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Preparation of cefpodoxime proxetil fine particles using supercritical fluids

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ABSTRACT

Fine particles of cefpodoxime proxetil (CPD) were prepared using an Aerosol Solvent Extraction System (ASES) with supercritical CO_2. The resulting primary particles were approximately 0.1–0.2 μm in size and were almost spherical in shape. The secondary particles were approximately 0.2–0.6 μm in size and had irregular shapes. The larger particle size and irregular shapes were due to the agglomeration of the primary particles. The effects of solvent type, CO_2-to-CPD solution weight ratio, and CPD solution concentration on the extent of agglomeration were investigated. As a result, the use of ethyl acetate and acetone as solvents also reduced the degree of agglomeration. The degree of agglomeration was reduced with the use of a high CO_2-to-solution weight ratio, and a low solution concentration. In particular, spherical particles, approximately 0.1–0.4 μm in size, were obtained when a 10.0 wt% CPD solution was used. As a result of dissolution study, almost 90% of the processed CPD had dissolved within 10 min. The recovery yield of the CPD powder reached approximately 80% using a membrane filter.

1. Introduction

Cefpodoxime proxetil (CPD, [6R-[6α, 7β(Z)]]-7-[[(2-aminothiazol-4-yl)(methoxyimino)-acetyl]-amino]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid 1-[[(1-methylethoxy)carbonyl]oxy]ethyl ester, $C_{21}H_{27}N_5O_9S_2$, MW. 557.61) is a third generation cephalosporin antibiotic and a pro-drug for oral administration (Fig. 1). CPD is a valuable antibiotic that is characterized by its broad-spectrum activity against gram-positive and gram-negative microorganisms. Several studies have been reported on the oral absorption and bioavailability of CPD (Borin, 1991; Crauste-Manciet et al., 1997; Nicolaos et al., 2003; Kakumanu et al., 2006; Date and Nagarsenker, 2007). The absolute bioavailability of CPD administered as a tablet relative to a cefpodoxime sodium intravenous infusion is approximately 50% (Borin, 1991). The low bioavailability of CPD is mainly attributed to the degradation of its ester side chain by cholinesterases present in the intestinal lumen. CPD's poor water solubility also contributes to its low bioavailability.

It has been disclosed that the amorphous forms of a number of drugs exhibit different dissolution characteristics and, in some cases, different bioavailability patterns compared to their corresponding crystalline forms. In the case of CPD, the amorphous form has been reported to have a higher bioavailability than the crystalline form (Kumar et al., 2003, 2006). The use of a solution crystallization process (batch-type) is one method for preparing amorphous CPD particles. In this method, concentrated CPD solution is dropped into a non-polar solvent (e.g. a hydrocarbon), which acts as an anti-solvent. This batch process cannot achieve uniform particle sizes because the crystallization conditions change continuously during the crystallization. Another method is the spray-drying technology for a continuous process (Kumar et al., 2003). Particles with relatively uniform sizes can be obtained using this method compared to those produced when using the batch process. In this process, the CPD is dissolved in an organic solvent and the solution is sprayed into hot air (or nitrogen gas), which is used as an anti-solvent. However, the high temperature used in this process increases the possibility of thermal decomposition. This decomposition can have negative effects on the assay, purity, color, and stability of the CPD particles.

Recently, there have been a number of publications on the preparation of fine particles using supercritical fluids (Reverchon, 1999; Jung and Perrut, 2001; Thiering et al., 2001; Beckman, 2004; Yeo and Kiran, 2005; Choi et al., 2006). Also, physicochemical properties and oral bioavailability of drug particles have been reported to compare supercritical anti-solvent (SAS) and spray-drying process (Kim et al., 2008a,b). In particular, the Aerosol

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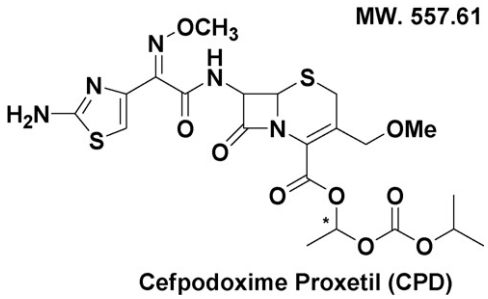

Fig. 1. Chemical structure of cefpodoxime proxetil (*: asymmetric carbon).

