Study on Dynamic Analysis and Optimization of Crystallization Process Using In-line Monitoring Technique

インライン分析技術を用いた
晶析操作の動的解析及び最適化に関する研究

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Chapter 1: Background & Outline of Present Thesis
In recent years, environmental problems such as global warming and exhaustion of fossil fuels have become international issues. In the manufacturing industry, development of low-energy manufacturing process as well as technologies for utilizing eco-friendly feedstock is active area of research. In general, target products, unreacted raw materials and by-products are generated through chemical synthetic process, and the resulting mixture is subjected to subsequent separation and purification process. Several separation and purification techniques such as methods utilizing distillation, chromatography and membrane have been developed. However these techniques are very useful and important in industry, they are relatively energy intensive and need larger amount of solvent. When adequate condition is set, high-purity solid of the target product can be obtained through crystallization process. That is, crystallization process has an advantage that purification and high-degree concentration are achieved simultaneously, and is eco-friendly separation and powderization process.

Crystallization process is widely used in the food, fine chemical and pharmaceutical industries, and I discuss crystallization process of pharmaceuticals in this paper. Crystallization processes are influenced by the following factors; solubility, supersaturation, seed loading, crystal shape factor, agitation, growth rate kinetics, nucleation rate kinetics, agglomeration kinetics, etc. However these factors, its effect on product and crystallization kinetics are systemized in the field of crystallization engineering, a crystallization model considering all possible factors is very complex and requires many kinetic and/or physical parameters, and it needs long-time and considerable experimental trial-and-error to estimate these
parameters. In many cases of pharmaceuticals key objectives of crystallization process are impurity rejection and polymorph control. It is often the case that controlling supersaturation and then avoiding the undesired nucleation throughout crystallization process are sufficient to achieve the objectives. It is one of needs of industry, especially pharmaceutical industry, that crystallization process is optimized (controlling the supersaturation throughout the process) with short time and minimal preliminary experimental runs for parameter estimations. I worked on this issue in this paper and developed an easy and quick optimization procedure to develop a robust and proper crystallization process of batch cooling crystallization and anti-solvent addition crystallization in Chapter 2 and 3 respectively. The developed optimization procedure has feature of use of in-line analytical techniques such as in-situ FTIR spectroscopy (React IR™) and in-situ measuring equipment of particle size distribution (FBRM). In Chapter 4, using FBRM real-time monitoring technique, a nucleation behavior of a pharmaceutical was investigated in semi-batch drowning-out crystallization.

In recent years, it has been reported that for pharmaceutical products with low aqueous solubility, fine particles are desirable for enhanced oral bioavailability. One of industrially useful ways to create fine particles is through crystallization while enhancing nucleation (bottom-up method) especially for compounds that have no effective milling techniques. Crystallization under the conditions of high mixing intensity, high-shear force, high magma density, and high supersaturation is a promising way to generate fine particles through a secondary nucleation process. In our previous study, we proposed a new crystallization semi-batch process that consists of a crystallizer with an external circulation line and
Conducted pilot-scale production of the API through the process. In Chapter 4, as a basic study of the process, behavior of secondary nucleation under a high degree of supersaturation is investigated by using a semi-batch crystallizer and in-line monitoring technique FBRM.
Chapter 2: Design of Constant Supersaturation Cooling

Crystallization of a Pharmaceutical

: A Simple Approach
Introduction

Crystallization is an important separation and purification technique used in the food, fine chemical and pharmaceutical industries (Fujiwara et al., 2005). Especially in the pharmaceutical industry, crystallization processes are widely used as the final purification step in the manufacturing of active pharmaceutical ingredients (APIs). The operating conditions of the crystallization process strongly affect the physical properties of the final product such as purity, morphology (crystal shape), polymorphic form (crystal form) and particle size distribution (PSD). These properties could significantly impact the downstream operations, such as filtration, drying and formulation, and the drug efficacy, such as bioavailability. A crystallization process, in which crystal growth predominates over nucleation, is preferred because an increase of nucleation may result in decrease in impurity rejection, generation of undesired polymorph and occlusion of solvent. Especially polymorph control is one important issue in this study since the solubility of one undesired polymorph was relatively close to that of the desired polymorph. Therefore to design a crystallization process, a critical factor is how to control the supersaturation to avoid the undesired nucleation, and its quantification is essential for a development of a scaleable process.

Batch cooling crystallization, along with anti-solvent addition crystallization, is extensively used in the pharmaceutical industry because of its simplicity and flexibility in the operation. The level of supersaturation can be controlled by adjusting the cooling rate and/or the seed loading. There are three types of cooling trajectory are shown in Figure 1 (Mullin, 2001).
**Fig. 1** Typical cooling trajectories employed in batch cooling crystallization.
Natural cooling represents the case where a crystallizer is cooled down naturally. And its trajectory is determined by the temperature of the coolant and the heat transfer capability of the crystallizer, and is expressed as the following Eq. (1), where $T_{in}$, $T_{fin}$ and $T$ are temperatures at the beginning, at the end and at any time $t$ in the process, and $\tau$ is the dimensionless time. Natural cooling is the simplest method but results in an exponential fall in temperature with time and produces a supersaturation peak in the early stages of the process that might cause undesired secondary nucleation.

$$\frac{T - T_{fin}}{T_{in} - T_{fin}} = \exp \left( -\frac{t}{\tau} \right)$$

The basic concept of controlled cooling was proposed and experimentally verified by Mullin and Nyvlt (1971). They derived Eq. (2) for an approximate optimal cooling trajectory, which is shown and named as controlled cooling in Figure 1. In short, it considers the supersaturation balance equation on the assumptions such as supersaturation is low and constant enough so that crystal growth occurs only on the added seeds with negligible nucleation. It also assumes that the crystal growth rate depends neither on the crystal size nor on the temperature.

$$\frac{T_{in} - T}{T_{in} - T_{fin}} = \left( \frac{t}{t_{end}} \right)^3$$

$t_{end}$ is batch operating time.
Jones and Mullin (1974) showed in their research based on computer simulations the potential advantages of controlled cooling in compared with natural or linear cooling. Rohani and Bourne (1990) also derived theoretically a temperature trajectory at constant supersaturation, and the derived temperature trajectory was similar to that of controlled cooling trajectory by Mullin and Nyvlt.

Batch cooling crystallization processes are influenced by many factors such as solvent, solubility, cooling trajectory, seed amount and size, agitation, growth and nucleation rate kinetics, agglomeration kinetics etc. A crystallization model considering all possible factors is very complex and requires many kinetic and/or physical parameters. Choong and Smith (2004) developed the methodology of optimizing a highly non-linear mathematical model of batch cooling crystallization, and optimized variables simultaneously including the cooling trajectory, seed size and amount, batch operating time, initial and final temperatures.

Therefore, the first goal in this study is to find out the "optimal cooling" trajectory, that can keep the supersaturation at a certain level throughout the process. And the second goal is to develop a simplified procedure to develop a robust crystallization process in a short period of time.
1. Methodology

In order to model the crystallization processes, basic mass balance equations are employed. A few assumptions are made in order to simplify the crystallization model. The assumptions are

i. Negligible nucleation, agglomeration and breakage of crystals

ii. Growth only on the seed and constant crystal shape factors

iii. Size independent crystal growth and no growth rate dispersion

iv. Uniform suspension of slurry in the crystallizer

Concentration $C$ in the liquid phase and the solubility $C_s$ are represented with the unit of g/g(total solvent weight $W$). In the liquid phase, the dissolving solute $M \ [g]$ is expressed by multiplying the concentration $C \ [g/g$-solvent] and the solvent weight $W \ [g]$.

$$M = WC \quad (3)$$

The Burton-Cabrera-Frank (BCF) surface diffusion theory is often employed for the crystallization kinetic expression and is given by

$$(\text{crystal growth rate}) \propto \sigma^g \quad (4)$$

where $g = 1$ at high supersaturation, $g = 2$ at low supersaturation and $\sigma$ is the relative supersaturation, which is defined by
\[ \sigma = \frac{C - C_s}{C_s} \]  \hspace{1cm} (5)

In this study, the power \( g \) is set to 2. Description of the BCF growth equation can be found in Mohan and Myerson (2002). The crystal surface area \( A \) [m\(^2\)] should be taken into consideration in the expression of overall crystal growth rate. In this study, the rate of solute decrease, which is equal to the crystal growth rate, is given by

\[ -\frac{dM}{dt} = k_g A \left[ \ln \left( \frac{C}{C_s} \right) \right]^2 \]  \hspace{1cm} (6)

where \( k_g \) [g/m\(^2\)/h] is the growth rate coefficient, which is a function of temperature \( T \) [\(^\circ\)C]. The crystal surface area can be expressed by Eq. (7) based on the assumption of growth only on the seed.

\[ A = A_0 \left( \frac{P}{P_0} \right)^{2/3} \]  \hspace{1cm} (7)

where \( A_0 \) [m\(^2\)] is the initial surface area of seed, \( P \) [g] is weight of crystal and \( P_0 \) [g] is initial solid weight which is equal to the initial mass of seed.

