Repeated Cooling Crystallization for Production of Microcrystals with a Narrow Size Distribution

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The batch cooling crystallization of an organic compound, *p*-acetanisidide, was carried out using methanol as a solvent and no seed crystal. The crystallization needed an induction period of 10–200 min before nucleation, and the size distribution of the product crystals was broad with sizes ranging from 10 to 270 μm determined as the diameter of a circle with equal projection area. In order to control the nucleation process and obtain microcrystals with a narrow size distribution, the dissolution of crystals obtained by cooling crystallization, followed by recrystallization was adopted. Thus, the crystals once obtained by cooling crystallization were completely dissolved by heating the slurry to a temperature 17°C that was higher than the saturation temperature by 1.5°C. After the solution was maintained at that temperature for a given time, it was cooled again. In the case of repeated crystallization, the induction period observed in the first crystallization disappeared, and small crystals with a mean diameter of 40 μm and a narrow size distribution were obtained. However, the effect of the complete dissolution of crystals on the production of microcrystals with a narrow size distribution disappeared when the solution was incubated at 17°C over 90 min. These results were explained on the basis of the history of the solution structure.

Introduction

Many characteristics of crystals, e.g., polymorphs and shape, should be controlled in industrial crystallization. The crystal size and crystal size distribution (CSD) are also important characteristics. A bimodal or broad CSD may cause severe problems in filtration, drying, filling, dissolution, etc. Often, the production of fine crystals is required, in particular, in pharmaceuticals. In such cases, we must simultaneously satisfy two requirements, namely, unimodal and narrow CSD and production of fine crystals.

Extensive efforts have been made to obtain a unimodal and narrow CSD. One of the earliest studies on the control of CSD in batch cooling crystallization was performed by Griffiths (Griffiths, 1925). Griffiths proposed maintaining the supersaturated solution in the meta-stable zone where only the growth of seed crystals could be expected without nucleation. In order to realize Griffiths' strategy, a programmed cooling operation (Mullin and Nývlt, 1971) and a controlled seeding operation (Chivate *et al.*, 1979) have been developed. However, the controlled cooling technology is not always successful in producing crystals with a unimodal CSD (Moore, 1994). Another way to obtain crystals with a unimodal CSD is to dissolve fine crystals, for example, by using a batch cooling crystallizer with an outside-circulation type of fine crystal dissolver or by using a novel batch crystallizer named the WWDJ batch crystallizer (Jones and Chianese, 1987; Ooshima *et al.*, 2002; Shan *et al.*, 2002). However, unfortunately, these methods are not suitable for producing fine crystals.

The fundamental strategy for obtaining a narrow CSD and fine crystals is to control the timing of nucleation. However, it is difficult to control the timing of nucleation without using seed crystals. In the present study, we attempted to control the solution structure. In other words, we attempted to control the state of molecular association in a solution. We propose the repeated cooling crystallization process that involves the repeated complete dissolution of crystals obtained by the cooling crystallization, which is again followed by crystallization.

1. Experiments

1.1 Materials

Reagent-grade *p*-acetanisidide (PAC) was purchased from Tokyo Kasei Kogyo Co., Ltd. and it was used without further purification. The chemical structure of PAC is shown in **Figure 1**. NMR shows that the chemical environment of the five hydrogen atoms labeled from 'A' to 'E' are different. Methanol-d4 (deuterium) containing 0.05% (v/v) tetramethylsilane (TMS) was purchased from Cambridge Isotope Laboratories, Inc. Unless otherwise noted, methanol herein means methanol-h4. Methanol with 99.8% purity was purchased from Wako Pure Chemical Industries, Ltd.

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1.2 Apparatus for crystallization

The apparatus for the repeated batch crystallization was designed, and it is shown in **Figure 2**. A crystallization solution in a screw-capped glass cell is heated or cooled by two pieces of Peltier element controlled by a computer. The solution temperature is detected by a thermocouple and the formation or dissolution of crystals is optically detected using a photodiode system using a laser (650 nm) by recording the scattered light (not the transmitted light). The photodiode system can be replaced with a digital microscope system for the visual analysis of the formation and dissolution of crystals. The solution was agitated with a magnetic stirrer.

