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The Stability of the Supersaturation State in Optical Resolution by the Preferential Crystallization Procedure

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In the optical resolution process of DL-serine *m*-xylene-4-sulfonate dihydrate by the preferential crystallization procedure, the stability of the supersaturation state of the unseeded isomer was investigated in connection with the cooling conditions. The experiment was initiated with the model solution and was then extended to the actual resolution process. The metastable region of the unseeded isomer on a two-dimensional diagram with the supersaturation degree and cooling time could be divided into the first and the second metastable regions. The limits of the first and the second metastable regions were expressed by a supersaturation degree and by an area on the diagram respectively. Both limiting values were constant, independent of cooling speed and the lowest arrival temperature, if the conditions for the crystallizer, the initial concentration of the DL-form, the amount of the seed crystals, and the degree of stirring were fixed.

The advantages of the optical resolution of DL-amino acids by the preferential crystallization procedure have been well recognized, and many DL-amino acids have been resolved successfully by this procedure.[1–5] Although several requirements must be fulfilled to achieve the resolution by this procedure, it is most important that the racemic modification be crystallized as a racemic mixture and not as a racemic compound. The resolution is carried out in the following manner. The crystals of a desired isomer (for example, L-isomer) are seeded into a supersaturated solution of a racemic modification. Only the L-isomer crystallizes preferentially, and the crystallized L-isomer is separated in an appropriate manner from the mother liquor before the spontaneous crystallization of the unseeded D-isomer. During the preferential crystallization, the same amount of the D-isomer as that of the crystallized L-isomer remains in the liquid phase as a supersaturation state. After a certain time, however, the unseeded D-isomer also begins to crystallize spontaneously because of the limitation of the stability of the supersaturation state. The situation in this process is shown in Fig. 1.

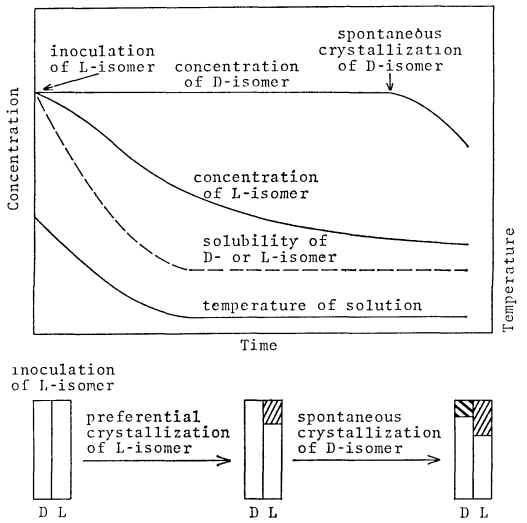

Fig. 1. Time course of optical resolution process by preferential crystallization procedure.

□ : D- or L-Isomer in solution, ▨ : crystals of L-isomer, ▧: crystals of D-isomer.

To avoid the crystallization of the unseeded D-isomer, and to crystallize only the desired L-isomer in an optically pure form, it is also important to know the limitations of the stability of the supersaturation state of the D-isomer, especially the starting time of the spontaneous crystallization of the unseeded D-isomer. Although a few non-quantitative descriptions of the stability of supersaturation in the preferential crystallization process have appeared,[6–8] no quantitative study of the prediction of the waiting time for the spontaneous crystallization of the unseeded isomer has yet been reported.

In our previous report on the optical resolution of DL-serine,[1] DL-serine *m*-xylene-4-sulfonate dihydrate (DL-Ser·mXS·$2H_2O$) was found to form a racemic mixture and to be easily resolved by the preferential crystallization procedure. In the present experiment, the stability of the supersaturation state of the unseeded isomer was investigated in order to predict the waiting time for the spontaneous crystallization of the unseeded isomer in this optical resolution process. Although the stability of the supersaturation state is influenced by various factors, it was examined under a variety of cooling conditions, *i.e.*, cooling speed and the lowest arrival temperature, which are the most important factors in industrial operations. The experiments were initiated with the model solution, assuming a situation of the resolution process, and then extended to the actual resolution process.

