Particle Characterization of Poorly Water-Soluble Drugs Using a Spray Freeze Drying Technique

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A spray freeze drying (SFD) method was developed to prepare the composite particles of poorly water-soluble drug. The aqueous solution dissolved drug and the functional polymer was sprayed directly into liquid nitrogen. Then, the iced droplets were lyophilized with freeze-dryer to prepare solid particles. Tolbutamide (TBM) and hydroxypropylmethylcellulose (HPMC) were used as a model drug and water-soluble polymeric carrier in this study, respectively. The morphological observation of particles revealed that the spherical particles having porous structure could be obtained by optimizing the loading amount of drug and polymer in the spray solution. Especially, SFD method was characterized that the prepared particles had significantly larger specific surface area comparing with those prepared by the standard spray drying technique. The physicochemical properties of the resultant particles were found to be dependent on the concentration of spray solution. When the solution with high content of drug and polymer was used, the particle size of the resulting composite particles increased and they became spherical. The specific surface area of the particles also increased as a result of higher concentration of solution. The evaluation of spray solution indicated that these results were dependent on the viscosity of spray solution. In addition, when composite particles of TBM were prepared using the SFD method with HPMC as a carrier, the crystallinity of TBM decreased as the proportion of HPMC increased. When the TBM : HPMC ratio reached 1 : 5, the crystallinity of the particles completely disappeared. The dissolution tests showed that the release profiles of poorly water-soluble TBM from SFD composite particles were drastically improved compared to bulk TBM. The 70% release time T_{70} of composite particles prepared by the SFD method in a solution of pH 1.2 was quite smaller than that of bulk TBM, while in a solution of pH 6.8, it was slightly lower. In addition, the release rates were faster than those of standard spray dried (SD) composite particles for solutions of pH 1.2 and 6.8, respectively. When composite particles were prepared from mixtures with various composition ratios, T_{70} was found to decrease as the proportion of HPMC increased; the release rate was faster than that of bulk TBM in a solution of pH 6.8, as well as solution of pH 1.2.

Key words tolbutamide; hydroxypropylmethylcellulose; spray freeze drying; composite particle; release rate; liquid nitrogen

Many newly developing compounds have been dropped out during the early stages of drug development process because of poorly water-soluble property. Poorly water-soluble drugs often demonstrate the low bioavailability when administered orally due to the low dissolution and absorption rate in the gastrointestinal tract. Therefore, improvement of the solubility of poorly water-soluble compounds is an important mission in drug development.

Increasing the solubility and dissolution rate of poorly water-soluble drugs is a significant challenge to pharmaceutical scientists. Technologies that have been commonly used to achieve this task include mechanical milling,[1] coprecipitation,[2,3] spray-drying,[4] the complex formation with water-soluble excipients,[5] and freeze-drying.[6] Spray drying has also been widely used as a technique to improve water solubility. However, it is not always appropriate for thermolabile compounds because the spray-drying process requires elevated temperatures. Although lyophilization or freeze-drying is a promising technique to produce pharmaceutical powders with improved solubility, the freezing rate is so slow that this technique is often difficult to apply in the pharmaceutical industry.

Spray freezing into liquid nitrogen (SFL) is a novel cryogenic atomization technology, developed by Williams *et al.*[7—11] in which either an aqueous or an aqueous-organic cosolvent solution containing an active pharmaceutical ingredient (API) and a pharmaceutical excipient is atomized directly into cryogenic liquid nitrogen. This method *via* atomization resulted in production of fine particles with a significant large specific surface area, with high yields, and has also been found to prevent interparticle aggregation. In addition, when insulin was atomized by this method, it was possible to obtain lower crystallinity while preventing protein aggregation.

