

Improvement of Dissolution Property of Poorly Water-Soluble Drug by Supercritical Freeze Granulation

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The dissolution property of the poorly water-soluble drug, flurbiprofen (FP) was improved by a novel supercritical freeze granulation using supercritical carbon dioxide. Supercritical freeze granulation was defined as a production method of the granulated substances by using the dry ice to generate intentionally for the rapid atomization of the supercritical carbon dioxide to the atmospheric pressure. This process utilized a rapid expansion of supercritical solutions (RESS) process with the mixture of the drug and lactose. In the supercritical freeze granulation, needle-like FP fine particles were obtained which adhered to the surface of lactose particles, which did not dissolve in supercritical carbon dioxide. The number of FP particles that adhered to the surface of particles decreased with an increase in the ratio of lactose added, leading to markedly improve the dissolution rate. This improvement was caused not only by the increase in the specific surface area but also the improvement of the dispersibility of FP in water. It is thus concluded that the supercritical freeze granulation is a useful technique to improve the dissolution property of the poorly water-soluble flurbiprofen.

Key words poorly water-soluble drug; supercritical freeze granulation; micronization; dissolution rate

Recently, many of the drug candidate compounds newly synthesized by pharmaceutical industries have a large molecular weight and are poorly soluble in water because of the development of synthetic and screening techniques and the patent strategy.¹⁾ The poor solubility of drugs leads to the low absorbability of oral administrations and an increase in the dose, whereas an improvement in the solubility is necessary to reduce adverse effects and manufacturing costs.

As a method to improve the solubility of poorly water-soluble drugs, the micronization of drug particles by physical pulverization or crystallization is being attempted to improve the dissolution property by increasing the specific surface area. Particularly, drug particles in a nanometer order are attracting much attention because of a marked improvement in their bioavailability and usefulness in drug delivery systems (DDS), and so research on methods for their preparation is being conducted enthusiastically.

Conventional drug particle-designing techniques can be divided into solid and liquid techniques depending on the environment of crystallization, and into chemical and physical techniques according to the presence or absence of the involvement of chemical reactions in crystallization. By the solid technique based on physical pulverization, the particle minimum size is limited to a micrometer order because of the enhancement of adhesive-agglomerative property among drug particles due to activation of the drug particle surface during the pulverization process, with their consequent adhesion to the wall or parts of the pulverizer. The quality of the drug may also be affected by the heat generated during pulverization. The liquid method, in which organic solvents are often used as dissolution media, is associated with problems such as the residual solvent in crystals, necessity of the solid-liquid separation process, and aggregation.

Recently, supercritical carbon dioxide has attracted attention as an environmentally friendly solvent that may replace conventional organic solvents as it may allow the precise control of solvent properties, is harmless, and tolerates rela-

tively moderate processing conditions. Supercritical carbon dioxide has various specific solvent properties different from those of common liquid solvents, and its application to separation and purification, reactions, materials development, and analysis has been attempted.

Among the particulate design techniques using supercritical carbon dioxide, a rapid expansion of supercritical solutions (RESS) using supercritical carbon dioxide as a good solvent and supercritical anti-solvent process (SAS) using it as a poor solvent are employed as physical methods. So far, the RESS has been considered to be very promising, because a tremendous high degree of supersaturation can be established in a very short time, allowing the manufacturing of nanoparticles. In addition, the principles and apparatus are simple, and the method can be performed inexpensively. Also, concerning the application of supercritical fluid techniques to the treatment of poorly water-soluble drugs, many studies have been performed to improve the solubility by reducing the particle size,^{2,3)} formation of solid dispersions,⁴⁾ and clathration with cyclodextrin.⁵⁾

Recently, the supercritical freeze granulation technique⁶⁾ based on the RESS has been developed, and it has been confirmed that the drug dissolution rate can be improved by preparing granules as follows: drug particles are extracted with intentionally generated dry ice during the spraying of supercritical carbon dioxide using a mixture of a drug (ibuprofen) and an excipient.

In this study, to improve the dissolution property of a poorly water-soluble drug (flurbiprofen (FP)), micronization of the drug by the RESS was evaluated, and it was found that its dissolution property can be improved by supercritical freeze granulation using a mixture of the drug and an excipient.