Materials and Methods

Materials. Optically active and racemic Ser·mXS·$2H_2O$ were prepared according to the previous report[1] and dried at 35 °C in a chamber with a relative humidity of 67%.

Apparatus and Cooling Methods. A 2*l*-cylindrical glass vessel with a jacket was employed as the crystallizer. Schematic drawings of the crystallizer and their dimensions are shown in Fig. 2. The solution was stirred at 210 rpm with poly(vinyl chloride)-paddle and cooled under the various cooling patterns shown in Fig. 3, which were achieved by using a Chino

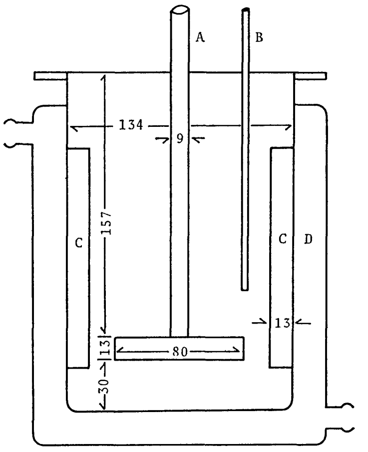

Fig. 2. Schematic drawings of the crystallizer and their dimensions (in millimeter).
A: Poly(vinyl chloride)-agitator, B: glass-thermistor (diameter, 6 mm), C: glass-baffle plate, D: jacket.

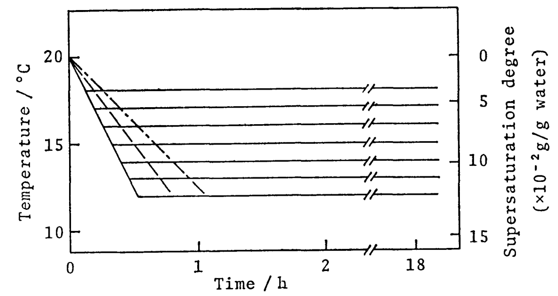

Fig. 3. Cooling patterns and supersaturation degree. The solution is saturated with D-Ser·mXS·$2H_2O$ at 20 °C (C_D=0.360 g/g water). Cooling speed is 1/4 °C per min (——), 1/6 °C per min (— — —), or 1/8 °C per min (------).

program setter, PMS-2, and a Chino electronic recording controller, ET-2p (Chino Works Ltd., Japan). The ranges of experimental variables for cooling were as follows: cooling speed, 1/4, 1/6, and 1/8 °C per min; the lowest arrival temperature, 12, 13, 14, 15, 16, 17, and 18 °C.

Analysis of Solution. The total concentration of D- and L-isomers in the sample solution, C_D+C_L [g/g(water)], was determined from the standard curve and the refractive index which was measured with a Karl Zeiss immersion refractometer, where C_D and C_L are the concentrations of D- and L-isomers [g/g(water)] respectively.

The concentration of the optical isomer (for example, D-isomer) existing in excess, C_D-C_L [g/g(water)], was obtained by the measurements of the rotation angle of the sample solution, its density, and the total concentration of D- and L-isomers according to the following calculation. The specific rotation of the D-isomer existing in excess in the solution is expressed as:

$$[\alpha]^{35}_{D} = 100 \times \frac{\alpha}{c}, \tag{1}$$

where α is the rotation angle and where c is the weight in grams of the D-isomer existing in excess per 100 ml of solution. c is expressed as:

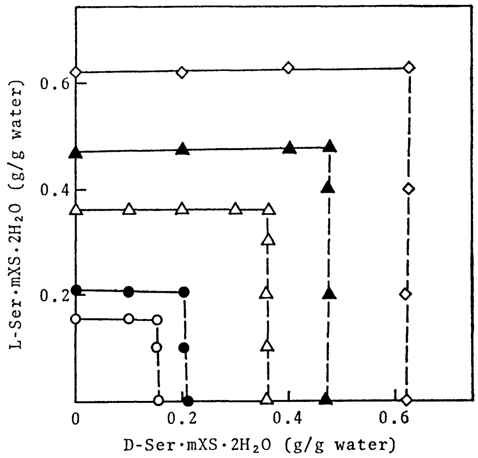

Fig. 4. Solubility of L- and D-Ser·mXS·$2H_2O$ in the coexisting system.
——: Solubility curve for L-isomer, — —: solubility curve for D-isomer, ○: at 5 °C, ●: at 10 °C, △: at 20 °C, ▲: at 25 °C, ◇: at 30 °C.

$$c = 100 \times \frac{C_D - C_L}{(1+C_D+C_L)/d}, \tag{2}$$

where d is the density of the solution.