Solvent Extraction System (ASES) technique has been reported to be useful for preparing fine particles (Reverchon, 1999; Li et al., 2006). This process may be a favorable alternative to the previous methods because the crystallization process is continuous and the operation temperature required is moderate compared to those used in other methods. In particular, the use of sc-CO_2 has some potential advantages. For example, the moderate critical temperature and pressure (31 °C, 73 MPa) required do not result in thermal degradation, which could otherwise affect the quality and purity of the produced CPD. Also, it is possible to remove the residual solvent almost completely because the diffusivity and penetration ability of CO_2 into the particles are greatly enhanced under the supercritical conditions. This is important because minimizing the amount of residual solvent can improve the quality of the final drug. It has also been reported that ultra-fine particles can be obtained (with

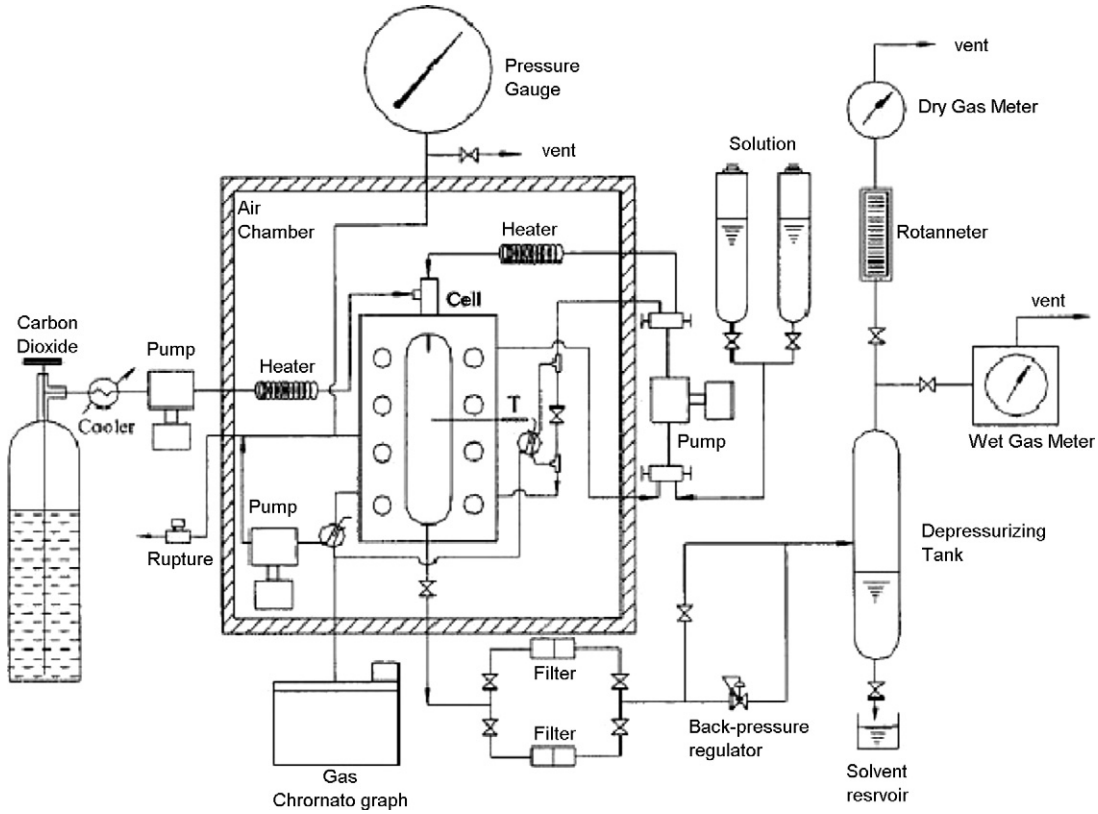

Fig. 2. Schematic diagram of the ASES apparatus.

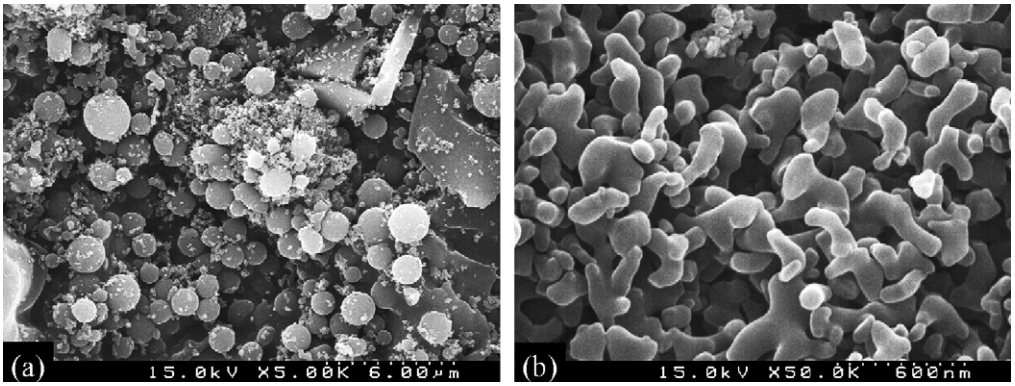

Fig. 3. SEM images of the raw and micron-sized CPD particles produced by ASES. (a) Raw CPD (×5000) and (b) micronized CPD (×50,000).

