

CRYSTALLIZATION BEHAVIOR AND TRANSFORMATION KINETICS OF L-HISTIDINE POLYMORPHS

MITSUTAKA KITAMURA

Department of Chemical Engineering, Hiroshima University, 4-1,
Kagamiyama 1 chome, Higashihiroshima 724

Key Words: Crystallization, Polymorph, Transformation, Amino Acid, Histidine, Crystal Growth, Transformation Kinetics

The crystallization and transformation behavior of the polymorphs of *L*-histidine (A,B) and the transformation kinetics were investigated in aqueous solutions. Both polymorphs precipitate in almost the same ratio from solutions in a wide concentration range. The ratio of the polymorphs in the precipitate was scarcely influenced by temperature, unlike the behavior of *L*-glutamic acid. Transformation from B to A with a solution-mediated transformation mechanism occurred. The activation energy for the overall transformation was estimated as about 38 kJ/mol. From measurements of the solubilities at temperatures between 283 and 333 K, it was confirmed that A is a stable and B a metastable form. From a van't Hoff plot the heat of fusion of *L*-histidine polymorphs was obtained as 15 kJ/mol, which is about half that of *L*-glutamic acid. No seed effect of either A or B crystals on the precipitation behavior was observed. A kinetic study of the transformation process was carried out and both the rate constants of growth of A (k_G) and dissolution of B crystals (k_D) were estimated simultaneously. At 313 K k_D was nearly six times larger than k_G , indicating that the transformation process is growth-controlled.

Introduction

Many kinds of amino acids have polymorphs, and in amino acid industries these polymorphs are connected with various problems¹⁻⁵. For example, of the two polymorphs of α and β (both are monoclinic P2₁) of *L*-glutamic acid precipitation of the former is preferred in industry because of the advantage in solid-liquid separation efficiency^{1,2}. In the previous paper⁵ it was shown that the crystallization behavior of *L*-glutamic acid polymorphs predominantly depend on the crystallization temperature in comparison with supersaturation degree and stirring rate, and the transformation mechanism in the crystallization process was also clarified. Furthermore, it was suggested that the characteristic temperature dependence of the crystallization behavior is explainable in relation with the concentrations of the conformers of *L*-glutamic acid, which are present in the solutions.

It is considered that the precipitation behavior of the polymorphs of each amino acid is related to the crystallographic structures of the polymorphs and the molecular formula, i.e. the substituent on the α carbon in amino acids. *L*-histidine, HOOCCH(NH₂)CH₂(C₃N₂H₃), has two polymorphs, orthorhombic (P2₁2₁2₁)⁷ and monoclinic(P2₁)⁸ respectively, which possess a bulky functional group of imidazole and fewer CH₂ groups in the carbon chain than *L*-glutamic acid. In this paper, for the polymorphs of *L*-histidine, crystallization behavior and kinetics of the transformation were investigated and compared with those obtained previously in the *L*-

glutamic acid system.

1. Experimental Procedure

A 50-ml cylindrical jacketed glass vessel equipped with an impeller was used as the crystallizer. The crystallization of *L*-histidine (special grade, Ajinomoto Co.) was carried out by cooling an aqueous solution, which is at the isoelectric point, rapidly to the crystallization temperatures between 283 and 323 K. The stirring rate was 150 rpm.

The two polymorphs of *L*-histidine in orthorhombic (P2₁2₁P₁) and monoclinic (P2₁) structures are denoted as A and B, respectively, in this paper and the crystal data are shown in **Table 1**. The pure each polymorph was prepared by the following methods. A was obtained when the transformation from B to A was completed in aqueous solution as shown in section 2.2, and pure B was crystallized from a mixed solution of water and ethanol (40 volume percent)⁴. The composition of the precipitated crystals was determined from X-ray diffraction measurement (CuK α line) (**Figs.1(1)(A)** and **(2)(B)**) using the characteristic peaks of A ($2\theta = 17.6^\circ$) and B ($2\theta = 17.2^\circ$) shown by arrows in the figures.