The value of the specific rotation of D-Ser·mXS·$2H_2O$ was observed to be $[\alpha]^{35}_{D}=+5.787°$ under the conditions employed ($0.5<C_D+C_L<1.0$, $0<C_D-C_L<0.12$ g/g(water)). From Eqs. 1 and 2, C_D-C_L is expressed as:

$$C_D - C_L = \frac{\alpha(1+C_D+C_L)}{5.787\,d}. \tag{3}$$

From Eq. 3, C_D-C_L can be obtained by measuring the values of α, d, and C_D+C_L. The optical rotation was measured in a 1-dm tube at 35 °C with sodium light by means of a Perkin-Elmer 141 automatic polarimeter. The density was determined with a hydrometer.

The concentrations of the D- and L-isomers, C_D and C_L, were calculated from the values of C_D+C_L and C_D-C_L respectively.

Determination of Waiting Time. The waiting time for the spontaneous crystallization of the D-isomer was determined by measuring the concentration of the D-isomer, and the time when the concentration decreased by 0.002 [g/g(water)] was taken as the starting time of the spontaneous crystallization. Also, a sudden spontaneous crystallization could be determined by visual observation.

Results and Discussion

Solubility of L- and D-Ser·mXS·$2H_2O$ in the Coexisting System. Mixtures of L- and D-isomers in various proportions were dissolved in water at an elevated temperature and equilibrated at different temperatures. The concentrations of both the isomers in the equilibrium solution were determined. The equilibrium compositions of the coexisting system are shown in Fig. 4. The figure shows that: (i) the solubility of an optical isomer is little influenced by the concentration of the opposite isomer coexisting in the solu-

Table 1. Composition of model solution

Solution No.	I	II	III	IV
D-Ser·mXS·$2H_2O$ (g)	468	468	468	468
L-Ser·mXS·$2H_2O$ (g)	468	393	351	295
Water (g)	1300	1300	1300	1300
Ratio of L/D	1.00	0.84	0.75	0.63

Each solution was saturated with the D-isomer in the presence of various amounts of the L-isomer at 20 °C.

tion, (ii) the solubility of the racemic modification is nearly equal to the sum of the individual solubilities of both isomers, and (iii) the saturated solution of the racemic modification no longer dissolves an optical isomer. These properties show that DL-Ser·mXS·$2H_2O$ crystallized from water forms a typical racemic mixture and that there is no appreciable interaction between the two isomers on the respective solubilities. Therefore, the supersaturation degree for each optical isomer was expressed as $C_D - C_S$ (for the D-isomer) or $C_L - C_S$ (for the L-isomer) [g/g(water)], regardless of the concentration of the coexisting opposite isomer, where C_S [g/g(water)] is the solubility of the D- or L-isomers at a given temperature. From Fig. 4, the solubilities of optically active and racemic Ser·mXS·$2H_2O$ could be expressed as a function of the temperature, t, by following empirical formulas over a range from $t=5$ to 30 °C respectively:

$$\log C_S = 2.34 \times 10^{-2} t - 9.12 \times 10^{-1},$$

$$\log C_{SR} = 2.48 \times 10^{-2} t - 6.39 \times 10^{-1},$$

where C_{SR} [g/g(water)] is the solubility of DL-Ser·mXS·$2H_2O$.

