Korean J. Chem. Eng., **25**(3), 575-580 (2008)

Recrystallization of phenylbutazone using supercritical fluid antisolvent process

Su-Jin Park and Sang-Do Yeo†

Department of Chemical Engineering, Kyungpook National University, Daegu, 702-701, Korea
(*Received 21 March 2007 • accepted 5 August 2007*)

Abstract−Phenylbutazone was recrystallized from its solutions by using a supercritical fluid antisolvent process. It was dissolved in acetone and supercritical carbon dioxide was injected into the solution, thereby inducing supersaturation and particle formation. Variation in the physical properties of the recrystallized phenylbutazone was investigated as a function of the crystallizing temperature and the carbon dioxide injection rate. The recrystallized particles showed cleaner surfaces and more ordered morphology compared to the particles obtained by other methods such as solvent evaporation. X-ray diffraction patterns indicated that the crystallinity of the particles had been modified upon the recrystallization. Differential scanning calorimetry measurement revealed that the crystallizing temperature influenced the thermal stability of the resulting crystals. Larger crystals were produced when the carbon dioxide injection rate was reduced.

Key words: Antisolvent, Nucleation, Phenylbutazone, Recrystallization, Supersaturation

INTRODUCTION

Supercritical fluids are widely used to generate particles for many pharmaceutical compounds [1-3]. In these particle formation processes, the supercritical fluids are used not only as solvents but also as antisolvents. If a drug compound is soluble in a supercritical fluid, the drug can be dissolved in the supercritical fluid and then the high pressure fluid is expanded to a lower pressure, generating precipitated drug particles. If a drug is insoluble in a supercritical fluid, the drug is first dissolved in an organic solvent, and the solution is brought to contact with a supercritical fluid that acts as an antisolvent toward the dissolved drug compound; the particles are then precipitated from the solution. In fact, most pharmaceutical compounds are practically insoluble in a supercritical fluid, and therefore supercritical fluid is now more popularly used as an antisolvent rather then as a solvent in order to recrystallize drug compounds from their solutions [4-8].

The supercritical fluid antisolvent (SAS) process involves the precipitation of a solute from its solution when the solution is mixed with a supercritical fluid. The mixing process brings about the volumetric expansion of the solution, and hence the solubility power towards the solute decreases and precipitation occurs. The main feature of the SAS process is the use of highly compressible antisolvent that is a supercritical fluid. When this compressible fluid is mixed with a solution, process parameters such as the expansion rate of the solution, the degree of supersaturation, and the rate of nucleation and crystal growth, can be manipulated to a large extent. These effects may lead to the variation of physical properties of resulting crystals that in turn may control the bioavailability of the solid state drug compounds.

Many SAS processed drug compounds have experienced modifications of their physical properties [9]. Changes in external characteristics have been observed including crystal habit and particle size as well as variations in internal structures such as crystallinity and thermal stability. Such modifications in drug characteristics can be achieved because the SAS process has versatile operational variables that facilitate control of the mechanism of nucleation and crystal growth [8]. The operational variable of the SAS process includes crystallizing temperature and pressure, concentration of solutions, the type of solvent and antisolvent, the rate of contact between solution and antisolvent, and the flow configurations of the streams.

The current study investigated the effect of two operational variables (temperature and antisolvent injection rate) on the solid state properties of a drug compound that was crystallized in the SAS process. Phenylbutazone was selected as a model compound. Phenylbutazone, which is used for the relief of pain and inflammation caused by arthritis, is slightly soluble in water at the concentration of 0.7 mg/ml at 22.5 °C. We selected it as a model drug compound because its polymorphic behavior has been well documented [10]. The variations of crystal habit, particle size, crystallinity, and thermal stability of the recrystallized drug compound were examined. We focused on how these properties of drug crystals varied with crystallizing temperature and with the rate of contact between the solution and antisolvent. Here, we recrystallized phenylbutazone using acetone as a solvent and carbon dioxide as an antisolvent. The generated particles were analyzed to examine the external and internal characteristics of the crystals.

EXPERIMENTAL

1. Materials

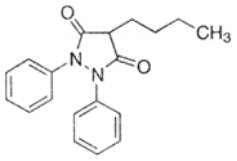
Phenylbutazone (Cat. No. P8386) was purchased from Sigma. Acetone (99.8%) was obtained from Aldrich. Carbon dioxide (99.5%) was supplied from Daesung Gas Co. All chemicals were used without further purification. Properties of phenylbutazone are summarized in Table 1.