An overall mass balance equation is given by Eq. (8).

\[ P = P_0 + M_0 - M \]  \hspace{1cm} (8)
From Eqs. (3), (7) and (8), the growth rate Eq. (6) can be expressed as a function of concentration in the liquid phase, the growth rate coefficient and the solubility.

\[
\frac{dM}{dt} = k_g \left(\frac{P_0 + M_0 - WC}{P_0}\right)^{\frac{2}{3}} \left[\ln\left(\frac{C}{C_s}\right)\right]^{\frac{2}{3}}
\]

(9)

where \( k_g A_0 \) is replaced by \( k_g' \).

The solubility \( C_s \) of compound of interest is expressed as a function of temperature \( T \) and the supersaturation \( S_s \) [•] is defined by

\[
S_s = \frac{C - C_s}{C_s}
\]

(10)
2. Experimental

2.1. Experimental Apparatus

All the experiments have been performed in a Mettler-Toledo RC1 reaction calorimeter equipped with ReactIR™ 1000 system, the lasentec focused beam reflectance measurement (FBRM) D600 system, a thermocouple and an overhead blade stirrer. RC1 Mettler-Toledo reaction calorimeter provides an accurate control of the batch temperature and the agitation speed. A schematic of the experimental apparatus is shown in Figure 2.

Dunuwila and Berglund (1997) pointed out in their research the feasibility of infrared spectroscopy for the in-situ measurement of supersaturation in crystallization processes. In this study the batch was monitored by ReactIR™ and FBRM. The ReactIR™ monitors real-time mid-infrared spectroscopy from 4000 to 650 cm⁻¹ at 2-min intervals during the crystallization. Laser backscattering, also known as Focused Beam Reflectance Measurement (FBRM), is widely used in the pharmaceutical industry to measure changes in the crystal size and amount. It measures the chord length distribution (CLD) of particles in the slurry. In this study FBRM was used for the monitoring of fine particles generation, i.e. nucleation, and fine particles dissolution.
Fig. 2 A schematic of the experimental apparatus.
2.2. Experimental Procedure

In this study, re-crystallization of compound A, which is a pharmaceutical organic compound with molecular weight ca. 400 and both aromatic and heteroaromatic rings, from its solution in DMF/water was investigated. Crystallization experiments were carried out in one liter jacketed glass vessel. The stirrer speed was kept at 500 ppm so that the batch was well-mixed. To it were added compound A (51.0 g) and DMF/water mixture (410 g, water 7.8 wt%) successively. The batch was heated to 60 °C in order to dissolve compound A completely. The solution was cooled to 50 °C resulting ca. 13 % supersaturated solution. Seed (1.0 g, 2 wt% and 3-5 μm mean particle size) was added and resulting slurry was aged for one hour at 50 °C. A relatively large amount of milled fine seed was introduced to the batch to avoid the secondary nucleation. And then the batch was cooled to 20 °C following several types of cooling trajectories including natural, controlled and multiple-step cooling. Multiple-step cooling was applied in the experiment that was designed for the estimation of crystal growth parameters (see Section 4.3).

The batch was monitored by in-situ IR every two minutes during crystallization and small aliquots were also sampled intermittently for HPLC analysis. The smoothed second derivative of IR spectra was then calibrated with concentration data by HPLC.
3. Computation

Numerical simulation was carried out using MATLAB®. A simulation program was made based on the model described in Chapter 1.

The simulation program consists of simple step-forward calculation. Provided the initial conditions, \( P_0, M_0, W, C_0, T_{in} \) and a temperature profile as the function of time \( T_t \), concentration \( C \), supersaturation \( S_s \) and the growth rate \(-dM/dt\) can be predicted by computation following the program algorithm, which is shown in Figure 3.

At each moment, the change of concentration in a small increment time \( \Delta t \) can be calculated from Eq. (9), then \( C_{t+\Delta t} \) is derived by Eq. (11) as below and \( S_s \) is derived from Eq. (10).

\[
C_{t+\Delta t} = C_t\left(-\frac{dM}{dt}\right)\frac{\Delta t}{W}
\]  

(11)

The program calculates repeatedly until the end which gives the profiles of \( C, S_s \) and the growth rate throughout a process.
Fig. 3. A schematic of algorithm for the $S_s$ profile calculation.
4. Results and Discussion

4.1. Quantification of Solution Concentration by IR spectroscopy

Infrared spectra were recorded using ReactIR™ during crystallization and the smoothed second derivative of spectra in the region of interest is shown in Figure 4(a). Data processing was performed using the mathematical software MATLAB®. Peaks at 1650, 1385, 1255 and 1090 cm\(^{-1}\) shown in Figure 4(a) are typical to the solvent, DMF, while small peaks in the range from 1000 to 900 cm\(^{-1}\) are expected to be from compound A because DMF has no specific peaks in that range of wavenumber.

The calibration curve was determined utilizing two selected peaks in the second derivative of infrared spectra. While the peak at 920 cm\(^{-1}\) shown in Figure 4(b) monitors the concentration of compound A, the reference peak at 1385 cm\(^{-1}\) shown in Figure 4(c) acts as an internal standard. The reference peak remains essentially constant when batch temperature is kept constant. In the case of Figure 4(c), the reference peak has approximately three reference lines because the batch was cooled down following three steps in the multiple-step cooling trajectory.

Calibration curve is shown in Figure 5. It was established by plotting the ratio of peaks of second derivative of IR absorbance at 920 and 1385 cm\(^{-1}\), which are expressed as \(A''_{920}\) and \(A''_{1385}\) respectively, versus the concentration of compound A and it shows a good linear relation between IR spectra and HPLC assay. Supersaturation is not directly measured but is calculated using this calibration line.
Fig. 4. IR absorbances data processing: (a) the smoothed second derivative (b) the concentration related peak (c) the reference peak.
Fig. 5. A calibration plot for concentration.
4.2. Seed Age

In the experimental runs, the batch was monitored using FBRM before seed was added to a supersaturated solution in order to confirm that the solution remained clear without nucleation. Decrease in concentration in one hour seed age period is shown in Figure 6.

At time zero seed was added. Supernatant concentration is ca. 0.116 g/g and supersaturation is 5–7 % after one hour of seeding with good reproducibility, which was set as initial conditions of simulation in this research.
Fig. 6. Decrease in concentration during seed aging.
4.3. $k_g$ and $C_s$ Estimation by Stepwise Cooling Experiment

In order to obtain growth rate coefficient $k_g$ and the solubility $C_s$ in the temperature range of interest, a multiple-step cooling experiment was conducted. The batch was cooled down following the multiple-step cooling trajectory shown in Figure 7.

The concentration was monitored in the experimental run utilizing IR absorbances as described in Section 4.1. And a growth rate $-dM/dt$ at each time can be derived from Eq. (11), where $\Delta t$ equals 2 minutes because infrared spectra were monitored at 2-min intervals.

Relationship between the experimental growth rate $-dM/dt$ and the temperature trajectory are also shown in Figure 7. At the points of step cooling, $-dM/dt$ increased steeply. On the other hand, when temperature was kept constant, $-dM/dt$ decreased gradually, and so were the concentration and supersaturation. Equation (9) was fitted to this desupersaturation curve at each temperature considering $k_g$ and $C_s$ as fitting parameters, and results are shown in Figure 8 and Table 1.
Table 1. Estimated values of $k_g$ and $C_s$ at each temperature.

<table>
<thead>
<tr>
<th>temp [°C]</th>
<th>fitting parameter</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_g$ [g/h]</td>
<td>$C_s$ [g/g]</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>12.2</td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>26.7</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>55.0</td>
<td>0.085</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>172.7</td>
<td>0.110</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. A growth rate profile in the multiple-step cooling crystallization.
Fig. 8. A parameter estimation by curve fitting: (a) at 50°C (b) at 40°C (c) at 30°C (d) at 20°C.
$k_g$ and $C_s$ are expressed as a function of $T [^\circ C]$ as shown in Eqs. (12) and (13), and also shown in Figure 9.

\[
k_g' = 1.54 \times 10^{13} \exp \left( -\frac{8191.7}{T + 273} \right) \tag{12}
\]

\[
C_s = 3.05 \times 10^{-2} + 1.20 \times 10^{-3} T - 1.10 \times 10^{-5} T^2 + 3.71 \times 10^{-7} T^3 \tag{13}
\]
Fig. 9. Comparison of estimated values listed in Table 1 with Eqs. (12) or (13): (a) the growth rate coefficient (b) the solubility data of compound A in DMF/water (water 7.8 wt%).
$k_g'$ and $C_s$ of the compound and the solvent system of interest, which were essential parameters in the crystal growth model as described in Chapter 1, were obtained simultaneously by a single multiple-step cooling experiment. Equations (12) and (13) are utilized in the following simulation sections.

