Regular Article

Highlighted Paper selected by Editor-in-Chief

Study on Control of pH-Modulation Crystallization Utilizing Concept of Dissociation Equilibrium

Eriko Sato,* a,b Shoji Kudo, b and Hiroshi Takiyama b

a Technology Development Department Production Headquarters, Otsuka Chemical; 463 Kagasuno Kawachi-cho, Tokushima 771–0193, Japan; and b Department of Chemical Engineering, Tokyo University of Agriculture and Technology (TUAT); 2–24–16 Naka-cho, Koganei, Tokyo 184–8588, Japan.

Received December 20, 2018; accepted February 1, 2019

Crystallization by pH adjustment, as a type of reaction crystallization, is a solid–liquid separation method widely used in the area of pharmaceutical and pharmaceutical intermediate manufacturing. On the other hand, 3-alkenyl cephem compound is a typical zwitterionic pharmaceutical intermediate that possesses both an amino group and a carboxylic acid group. Such structure affords three main pH regions in solution and results in difficulties using crystallization by pH adjustment for isolation. As a consequence, 3-alkenyl cephem compound is usually crystallized at the point away from the solubility curve, causing unrestricted nucleation and flocculation behavior for the deposited particles which is difficult to filtrate. In this study, the pK a of 3-alkenyl cephem compound was intensively investigated to inhibit the nucleation. An optimal pH level point was also sought to make monodisperse particles. In particular, during crystallization by pH-modulation operation, the key point was identified to be the number of primary particles aggregated in the secondary particles. It was revealed that the increment number of primary particles led to the generation of larger monodisperse particles. This investigation, combined with solid–liquid equilibrium, enabled the acquisition of target species with good operability for filtration process. This present investigation becomes the prosperity in the zwitterion compound production that it has hardships to crystallize and filtrate.

Key words pH-modulation crystallization; dissociation equilibrium; metastable zone width; aggregate; primary particle; filtration

Introduction

Increasing the yield and improving the productivity in the manufacturing of pharmaceutical products are an eternal challenge for pharmaceutical or pharmaceutical intermediate companies facing intensive competition nowadays. 1–3 Still, the quality of products is timelessly of utmost importance. 4 Therefore, striking a delicate balance between manufacturing efficiency and good quality is highly significant. 5 Typically among all the manufacturing processes, the separation process is one of such crucial processes that can significantly affect the overall profitability. 6 Crystallization, as one kind of separation processes of final products, could be inexpensive and efficient and therefore has been widely employed to lessen the possible damages caused by the presence of heat and light. 7 3-Alkenyl cephem compound ((6R,7R)-7-amino-3-[2-(4-methyl-5-thiazolyl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid), Fig. 1 7 is a pharmaceutical intermediate of cefditoren pivoxil. 9 In general, cephem compounds are unstable by heat. 10,11 In order to prevent degradation, 3-alkenyl cephem as shown in Fig. 1 is often treated at a low temperature. 12 And it is typically crystallized by pH adjustment method. 8 In general, various methods use supersaturation during the crystallization process to control the quality of products. 12,13 This is true for the pH adjustment method as well. 14–16 However, regarding the pH adjustment method, few papers discuss the relationship with supersaturation and quality control. This study considers the importance of supersaturation in the pH adjustment method along the lines of crystallization engineering and dissociation equilibrium.

Furthermore, the control of crystal morphology is important from the viewpoint of production efficiency in pharmaceutical manufacturing. 17 Since the filtration time in the solid–liquid separator is a bottleneck in the production of 3-alkenyl cephem compound, 18 there is a need to control crystal morphology while changing the pH. It has been reported that as the crystal morphology changes by supersaturation, the physical properties of the crystal structure (i.e., aggregate size) are different even though the zwitterion are the same. 19 It has also been reported that differences in zwitterion led to different crystal form and diffraction pattern in X-ray diffraction (XRD). 20 Recently, Kong and Leong, and Dutcher and colleagues have clarified the degree to which aggregation is affected when pH is changed. 21,22 These results suggest that supersaturation and dissociation equilibrium play important roles in controlling the degree of aggregation. Aggregates

---

* Present address: Research and Development Department, Otsuka Chemical India; SP-3 10&amp;11 RIIICO Industrial Area Keshwana Rajpoot Kotputli Jaipur Rajasthan, India.

† Present address: Technology Development Department Production Headquarters, Otsuka Chemical; 463 Kagasuno Kawachi-cho, Tokushima 771–0193, Japan: and b Department of Chemical Engineering, Tokyo University of Agriculture and Technology (TUAT); 2–24–16 Naka-cho, Koganei, Tokyo 184–8588, Japan.