2. Apparatus and Procedure

The experimental apparatus for recrystallization of phenylbutazone is shown in Fig. 1. Details of the apparatus and experimental

†To whom correspondence should be addressed.
E-mail: syeo@knu.ac.kr

Table 1. Properties of phenylbutazone

Synonym	4-Butyl-1,2-diphenyl-3,5-pyrazolidinedone
Molecular formula	$C_{19}H_{20}N_2O_2$
Molecular weight	308.38
Structure	
Melting point	105 °C
Organic solvents	Acetone, Ethanol
Solubility in water	0.7 mg/ml at 22.5 °C

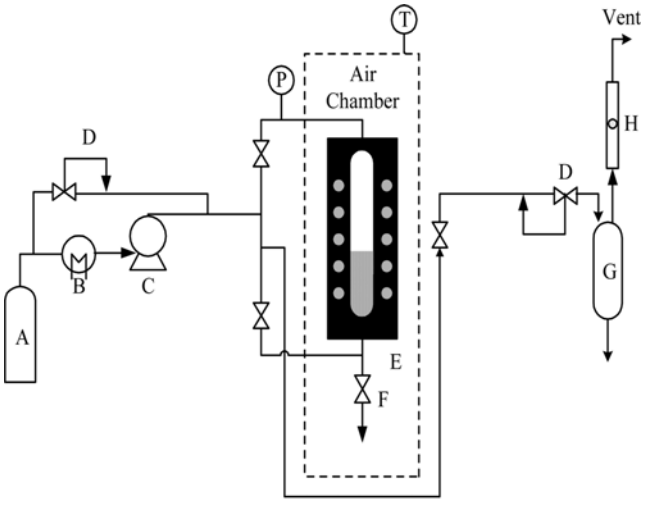


Fig. 1. Experimental apparatus for recrystallization of phenylbutazone by SAS process.

A. Carbon dioxide cylinder E. Crystallizing chamber
B. Cooler F. Ventilation valve
C. High pressure pump G. Solvent trap
D. Back pressure regulator H. Rotameter

procedure are illustrated in our previous report [8], and only a brief description is given here. The equipment mainly consists of 1) a carbon dioxide supplying part, 2) a crystallizing chamber (Jerguson Gauge, Model 19-T-40), and 3) a depressurizing section. The crystallizing chamber is placed in an air bath to maintain constant temperature during crystallization. The crystallizing chamber has two windows through which one can observe the phenomenon of particle precipitation and crystal growth inside the chamber. First, solutions of 30.0 wt% phenylbutazone in acetone were prepared. Normally, 10 ml of the prepared solution was loaded into the crystallizing chamber. The temperature of the air bath was increased as desired. The experiments were conducted at temperatures of 25, 30, and 35 °C. Next, carbon dioxide was injected from the bottom of the chamber to achieve a mixing between the solution and antisolvent. Injection was made so as to increase the pressure of crystallizing chamber from atmospheric pressure to 95 bar. When the carbon dioxide injection was conducted, two different injection rates were used, slow injection and rapid injection. In the slow and rapid injection modes, the pressures of crystallizing chamber increased

Table 2. Name of experimental runs and operating conditions for the recrystallization of phenylbutazone

Experiments	Temperature (°C)	CO_2 injection rate (bar/min)
PBS1	25	0.35
PBS2	30	0.49
PBS3	35	0.61
PBR1	25	16.62
PBR2	30	16.55
PBR3	35	16.70

at rates of 0.35-0.61 bar/min and 16.62-16.70 bar/min, respectively. The operating conditions and the name of experimental runs are summarized in Table 2.

