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Micronization and Polymorphic Conversion of Tolbutamide and Barbital by Rapid Expansion of Supercritical Solutions

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ABSTRACT Rapid expansion of supercritical solutions (RESS) was applied to tolbutamide and barbital. The solubility in supercritical CO_2 was determined to estimate the extraction efficiency roughly by a simple method and accurately by a direct spectrophotometric technique. The latter revealed that the solubility of tolbutamide was a function of applied pressure and temperature and was proportional to the pressure. No significant difference in solubility between polymorphic Forms I and II of tolbutamide was detected. Tolbutamide and barbital particles produced by the RESS were characterized by size distribution measurement, polymorph identification and morphological evaluation. Significant size reduction to micron or sub-micron level with narrow size distribution was achieved, while conventional mechanical grinding had only slight effect. The particle size was greatly affected by both extraction and expansion conditions. The lower the extraction temperature was, the smaller was the mean particle size. Higher extraction pressure resulted in smaller mean particle size when compared at the same extraction temperature. The mean particle size was reduced by lowering the spray nozzle temperature, by lowering the expansion chamber temperature, by increasing the CO_2 amount per spray, and by increasing the exhaust gas flow rate. The RESS processing realized the polymorphic conversion as well. As for tolbutamide, three polymorphs (Forms I, II, and IV) out of four could be produced by changing the extraction conditions, and in the case of barbital, one polymorph (Form II) out of three was produced consistently.

KEYWORDS Supercritical fluid, RESS, Micronization, Polymorphic Conversion, Tolbutamide, Barbital

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INTRODUCTION

Most of the newly developed active pharmaceutical ingredients are poorly soluble or insoluble in aqueous media. Increasing surface area of such pharmaceuticals by particle size reduction is one of the clues to improve the dissolution, absorption, and therefore bioavailability (Hu et al., 2004; Leuner & Dressman, 2000). Grinding and spray-drying are the major techniques for the size reduction; however, heat- or mechanical stress-induced degradation of the material and the difficulty of removing residual organic solvents often limit the application, respectively. Furthermore, the size distribution is usually broad and hardly reach to micron or sub-micron level.

Supercritical fluid has been applied to various industries utilizing the unique characteristics. It can dissolve solid materials due to the high density close to liquid. On the other hand, the density can vary depending on the applied pressure and temperature, and rapid diffusion like gas can occur (Tom & Debenedetti, 1991). Recently, particle design using supercritical fluid is of great interest in the pharmaceutical industry (Jung & Perrut, 2001)–such as microencapsulation of peptides or genes (Koushik & Kompella, 2004; Okamoto et al., 2005), suspension preparation (Young et al., 2000), and fluidized-bed coating (Tsutsumi et al., 1995).

Several techniques to micronize the material utilizing supercritical fluids are known (Jung & Perrut, 2001) – rapid expansion of supercritical solutions (RESS), gas anti-solvent (GAS), aerosol solvent extraction system (ASES), and solution-enhanced dispersion of solids (SEDS). Among them, the RESS method is the only way to process the material without organic solvents. In the RESS process, the solute is dissolved in a supercritical fluid, and the supercritical solution is rapidly expanded by spraying it into normal pressure throughout a nozzle. Rapid change of the supercritical solution into gas phase induces high supersaturation of the solute and therefore micronized material will precipitate. The level of supersaturation on the RESS process is much higher than that on spray-drying or GAS (Helfgen et al., 2001; Tom & Debenedetti, 1991). The high level of supersaturation predominantly induces the nucleation over the crystal growth, thus resulting in small particles with narrow size distribution. As a supercritical fluid, carbon dioxide (CO_2) has commonly been used because of its mild critical pressure and temperature (7.4 MPa, 31°C). 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 (Sarrade et al., 2002). Micronization of pharmaceuticals by the RESS method was reported on griseofulvin (Türk et al., 2002), ibuprofen (Kayrak et al., 2003), progesterone, and medroxyprogesterone (Alessi et al., 1996). The mean particle size of the drugs produced by the RESS technique drastically reduced to micron order or sometimes to sub-micron level. The limitation of the RESS method is that the material must dissolve in the supercritical fluid.

An important factor influencing dissolution behavior other than the particle size is the polymorphic form. In general, metastable forms show higher solubility than the stable form. Therefore polymorphic conversion to a metastable form is often taken to improve the dissolution behavior of poorly water-soluble drugs, where recrystallization from organic solution is commonly used. A unique approach utilizing supercritical fluids is the preparation of polymorphs. Carbamazepine microparticles were produced by the RESS method, and different polymorphs were obtained depending on the operating conditions (Gosselin et al., 2003). Micronization of phenylbutazone by the RESS processing resulted in the formation of a metastable form (Moribe et al., 2005).

The objective of this study was to gain an insight into the RESS method as one-step processing for micronization and polymorphic conversion. For this purpose, the influence of operating conditions during the RESS process on particle size and polymorphic form was investigated. Tolbutamide and barbital were employed as model compounds since both drugs are poorly water-soluble and the advantages of the metastable forms in solubility and dissolution behavior have been reported (Grabowska & Kaliszan, 1976; Kimura et al., 1999).

MATERIALS AND METHODS

Materials

Tolbutamide was purchased from Nacalai Tesque, Inc. (Kyoto, Japan), Sigma Aldrich Japan K.K. (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used as received. The intact tolbutamide was Form I (Nacalai and Sigma) or Form II (Wako). Form I from Nacalai and Form II from Wako were used for the solubility determination, and Form I from Sigma was used as starting material for the RESS experiments. Barbital was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and was a mixture of Forms I and II. For the RESS experiments the intact barbital was converted to pure Form I by storing in supercritical CO_2 atmosphere at 26 MPa and 60°C for 3 h before use. Liquid CO_2 ($>$ 99.5 %) was purchased from Ozawa Sanso Co., Ltd. (Chiba, Japan). Ethanol was of reagent grade and used as received. To compare with the RESS processing, ground samples of intact drugs were prepared by using a vibration rod mill (TI-200, CMT, Tokyo, Japan).

Apparatus and Settings

A supercritical fluid operating system, based on the rapid expansion of supercritical solutions (RESS) method, was used (Fig. 1: SC-Sprayer, Nikkiso Co., Ltd., Tokyo, Japan). The maximum tolerable pressure and temperature of the whole system were 29 MPa and 80°C, respectively. The starting material was loaded in a temperature-controlled reaction vessel of 90 mL inner volume. Liquid CO_2 was compressed and heated to above the critical pressure and temperature, 7.4 MPa and 31°C, and then introduced into the reaction vessel by using a pump (NP-AX-40J, Nihon Seimitsu Kagaku Co., Ltd., Tokyo, Japan). The material was kept under supercritical CO_2 atmosphere at a constant pressure and temperature while magnetic-stirring (PC-420, Corning, Inc., NY, USA). After the extraction, a constant amount of supercritical CO_2 containing dissolved material was sprayed, via a stainless steel tubing and a tungsten carbide spray tip (UniJet®, Spraying Systems Co., Japan, Tokyo, Japan: internal diameter = 0.23, 0.33 or 0.66 mm, length = 9.5 mm), into an expansion chamber of 12 L inner volume. Rapid change of the supercritical solution into gas phase could induce high supersaturation of the solute and therefore micronized material would precipitate. The CO_2 gas was exhausted by using an air compressor (0.75LP-7S·T, Hitachi, Ltd., Tokyo, Japan) and the flow rate was adjusted with an ejector cock to collect precipitated material on a 0.8 μm glass fiber filter (Millipore Corporation, MA, USA) or a 0.45 μm membrane seal (MilliWrap™, Millipore Corporation, MA, USA) placed on the bottom flange of the expansion chamber. The temperature of the expansion chamber was controlled by covering with a silicone rubber heater (SR, Mitutoyo Tusyo Corporation, Tokyo, Japan), if need be.