Stability of Supersaturation in Model Solution. *The First Metastable Region and Its Width, S_I*: Assuming, in the resolution process of DL-Ser·mXS·$2H_2O$, that the L-isomer is preferentially crystallized, while the D-isomer remains in the initial concentration as a supersaturation state, the model solutions were prepared as is shown in Table 1. The mixtures of the D-isomer and the L-isomer in different proportions were dissolved by heating at 35 °C. The solutions were saturated at 20 °C with the D-isomer in the presence of various amount of the L-isomer. The solutions were cooled at various speeds (1/4, 1/6, and 1/8 °C per min) to given temperatures ranging from 12 °C to 18 °C, as is shown in Fig. 3, under a constant stirring speed; 0.2 g of D-Ser·mXS·$2H_2O$ (28—35 meshes) was added at 20 °C in the course of cooling. The addition of the crystals was preferable for the reproducibility of the waiting time. No significant decrease in the concentration of the D-isomer attributable to the growth of the added crystals was observed until the spontaneous crystallization of the supersaturated D-isomer took place. In other words, the first significant decrease in the concentration was mainly attributed to the spontaneous crystallization of the D-isomer. Under the above cooling conditions, the temperature of the solution was recorded and the waiting time for the D-isomer was determined. The range in the waiting times observed in these experiments was from 30 min to about 15 h. One example of the time course for model solution I is shown in Fig. 5, indicating that the supersaturation degree of the D-isomer, $(C_D - C_S)$, increases with a decrease in the temperature and that a large spontaneous crystallization of the D-isomer begins suddenly at the point N (time θn, 50 min). This point, N, varied with the cooling speed and with the lowest arrival temperature. That is to say, it varied not only with the cooling speed, but also with the maximum supersaturation degree, $(C_D - C_S)_{max}$, depending on the lowest arrival temperature.

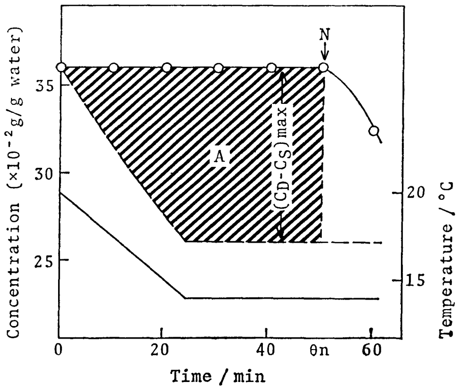

Fig. 5. Time course of supersaturation degree of D-isomer in model solution. The model solution I described in Table 1 was cooled at 1/4 °C per min to 14 °C and maintained at 14 °C
——: Temperature of solution, — — —: solubility of D-isomer (C_S) at the temperature of solution, —O—O—: concentration of D-isomer (C_D), N: the point occurring a large spontaneous crystallization, A: the area given by $A=\int_0^{\theta n}(C_D - C_S)d\theta$.

To obtain the relationship between the waiting time, θn, and the cooling conditions described above, a certain area, A, expressed as a shaded part in Fig. 5 was considered. The area may be expressed mathematically as follows:

$$A = \int_0^{\theta n} (C_D - C_S) d\theta \quad [\text{g}\cdot\text{min/g(water)}], \qquad (4)$$

where θ is the time and where θn is the time when spontaneous crystallization is observed at point N. In this study, the A area was graphically evaluated.

For each model solution shown in Table 1, the evaluation of A was carried out under various cooling conditions. The logarithm of A was plotted against $(C_D - C_S)_{max}$ on semilogarithmic graph paper. The results obtained when the cooling speed was 1/4 °C per min are shown in Fig. 6. In the case of any model solution, the log A decreased linearly with $(C_D - C_S)_{max}$, but the slope of the straight line changed at the point of $(C_D - C_S)_{max} = 6.6 \times 10^{-2}$ [g/g(water)], expressed as S_I in Fig. 6. Furthermore, the figure shows that the value of A is markedly large when $(C_D - C_S)_{max}$ is lower than S_I. In this range, the spontaneous crystallization of the D-isomer did not occur for a longer time, and the supersaturation state was more stable than in the other range. Thus, the region where the supersaturation degree is within S_I was termed "the first metastable region".