© 2019 The Pharmaceutical Society of Japan
which is composed primary particles affect the industrial parameters of filtration time, contamination, drying time, and physical properties in solid-state. Among the aggregated crystals, a single dispersion in which strongly attached fine particles of nano order are quite distinct in chemical and physical properties from a flocculation assembled of micro order particles under weak van der Waals force. The former is formed in the case of low density of primary particles, while the latter gathers indefinitely in the case of high density of secondary particles. These high density secondary particles can constitute a network structure, leading to worse dispersion and more increased cake. In order to overcome these issue, in this present study, the relationship between dissociation equilibrium with aggregation under the pH change is demonstrated. We discovered that the primary particle aggregated rescue the poor filterability.

Experimental

Crystallization Experiment 3-Alkenyl cephem powder as shown in Fig. 1 was prepared according to the practical example in the patent. The solubility curve represents the saturation solubility of 3-alenyl cephem compound at each pH level. In addition, the supersolubility curve indicates the point of supersolubility, which is determined by the point that becomes cloudy when operating the pH adjustment. The zone between the solubility curve and supersolubility curve determines the metastable zone width (MSZW). In crystallization experiment, 3-alenyl cephem powder (7.1 g, 0.22 mol) was added to a 1L separable flask and 500 mL of 1.8 mass% sodium bicarbonate water and 100 mL of acetone were added to a 1L separable flask and 500 mL of 1.8 mass% sodium bicarbonate water and 100 mL of acetone were charged at under 278 K. The slurry was stirred at 200 rpm by using Pfaudler paddle for 10 min. After dissolution at pH 7.0, 39 mL of 6 mol/L HCl was dropped over a period of 30 min. After dissolution at pH 0.8, 25 mass% of NaOH was added dropwise at each dropping speed into the solution and finally adjusted around the isoelectric point around pH 5 at 200 rpm. Three different processes were examined in the experiment. In Exp.-1, the dropping rate was varied. In Exp.-2, seed (1.0 mass%) was added at pH 1.7 and the resulting slurry was aged for an extended period of time. In Exp.-3, after addition of 1.0 mass% seed at pH 1.7, 25 mass% NaOH(aq.) was intermittently dropped into the solution so as to perform the crystallization operation in the MSZW. Regarding on Exp.-2,3, nucleation was not happened without seed. Crystallization ratio Cr. (%) was quantified by using the concentrations of 3-alenyl cephem solution (C₀) and after crystallization (Cᵢ). Cr. can be described by Eq. 1. The concentration was measured at each pH level by sampling 0.5 g of the solution, diluting to 50 mL with water, and then measuring by HPLC (high performance liquid chromatography, Shimazu LC 20 AT) with a wavelength set at 254 nm. The crystallization ratio was observed to be of three values: Cr.ini is the value measured after confirming nucleation and 10 min have passed; Cr.mod is the value when seed is added and aged for each aging time in Table 1; and Cr.mod is that measured after intermittent pH operation by 25 mass% NaOH. Finally, total Cr. achieved 98 mass% after adjusting the isoelectric point.

\[ \text{Cr.} = \left( \frac{C_0 - C_i}{C_0} \right) \times 100 \] (1)

C₀: concentration of 3-alenyl cephem solution before crystallization (mol/L)

Cᵢ: concentration of 3-alenyl cephem solution after crystallization (mol/L)

The slurry after pH adjustment at pH 5 was filtrated with stirring at 200 rpm at atmospheric pressure. A buchner funnel with a diameter of 7 cm and filter paper of ADVANTEC No. 2 (pore size: 5 μm) were used as the filtration system. At the same time, the filtration time was measured from filling of slurry until disappearing of liquid surface on wet cake. After slurry filtration, the obtained wet cake was washed with 110 mL of water and 65 mL of acetone. The dried powder was finally acquired by vacuum drying at 67 kPa for 12 h.