The injection of carbon dioxide and the subsequent pressure increase caused a concomitant expansion of the solution and nucleation of the dissolved compound. During expansion, the solution became cloudy at a certain pressure, and it was recorded as a nucleation pressure. The system was pressurized until the carbon dioxide and acetone phases almost merged, at which stage the recrystallization process was assumed to be completed. The precipitated crystals fell down to the bottom of the chamber and were collected by a metal filter. The carbon dioxide and acetone mixture was then removed from the chamber by using the ventilation valve. During ventilation, the pressure inside the chamber was maintained constant at 95 bar by introducing pure carbon dioxide from the top of the chamber. The residual solvent on the crystal surface was removed by pure carbon dioxide flowing continuously through the chamber for 10 min. Finally, the crystallizing chamber was depressurized and the crystals were collected for analysis. The phenylbutazone crystals were also obtained by simple solvent evaporation method. The 10 ml of the prepared phenylbutazone solution in acetone was placed in a fume hood until all the solvent was evaporated, and the residual phenylbutazone particles were collected for analysis.

In this study, the supersaturation of the solution and the subsequent nucleation is induced by the injection of carbon dioxide from the outside of the crystallizing chamber. In general, the supercritical antisolvent process has been operated in two different modes which are 1) the injection of solution into the antisolvent and 2) the injection of antisolvent into the solution. In the first mode, the solution is continuously supplied to the crystallizing chamber and the mass production of drug particle is possible. In the second mode, only a fixed amount of particle can be generated. However, the second operational mode allows the control of rate of nucleation and crystal growth by regulating the injection rate of carbon dioxide. We adopted the second mode to investigate how the rate of contact between the solution and antisolvent influences the characteristics of resulting particles.

3. Crystal Analysis

The morphology of the crystals was examined by a scanning electron microscope (SEM, Hitachi). The crystallinity of the particles was analyzed by a powder X-ray diffractometer (XRD, Philips). The thermal stability of the crystal was examined by a differential scanning calorimetry (DSC, Rhometric). The particle size distribution was measured by a particle size analyzer (PSA, Ankersmid).

RESULTS AND DISCUSSION

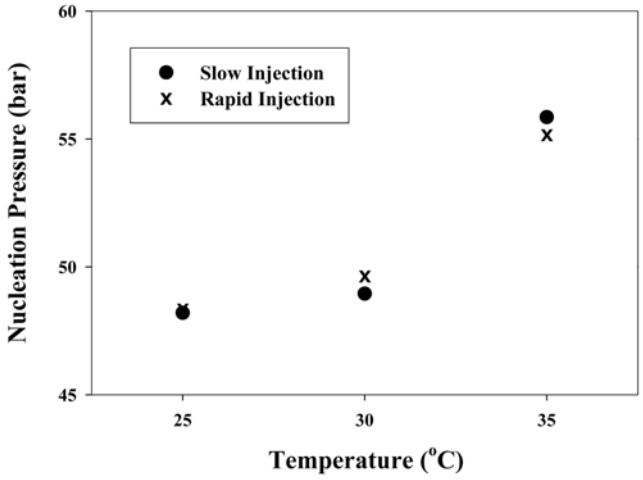


Fig. 2. Nucleation pressure of phenylbutazone solution in acetone as a function of crystallizing temperature.

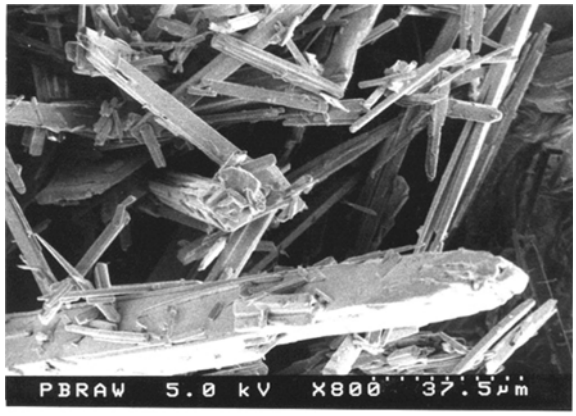
1. Nucleation Pressure

Nucleation pressure was defined as the pressure at which the drug solution inside the crystallizing chamber became cloudy when the carbon dioxide was injected into the solution. The nucleation pressure is the phase separation boundary at which the solid phase is formed in the mixture of acetone and carbon dioxide. Information on this phase separation boundary is important because the nucleation pressure stands for the minimum pressure that should be maintained inside the crystallizing chamber in order to prevent the redissolution of the precipitated crystals back into the solution. In this study, the nucleation pressure was measured at three temperatures when the carbon dioxide injections were made by using two different injection rates. Fig. 2 shows the variation of nucleation pressure as a function of crystallizing temperature. The nucleation pressure increased with temperature, and the change of carbon dioxide injection rate rarely influenced the nucleation pressure. These results indicate that as temperature increases, higher pressure is required to dissolve a necessary amount of carbon dioxide in the acetone solution to bring about the nucleation of phenylbutazone. At low temperatures, the nucleation easily occurs at low pressure because of the high solubility of carbon dioxide in acetone. The effect of temperature on the nucleation pressure follows the principle of high-pressure vapor-liquid equilibrium. At an elevated temperature, a higher pressure is required to maintain a sufficient carbon dioxide concentration in the acetone phase to achieve a necessary supersaturation of the solution.