In our previous work,[12] the solid dispersions were prepared by rapidly cooling of melt drugs in ice or in liquid nitrogen, and it was found that liquid nitrogen was appropriate as a cryogenic source to prepare particle-shaped solid dispersion with amorphous drug. Based on these previous results, we devised new atomization with quick freezing drying (a novel spray freeze drying: SFD) process which combined the spray dryer with the freeze drying equipment. This SFD method is spraying the drug solution over the surface of liquid nitrogen using the nozzle of the spray drier unlike the SFL method. Mumenthaler and Leuenberger[13] use the spray dryer as well as us, however, the freezing temperature makes it freeze at −70—−90 °C, comparatively high temperature.

The objective of this study was to utilize SFD technology for the preparation of composite particles containing a water-soluble polymer and a poorly water-soluble drug. In addition, the particles obtained by SFD were physicochemically characterized in the viewpoint of solubility improvement.

Experimental

Materials Tolbutamide (TBM), which was commercially purchased from Wako Pure Chemical Industries Ltd., was applied as a model drug in this study. Hydroxypropylmethylcellulose (HPMC 2910, Metolose, 60SH-

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4000), which was used as a hydrophilic carrier, was supplied by Shin-Etsu Chemical Co., Ltd. Tyloxapol of the nonionic detergent was commercially purchased from Sigma Life Science.

Spray Freeze Drying (SFD) Process A schematic diagram of the SFD apparatus is shown in Fig. 1, and composition of the aqueous feed solution is shown in Table 1. In a typical SFD process, the aqueous feed solution was prepared by dissolving TBM bulk powder and HPMC in 1% aqueous ammonia, and to form a spray solution containing TBM : HPMC = 1 : 5. The concentration in aqueous solution was changed to set to 0.9, 1.8, 9.0 and 13.5% to investigate the effect on physicochemical characteristics of composite particles. Furthermore, by a similar method, solutions containing TBM : HPMC = 1 : 1 and 1 : 3 was prepared. The solutions were supplied to the spray dryer (Model SD-1000, Tokyo Rikakikai Co., Ltd., Japan) at a rate of 20 ml/min and atomized through two-fluid nozzle at a set pressure of 50 kPa over the surface of the liquid nitrogen.

The frozen particles were collected and lyophilized in a freeze dryer (Model FD-550, Tokyo Rikakikai Co., Ltd., Japan) at −35 °C for 24 h. After freeze-dray process, the SFD was stored in glass vials in a vacuum desiccators at room temperature before characterization.

Standard Spray Drying (SD) Process The composite particles were prepared by spray drying using a SD-1000 instrument (two-nozzle fluid spray drier, Tokyo Rikakikai Co., Ltd., Japan). The spray drying was carried out under the following conditions: the solution sending rate was 10 ml/min, inlet temperature was 125 °C, the drying air flow was 0.77 ml/min, the atomizing air pressure was 50 kPa, and the outer temperature was 80—85 °C.

Physicochemical Measurement of Composite Particles The physicochemical properties of the SFD particles were evaluated by comparison with a physical mixture of TBM and HPMC with the same composition as the SFD products. A scanning electron microscope (SEM, JSM-6060, JEOL, Japan) was used to observe the morphology and particle size of the composite particles. The sample was fixed to a SEM sample stage and platinum sputter coated.

The particle size distribution of the sample powders was measurement by a laser diffraction scattering method using the diffractometer with the dry dispersing unit (LSM-30, Seishin Enterprise Co., Ltd., LSM-30). The particles were dispersed into dry air at constant pressure air of 0.4 MPa. The volume median diameter was represented as mean particle size of composite particles and bulk TBM.

A Nova-1000 surface area analyzer (Yuasa Ionics, Japan) was used to determine specific surface area using argon gas sorption process at −195.6 °C. The surface area per unit powder mass was calculated based on the fit of the adsorption data to the BET equation.

Powder X-ray diffraction (PXRD) analysis was performed using a Rigaku Geiger-Flex diffractometer (type RAD-2CV) with a Ni filter, CuKα radiation, a voltage of 30 kV, and a current of 20 mA. The scanning rate was 5°/min over a 2 θ range of 5°—45°.