Determination of Solubility of Tolbutamide and Barbital in Supercritical CO_2

A sufficient amount of the drug (0.2 g) was put into a glass vial and set in the reaction vessel. The drug was kept under supercritical CO_2 atmosphere at 26 MPa and 60°C for 1 h, and 0.2 mol CO_2 equivalent was sprayed into ethanol, which was used as a solvent for assay. Solubility of drugs in supercritical CO_2, expressed as a mole fraction, was determined spectrophotometrically (UV-160, Shimadzu Corporation, Kyoto, Japan).

Separately, the solubility was determined by a direct spectrophotometric technique using a pressure-resistant

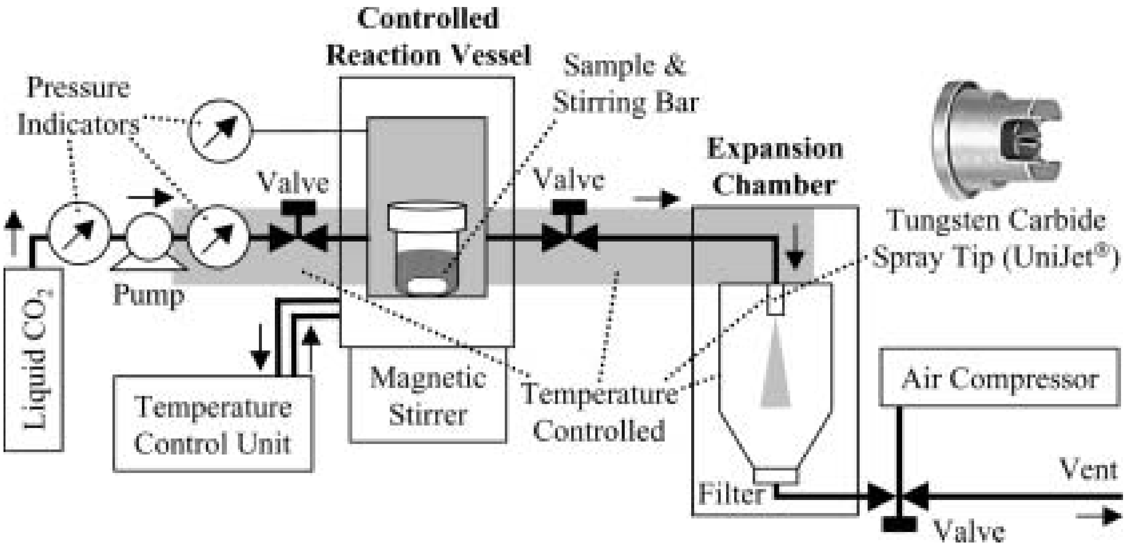

FIGURE 1 Schematic Diagram of RESS Apparatus (SC-Sprayer). The Gray Zone Indicates Supercritical CO_2 Atmosphere.

optical cell unit. UV-visible spectra of the drug supercritical CO_2 solution in an optical cell, made of SUS 316, were measured by a spectrophotometer (V-550, Jasco Corporation, Tokyo, Japan) through a pair of sapphire windows and fiber optics. The optical path length was 2 mm and the inner volume was 5 mL. The maximum working pressure and temperature were 30 MPa and 100°C, respectively. After the sample loading, the cell was evacuated for 1 h to remove inside air completely, then CO_2 was introduced. After saturation was reached (2 h each), UV-visible spectra at various pressure (10–30 MPa) and temperature (40–60°C) were measured. The solubility was determined based on the integrated Lambert-Beer's law:

$$\int_{\lambda_1}^{\lambda_2} A d\lambda = Cl \int_{\lambda_1}^{\lambda_2} \varepsilon d\lambda$$

where A is an absorbance, C is a solute concentration, l is an optical path length, ε is a solute molar absorptivity and λ is a wavelength. λ_1 and λ_2 are lower and upper limits for integration, respectively (Suzuki et al., 2003). By integrating the specific spectra for the calculation, accurate solubility could be determined when compared to the conventional equation at a single and fixed wavelength. This calculation method also enabled to reduce the influence of the CO_2 density on the spectrum shift and the change in the solute molar absorptivity.

Micronization of Tolbutamide and Barbital by Rapid Expansion of Supercritical Solutions (RESS)

A sufficient amount of the drug (1.0 g) was put into a 30 mL glass vial, covered with a 0.45 μm membrane seal (MilliWrap™, Millipore Corporation, MA, USA), and set in the reaction vessel. The drug was kept under supercritical CO_2 atmosphere at a constant pressure and temperature until solubility saturation was reached (tolbutamide: 3 h; barbital: 1 h). A constant amount of supercritical CO_2 containing dissolved drug was sprayed into the expansion chamber. Pressure loss on spraying inside the reaction vessel was compensated by introducing pressure and temperature-controlled supercritical CO_2 at a flow rate of 40 mL/min. It was not possible to visually observe any potential precipitation in the reaction vessel due to the pressure loss because an excess amount of starting material was put into the vessel and therefore a solid fraction always existed together. Nevertheless, it could be assumed that there would be no precipitation before and during pressure recovery since the inside of the reaction vessel was in a supercritical condition even after spraying, and pressure loss on spraying was recovered in several seconds. Thirty seconds after the pressure recovery another part of supercritical solution was sprayed. Repeating this cycle 100 times, micronized drug particles were collected. The influence of extraction conditions on particle size and polymorphic form was investigated for both drugs, and the size reduction effect was further optimized using barbital by varying the expansion conditions. Process variables on extraction were pressure (18–26 MPa) and temperature (32–80°C), and those on expansion were spray nozzle diameter (0.23–0.66 mm), spray nozzle temperature (32–80°C), CO_2 amount per spray (0.07–0.28 mol equivalent), expansion chamber temperature (30–80°C), distance between spray nozzle and filter (7–30 cm) and exhaust gas flow rate (0–4.3 m^3/h).

Characterization of Tolbutamide and Barbital Particles

Size Distribution Measurement by Laser Diffractometry and Dynamic Light Scattering

Particle size based on the volume distribution was measured by laser diffractometry on Microtrac® FRA, ranging 0.1–700 μm (Nikkiso Co., Ltd., Tokyo, Japan) or by dynamic light scattering on Microtrac® UPA150, ranging 0.003–6 μm (Nikkiso Co., Ltd., Tokyo, Japan). Appropriate amounts of specimens were dispersed in water by ultrasound for the measurement.

Polymorph Identification by X-Ray Powder Diffractometry (XRPD) and Differential Scanning Calorimetry (DSC)

Polymorphic forms were investigated by using an X-ray powder diffractometer (MiniFlex, Rigaku Corporation, Tokyo, Japan) equipped with a Ni-filtered Cu-anode (A-20Cu, Toshiba Corporation, Tokyo, Japan; 30 kV, 15 mA). Specimens were gently ground with an agate mortar and filled in a glass sample plate. The XRPD patterns were recorded at a scanning speed of 4°2θ/min over a range of 2–35°2θ.

Supplemental evidence on the polymorphic form was taken with a differential scanning calorimeter (DSC6200, Seiko Instruments Inc., Chiba, Japan). About 3 mg of specimens were filled in a crimped aluminum pan and the DSC traces were recorded at a heating rate of 10°C/min over a range of 30–220°C (tolbutamide) or 5°C/min over a range of 30–250°C (barbital) under the nitrogen atmosphere flowing at 60 mL/min. The onset temperature and enthalpy of melting and transition peaks were calculated.

Morphological Evaluation by Scanning Electron Microscopy (SEM)

Particle morphology was investigated by using a scanning electron microscope (S-4300, Hitachi, Ltd., Tokyo, Japan). Specimens were mounted on a metal stub with a conductive double-sided adhesive tape and coated with Au-Pd (6:4) by an ion sputter (JEC-550, JEOL Ltd., Tokyo, Japan) prior to the observation. The SEM photographs were taken at 10–20 kV and magnifications of 60–5000 x.