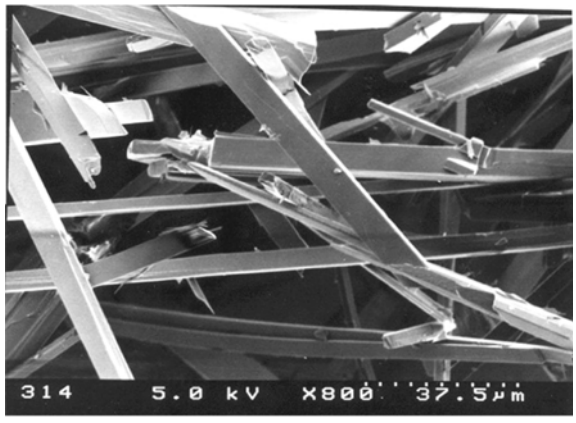
The nucleation pressure was not affected by the rate of carbon dioxide injection, indicating that the pressure at which the nucleation occurs is constant regardless of the rate of pressure increase inside the crystallizing chamber. In other words, the rate of antisolvent addition does not influence the pressure at which the supersaturation takes place, and nucleation should occur only when a sufficient amount of antisolvent is added to the drug solution.

2. Crystal Morphology

The morphology of phenylbutazone crystals is shown in Fig. 3. Fig. 3(a) is the SEM image of unprocessed phenylbutazone crystals. Fig. 3(b) shows the phenylbutazone that was recrystallized by the SAS process. Fig. 3(c) shows the crystals obtained from acetone



(a)



(b)

(c)

Fig. 3. Crystal morphology of phenylbutazone, (a) unprocessed material, (b) recrystallized from acetone by SAS process, (c) recrystallized from acetone by solvent evaporation method.

solutions by a simple solvent evaporation method. All the phenylbutazone crystals exhibited an acicular crystal habit. Among the three phenylbutazone samples, the crystals obtained via the SAS process have better external shape compared to other samples. The unprocessed phenylbutazone consists of particles with irregular sizes and rough surfaces, and the crystals obtained by solvent evaporation show significant agglomeration problem. However, crystal particles

obtained with the SAS process exhibit a regular external shape with a clean surface texture and sharp angles. These results indicate that SAS process provides a proper environment for the good growth of phenylbutazone crystals, minimizing the conditions for growth-related imperfections and solvent occlusion into the crystal faces [11]. This may stem from the enhanced mass transfer of drug molecules from the solution phase to the crystal surface which is due to the presence of supercritical fluid in the crystal growing system.

3. Crystallinity

Information on crystallinity and crystal orientation of drug particles can be obtained from XRD patterns. We measured the XRD patterns of three phenylbutazone samples that include unprocessed material and crystals obtained from two experiments using different carbon dioxide injection rates. Fig. 4 shows the XRD patterns

