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Crystallization Behavior of Polymorphic Mixtures of MPPO

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Abstract:

4-(1-(2-(3-Methoxyphenethyl)phenoxy)-3-(dimethylamino)propan-2-yloxy)-4-oxobutanoic acid (MPPO), a serotonin receptor blocker, is known to have two polymorphs, Form A and Form B. Crystallization of MPPO was carried out from aqueous 2-butanone solutions by the further addition of 2-butanone as a poor solvent and by cooling to determine the operating conditions, in particular, the seeding effect under which polymorphic mixtures of desired compositions were produced. Seed particles were prepared by comminuting both polymorphs and mixing them at given compositions X_{As}. The product polymorphic compositions (X_{Ap}) were well controlled by the seed composition as well as by the initial solvent composition (w) and seeding temperature in such a way that X_{Ap} increased as the supersaturation at the moment of seeding decreased. In addition, size-dependent product compositions were analyzed to reveal the crystallization behavior of the polymorphs.

1. Introduction

The polymorphism of active pharmaceutical ingredient (API) crystals can generally influence their bioavailability[1] because different polymorphs usually have different solubilities and this may lead to problems in that required product properties may not be satisfied.[2] Therefore, many studies have focused on polymorphic crystallization behavior in the production of APIs.[3−6]

The crystallization behaviors of polymorphic systems are considerably influenced by the solvent used because the solvent type and composition may affect the interaction with the solute. For example, tartirelin[7] crystallized as an unstable form from water and an aqueous methanol solvent containing less than 10% methanol, whereas it crystallized in a stable form from solvents containing more than 30% methanol. Similar crystallization behaviors of four polymorphs (including two pseudopolymorphs) caused by the solvent composition were reported for 2-(3-cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid (BPT).[8]

In the pharmaceutical industry, most crystallizations are carried out batchwise; the techniques used for the same include cooling, evaporative, drowning-out, or reactive crystallization. These crystallizations are usually carried out without seeding so that crystals can precipitate spontaneously; therefore, unstable forms often result. For substances in which spontaneous crystallization is difficult or two liquid phases appear upon cooling, seeding is carried out to initiate crystallization or to obtain the required polymorphs. A review concerning seeding crystallization is available.[9]

The crystallization of eflucimibe[10] and cimetidine[11] reported that polymorphs that differed from the seed or polymorphic mixtures were obtained in certain supersaturation ranges. In the crystallization of avecanil[12] to obtain an unstable polymorphic form by seeding, seeds contaminated with 5% of a more stable polymorph induced a solution-mediated transformation, resulting in an undesirable 50:50 mixture of the two polymorphic forms.

Drowning-out crystallization has been applied in the pharmaceutical industry to generate high supersaturation by the addition of a poor solvent that substantially reduces the solubility. This method is used to produce unstable forms, improve yield, or reduce process time. In the manufacture of AE1-923,[13] drowning-out was applied to obtain an unstable form, since cooling at a very rapid rate of 10 K/min was required to obtain this unstable form if cooling crystallization was applied.

For these reasons, APIs are generally crystallized as single polymorphs. However, if the product composition can be well controlled and if polymorphic transformation during preparation and storage can be prevented, a polymorphic mixture may also be a possible candidate as an API. To demonstrate this possibility, the crystallization of 4-(1-(2-(3-methoxyphenethyl)phenoxy)-3-(dimethylamino)propan-2-yloxy)-4-oxobutanoic acid (MPPO), a serotonin receptor blocker, was carried out. MPPO has two polymorphs, Form A and Form B. In preliminary experiments of spontaneous crystallization of MPPO without seeding, polymorphic mixtures with high contents of the unstable form (Form B) were obtained, but the composition

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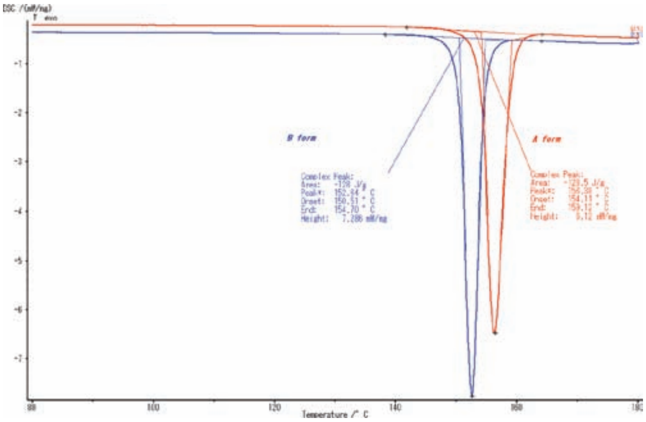