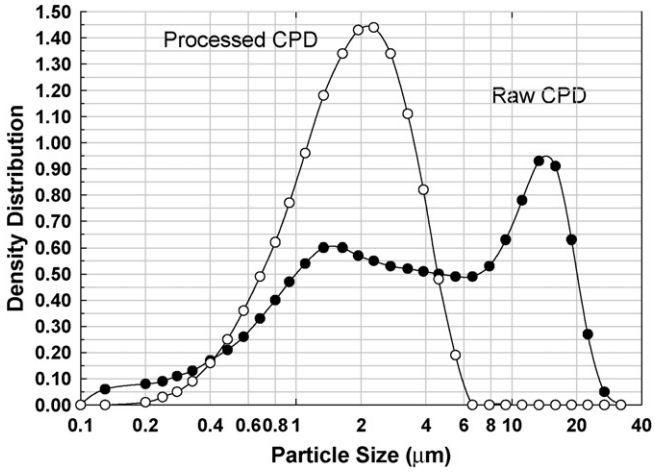

Fig. 4. Particle size distribution data of CPD (○: processed, ●: raw).

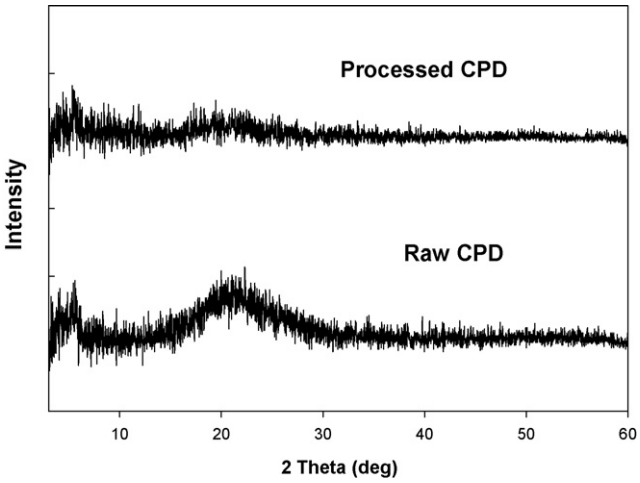

Fig. 5. PXRD patterns of the raw and processed CPD.

a very narrow particle size distribution (PSD)) as result of a rapid mass transfer between the solvent and anti-solvent. To obtain such fine particles, the solution containing CPD is sprayed into a high-pressure precipitator through a capillary nozzle. Dissolution of the supercritical fluid into the liquid droplets is accompanied by a large volume expansion and a decrease in the organic solvent power. This causes a sharp increase in the level of supersaturation within the liquid mixture, resulting in the formation of small and uniformly sized particles.

This study prepared fine CPD particles with a narrow particle size distribution using supercritical fluid technology to enhance the quality of the CPD. The effects of CPD solution concentration, CO_2-to-CPD solution weight ratio, and type of solvent on the particle size and morphology of the CPD were examined during ASES crystallization.

2. Materials and methods

2.1. Materials

Cefpodoxime proxetil was supplied by Hanmi Fine Chemicals Co. Ltd. (Korea). Carbon dioxide (99.0%) was purchased from Shin Yang Co., Korea. The solvents methylene chloride (MC, 99.8%, HPLC grade, J.T. Baker), acetone (AC, 99.5%, GR grade, Merck), ethanol (EtOH, 99.5%, GR grade, Samchun), and ethyl acetate (EA, 99.5%, GR grade, Merck) were used without further purification.

2.2. Apparatus

The CPD was precipitated using self-designed and manufactured ASES apparatus, as shown in Fig. 2. The apparatus consisted of a