Experimental

Materials Flurbiprofen manufactured by BASF AG (Ludwigshafen, Germany) (abbreviated as FP, mean particle size: 9.2 μm, melting point: 114–117 °C, solubility in water: 38 μg/ml at 37 °C) was used as a poorly

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water-soluble model compound. Lactose, employed as the excipient, was a product of DMV International (Veghel, Netherlands) (pulverized in a hammer mill, mean particle size: about 12 μm). The other reagents were those for high-performance liquid chromatography or special grade products.

Figure 1 shows the solubility of FP in supercritical carbon dioxide.⁷⁾ The solubility of lactose in supercritical carbon dioxide is extremely low, and it hardly dissolves under the experimental conditions of this study.

Preparation of Particles by the RESS Figure 2 shows the schematic diagram of the apparatus used in this experiment. The apparatus consisted of a supercritical carbon dioxide feed system with its flow rate controlled, stirring bath (capacity: 622 ml), and a spray nozzle. A visualization cell was attached to the stirring bath, allowing observation of the interior of the vessel.

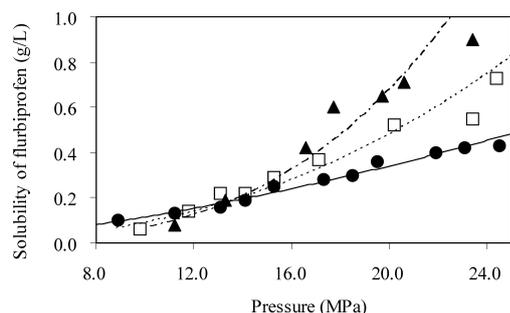


Fig. 1. Solubility of Flurbiprofen in Supercritical Carbon Dioxide as a Function of Pressure

●: 303 K, □: 313 K, ▲: 323 K.

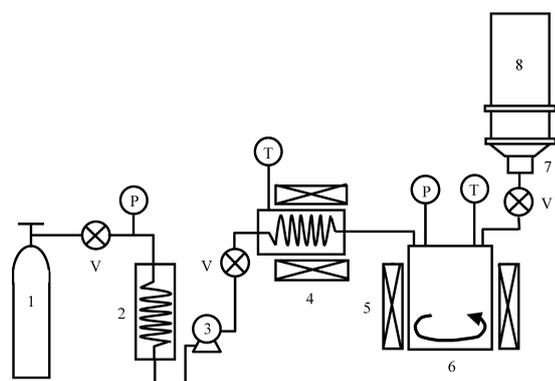


Fig. 2. Schematic Diagram of Experimental Set-Up

1: carbon dioxide cylinder, 2: cooler, 3: pump, 4: pre-heater, 5: heater, 6: stirring bath, 7: spray nozzle, 8: bag filter, V: valve, P: pressure gage, T: thermometer.

Also, a needle valve with an orifice size of 4 mm and an external diameter of 1/4 inches (Swagelok) was used as the spray nozzle, and 3/8- and 1/8-inch stainless steel pipes (Swagelok) were used for piping. To evaluate the particle surface condition by a field emission scanning electron microscope (FE-SEM), the particles were collected directly on the SEM sample table. Tables 1 and 2 show the manipulation conditions of this experiment.

Evaluation of Physicochemical Properties of the Particles Prepared by the RESS The surface condition of particles prepared by the RESS was examined under a field emission scanning electron microscope (FE-SEM, JSM-6700F, JEOL). The crystallinity of FP in the FP particles prepared by the RESS was studied, using a differential scanning calorimeter (SDA 2960 Simultaneous DSC-TGA, TA Instruments). For the measurement, the temperature was raised at 4 K/min⁻¹, the measurement range was 293–473 K, argon gas was used as the environmental gas, and the flow rate was 100 ml/min⁻¹.

Measurement of the FP Content in the Granules Prepared by Supercritical Freeze Granulation The FP content in the granules prepared by supercritical freeze granulation was determined by ultraviolet-visible absorption spectrometry. Ethanol (99.5) was added to 15 mg of granules prepared by supercritical freeze granulation to make the total volume of 200 ml. To remove lactose diffused in this solution, the solution was filtered through a hydrophilic 0.20- μm cellulose acetate filter (DISMIC-25CP, Toyo-Roshi), and the filtrate was used as the sample solution. The absorbance of the sample solution was measured using an ultraviolet-visible spectrophotometer (UV-1200, Shimadzu) (measurement wavelength: 246 nm), and the FP content in the granules was calculated using a calibration curve prepared in advance.