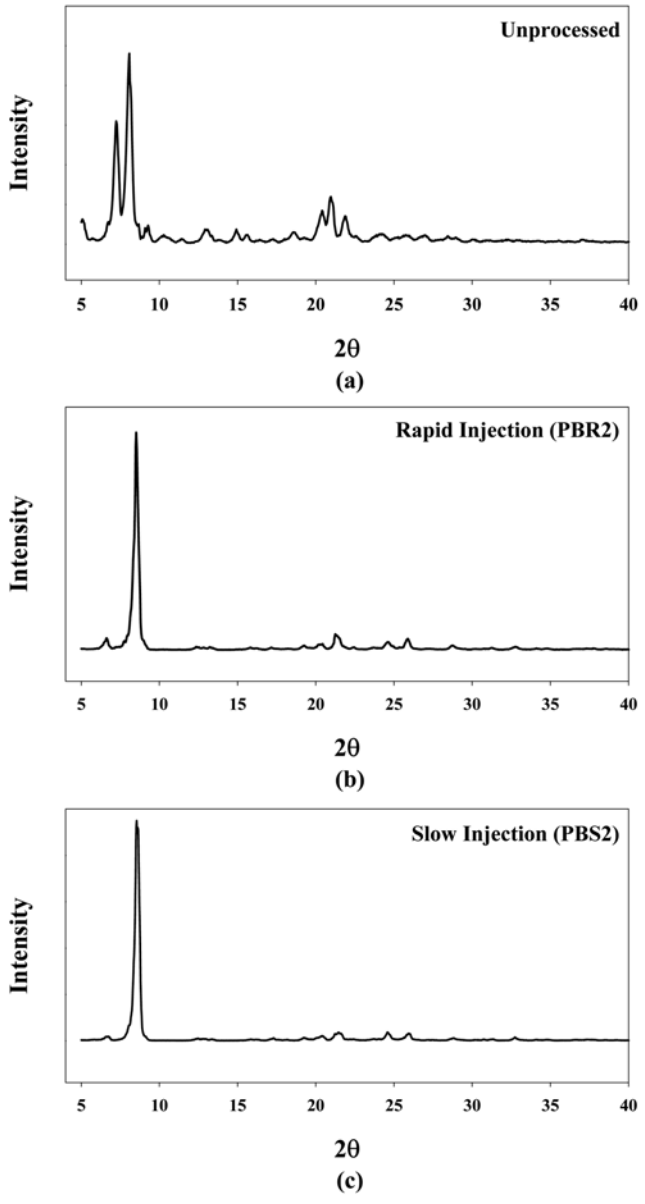


Fig. 4. XRD patterns of phenylbutazone, (a) unprocessed material, (b) processed with rapid injection experiment at 30 °C, (c) processed with slow injection experiment at 30 °C.

of unprocessed (Fig. 4(a)) and processed phenylbutazone crystals obtained from rapid injection (Fig. 4(b)) and slow injection (Fig. 4(c)) experiments, respectively. These XRD patterns show that the crystallinity of the phenylbutazone as purchased has been modified by SAS process, and that the experimental conditions of SAS process can affect the degree of crystallinity of the resulting crystals. Observation of the three XRD patterns indicates that the reflection peaks are located at diffraction angles (2θ) of 6.3, 8.5, 13.5, 15.1, 21.9, 24.5, and 25.9. All reflection peaks at these locations were detected in the three patterns. However, the relative intensity of the peaks, however, has been changed. In the patterns of SAS processed samples, the intensity of all the peaks except a peak at 2θ=8.5 has been significantly minimized compared to the pattern of unprocessed material. In addition, the peaks were further minimized as the carbon dioxide injection rate decreased (Fig. 4(b) and 4(c)).

The relative intensity of a peak in XRD patterns may represent the degree of ordered arrangement of molecules in a particular orientation. Therefore, the greater relative intensity of the peak at 2θ= 8.5 means that an ordered molecular arrangement which corresponds to that diffraction angle becomes dominant compared to other molecular orientations. This phenomenon, called preferred orientation [12], is a condition in which the distribution of crystal orientations is ordered, and a specific crystalline frame tends to cluster to a greater degree about some particular orientation. The XRD patterns in Fig. 4 indicate that the orientation of one particular arrangement of molecules becomes preferred by processing the purchased phenylbutazone by the SAS process. Moreover, the degree of preferred orientation could be controlled by varying the carbon dioxide injection rate. In other words, the orientation of a given molecular arrangement becomes more preferred in the crystals produced in the slow injection experiment (Fig. 4(c)). These results indicate that the slow rate of nucleation and crystal growth, which is due to the slow anti-solvent injection, may facilitate the molecules to arrange in a particular orientation during the packing inside the crystalline lattice.

The crystallinity may affect the drug release rate of crystals. It has been known that amorphous particles show higher release rate than crystalline particles. Therefore, the preferred orientation observed in the SAS processed phenylbutazone crystals may diminish the release rate of drug. If this is the case, SAS process can be used to increase the crystallinity of highly soluble drugs whose solubility and release rate need to be reduced in order to achieve controlled release and prolonged drug effect.