The solubility of the polymorphs was measured by a method described elsewhere⁵. The concentration of *L*-histidine in the solution was determined by UV spectrophotometry using the absorbance at 225 nm.

The kinetic study of the transformation process of the polymorphs was also done by adding sieved crystals (210-297 μ m) in 50 ml solution at 313K using the same

* Received January 8, 1993. Correspondence concerning this article should be addressed to M. Kitamura.

Table 1. Crystal data of L-histidine, $C_6H_7N_3O_2$
(M. W. 155. 2)

(A)	(B)
Orthorhombic ⁷⁾	Monoclinic ⁸⁾
$P2_12_12_1$	$P2_1$
$a = 5.177(5) \text{ \AA}$	$a = 5.172(5) \text{ \AA}$
$b = 7.322(7) \text{ \AA}$	$b = 7.384(7) \text{ \AA}$
$c = 18.87(2) \text{ \AA}$	$c = 9.474(1) \text{ \AA}$
$Z = 4$	$\beta = 97.162^\circ$
$V = 637.4 \text{ \AA}^3$	$Z = 2$
$d = 1.428 \text{ g/cm}^3$	$V = 359.0 \text{ \AA}^3$
	$d = 1.446 \text{ g/cm}^3$

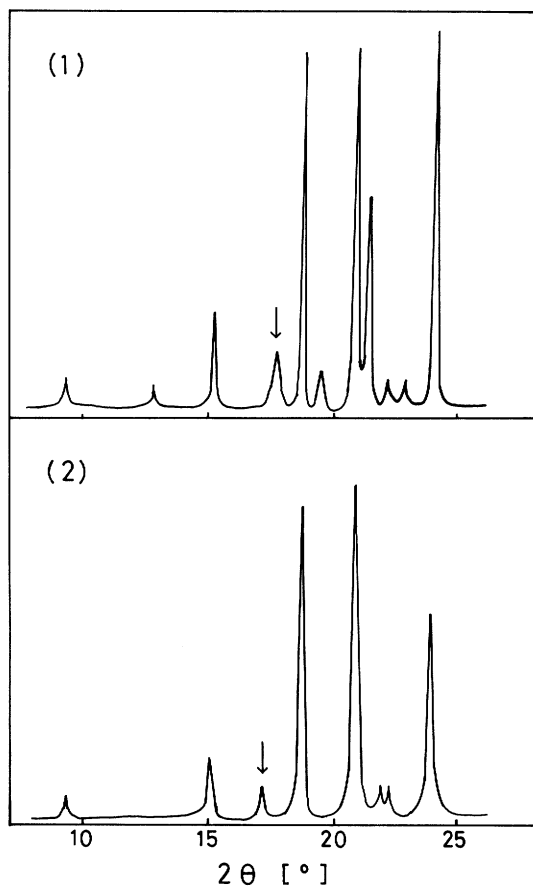


Fig. 1 X-ray powder diffraction patterns of A(1) and B(2) crystals

crystallizer. Time dependencies of the solution concentration and the mass of each polymorph in solutions were measured in the course of the transformation. Details are provided in the following section along with the results.

2. Results and Discussion

2.1 Effect of concentration and temperature on the crystallization behavior

Crystallization was carried out in aqueous solutions at various concentrations of L-histidine (0.35-0.50 mol/l) and the composition of the precipitates at the initial stage of the crystallization was analysed (we call this method "differential crystallization"). In **Fig. 2** the result obtained at 293 K is shown. It was observed that from aqueous

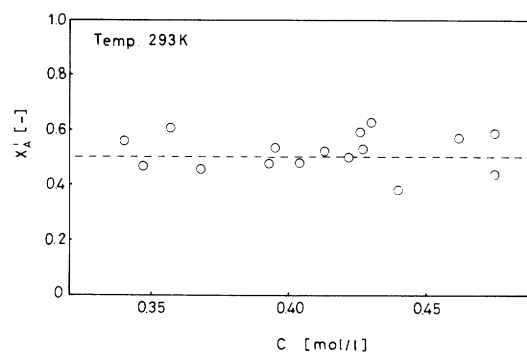


Fig. 2 Relationship between fraction of A in the precipitates at the initial stage of crystallization, X_A^i and concentration in solution