Figure 1. **DSC charts of both polymorphs. Form A and Form B showed a single endothermic peak at 156.38 and 152.64 °C, respectively. The heating rate was 10 °C/min under nitrogen purge.**

was scattered. Therefore, to obtain a desired composition, seeding was studied in greater detail, and the crystallization behavior of the polymorphs was clarified. Consequently, this study aims to understand, in particular, the effectiveness of seeding on the product composition in terms of seed composition and seeding temperature, as well as the effect of solvent composition.

2. Experimental Methods and Procedures

2.1. Material Used in Experiment and Identification of Polymorphs. MPPO was synthesized by OHARA Pharmaceutical Co., Ltd. Its chemical purity as analyzed by HPLC was >99%, and any impurities that exceeded 0.1% were not included. 2-Butanone (Nacalai Tesque, Inc.; gas chromatography purity: >98%) and distilled water (Wako Pure Chemical Industries, Ltd.) were used without further purification. To obtain Form A of MPPO, cooling crystallization of aqueous 2-butanone solutions (water content in mass fraction, $w = 0.073$) was adopted, while Form B was crystallized from aqueous acetone solutions ($w = 0.060$). The polymorphic purities of both polymorphs were confirmed to be sufficiently high by powder X-ray diffraction (PXRD) analysis using Cu Kα radiation (Bruker Model DISCOVER with GADDS; operating current: 40 mA, operating voltage: 45 kV), infrared absorption spectrometry (Shimadzu FTIR 8400), and differential scanning calorimetry (NETZSCH DSC 204 F1). The polymorphic composition was measured by PXRD, as mentioned later.

Figure 1 shows the differential scanning calorimetry (DSC) charts of both polymorphs. At the heating rate of 10 °C/min under nitrogen purge, the DSC curve of Form A and Form B present sharp endothermic peak at 156.38 and 152.64 °C, respectively.

Panels a and b of Figure 2 show typical SEM images of Form A and Form B, respectively, prepared by the above-mentioned procedure. The figure shows that both forms had significantly different morphologies; Form A crystals are rodlike and less agglomerated, whereas Form B crystals are strongly agglomerated and have well-faceted features. These morphologies of Form A and Form B were also observed in most crystallizations conducted in the present study.

2.2. Solubility. The solubility of each polymorph in aqueous 2-butanone solutions was measured in the temperature range of 273−333 K. Since the maximum water content in 2-butanone at 298 K is $w = 0.109$, the compositions of the mixed solvents were chosen as one-third and two-thirds of saturation; the water content of the mixed solvent in terms of mass fraction w was therefore varied as $w = 0.037$, 0.073, and 0.109.

To measure the solubility of MPPO, 20 g of each polymorph and 50 mL of a mixed solvent were placed in a 100-mL glass vessel and stirred with a four-blade impeller for 1 h at a predetermined temperature (273, 283, 293, 303, 313, 323, or 333 K). Then, approximately 2 mL of the supernatant solution was passed through a filter having a pore size of 0.45 μm, and 200 μL of the filtrate was weighed accurately. Its solute concentration was determined by a gravimetric method and then converted into units of mole fraction. After the solubility measurement, the remaining crystals were collected and analyzed by PXRD to confirm that solution-mediated transformation had not occurred.