As mentioned above, the growth rate constant $k_g'$ is a function of temperature $T$ and, in general, expressed as following,

$$k_g' = k_0 \cdot \exp\left(\frac{-E_a}{R(T + 273)}\right)$$

(14)

where $k_0$ and $R$ are the frequency factor and the gas constant respectively. Activation energy is derived by comparing Eq. (12) with Eq. (14), and it is found that $E_a$ of compound A in this solvent system is 68 kJ/mol. Tavare (1994) mentioned that, in general, a common range of $E_a$ is 10–80 kJ/mol and at a range of 40–80 kJ/mol, crystal surface integration is considered to be the growth rate determining step. We like to point out that the crystal growth rate of compound A in DMF/water (water 7.8 wt%) has a high temperature dependency.
4.4. Natural and Controlled Cooling

Natural and controlled cooling crystallization experiments were carried out. A batch was cooled down following Eq. (1) for natural cooling ($\tau = 1$) or Eq. (2) for controlled cooling. Experimental profiles of the supersaturation $S$, the growth rate $-dM/dt$ and the temperature trajectory are shown in Figure 10 and Figure 11 respectively.

It was found that natural cooling experiments produced a supersaturation peak at the early stage of the process, whereas controlled cooling experiments peaked at the late stage of the process. These results showed that natural or controlled cooling is not adequate to keep the supersaturation at a certain level in the case of compound A and the solvent system chosen. Especially it is pointed out that controlled cooling could not be applied for general use to keep constant supersaturation even though it is derived based upon the assumption of constant supersaturation.

Corresponding numerical simulations were conducted with the crystal growth model and computation algorithm described above (see Chapter 1 and 3). Simulation results of profiles of the supersaturation and the growth rate through natural and controlled cooling crystallization are also shown in Figure 10 and Figure 11 respectively. The numerical simulations gave good agreement with experimental results.

It was confirmed that an adequate simulation could be possible with this simplified crystal growth model and with physical and kinetic properties estimated by the multiple-step cooling experiment. Once the model was sufficiently
accurate, it was used for computing the optimal cooling trajectory in the following section.
Fig. 10. Experimental data and simulation results (natural cooling).
**Fig. 11.** Experimental data and simulation results (controlled cooling).
4.5. Optimal Cooling

4.5.1. Computation of the Optimal Cooling Trajectory

In this study, it is considered that an “optimal cooling” trajectory means a trajectory which keeps the supersaturation at a certain level throughout the process. Based on the model above (see Chapter 1), an optimal cooling profile was obtained by the algorithm shown in Figure 12.

Provided the initial conditions, \( P_0, M_0, W, C_0, T_{in} \), temperature \( T \) at \( t = t + \Delta t \) can be calculated to maintain the supersaturation at the set value \( S_{s, set} \) of 17%. In detail, temperature \( T \) at \( t = t + \Delta t \) can be calculated from both Eqs. (13) and (15) by Newton’s Method, where \( S_{s, set} = 17 \% \) and concentration \( C \) at \( t = t \).

\[
C_s(T) - \frac{C}{1 + S_{s, set}} = 0
\]  

(15)

And then concentration \( C \) at \( t = t + \Delta t \), is calculated from Eqs. (9), (11) and (12). The program calculates repeatedly until temperature reaches the end temperature, \( T_{\text{fin}} = 20 \, ^\circ\text{C} \), and gives the optimal cooling trajectory throughout a process. The obtained trajectory is shown in Figure 13, line (1).

After 1 hour of seeding, that is, at the starting point of simulation, the supersaturation level is ca. 7% as described in Section 4.2. Therefore at the initial calculation step, \( i.e. \) at \( t = \Delta t \), this simulation results a sharp drop in the temperature in order to raise the supersaturation to the target degree, 17%. In
order to avoid the unrealistic temperature drop, the maximum cooling rate is set at 
-0.5 °C/min and the optimal cooling trajectory for constant supersaturation 
experiment is recalculated. A resulting modified optimal cooling trajectory for 
constant supersaturation experiment is shown Figure 13, line (2). 
The obtained trajectory is sub-linear and unlike natural or controlled cooling 
trajectory as shown in Figure 13.
Fig. 12. A schematic diagram of algorithm for the optimal temperature trajectory calculation
Fig. 13. An optimal cooling trajectory obtained by numerical simulation.
4.5.2. Constant Supersaturation Experiment

A crystallization experiment was carried out in order to demonstrate the cooling trajectory obtained by numerical simulation as described in the previous section. After 1 hour seed aging, the resulting slurry was cooled following the modified optimal cooling trajectory shown in Figure 13, line (2). The batch was monitored by ReactIR™ and obtained infrared spectra were processed to calculate the supernatant concentration $C$ profile. Growth rate $-dM/dt$ and supersaturation $S_s$ at each time were calculated by Eqs. (10), (11) and (13), and these profiles are exhibited in Figure 14.

As shown in Figure 14, in the first 30 min of the experimental run, supersaturation was raised steeply to the target value of 17 % from the initial value of 7 %. After this period, as intended by numerical simulation, the supersaturation was kept constant to the end. It was confirmed experimentally that by following the cooling trajectory estimated by the numerical simulation in Section 4.5.1, supersaturation was kept at a certain level throughout the crystallization process.
Fig. 14. Results of the constant supersaturation experiment; the supersaturation and the growth rate profiles.
5. Chapter Conclusion

In this study, we developed a simplified procedure to design a temperature trajectory for maintaining the supersaturation of a batch cooling crystallization. An overall view of the procedure is illustrated in Figure 15.

The simplified crystal growth model requires only two parameters, crystal growth rate constant and solubility, which are functions of temperature and could be obtained simultaneously by conducting a single experiment with multiple-step cooling. To validate the model, natural cooling and controlled cooling experiments were conducted. The numerical simulations based on the model gave good agreement with experimental results of natural cooling and controlled cooling experiments. Finally, it was confirmed experimentally that supersaturation throughout the crystallization process was kept almost constant by following the cooling trajectory predicted by the numerical simulation for constant supersaturation.

This simplified procedure could be applied generically to other pharmaceutical and fine chemical compounds.
Fig. 15. A schematic of the procedure for optimal cooling crystallization process.
Nomenclature

$A$    total surface area of crystal [m$^2$]

$A_0$  surface area of seed crystal [m$^2$]

$C$    concentration [g(g$_{solvent}$)$^{-1}$]

$C_s$  solubility [g(g$_{solvent}$)$^{-1}$]

$g$    growth order [-]

$k_g$  growth rate coefficient [gh$^{-1}$m$^2$]

$k_g'$ growth rate coefficient [gh$^{-1}$]

$M$    weight of compound A dissolving in solution [g]

$P$    weight of compound A in solid phase [g]

$P_0$  weight of seed [g]

$S_s$  supersaturation [%]

$t$    operation time [h]

$t_{end}$ end time of operation [h]

$T$    temperature [°C]

$T_{in}$ initial temperature of cooling [°C]

$T_{fin}$ final temperature of cooling [°C]

$W$    weight of solution [g]

$\sigma$ relative supersaturation [-]

$\tau$ dimensionless time [h]

<Subscripts>

0 = initial condition
\( t = \text{at time } t \)

\( \Delta t = \text{increment time} \)
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Chapter 3: Constant Supersaturation Control of Antisolvent-Addition Batch Crystallization
Introduction

Antisolvent-addition batch crystallization, sometimes referred to as dilution crystallization, is commonly used for the small-scale production of fine chemicals, active pharmaceutical ingredients (API), and their intermediates. Robustness and accurate quality control are required to manufacture these compounds. Especially in the production of final API crystals, the quality requirements with regard to purity, size distribution, and crystal form are very high. We are working on APIs in the preclinical development stage where we often need to develop and scale up crystallization processes in a short period of time for on-time bulk supply and for larger-scale manufacturing. Therefore, an easy and quick procedure to develop a robust and proper crystallization process is needed.

The crystallization process for the compound of interest had several key objectives, such as impurity rejection and polymorph control. Of these issues, polymorph control was the primary driving force in this study, since the solubility of one undesired polymorph was only 10-20% higher than that of the desired polymorph. For this compound, introduction of the undesired polymorph must be avoided. Therefore, an initial goal was to keep the supersaturation below a certain level throughout the process to avoid nucleation of the undesired polymorph.

Although crystallization is a common subject of investigation, considerable experimental trial and error is still needed to develop a robust process due to the complexity and variety of crystallization behaviors displayed by organic compounds. Crystallization processes are influenced by the following factors; solubility,
supersaturation, seed loading, crystal shape factor, agitation, growth rate kinetics, nucleation rate kinetics, agglomeration kinetics, etc. (Mullin, 2001). Incorporation of all possible factors into a model can lead to the need to estimate many parameters. In practice, it is convenient to describe a crystallization process using as few parameters as possible while retaining a reasonable degree of model accuracy. Our purpose is to develop a simple numerical simulation of crystallization to understand and design a crystallization process with practical accuracy.