Differential scanning calorimetry was carried out using a DSC-60 instrument (Shimadzu Co., Ltd., Japan). Around 5 mg of each test sample was placed in an aluminum pan. The heating program was carried out using a modulated setting at 10 °C/min over the range 20—200 °C. The heat of fusion was calculated by a peak area of thermogram at melting point.

Viscosity Measurement The viscosity of the sample solutions was measured using a cone and plate-type rotational viscometer (LVDV-II+ProCP, Brookfield). Measurements were carried out at a solution temperature of 25 °C using 0.5 ml of a sample solution and with a shear rate of 7.5 N.

Dissolution Studies Dissolution tests were performed using the Japanese Pharmacopoeia (JP) XV edition paddle method for sample powders, using 10 mg of the drug and 900 ml of the dissolution medium at pH 1.2 or pH 6.8 with holding at a temperature of 37±0.1 °C. The rotation speed of the paddle was set to 50 rpm. The quantity of TBM dissolved was assayed by HPLC (LC-10, Shimadzu Co., Ltd.) at 226 nm. The mobile phase was 10 mM KH_2PO_4 solution with acetonitrile (40 : 60, v/v), which flowed through an octadecylsilyl (ODS) column (Inertsil ODS-2, 4.6×150 mm, GL Science Inc., Japan) at a rate of 1.0 ml/min.

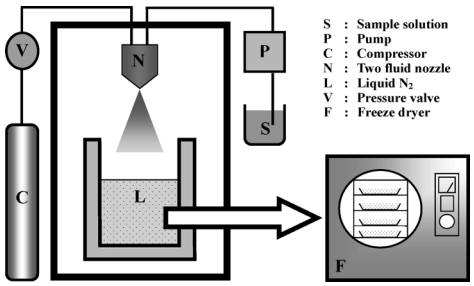

Fig. 1. Schematic Diagram of Spray Freeze Drying (SFD) Apparatus with Two-Fluid Nozzle

Table 1. Composition of the Aqueous Feed Solution

Process	Mixture ratios		Solution concentration
	TBM	HPMC	
SFD	1	5	0.9%
	1	5	1.8%
	1	5	9.0%
	1	5	13.5%
	1	1	9.0%
	1	3	9.0%
	1	0	9.0%
	0	1	9.0%
SD	1	5	9.0%

Results and Discussion

Preparation of Composite Particles in Solutions of Various Concentrations SEM photographs of SFD composite particles of TBM : HPMC = 1 : 5 obtained from various concentrations of spray solution are shown in Fig. 2. It was found that the composite particles prepared by SFD had unique structure having many small pores. The structure of the composite particles obtained in this study became a case in which the spray drug concentration was low with the rough and porous particles with large cavity (see Fig. 2, C and D), on the other hand it showed the tendency as a rigid and spherical particles the drug concentration is high cavity (see Fig. 2, E and F). It was clarified that the particles from higher concentration E and F had numerous pores in the surface as well as the inside as shown in magnified photograph G (surface) and I (cross section). Therefore, it was clear that the specific surface area considerably increases further than the particle of C and D. The sprayed droplets were frozen immediately after immersing in liquid nitrogen while keeping their shapes and sizes. It was assumed that the rigidness that of particle was dependent upon the amount of solute material (TBM and HPMC) in the droplets. Based on this fact, it was confirmed that the solution concentration had an strong influence on particle morphology in the SFD process.

In addition, the effect of the viscosity of spray solution, which was measured by rotational viscometer, on particle morphology was examined. The viscosity was found to increase exponentially with increasing solution concentration as shown in Table 2. The pores sizes of the particles E and F prepared from higher concentration, which had high viscosities, are considerably smaller than those of particles C and D (low viscosity). The high viscosity in the droplets might interrupt the crystal growth of ice crystal during freezing process. This hypothesis also explains that the particles from higher solution had a large number of small pores. On the other hand, the small block of ice disperses, because it is little for the quantity of the water which exists between drug molecules, when the solution concentration is high (E and F), and small pores are possible.