2.3. Calibration Curve of Polymorphic Mixtures. In PXRD patterns of MPPO, characteristic sharp peaks of Form A were present at $2\theta = 10.3°$ and 11.1°, and those of Form B were observed at $2\theta = 10.7°$ (Figure 3). To prepare a calibration curve to determine the polymorphic composition of a mixture, comminuted powders of Form A and Form B having a mean size of approximately 10 μm were mixed such that the mass fraction of Form A would be 0.10−0.90, and their PXRD patterns were measured. From the measured patterns, the characteristic peaks of both polymorphs were separated using TOPAS software (Bruker AXS TOPAS, version 2.1), and the peak heights from the baseline were read from the chart. Then, the polymorphic composition I_A was calculated using eq 1, and the calibration curve (eq 2, the correlation factor is 0.9984) for Form A between the sample composition (X_A) and the calculated one (I_A) was prepared; H_{A1} and H_{A2} in eq 1 are the peak heights at $2\theta = 10.3°$ and 11.1° in the case of Form A, and H_{B1} is that at $2\theta = 10.7°$ in the case of Form B. The polymorphic composition of the crystals (X_A) was thus determined on the basis of this calibration curve. Hereafter, X_{Ap} and X_{As} denote the polymorphic composition of the product and the seed, respectively. Error of the polymorphic composition of the product was within ±1%.

$$I_A = \frac{H_{A1} + H_{A2}}{H_{A1} + H_{A2} + H_{B1}} \tag{1}$$

$$X_A = 1.01 I_A \tag{2}$$

2.4. Crystallization Procedure. A 2-L glass reactor (inside diameter: 14 cm, depth: 15 cm) with a jacket and equipped with a three-blade retreat agitator (diameter: 90 mm) and a beaver tail baffle was used as a crystallizer. To prepare a mother liquor, 150 g of MPPO was dissolved in 619 g of aqueous 2-butanone solution ($w = 0.073$) at 348 K and then stirred at 215 rpm. The composition of this solution in mole fraction was 0.0298, and its saturation temperature was 338 K. After complete dissolution, it was then cooled to 323 K in about 25 min, where the temperature was controlled manually.

In most experiments, when the temperature reached 323 K, 0.15 g of seed was added as a dry powder; this amount corresponded to 0.1 mass % of the dissolved MPPO. After

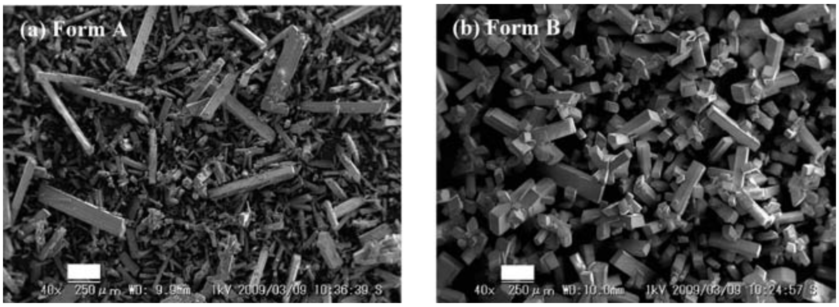

Figure 2. **(a) SEM image of Form A. (b) SEM image of Form B. Scale = 250 μm.**

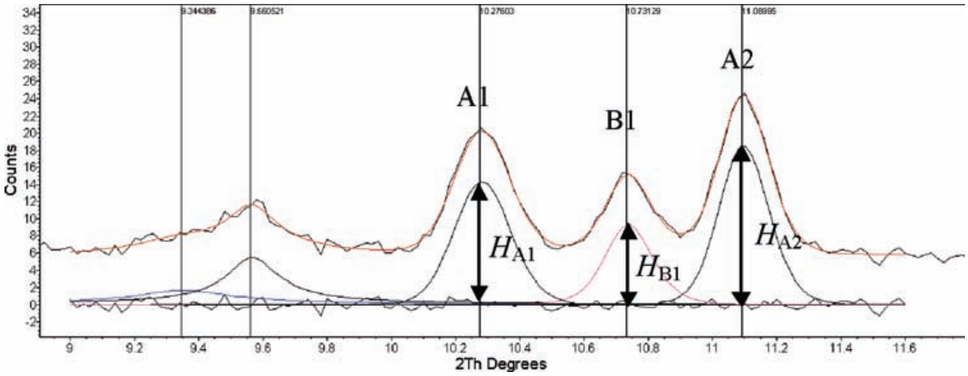

Figure 3. **Powder X-ray diffraction patterns of mixed crystal. H_{A1} and H_{A2} are the characteristic peak heights of Form A, and H_{B1} is that of Form B.**