RESULTS AND DISCUSSION

Solubility of Tolbutamide and Barbital in Supercritical CO_2

Solubility of drugs in supercritical CO_2 affects the extraction efficiency of micronized products on the RESS process, while the relationship between the solubility and chemical structure has not fully been understood. To estimate the solubility, the same apparatus as the RESS processing was employed. Table 1 summarizes the solubility of tolbutamide and barbital in supercritical CO_2 measured after storing at 26 MPa and 60°C for 1 h, combined with the data of some other drugs obtained in-house under the same conditions. Drugs having lower melting temperature tended to have higher solubility in supercritical CO_2, although there were some exceptions. Tolbutamide and barbital showed relatively high solubility (6.89×10^{-5} and 3.57×10^{-5}, respectively) and appeared to be suitable for the RESS experiments.

Tolbutamide, commonly used as an antidiabetic or a hypoglycemic agent, is known to exist as at least four polymorphs, Forms I, II, III, and IV (Kimura et al., 1999). The crystal forms of tolbutamide available on the market were Form I (stable form) and Form II. To determine the starting material for the RESS experiments, the solubilities of tolbutamide Forms I and II were investigated in detail by a direct spectrophotometric technique. The solubility of tolbutamide in supercritical CO_2 was a function of applied pressure and temperature and was proportional to the pressure as shown in Fig. 2 No significant difference in solubility between Forms I and II of tolbutamide was detected. Meanwhile, gradual transition of Form II into Form I under the supercritical CO_2 atmosphere was found. Taking those findings into consideration we decided to use stable Form I for a series of the RESS experiments.

TABLE 1 **Solubility of Drugs in Supercritical CO_2**

Drug	Mol. weight (g/mol)[a]	Melting temp. (°C)[a]	Solubility in supercritical CO_2 (mole fraction, $\times 10^{-5}$)[b]
Phenytoin	252.27	297	0.26
Barbituric acid	128.09	248	0.46
Naproxen	230.26	153	0.54
Flurbiprofen	244.27	111	1.04
Aspirin	180.16	135	1.40
Phenobarbital	232.24	176	2.20
Mephobarbital	246.27	176	2.71
Barbital	184.19	190	3.57
Allobarbital	208.22	172	4.21
Hexobarbital	236.27	146	4.25
Phenylbutazone	308.38	105	6.78
Tolbutamide	270.35	129	6.89
Ibuprofen	206.28	76	11.30

[a] Quoted from The Merck Index, 12th Edition (1996). The mean of the upper and lower limits, if provided as a range.

[b] Measured after storing at 26 MPa and 60°C for 1 h.

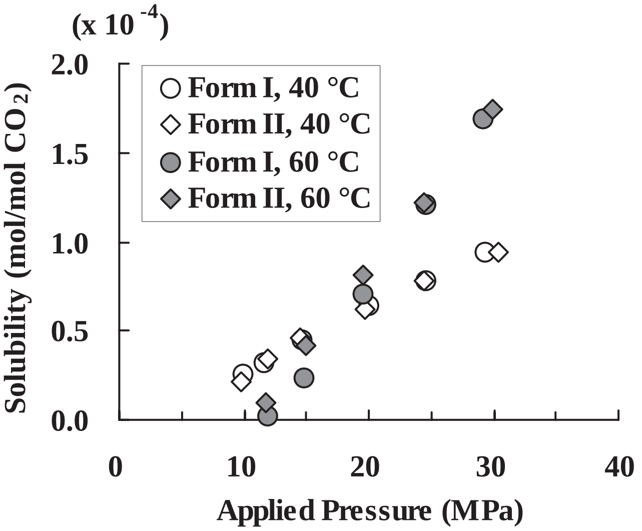

FIGURE 2 **Solubility of Tolbutamide in Supercritical CO_2 (Direct Spectrophotometric Measurement).**

Barbital, a sedative or a hypnotic, exists as at least three polymorphs (Sekiguchi et al., 1975). The crystal form of barbital purchased was found as a mixture of Form I (stable form) and Form II. For the RESS experiments the intact barbital was converted to pure Form I by storing in supercritical CO_2 atmosphere at 26 MPa and 60°C for 3 h before use.

Characterization of Tolbutamide and Barbital Particles Micronized by RESS Processing

Influence of Extraction Conditions on Particle Size

The influence of extraction pressure (18–26 MPa) and temperature (32–80°C) on particle size of tolbutamide and barbital was investigated under the fixed expansion conditions. The results for tolbutamide and barbital are summarized in Tables 2 and 3, respectively. Significant size reduction was achieved by the RESS processing. The mean particle size of intact tolbutamide and barbital was 126 and 204 µm, respectively, and the size of RESS processed particles was all micron or sub-micron level while the mechanical grinding had only slight effect. Figures 3 and 4 represent typical patterns of the particle size distribution of tolbutamide and barbital, respectively. It is apparent that the RESS processing made the size distribution narrower. It was found for both drugs that the lower the extraction temperature was, the smaller was the mean particle size. Higher extraction pressure resulted in smaller mean particle size when compared at the same extraction temperature. The smallest particles were thus obtained when the RESS process was operated at 26 MPa and 32 °C (mean particle size: tolbutamide = 1.64 µm, barbital = 0.69 µm).

In general, higher solubility of the solute in supercritical fluid is given at higher extraction pressure and temperature (Helfgen et al., 2000; Kayrak et al., 2003;

TABLE 2 Influence of Extraction Conditions on Particle Size and Polymorphic Form of Tolbutamide

	Extraction conditions		Mean particle size	XRPD	DSC					
					IV - II transition		II - I transition		Form I melting	
Sample	Pressure (MPa)	Temp. (°C)	(µm)	Polymorphic form	Onset (°C)	Enthalpy (J/g)	Onset (°C)	Enthalpy (J/g)	Onset (°C)	Enthalpy (J/g)
Intact, fromSigma (Starting material[a])	–	–	126[d]	I	n.d.	n.d.	n.d.	n.d.	127.1	99.9
Ground[b]	–	–	29.7[d]	I	n.t.					
	18	32	n.t.[e]	n.t.[e]	n.t.[e]					
	18	40	1.98[f]	(I +) II[g]	n.d.	n.d.	56.4	1.1	115.5	18.2
	18	60	3.58[f]	I + II	n.d.	n.d.	73.1	0.1	117.8	20.0
	18	80	3.74[f]	I (+ II)[h]	n.d.	n.d.	71.7	0.1	119.2	19.2
RESS[c]	26	32	1.64[f]	II	n.d.	n.d.	63.5	1.7	113.8	17.0
	26	40	1.91[f]	II	n.d.	n.d.	56.7	1.1	116.9	19.3
	26	50	1.95[f]	II	n.d.	n.d.	66.6	9.0	120.3	78.0
	26	60	2.13[f]	IV	n.d.	n.d.	61.5	9.5	119.0	81.1
	26	70	2.25[f]	IV	n.d.	n.d.	59.4	1.7	121.4	19.5
	26	80	3.30[f]	IV	49.1	−1.4	76.5	0.1	116.4	19.1
Intact, from Wako	–	–	n.t.	II	n.d.	n.d.	97.4	10.8	125.4	88.6

[a] The intact tolbutamide from Sigma was used as the starting material of the RESS process.

[b] The intact tolbutamide was mechanically ground for 30 min by using a vibration rod mill.

[c] The expansion conditions were fixed: spray nozzle diameter = 0.33 mm, spray nozzle temperature = same temperature as extraction, CO_2 amount per spray = 0.19 mol equivalent, expansion chamber temperature = 30°C, distance between spray nozzle and filter = 30 cm, exhaust gas flow rate = 4.3 m^3/h.

[d] Measured by laser diffractometry.

[e] Not tested, due to the low yield.

[f] Measured by dynamic light scattering.

[g] Form II dominant.

[h] Form I dominant.

n.t.: Not tested; n.d.: Not detected.

