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Recrystallization of tetracycline hydrochloride using supercritical anti-solvent process

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Abstract−Tetracycline hydrochloride (TTC) was micronized by an Aerosol Solvent Extraction System (ASES) using supercritical CO_2. The effects of solvent, pressure and temperature of CO_2, solution concentration, and solution feed rate on particle size were investigated. Mean particle sizes of processed TTC were 0.16-0.31 μm, but the morphologies of processed particles were affected by agglomeration between the primary particles. Mean particle sizes of unprocessed TTC were *ca*. 200 μm and the shapes were irregular with rough surfaces. Especially, particle sizes increased from 0.18 to 0.31 μm as CO_2 temperature increased. In addition, particle sizes increased from 0.18 to 0.23 μm as TTC concentration increased. Powder X-Ray diffractometry revealed that processed particles were amorphous whereas unprocessed particles showed strong crystallinity.

Key words: Tetracycline Hydrochloride, Supercritical Carbon Dioxide, ASES, Recrystallization

INTRODUCTION

Supercritical fluid technology has been applied for micronization, extraction, coating, separation, chemical reaction, and other [1-9]. Recently, a number of reports have been issued concerning the preparation of various powdered drugs using this technology [7-9]. Several methods have been used to produce different particle sizes and morphologies, and Rapid Expansion of Supercritical Solution (RESS) and Supercritical Anti-Solvent (SAS) are most widely used to prepare fine particles using supercritical fluids. The simplest SCF technique for preparing fine particles is the RESS process, though this process is feasible when a drug can be dissolved in supercritical fluids [10,11]. According to this method, a drug is dissolved in supercritical fluids and the solution expands at ambient pressure through a nozzle. Submicron and nano-sized dry particles with a narrow particle size distribution can be obtained for various pharmaceutical compounds and polymers using this process. On the other hand, the SAS process can be used to produce fine particle when a drug has a low solubility in supercritical fluids [12,13]. In the SAS process, the drug is dissolved in a solvent miscible with supercritical fluid, which acts as an anti-solvent, and the drug solution is mixed with supercritical fluids in a precipitator to make a fine particle.

In the present study, the Aerosol Solvent Extraction System (ASES), which is one kind of the SAS process, was used [8,13]. In this process, a drug solution is sprayed into a high pressure precipitator through a capillary nozzle. The dissolution of the supercritical fluid into the liquid droplets is accompanied by a large volume expansion of solution and a reduction in solvent power, which causes supersaturation within the liquid mixture and the consequent formation of small and uniform particles. The use of sc-CO_2 as an anti-solvent has some advantages; for example, it is possible to remove residual solvent almost completely since the ability of CO_2 to diffuse into particles is greatly enhanced under supercritical conditions. Therefore, problems associated with residual solvent can be minimized, and the quality of drugs improved when sc-CO_2 is used as an anti-solvent.

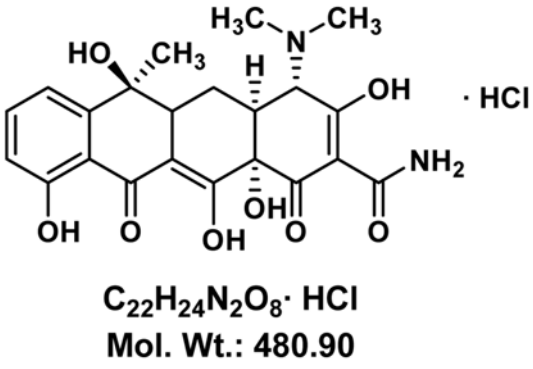

Fig. 1. The chemical structure of tetracycline·HCl (TTC).

Tetracycline hydrochloride (TTC, $C_{22}H_{24}N_2O_8$·HCl, Fig. 1) is used to treat bacterial infections, such as, infections of the respiratory tract and lungs, skin, genital and urinary systems, and of the stomach, including those caused by Helicobacter pylori. The micronization of TTC using Supercritical Assisted Atomization (SAA) and Supercritical Anti-Solvent (SAS) techniques has been reported [14, 15]. However, in these studies, the effects of process parameters on TTC particles were not investigated sufficiently. Accordingly, the aim of the present study was to micronize TTC using an ASES process and to determine the effects of solvent, pressure and temperature of CO_2, solution concentration, and solution feed rate on particle sizes and morphologies.