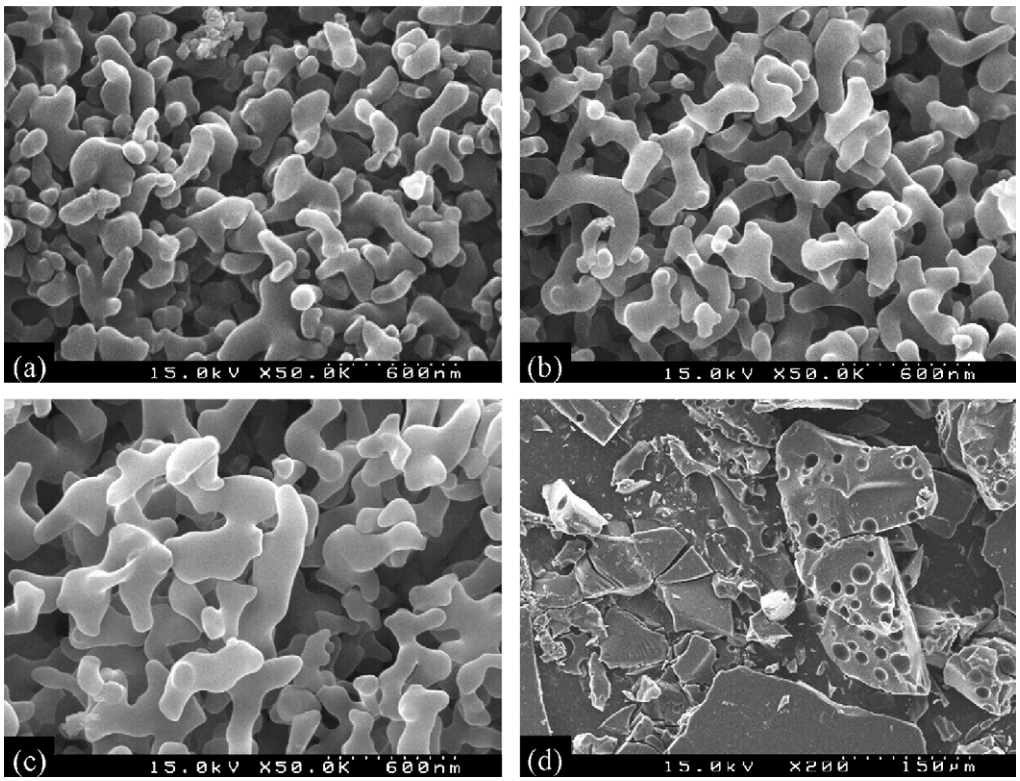

Fig. 6. The effect of the solvent type on the CPD particles at 35 °C, 10.0 MPa, concentration: 0.7 wt%. (a) Ethyl acetate, (b) acetone, (c) methylene chloride and (d) ethanol.

Table 1
ASES experimental conditions and PSD data (35 °C, 10.0 MPa).

Run number	Solvent	Solution concentration (wt%)	CO_2-to-CPD solution weight ratio	Particle size (μm)		
				x_{10}	x_{50}	x_{90}
1	EA	0.7	22	0.84	2.16	4.39
2	AC	0.7	22	0.66	1.90	4.13
3	MC	0.7	22	0.66	2.23	10.52
4	EtOH	0.7	22	NA	NA	NA
5	MC	0.3	88	0.11	0.15	0.20
6	MC	0.3	44	0.14	0.75	1.40
7	MC	0.3	22	0.73	2.02	3.85
8	MC	0.5	22	0.79	2.24	4.96
9	MC	1.0	22	0.71	2.63	19.90
10	MC	10.0	22	0.41	1.26	4.02
11	EA	0.3	22	0.45	1.42	4.60
12	EA	0.5	22	0.76	2.22	5.06
13	EA	1.0	22	0.87	2.37	5.81
14	EA	10.0	22	0.39	1.12	3.65

solvent and anti-solvent supplying section, a precipitation and particle collection section, and a depressurizing and solvent separation section. The volume of the precipitator was 34 cm^3, and it was installed with a water jacket to maintain the appropriate temperature. A glass window was installed outside the precipitator in order to observe the particle formation process. The pressure was adjusted using a back pressure regulator (Tescom; 26-1721-24) placed after a stainless steel filter (Tee-type filter, 0.5 μm, Swagelok). Carbon dioxide, used as an anti-solvent, was supplied to the cell through a diaphragm metering pump (PULSA 680, Pulsafeeder Inc., USA), and the desired temperature was reached using a heat exchanger. CPD solution entered the cell through a reciprocation pump (Milton Roy, USA). A capillary tube (stainless steels, 254 μm I.D., 1.59 mm O.D.), used as the nozzle, was placed on the top of the precipitator to spray in the CPD solution. A membrane (polytetrafluoroethylene (PTFE, 0.5 μm pore size), polyvinylidene fluoride (PVDF, 0.45 μm pore size), Millipore) was used as a filter when the recovery yield was measured.

2.3. Experimental procedure

CPD was dissolved in 20 mL of methylene chloride, acetone, ethanol, or ethyl acetate. Before injecting the solution, the precipitator was preheated to the desired temperature. CO_2, used as an anti-solvent, was introduced continuously to the precipitator from the top of the vessel through a diaphragm metering pump. When the desired temperature and pressure had been achieved, the CPD solution was sprayed into the precipitator through the nozzle, using the reciprocation pump. The flow rates of the sc-CO_2 and the CPD solution were 12.8 g/min and 0.3–0.6 mL/min, respectively. The CPD particles formed as soon as they were sprayed into the sc-CO_2 and were then collected on the filter. After the injection was complete, the sc-CO_2 was passed through for a further 10 min to eliminate the residual solvent in the particles.

2.4. Analysis and characterization

2.4.1. Particle size and particle size distribution

The particle size and particle size distribution were obtained using a Particle Size Analyzer (Sympatec HELOS/BF, Germany). This instrument could measure particle sizes ranging from 0.1 to 35 μm. The powder was placed in the PSA system and was allowed to flow into the particle size analyzer by RODOS/M using an ASPIROS dispersing system. The dispersion of the dry powder was performed using ASPIROS, using compressed air.