1.3 Cooling crystallization

PAC of 475 mg was dissolved in 5 mL methanol. Then, the solution was incubated at 45°C for 1 h and filtered using a membrane filter of 0.45 µm to remove dust particles. The saturation temperature of the solution is 15.5°C. 4 mL of the solution was placed in a glass cell with a magnetic stirring bar and was pre-incubated at 40°C for 12 h to ensure a constant initial condition of the solution even in all experiments. Then, the cooling was started at a constant cooling rate. The changes in the temperature and light intensity were recorded as shown in **Figure 3**. In the first cooling crystallization process, the cooling was stopped at 17°C, the solution was incubated at that temperature for 30 min with stirring, and then, cooling was re-started. When the solution temperature reached 5°C, the temperature was maintained for 30 min after crystals first appeared, and then, it was increased to 17°C. The slurry was incubated at that temperature for a given time varying from 5 to 240 min to completely dissolve the crystals obtained. This process of cooling crystallization that is followed by the dissolution was carried out five times. These processes were automatically carried out. The crystals were recovered after cooling crystallization performed for the fifth, and compared with crystals obtained by one-time cooling (namely, the conventional cooling crystallization).

1.4 NMR measurements

PAC (75.92 mg) was dissolved in 800 µL methanol-d4 containing 10% methanol. The solution was incubated at 45°C for 30 min. 600 µl of the solution was placed in a 5 mm NMR tube after filtration using a membrane filter of 0.45 µm. The solution was degassed by repeating the freezing-vacuum method four times, and then, the NMR tube was flame-sealed.

The crystallization process as shown in Figure 3 was replicated for PAC solution in an NMR tube that was prepared as described above. First, to realize the same initial condition of the solution in all experiments, the flame-sealed sample was incubated at 45°C for 24 h and cooled to 5°C. After crystals appeared at 5°C, the slurry was heated to 17°C. The total time for the increase in temperature from 5 to 17°C and for the complete dissolution of crystals was 6 min. As a reference sample, a solution from which no crystal appeared due to the short incubation at 5°C was prepared. Then,

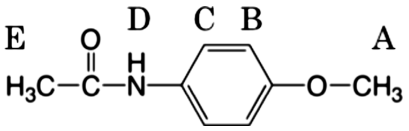

Fig. 1 Chemical structure of *p*-acetanisidide; hydrogen atoms are labeled from A to E

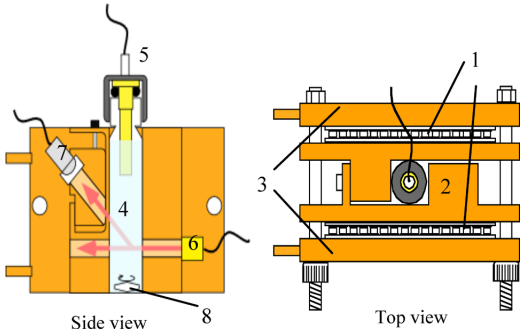

Fig. 2 Diagram of the crystallizer. 1, Peltier element; 2, Copper blocks; 3, Cooling blocks; 4, Glass cell; 5, Thermocouple, 6, Laser diode; 7, Laser detector; 8, Magnetic stirrer

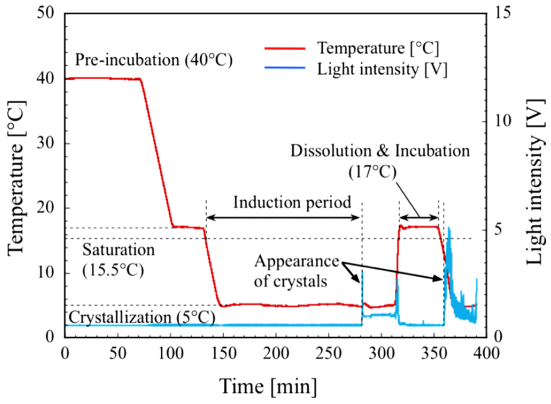

Fig. 3 Example of the changes in the solution temperature and the light intensity during the repeated cooling crystallization

each solution in the NMR tube was set into, an NMR spectrometer (Varian Unity 500) using the standard software, pre-cooled at 17°C, and the NMR measurement was immediately started.

The spin-lattice relaxation time T_1 was determined using the standard inversion recovery sequence, *p*-*t*-*p*/2. Nine variable time delays of t were used per sample. The T_1 values were calculated by a method of nonlinear least-squares fit to the exponential magnetization recovery,

$$M_z = M_0\{1 - \exp(t / T_1)\}$$

where M_z is the magnetization vector at time t, and M_0 is the initial magnetization vector. An integration of 8 times was used in the T_1 measurements to obtain a minimum determination time of 280 s (4.67 min).