MATERIALS AND METHODS

1. Materials

Tetracycline hydrochloride (TTC, min. 95%) was purchased from Sigma-Aldrich. *N*-methyl-2-pyrrolidone (NMP, 99.0%), methanol (MeOH, 99.8%), ethanol (EtOH, 99.0%), dimethylsulfoxide (DMSO,

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99.0%), and *N,N*-dimethylformamide (DMF, 99.0%), which were used as solvents, were purchased from Samchun Pure Chemicals (Korea) without further purification. Carbon dioxide (99.0%) was purchased from the Shin Yang Co. (Korea).

2. Experimental Apparatus

TTC precipitation was performed using a self-designed ASES apparatus. The apparatus consisted of a solvent and anti-solvent supplying section, a precipitation and particle collection section, and a depressurizing and solvent separation section (Fig. 2). The volume of the precipitator was 34 cm^3, and it was surrounded by a thermostatically controlled water jacket for temperature control. Temperature in the precipitator was controlled using two K-type thermocouples and water jacket, which was connected to a circulating bath. A glass window was installed in the wall of the precipitator to allow processes to be observed. Pressure was adjusted using a back pressure regulator (Tescom; 26-1721-24) and monitored using a pressure gauge (Millipore). Carbon dioxide was supplied to the precipitator using a diaphragm metering pump (PULSA 680, Pulsafeeder, Inc, USA), and required temperature was obtained using a heat exchanger. TTC solutions were pumped into the precipitator using a reciprocation pump (Milton Roy, USA). A capillary tube (stainless steel, 254 μm I.D., 1.59 mm O.D.), which was used as a nozzle, was placed on the top of the precipitator to spray the TTC solutions. A stainless steel filter (Tee-type filter, 0.5 μm, Swagelok) was used to collect the TTC particles. Heating tape was used to warm the gas/liquid separator when supercritical solution was depressurized.

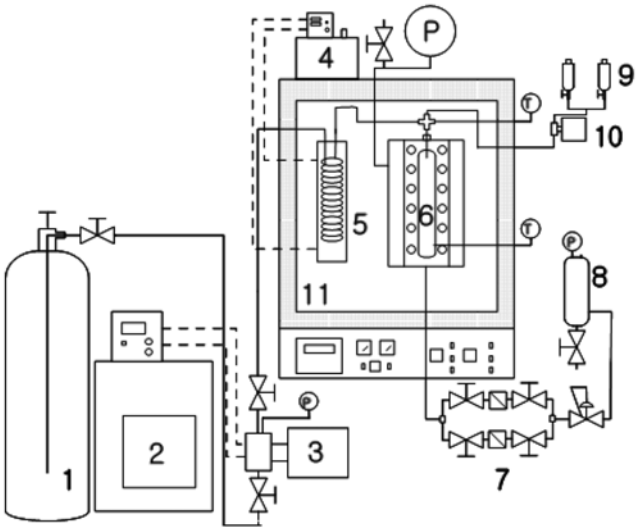

Fig. 2. Schematic diagram of the ASES apparatus.

1. CO_2 cylinder
2. Cooling bath
3. CO_2 pump
4. Heating bath
5. Pre-heater
6. Precipitator
7. T-type filters
8. G/L separator
9. Solution reservoir
10. Solution pump
11. Thermostat air bath

3. Experimental Method

TTC was dissolved in NMP, MeOH, EtOH, DMSO, or DMF. Before injecting the solution, the precipitator was preheated to the desired temperature (35-45 °C). CO_2 was introduced continuously to the precipitator from the top of the vessel using a diaphragm metering pump. A backpressure regulator was used to control the pressure of the system (10.0-15.0 MPa). When the desired pressure and temperature had been achieved, TTC solution was sprayed into the precipitator through the capillary nozzle using a reciprocation pump. The injection flow rates of sc-CO_2 and TTC solution were 16.2 g/min and 0.32-0.76 mL/min. After completing the injections, sc-CO_2 was fed for 15 minutes continuously to remove residual solvent from particles. TTC particles were recovered from the stainless steel filter after releasing the pressure of the apparatus.