TABLE 3 **Influence of Extraction Conditions on Particle Size and Polymorphic Form of Barbital**

Sample	Extraction conditions		Mean particle size (μm)	XRPD	DSC					
					IV-II transition		II-I transition		Form I melting	
	Pressure (MPa)	Temp. (°C)		Polymorphic form	Onset (°C)	Enthalpy (J/g)	Onset (°C)	Enthalpy (J/g)	Onset (°C)	Enthalpy (J/g)
Intact	–	–	204[d]	I + II	n.d.	n.d.	130.3	4.1	189.6	142.4
Ground[a]	–	–	110[d]	I + II	n.d.	n.d.	130.3	3.6	189.9	127.1
Starting material[b]	–	–	n.t.	I	n.d.	n.d.	n.d.	n.d.	189.5	137.7
RESS[c]	18	32	1.27[e]	II	n.d.	n.d.	115.3	2.3	188.9	105.9
	18	40	1.31[e]	II	n.d.	n.d.	123.8	3.5	188.9	135.8
	18	50	1.81[e]	II	n.d.	n.d.	121.2	3.7	186.2	114.9
	18	60	4.18[e]	II (+ IV)[f]	n.d.	n.d.	119.3	3.2	188.4	133.2
	26	32	0.69[e]	II	n.d.	n.d.	112.0	2.4	187.9	92.9
	26	40	0.84[e]	II	n.d.	n.d.	114.3	3.2	187.3	96.2
	26	50	1.32[e]	II	n.d.	n.d.	119.0	2.9	187.7	110.2
	26	60	2.85[e]	II	n.d.	n.d.	130.6	1.9	187.5	90.4

[a] The intact barbital was mechanically ground for 30 min by using a vibration rod mill.

[b] The intact barbital, a mixture of Forms I and II, was converted to pure Form I by storing in supercritical CO_2 atmosphere at 26 MPa and 60°C for 3 h and used as the starting material of the RESS process.

[c] The expansion conditions were fixed: spray nozzle diameter = 0.33 mm, spray nozzle temperature = same temperature as extraction, CO_2 amount per spray = 0.19 mol equivalent, expansion chamber temperature = 30°C, distance between spray nozzle and filter = 30 cm, exhaust gas flow rate = 4.3 m^3/h.

[d] Measured by laser diffractometry.

[e] Measured by dynamic light scattering.

[f] Form II dominant.

n.t.: Not tested; n.d.: Not detected.

Türk, 2000). Actually, the highest solubility of tolbutamide in supercritical CO_2 was given at the highest pressure and temperature (Fig. 2). The high solubility in supercritical CO_2 means high level of supersaturation during the expansion. The high level of supersaturation predominantly induces the nucleation over the crystal growth, thus results in small particles with narrow size distribution. In this context, it seemed reasonable that higher extraction pressure resulted in smaller particles. Moreover, higher pressure should contribute producing smaller droplets when sprayed. On the other hand, another explanation would be needed for the influence of extraction temperature since the results were the opposite. Rapid expansion of supercritical fluid induces rapid drop in temperature (adiabatic expansion), which is also related to the level of supersaturation. Reverchon and Pallado (1996) reported that lower expansion temperature was favorable to produce larger temperature difference before and after expansion. The impact of the temperature difference, which could differ with the expansion conditions, was considered dominant over the solubility difference under the apparatus and settings employed in this study.

Influence of Expansion Conditions on Particle Size

The influence of expansion conditions on particle size was investigated using barbital. The process variables were spray nozzle diameter, spray nozzle temperature, CO_2 amount per spray, expansion chamber temperature, distance between spray nozzle and filter, and exhaust gas flow rate. The extraction pressure and temperature were fixed at 26 MPa and 32°C, respectively. The results are summarized in Table 4.

The mean particle size of barbital was reduced by lowering the spray nozzle temperature, by lowering the expansion chamber temperature, and by increasing the CO_2 amount per spray. High spray nozzle temperature or high expansion chamber temperature was reported to increase the particle size (Domingo et al., 1997; Helfgen et al., 2001). Heating the spray nozzle or expansion chamber can inhibit rapid drop in temperature during the expansion of supercritical solution. Increasing the CO_2 amount per spray should be related to the improvement of the diffusion efficiency of sprayed supercritical solution.

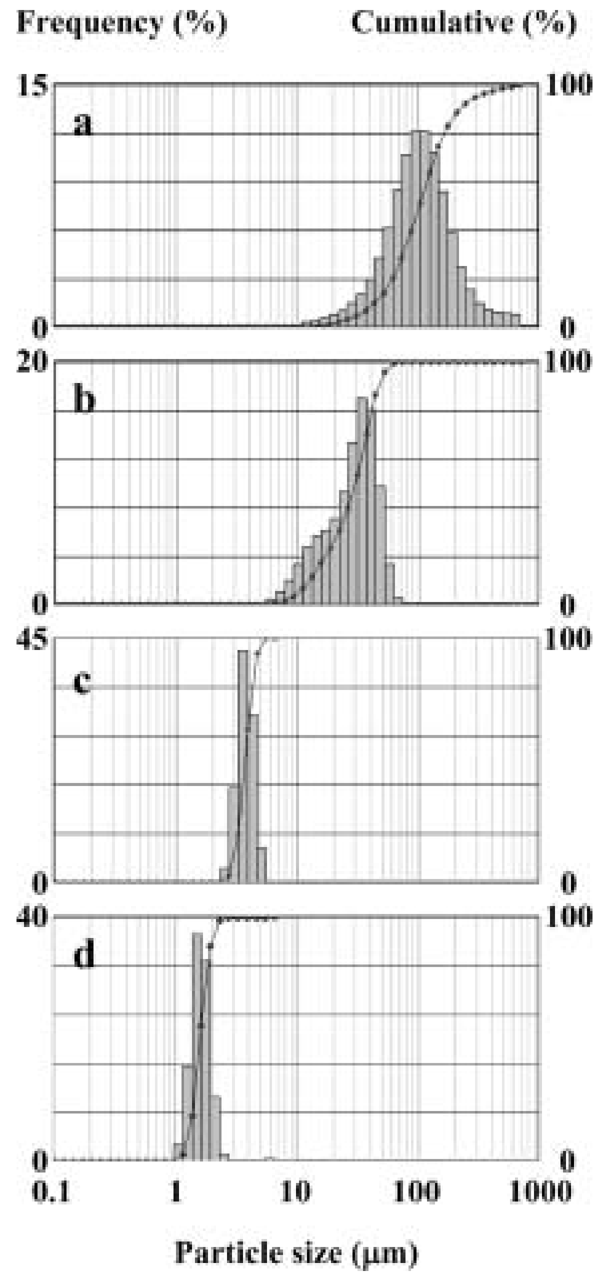

FIGURE 3 **Particle Size Distribution Patterns of Tolbutamide. a: Intact. b: Ground for 30 min by Using a Vibration Rod Mill. c: RESS-Processed at Extraction Conditions of 18 MPa and 80°C. d: RESS-Processed at Extraction Conditions of 26 MPa and 32°C.**