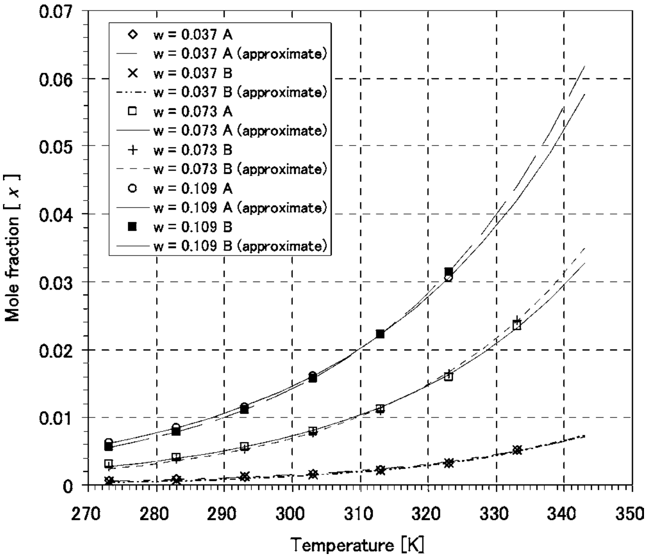

Figure 4. **Measured solubility and correlations of both polymorphs at different water contents. Curves were drawn using eq 3.**

Table 1. **Values of constants in solubility correlation by eq 3**

polymorph	water fraction (w)	A	B	C
Form A	0.037	−122.19	2368.68	18.90
	0.073	−121.07	2457.66	18.93
	0.109	−121.39	2779.67	18.92
Form B	0.037	−125.19	2249.17	19.48
	0.073	−121.57	2263.73	19.12
	0.109	−121.11	2568.75	18.99

seeding, the solution was kept at the same temperature for 1 h; then 589 g of a second solvent, 2-butanone warmed to 323 K, was added, making the solvent composition $w = 0.037$ and the corresponding saturation composition in mole fraction of 0.0035. Thereafter, the solution was stirred for another 30 min at the same temperature and then cooled down to 293 K in about 30 min. After this solution was kept at 293 K for 1 h, the product was taken out, and the polymorphic composition was determined. The obtained product was halved (approximately 70 g each). One half was comminuted using a cutter mill (Wonder Crush/Mill, D3 V-10) to measure the polymorphic composition, whereas the other half was used to measure the particle size distribution (PSD) using a sieve shaker (NITTO ANF-30).

2.5. Measurement of Particle Size Distribution and Preparation of Seeds. The PSD was measured using the sieve shaker. The sieve openings used were 710, 600, 500, 425, 355, 297, 250, 177, 149, 125, 106, 75, and 53 μm. The mass of the crystals (ΔW) on each sieve was measured and divided by the difference ($L_1 - L_2 = \Delta L$) between successive sieve openings (L_1 and L_2). The obtained values of $\Delta W/\Delta L$ were plotted against the arithmetic mean particle size ($(L_1 + L_2)/2$). The particle size at the 0.50 cumulative value was defined as the mean particle size, L_p.

To prepare the seed, both polymorphs were comminuted and then mixed to yield the desired polymorphic composition X_{As}. The comminuted crystal was reanalyzed by PXRD to confirm that the polymorph did not change during the comminute operation. Since the comminuted powders were easily charged with static electricity and they could not be separated by sieving, classification was not carried out. Instead, the PSDs were measured using a dry particle size analyzer (BECKMAN Coulter, LS13 320, Laser Diffraction Particle Size Analyzer).

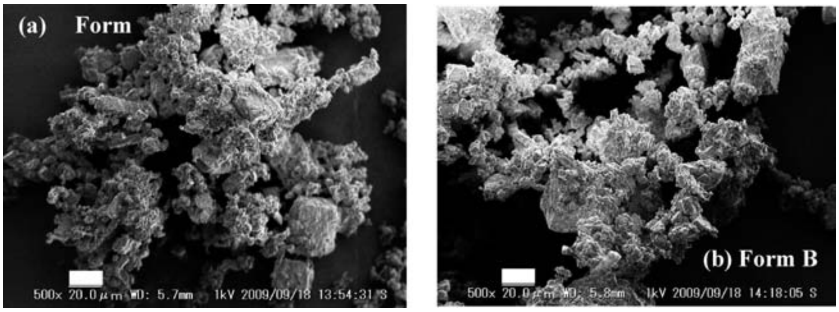

Figure 5. **(a) SEM image of comminuted seed of Form A. (b) SEM image of comminuted seed of Form B. Scale = 20 μm.**