4. Characterization

Particle morphologies were analyzed using a field emission scanning electron microscope (FE-SEM) (Hitachi S-4200, Japan). Sample preparation for SEM involved spreading particles onto a piece of carbon tape glued to an aluminum stub. Samples were then sputter

Table 1. ASES experimental conditions and achieved particle sizes

Run no.	Solvent	CO_2		TTC Solution		Mean particle size (μm)	Particle size distribution
		°C	MPa	Conc. (wt%)	Feed rate (g/min)		
1	NMP	35	10.0	1.0	0.32	0.18	1.39
2	DMSO	35	10.0	1.0	0.32	0.23	1.48
3	EtOH	35	10.0	1.0	0.32	0.16	1.42
4	DMF	35	10.0	1.0	0.32	0.17	2.35
5	MeOH	35	10.0	1.0	0.32	0.19	1.43
6	NMP	40	10.0	1.0	0.32	0.29	1.70
7	NMP	45	10.0	1.0	0.32	0.31	1.47
8	NMP	35	12.5	1.0	0.32	0.23	1.82
9	NMP	35	15.0	1.0	0.32	0.20	1.46
10	NMP	35	10.0	0.5	0.32	0.18	1.46
11	NMP	35	10.0	2.0	0.32	0.22	2.44
12	NMP	35	10.0	4.0	0.32	0.23	2.12
13	NMP	35	10.0	1.0	0.44	0.20	1.72
14	NMP	35	10.0	1.0	0.61	0.20	1.50
15	NMP	35	10.0	1.0	0.76	0.18	1.45

coated for 3 minutes with platinum (sputter coater, GATAN 682, Japan). The particle size and particle size distribution were measured with the TDI Scope Eye software (Techsan, Korea) in the SEM images. The particle size distributions were reported by the polydispersity index (PDI; PDI=D_w/D_n). Number (D_n) and weight (D_w) of average particle diameters were calculated from the following equations:

$$D_n = \frac{\Sigma_{i=1}^{N} d_i}{N} \quad (1)$$

$$D_w = \frac{\Sigma_{i=1}^{N} d_i^4}{\Sigma_{i=1}^{N} d_i^3} \quad (2)$$

where d_i is the diameter of particle i and N is the total number of particles measured in the SEM images. X-ray diffraction (XRD, Bruker, D5005, Germany) was performed to determine crystal structures. Each XRD pattern was recorded from 3 to 50° (2θ), at a scanning speed of 1°/min.

RESULTS AND DISCUSSION

This study was undertaken to determine the effects of solvent, pressure and temperature of CO_2, solution concentration, and solution feed rate on the sizes and morphologies of TTC particles obtained using the ASES process. The experimental conditions and results are summarized in Table 1.

SEM images of unprocessed TTC showed that particles were irregular with rough surfaces and mean particles size was 195 μm (Fig. 3). SEM images of ASES processed particles obtained using a temperature of 35 °C, a pressure of 10.0 MPa, and solution concentration of 1.0 wt% for the various solvents are shown in Fig. 4. Also, particle size distributions were shown in Fig. 5 and Table 1. In all cases, particle sizes were reduced from 195 to 0.2 μm after ASES process. But, particle morphology of processed TTC was affected by agglomeration between the primary particles. To select the processing solvent, mean particle size and particle size distribution were considered. As can be seen in Fig. 4 and Table 1, mean particle sizes were 0.16 and 0.17 μm in the case of ethanol or DMF were used as a solvent, but particle size distributions were 1.42 and 2.35. In the case of DMSO, particle size distribution was 1.48, but

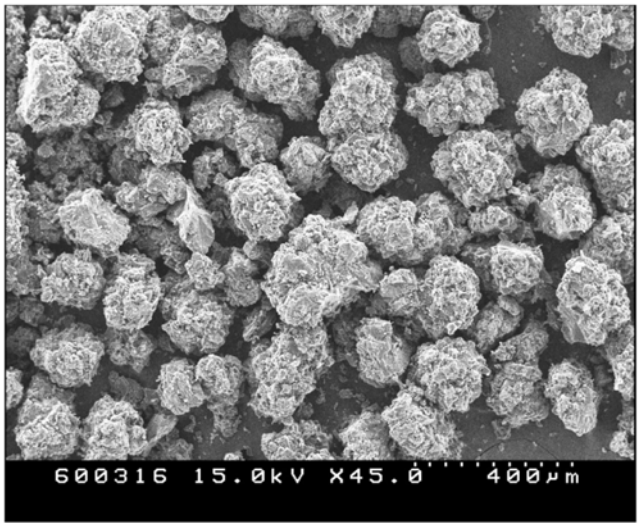

Fig. 3. SEM image of unprocessed TTC particles.