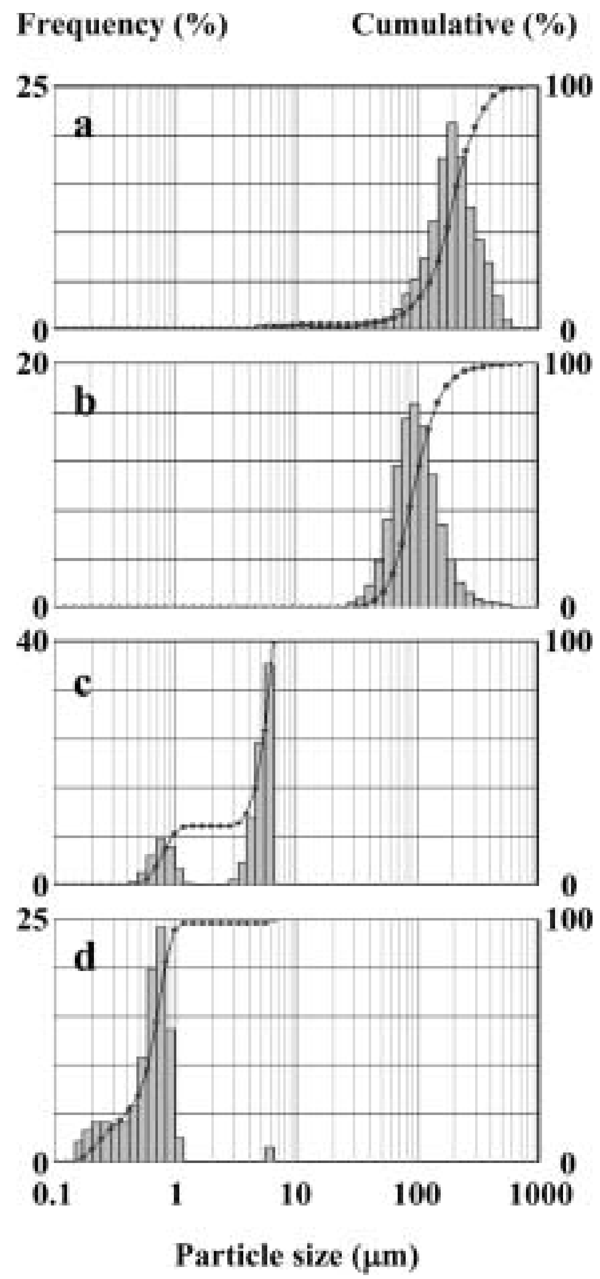

FIGURE 4 **Particle Size Distribution Patterns of Barbital. a: Intact. b: Ground for 30 min by Using a Vibration Rod Mill. c: RESS-Processed at Extraction Conditions of 18 MPa and 60°C. d: RESS-Processed at Extraction Conditions of 26 MPa and 32°C.**

Increasing the exhaust gas flow rate resulted in particle size reduction. Reducing the pressure inside the extraction chamber has been reported to reduce the particle size (Alessi et al., 1996). Consecutive spraying may accumulate residual CO_2 inside the extraction chamber, which can inhibit the rapid expansion of supercritical solution.

The influence of the other two process variables, spray nozzle diameter and distance between spray nozzle and filter, were not significant. The size of spray nozzle has been reported less influential when compared to the shape (Domingo et al., 1997; Jarmer et al., 2003). It probably means that the diffusion manners of sprayed supercritical solution were almost the same within the tested range of 0.23–0.66 mm. The distance between spray nozzle and filter can affect the crystal growth and agglomeration behavior of primary particles. A large number of mathematical and experimental models have been reported (Helfgen et al., 2003; Reverchon & Pallado, 1996; Weber et al.,

TABLE 4 **Influence of Expansion Conditions on Particle Size of Barbital**[a]

Expansion conditions						
Spray nozzle diameter (mm)	Spray nozzle temp. (°C)	CO_2 amount per spray (mol)	Expansion chamber temp. (°C)	Distance spray nozzle vs. filter (cm)	Exhaust gas flow rate (m^3/h)	Mean particle size (μm)[b]
0.23	32	0.19	30	30	4.3	0.77
0.33	32	0.19	30	30	4.3	0.69[c]
0.66	32	0.19	30	30	4.3	0.79
0.33	32	0.19	30	30	4.3	0.69[c]
0.33	40	0.19	30	30	4.3	1.22
0.33	60	0.19	30	30	4.3	1.57
0.33	32	0.07	30	30	4.3	1.77
0.33	32	0.19	30	30	4.3	0.69[c]
0.33	32	0.28	30	30	4.3	0.54
0.33	32	0.19	30	30	4.3	0.69[c]
0.33	32	0.19	40	30	4.3	1.19
0.33	32	0.19	60	30	4.3	1.57
0.33	32	0.19	80	30	4.3	1.77
0.33	32	0.19	30	7	4.3	0.75
0.33	32	0.19	30	14	4.3	0.54
0.33	32	0.19	30	30	4.3	0.69[c]
0.33	32	0.19	30	30	0.0	1.83
0.33	32	0.19	30	30	2.2	0.93
0.33	32	0.19	30	30	4.3	0.69[c]

[a] The intact barbital, a mixture of Forms I and II, was converted to pure Form I by storing in supercritical CO_2 atmosphere at 26 MPa and 60°C for 3 h and used as the starting material of the RESS process. The extraction conditions were fixed: extraction pressure = 26 MPa, extraction temperature = 32 °C.

[b] Measured by dynamic light scattering.

[c] The same data as in Table 3.

n.t.: Not tested.

2002). Although the influence of distance between spray nozzle and filter on particle size was not so significant in this study, the best result was obtained at the intermediate distance of 14 cm, indicating the existence of optimum distance.

The influence of expansion conditions as well as extraction conditions on particle size presented here was similar to our previous report on phenylbutazone (Moribe et al., 2005). The mean particle size of phenylbutazone was reduced by lowering the spray nozzle temperature, by increasing the CO_2 amount per spray, and by increasing the exhaust gas flow rate.

The mean particle size of barbital was successfully reduced to 0.54 μm by the process optimization: extraction pressure = 26 MPa, extraction temperature = 32°C, spray nozzle diameter = 0.33 mm, spray nozzle temperature = 32°C, CO_2 amount per spray = 0.28 mol equivalent, expansion chamber temperature = 30 °C, distance between spray nozzle and filter = 30 cm, and exhaust gas flow rate = 4.3 m^3/h.

Influence of Extraction Conditions on Polymorphic Form of Tolbutamide

The influence of extraction pressure (18–26 MPa) and temperature (32–80°C) on polymorphic form of tolbutamide was investigated. The results are summarized in Table 2, where the polymorphic forms were assigned based on the XRPD and DSC data reported by Kimura et al. (1999). It was found that the crystalline forms could differ with the extraction conditions on the RESS process. Figure 5 represents the XRPD patterns of tolbutamide, combined with the DSC traces. At a low extraction pressure of 18 MPa, the RESS processed samples exhibited characteristic X-ray diffraction peaks of both Form I (2θ = 8.7, 12.1, and 19.9°) and Form II (2θ = 10.3, 11.3, and 19.6°). From the comparison of X-ray diffraction intensities, the RESS processed samples were recognized as mixtures of Forms I and II, even Form II was dominant at lower temperature and Form I at higher. The samples prepared at 40 and 80°C were mainly consisted of Forms II and I, respectively.