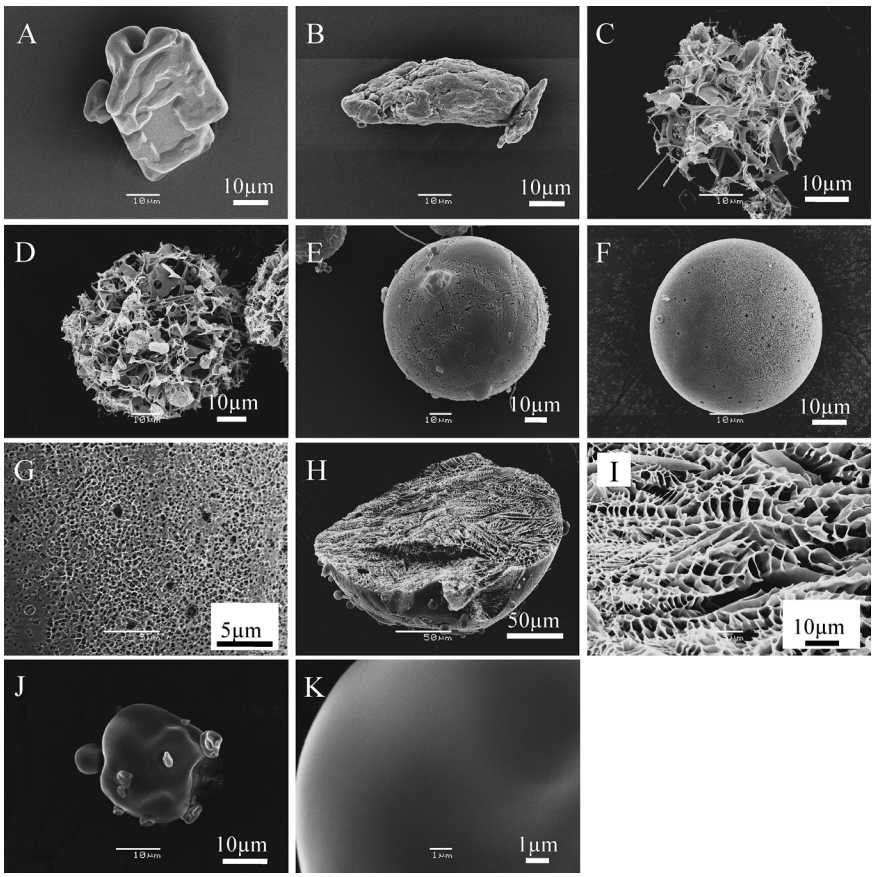

Fig. 2. SEM Photographs of Bulk Materials and SFD Composite Particles with Varying Solution Concentrations

A, TBM bulk; B, HPMC bulk; C, 0.9% SFD; D, 1.8% SFD; E, 9.0% SFD; F, 13.5% SFD; G, close-up photograph of F; H, internal structure of F; I, close-up photograph of H; J, 9.0% SD; K, close-up photograph of J.

Table 2. Viscosity of Sample Solutions

Concentration	0.9%	1.8%	9.0%	13.5%
Viscosity (mPa·s)	1.75	3.09	71.49	248.12

Table 3. Physicochemical Properties of Samples Prepared Using Solutions of Various Concentrations

Sample	Particle size (μm)	Specific surface area (m^2/g)[a)]
TBM bulk	32.9	0.04
HPMC bulk	45.1	0.22
0.9% SFD	55.2	18.07
1.8% SFD	72.3	22.13
9.0% SFD	99.4	28.32
13.5% SFD	178.7	27.47
9.0% SD	18.4	0.35

a) n=3.