4. Thermal Stability

The thermal behavior of crystals reflects the degree of crystallinity and stability of solid-state drug compounds. DSC measurement provides information on the temperatures at which the phase transitions occur and the accompanying heat of the transitions. Fig. 5 shows typical DSC scans of phenylbutazone crystals. The heating rate for the DSC measurement was 10 °C/min. Phenylbutazone exhibits endothermic behavior and shows two endothermic transitions during the temperature increase. Fig. 5(a) shows DSC scans of unprocessed material along with those of processed crystals obtained from slow injection experiments at 25, 30, 35 °C. Fig. 5(b) shows DSC scans of processed crystals form the rapid injection experiments. The DSC curves of all processed crystals show two endothermic peaks where the first peak corresponds either to the solid-solid transition between the two different solid states or to the solid-liquid tran-

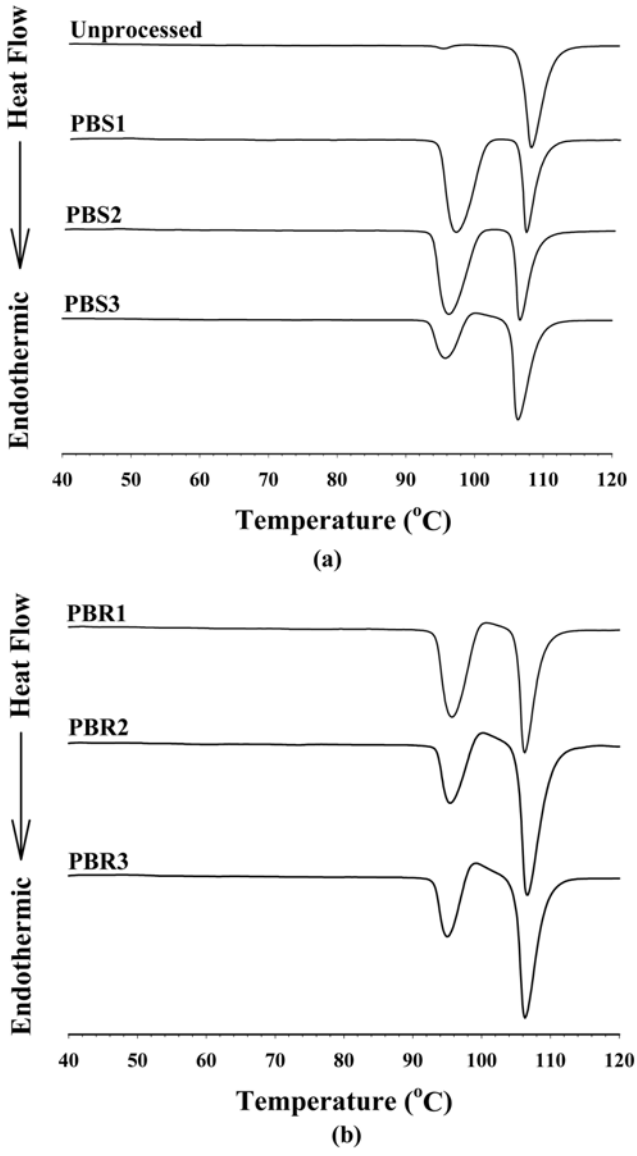


Fig. 5. DSC scans of phenylbutazone, (a) scans of unprocessed materials and processed crystals with slow injection experiments at 25, 30, and 35 °C, (b) scans of processed crystals with rapid injection experiments at 25, 30, and 35 °C.

sition of a part of the crystalline mixture. In this case, the solid-solid transition is more likely to happen [10]. The second peak of each curve corresponds to the complete fusion from the solid to liquid state. However, the DSC scan of unprocessed material shows only one fusion peak with a very minor transition peak at 94.5 °C. Table 3 summarizes the DSC data of unprocessed and processed phenylbutazone crystals. Table 3 includes the onset and peak maximum temperatures for each transition and fusion peaks, and also provides the information on the amount of heat released when transition and fusion take place.

The temperatures at which the solid state transition and fusion took place were not influenced by changing the crystallizing temperature and carbon dioxide injection rate. However, the heats of transition and fusion were significantly different depending on the experimental conditions (Table 3). The heat of solid state transition of unprocessed material was very small compared to the heat of fusion. The appearance of a major solid state transition peak in the processed crystals implies that the SAS process modified the internal structure of phenylbutazone crystals. This means that the SAS processed crystals have a mixture of different crystalline forms in the same sample, and that upon heating of the crystals, part of the crystalline form first undergoes phase transition followed by the complete fusion of crystals. These results show that phenylbutazone crystals become more thermally unstable after SAS recrystallization.