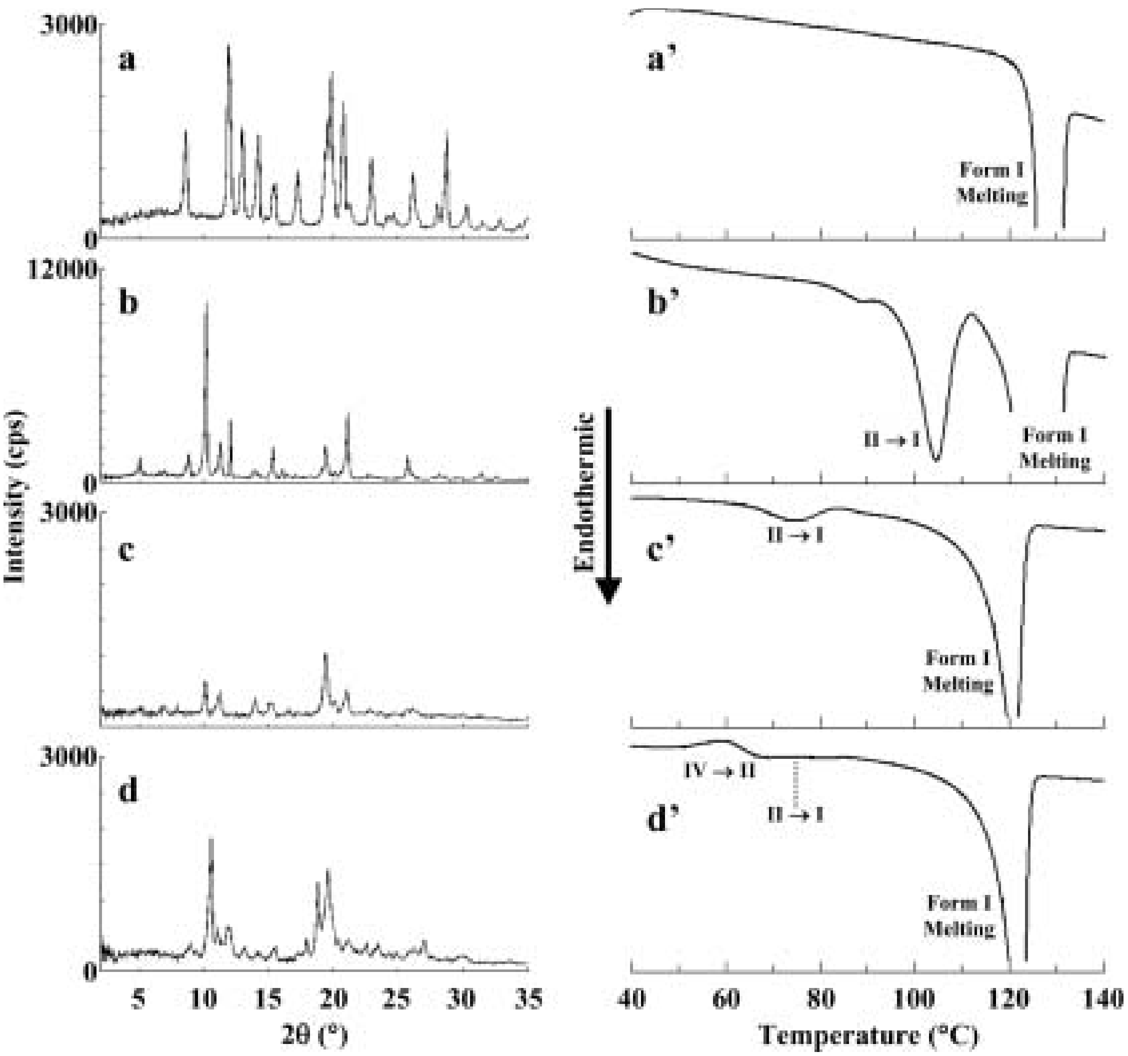

FIGURE 5 XRPD Patterns (Left) and DSC Thermograms (Right) of Tolbutamide. a, a': Intact from Sigma (Form I; Starting Material of the RESS Process). b, b': Intact from Wako (Form II). c, c': RESS-Processed at Extraction Conditions of 26 MPa and 32°C. d, d': RESS-Processed at Extraction Conditions of 26 MPa and 80°C. The Y-axes of the DSC traces were normalized by the weight of each sample.

Metastable forms (Forms II and IV) were possible to be produced at a high extraction pressure of 26 MPa. Form II characteristic X-ray diffraction peaks were observed when prepared at 50°C or lower (Fig. 5c), as seen in the intact tolbutamide purchased from Wako (Fig. 5b: Form II). On the other hand, Form IV characteristic X-ray diffraction peaks, $2\theta = 10.6$, 18.0 and 18.9°, were given when prepared at 60°C or higher (Fig. 5d).

The DSC data were consistent with the results on XRPD. The RESS processed samples prepared at low pressure (18 MPa) or low temperature (50°C or lower) showed Form II characteristic two endothermic peaks (Fig. 5c'), as seen in the DSC trace of intact tolbutamide purchased from Wako (Fig. 5b': Form II). The first small peak observed at 56.4–73.1°C was considered to be the transition of Form II to I. The second peak detected at 113.8–120.3°C corresponded to the melting of Form I, as seen at 127.1°C in intact tolbutamide purchased from Sigma (Fig. 5a': Form I). In addition to those two endothermic peaks, the RESS sample prepared at 26 MPa and 80°C exhibited an exothermic peak at 49.1°C (Fig. 5d). This peak was due to the transition of Form IV to II. Although the RESS samples prepared at 26 MPa and 60 or 70°C were supposed to show the same exothermic peak considering the results on XRPD (Form IV), the peak was not detected as it was very small and close to the detection limit under the operation conditions in this study. The RESS processed samples showed lower temperature and smaller enthalpy on both the transition of Form II to I and the melting of Form I when compared with intact tolbutamide. In general, size reduction sometimes results in melting point depression. Actually, Bettini et al. (2003) reported that the melting point depression of acetylsalicylic acid observed for RESS powders with respect to the commercial material could be attributed to the dramatic decrease of particle size as a consequence of micronization. Similarly, significant size reduction of tolbutamide on the RESS processing in this study could be a reason for the lower melting temperature. The lower transition temperature might be also related to the particle size. The enthalpies of Form I melting of the RESS samples were about 20% of those of the commercial products. Considering the difference in overall X-ray diffraction intensities, the degree of crystallinity of the RESS samples might be low. Exceptions were the RESS samples prepared at 26 MPa and 50°C (Form II) or 60°C (Form IV). The enthalpies of the Form I melting as well as the Form II to I transition were almost equivalent to those of the commercial products. It is interesting that those samples were prepared around the borderline determining Form II or IV, but the reason has not been clarified yet. The reduced crystallinity was not always regarded as worse since enhanced dissolution could be expected (Charoenchaitrakool et al., 2000).

Three polymorphs out of four could be produced by the RESS processing. The level of supersaturation of the solute in supercritical fluid during the expansion could affect not only the particle size but also the polymorphic form (Gosselin et al., 2003; Moribe et al., 2005). In this study, Form II was dominant at lower extraction temperature where the level of supersaturation was considered higher and thus smaller particles were produced. Form I came into existence at low pressure and high temperature (18 MPa, 80°C) where the level of supersaturation was considered lowest among the tested conditions. Considering these findings, rapid precipitation due to the high level of supersaturation may be preferable to produce Form II (metastable form), while slow precipitation kinetic would be advantageous to produce Form I (stable form). Form IV (metastable form) could be produced at intermediate conditions (26 MPa, 60–80°C). The residue in the reaction vessel stayed Form I regardless of the extraction conditions.

Influence of Extraction Conditions on Polymorphic Form of Barbital

While there are various opinions on the polymorphism of barbital, at least three polymorphs, Forms I, II, and IV, seem to exist (Sekiguchi et al., 1975). In the same way as tolbutamide, the influence of extraction pressure (18–26 MPa) and temperature (32–60°C) on polymorphic form of barbital was investigated. The results are summarized in Table 3, where the polymorphic forms were assigned referring to the XRPD and DSC data reported by Sekiguchi et al. (1975). Figure 6 represents the XRPD patterns of barbital, combined with the DSC traces. The intact barbital exhibited a mixed X-ray diffraction pattern of Forms I and II (Fig. 6a). The sample used as the starting material of the RESS process proved to be Form I as the characteristic X-ray diffraction peaks of Form II, $2\theta = 15.4$, 16.6 and 31.2°, were not detected while Form I characteristic peaks, $2\theta = 6.5$, 11.4, 16.3 and 17.3°, were clearly seen (Fig. 6b). All the RESS processed samples gave Form II characteristic peaks at $2\theta = 15.4$ and 16.6° (Fig. 6c). Only the sample prepared at 18 MPa and 60°C showed small X-ray diffraction peaks at $2\theta = 7.7$ and 13.5°, which were characteristic of Form IV, besides the Form II origin (Fig. 6d).