There have been only a few reports on antisolvent-addition crystallization (e.g. Doki et al., 2002; Holmbäck and Rasmuson, 1999; Kaneko et al., 2002). Tavare (1995) briefly described an optimal antisolvent-addition profile. Otherwise, there have been no reports on general optimization methodologies. He treated antisolvent-addition crystallization in a similar manner as cooling crystallization, and obtained an antisolvent concentration curve by maintaining a constant supersaturation, as described in Eq. (1)

\[
\frac{U - U_{\text{initial}}}{U_{\text{final}} - U_{\text{initial}}} = \left(\frac{t}{t_{\text{final}}}ight)^4
\]

(1)

This curve was derived under the following assumptions: no seed, linear solubility relation, and simple power-low growth and a nucleation rate that is independent of the solution composition. Unfortunately, these assumptions are not in agreement with our compound of interest, for which the growth rate is a strong function of solvent composition and the solubility is not linear. Therefore, we cannot use this curve for our compound.
In this, we describe a sequence of experiments and a simple numerical simulation that predicts the experimental results, and we also calculate the optimal antisolvent-addition profile. Through the recent development of an *in situ* monitoring technique (Togkalidou et al., 2002; Yu et al., 2004), it has become easier to obtain kinetic data. We can quickly and reliably design a reasonable crystallization process with minimal experiments using on-line monitoring tools. This method can be applied to the antisolvent-addition batch crystallization of a wide range of compounds.
1. Modeling

The model is based on basic crystallization equations. From a practical perspective, to maintain a reasonable degree of accuracy, the model was simplified by making the following assumptions:

• size-independent crystal growth
• no nucleation
• no agglomeration
• no breakage
• self-symmetrical crystal growth
• uniformity within the vessel

1.1. Liquid Phase

Concentration \((C)\) and solubility \((Cs)\) are shown in units of \([g/g(\text{total solvent weight } W)]\), which simplify the calculation since there is no need to know volume or density. The dissolved solute weight \((M)\) is thus the product of concentration \((C)\) and total solvent weight \((W)\).

\[ M = WC \] \hspace{1cm} (2)

The solubility is expressed as a function of solvent composition \((Q)\) at a constant
temperature.

\[ Q = \frac{\text{Weight of water}}{\text{Total solvent weight}} \]  \hspace{1cm} (3)

\[ Cs = Cs(Q, T) \]  \hspace{1cm} (4)

The rate of solute decrease, equal to the crystal growth rate, is proportional to the crystal surface area \((A)\) and the driving force of supersaturation \((\Delta C)\), where the crystal growth constant \((k_g)\) is a function of solvent composition \((Q)\), temperature, agitation, size, etc. In this study, all experiments were carried out at the same temperature and agitation speed, and, by assuming size-independent growth, we considered \(k_g\) to be a function of only the solvent composition \((Q)\).

\[ \frac{dM}{dt} = -k_g A \Delta C \]  \hspace{1cm} (5)

\[ k_g = k_g(Q, T, \text{agitation, size,...}) \]  \hspace{1cm} (6)

We used Eq. (7) for the driving force \((\Delta C)\) for crystallization, as suggested by Mohan and Myerson (2002) from a thermodynamic perspective and in combination with the Burton-Cabrera-Frank crystal growth model.

\[ \Delta C = \left(\ln\left(\frac{C}{Cs}\right)\right)^2 \]  \hspace{1cm} (7)
\[ \Delta C = (C - C_s)^g \]  \hspace{1cm} (8)

Equation (8) presents the well-known power low form; however, it was not suitable for this study. When Eq. (8) is used instead of Eq. (7), there is so much variation in \((C-C_s)^g\) with changes in \(C\) and \(C_s\) over a wide range that the value of \(k_g\) is unstably dispersed.

1.2. Solid Phase

The weight of crystal \((P)\) can be calculated from the initial solid weight \((P_0, \text{ equal to the seed amount})\), the initial solute amount \((M_0)\), and the dissolved solute \((M)\)

\[ P = P_0 + M_0 - M \]  \hspace{1cm} (9)

The surface area of the crystal is expressed as Eq. (10) based on the assumption of self-symmetrical crystal growth.

\[ A = A_0 \left( \frac{P}{P_0} \right)^{2/3} \]  \hspace{1cm} (10)

1.3. Simulation Algorithm
We made a simulation program based on the above model. Given the initial conditions $P_0$ and $M_0$, and a solvent composition profile as a function of time ($Q(t)$), this program can be used to predict the concentration ($C$), supersaturation (($C-C_s)/C_s$), and growth rate ($dM/dt$). The simulation program consists of simple step-forward calculation. At each moment, the change in solute weight ($\Delta M$) over a small-increment time-step ($\Delta t$) can be calculated from Eq.(11), in which all values are derived from Eqs. (2)-(4), (6), (7), (9), and (10).

$$\Delta M = -k_x \Delta C \cdot \Delta t$$  \hspace{1cm} (11)

$$M(t + \Delta t) = M(t) + \Delta M$$ \hspace{1cm} (12)

The program calculates repeatedly until the end and gives the profiles of $C$, supersaturation, and $dM/dt$ throughout the process.
2. Experiments

Typical Crystallization Procedure

Crystallizations were carried out in a 1-L jacketed glass vessel equipped with ReactIR (Mettler-Toledo K.K.), FBRM (Mettler-Toledo K.K.), a thermocouple, and an overhead blade stirrer. Compound P (50 g) was dissolved in solvent (410 g) and heated to 60°C. The solution was cooled to 50°C, resulting in a ca. 15% supersaturation solution. Seed (1.0 g, 2 wt %, surface area = 3.0 m²/g) was introduced and the mixture was stirred for 30 min. Water (200 g) was added as an antisolvent for several hours at a controlled rate, as described in the next section. The temperature and stirring speed were kept constant at 50 °C and 500 rpm throughout the process. The slurry was filtered and washed with water (100 g x 2). The cake was blown with N₂ and dried overnight under vacuum at 50°C. Particle sizes were analyzed with MICROTRAC (model 9320-X100). Crystal forms were analyzed with X-ray powder diffraction (X’Pert PRO MPD), and the peaks of undesired form were not detected in all experiments.

The batch was monitored by ReactIR and FBRM every two minutes. Small aliquots were also sampled intermittently for HPLC analysis. IR absorbance data were processed, second-derivatives were taken, and smoothing was applied, and the results were calibrated with concentration data obtained from HPLC. One of the peaks that showed a nice linear relation to the concentration was used to convert IR absorbance to concentration.
3. Results and Discussion

3.1. Introduction

Compound P is a typical pharmaceutical organic compound with a molecular weight of ca. 400 and both aromatic and heteroaromatic rings. It dissolves well in polar organic solvents and scarcely dissolves in water. Thus, in this study, the compound was first dissolved in polar organic solvent, and then water was added as an antisolvent.

Some of the physical properties of compound P need to be obtained from experiments for the model calculation. Agglomeration and nucleation were neglected in this study, and thus only the growth rate constant \( (k_g) \) and solubility \( (C_s) \) are required. First, we measured \( C_s \) and outlined the crystallization process: start, seeding, and end points (section 3.2). Next, throughout the entire crystallization process, \( k_g \) was obtained from an antisolvent spiking addition experiment (3.3). Using the obtained physical properties, simulations were carried out under various conditions and the results were compared with the corresponding experimental results (3.4). In addition, the ideal antisolvent-addition profile to maintain a constant supersaturation was calculated based on the same model, and minimal time cycles were estimated under several conditions (3.5).
3.2. Solubility Measurement and Outline of the Process

Excess compound P was placed in a series of vials of various solvent compositions. The resulting vials were stirred overnight at 50 °C and the supernatants were analyzed by HPLC. The results are shown in Figure 1. A fourth-polynomial curve was fitted to the experimental points and the correlation between $C_s$ and $Q$ was obtained.

$$C_s = 8.0004Q^4 - 12.1640Q^3 + 7.1961Q^2 + 1.9982Q + 0.2251$$ (13)

From the solubility curve, we chose the start and end points at $Q = 0.08$ and 0.35, respectively, where the solubility decreases from 0.107 to 0.004 g/g. To achieve moderate supersaturation at the seed point, the initial concentration of compound P was set at 0.122 g/g.
Fig. 1. Growth rate constants \((k_9)\) obtained from an antisolvent spiking addition experiment and power curve fitting.
3.3. Antisolvent Spiking Addition Experiment to Obtain $k_g$

To obtain $k_g$ throughout the entire process, antisolvent spiking addition experiments were carried out. Some amount of water was added in one minute to attain 15-30% supersaturation. The de-supersaturation curve was then monitored for a couple of hours. This procedure was repeated at several points over the entire range of solvent composition. From each de-supersaturation curve, $k_g$ at each solvent composition was calculated based on Eq. (5). The results were fitted to a power function curve to give an equation for $k_g$ as a function of $Q$ (Eq. 14). This relation was used for the following simulations.

$$k_g(Q) = 4.23Q^{-1.54}$$

(14)
3.4. Continuous antisolvent-addition experiments

To verify the simulation program, three experiments with different antisolvent addition profiles were carried out. The concentration was monitored with ReactIR, and the results were compared to the corresponding simulation output. In all three experiments, no significant nucleation was observed on FBRM and crystals seemed to grow smoothly. The three experiments had the same initial and final conditions as described above, i.e. the same amounts of solvent (410 g), compound P (50 g), and seed (1.0 g) were used, and the same amount of antisolvent (200 g) was then added at different rates.