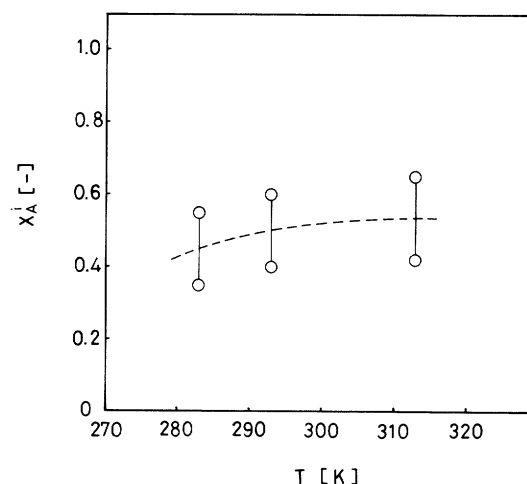


Fig. 3 Dependence of X_A^i on crystallization temperature

solutions in the concentration range examined, both polymorphs precipitate and the fraction of A in the precipitate at the initial stage of the crystallization, X_A^i , was between 0.4 and 0.6. In crystallization at two different temperatures (283 K, 313 K), similar results were obtained, i.e. X_A^i depends little on temperature, although a very slight increase is observed (**Fig. 3**).

Such temperature dependence is very much different from that of L-glutamic acid, in which case the relative amount of α polymorph in the precipitate rapidly decreased with increasing temperature (**Fig. 6** in Ref. 5). In the previous paper⁵⁾ we pointed out that the concentration of L-glutamic acid conformers in solutions may be connected with the temperature dependence of the precipitation behavior. From this point of view, conformational freedom in the structure of L-histidine is considered to be small and it may hardly take various conformers, because L-histidine has the fewer CH_2 groups in a chain, which provide flexibility to the molecule, than does L-glutamic acid. Furthermore, L-histidine has a bulky imidazole ring. Actually the difference in the crystallographic structures^{7,8)} between the polymorphs of L-histidine is much smaller than that of the polymorphs of L-glutamic acid. It is supposed that the potential energy difference between the conformers corresponding to each polymorph is also very small and on

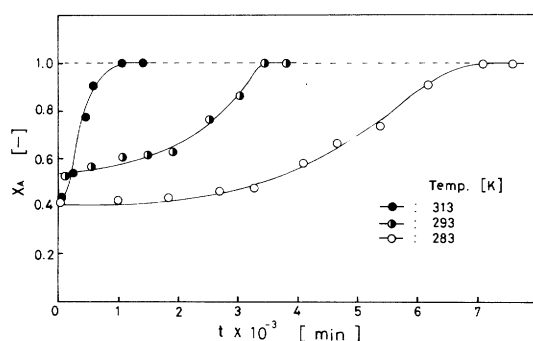


Fig. 4 Change of composition of precipitates, X_A with elapsed time in solutions

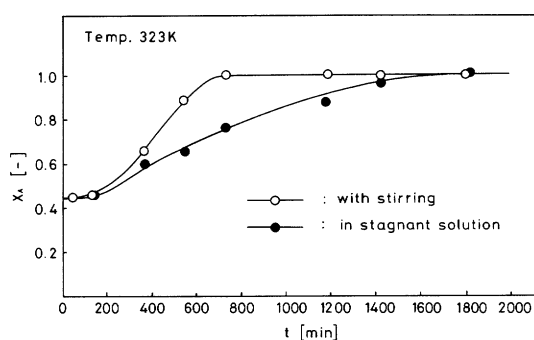


Fig. 5 Effect of stirring on the transformation rate at 323 K

this account both conformers may be present equally in the solution. This could be the reason for the precipitation of the polymorphs with the same probability at each temperature.

