Micronization of Phenylbutazone by Rapid Expansion of Supercritical CO_2 Solution

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Rapid expansion of supercritical solutions (RESS) technique was applied for the preparation of phenylbutazone fine particles. The operating temperature and pressure affected the yield of the drug fine particles, which was evaluated by dissolving the sprayed product of drug into ethanol. Effect of pre- and post-expansion conditions on the particle size distribution of phenylbutazone was investigated and the smallest sample (mean particle size: 1.59 μm) was obtained when the RESS method was operated at a pressure of 26 MPa combined with a temperature of 32 °C. Physicochemical properties of the fine particles were investigated by powder X-ray diffraction and differential scanning calorimetry. It was found that the phenylbutazone fine particles obtained were metastable β form under the experimental conditions tested, suggesting polymorphic transformation during the RESS process.

Key words supercritical fluid; micronization; submicron crystal; phenylbutazone; polymorph

Most of the newly developed active pharmaceutical ingredients are poorly soluble or insoluble in aqueous media. Particle size reduction of such pharmaceuticals is one of the clue to improve the dissolution, absorption and subsequent bioavailability.[1,2] Grinding and spray drying are the major techniques for the size reduction, however, heat- or mechanical stress-induced degradation of the material and residual organic solvent often limit the application, respectively. Furthermore, the resultant particle size distribution is usually broad and does not reach to the few micron or sub-micron level.

Supercritical fluids have been used for particle size reduction in chemical, cosmetic and pharmaceutical industries.[3] Rapid expansion of supercritical solutions (RESS), gas antisolvent (GAS), aerosol solvent extraction system (ASES) and solution enhanced dispersion of solids (SEDS) are known as the preparation methods of drug fine particles and among them, RESS method is the only way to prepare the powder without using organic solvents.[3,4] In the RESS process, the solute is dissolved in a supercritical solvent and the supercritical solution is rapidly expanded through a nozzle. Rapid phase change from supercritical fluid to the gas state induces the high supersaturation of the solute and results in the formation of very small particles. As a supercritical fluid, carbon dioxide (CO_2) has commonly been used because of its mild critical temperature (304.2 K) and pressure (7.39 MPa). Supercritical CO_2 is advantageous to the environment due to the non-toxic and easily recycled properties and to the application for heat-sensitive pharmaceuticals.[5,6]

The RESS method has been applied for polymer coating, microencapsulation and micronization. For the purpose of micronization, some active pharmaceutical ingredients, such as salicylic acid,[7,8] griseofulvin,[9] ibuprofen,[9,10] have been used. The mean particle size of the drugs obtained by the RESS technique drastically reduced to micron order. In some cases, the particle size reached to sub-micron, however, it was very difficult to keep the size because of the crystal growth and agglomeration simultaneously occurred with the fine particle formation.

A unique approach utilizing RESS is preparation of polymorphs. Deoxycholic acid[11] and carbamazepine[12] polymorphs would be examples which have been reported. A meta-stable form of deoxycholic acid was obtained by storing a sample in a vessel filled with CO_2 at 12 MPa, 60 °C. The purpose of this study is to prepare phenylbutazone fine particles by the RESS method and to characterize the physicochemical properties. Phenylbutazone, a non-steroidal anti-inflammatory drug, was chosen as a model compound because the solid state stability depended on the polymorphic forms where five kinds of the polymorphs, within which form δ was the most stable, and two pseudopolymorphs have been reported.[13] Effect of pre- and post-expansion conditions on the drug fine particle formation was investigated to optimize the conditions to prepare fine particles. The product yield was determined to estimate the solubility of phenylbutazone in supercritical CO_2.

Experimental

Materials Phenylbutazone (form δ) was purchased from Lancaster Synthesis (Lancashire, U.K.). The intact phenylbutazone was needle-like crystals. Ethanol was of reagent grade and used as received.

Apparatus and Settings Supercritical fluid operating system based on the rapid expansion of supercritical solutions (RESS) method (SC sprayer®, Nikkiso, Co. Ltd., Japan) was schematically shown in Fig. 1. The setup consists of an extraction unit and a precipitation unit. The solvent CO_2 was introduced to a temperature-controlled reaction vessel (internal volume: 90 ml) in the extraction unit by a pump NP-AX-403 (Nihon Seimitsu Kagaku Co., Ltd., Japan) up to a desired upper limit pressure (max. 29 MPa). Because CO_2 density varies depending on the temperature,[14] lower limit of the pressure was adjusted to the values at which single-sprayed amounts of supercritical CO_2 became a fixed amount (0.19 mol). After the extraction, definite amounts of sample/supercritical CO_2 mixtures passed through a high-pressure stainless steel tubing and were expanded from a spray nozzle, which was composed of a tungsten carbide orifice UniJet flat spray tip (Spraying Systems Co., Japan), in the precipitation unit. Spraying period is less than 0.5 s. Temperature of the tubing and nozzle was usually the same as that of the extraction unit. At the precipitation unit, rapid phase change of sprayed supercritical CO_2 into the gas state induced the high supersaturation of the