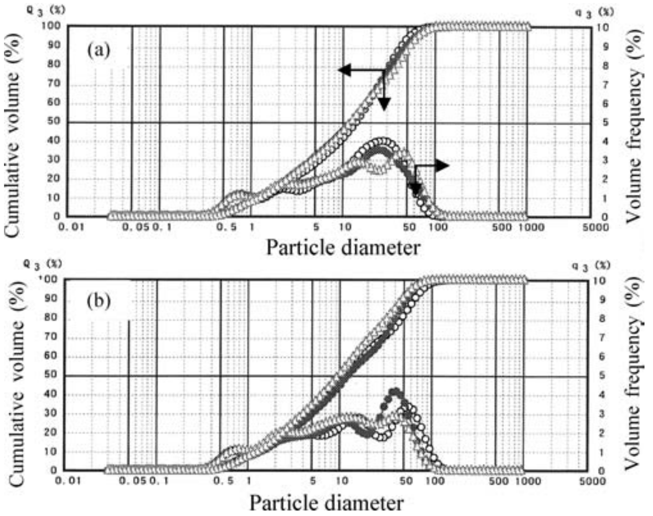

Figure 6. **(a) Particle size distribution of comminuted Form A. (b) Particle size distribution of comminuted Form B.**

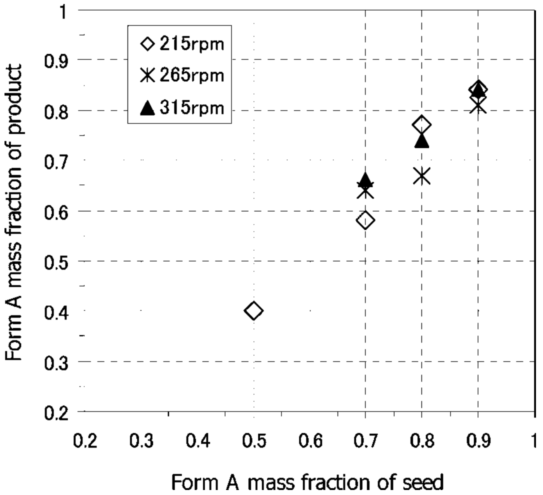

Figure 7. **Polymorphic composition vs seed composition at different agitation speed for comminuted seeds. (T_s = 323 K.)**

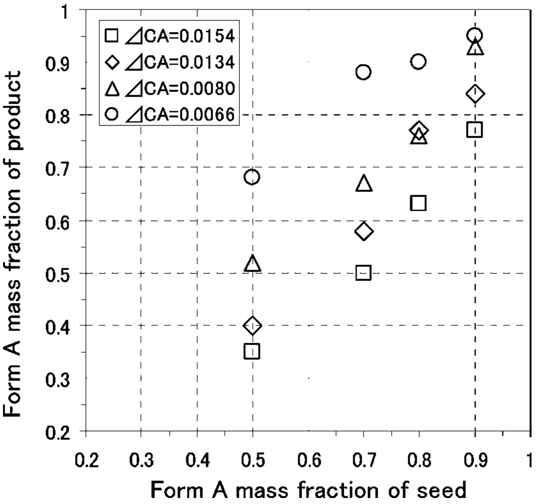

Figure 8. **Polymorphic composition vs seed composition at different initial supersaturation ΔC_A. (Agitation speed = 215 rpm.)**

Table 2. **Initial supersaturation in each operating condition**

initial mole fraction	seeding temp (K)	water mass fraction	$\Delta C_A{}^a$	$\Delta C_B{}^a$	$C_B^* - C_A^{*a}$
0.0298	323	0.063	0.0154	0.0151	0.0003
		0.073	0.0134	0.0131	0.0003
		0.083	0.0080	0.0075	0.0005
	333	0.073	0.0066	0.0056	0.0010

[a] ΔC_A, ΔC_B = initial supersaturation; C_A^*, C_B^* = saturated concentration.