2.2 Transformation of the polymorphs

The composition of polymorphs in the precipitates was analysed continuously even after the crystallization was completed. It was observed that the ratio of the polymorphs slowly changes, i.e. the transformation from *B* to *A* occurs with elapsed time in solutions, and pure *A* was obtained after 20 hours at 313 K, after 3 days at 293 K and after 5 days at 283 K (**Fig.4**). Under a microscope the dissolving of *B* and growing of *A* were hardly discriminated, but the transformation rate was clearly retarded at 323 K when stirring was avoided, as shown in **Fig.5**. Furthermore, the transformation did not proceed when the crystals were kept in air at the same temperature. These results indicate that the transformation mechanism is "solution-mediated" in the same manner as in the case of *L*-glutamic acid⁵⁾. However, the transformation rate is much slower in comparison with that of *L*-glutamic acid at the same temperature.

The amounts of precipitated crystals in solutions and the crystal size distributions were nearly the same for runs at different temperatures. Furthermore, the composition of the polymorphs precipitated at each temperature are very similar, as shown previously. Consequently, the reciprocal of the total time (τ_t) needed for the transformation to be completed could correspond to the overall transformation rate and could be proportional to the kinetic constant of the transformation. In **Fig.6** $1/\tau_t$ is

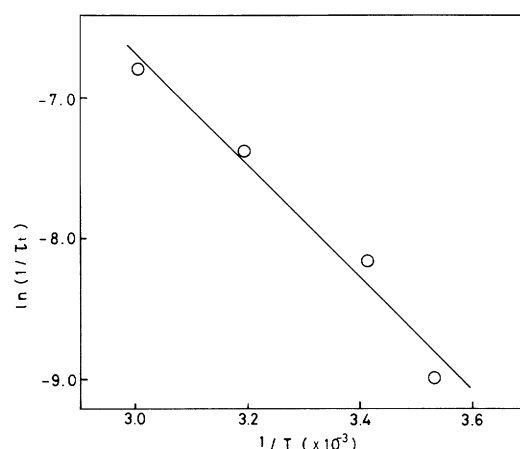


Fig. 6 Relationship between $1/\tau_t$ and $1/T$

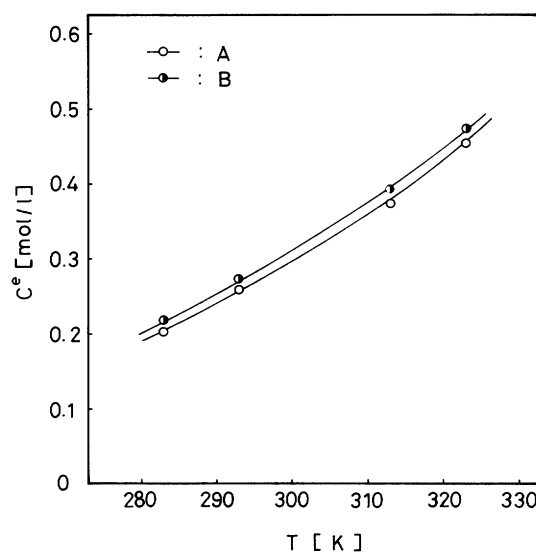


Fig. 7 Solubility curves of the polymorphs of *L*-histidine

plotted against the reciprocal of temperature ($1/T$) and the activation energy was estimated from the inclination of the straight line to be about 38 kJ/mol. This value seems reasonable for the activation energy of the growth process of crystals from solutions⁹⁾ and does not to contradict the solution-mediated mechanism.