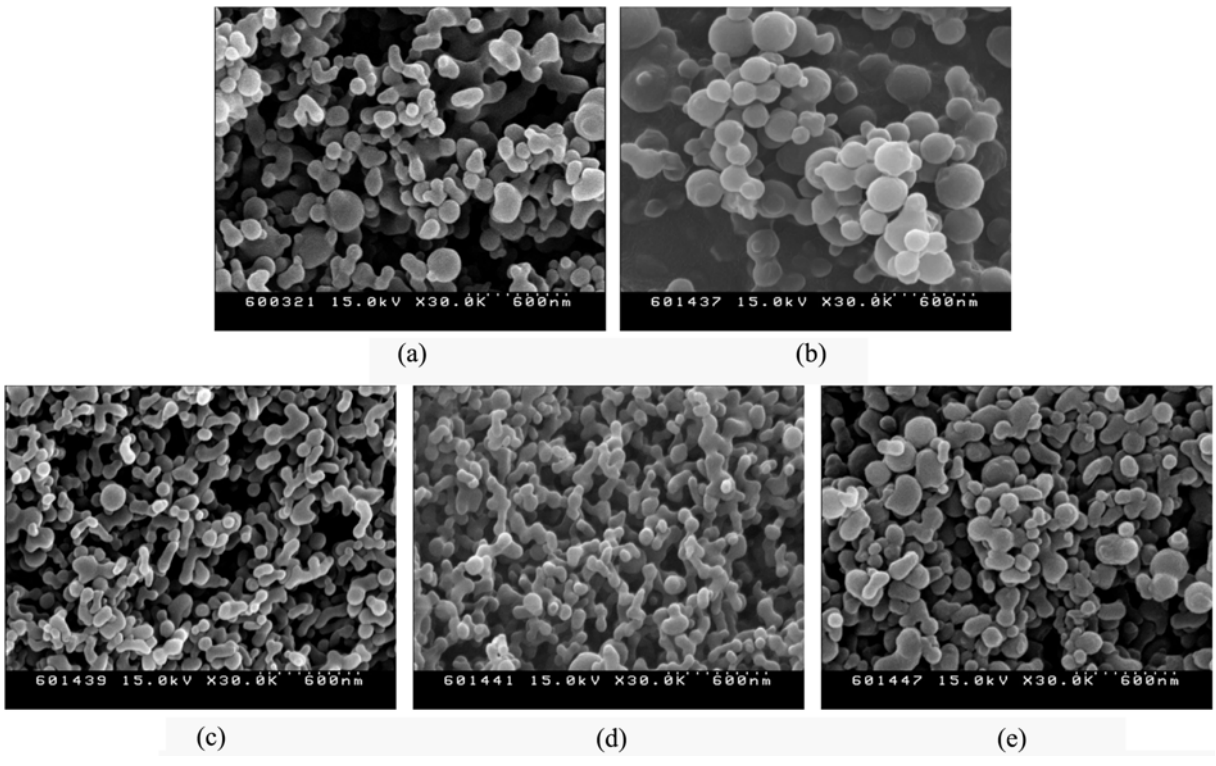

Fig. 4. SEM images of processed TTC particles (Run 1-5): (a) NMP, (b) DMSO, (c) EtOH, (d) DMF, (e) MeOH.

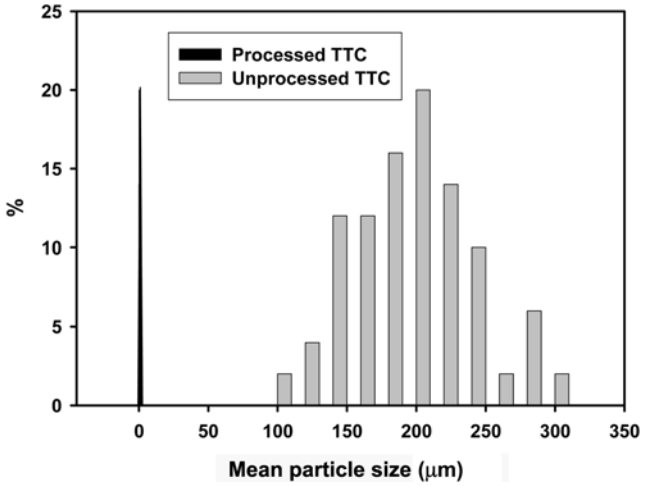

Fig. 5. Particle size distributions of unprocessed and processed TTC.