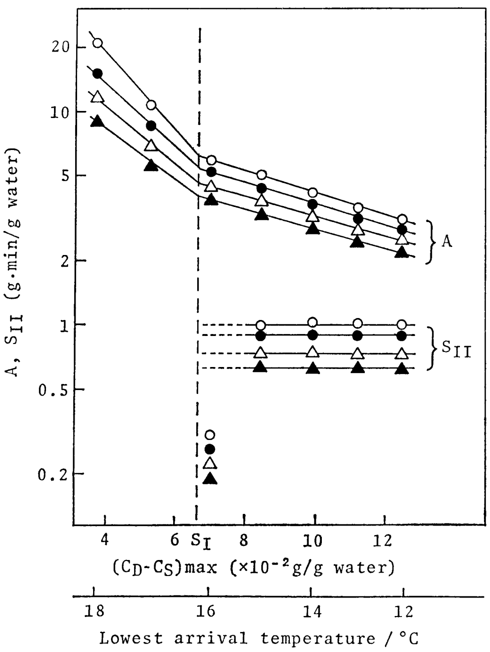

Fig. 6. Relationship among A, S_I, S_{II}, and $(C_D-C_S)_{max}$ in model solution. The solutions were cooled at 1/4 °C per min to given temperatures ranged from 12 °C to 18 °C.

$A=\int_0^{\theta n}(C_D-C_S)d\theta$, $S_{II}=\int_0^{\theta n}(C_D-C_S-S_I)d\theta$.

○: Model solution I ($C_L/C_D=1.00$), ●: model solution II ($C_L/C_D=0.84$), △: model solution III ($C_L/C_D=0.75$), ▲: model solution IV ($C_L/C_D=0.63$).

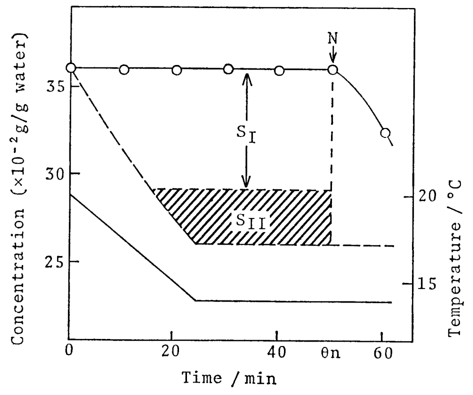

Fig. 7. Illustration of the first and the second metastable regions. The experiment was the same as that in Fig. 5. S_I represents the width of the first metastable region. S_{II} represents the area of the second metastable region.

The Second Metastable Region and Its Area, S_{II}: In the above experiments, the supersaturation was relatively unstable in the region where the supersaturation degree was higher than S_I. Then, an area, S_{II}, expressed as a shaded part in Fig. 7, was considered. This may be expressed mathematically as follows:

$$S_{II}=\int_0^{\theta n}(C_D-C_S-S_I)d\theta \quad [\text{g·min/g(water)}], \tag{5}$$

where the integration is restricted to cases of $C_D-C_S \geqq S_I$.

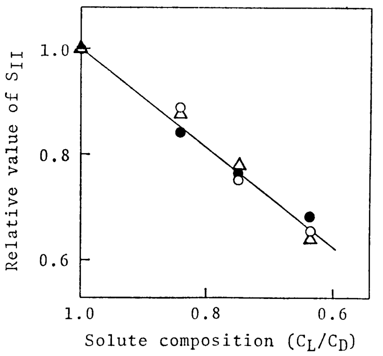

Fig. 8. Relationship between the value of S_{II} and C_L/C_D.
Cooling speed: ○; 1/4 °C/min, ●; 1/6 °C/min, △; 1/8 °C/min.

In this paper, the value of S_{II} was graphically evaluated. The logarithm of S_{II} is plotted against $(C_D-C_S)_{max}$ in Fig. 6. The figure indicates that the values of S_{II} for the solution with a constant ratio of C_L/C_D are constant independent of the maximum supersaturation degree, with the exception of the case when $(C_D-C_S)_{max}$ closely approaches S_I. However, these values decreased with a decrease in the ratio of C_L/C_D. The relationship between S_{II} and the ratio of C_L/C_D is shown in Fig. 8.