1.5 Crystal size distribution (CSD)

Images of crystals that were recovered after repeated

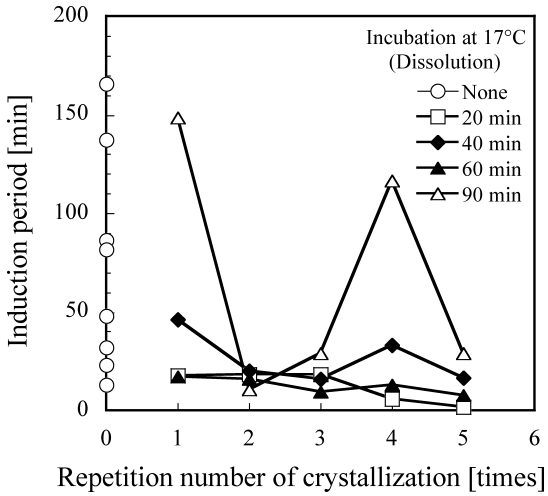

Fig. 4 Induction period in the repeated cooling crystallization

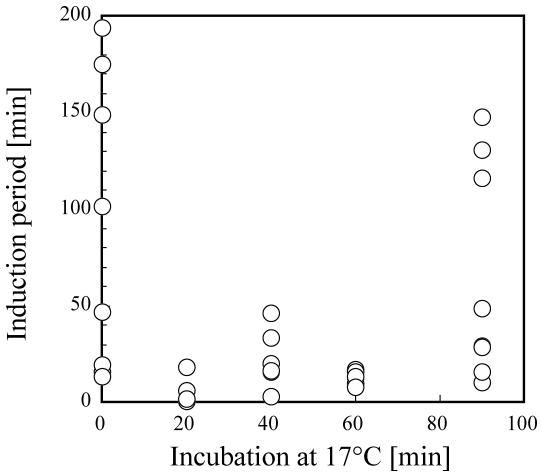

Fig. 5 Effects of incubation at 17°C in the repeated crystallization on the induction period before nucleation

cooling crystallization were taken with a scanning electron microscope (Hitachi High-Technologies Corp.) to determine CSD. The sample crystals were coated with an osmium plasma coater (Filgen, Inc.), and the crystal size was determined as the diameter of a circle with an equal projection area.

2. Results and Discussion

2.1 Ascertainment of complete dissolution of crystals in repeated batch crystallization

In the repeated crystallization, the crystals once obtained must be completely dissolved before the next cooling. First, we ascertained the dissolution of crystals at 17°C, which is higher than the saturation temperature by 1.5°C. The complete dissolution was ascertained by the fact that the diffuse reflection of laser due to fine particles disappeared in the solution at 17°C. A digital microscope was used to observe the crystal slurry in the repeated cooling crystallization. The average time required for the complete dissolution of crystals obtained by first cooling crystallization in experiments in which crystallization is performed five times was 12.8± 2.0 min. This dissolution time includes the 3.2 min that is required for the change from 5 to 17°C. On the basis of this result, we set the incubation time at 17°C to over 15 min.

2.2 Induction period before nucleation and effect of repeated cooling crystallization on induction period

When PAC was crystallized without repetition, a long induction period before nucleation was observed. The length of the induction period was poorly reproducible, and it varied in the range of 10 to 170 min, as shown in **Figure 4** (at repetition number of crystallization, =0). When the crystallization was repeated after the incubation at 17°C for 20 to 60 min for the complete dissolution of the obtained crystals and aging of the solution, the induction period was shortened and became uniform at around 15 min (repetition number of crystallization, =1 to 5). However, when the incubation at 17°C was carried out for 90 min, the induction