The cumulative size distribution curves and mean particle sizes of the composite particles obtained in the experiments are shown in Fig. 3 and Table 3, respectively. The sizes of composite particles were larger that those of raw material (TBM, HPMC), increased with increasing the concentration of spray solution. It was found that the mean particle diameter had linear relationship with the solution viscosity, as shown in Fig. 4.

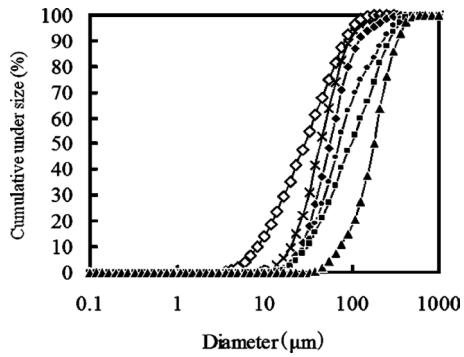

Fig. 3. Particle Size Distribution of Composite Particles Prepared with Varying Solution Concentration

◇, TBM bulk; ×, HPMC bulk; ◆, 0.9%; ●, 1.8%; ■, 9.0%; ▲, 13.5%.

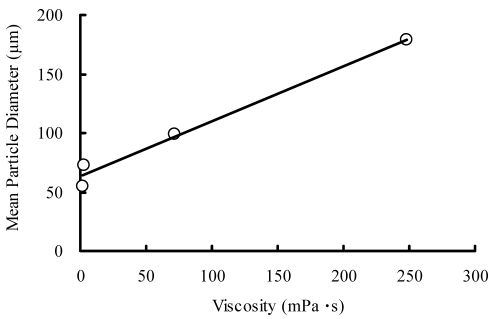

Fig. 4. Relationship between Particle Diameter and Solution Viscosity

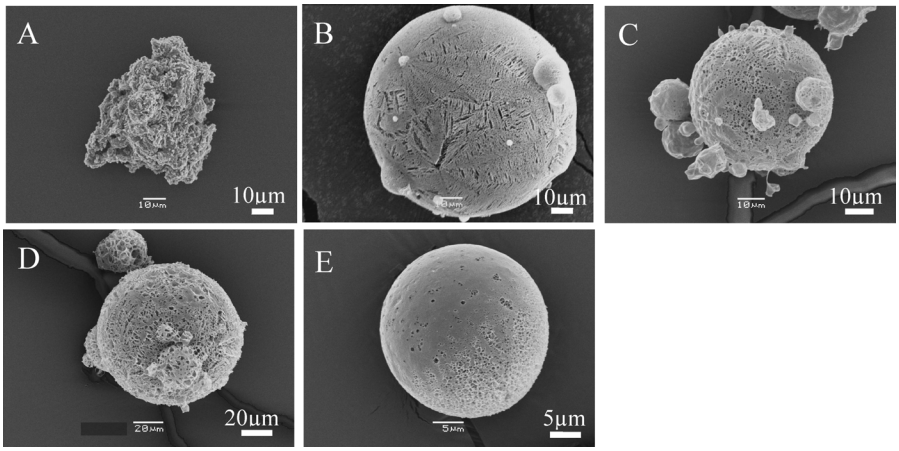

Fig. 5. SEM Photographs of Samples with Varying Mixture Ratio
A, TBM SFD; B, HPMC SFD; C, TBM : HPMC = 1 : 1 SFD; D, TBM : HPMC = 1 : 3 SFD; E, TBM : HPMC = 1 : 5 SFD.