Evaluation of the Dissolution Property of FP from the Granules Prepared by Supercritical Freeze Granulation Dissolution testing was performed according to dissolution test method 2 (paddle method) described in the 15th edition of the Japanese Pharmacopoeia. The dissolution medium was 900 ml of water adjusted to 37 \pm 0.1 $^{\circ}\text{C}$, and the paddle was rotated at 100 rpm. An amount of granules equivalent to 10 mg of FP was added to the dissolution medium, the solution was sampled at predetermined intervals, and the amount of dissolved FP ($n=3$) was calculated from the absorbance at a wavelength of 246 nm.

Results and Discussion

Effects of the Temperature and Pressure of Supercritical Carbon Dioxide on the Particle Morphology Figure 3 shows SEM images of FP particles obtained under experimental condition Nos. 1 and 2, and Fig. 4 shows SEM images of FP particles obtained under experimental condition Nos. 3–6. Under condition No. 1, aggregates of spherical particles with a diameter of 1 μm or less were observed (Figs. 3A, B). These spherical particles were obtained because of the high degree of supersaturation and the short period of crystal growth around the generated nuclei. They are

Table 1. Manipulation Conditions of RESS Process

Experimental condition No.	Pressure (MPa)	Temperature (K)	Flurbiprofen (g)	Spray flow rate (ml/min)	Entrainer (ethanol, g)
1	20	328	0.5	2	—
2	10	328	0.5	2	—
3	20	318	0.5	2	—
4	20	328	0.1	2	—
5	20	328	0.1	80	—
6	20	328	1.0	2	10

Table 2. Manipulation Condition of Supercritical Freeze Granulation

Experimental condition No.	Pressure (MPa)	Temperature (K)	Spray flow rate (ml/min)	Flurbiprofen : lactose (Additive ratio)
1	20	318	2	100 : 0
2	20	318	2	50 : 50
3	20	318	2	30 : 70

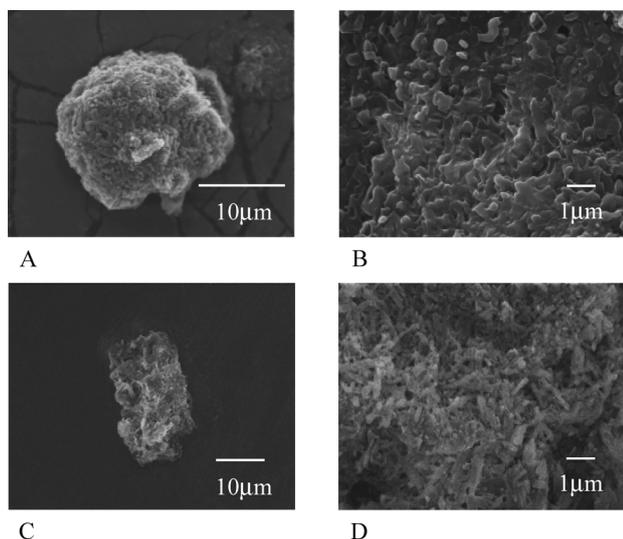


Fig. 3. SEM Images of Flurbiprofen Particles Produced by Different Operating Conditions (Experimental Numbers 1, 2)

(A, B) Condition number 1, (C, D) condition number 2.

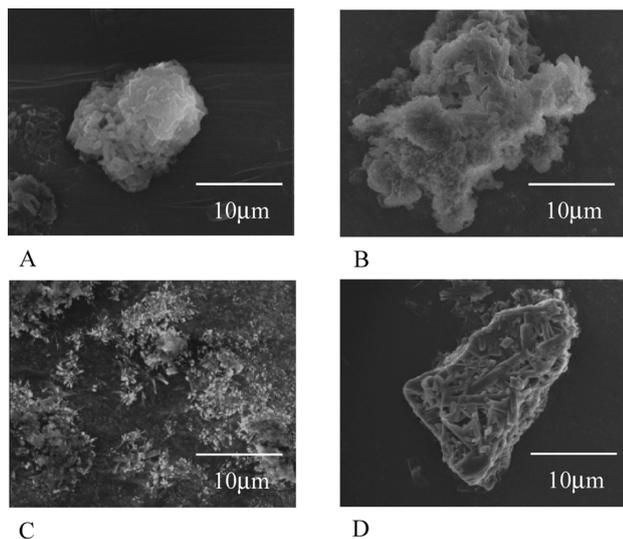


Fig. 4. SEM Images of Flurbiprofen Particles Produced by Different Operating Conditions (Experimental Numbers 3—6)

