthalpy of 24.29 J/g) could be attributed to the removal of three molecule water, which is consistent with the result

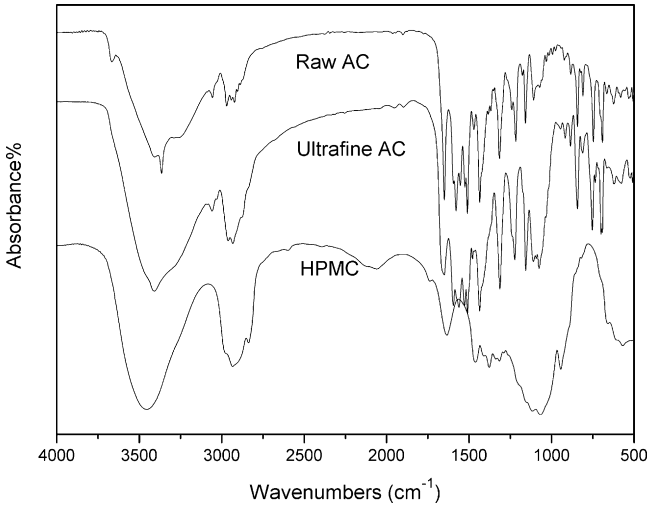


Fig. 6. FT-IR spectra of raw AC, ultrafine AC and HPMC.

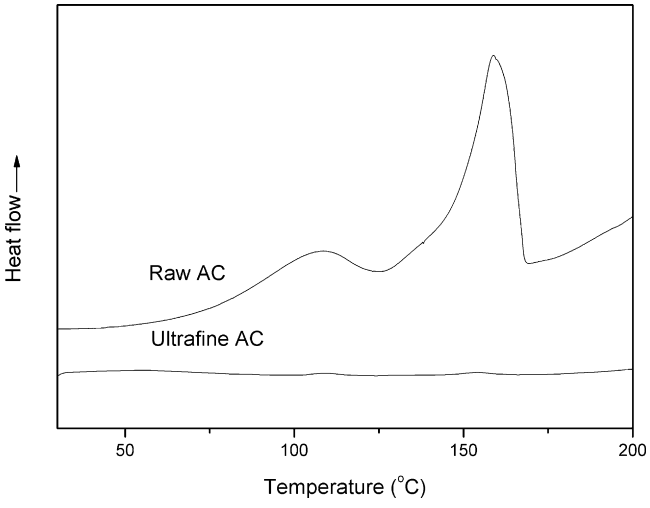


Fig. 8. DSC curves of raw AC and ultrafine AC.

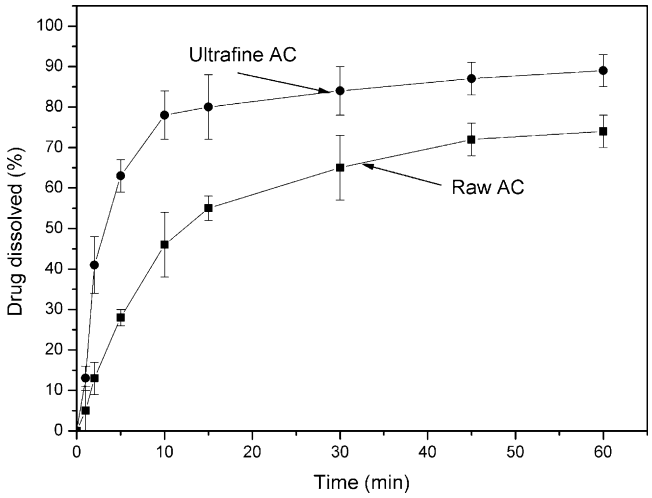


Fig. 9. Dissolution profiles of raw AC and ultrafine AC.

of TG. The sharp endotherm of raw AC at 158.8 °C (with an enthalpy of 86.85 J/g) corresponds to its melting point, confirming that the raw AC consisted solely of form I. However, no endothermic peak was observed in the DSC curve of ultrafine AC. The DSC study confirmed that the physical state of raw AC changed from crystalline to amorphous form.

3.9. Dissolution results

The profiles presented in Fig. 9 illustrated the dissolution rates of raw AC and the as-prepared ultrafine AC ($n = 3$). Ultrafine AC reached 63% drug dissolution within 5 min. However, only 28% of raw AC dissolved during the same period. After 60 min, about 89% of ultrafine AC was dissolved, but there was only 74% of raw AC was dissolved. The results indicated that ultrafine AC exhibited better dissolution property than the raw AC. The increased dissolution rate of ultrafine AC could be attributed to the great reduction of particle size, the corresponding increased specific surface area (from 4.8 m^2/g of raw AC to 9.5 m^2/g of ultrafine AC) and the amorphous nature of the product.

4. Conclusions

In this study, amorphous atorvastatin calcium ultrafine powder was successfully prepared using antisolvent precipitation and spray drying method. In the process, methanol and water were selected as solvent and antisolvent, respectively. HPMC was used as a hydrophilic polymer to inhibit the particle growth and control the particle morphology. The particle size exhibited an increasing tendency with the increase of stirring time, but decreased with the increase of drug concentration. Under the conditions of stirring time of 30 s, drug concentration of 40 mg/mL and spray drying process, ultrafine AC powder with narrow particle size distribution of 1–3 μm was prepared. In addition, XRD, IR, TG and DSC analysis indicated that the prepared ultrafine AC was amorphous. In the dissolution study, about 89% of amorphous AC ultrafine powder was dissolved after 60 min, while there was only 74% of raw AC dissolved. The as-prepared ultrafine AC demonstrated the enhanced dissolution rate owing to their decreased particle size, the formation of amorphous state and the increasement of specific surface area from 4.8 m^2/g to 9.5 m^2/g. Therefore, it can be concluded that antisolvent precipitation process is a facile pathway to prepare ultrafine drug particles.

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