Variations of the heats of transition and fusion were observed depending on the crystallizing temperature and carbon dioxide injection rate. As shown in Table 3 and Fig. 5(a), the heat of solid state transition for crystals obtained from the slow injection experiments tended to decrease as the crystallizing temperature increased, while the heat of fusion increased with temperature; as a result, the total of the two heat values increased with temperature. These results indicate that the phenylbutazone which recrystallizes at higher temperatures requires more heat to turn from solid to complete liquid state. A similar effect of temperature on the heat of transition and fusion was also observed in crystals obtained from the rapid injection experiments (Fig. 5(b)). Comparison of the two samples that were obtained from two different injection rates showed that the crystals from the rapid injection experiments have absorbed more heat when melting took place (Table 3). The amount of heat involved in phase transformation has been used as a standard to estimate the degree of crystallinity [13]. Therefore, these results indicate that crystals with higher crystallinity tend to form when phenylbutazone is recrystallized by using the SAS process with a rapid injection of carbon dioxide at elevated temperatures.

5. Particle Size

The particle size distribution of phenylbutazone crystals was meas-

Table 3. DSC data of phenylbutazone crystals obtained from the experimental runs described in Table 2

Experiments	Transition (°C)		Fusion (°C)		Heat of transition or fusion (cal/g)		
	Onset	Peak	Onset	Peak	Transition	Fusion	Total
Unprocessed	92.3	94.5	105.7	107.3	0.44	22.65	23.09
PBS1	93.7	96.1	105.4	106.5	16.18	8.95	25.13
PBS2	93.5	95.9	105.2	106.2	17.43	11.07	28.50
PBS3	93.4	95.8	105.3	106.4	10.64	20.40	31.04
PBR1	93.4	95.7	105.1	106.3	13.69	13.98	27.67
PBR2	93.4	95.6	105.1	106.7	8.53	22.83	31.36
PBR3	93.0	95.0	104.9	106.3	10.74	22.72	33.46

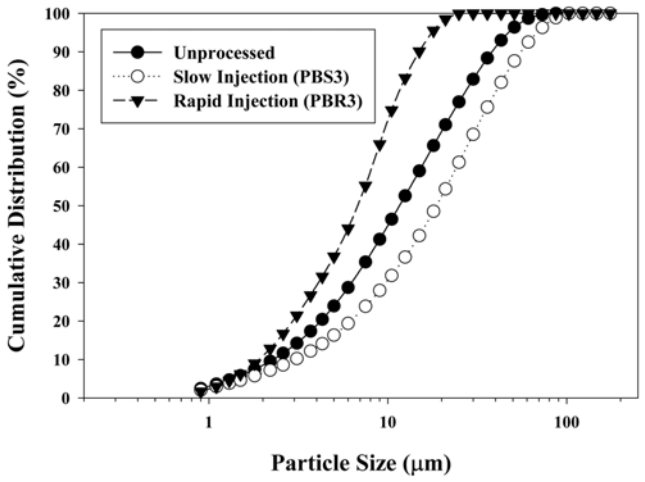


Fig. 6. Particle size distribution of phenylbutazone crystals. The particle size of unprocessed crystals is compared with that of processed crystals obtained from slow and rapid injection experiments at 35 °C.

ured to investigate the possible effect of carbon dioxide injection rate on particle size. The change of the carbon dioxide injection rate involves the change of the addition rate of antisolvent to the solution. Since the supersaturation of the solution is proportional to the amount of antisolvent added, the carbon dioxide injection rate governs the degree of supersaturation. Moreover, the rate of nucleation is a strong function of supersaturation; therefore, the carbon dioxide injection rate is directly related to the number of nuclei formed per volume of the solution. In addition, the rate of antisolvent addition controls the growth rate of each nucleus. The rate of crystal growth reflects the mass transfer rate of drug molecules from the solution phase to the crystal surface, and this mass transfer rate depends on how rapidly the antisolvent is added to the solution. Therefore, the carbon dioxide injection rate influences the rate of crystal growth and the resulting particle size.