3. Results and Discussion

3.1. Solubility Curves. The solubility in terms of mole fraction x was found to increase with the water content in the mixed solvents (Figure 4), and the solubility difference between the two polymorphs was quite small; in particular, the difference was negligible (<0.0001) in mixtures having low water content (w = 0.037).

The measured solubilities agreed well with the empirical correlation given by eq 3, and the values of the constants were determined by the Marquardt method. These are listed in Table 1 for each polymorph and w. The solubility curves suggest that the MPPO aqueous 2-butanone system exhibits enantiotropic behavior, and the intersection temperatures of the polymorphs obtained from the correlations are 312, 317, and 332 K for w = 0.109, 0.073, and 0.037, respectively.

$$x = \exp\left(A + \frac{B}{T} + C \ln T\right) \tag{3}$$

3.2. Crystallization Behavior of Comminuted Seeds. The comminuted seeds of both polymorphs were finely comminuted as shown in Figure 5, a and b. In addition, the comminuted seeds exhibited very broad distributions ranging from approximately 0.5−100 μm, as shown in Figure 6, a and b, whereas the mean particle sizes were approximately 10 μm for the two polymorphs.

Using the comminuted seeds, the effect of agitation on the product polymorphic composition (X_{Ap}) was first examined for

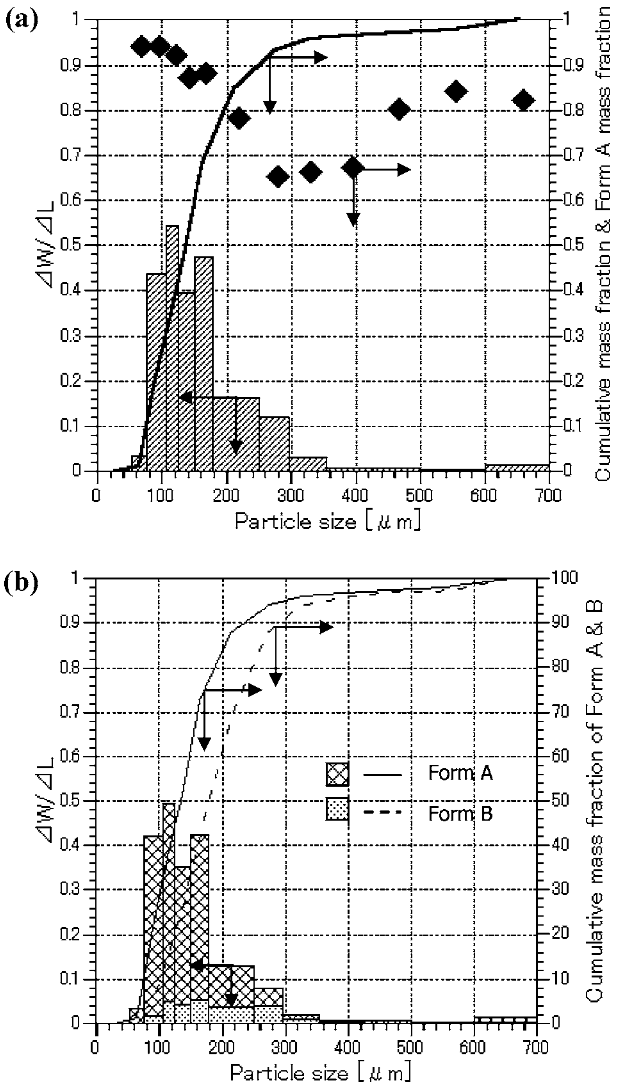

Figure 9. **(a) Particle size distribution of product and polymorphic composition for the case of comminuted seeds. (X_{As} = 0.90, L_s = 10 μm, and T_s = 323 K, 215 rpm.) (b) Calculated particle size distributions of both polymorphs based on particle size distribution in graph a.**

agitation speeds of 215, 265, and 315 rpm. Although agitation speed may be expected to enhance secondary nucleation by crystal breakage, X_{Ap} was not affected at the above-mentioned agitation speeds, as shown in Figure 7. The breakage of particles was not likely to occur because the seed was so small that the relative velocity from the solution was low, and collisions between the particles and the stirrer blades hardly occurred.