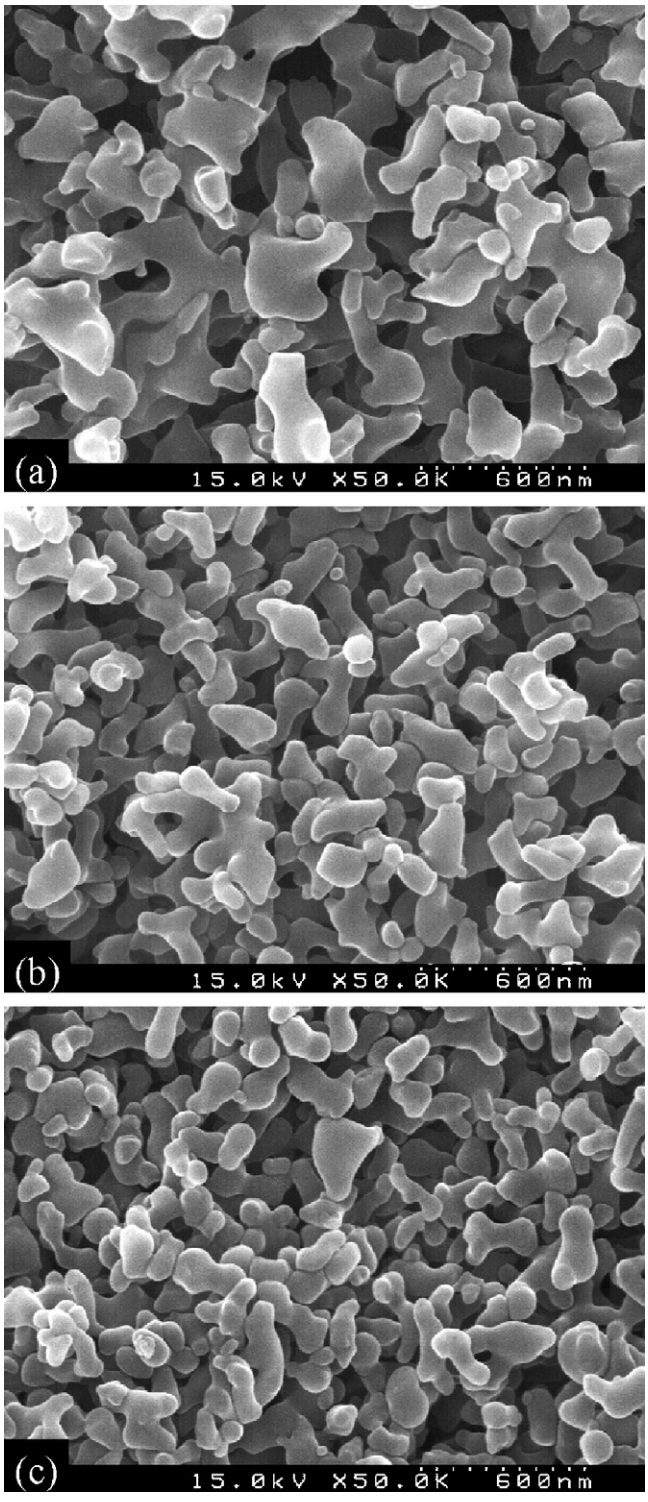

Fig. 7. The effect of the weight ratio (CO_2/CPD solution) on the CPD particles at 35 °C, 10.0 MPa, MC, concentration: 0.3 wt%. (a) 22, (b) 44 and (c) 88.

2.4.2. Scanning electron microscopy

The particle morphology was analyzed using field emission scanning electron microscopy (FE-SEM) (Hitach S-4200, Japan). Sample preparation for the SEM involved spreading the particles onto a piece of carbon tape that had been glued to an aluminum stub. The sample was then sputter coated for 3 min with platinum (sputter coater, GATAN 682, Japan) to make the surfaces