Also, in case where the cooling speed was 1/6 or 1/8 °C per min, the values of S_I and S_{II} themselves were the same as those at the cooling speed of 1/4 °C per min, as is shown in Fig. 8. Generally, it may be concluded that the values of S_I and S_{II} are constant, independent of the cooling speed and the lowest arrival temperature. Thus, the region shown as a shaded part in Fig. 7 was termed "the second metastable region," and its area was expressed as S_{II}.

Ting and McCabe[9] and also Tanimoto *et al.*[10] found that two points existed in the supersaturation region for magnesium sulfate or copper sulfate. One was the first supersaturation point, where new nuclei was first observed, while the other was the second supersaturation point, where a sudden increase in the rate of formation of new crystals was observed. The conception of the first and second metastable regions in the present paper seems to correspond to the conception proposed by them. In fact, at a supersaturation degree lower than S_I, the spontaneous crystallization did not occur for a long time. In the second metastable region, where the integral in Eq. 5 is within S_{II}, the spontaneous crystallization was rarely observed for a period sufficient to achieve the optical resolution by the preferential crystallization procedure. In the supersaturation region where the integral in Eq. 5 is beyond S_{II}, a large amount of crystals appeared suddenly and grew rapidly.

Stability of Supersaturation in Actual Resolution Process. A Correction of the S_{II} *Value by the Ratio of the* L*-Isomer to the* D*-Isomer*: The above experiments with the model solutions were carried out when the initial ratios of C_L/C_D were different, as is shown in Table 1, but the ratio in each individual solution was kept constant during the experiment. Under such conditions, the

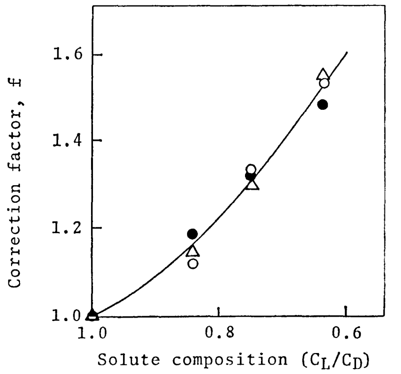

Fig. 9. Relationship between correction factor f and C_L/C_D.
Cooling speed: ○; 1/4 °C/min, ●; 1/6 °C/min, △; 1/8 °C/min.

value of S_{II} was found to be constant, independent of the cooling speed and the lowest arrival temperature. However, it decreased with a decrease in the initial ratio of C_L/C_D, as is shown in Fig. 8. On the other hand, in the actual resolution process, the ratio of C_L/C_D is the solution decreases gradually with the progress of the preferential crystallization of the L-isomer. Therefore, in order to estimate the value of S_{II} in the actual resolution process, the S_{II} shift resulting from the change in the ratio of C_L/C_D should be compensated for. From the data of Fig. 8 showing the relationship between S_{II} and C_L/C_D, the correction factor, f, was determined so that the values of $f_i \cdot S_{II,i}$ became constant:

$$f_i \cdot S_{II,i} = S_{II,1},$$

where f_i and $S_{II,i}$ are the values of f and S_{II} respectively when the ratio of C_L/C_D is i, and where $S_{II,1}$ is the value of S_{II} when the ratio of C_L/C_D is 1.00. The relationship between the correction factor, f, and the ratio of C_L/C_D is shown in Fig. 9. In order to extend the rule obtained on the model solutions to the actual resolution process, in which the ratio of C_L/C_D changes from moment to moment, the evaluation of the S_{II} value in the actual resolution process should be carried out by using the correction factor, f, and the S_{II} value in the actual resolution process should be expressed by the following equation:

$$S_{II} = \int_0^{\theta n} f(C_D - C_S - S_I)\mathrm{d}\theta \quad [\mathrm{g \cdot min/g(water)}], \qquad (6)$$

where the integration is restricted to cases of $C_D - C_S \geqq S_I$ and where f is a function of the ratio of C_L/C_D, which changes from moment to moment with the progress of the preferential crystallization of the L-isomer. In this paper, the S_{II} value may be graphically evaluated from the sum of the individual $f_i \cdot (C_D - C_S - S_I) \cdot \Delta\theta$ values between zero and θn by using the f_i shown in Fig. 9.