Effect of Solution Concentration on Specific Surface Area The specific surface areas of the composite particles, measured by the gas adsorption method, are shown in Table 3. Both specific surface area of bulk TBM and bulk HPMC was small because they have a smooth surface with no pore as shown in Fig. 2. The specific surface areas of the composite particles prepared by the SFD method increased 450—700 times greater than that of the bulk material of TBM. The specific surface area also increased with increasing solution concentration, in this study, showing a maximum when the solution concentration was around 9.0%. From the morphological view point, the particle prepared from lower solution (Fig. 2, C and D) looks higher specific surface area than those from higher solution (Fig. 2, E and F), but the adsorption data showed the opposite results. It means that the particles (E and F) with rigid and smooth surface have also a lot of pores within internal structure. In addition, HPMC SFD particles with no drug have much larger specific surface area (35.20 m^2/g) than that of TBM SFD particles with no polymer (0.28 m^2/g). That is to say, the fine network structure is mainly formed by the polymer, when polymer aqueous solution is freeze-dried, and the fineness of network formed with the higher polymer concentration increase. Therefore, in this four solutions, 9.0% or more was taken to be the optimum solution concentration to obtain the particles with higher specific surface area and smaller pore size. Whereas, composite particles produced by spray drying (SD) process did not have such large specific surface area as SFD particles. SEM photograph revealed that the SD particle has rigid surface structure with no pore. Comparing to SD particles, it was clarified that SFD particles have unique and specific structure.

Effect of Carrier–Drug Ratio on the Physicochemical Properties of Composite Particles In order to increase the loading amount of TBM, it was projected that the ratio of TBM to HPMC in the spray solution was changed in SFD process. As mentioned earlier, it was found that the maximum specific surface area was obtained in particles prepared from a solution with a concentration of 9.0% or more. Therefore, the HPMC concentration in the spray solution was decreased with holding 9.0% of TBM concentration in the spray solution to provide the 1 : 1, 1 : 3 and 1 : 5 of TBM : HPMC ratio. The SEM of the SFD products is shown in Fig. 5. The mean particle diameters and specific surface areas of these particles are shown in Table 4. The specific surface area and mean particle size increased as the amount of HPMC increased. It was considered that the fineness inside the composite particles increased with increasing amounts of HPMC because the polymeric carrier strongly contributed to the formation of fine network structure explained above. The surface area of the composite particles prepared using a mixture ratio of 1 : 5 was 700 times greater than that of the bulk TBM.

Table 4. Physicochemical Properties of Samples with Various Mixture Ratios

Sample	Particle size (μm)	Specific surface area (m^2/g)[a)]
TBM bulk	32.9	0.04
HPMC bulk	45.1	0.22
TBM SFD	157.5	0.28
HPMC SFD	129.4	35.20
TBM : HPMC = 1 : 1 SFD	61.8	3.56
TBM : HPMC = 1 : 3 SFD	71.0	21.68
TBM : HPMC = 1 : 5 SFD	99.4	28.32

a) $n=3$.

The crystallinity of the composite particles was examined by powder X-ray diffraction and DSC. The results of powder X-ray diffraction are shown in Fig. 6, including the results of the physical mixture (PM) as a reference. The diffraction peaks which appeared in the bulk material at 8° and 20° shifted to 10° and 19° in the TBM SFD sample. It was concluded that a polymorphic transition had taken place from α-type of original form to another crystal form. Based on the reports of Simmons[14] and Kimura,[15] the newly prepared crystal form in SFD products was identified as β-type crystalline. In addition, the diffraction peaks decreased as the amount of HPMC in the composite particles increased. In the 1 : 5 SFD composite particles, amorphization was considerable, with almost no diffraction peaks observed.

The results of DSC thermal analysis, which are shown in Fig. 7, that revealed similar results to those obtained by X-ray diffraction: the endothermic peak decreased as the amount of HPMC carrier increased and disappeared in the

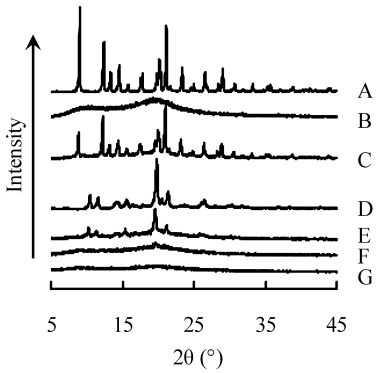

Fig. 6. Powder X-Ray Diffraction Patterns of Samples

A, TBM bulk; B, HPMC bulk; C, TBM : HPMC = 1 : 1 PM; D, TBM SFD; E, TBM : HPMC = 1 : 1 SFD; F, TBM : HPMC = 1 : 3 SFD; G, TBM : HPMC = 1 : 5 SFD.