Fig. 6 shows the particle size distributions of unprocessed and processed phenylbutazone crystals obtained from slow and rapid injection experiments at 35 °C. The particles produced from the slow injection experiments were larger than the unprocessed material, while the crystals from the rapid injection experiments were much smaller than others. Fig. 6 shows that 90% of the unprocessed particles were smaller than 38.2 μm and that 20% were smaller than 4.3 μm. For the crystals from the slow injection experiment, 90% of the particles were smaller than 56.5 μm and 20% were smaller than 6.8 μm. For the crystals from rapid injection experiments, 90% of the particles were smaller than 15.5 μm and 20% were smaller than 3.2 μm. These results can be interpreted by the dependence of rate of nucleation and crystal growth on the antisolvent addition rate. At high rates of antisolvent addition, the supersaturation is accelerated and hence the nucleation rate will increase. This induces the formation of a larger number of nuclei in the initial stage of nucleation process, and therefore the size of each crystal will be reduced. Another factor that can influence the nucleation rate is the turbulent flow that is generated by the fast injection of carbon dioxide into the solution. The effect of vigorous mixing with the high turbulence also facilitates the formation of a nucleus and accelerates the rate of mass transfer for crystal growth. Therefore, the larger number of nuclei will grow to complete crystals, and hence the number of resulting crystals will increase and their size will be reduced, accordingly. These results suggest that the carbon dioxide injection rate can be manipulated in the SAS process for the effective size control of drug particles.

CONCLUSIONS

Phenylbutazone was recrystallized by using supercritical carbon dioxide as an antisolvent. Variations of physical properties of the processed crystals were observed with the change of experimental variables, such as crystallizing temperature and carbon dioxide injection rate. SAS processed crystals exhibited a regular external shape with clean surface texture and sharp angles, indicating the proper environment for the good growth of crystals that minimizes the conditions for growth-related imperfection. The SAS process modified the internal structure of the crystals, and the crystallinity and thermal stability of phenylbutazone particles were significantly influenced by the experimental conditions. The carbon dioxide injection rate showed great impact on the particle size distribution of the SAS processed crystals.

ACKNOWLEDGMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2004-041-D00157).

REFERENCES

1. J. Jung and M. Perrut, *J. Supercrit, Fluids*, **20**, 179 (2001).
2. N. Foster, R. Mammucari, F. Dehghani, A, Barrett, K. Bezanehtak, E. Coen, G. Combes, L. Meure, A. Ng, H. L. Regtop and A. Tandya, *Ind. Eng. Chem. Res.*, **42**, 6476 (2003).
3. G. Li, J. Chu, E. S. Song, K. H. Row, K. H. Lee and Y. W. Lee, *Korean J. Chem. Eng.*, **23**, 482 (2006).
4. G. A. Sacha, W. J. Schmitt and S. L. Nail, *Pharm. Dev. Technol.*, **11**, 187 (2006).
5. F. Miguel, A. Martin, T. Gamse and M. J. Cocero, *J. Supercrit. Fluids*, **36**, 225 (2006).
6. E. Reverchon and I. De Marco, *Powder Technol.*, **164**, 139 (2006).
7. M. Gimeno, N. Ventosa, Y. Boumghar, J. Fournier, I. Boucher and J. Veciana, *J. Supercrit. Fluids*, **38**, 94 (2006).
8. S. J. Park, S. Y. Jeon and S. D. Yeo, *Ind. Eng. Chem. Res.*, **45**, 2287 (2006).
9. D. J. Jarmer, C. S. Lengsfeld, K. S. Anseth and T. W. Randolph, *J. Pharm. Sci.*, **94**, 2688 (2005).
10. Y. Matsuda, E. Tatsumi, E. Chiba and Y. Miwa, *J. Pharm. Sci.*, **73**, 1453 (1984).
11. S. D. Yeo, M. S. Kim and J. C. Lee, *J. Supercrit. Fluids*, **25**, 143 (2003).
12. B. D. Cullity, *Elements of X-ray diffraction*, 2nd Ed., Addison-Wesley Publishing Co., Massachusetts (1978).
13. J. L. Ford and P. Timmins, *Pharmaceutical thermal analysis*, Ellis-Horwood Ltd., Chichester, England (1989).