Supersolubility in Actual Resolution Process: According to the above manner, the values of S_{II} were determined in the actual resolution process, in which the L-isomer was preferentially crystallized from the supersaturated solution of DL-Ser·mXS·$2H_2O$ and the D-isomer remained in a supersaturated state.

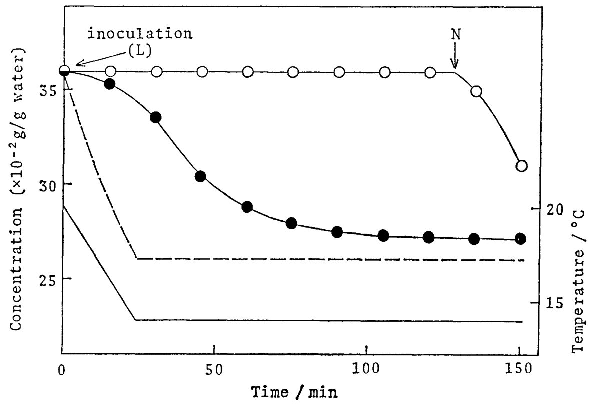

Fig.10. An example of time course of actual resolution process. The solution consisting of 936 g of DL-Ser·mXS·$2H_2O$ and 1300 g of water was seeded with 2 g of L-Ser·mXS·$2H_2O$ (100—200 meshes) at 20 °C, and was cooled at 1/4 °C per min to 14 °C. ——: Temperature of solution, — — — —: solubility of D-isomer at the temperature of solution, —●—●—: concentration of L-isomer, —○—○—: concentration of D-isomer, N: the point occurring a large spontaneous crystallization.

A mixture of DL-Ser·mXS·$2H_2O$ (936 g) and water (1300 g) was dissolved by heating it at 35 °C for 1 h. The saturation temperature of each isomer in the solution was 20 °C. The solution was then seeded with 2.0 g of L-Ser·mXS·$2H_2O$ (100—200 meshes) at 20 °C and subsequently cooled at the cooling speed of 1/4 °C per min to a given temperature at a constant stirring speed. During the preferential crystallization of the L-isomer, the temperature was recorded and the concentration of each individual isomer was measured. In this case, a good reproducibility of the waiting time was obtained, though the crystals of the D-isomer were absent, because the crystals of the L-isomer were always present during the preferential crystallization of the L-isomer. The range of the waiting times observed in these experiments was from 30 min to about 15 h. One example of the time course of an actual resolution process is shown in Fig. 10. In the same manner as was described in connection with the model solution, the A area in Eq. 4 was graphically determined. The logarithm of A obtained under various cooling conditions was then plotted against the maximum supersaturation degree, $(C_D - C_S)_{max}$, on semilogarithmic graph paper. The results are shown in Fig. 11. The log A changed linearly with $(C_D - C_S)_{max}$, but the slope of the straight line was changed at the point of 6.6×10^{-2} [g/g(water)], which is shown as S_I in the figure. This result was similar to that obtained for the model solution.

In the region where the supersaturation degree was higher than 6.6×10^{-2} [g/g(water)], the value of S_{II} expressed by Eq. 6 was graphically evaluated in individual experiments. The values were then plotted against $(C_D - C_S)_{max}$ in Fig. 11, much as in the case of the model solution. The figure shows that the S_{II} values thus obtained were constant 4.49 [g·min/g (water)], independent of $(C_D - C_S)_{max}$, with the exception of the case where $(C_D - C_S)_{max}$ closely approached