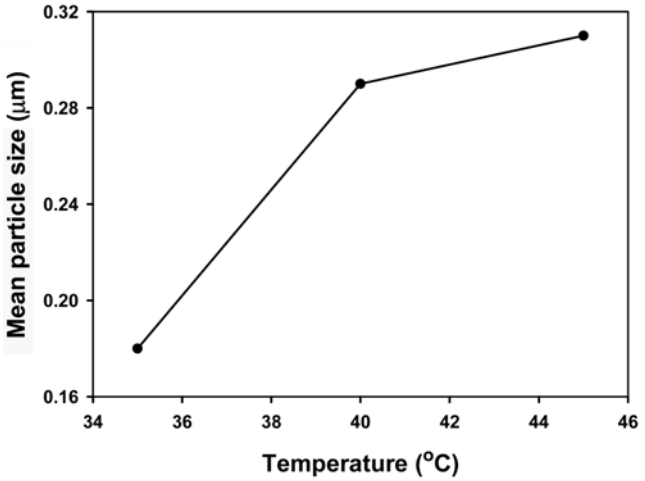

Fig. 7. Effect of CO_2 temperature on mean particle size of TTC.

the sizes of particles were up to 0.2 μm. To determine the effects of process parameters, NMP was selected because it was associated with a moderate mean particle size and its distribution.

The effects of CO_2 temperature on particle size were investigated over the range of 35 to 45 °C. SEM images of resulting particles showed that an increase in temperature increased mean particle size (from 0.18 to 0.31 μm) with less agglomeration between primary particles (Figs. 6-7). Two competing phenomena should be considered when considering the effect of temperature on particle formation. First, the reduced density of CO_2 with temperature causes increase in diffusion rate between CO_2 and NMP and rapid achievement of supersaturations, and results in small particle size. Second, the solubility of solutes in the solvent increases, and the level of supersaturation reduces as temperature increases. As a result, particle size increases. In the present study, particles size increased because the second phenomenon may dominate [16]. In particular, the vapor pressure of NMP increased as temperature increased, which reduced residual solvent levels on the particles, and thus, the degree of agglom-

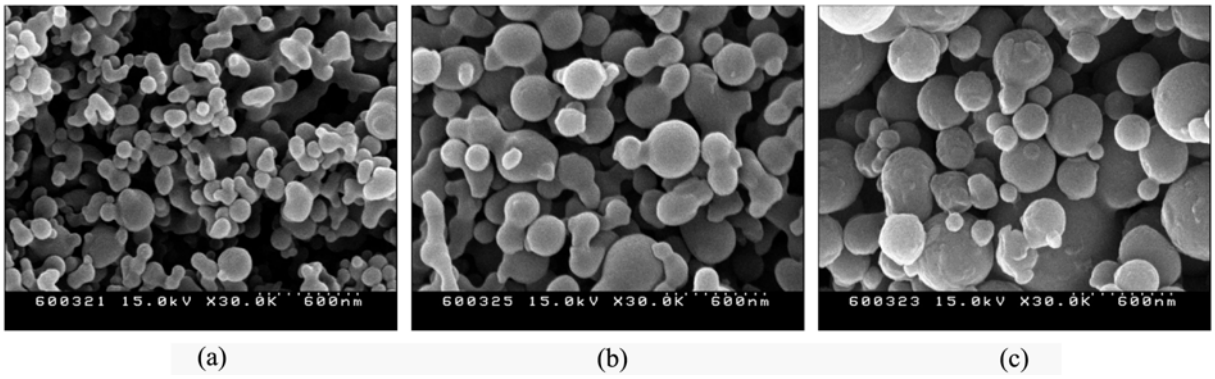

Fig. 6. SEM images of processed TTC particles (Run 1, 6-7): (a) 35 °C, (b) 40 °C, (c) 45 °C.