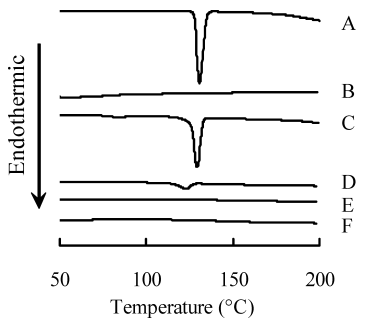

Fig. 7. DSC Profiles of Samples

A, TBM bulk; B, HPMC bulk; C, TBM SFD; D, TBM : HPMC = 1 : 1 SFD; E, TBM : HPMC = 1 : 3 SFD; F, TBM : HPMC = 1 : 5 SFD.

1 : 5 SFD composite particles completely. Calculations of the crystallinity of each sample, based on the endothermic peak area, from the equation below are shown in Table 5. The crystallinity decreased as the amount of HPMC increased, and in the 1 : 5 SFD particles, crystallinity could not be calculated. Based on this, it was concluded that the 1 : 5 SFD particles form a solid dispersion in which the drug is dispersed perfectly in the polymer molecules with no crystal.

The crystallinity Xc was calculated according to the following equation.

$$Xc = (\Delta H / \Delta H_0) \times 100 \quad (1)$$

where ΔH_0 is the heat of fusion of the crystalline form and ΔH is the heat of fusion of samples.

In addition, the residues of ammonia were measured in prepared particles by gas chromatography, because the ammonia aqueous solution was used in this study in order to dissolve TBM. As the result, the ammonia was not detected in the detection limit of the equipment.

Release Profiles from Composite Particles The release of TBM from composite particles prepared by the SD and SFD methods using a 9.0% sample solution was examined. Media adjusted of pH 1.2 and pH 6.8 were used to simulate the environments of the stomach and small intestine, respectively. The results for composite particles are shown in Fig. 8. In addition, the times required 70% release of TBM (T_{70}) are shown in Table 6. For the pH 1.2 medium, using both the SD and SFD methods, the release of TBM from the composite particles was improved considerably compared to the results obtained for bulk TBM. However, for the pH 6.8 medium, the composite particles prepared by the SD method showed delayed release in comparison with bulk TBM. The release rate in the pH 6.8 medium was greater than those in the pH 1.2 medium, because TBM is more soluble in alkaline solution.[15] Both release profiles showed that the composite particles by SFD method had rapid release performance compared to those by SD method. The rapid dissolution behavior of SFD particles seems to be caused by considerable higher surface area of SFD than that of SD, because the TBM included in both SFD and SD particles were amorphized.

Next, we examined drug release from composite particles with various ratio of TBM : HPMC into pH 1.2 and 6.8 test solutions. The results are shown in Fig. 9. The release profile

Table 5. Melting Point, Heat of Fusion and Crystallinity

Sample	Melting point (°C)	Heat of fusion (J/g)	Crystallinity (%)
TBM bulk	128.3	82.5	100
TBM SFD	124.8	81.6	99.0
TBM : HPMC = 1 : 1 SFD	115.8	30.7	37.2
TBM : HPMC = 1 : 3 SFD	113.7	2.2	2.7
TBM : HPMC = 1 : 5 SFD	—	0.0	0.0

— Not obtained.