2.3 Solubility of the polymorphs

Results of solubility measurement of the polymorphs at several temperatures are shown in **Fig.7**. It can be seen that the solubility of *A* is lower by 4-8% than that of *B* at temperatures between 283 and 333 K, indicating that *A* is stable form. The difference between these solubilities is about one-fifth of that for the polymorphs of *L*-glutamic acid⁵⁾. Since for the solution-mediated transformation the difference in solubilities of the polymorphs is the maximum driving force, the slow transformation rate of *L*-histidine must be caused by the small difference between the solubilities. The heat of fusion of the polymorphs was estimated by a van't Hoff plot from the solubility curves of *L*-histidine and *L*-glutamic acid⁵⁾. The heats of fusion of both polymorphs of *L*-histidine were almost the same (15 kJ/mol) and about half that of *L*-glutamic acid (29 kJ/mol for both

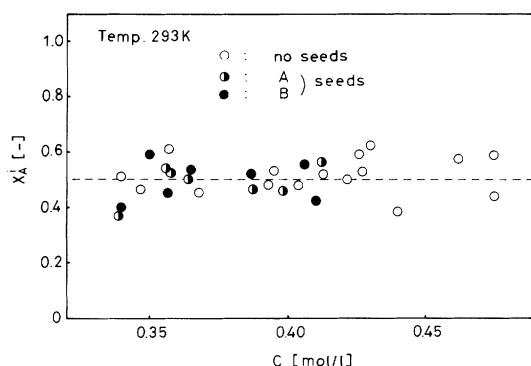


Fig. 8 Effect of polymorphous seed crystals on crystallization behavior

polymorphs). The difference in heats of fusion suggests that the interaction between the molecules in crystal (hydrogen bond is dominated) is greater for *L*-glutamic acid.

2.4 Effect of seed crystals on precipitation behavior

The seed crystals of each polymorph (1mg, 105–149 μm) were added to the solution (50ml) just after the solution temperature attained the set value in the crystallization operation before the primary nucleation occurred, and the composition of the precipitates was examined (Fig. 8). Neither A nor B seeds influenced the precipitation behavior of the polymorphs. On the other hand, seed effects of each polymorph (α and β) were observed in the case of *L*-glutamic acid⁽⁶⁾. It is considered that as the potential energy difference between the conformers corresponding to each polymorph may be very small for *L*-histidine, the relative concentration of the conformers may be little influenced by the seeds.

2.5 Kinetics of the transformation

The solution-mediated transformation process is composed of the growth of the stable form A and the dissolution of the metastable form B. The increasing rate of the mass of A, R_A (mol/(sl)) and decreasing rate of the mass of B, R_B (mol/(sl)) in the transformation process can be written as follows, assuming the power law of the supersaturation degree for R_A and the diffusion-controlled process for R_B ,

$$R_A = k_G z_A n_A^{1/3} M_A^{2/3} \Delta C_A^p \quad (1)$$

$$\Delta C_A = C - C_A^e \quad (2)$$

$$M_A = n_A \rho_A f_A^v L_A^3 \quad (3)$$

$$z_A = f_A^s / (\rho_A f_A^v)^{2/3} \quad (4)$$

$$R_B = -k_D z_B n_B^{1/3} M_B^{2/3} \Delta C_B \quad (5)$$

$$\Delta C_B = C_B^e - C \quad (6)$$

$$M_B = n_B \rho_B f_B^v L_B^3 \quad (7)$$

$$z_B = f_B^s / (\rho_B f_B^v)^{2/3} \quad (8)$$

where k_G and k_D are rate constants of the growth of A and dissolution of B, p is the power constant, f^v and f^s are volume and surface shape factors, n is the number of crystals in one liter solution, ρ is solid density, M is mass of crystals in the unit of mol/l and C_A^e and C_B^e (mol/l) are

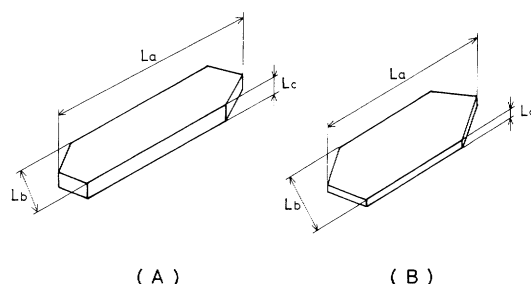


Fig. 9 Crystal shape of A and B crystals

solubilities of A and B at 313K.