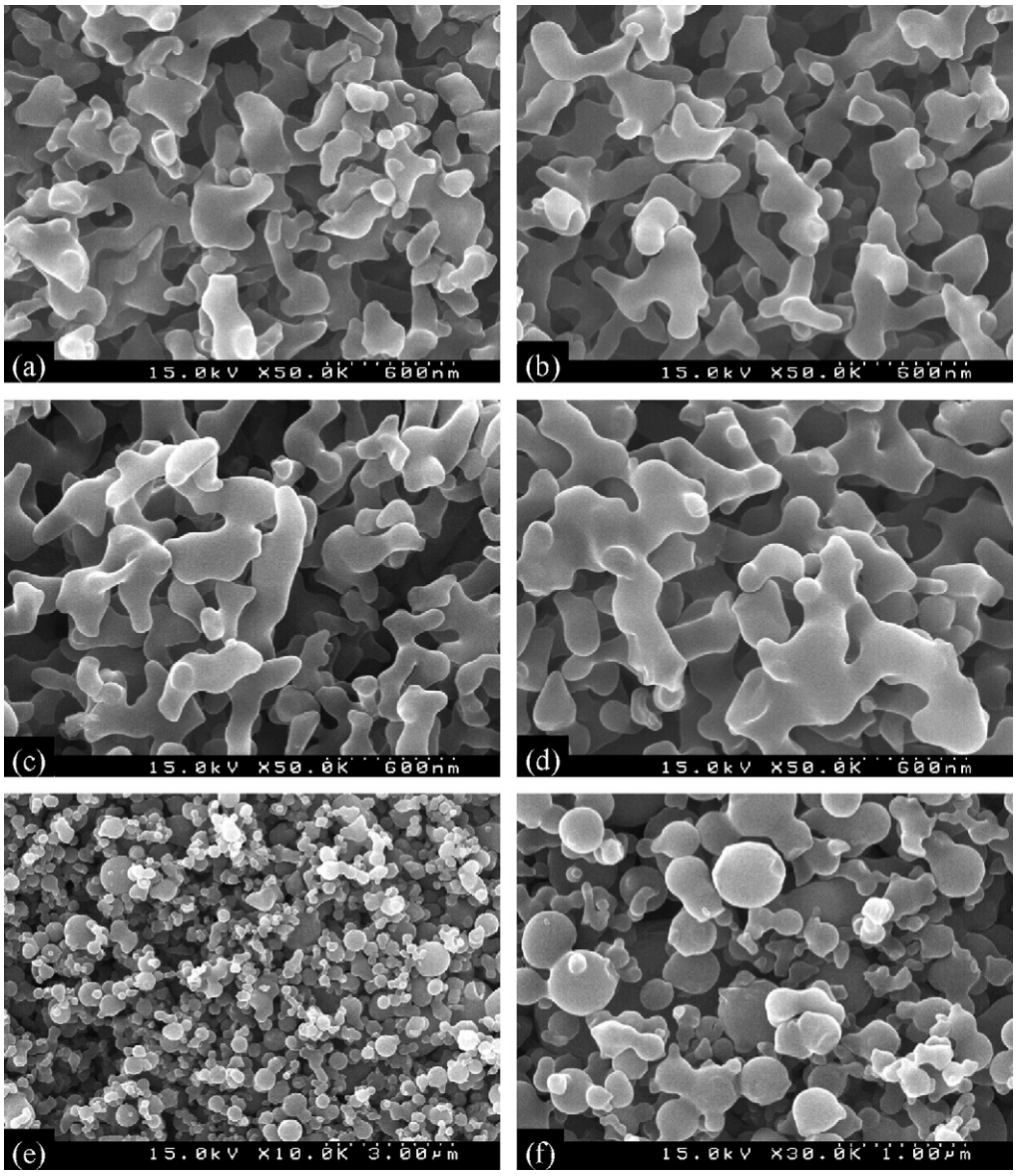

Fig. 8. The effect of the solution concentration on the CPD particles at 35 °C, 10.0 MPa, MC. (a) 0.3 wt%, (b) 0.5 wt%, (c) 0.7 wt%, (d) 1.0 wt%, (e) 10 wt% and (f) 10 wt%.

of the particles conducting. The particles were then observed by SEM.

2.4.3. Powder X-ray diffraction

Powder X-ray diffraction (PXRD, Bruker, D5005, Germany) was performed to assess the crystal structures of the particle. Each XRD pattern was recorded from 3 to 60° (2θ), at a scanning speed of 15°/min.

2.4.4. Dissolution profiles

The dissolution profiles of the raw and processed CPD samples were tested using a USP paddle method with a dissolution tester (DST-810, LABFINE INC., Korea). Each dissolution test was performed in 900 mL of simulated gastric juice (pH 1.2), using 100 mg of CPD. The measurements were carried out at 37 °C, and the paddle rotated at 50 rpm. For each CPD solution, a sample (5 mL) was taken after 1, 5, 10, 20, 30, 60, and 90 min, and filtered through a membrane filter (0.45 μm). The CPD concentration was determined by UV–vis spectroscopy (Helios α UV–vis spectrophotometer, England) at a wavelength of 235 nm.

3. Results and discussion

The CPD particles prepared by the ASES process were free-flowing particulates. Fig. 3 shows SEM images of the raw and micronized CPD particles. The raw CPD particles, which were produced by batch process using an organic solvent, show three types of morphologies, which include spherical nano-size particles, spherical micro-size particles, and large plate particles. The size of

Table 2
The precipitation conditions and recovery yields of CPD.

Run number	CPD solution concentration (wt%)	Solvent	Membrane	Yield
15	1.0	MC	PTFE	79%
16	2.0	MC	PVDF	82%
17	1.0	EA	PVDF	78%