Case 1: Addition over 3 h in One Step

To a supersaturated solution, seed (1.0 g) was added and the mixture was stirred for 30 min. Antisolvent (200 g) was then added at a constant rate for 3 h. The experimental results are shown in Figure 2. Supersaturation exceeded 30% in the early stage due to a sharp drop in solubility at a low water composition. The dashed line in Fig. 2 shows the output of the simulation. The results agree nicely with the experimental results. Thus, from the initial conditions and the antisolvent-addition profile, the simulation program could accurately predict the experimental results.
Case 2: Addition over 3 h in Three Steps

The initial conditions and seeding were the same as in case 1, but antisolvent was added in three phases at different rates over a total of 3 h. The rate of addition was initially slow (30 g/h) and then increased (60 g/h and 110 g/h). The experimental results are shown in Figure 3. Supersaturation was kept constant at about 20% throughout the addition of antisolvent. The corresponding simulation results (dashed line in Fig. 3) agreed well with the experimental results. With the slow addition of antisolvent at the initial stage, solubility drops moderately, and thus the rapid increase to a high supersaturation, as observed in case 1, is avoided. Although the same amount of antisolvent was added over the same time period as in case 1, supersaturation remained low in case 2. In case 2, the possibility of nucleation is lower than in case 1 for a lower supersaturation. Accordingly, the profile for case 2 is preferable to that of case 1 for avoiding nucleation.

Case 3: Addition over 5 h in Five Steps

The initial conditions and seeding in case 3 were again the same as in cases 1 and 2. Antisolvent was added in five phases at different rates over a total of 5 h. Addition was started at a slow rate and then gradually accelerated (10, 20, 30, 50, and 90 g/h). The total amount of antisolvent added was the same as in cases 1 and 2, but the addition occurred over a longer period. The experimental results are shown in Figure 4. The simulation results (dashed line in Fig. 4) roughly
correspond to the experimental results. Supersaturation was kept constant at about 15% throughout the addition of antisolvent. With the initial very slow addition and longer addition period, the supersaturation profile was preferable to those in cases 1 and 2 with respect to the avoidance of nucleation.

As described in the above three cases, the experimental and simulation results showed good agreement. The signal-noise ratio of IR signal at low substance concentration range is relatively large, therefore, the error of substance concentration seems large in the later stage of each case. Although the effects were not observed in these three cases, above a certain level of supersaturation, nucleation and agglomeration cannot be ignored and may deteriorate the accuracy of the simulation. While this is certainly a limitation of this model, we would try to avoid such a high supersaturation in our crystallization process to minimize nucleation. Thus, this simulation is not intended to be used under such conditions.

Particle size data obtained with MICROTRAC and FBRM at the final stage of each experiment are shown in Table 1. Assuming neiher nucleation nor aggregation, the calculated value of ideal growth from 4 μm seed crystals was 14.7 μm. The mean values of the resulting PSDs in cases 1-3 were close to that of the ideal growth model, which suggests that there was no significant nucleation in these experiments.
Fig. 2. Experimental and simulation results for case 1

Fig. 3. Experimental and simulation results for case 2

Fig. 4. Experimental and simulation results for case 3
**Table 1. Particle size information**

<table>
<thead>
<tr>
<th></th>
<th>MICROTRAC</th>
<th>FBRM chord length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (µm)</td>
<td>d95% (µm)</td>
</tr>
<tr>
<td>seed</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>case 1</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>case 2</td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>case 3</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>model</td>
<td>14.7</td>
<td></td>
</tr>
</tbody>
</table>

*a Dispersed with sonication*
3.5. Calculation of Antisolvent-Addition Profile To Maintain a Constant Supersaturation

Procedures for the crystallization of API compounds are usually required to reproducibly yield uniform-quality crystals in a minimal time cycle, i.e. contamination by an undesired polymorphs must be avoided, and they should always give a similar particle-size distribution. To achieve these characteristics, nucleation should be avoided. If a compound has polymorphs, the nucleation of some forms may occur above a certain level of supersaturation, and crystals contaminated with the undesired polymorphs may be obtained. When nucleation occurs, the particle-size distribution becomes irregular and is difficult to control.

To avoid nucleation, supersaturation should be kept in the metastable zone throughout the process. However, it is usually very difficult to find the metastable zone for secondary nucleation. While a very low supersaturation can be used to avoid nucleation, it can be very time-consuming and impractical. Accordingly, it is reasonable to maintain moderate supersaturation throughout the process to avoid nucleation and minimize the time required for the process.

The present model was used to calculate the optimal rate of antisolvent addition to maintain a constant supersaturation. Given the initial conditions, $Cs$ is calculated under the restriction that supersaturation $((C-Cs)/Cs)$ is constant. Next, $Q$ is calculated from a polynomial function of $Cs(Q)$. The subsequent sequence is the same as that described in section 1.3. Thus, we obtain a concentration profile $(C(t))$ and an antisolvent-addition profile $(Q(t))$. Figure 5 shows the results of the
calculation when the supersaturation was kept constant at 15% under the same initial conditions as in the above three experiments (section 3.4). At the beginning of the process, the solubility curve in the region of low water content is steep and the crystals have a small surface area. Therefore, the program indicated that the antisolvent should be added very slowly to gradually decrease the batch solubility. In a later stage, the solubility curve with higher water content is flatter and the surface area is greater, and thus antisolvent can be added more rapidly. If the process follows this antisolvent-addition profile, supersaturation will be kept constant at 15%, and hence it shows a minimal batch time cycle under this restriction and the initial conditions. In practice, the addition curve can be approximated by several steps of linear addition, as in case 3 in section 4.4.

Antisolvent-addition profiles were calculated under various conditions (Table 2). While the initial amounts of solvent and solute were the same as in the above experiments, the amount of seed and supersaturation were altered. The seed is assumed to have the same surface area per gram as the seed used in the above experiments. Entries 1-3 in Table 2 compare differences in the amount of seed at the same supersaturation level. The duration of antisolvent addition is reduced with an increase in the amount of seed. Entries 2, 4, and 5 show the antisolvent-addition time with varying allowance of supersaturation. When supersaturation is limited to 10%, antisolvent addition takes 6.5 hours; while in the case of 20% supersaturation, it takes only 2 hours. If the metastable zone is wide and no nucleation occurs, the use of a high supersaturation can shorten the process cycle time.
Fig. 7. Calculated ideal antisolvent-addition profile to maintain supersaturation at 15%

Table 2. Estimation of the batch cycle time under various conditions

<table>
<thead>
<tr>
<th>entry</th>
<th>seed (wt%)</th>
<th>supersaturation (%)</th>
<th>time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>15</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>15</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
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<td>15</td>
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</tr>
<tr>
<td>5</td>
<td>4</td>
<td>20</td>
<td>2.0</td>
</tr>
</tbody>
</table>
4. Chapter Conclusion

The antisolvent-addition crystallization of API was optimized by using a simple simulation program. This process was designed to avoid nucleation by keeping supersaturation below a certain level. This process was successfully scaled-up to a pilot-plant scale and gave more than 100 kg of API crystals. In pilot-plant production, an antisolvent-addition procedure was designed to keep supersaturation under 10% to minimize the risk of generating wrong polymorphs which have only 10-20% higher solubility under these experimental conditions. As a result, crystals of the desired form were obtained exclusively, and similar particle-size distributions were obtained reproducibly.

As described above, a rapid procedure for the optimization of antisolvent-addition crystallization was established. In conventional process development, many experiments are needed to identify a good process, which may not be optimal. In this study, by measuring the growth rate constant and solubility curve in just a few experiments, we obtained an optimal antisolvent-addition profile by using a simulation. We were also able to estimate the batch cycle time under arbitrary conditions. This procedure can be easily applied to develop an optimal crystallization process for a wide variety of compounds.
Nomenclature

$A$  Total surface area of crystal (m$^2$)
$A_0$  Surface area of seed crystal (m$^2$)
$C$  Concentration (g/g(solvent))
$C_s$  Solubility (g/g(solvent))
$g$  Growth rate order
$k_g$  Growth rate coefficient (g/h)
$M$  Weight of compound P dissolved in solution (g)
$Q$  Weight fraction of antisolvent
$W$  Weight of solution (g)
$P$  Weight of compound P in the solid phase (g)
$P_0$  Weight of seed (g)
$T$  Temperature
$U$  Antisolvent concentration

* Subscript 0 represents the initial condition
Literature Cited


Chapter 4: Observation of Secondary Nucleation in Drowning-Out Crystallization Using FBRM
Introduction

For pharmaceutical products with low aqueous solubility, fine particles with narrow particle size distribution are desirable for enhanced oral bioavailability or for inhalation therapy. One way to create fine particles is through crystallization while enhancing nucleation especially for compounds that have no effective milling techniques.

Nucleation can be classified into primary and secondary. The secondary nucleation is a nucleation process that takes place only because of prior presence of crystals of the material being crystallized, and has a predominant influence on the overall performance in industrial practices.

Various mechanisms of secondary nucleation have been proposed, e.g., shear nucleation and contact nucleation. It has been confirmed experimentally by a number of researchers that fluid shearing forces on growing seed crystal surface affect the generation of secondary nuclei. Shamlou et al. (1990) examined particle size of samples of potassium sulfate removed periodically from an agitated batch crystallizer and concluded attrition of crystals can occur by purely hydrodynamic effects. It was also well established experimentally that collisions between crystals, crystal-impeller and crystal-vessel internals generate secondary nuclei. Garside and Jarson (1978) conducted direct microscopic observation of impact-induced secondary nucleation by collision between potash alum crystals and a solid rod in both supersaturated and undersaturated solutions. They indicated in their paper that the majority of the fragments produced were in the 1–10 μm size
range and many of the nuclei smaller than about 10 μm appear not to grow or to grow very slowly.