Table 6. 70% Drug Release Time (T_{70}, min) of Samples Prepared Using SD and SFD Technique

Sample	pH 1.2	pH 6.8
TBM bulk	182	4.93
TBM : HPMC = 1 : 5 SD	43.52	23.02
TBM : HPMC = 1 : 5 SFD	3.27	2.28

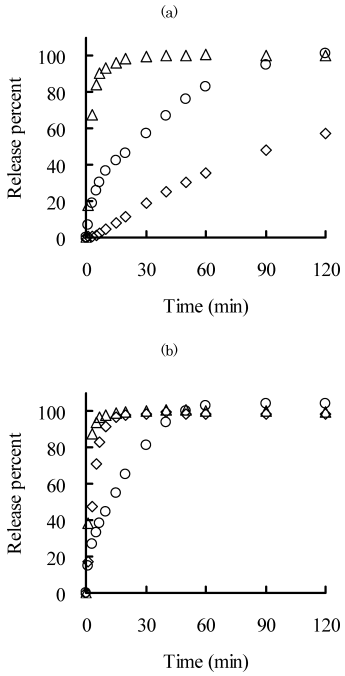

Fig. 8. Release Profiles of TBM from Bulk TBM and Composite Particles

(a) pH 1.2; (b) pH 6.8. ◇, TBM bulk; ○, TBM : HPMC = 1 : 5 SD; △, TBM : HPMC = 1 : 5 SFD.

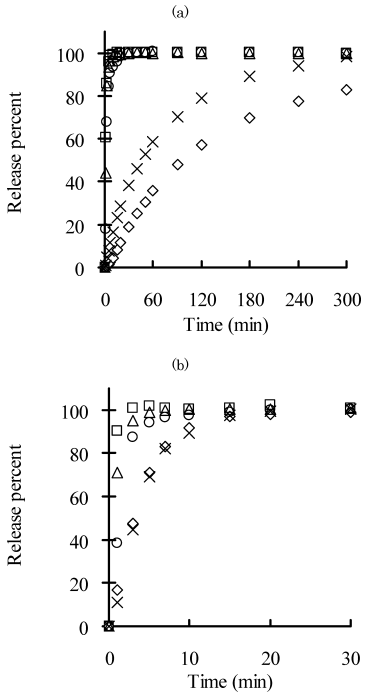

Fig. 9. Release Profiles of Samples

(a) pH 1.2; (b) pH 6.8. ◇, TBM bulk; ×, TBM SFD; □, TBM : HPMC=1 : 1 SFD; △, TBM : HPMC=1 : 3 SFD; ○, TBM : HPMC=1 : 5 SFD.

of TBM bulk in the pH 1.2 medium showed that release percentage reached the only 80% even after 300 min of test, while the composite particles showed perfect dissolution within 30 min. In contrast, drug release from the SFD composite particles in pH 6.8 solution was considerably faster than in pH 1.2 solution. The 70% release time, T_{70}, was calculated in order to compare the drug release properties of each sample, and the results are shown in Table 7. The dissolution rate was improved, because specific surface area increases. However, when the quantity of HPMC in composite particle increases, the dissolution rate slowed down in this case. It is considered to be caused by swelling of HPMC in the solution.

Conclusions

In the SFD method, the spherical particles were obtained, and those particle sizes were controlled by solution concentration. The SFD particles had quite characteristic internal structure with marvelous large specific surface area, which could not be produced by standard spray drying process. It was found that TBM became amorphous dispersed in the polymeric carrier over 1 : 5 of loading ratio. The release profiles of the active ingredient from composite particles prepared by the SFD method were considerably improved than that of either TBM bulk or composite particles prepared by the SD method. Those results indicated that the SFD method was useful technique as a new formulation method for the solubilization of the poorly water soluble drug.

Table 7. 70% Drug Release Time (T_{70}, min) of Samples with Various Mixture Ratios

Sample	pH 1.2	pH 6.8
TBM bulk	182	4.93
TBM SFD	88.9	5.13
TBM : HPMC=1 : 1 SFD	1.74	0.77
TBM : HPMC=1 : 3 SFD	2.28	0.99
TBM : HPMC=1 : 5 SFD	3.27	2.28

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