The shape of each polymorph is shown in Fig. 9. The shape factors were obtained by measuring each length in three directions (L_a , L_b and L_c) for about a hundred crystals using a microscope with gauge. The volume (v) and surface area (s) of A and B crystals are expressed with the representative length of L_b as follows.

(A crystal)

$$v = f_A^v L_b^3 = 2.78 L_b^3 \quad (9)$$

$$s = f_A^s L_b^2 = 22.47 L_b^2 \quad (10)$$

(B crystal)

$$v = f_B^v L_b^3 = 0.32 L_b^3 \quad (11)$$

$$s = f_B^s L_b^2 = 8.62 L_b^2 \quad (12)$$

A solution of a concentration between the solubilities of A and B was prepared and thermostated at 313K. The mixed crystals of A and B (about 800 mg) ($x_A(0) = 0.5$) were added to the solutions (50 ml) in the crystallizer. Then the composition of the solid ($X_A(t)$) and concentration in the solution ($C(t)$) were measured continually. The mass of each polymorph was calculated by the following equations.

$$M_T(t) = C(0) - C(t) + M_T(0) \quad (13)$$

$$M_A(t) = M_T(t) X_A(t) \quad (14)$$

$$M_B(t) = M_T(t) (1 - X_A(t)) \quad (15)$$

where M_T is total mass, and 0 and t are the values at start and time t .

Typical results are shown in Figs. 10 and 11. It appears that the concentration ($C(t)$) initially increases due to dissolving of B and decreases at the final stage of the transformation (Fig. 10). The values of M_A and M_B were calculated by Eqs. (14) and (15) (Fig. 11), and it can be seen that M_A increases and M_B decreases smoothly during the transformation.

The numbers of crystals, n_A and n_B , were calculated by Eqs. (3) and (7), and were assumed to be constant in the course of the transformation, because the sizes of crystals of both A and B were nearly the same, breakage of the crystals was negligibly small and nucleation of A was scarcely detected. From the results in Fig. 11 the increasing rate $R_A (= dM_A/dt)$ and decreasing rate $R_B (= dM_B/dt)$ were estimated, then the least-squares method was applied to R_A and R_B in Eqs. (1) and (5), and k_G , k_D and p were calculated with a computer. The following results were obtained: $k_G = 3.4 \times 10^{-8}$ m/s, $k_D =$

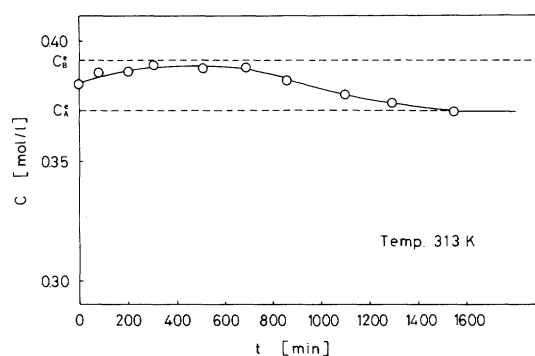


Fig. 10 Concentration change of *L*-histidine in solution during transformation

2.1×10^{-7} m/s, $p=1.0$. It can be seen that k_D is about 6 times k_G , indicating that the transformation process is controlled by the growth rate of A crystals.

Conclusions

The crystallization and transformation behavior of the polymorphs of *L*-histidine (A,B), and the transformation kinetics were investigated in aqueous solutions at the isoelectric point and the following results were obtained.

(1) Both polymorphs precipitate with almost the same ratio from solutions in a wide concentration range. The ratio of the polymorphs in the precipitate was scarcely influenced by temperature, in contrast to the behavior of *L*-glutamic acid.

(2) Transformation from B to A with solution-mediated transformation was observed. The rate is very slow in comparison with *L*-glutamic acid and the activation energy for the overall transformation process was estimated as about 38 kJ/mol.