Crystallization under the conditions of high mixing intensity, high-shear force, high magma density, and high supersaturation is a promising way to generate fine particles through a secondary nucleation process. In our previous study (Kamahara et al., 2007), we proposed a new crystallization semi-batch process that consists of a crystallizer with an external circulation line and conducted pilot-scale production of the API through the process. A schematic view of the process is illustrated in Figure 1. In the middle of the anti-solvent circulation line, a t-mixer and high-shear rotor-stator mixing device are provided. A solution of compound to be crystallized is fed through the t-mixer into the recycle loop of product slurry. The t-mixer is located adjacent to the inlet of a high-shear rotor-stator mixer in order to accomplish a high degree of supersaturation in the mixer. In this paper, as a basic study of the process, behavior of secondary nucleation under a high degree of supersaturation is investigated by using a semi-batch crystallizer and in-line monitoring technique.

Laser backscattering technique, also known as focused beam reflectance measurement (FBRM), was used in situ for monitoring of population trends of fine particles in a slurry. It measures the chord length distribution (CLD) of particles in slurry, which is a function of the true particle diameter distribution.

This technique is widely used in the pharmaceutical industry to measure changes in the crystal size and amount. Fujiwara et al. (2002) applied it to detect the onset of nucleation and determine metastable zone. Kougioumdis et al. (2005) applied it to monitor the steady-state operation of crystallization kinetics for organic fine
chemicals in a modified mixed suspension mixed product removal crystallizer.

In this study, FBRM is applied to detect the onset of nucleation, to estimate the rate of the nucleation and to observe population trends of fine particles during secondary nucleation.
Fig. 1 High-shear mixing process
1. Experimental

1.1. Materials

The API investigated in this study is a common pharmaceutical organic compound with a molecular weight of ca. 450 and both aromatic and hetero rings.

Solubility of the API in water and in aqueous solutions of methanol is represented in Figure 2. Solubility was determined by the isothermal method and the API was assayed using HPLC. As shown in Figure 2(a), the API is insoluble in water, that is, water can be used as an anti-solvent in drowning-out crystallization of the API. Solubility of the API is 0.121 mg/ml, as shown in Figure 2(b) under the experiment at conditions in this study with the batch temperature of 2°C and the volume fraction of water/methanol is 10/1.
Fig. 2 Solubility of the API used in this study: (a) 5°C and (b) Water/MeOH: 10/1 (v/v)
1.2. Experimental apparatus

All the experiments were performed in a Mettler Toledo RC1 reaction calorimeter equipped with a Lasentec FBRM D600 system, a thermocouple and a pitched blade stirrer. A schematic of the experimental apparatus is shown in Figure 3. The RC1 Mettler-Toledo reaction calorimeter provides accurate control of the batch temperature and the agitation speed. FBRM probe was inserted into the turbulent zone adjacent to the agitation blade.
**Fig. 3** A schematic of the experimental apparatus
1.3. Experimental procedure

In this study, re-crystallization of the API from its solution in methanol was investigated. Crystallization experiments were carried out in a one-liter jacketed glass vessel. Distillate water (500 mL) as an anti-solvent was added to the vessel, and then it was cooled to 2°C. The stirrer speed was kept at 300 rpm so that the batch was mixed well. Seed (0.1–1.0 g, 173 μm mean particle size measured using laser diffraction analyzer (HELOS & RODOS, Japan Laser)) was added and the resulting slurry was aged for 10 min. Then, 50 mL of methanol solution of the API (14.0–28.0 mg/mL, here in after referred to as the feed concentration $C_f$) was injected into the seed slurry adjacent to the agitating blade using a syringe with a long needle. The feed of the API solution was completed in a short period of around 7 s.

Nucleation took place after the induction period in some experimental runs. The slurry was filtered and dried under vacuum at 40°C over night. The obtained dried powder was subjected to measurement of volume mean diameter, $D_{av}$, using a laser diffraction particle size analyzer.
1.4. FBRM data processing

FBRM chord count in the size class 1–500 \(\mu m\) was measured, and the count in the size class 1–10 \(\mu m\) was considered as a representative value of fine particles in this study. Induction time, nucleation rate and chord count at the end of nucleation were calculated from time course of FBRM chord count in the size class 1–10 \(\mu m\) through the following data processing.

A sigmoid curve was fit to the time course of FBRM chord count in the size class 1–10 \(\mu m\) with time at each experimental runs considering \(a, b, f_0\) and \(t_0\) as fitting parameters for Eq. (1)

\[
f(t) = f_0 + \frac{a}{1 + \exp\left[b(t_0 - t)\right]}
\]  

Eq. (1) has an inflection point at \(t = t_0\). A slope at \(t_0\) was derived by differentiation of Eq. (1) with respect to \(t\) and is represented by Eq. (2). In this study, this slope was defined to be the rate of nucleation \(J\).

\[
J = f(t_0)' = \frac{ab}{4}
\]  

The induction period \(t_{\text{ind}}\) was derived from the point of intersection of the line tangent to the curve (1) at the inflection point and the line \(f(t) = f_0\).
\[ t_{\text{ind}} = t_0 - \frac{4}{ab} \left( f_0 - \frac{a}{2} \right) \]  

(3)

The chord count at end of nucleation, \( N_f \) was calculated using Eq. (4).

\[ N_f = f(\infty) = f_0 + a \]  

(4)
2. Results and Discussion

2.1. Nucleation behavior

A typical example of time courses of FBRM chord count in the size class 1–10 μm and 1–500 μm is shown in Figure 4. In this case, 0.1 g of seed crystals were added to 500 mL of 2°C water, and then 50 mL of 18 mg/mL API methanol solution was fed to the resulting dilute seed bed.

While little to no increase in chord count was detected just after feeding, chord count started to increase after 50 min from feeding. This means that secondary nucleation took place after the 50-min induction period. The time course of chord count in the size class 1–10 μm is well fit to a sigmoid curve as shown by the dashed line in Figure 4. In this experiment, \( t_{ind} \) is 58 min, \( N_0 \) is 35940 1/s and \( J \) is 906 (1/s)/min.

In general, the induction time has been related to unseeded crystallization, but it has been reported that an induction period can also be observed when seeds are added in the solution (Mullin, 2001). The nucleation just after feeding and after the induction period will be referred to as “first nucleation” and “second nucleation”, respectively, hereafter. Both “first nucleation” and “second nucleation” will be defined as secondary nucleation because of the presence of the seeds. However, especially in the case of “second nucleation”, its kinetics, a sudden burst of nuclei, is similar to that of primary nucleation. The data processing, which is described in Section 2.4, was applied twice for each experimental run. A series of data points
for 20 min from the API feeding was selected and fit to the sigmoid curve in order to estimate kinetic parameters of “first nucleation”, while the complete set of data points of each experimental run was fit for “second nucleation”.

In all experimental runs in this study, no polymorphic change was observed by the powder X-ray diffraction patterns and the crystal form of crystals generated through the nucleation was the same as that of seed crystal (data not shown).
**Fig. 4** An experimental example of time course of chord count (solid line) and a sigmoid curve fitting (dashed line, ref. Section 2.4)
2.2. Effect of crystallization conditions on the nucleation behavior

2.2.1. Effect of amount of seed crystals

The effect of the amount of seed crystals on nucleation behavior was examined. No seed or 0.1, 0.5, 1.0 g of seed was added to the vessel and then 50 mL of 20 mg/mL methanol solution of the API was injected. The resulting time courses of FBRM chord count in the size class 1–10μm are shown in Figure 5 and the results of the experiments are listed in Table 1.

As shown in Figure 5, steep “second nucleation” was observed, while no “first nucleation” to indicate the nucleation just after feeding, was observed in the resulting time courses of FBRM chord count. It was found that the induction period for “second nucleation” decreases and its nucleation rate increases as the amount of seed crystals increases, as shown in Table 1. Verdoes et al. (2005) also compared in their paper the induction period obtained from both seeded and unseeded crystallization and proved the effect of seed addition on the decrease in induction time.

It was found that the addition of seed crystals is effective to control the induction period and to enhance nucleation to produce fine crystals.
Fig. 5 The time courses of chord count: (#1) no seed; (#2) 0.1 g; (#3) 0.5 g; and (#4) 1.0 g of seed crystals
2.2.2. The effect of the feed concentration (the degree of supersaturation)

The effect of the feed concentration on nucleation behavior was examined. The operating conditions are listed in Table 2. In this study, the degree of supersaturation, \( S_s \), was defined as Eq. (5).

\[
S_s = \frac{C_f \left( \frac{50}{550} \right)}{C_s}
\]  

(5)

Where, \( C_s \) is the solubility of the API in water/methanol (10/1 v/v) at 2°C. The resulting time courses of FBRM chord count of the experiments #2, #3, #5 and #7 are shown in Figure 6.