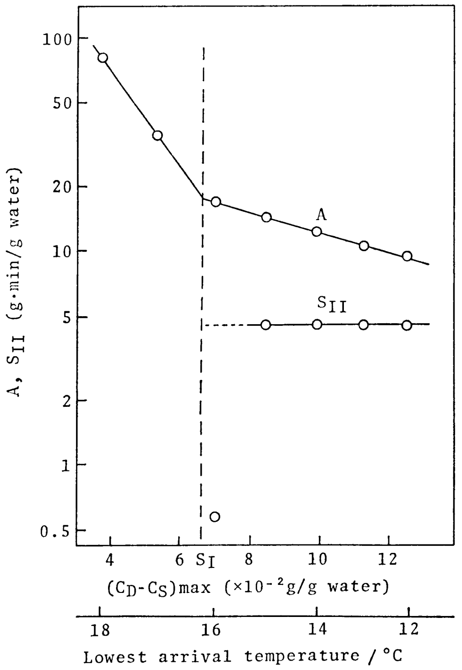

Fig. 11. Relationship among A, S_{I}, S_{II}, and $(C_{\text{D}}-C_{\text{S}})_{\text{max}}$ in actual resolution process.

$$A=\int_0^{\theta n}(C_{\text{D}}-C_{\text{S}})d\theta,\quad S_{\text{II}}=\int_0^{\theta n}f(C_{\text{D}}-C_{\text{S}}-S_{\text{I}})\text{d}\theta.$$

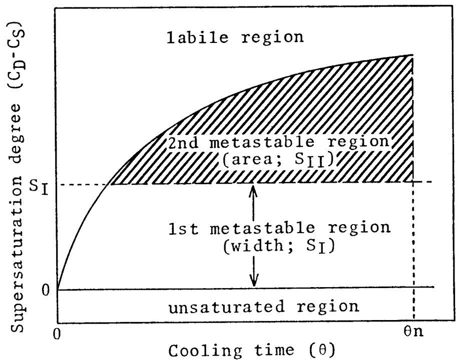

Fig.12. Time course of supersaturation degree of D-isomer and relationship between the first and the second metastable regions.

——: Increase in supersaturation degree of D-isomer due to cooling, θn: starting time of spontaneous crystallization of D-isomer. S_{I} and S_{II} are constant, independent of cooling speed and the lowest arrival temperature.

S_{I}. However, the value of S_{II} in the actual resolution process, 4.49 [g·min/g(water)], was different from that of the model solution, 1.00 [g·min/g(water)]. This discrepancy in the S_{II} value for the D-isomer is considered to arise from the following difference. In the case of the model solution, a small amount of crystals of the D-isomer was added to obtain the reproducibility. On the other hand, in the actual resolution process, the crystals of the D-isomer were absent, though the crystals of the L-isomer were present.

Consequently, the conception for the supersaturation state of the model solution was found to be applicable to the actual resolution process.

The results obtained in the actual resolution process of DL-Ser·mXS·$2H_2O$ can be summarized as follows; they are illustrated in Fig. 12.

(1) The metastable region on a two-dimensional diagram with the supersaturation degree of the unseeded D-isomer and cooling time could be divided in two regions, the first metastable region and the second metastable region.

(2) The first metastable region was the one where the supersaturation degree was lower than a constant value, $S_{\text{I}}=6.6\times10^{-2}$ [g/g(water)]. In this region, no spontaneous crystallization was observed for about 4 h or more.

(3) The second metastable region was the one where the supersaturation was stable for a certain time (within 4 h). This range was limited by the constant value of the integral in Eq. 6, $S_{\text{II}}=4.49$ [g·min/g(water)]. In other words, no spontaneous crystallization took place until the integrated area of the supersaturation degree as a function of the cooling time reached 4.49 [g·min/g(water)].

(4) The limiting values of both the first metastable region and the second metastable region were constant, independent of the cooling speed and the lowest arrival temperature.

Although the present work was carried out under fixed conditions with respect to type of crystallizer, the initial concentration of DL-form, the amount of seed crystals, and the degree of stirring, the metastable region of the unseeded isomer existing as a supersaturation state in the actual resolution process of DL-Ser·mXS·$2H_2O$ could be quantitatively defined. Then, the preferential crystallization should be achieved in the second metastable region. If the increase in supersaturation degree due to cooling the solution and the change in the ratio of the L- and D-isomers due to the preferential crystallization of L-isomer can be predicted, it is also possible to predict the waiting time for the spontaneous crystallization of the unseeded D-isomer. Our study of the prediction will be reported next.

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