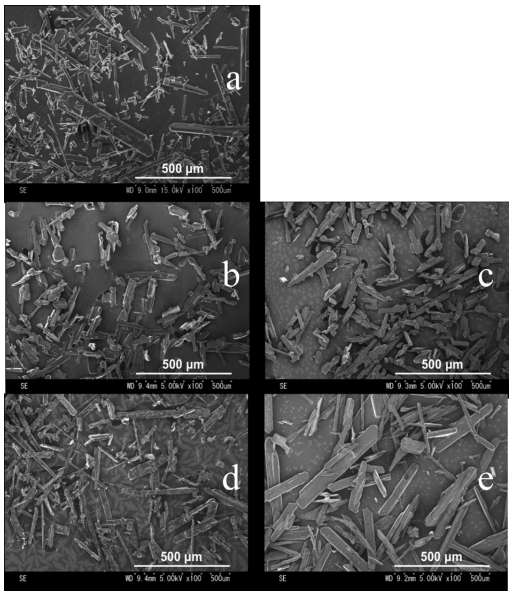

Fig. 6 SEM images of crystals obtained by the conventional crystallization (a) and the repeated cooling crystallization (b–e). Incubation time at 17°C [min]: 0 (a), 20 (b), 40 (c), 60 (d), 90 (e)

period appeared to become erratic again. This is represented in **Figure 5**. The figure shows the effects of the length of incubation at 17°C on the induction period, and several data apart from the ones presented in Figure 4 are shown. It was reconfirmed that the induction period for crystallization in a solution formed by the dissolution of once-formed crystals becomes uniform and short, at least in the solution whose incubation time at 17°C is less than 60 min. Figure 5 shows that an experience of PAC molecules having constructed crystals makes the nucleation of PAC easy and reproducible and also shows that the "crystal experience" of solute molecules continues at least for 60 min (i.e., 47 (=60−12.8) min after complete dissolution). In other words, the solution remembers the conformation of PAC molecule and the interaction between PAC molecules for a short time after dissolution of once-formed crystals.

2.3 Crystals obtained by repeated batch crystallization

The crystals obtained by repeated cooling crystallization were observed with a scanning electron microscope and compared with the crystals obtained by conventional crystallization without the dissolution of crystals at 17°C. **Figure 6** shows the SEM images.

The crystals obtained by the conventional crystallization (Panel-a) and the crystals obtained by repeated crystallization with 90-min incubation (Panel-e) were composed of a mixture of small and large crystals. On the other hand, crystals obtained by repeated crystallization with 20-min, 40-min, or 60-min incubation at 17°C were uniform in size. The CSD was determined on the basis of the diameter of a circle of equal projection area estimated from the SEM images. **Figure 7** presents the CSD. As expected from Figure 6, the crystals obtained by the repeated crystallization with 20-min incubation were small with a narrow CSD. Similar results were obtained in the case of 40-min and 60-min incubation, except for the fact that 150 μm crystals appeared in the case of 60-min incubation.

2.4 Association behavior of PAC molecules after complete dissolution at 17°C

Association behavior of PAC molecules in a methanol solution immediately after the complete dissolution of crystals at 17°C was examined by measuring the changes in the spin-lattice relaxation time (T_1) using NMR. The association of molecules influences the movability of molecules, because in the case of large associates in which many interactions exist between molecules, motion of the molecules becomes hard. The movability of molecules in solution can be evaluated from T_1. The larger the associates are, the more the movability of molecules forming the associates should be limited. T_1 of hydrogen-A to E of PAC molecules once crystallized at 5°C was measured and plotted in **Figure 8** (filled symbols; solid lines) with data obtained for molecules that were not crystallized (open symbols; dotted lines). T_1 values of the hydrogen-B and C increased by the dissolution of once-formed crystals. Figure 8 also shows that the T_1 values of hydrogen-A slightly decreased. These results indicate that phenyl group became easy to move by experiencing crystals and methoxy group became slightly hard to move. They also suggest that PAC molecules were set free from tight interactions between molecules. The free movement of molecules must have promoted nucleation. In the previous work, it was found in the crystallization of PAC dissolved in chloroform that the structure of associates (that are formed by the interactions between solute molecules) in the supersaturated solution was not the same as that of crystals but was similar, and then, it was implied that the transformation from the structure of associates to that of nuclei was required for nucleation (Saito *et al.*, 2002). The data of the relaxation time shown in Figure 8 show that the associates formed or remained after dissolution of crystals at 17°C are somewhat loosely bound and that their structure is close to that of nuclei.