It was found that “first nucleation” is negligible in the cases of experiments #2 and #3 when the feed concentration is relatively low, as shown in Figure 6. It was observed that distillate water around the tip of a syringe needle became clouded during feeding and the cloud disappear in a very short time. A clear supersaturated solution was obtained after the feeding of API solution. On the other hand, “first nucleation” is not negligible when the feed concentration is relatively high, as shown in the cases of experiments #5 and #7. Generation of nuclei during feeding was observed, and then, supersaturated slurry of fine particles was obtained.

It was also observed that chord count in experiment #5 increased slowly for around 70 min from feeding (see Figure 6). Garside and Jarson (1978) investigated
secondary nucleation by contact between a crystal and a solid rod by direct microscopic observation and concluded in their paper that many of nuclei smaller than about 10 μm in supersaturated solution appear not to grow or grow very slowly. In this study, we assume that fine particles under 1 μm generated during feeding, which is not detectable by FBRM, grow very slowly to be over 1 μm and chord count in the size class 1-10 μm appear to increase slightly during induction period of “second nucleation”.
Fig. 6 Time courses of FBRM chord count: (#2) 16.0 mg/mL; (#3) 18.0 mg/mL; (#5) 22.0 mg/mL; (#7) 26.0 mg/mL of the API solution
For more detailed observation of nucleation behavior, the time courses of FBRM chord count in different size classes are plotted in Figures 7 and 8. Figures 7 and 8 show the resulting time courses of FBRM chord count in experiments #3 and #5 respectively, in the size classes 1–10 μm, 10–50 μm, 50–100 μm and 100–500 μm.

In experiment #3, when feed concentration was relatively low, no “first nucleation” was observed, as shown in Figure 7(a), and after the 60-min induction period, the FBRM chord count in the size class 1–10 μm and 10–50 μm started to increase, while count in the size class 50–100 μm and 100–500 μm did not change, as shown in Figure 7(b). These kinetics of nucleation can be explained by the primary nucleation theory.

On the other hand, the nucleation behavior is more complicated in experiment #5 when the feed concentration is relatively high. “First nucleation” was not negligible as shown in Figure 8(a). A short time before the “second nucleation”, the chord count of fine particles in the size class 1–10 μm and 10–50 μm started to decrease, and the chord count of larger particles in the size class 100–500 μm started to increase slightly. At the moment of “second nucleation”, chord count of larger particles in the size class 100–500 μm started to decrease and the chord count of fine particles in the size classes 1–10 μm and 10–50 μm increased steeply.
Fig. 7 The time course of FBRM chord count when feed concentration is 18.0 mg/ml:
(a) full-figure; (b) close-up figure: the numbers in (b) show the size class of chords, 1:1–10 μm, 2:10–50 μm, 3: 50–100 μm, and 4:100–500 μm
Fig. 8 The time course of FBRM chord count when feed concentration is 22.0 mg/ml:
(a) full-figure; (b) close-up figure: the numbers in (b) show the size class of chords,
1:1~10 μm, 2:10~50 μm, 3: 50~100 μm, and 4:100~500 μm
Figure 9 shows appearance changes of the slurry in the glass vessel in experiment #5. Clear seed bed (see Figure 9(a)) turned to be a white slurry just after the feeding of the API solution, as shown in Figure 9(b). “First nucleation” was observed clearly. A short time before “second nucleation”, the slurry became dilute and a translucent white slurry was observed as shown in Figure 9(c). After the “second nucleation”, a denser slurry compared to the slurry obtained after “first nucleation” was obtained as shown in Figure 9(d).

We considered from the time courses of FBRM chord count (Figure 8) and the appearance change of the slurry in the vessel (Figure 9) that when feed concentration is relatively high, the crystallization process has the three following steps. When the API solution was fed into the dilute seed bed, several nuclei generated and then a supersaturated slurry of fine particles was obtained, which act as a seed crystal for “second nucleation”. The newly generated fine seed crystals grew very slowly in the supersaturated slurry during the induction period of “second nucleation”. Once fine particles under 50 μm in chord length formed an agglomerate with each other or original seed crystal, dispersion of agglomerates took place while generating an enormous amount of nuclei at the moment of “second nucleation”.

- 93 -
Fig. 9 The pictures of the vessel in the experiment #5: (a) seed aging; (b) the time of solution feeding; (c) several minutes before “second nucleation”; and (d) the end of the experiment
Figure 10 shows the chord length distribution (CLD) of the slurry when 110 min had passed from feeding in experiments #2, #3, #5 and #7. It was found that CLD had a peak at around 5 μm when the feed concentration was higher (#3, #5 and #7), while the slurry had a broad CLD when the feed concentration was low (#2). We assume that a peak at around 5 μm in CLD results is from “second nucleation”. The CLD in experiment #7 had a shoulder at around 30 μm, which we believe is related with grown nuclei generated at “first nucleation”.

Results of experiments are summarized in Table 2. Nucleation rate of “second nucleation” is much faster than that of “first nucleation”. Therefore, it was found to be important to enhance “second nucleation” to produce lots of fine particles using a semi-batch process as shown in this study. It was also found that the nucleation rate of “second nucleation” has a maximum against the feed concentration. It is thought that both an amount of newly born seed crystals at “first nucleation” and the actual degree of supersaturation when “second nucleation” determine the kinetics of “second nucleation”. In other words, the kinetics of “second nucleation” was not directly related to the amount of initially added seed crystals or the feed concentration in this drowning-out crystallization.
Fig. 10 Chord length distribution of the product slurry
Table 1 The effect of the amount of seed crystal

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<th>Results</th>
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<td>Seed ( C_f ) ( S_s )</td>
<td>( t_{nd} ) ( J ) ( D_{av} )</td>
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<td></td>
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<td>[min] [(1/s)/min] [μm]</td>
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Table 2 The effect of the feed concentration

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3. Chapter Conclusions

A nucleation behavior of the API was investigated in semi-batch drowning-out crystallization. The effect on nucleation behavior of an amount of seed crystals and concentration of API solution to be fed was investigated using FBRM.

Nucleation took place after the induction period from feeding in some experiments despite a presence of seed crystals in an anti-solvent into which the API solution was fed. Also, it was found that an addition of seed crystals is effective to control the induction period and to enhance nucleation after the induction period.

At a high feed concentration ($S_s$ is greater than 15), generation of nuclei during feeding was observed and then a supersaturated slurry of fine particles was obtained. It was found from the time courses of FBRM chord count that fine particles generated during feeding grew very slowly during the induction period and rapid nucleation took place accompanied with agglomeration of fine particles and subsequent dispersion with the generation of nuclei after the induction period.
Nomenclature

$A$ fitting parameter [1/s]

$b$ fitting parameter [1/min]

$C_s$ solubility [mg/mL]

$C_f$ feed concentration [mg/mL]

$D_{av}$ volume mean diameter [$\mu$m]

$f(t)$ sigmoid curve [1/s]

$f_0$ fitting parameter [1/s]

$J$ rate of nucleation [1/s/min]

$N_f$ FBRM chord count at end of nucleation [1/s]

$S_s$ degree of supersaturation [-]

$t_{ind}$ induction period [min]

$t_0$ fitting parameter [min]
Literature Cited


Shamlou, P. Y., A. G. Jones and K. Djamari; “Hydrodynamics of Secondary
Chapter 5: Conclusion
There are mainly two objectives of crystallization process in the pharmaceutical industry as following,

1) high-degree purification (impurity rejection) and polymorph control by avoiding undesired nucleation (supersaturation control)

2) fine particle production utilizing nucleation.

We worked on these objectives using in-line monitoring techniques as feature of research.

As a study for achieving the objective 1) above, we developed an easy and quick optimization procedure, which includes computation of optimal cooling and anti-solvent addition rate using crystal growth kinetics model with minimal parameters, to develop a robust and proper crystallization process of batch cooling and anti-solvent addition crystallization (Chapter 1 and 2). The developed optimization procedure is so simple and versatile that it can be applied in several research fields including the preclinical development stage of pharmaceuticals where it is often needed to develop and scale up crystallization processes in a short period of time for on-time bulk supply and for larger-scale manufacturing.

As a study for achieving the objective 2) above, in our previous study, we proposed a new crystallization semi-batch process that consists of a crystallizer with an external circulation line as a scalable process. As a basic study of the process, a nucleation behavior of the pharmaceutical was investigated in semi-batch drowning-out crystallization using in-line particle monitoring technique (FBRM). We could observe and report an interesting nucleation behavior that rapid nucleation after the induction period took place accompanied with
agglomeration of fine particles and subsequent dispersion with the generation of nuclei. It was demonstrated in this study that in-line monitoring technique such as FBRM is very useful and essential not only for purpose of in-line process control and product quality control in the industrial process but also for basic study of crystallization behavior such as rapid nucleation.
Acknowledgement

To write up this thesis, deepest respect and gratitude for Professor Izumi Hirasawa of Department of Applied Chemistry, School of Advanced Science and Engineering, Waseda University, for further help and encouragements of me.

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Finally, thanks to my wife, Junko, my sons, Keitaro and Seigo, and my parents for everything.