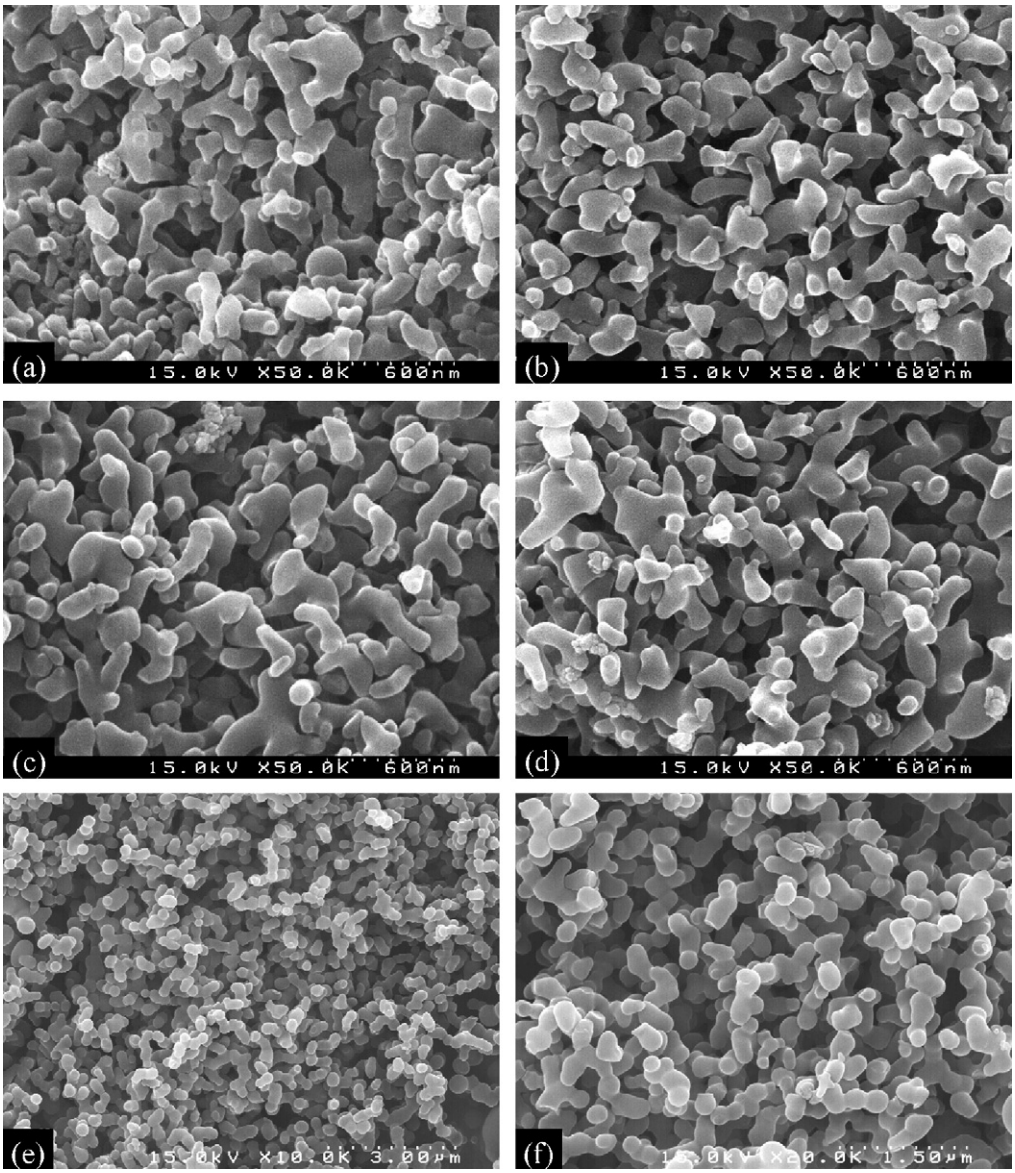

Fig. 9. The effect of the solution concentration on the CPD particles at 35 °C, 10.0 MPa, EA. (a) 0.3 wt%, (b) 0.5 wt%, (c) 0.7 wt%, (d) 1.0 wt%, (e) 10 wt% and (f) 10 wt%.

the large plate particle was >6 μm. This means that the crystallization condition is not consistent during the batch process. After the ASES process (35 °C, 10.0 MPa, EA, 0.7 wt%), there was noticeable sintering of the CPD fine particles. The sizes of the sintered particles were approximately 0.2–0.4 μm, as a result of particle agglomeration. Fig. 4 shows the volume-based particle size distribution of raw and processed CPD. The raw CPD shows a broad particle size distribution with three main peaks. In the case of the raw CPD, x_{10} and x_{90} were 0.64 and 16.2 μm, respectively. After the ASES process, x_{10} and x_{90} were 0.84 and 4.39 μm, respectively. The plot for the processed CPD shows only a single peak, which means that the particles have a consistent size and morphology. This is due to the continuous process preventing a change in the crystallization condition during the atomization, nucleation, and growth of the particles. PXRD analysis (Fig. 5) shows that the CPD particles prepared using both the batch and the continuous processes were amorphous.

In these experiments, the CPD particles were affected by the agglomeration of the primary particles. Therefore, the focus of this study was to reduce the level of agglomeration by adjusting the processing conditions. The experimental conditions and the volume-based particle size distribution data are summarized in Table 1. First, a series of experiments was carried out to determine the effect of using different solvents on particle agglomeration. The solvent used was varied between MC, AC, EA and EtOH, while keeping the other experimental conditions the same. As shown in Fig. 6 and Table 1, agglomeration was most severe when MC was used as the solvent. This was caused by the high solubility of CPD in methylene chloride. In the case of MC, the particles can agglomerate more easily during the ASES process in the precipitator and the filter. The SEM image also shows that the CPD agglomerates recovered from EtOH. Due to CPD's high solubility in ethanol and the fact that ethanol can interact with CPD through hydrogen bonding, the precipitated particles could be dissolved at the filter and recrystallize on the filter surface. The solvent is an important parameter for controlling the agglomeration during CPD precipitation.