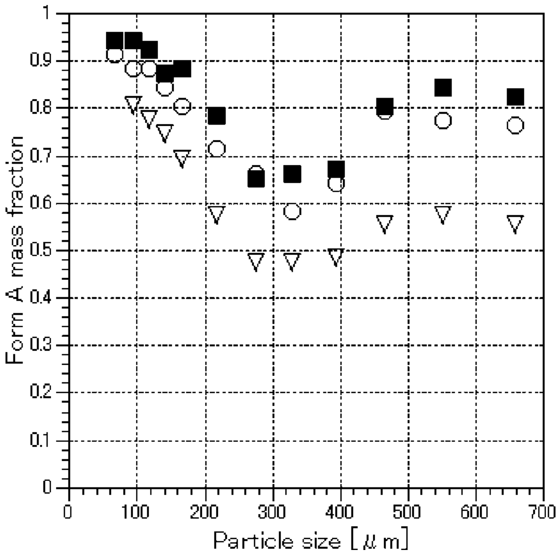

Figure 10. **Size-dependent polymorphic compositions for different seed compositions. Black squares, open circles, and open triangles indicate X_{As} = 0.90, X_{As} = 0.80, and X_{As} = 0.70, respectively.**

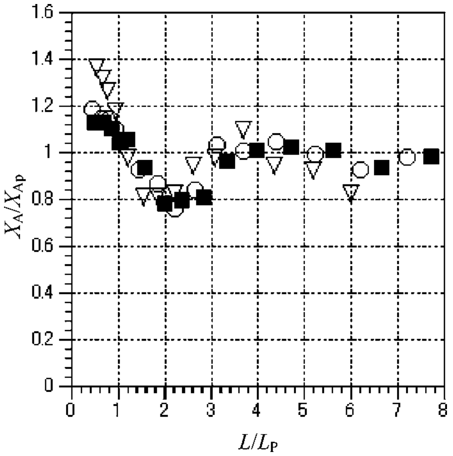

Figure 11. **Relative polymorphic composition distribution.**

The polymorphic compositions of the product obtained under conditions of different solvent composition and seeding temperature are shown in Figure 8. Varying the solvent composition and seeding temperature resulted in different levels of supersaturation at the moment of seeding (initial supersaturation). This indicates that the product composition was determined by the initial supersaturation and seed composition. The values of the initial supersaturation ΔC_A and ΔC_B in units of mole fraction are listed in Table 2. In the examined concentration and temperature ranges, the initial supersaturation was always larger for Form A than for Form B, although the difference was quite small, indicating that Form A is the stable polymorph. For T_s = 323 K, the mean product composition (X_{Ap}) increased with decreasing supersaturation. This tendency may be true at T_s = 333 K, for which the mean composition (X_{Ap}) even exceeded the seed composition (X_{As}). The product composition can thus be controlled by varying the initial supersaturation at the moment of seeding by varying the solvent composition and seeding temperature. It should be noted that at the end of crystallization, the final solution composition lay in a region in which Form B was stable; however, because of negligible concentration differences, its effect on the final product composition was also negligible.

3.3. Particle Size and Polymorphic Composition Distributions. Since the comminuted seed exhibited rather wide size distributions, as shown in Figure 4, and the product also exhibited wide size distributions, the product compositions shown in Figures 5 and 6 are averaged over the size distributions. Therefore, the size distribution as well as the composition distribution of the product was analyzed in detail to clarify the growth behavior of both polymorphs.

The effect of seed compositions was examined by using different seed compositions of X_{As} = 0.90, 0.80, and 0.70. The mean size (L_p) and the mean product composition (X_{Ap}) for these experiments corresponding to X_{As} (0.9, 0.8, and 0.7) were 140 and 0.84 μm, 150 and 0.77 μm, and 180 and 0.59 μm, respectively.

Figure 9(a) shows the PSD of the product for $X_{As} = 0.90$ as well as the distribution of polymorphic compositions as a function of particle size. For this case, the mean composition was $X_{Ap} = 0.84$. The PSDs of the two polymorphs were calculated on the basis of the data shown in Figure 7a, and these are shown in Figure 9b. From the cumulative mass fractions, indicated by the solid and broken curves for Form A and Form B, respectively, the mean size of Form A was $L_p =$ 125 μm and that of Form B was $L_p = 180$ μm, indicating that Form B was larger than Form A.