(A) Condition number 3, (B) condition number 4, (C) condition number 5, (D) condition number 6.

also considered to have aggregated before they reached the SEM sample table used for sampling. Under condition Nos. 2 (Figs. 3C, D) and 3 (Fig. 4A), aggregates consisting of needle-like particles with a diameter of $1\ \mu\text{m}$ or greater were observed. The solubility of FP in supercritical carbon dioxide decreases at a low temperature and pressure (Fig. 1). Therefore, the degree of supersaturation is considered to have decreased, needle-like particles to have been formed as crystal growth was promoted, and the particles to have aggregated before they reached the SEM sample table, under condition Nos. 2 and 3 with a lower temperature and pressure than under condition No. 1. From these results, micronized FP fine particles by the RESS were shown to form aggregates. Also, the temperature and pressure of supercritical carbon dioxide were suggested to affect the shape of the particles obtained.

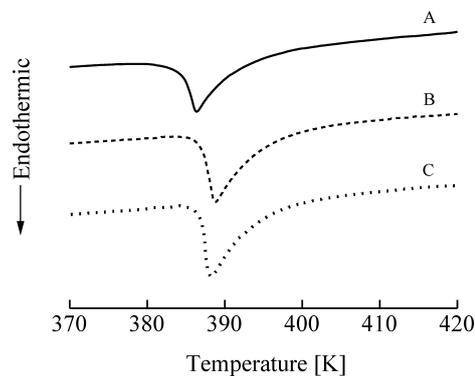


Fig. 5. DSC Profiles of Flurbiprofen Particles Produced by RESS Process

A: produced by RESS process (experimental condition No. 1), B: recrystallized flurbiprofen, C: original flurbiprofen.

Effects of the Amount of FP Dissolved in Supercritical Carbon Dioxide on the Particle Morphology

Figure 4B shows SEM images of FP particles obtained under condition No. 4. Under this condition, aggregates of needle-like particles of $1\ \mu\text{m}$ or greater in diameter were observed. Under this condition, the amount of FP dissolved in the stirring bath of the apparatus used was $0.456\ \text{g}/622\ \text{ml}$ (Fig. 1), and saturation was not reached. Therefore, the degree of supersaturation is considered to be low, leading to promote crystal growth and to form needle-like particles.

Effects of the Spray Flow Rate on the Particle Morphology

Figure 4C shows SEM images of FP particles obtained under condition of No. 5. Under this condition, needle-like particles about $1\ \mu\text{m}$ in diameter were observed. Similarly to condition No. 4, supercritical carbon dioxide was not saturated with FP, so crystal growth is considered to be promoted, leading to the formation of needle-like particles. However, the particles obtained under condition No. 5 did not form homogeneous three-dimensional aggregates as under condition Nos. 1—4, but they aggregated two-dimensionally on the SEM sample table. A large number of needle-like particles may have been formed and layered over the SEM sample table due to a high spray flow rate, or aggregates of needle-like particles may have collapsed when they collided with the SEM sample table and attached to it two-dimensionally. In order to solve this problem, it is necessary to examine the particle aggregation condition by changing the spray flow rate and spray time.

Effects of the Entrainer Effect on the Particle Morphology

Figure 4D shows SEM images of particles obtained under condition of No. 6. Under this condition, aggregates of needle-like particles of $10\ \mu\text{m}$ or greater in diameter were observed. Since ethanol is a good solvent, precipitated FP is considered to have been dissolved in ethanol and recrystallized. Also, the number of particles obtained increased by the addition of ethanol.