In addition, the CO_2-to-CPD solution weight ratio (grams of CO_2/grams of the CPD solution) was changed from 22 to 88 in order to reduce the level of agglomeration. As shown in Fig. 7, increasing the weight ratio decreased the extent of agglomeration, which resulted in smaller particles.

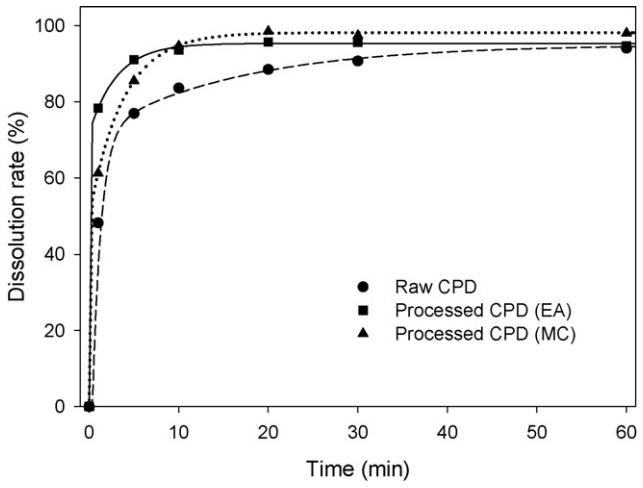

Fig. 10. Dissolution profiles of the raw and processed CPD (●: raw, ■: processed (EA), ▲: processed (MC)).

The concentration of the CPD solution was varied from 0.1 to 10.0 wt%. The temperature and pressure of the sc-CO_2, the type of solvent, the solution flow rate, and the sc-CO_2 flow rate were fixed to 35 °C, 10.0 MPa, MC, 0.37 mL/min, and 12.8 g/min, respectively. Fig. 8 shows the continuous increase in particle agglomeration with increasing solution concentration. It is possible that a single particle has a higher probability of coming into contact with other particles as the CPD concentration in the solution is increased. At a concentration of 1.0 wt%, large particles are generated and condensed quite readily. The CPD solution concentration was increased to 10.0 wt% in order to determine the effect of increasing the concentration. As shown in Fig. 8, the CPD particles were almost spherical in shape, and the particles were approximately 0.1–0.4 μm in size. Although a small extent of agglomeration was still observed, the agglomeration structure is quite different from those found previous experiments. The same experiments were performed using EA as a solvent instead of MC. As shown in Fig. 9, the use of EA resulted in the degree of agglomeration being decreased for all concentrations. The agglomeration structure is also different from other those found for other cases at 10.0 wt%.

For the dissolution studies, raw and processed CPD particles, obtained from the concentrated solutions, were tested. In this case, EA and MC were used as solvents, and the solution concentration of each test sample was 10.0 wt%. The dissolution rate was determined by the USP paddle method using a dissolution tester. Fig. 10 shows example dissolution profiles of the raw and processed CPD. The processed CPD particles showed a high dissolution rate compared to the raw CPD. The dissolution rate of the processed CPD was greater than that of the raw material, but only during the first 30 min. Almost 90% of the processed CPD had dissolved within the first 10 min.

Finally, the recovery yields of the CPD samples were measured. However, it was very difficult to measure the yields due to the powders experiencing static charging. Therefore, a cylindrical filter equipped with a membrane (PTFE, PVDF) was used to collect the powders. Except for the filter type, the precipitation conditions were same as those that were used in the previous experiments. As shown in Table 2, the recovery yields reached approximately 80%.

4. Conclusions

CPD particles were crystallized successfully using the ASES method using sc-CO_2. The primary particles were approximately 0.1–0.2 μm in size. The secondary particles were approximately 0.2–0.6 μm in size, as a result of the primary particles agglomerating. The degree of the agglomeration of the precipitated CPD was affected by the CO_2-to-CPD solution weight ratio, the solvent type, and the CPD solution concentration. The solvent type was found to be an important parameter for controlling the extent of agglomeration of the particles. In particular, the degree of agglomeration was minimized when either ethyl acetate or acetone was used. In addition, the extent of particle agglomeration decreased with increasing CO_2-to-CPD solution weight ratio. The use of a high CO_2-to-CPD solution weight ratio resulted in particles that were almost spherical. The extent of particle agglomeration continuously increased with increasing solution concentration. Spherical CPD particles were obtained when the solution concentration was 10.0 wt%. The particles were approximately 0.1–0.4 μm in size, with little agglomeration. The dissolution rate of the processed CPD was higher than that of the raw material, but only during the first 30 min. Moreover, almost 90% of the CPD had dissolved within the first 10 min. A membrane filter was used to recover the CPD particles due to the static charging of the powders. The recovery yield of CPD, when using these filters, was approximately 80%.

Acknowledgments

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