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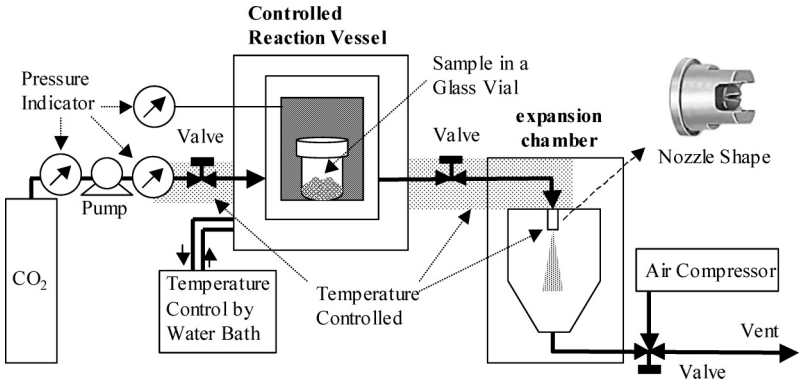

Fig. 1. Experimental Apparatus of Rapid Expansion of Supercritical Solutions (RESS)

solute and resulted in the formation of very small particles. The expansion chamber volume was 12 liter and the spraying distance from the nozzle to a 0.8 μm glass fiber prefilter (Millipore, MA, U.S.A.) placed on the bottom flange was 30 cm. The expanded gas was vented by using a compressor (Hitachi 0.75LP-7S·T, Japan) and the vacuum flow rate was adjusted by an ejector cock to collect precipitated particles on the filter. The process parameter variables were optimized to obtain drug fine particles.

Product Yield of Phenylbutazone Two grams of the drug in a glass vial was set at the extraction unit. After the pressure of supercritical CO_2 reached to a definite value at a definite temperature, the conditions were kept for 3 h and then sample/supercritical CO_2 mixtures were sprayed into ethanol until the pressure reached to the lower limit. The product yield of the drug was spectrophotometrically determined by using UV-160 spectrophotometer at 240 nm (Shimadzu, Japan) and was shown as drug/CO_2 molar ratio.

Preparation of Micronized Particles Two grams of the drug was filled in a 30 ml glass vial covered with Milliwrap (Millipore, MA, U.S.A.) and placed in a controlled reaction vessel. Temperature-controlled CO_2 was brought into the vessel at constant flow rate (40 ml/min). After the pressure of supercritical CO_2 reached to the upper limit, the extraction pressure and temperature was kept for 30 s and then sample/supercritical CO_2 mixtures were sprayed to the expansion chamber until the pressure reached to the lower limit. After repeating the spray at the definite intervals for 100 times, the micronized particles obtained on the filter were collected.

Particle Size Distribution Volumetric particle size distribution of each samples after dispersed in water was measured by laser diffraction on Microtrac® FRA (Nikkiso, Co. Ltd., Japan; measurement range, 0.1—700 μm) and by dynamic light scattering on Microtrac® UPA150 (Nikkiso, Co. Ltd., Japan; measurement range, 0.003—6 μm).

Powder X-Ray Diffraction Powder X-ray diffraction was measured by using Rigaku Miniflex diffractometer (Tokyo, Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; scanning speed, 2 min.

Differential Scanning Calorimetry A differential scanning calorimeter (DSC3100, MAC Science Co., Japan) was used for thermal analysis. The operating conditions in the closed-aluminum pan system were as follows: sample weight, 3 mg; heating rate, 5 °C/min; nitrogen gas flow, 50 ml/min.