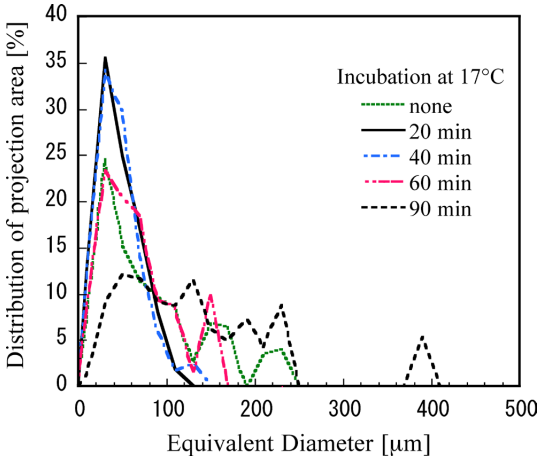

Fig. 7 Crystal size distribution obtained by the conventional or repeated crystallization

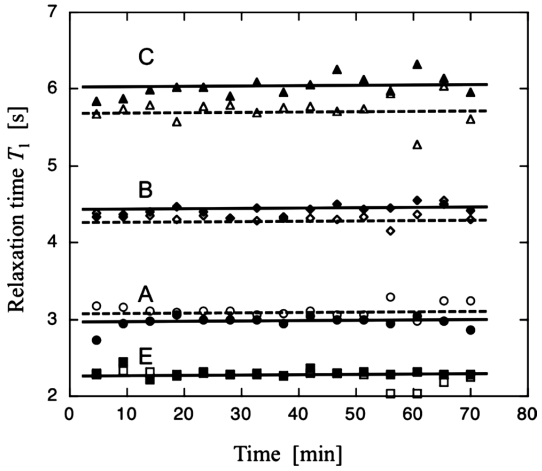

Fig. 8 Changes in relaxation time T_1 of hydrogen A, B, C, and E of *p*-acetanisidide once-crystallized at 5°C. T_1 measurement: at 17°C. Sample solutions: not crystallized (dotted lines), once-crystallized (solid lines)

Conclusion

In order to produce fine crystals with a unimodal and narrow CSD, repeated batch crystallization was proposed and evaluated using *p*-acetanisidide (PAC) as a model compound.

In conventional cooling crystallization, the long induction period ranging from 10 to 200 min was observed before nucleation. Crystals obtained in the conventional cooling crystallization had a broad and multimodal CSD. On the other hand, in repeated crystallization, the induction period was drastically shortened and the timing of nucleation was controlled. As a result, crystals obtained by the repeated crystallization became small and the CSD became uniform.

The spin-lattice relaxation time T_1 of hydrogen-A, B, C, and E suggested that PAC molecules were set free of the strong interactions between molecules by dissolution of once-formed crystals. Repetitive recrystallization and the complete dissolution at a temperature that is slightly higher

than the saturation temperature is not effective for achieving a good result. One repetition of crystallization, i.e., crystallization, complete dissolution, and then re-crystallization, is sufficient for obtaining a good result.

Acknowledgment

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Nomenclature

M_0	=	initial magnetization vector	
M_z	=	magnetization vector at time t	
t	=	time	[s]
T_1	=	spin-lattice relaxation time	[s]

Literature Cited

Chivate, M. R., B. G. Palwe and N. S. Tavare; "Effect of Seed Concentration in a Batch Dilution Crystallizer," *Chem. Eng. Commun.*, **3**, 127–133 (1979)

Griffiths, H.; "Mechanical Crystallization," *J. Soc. Chem. Ind.*, **44**, 7T (1925)

Jones, A. G. and A. Chianese; "Fines Destruction during Batch Crystallization," *Chem. Eng. Commun.*, **62**, 5–16 (1987)

Moore, W. P.; "Optimize Batch Crystallizer: Induction Seeding Offers a Quick and Easy Way to Robust Production Processes," *Chem. Eng. Prog.*, **90**(9), 73–79 (1994)

Mullin, J. W. and J. Nývlt; "Programmed Cooling of Batch Crystallizers," *Chem. Eng. Sci.*, **29**, 105–118 (1971)

Ooshima, H., K. Igarashi, Y. Sasaki, M. Azuma and H. Noda; "Production of Large Crystals of Glycine with a Narrow Size Distribution Using WWDJ-Batch Crystallizer," *Chem. Eng. Trans.*, **1**, 981–986 (2002)

Saito, A., K. Igarashi, M. Azuma and H. Ooshima; "Aggregation of *p*-Acetanisidide Molecules in the Under- and Super-saturated Solution and Its Effect on Crystallization," *J. Chem. Eng. Japan*, **35**, 1133–1139 (2002)

Shan, G., K. Igarashi, H. Noda and H. Ooshima; "Production of Large Crystals with a Narrow Crystal Size Distribution by a Novel WWDJ Batch Crystallizer," *Chem. Eng. J.*, **85**, 161–167 (2002)