(3) From measurement of the solubilities at 283-333 K, it was confirmed that A and B are respectively stable and metastable forms. From a van't Hoff plot the heat of fusion of *L*-histidine polymorphs was estimated to be 15 kJ/mol, which is about half that of *L*-glutamic acid.

(4) No seed effect of A and B crystals on the precipitation behavior was observed.

(5) A kinetic study of the transformation process was carried out and both the rate constants of growth of A (k_G) and dissolution of B crystals (k_D) were obtained simultaneously. At 313 K the value of k_D was nearly six times larger than k_G , indicating that the transformation is growth-controlled.

Nomenclature

C	= concentration	[mol/l]
C^*	= solubility	[mol/l]

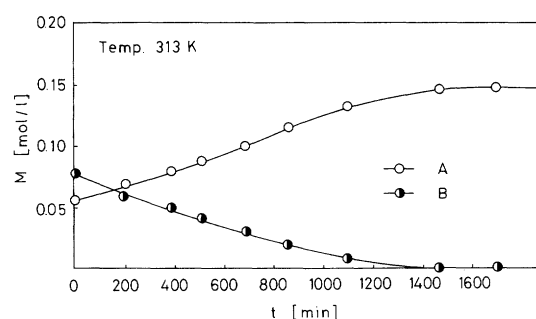


Fig. 11 Relationship between mass of the polymorphs and time during transformation

ΔC	= super- and undersaturation degree	[mol/l]
f^v	= volume shape factor	[-]
f^s	= surface shape factor	[-]
k_G	= rate constant of the growth of A	[m/s]
k_D	= rate constant of the dissolution of B	[m/s]
L_a	= length of crystal in direction a in Fig.9	[m]
L_b	= length of crystal in direction b in Fig.9	[m]
L_c	= length of crystal in direction c in Fig.9	[m]
M	= mass of crystals per a liter solution	[mol/l]
n	= number of crystals in one liter solution	[l ⁻¹]
p	= the power constant of supersaturation degree	[-]
R_A	= increasing rate of the mass of A per liter solution	[mol/(sl)]
R_B	= decreasing rate of the mass of B per liter solution	[mol/(sl)]
s	= surface area of crystal	[m ²]
τ_t	= total time to complete the transformation	[min]
T	= temperature	[K]
v	= volume of crystal	[m ³]
X_A	= fraction of A in crystals	[-]
z	= constants defined in Eqs.(4) and (8)	[m ² /mol ^{2/3}]
ρ	= solid density	[mol/m ³]
2θ	= reflection angle of X-ray	[°]

<Subscripts>

A	= A crystal
B	= B crystal
T	= total mass

<Superscript>

i	= initial stage of the crystallization
-----	--

Literature Cited

- 1) S. Hiramatsu: *Nippon Nogeikagaku Kaishi*, **51**, 27-37 (1977)
- 2) S. Hiramatsu: *Nippon Nogeikagaku Kaishi*, **51**, 39-46 (1977)
- 3) H. Sakai, H. Hosogai, T. Kawakita, K. Onuma and K. Tsukamoto: *J. Crystal Growth*, **116**, 421-426 (1992)
- 4) M. Kitamura: *J. Soc. Powder Technol., Japan*, **29**, 118-123 (1992)
- 5) M. Kitamura: *J. Crystal Growth*, **96**, 541-546 (1989)
- 6) M. Kitamura, to be submitted to *J. Chem. Eng. Japan*
- 7) J.J. Maddin, E.L. McGandy and N.C. Seeman: *Acta Cryst.*, **B28**, 2377-2382 (1972)
- 8) J.J. Maddin, E.L. McGandy, N.C. Seeman, M.M. Harding and A. Hoy: *Acta Cryst.*, **B28**, 2382-2389 (1972)
- 9) J. Garside and J.W. Mullin: *Trans. Instn. Chem. Engrs.*, **46**, T11-T18 (1968)