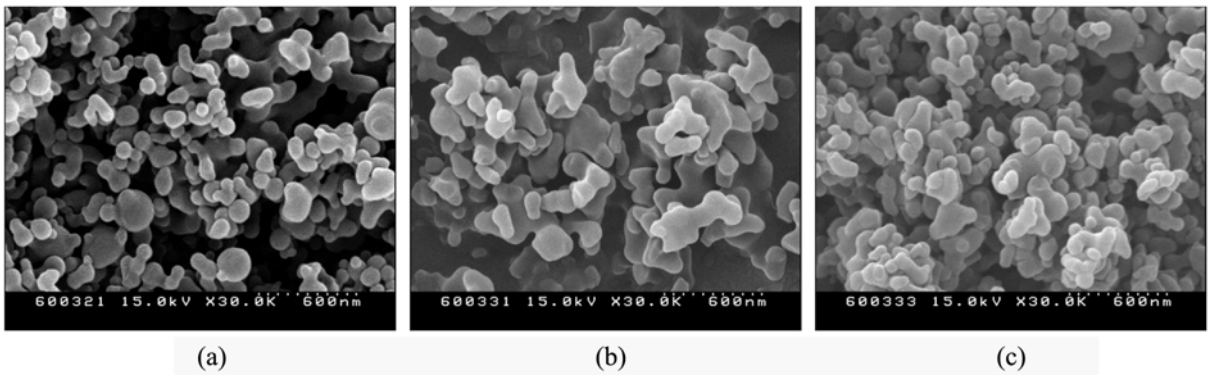

Fig. 8. SEM images of processed TTC particles (Run 1, 8-9): (a) 10.0 MPa, (b) 12.5 MPa, (c) 15.0 MPa.

eration reduced. The effects of pressure in the range 10.0 to 15.0 MPa at 35 °C on particle size were assessed using SEM images of processed TTC (Fig. 8), and this showed that no remarkable changes in particle size or morphology occurred when pressures were altered [17].

The effects of concentration of TTC in NMP (from 1.0 to 4.0 wt%) on particle size were also examined. As TTC concentrations were increased, mean particle size increased from 0.18 to 0.23 μm (Figs. 9-10). At higher concentrations, large particles are generated quite readily as a result of agglomeration because primary particles are more likely to contact other particles during precipitation process [18]. In addition, a series of experiments was carried out to determine the effect of TTC solution feed rate (Fig. 11). The result showed no significant change in size or morphology because the solution feed rates were sufficient to cause jet breakup of the solution.

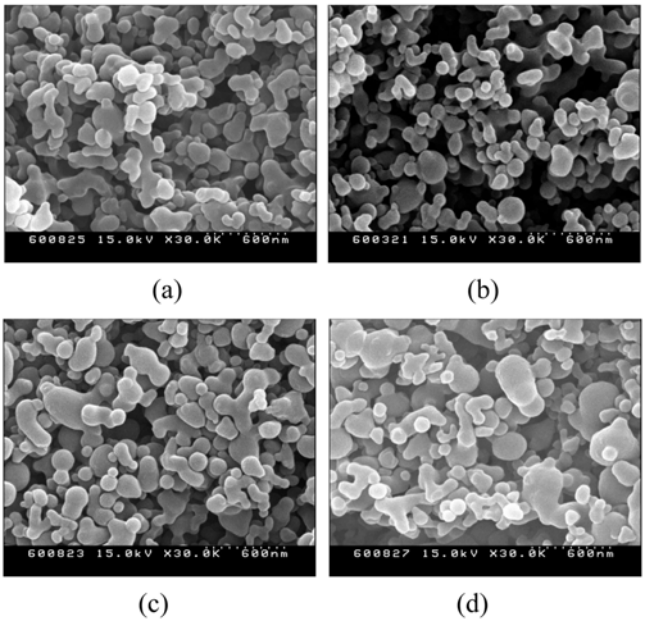

Fig. 9. SEM images of processed TTC particles (Run 1, 10-12): (a) 0.5 wt%, (b) 1.0 wt%, (c) 2.0 wt%, (d) 4.0 wt%.