Results and Discussion

Estimation of Yield by Spraying Drug/Supercritical CO_2 Mixture in Ethanol Solubility of the drug in supercritical CO_2 is known to influence on the yield of the drug fine particles on the RESS method. Solubility measurements of drugs in supercritical CO_2 are usually made by gravimetric[15] or spectroscopic techniques,[16] however, these experiments require much time and special equipments. To estimate the yield of phenylbutazone fine particles, total sprayed amounts of the drug were determined as a function of the extraction pressure and temperature. Figure 2 shows the relationship between the extraction pressure and sprayed drug amount at different extraction temperatures. The results clearly showed that sprayed amounts of phenylbutazone increased with increasing extraction pressure and temperature. Since adequate amount of drug powders has been obtained by using the RESS apparatus when single-sprayed amount of the drug was more than 1.0×10^{-4} (drug/CO_2 molar ratio) at the extraction pressure of 26 MPa (data not shown), phenylbutazone seemed to be suitable for the RESS experiments.

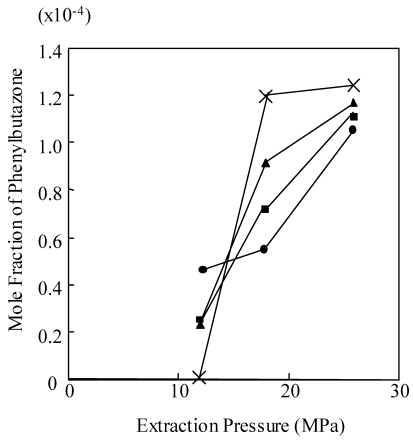

Fig. 2. Effect of Extraction Pressure and Temperature on the Sprayed Amounts of Phenylbutazone

●, 32 °C; ■, 40 °C; ▲, 50 °C; ×, 60 °C. The definite pressure and temperature of sample/supercritical CO_2 mixtures at the extraction unit were kept for 30 s before spraying to ethanol. Lower limit of the pressure was adjusted to the values at which single-sprayed amounts of supercritical CO_2 became a fixed amount (0.19 mol). Sprayed amounts of drugs in supercritical CO_2 were shown as the molar ratio.

Effect of Pre- and Post-expansion Conditions on the Mean Particle Size of Phenylbutazone Pre- and post-expansion conditions of the RESS method were optimized to obtain small-sized phenylbutazone particles. The process parameter variables were temperature (32, 40, 50, 60 °C) and pressure (upper limit: 18, 26 MPa) as pre-expansion conditions, and sprayed amount of CO_2 (0.07, 0.19, 0.33 mol), nozzle diameter (0.23, 0.33, 0.66 mm), nozzle temperature (32, 40, 60 °C) and vacuum flow rate (opening of an ejector cock: 0, 50, 100%) as post-expansion conditions. Effect of pre-expansion conditions on the mean particle size of phenylbutazone are shown in Table 1, where post-expansion conditions were fixed as follows; sprayed amount of CO_2: 0.19 mol, nozzle diameter: 0.33 mm, nozzle temperature: equal to the pre-expansion temperature, spraying distance: 30 cm and vacuum flow rate: 100%. The mean particle size

Table 1. Effect of Pre-expansion Condition of RESS Method on the Particle Size of Phenylbutazone[a)]

Extraction pressure (MPa)	Extraction temperature (°C)	Mean particle size (μm)
26	32	1.59
18	32	2.99
12	32	4.23
26	40	2.86
26	60	3.25
18	40	3.51
18	60	4.33

a) Post-expansion conditions: nozzle temperature was equal to the extraction temperature; nozzle diameter, 0.33 mm; sprayed amount of supercritical CO_2, 0.19 mol; spraying distance, 30 cm; vacuum flow rate, 100%.

Table 2. Effect of Post-expansion Condition of RESS Method on the Particle Size of Phenylbutazone[a)]

Nozzle diameter (mm)	Nozzle temperature (°C)	Sprayed amount (mol)	Vacuum flow rate (%)	Mean particle size (μm)
0.33	32	0.19	100	1.59
0.23	32	0.19	100	2.02
0.66	32	0.19	100	1.63
0.33	40	0.19	100	1.82
0.33	60	0.19	100	2.17
0.33	32	0.28	100	1.51
0.33	32	0.07	100	2.64
0.33	32	0.19	50	2.91
0.33	32	0.19	0	3.80

a) Extraction pressure, 26 MPa; extraction temperature, 32 °C; spraying distance, 30 cm.