Figure 10 shows a comparison of the resulting polymorphic composition distributions ($X_{Ap}(L)$) as a function of the particle size for different seed compositions ($X_{As} = 0.90$, 0.80, and 0.70). The composition of Form A was lower as the seed composition was lower, although it was high for sizes smaller than 200 μm; that of Form B was high in the range of 200−400 μm. This shows that Form B grows faster than Form A.[14] The polymorphic composition of Form A was higher for crystals having sizes greater than 400 μm, and such crystals were assumed to be of Form A by the SEM analysis shown in Figure 2a.

In Figure 10, a common pattern can be observed between $X_{Ap}(L_p)$ and the particle mean size L_p. $X_{Ap}(L)/X_{Ap}$ and L/L_p were calculated, and these are plotted in Figure 11, in which the composition distribution for the three runs is also shown. The fact that the three data indicate $X_A(L)/X_{Ap} = 1$ at $L/L_p = 1$ suggests that the mean product composition coincided with the polymorphic composition at the mean particle size. The curves for different seed compositions were correlated by a single curve in which products under different seed compositions exhibited similar distributions.

Figure 11 indicates that Form A is dominant in the region of $L/L_p < 1$, whereas Form B is dominant in the region of $L/L_p = 1-3$. The ratio $X_A(L)/X_{Ap}$ was almost unity in the region of $L/L_p > 3$.

4. Conclusions

To obtain a polymorphic mixture having a desired composition, the crystallization behaviors of MPPO, which has two polymorphs, Form A and Form B, were experimentally examined. Form A and Form B have different morphologies; Form A was rodlike, whereas Form B was platelike and agglomerated. From the measured solubility in three binary solvents of 2-butanone and water at different compositions and temperatures, the solubility difference was found to be quite small.

In batch cooling and drowning-out crystallizations of MPPO, comminuted seeds having a mean size of 10 μm were used. Among the many operating variables that were examined, the critical factors that determined the mean polymorphic composition of Form A, X_{Ap}, were found to be the supersaturation at the moment of seeding and the seed compositions. The initial supersaturation was determined by the solvent composition and seeding temperature. The mean composition increased with the initial supersaturation and the seed composition.

Since the seed exhibited broad size distribution, the size and composition distributions of the product particles were analyzed. The composition was found to vary with the size, and it exhibited a common trend in that Form A was dominant in particles smaller than the mean size whereas Form B was dominant in larger particles. This indicates that Form B crystallized faster than Form A. The composition at the mean size coincided with the mean composition.

In conclusion, the product composition was well controlled by seeding crystallization, and in particular, by the initial supersaturation and seed composition.

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Nomenclature

A = constant in eq 3
B = constant in eq 3
C = constant in eq 3
ΔC_A, ΔC_B = initial supersaturation
C_A*, C_B* = saturated concentration
H_{Ai}, H_{Bi} = characteristic peak heights of Form A and Form B, respectively, in PXRD pattern
I_A = fraction of Form A read obtained PXRD pattern
L = size
T = temperature
W = mass of crystal on sieve
w = water content in solvent in mass fraction
x = solubility in mole fraction
X_A = fraction of Form A
$X_{Ap}(L)$ = product composition as a function of particle size

Superscripts and Subscripts

p = product
s = seed or seeding

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[14] To explain these results, the volume shape factor ϕ_v defined by $\phi_v = W/(n\,\rho_s\,L^3)$ was calculated for both polymorphs. The n is the number of particles of particle size L, and the W is those weights. When the volume shape factors of particle sizes of $L = 163$ μm, 326 μm, and 550 μm and those ratios were calculated, they were $\phi_{v,A/B} = 2.27$, 1.03, and 1.04, respectively. In the mean particle size of 326 μm or more, those ratios are approximately 1, and this indicates that the growth of Form B is faster because the mass of both polymorphs of the same particle size is the same. On the other hand, Form A was abundant in smaller particle size because the mass of Form A was more than that of Form B; however, further works will be required to clarify the solution concentration transition and the behavior of secondary nucleation of both polymorphs.