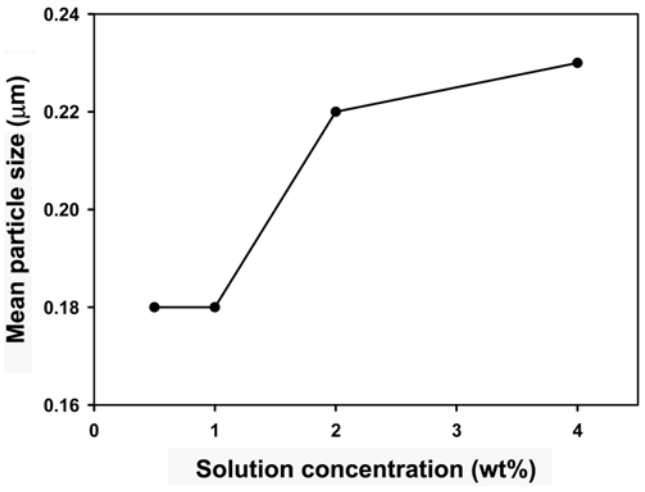

Fig. 10. Effect of solution concentration in NMP on mean particle size of TTC.

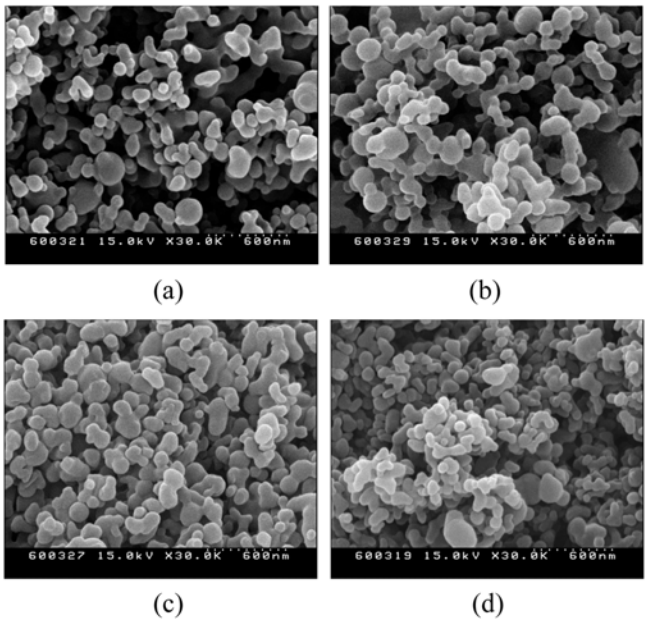

Fig. 11. SEM images of processed TTC particles (Run 1, 13-15): (a) 0.32 g/min, (b) 0.44 g/min, (c) 0.61 g/min, (d) 0.76 g/min.

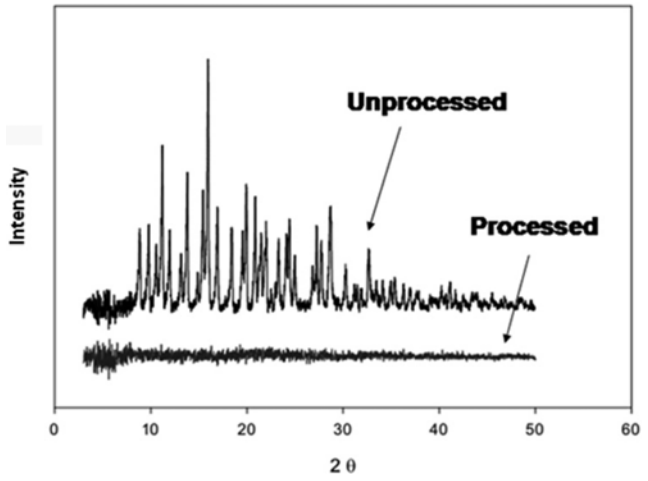

Fig. 12. XRD data of unprocessed and processed TTC particles.

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The X-ray diffraction patterns of unprocessed and processed TTC particles are shown in Fig. 12. Processed particles were found to be amorphous whereas unprocessed particles had crystalline characteristics.

CONCLUSIONS

Tetracycline hydrochloride (TTC) was micronized by ASES using supercritical CO_2. Mean particle size of processed TTC by SEM was 0.16-0.31 μm, whereas particle size of unprocessed TTC was 195 μm. Furthermore, the sizes and shapes of processed particles were found to be affected by primary particle agglomeration. Most strikingly, processed particles were almost spherical and their sizes increased from 0.18 to 0.31 μm as CO_2 temperature increased. In

addition, processed particle sizes increased from 0.18 to 0.23 μm as TTC concentration increased. However, CO_2 pressure and TTC solution feed rate did not significantly affect particle size. Furthermore, powder X-ray diffractometry revealed that processed particles were amorphous whereas unprocessed particles showed obvious crystalline characteristics.

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