Figure 5 shows the relationship between the solubility of FP in supercritical carbon dioxide and the amount of ethanol added when the temperature and pressure were set at $313\ \text{K}$ and $18\ \text{MPa}$, respectively. Here, ethanol was added as an entrainer.⁸⁾ With an increase in the amount of ethanol added, the solubility of FP is considered to have improved due to the entrainer effect, resulting in an increase in the number of FP particles obtained.

Crystallinity of FP Particles Obtained by the RESS

Figure 5 shows DSC curves of particles obtained by the RESS with FP alone and untreated FP. It also shows the DSC curve of FP after recrystallization by adding its ethanol solution to water for comparison. The melting points of the untreated and recrystallized samples were about 388 K, and that of the FP particles obtained by the RESS was about 386 K. Also, the heat absorption peak of the recrystallized sample showed the same shape as that of the untreated sample, but that of the FP particles obtained by the RESS was slightly broader. These results suggest that the crystallinity of FP was reduced by the RESS. Concerning this decrease in crystallinity, evaluations not only by DSC to examine changes in thermal behavior but also by powder X-ray diffraction analysis and other techniques are necessary. And also, further evaluation is necessary to determine the stability of crystallinity after storage.

In the above evaluations, the particles obtained by the RESS with FP alone were aggregates of FP fine particles. In addition, FP such as hydrophobic drug has extremely poor aqueous wettability and dispersibility of FP fine particles, and it was considered difficult to improve its dissolution property by increasing the specific surface area. We, therefore, decided to investigate the possibility of improving the dissolution property of FP by supercritical freeze granulation using FP and lactose. Granules were prepared by changing the ratio between FP and lactose under experimental conditions similar to those for the evaluation with FP alone (Table 2).

Morphology of Granules Obtained by Supercritical Freeze Granulation Figure 6 shows SEM images of granules obtained by adding lactose to FP at various ratios. Needle-like FP particles adhered to the surface of lactose particles, and the number of FP particles that adhered to the surface of particles decreased with an increase in the ratio of lactose added. Lactose does not dissolve in supercritical carbon dioxide, and its particle size is not reduced by the RESS. Therefore, FP dissolved in supercritical carbon dioxide is considered to have been crystallized into needle-like particles and to have attached to the surface of lactose particles. Also, the FP attached to lactose comprised needle-like particles about $3\ \mu\text{m}$ in diameter, which was similar to the diameter of the particles obtained by the RESS with FP alone (Fig. 4A).

FP Content in Granules Obtained by Supercritical Freeze Granulation Figure 7 shows the FP content in granules obtained by supercritical freeze granulation. The FP content and theoretical FP content in the amount of power sample placed in the vessel are shown along the vertical and horizontal axes, respectively. The figure also shows the results obtained using physical mixtures. The theoretical and obtained FP contents in physical mixtures showed a good linear relationship. However, the FP content in the granules obtained by supercritical freeze granulation widely differed from the theoretical values. These differences are considered to have been caused by the use of a needle valve in the nozzle. In supercritical freeze granulation using ibuprofen and lactose, a diaphragm valve was used, and a close relationship was observed between the theoretical and observed contents.⁶⁾ While a diaphragm valve leaves a wide flow channel, a needle valve considerably narrows it, making the passage

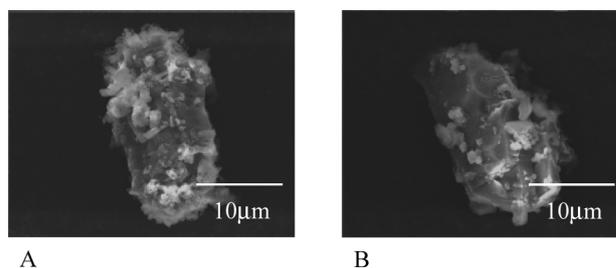


Fig. 6. SEM Images of Granules Produced by Supercritical Freeze Granulation

(A) Flurbiprofen/lactose (50/50), (B) flurbiprofen/lactose (30/70).