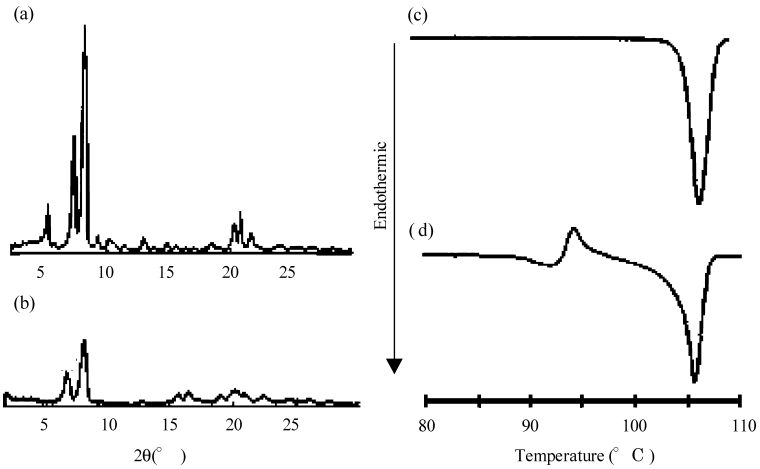

Fig. 3. Powder X-Ray Diffraction Patterns (a, b) and Differential Scanning Calorimetry Curves (c, d) of RESS-Treated Phenylbutazone
(a, c) Intact, (b, d) RESS-treated sample with pre-expansion condition of 26 MPa and 32 °C.

of phenylbutazone increased with increasing extraction temperature. Higher pressure was more suitable to obtain small-sized drug particles. The smallest sample (mean particle size: 1.59 μm) was obtained when the RESS method was operated at 26 MPa and 32 °C. The appearance of micronized particles was recognized as spherical and smooth from SEM observation.

Pre-expansion pressure and temperature strongly affected the supersaturation of the expanding solution which would contribute to the particle size reduction. Generally, dissolved amounts of a drug in supercritical fluid relate to the level of supersaturation of the expanding solution, and pre-expansion conditions of higher pressure and higher temperature contribute to the higher solubility of the drug in supercritical fluid. Another important factor which contributes to the particle size reduction is large temperature difference before and after expansion of the supercritical solution. Reverchon *et al.* reported that pre-expansion conditions of lower temperature were preferred to produce the larger temperature difference.[17)] Since the contribution of temperature difference caused by lower pre-expansion temperature more largely influenced on the high supersaturation than that of the higher solubility at higher pre-expansion temperature, smaller-sized phenylbutazone particles would be produced when the pre-expansion condition was set at 26 MPa and 32 °C.

Effect of post-expansion conditions on the particle size distribution of phenylbutazone are summarized in Table 2, where pre-expansion pressure and temperature were fixed at 26 MPa and 32 °C, respectively. The mean particle size was almost same when wide nozzle diameter was 0.33 and 0.66 μm. Since 0.23 μm nozzle was easily stuffed by the spraying process, drug powders with larger particle size would be obtained by using it. The higher nozzle temperature setting than that of pre-expansion temperature (32 °C) resulted in the size increment. Mean particle size of phenylbutazone decreased with increasing sprayed amounts of supercritical CO_2 and vacuum flow rate. It was considered that higher vacuum flow rate contributed to the rapid diffusion of the supercritical solution and consequently the high level of the supersaturation as reported by Alessi *et al.*[18)]

Physicochemical Properties of Micronized Phenylbutazone Physicochemical properties of RESS-treated phenylbutazone particles were investigated by powder-X-ray diffraction and differential scanning calorimetry as shown in Fig. 3. Polymorphic transformation by RESS treatment from the stable form of intact phenylbutazone (δ form) to a metastable β form, which was assigned by powder-X-ray diffraction data reported by Matsuda *et al.*,[13)] was observed (Figs. 3a, b). The obtained crystal form was also β form when the RESS treatment was operated at 18 MPa and 60 °C (data not

shown). The polymorphic transformation was supported by differential scanning calorimetry measurements (Figs. 3c, d). Intact phenylbutazone showed a melting peak at 107 °C corresponding to stable δ form. In the case of RESS-treated sample, small endothermic and exothermic peaks at 91 and 94 °C, followed by the melting peak at 107 °C, which was assigned to β form,[13] were observed. The first and second peaks corresponded to the melting of β form and crystallization of δ form, respectively.

From the results, we concluded that RESS method was successfully applicable for fine particle production of phenylbutazone as a single crystal form. Pre-expansion conditions of higher pressure combined with lower temperature provided particles of smaller size. The RESS treatment of phenylbutazone also contributed to obtain the micronized particles as a single meta-stable crystal form at definite operating pressure and temperature. Micronized phenylbutazone particles obtained (β form) showed improved wettability and dispersibility in water. Since the size reduction and the polymorphic transformation to meta-stable form improve the solubility, the phenylbutazone fine particles by RESS would contribute to improve the bioavailability by oral administration. These results indicate the advantages of the RESS method over conventional processes like mechanical milling and spray-drying.

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