October, 2010

Keigo HANAKI
Appendix

（博士論文概要書）
近年、地球温暖化、石油資源枯渇などに代表される地球環境問題が注目され、製造業においては、低環境負荷な原料の利用技術開発や、低エネルギー製造プロセス開発が活発化している。化学合成プロセスでは一般に目的化合物、未反応原料、副生成物の混合物が生産され、目的化合物を得るには、その後の分離・精製プロセスが必要となる。目的化合物を分離、精製する溶媒的な方法として、蒸留法、クロマト分離法、膜分離法などが考えられるが、投入エネルギーが大きいなど、環境負荷が大きい場合がある。晶析法では、適切な結晶化条件が設定できれば、高純度な目的化合物固体が得られる。これは、つまり、晶析法は先に述べた手法と比較した場合、精製、濃縮（固液分離）を同時に達成できる点で優位な単位操作であり、低環境負荷な分離、粉末化プロセスと言える。本論文は五章から構成されており、第一章では医薬品に代表される有機結晶製造プロセスにおける課題とそれらに対する本論文でのアプローチをまとめた。第二章から第四章では、具体的な検討結果を示し、第五章では本論文を総括した。晶析法は食品、ファインケミカル、医薬品分野等で幅広く利用されており、本論文も医薬品の晶析プロセスに関する研究である。晶析プロセスの操作条件は目的化合物の溶解度、過飽和度、種結晶、結晶形、摂拌、結晶成長速度、結晶核発生速度、凝集などさまざまな因子により影響を受ける。これら因子が結晶生成物に与える影響やその速度論の多くは晶析工学で体系化されているが、これらすべての因子を考慮して晶析プロセスをモデル化するには、パラメーター算出のために多くの実験数を必要とするとともに、計算過程も煩雑になる。特に医薬品の晶析プロセスでは、必要とされる要求品質は不純物の除去（高純度化）、結晶多形制御などであり、溶媒種等の晶析条件がある程度適切であれば、これらは晶析過程における過飽和度を十分に制御することで達成される場合が多い。従って、できるだけ少ない予備検討（パラメーター算出実験など）により、迅速に晶析プロセスを最適化（過飽和度制御）できるのは、工業晶析プロセスに関するニーズの一つである。

本論文第二章、第三章ではこの課題に焦点をあて、バッチ式冷却晶析（第二章）および貯溶媒晶析（第三章）について過飽和度制御を目的とした場合の適切な操作条件を迅速に提供できる最適化手法の開発について報告した。本手法は対象とする系において、温度または溶媒組成をステップ状に変化させる多段ステップ応答実験を1回実施し、結晶成長速度を表現するのに必要なパラメーターを推算し、それらを利用して、適切な冷却プロファイルや、貯溶媒添加プロファイルを推算するフィードフォワード型最適化手法である。核化、凝集、結晶破砕などは観察し、結晶成長は結晶サイズに依存せず、種結晶が均一に成長するなどさまざまな仮定を導入した結晶成長速度式を用いた。結晶成長速度を表現するのに必要なパラメーターは結晶成長速度係数と溶解度であるシンプルな速度式である。多段ステップ応答実験では、温度及び溶媒組成をステップ状に変化（冷却及び貯溶媒の増加）させた直後から減少する溶質濃度をオンライン分析技術（ReactIR）でモニタリングし、溶質濃度変化から算出される結晶成長速度をモデル化
度式にフィッティングすることでパラメーター推算を行った。
本論文では薬品原液（分子量約 400）の冷却晶析（DMF/water 溶媒系で 50℃から 20℃への冷却晶析）および溶媒晶析（過溶媒重量比率を 8%から 38%程度まで増加）について検討した結果、晶析過程での結晶成長速度と過飽和度は計算値と実験値で良好な一致を示した。次に示したように仮定の多い結晶成長速度式を用いているが、検討した条件範囲では晶析過程での結晶成長速度及び過飽和度を正確に表現することができた。第 2 章では過飽和度を一定値（過飽和度 17%）に制御することを目的に最適冷却プロファイルを算出し、算出されたプロファイルに沿って晶析実験を实施したところ、晶析過程での過飽和度を目的値で一定にできることも確認できた。本薬品原液の冷却晶析に関しては従来型の過飽和度制御冷却プロファイル（上に凸な冷却プロファイル）では不十分であることを示し、算出された最適冷却プロファイルは直線的な冷却プロファイルであった。溶媒晶析に関する検討（第 3 章）では、結晶成長モデルから計算された理想的な粒子径と、晶析実験後の結晶粒子径は概ね一致（体積平均径 15～17μm）しており、極端な核生成を伴わなければ、粒子径に関してもある程度正しく表現できることがわかった。また、溶媒晶析に関しては、その最適化手法に関する研究例は少なく、本研究は学問的にも有意義であると考える。
この最適化手法検討では反応器温度を迅速かつ精密に制御できる攪拌式ガラス反応器（RC1）と、in situ FTIR スペクトルスコピー（ReactIR）や in situ 粒度分布測定装置（FBRM）などのインライン分析技術などを活用することが一つの特徴になっている。特に粒度分布測定に関して、従来の測定装置にはレーザー回折式や遠心沈降式などがあるが、これらはサンプリングし、希釈、分散などの試料調整後に測定するのが一般的であり、その際に実際の系とは粒子状態（サイズ、凝集体など）に変化してしまうことが懸念される。そこで結晶化過程を解析することは困難である。FBRM は晶析プロセスの観察に関して、核化現象や凝集中の結晶など不安定な粒子を観察するのに優れた測定装置であると言える。
第四章では FBRM インライン分析技術を活用した、微結晶製造をターゲットとした薬品添加晶析プロセスの基礎的検討を行った。近年まで難溶性薬品の吸収性、有効性を向上させる手法は段階的検討されている。界面活性剤などの可溶化剤を用いない場合、対象化合物を非晶化する、準安定な結晶形にする、微細な結晶にすることなどで吸収性、有効性の向上が期待される。工業的視点から二次核生成を促進させて微結晶を製造する方法は、粉砕技術を利用しない、ボトムアップ式手法として有効である。二次核生成に際しさまざまなメカニズムが報告されており、流体せん断力や結晶間衝突などが挙げられている。定性的には高過飽和度、高せん断力、高懸濁濃度での晶析操作が有効であると考えられ、筆者らは T 型ミキサーと高せん断ミキサーをこの順に連結させた工業晶析プロセスを提案している。T 型ミキサーで目的化合物の溶液と種結晶が分散した難溶薬を衝
突混合させ、直下の高せん断ミキサーでせん断力を付与することで二次核の生成が期待される。微結晶製造プロセスへの要求はあるものの、提案プロセスのようなスケールアップ可能な、また、汎用性のあるものに関する研究、また、その結晶化現象に関する研究は少ない。

提案プロセスの基礎検討として、パッチ晶析槽を用いて、種結晶が分散した溶媒中に薬品原薬溶液を短時間で投入する高濃度晶析について、その二次核生成挙動をFBRMで観測した。検討した系では種結晶の存在下であるが、FBRM コード長カウントのトレンドより、誘導期間（30分から80分程度）が観測され、また、誘導期間の長さは種結晶量が多いほど短く、種結晶量で誘導期間を制御できることが判った。投入する溶媒濃度が高いため、つまり溶液投入直後の過飽和度が高い場合（溶解度の15倍以上高濃度の場合）、投入後すぐに様々な核生成が観測される（First nucleation）、その後誘導期間が観測され、誘導期間後は投入直後よりFBRMコード長カウントで10倍程度多い核生成が観測された（Second nucleation）。溶液投入直後の過飽和度が19.5倍の時、二段階核生成後のFBRMコード長分布最頻径は4μm程度であった。さらに興味深いことに、このような二段階での核生成が観測された場合のFirst nucleationで生成した微小粒子は誘導期間中ごくゆっくり成長し、Second nucleationではこれらが一旦凝集したのち、凝集体が分散するのと同時に多量の核生成がFBRMコード長変化から観測された（核生成時に100μm以上のFBRMコード長が増加、減少）。二次核生成誘導期間での微結晶の表面積（結晶成長）過程と誘導期間後の核生成に関する興味深い挙動を観測、考察することが出来た。

本論文では古典的なパッチ式晶析操作に関する操作条件最適化手法の研究と、近年まで鋭意検討されている微結晶製造プロセスに関する核生成挙動の解析研究を実施した。パッチ式晶析操作の最適化手法研究では、必要最小限のパラメータを用いた最適化を利用し、過飽和度制御を目標に、迅速かつ適切な操作条件を導くことができる最適化手法を提案した。本手法は薬品原薬の臨床開発段階など、開発スピードが要求される分野でも十分に適用できる汎用性のある手法であり、工業プロセスにおいてその貢献度は大きいと考えられる。核生成挙動の解析研究では、インライン測定装置を活用することで、これまで観察することが困難であった二次核生成挙動を詳細に観測し、二次核生成に関する新たな知見を見出した。このようなインライン測定技術は工業プロセスのインライン制御の目的だけでなく、研究レベルの検討についても現象理解に有効なツールであることを明示できた。これは新たな研究、解析方法の一端を提示しており、晶析工学の発展に少なからず貢献できたと考える。
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