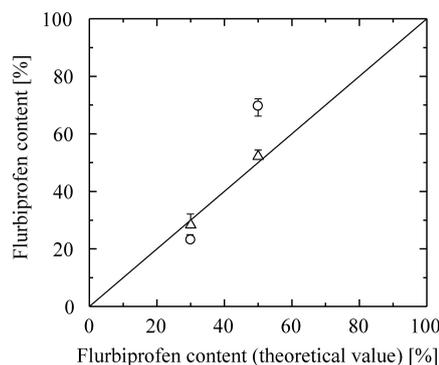


Fig. 7. Flurbiprofen Contents of Granules Produced by Supercritical Freeze Granulation

○: granules produced by supercritical freeze granulation, △: physical mixture. Each point represents the mean \pm S.D. ($n=3$).

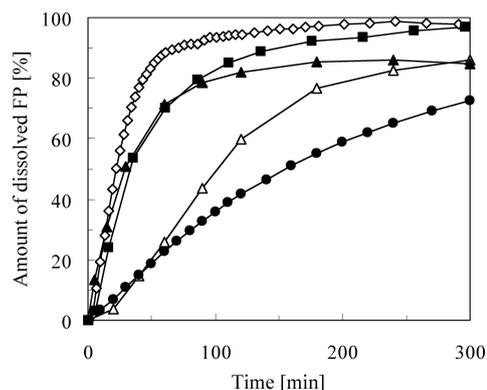


Fig. 8. Dissolution Profiles of Granules Produced by Supercritical Freeze Granulation

●: flurbiprofen particles produced by RESS process, △: original flurbiprofen, ▲: physical mixture (flurbiprofen/lactose (30/70)), ■: flurbiprofen/lactose (50/50), ◇: flurbiprofen/lactose (30/70). Each point represents the mean \pm S.D. ($n=3$).

of lactose, which does not dissolve in supercritical carbon dioxide, difficult. This is considered to result in the adhesion of a larger amount of FP to the surface of lactose particles than the theoretical value. This suggests the importance of the nozzle shape to obtain the expected FP contents in granules prepared by supercritical freeze granulation.

Dissolution Properties of FP from Granules Prepared by Supercritical Freeze Granulation Figure 8 shows the results of dissolution tests of granules obtained by supercritical freeze granulation and untreated FP. The dissolution rate was slower in the particles obtained by the RESS with FP alone (●) than in the untreated sample (△). This is consid-

Table 3. Percent Dissolution at 15 min

Supercritical freeze granulation	Percent dissolved at 15 min	Ratio of improvement
Flurbiprofen : lactose (30 : 70)	33.2	11.1
Flurbiprofen : lactose (50 : 50)	21.8	7.3
Flurbiprofen : lactose (100 : 0)	5.3	1.8
Original flurbiprofen	3.0	1.0

ered to have been a result of an apparent decrease in the specific surface area due to the aggregation of FP particulates as shown in Fig. 3. In the granules prepared by supercritical freeze granulation (■, ◇), the dissolution rate of FP was found to be accelerated with an increase in the ratio of lactose added (about 7—11 times, Table 3) as compared with the untreated sample (15 min). This can be explained by an improvement in the dispersibility of FP particles in water and consequent increase in the contact area with water associated with increases in lactose present among FP fine particles. Also, the DSC curves shown in Fig. 5 suggest the contribution of a decrease in the crystallinity of FP to the improvement in the dissolution rate.

From these results, the improvement in the dissolution property of FP is considered to be ascribed to an increase in the specific surface area due to a reduction in the particle size, decrease in the crystallinity, and improvement in the dispersibility in water. Among them, improvement in the dispersibility in water is considered to have contributed most to the improvement in the dissolution property of FP.

Conclusion

When the particle size of FP was reduced by RESS using supercritical carbon dioxide, the obtained fine FP particles formed aggregates, and the dissolution rate could not be improved. As a countermeasure, a novel supercritical freeze granulation was used to produce fine FP particles with the adding of lactose. It was found that the dissolution rate was markedly improved as fine particulate FP adhered to the surface of lactose particles, which do not dissolve in supercritical carbon dioxide, and not only increased the specific surface area but also improved the dispersibility of FP in water.

Therefore, supercritical freeze granulation using supercritical carbon dioxide is considered to be a useful technique to improve the dissolution property of FP.

In the next contribution, further studies are needed to clarify the mechanism of the improvement of dissolution property of flurbiprofen by supercritical freeze granulation. We will further evaluate the possibility of the practical application of this method by optimizing the temperature and pressure of supercritical carbon dioxide, amount of the drug added, and nozzle morphology.

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