Supplemental evidence on the polymorphic form was taken with DSC. The sample used as the starting material of the RESS process showed a single endothermic peak at 189.5°C on the DSC trace, which corresponded to the melting of Form I (Fig. 6b'). All the RESS processed samples as well as the intact and ground barbital, mixtures of Forms I and II, showed a small endothermic peak at 112.0–130.6°C followed by another endothermic peak at 186.2–188.9°C (Figs. 6a', 6c', and 6d'). The first and second peaks corresponded to the transition of Form II to I and the following melting of Form I, respectively. Form IV characteristic endothermic peak was not detected even on the DSC trace of the sample prepared at 18 MPa and 60°C, probably due to the small existence ratio.

The extraction conditions hardly affected the polymorphic form of barbital. Metastable Form II was dominant on the RESS processing under a wide range of operating conditions, although slight contamination of another metastable form, Form IV, could occur at 18 MPa and 60°C. All the RESS conditions employed in this study were considered rapid enough to produce metastable forms. Especially, higher level of supersaturation seemed to be preferable to produce Form II, and relatively lower level of supersaturation, namely a combination of low pressure and high temperature on extraction, would be advantageous to produce Form IV. The residue in the reaction vessel was determined as Form I.

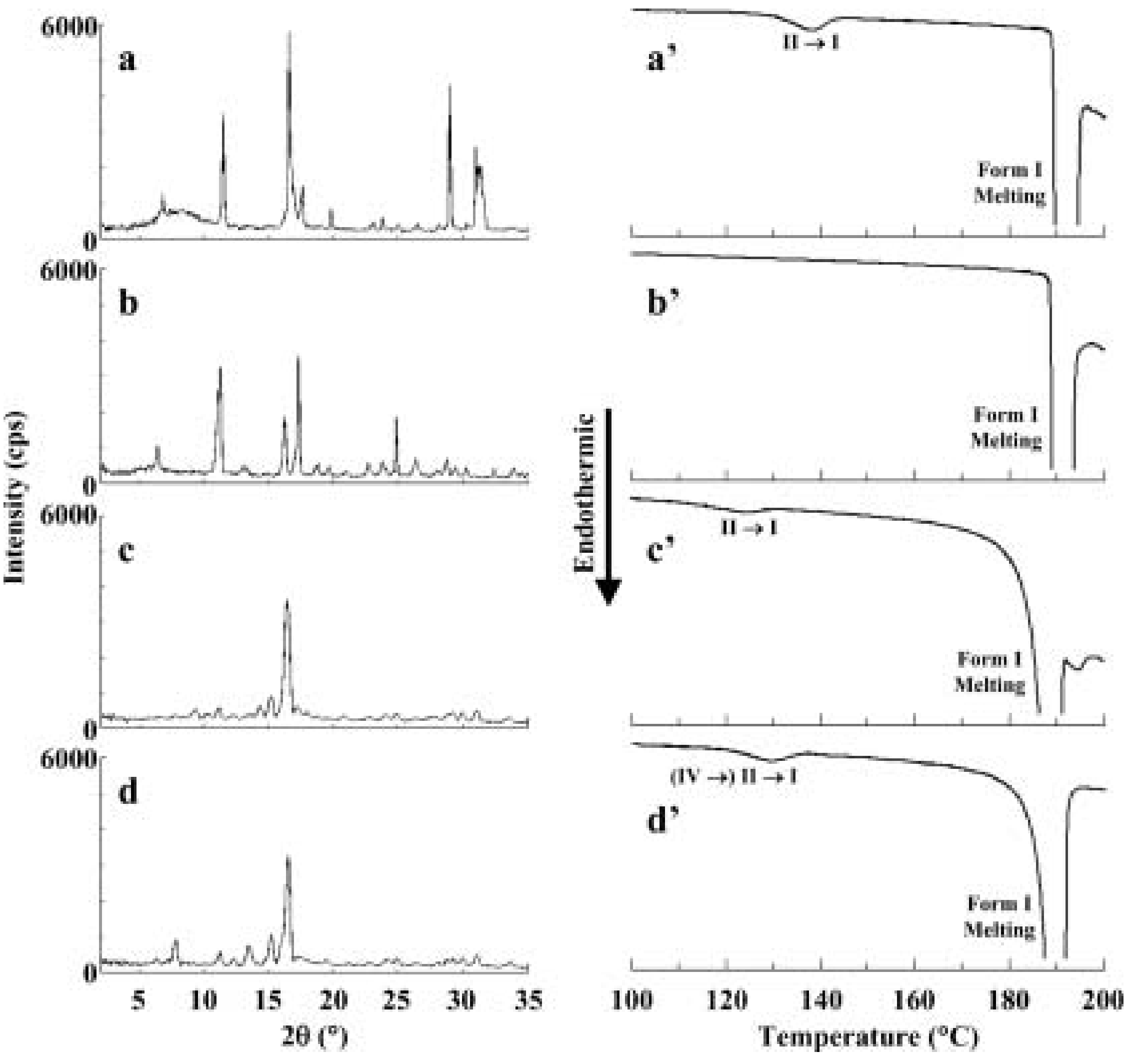

FIGURE 6 XRPD Patterns (Left) and DSC Thermograms (Right) of Barbital. a, a': Intact (Forms I + II). b b': Stored in Supercritical CO_2 Atmosphere at 26 MPa and 60°C for 3 h (Form I; Starting Material of the RESS Process). c, c': RESS-Processed at Extraction Conditions of 26 MPa and 32°C. d, d': RESS-Processed at Extraction Conditions of 18 MPa and 60°C. The Y-axes of the DSC traces were normalized by the weight of each sample.

Apart from the micronization, it was possible to convert a mixture of Forms I and II of barbital into pure Form I by simply storing in supercritical CO_2 atmosphere. Grabowska & Kaliszan (1976) mentioned that individual polymorphic forms evolved into the stable form in given conditions. The treatment might be of great interest for pharmaceutical industry to produce a pure form or to prevent polymorphic transition during storage, which could alter the solubility and therefore bioavailability. Bettini et al. (2001) reported the polymorphic transition of carbamazepine from a mixture of Forms I and III into pure Form III in supercritical CO_2. They ascribed it to the solubilization of Form III followed by the crystallization of Form I, as Form III had higher solubility in supercritical CO_2 than Form I. Tozuka et al. (2003) reported the formation of a new polymorph of deoxycholic acid by supercritical CO_2 treatment. They suggested the possibility of solution-mediated physical transformation among polymorphs and/or crystallization after dissolution in supercritical CO_2. Polymorphic transition of barbital might occur during extraction or during depression to normal pressure at the end of experiments. Anyway, polymorphic transition and/or precipitation in the reaction vessel should be a slow reaction when compared to those at the RESS spraying, and therefore stable Form I would be favorable for the residue in the reaction vessel.

Influence of RESS Processing on Particle Morphology

Figure 7 shows the SEM photographs of tolbutamide particles. The intact tolbutamide particles were large irregular-shaped agglomerates (Fig. 7a). Some huge agglomerates were broken by the mechanical grinding, but the particle size stayed large (Fig. 7b). Particles prepared by the RESS processing were small homogeneous thin-plates regardless of the polymorphic form (Fig. 7c: Form I; Fig. 7d: Form II).

Figure 8 shows the SEM photographs of barbital particles. The intact barbital particles were large polyhedral crystals (Fig. 8a). The mechanical grinding had only slight effect on the morphology (Fig. 8b). Form II particles prepared by the RESS processing were small needles (Figs. 8c & Figs. 8d), even some large particles were seen. These observations were in agreement with the size distribution, where two peaks at around 0.7 and 6 μm were seen (Figs. 4c & Figs. 4d). The reason for this phenomenon has not been clarified yet.

CONCLUSIONS

The RESS processing was successfully applied to tolbutamide and barbital to produce micronized particles. Significant size reduction to micron or submicron level with narrow size distribution was achieved, while conventional mechanical grinding had only slight effect.

The particle size was greatly affected by the extraction conditions. It was found for both drugs that the lower the extraction temperature was, the smaller was the mean particle size. Higher extraction pressure resulted in smaller mean particle size when compared at the same extraction temperature. It was possible to reduce the mean particle size of tolbutamide (126 μm) and barbital (204 μm) to 1.64 and 0.69 μm, respectively.

The size reduction effect was further optimized using barbital by varying the expansion conditions. The mean particle size was reduced by lowering the spray nozzle temperature, by lowering the expansion chamber temperature, by increasing the CO_2 amount

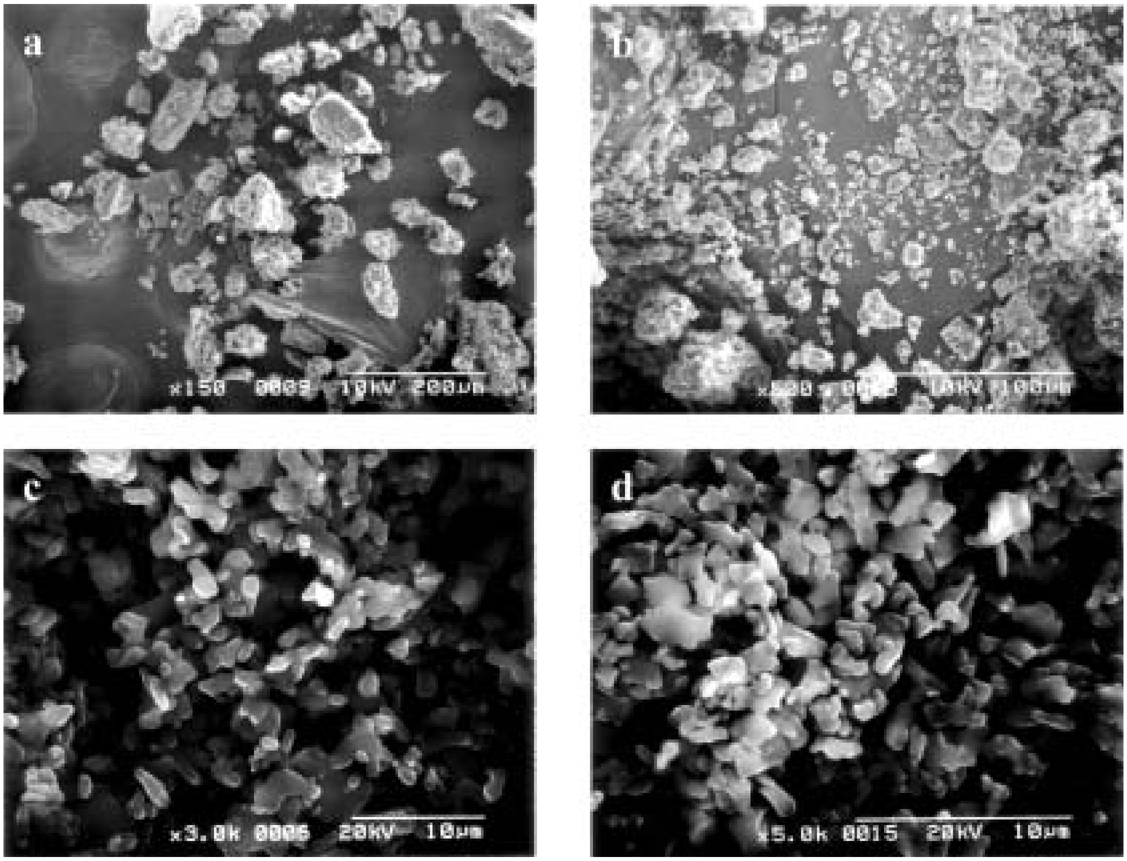

FIGURE 7 SEM Photographs of Tolbutamide. a: Intact. b: Ground for 30 min by Using a Vibration Rod Mill. c: RESS-Processed at Extraction Conditions of 18 MPa and 80°C. d: RESS-Processed at Extraction Conditions of 26 MPa and 32°C.

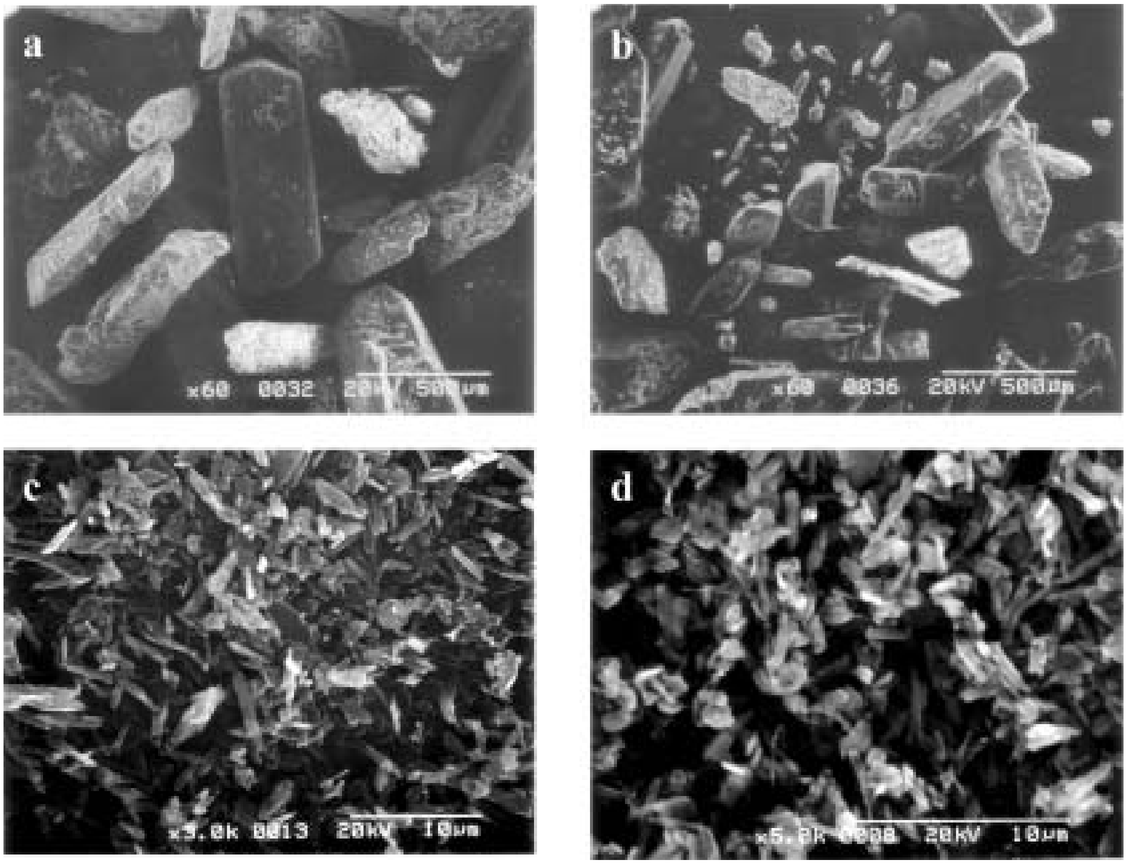

FIGURE 8 SEM Photographs of Barbital. a: Intact. b: Ground for 30 min by Using a Vibration Rod Mill. c: RESS-Processed at Extraction Conditions of 18 MPa and 60°C. d: RESS-Processed at Extraction Conditions of 26 MPa and 32°C.

per spray, and by increasing the exhaust gas flow rate. The mean particle size of barbital was successfully reduced to 0.54 µm by the process optimization.

The RESS processing realized not only the micronization but the polymorphic conversion as well. As for tolbutamide, three polymorphs (Forms I, II, and IV) out of four could be produced by changing the extraction conditions, and in the case of barbital one polymorph (Form II) out of three was produced consistently.

The RESS processing proved to be a promising method to improve the bioavailability of poorly water-soluble drugs due to the significant size reduction effect and the polymorphic conversion to the metastable form. The method offers an attractive alternative to contribute green chemistry.

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