Abundance Ratio of the Different Species 3-Alkenyl cephem has two dissociation points and thereby affords three types of zwitterion, represented by \( \left\{ \text{NH₃}–\text{C}_12\text{H}_10\text{N}_2\text{OS}_2–\text{CO}_2\text{H}, \text{NH}_2–\text{C}_12\text{H}_10\text{N}_2\text{OS}_2–\text{CO}_2\text{H}, \text{and NH}_3–\text{C}_12\text{H}_10\text{N}_2\text{OS}_2–\text{CO}_2^- \right\} \). They are named by the (+) form, the non-dissociated form, and the (−) form, respectively as shown in Fig. 2. Substrate (1.2 g, 0.004 mol) was added into each of the aqueous solutions that were individually adjusted to each pH point from 0.7 to 8.1 by HCl(aq.) or Na₂CO₃(aq.) in 100 mL of 20 vol% acetone aqueous solution. Then the abundance ratio of the zwitterion was investigated. The (+) form was defined as dissolved substrate in acidic side. And the (−) form was defined as dissolved substrate in basic side. The non-dissociated structure was remaining as solids. Amount of dissolved substrate was measured by HPLC (Shimadzu LC 20 AT) with a

<p>| Table 1. Result of Crystallization Experiments |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Entry</th>
<th>Filtration time (min)</th>
<th>NaOH dropping (mL/min)</th>
<th>Seeding pH</th>
<th>Aging (h)</th>
<th>Cr.ini (%)</th>
<th>Cr.mod (%)</th>
<th>pH Range in modulation operation</th>
<th>pH Modulation times</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>1.4</td>
<td>1.7</td>
<td>4</td>
<td>57</td>
<td>56</td>
<td>1.70–1.75</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>1.65–1.75</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>0.1</td>
<td>1.7</td>
<td>6</td>
<td>27</td>
<td>2</td>
<td>1.40–1.70</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>81</td>
<td>2</td>
<td>1.70–1.75</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>84</td>
<td>2</td>
<td>1.65–1.75</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>88</td>
<td>2</td>
<td>1.40–1.70</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>81</td>
<td>2</td>
<td>1.70–1.75</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>84</td>
<td>2</td>
<td>1.65–1.75</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>0.5</td>
<td>1.7</td>
<td>2</td>
<td>88</td>
<td>2</td>
<td>1.40–1.70</td>
<td>4</td>
</tr>
</tbody>
</table>

Cr.ini: Crystallization ratio after confirming nucleation and 10 min have passed (%). Cr.mod: Crystallization ratio when seed is added and aged for each aging time in Table 1 (%). Cr.mod: Crystallization ratio after intermittent pH operation (%).
wavelength set at 254 nm. The abundance ratio was assumed to be the point where the equilibrium incline curves intersected as can be seen in Fig. 2. Two intersection points of these curves were shown as \( pK_{a1} \) and \( pK_{a2} \) and the abundance ratio of each zwitterion was 50%.

**Powder Test** In this study, final products after drying were called secondary particles. The resulting secondary particles powder was examined by Scanning Electron Microscope (SEM, Hitachi High-Technologies S-4800). Median diameter \( d_{50} \) and coefficient of variation \( CV \) were calculated from a count of one hundred particles using Krumbein diameter by SEM images. Primary particles were defined as those secondary particles that were changed to a dispersed aggregate state after being subjected to an ultrasonic wave charge. 100 mg of 3-alkenyl cephem primary particles was then added to 200 mL of water as a dispersant for 5 min. The resulting slurry of primary particles was measured by laser diffraction method using a wet type particle size distribution meter of Shimazu SALD-2100. Median diameter and \( CV \) were estimated. In addition, the number of primary particles \( N \) was estimated by Eq. 2.

\[
N = \frac{d_{50(2)}}{d_{50(1)}}, \quad (2)
\]

\( d_{50(1)} \): Median diameter of primary particles (\( \mu m \)).
\( d_{50(2)} \): Median diameter of secondary particles (\( \mu m \)).

**Results and Discussion**

When producing 3-alkenyl cephem in the manufacturing process, filterability is often to be poor and the separation process is the bottle-neck of the total process. In order to solve this problem, the relationship between pH and supersaturation in crystallization was examined in this study.

**Exp.-1 Dropping Rate Change of NaOH** Dropping rate of NaOH was set as 1.4, 0.5, and 0.1 mL/min in entry 1, 2 and 3, respectively. The experiment condition and the crystallization ratio \( Cr_{ini} \) is shown in Table 1. As the dropping rate was varied, turning points of pH differed during the dropping NaOH and are shown as \( \bigcirc \), \( \bullet \), \( \square \) in Fig. 3. At the turning points at around 3 mol/mol of NaOH addition/3-alkenyl cephem in each dropping rate, the solution turned cloudy immediately. When the dropping rate was adjusted to 0.1 mL/min in entry 3, the filtration speed was shortened. The lower cloudy point was, the faster filtration speed became. It is possible that the amount of \( Cr_{ini} \) can be controlled filtration speed. However, considering the amount of \( Cr_{ini} \) and the results shown in Fig. 4, it seems that the rapid nucleation occurs at around pH 1.9/\( C_0 \) (0.034 mol/L) that the point most distant from the...
Seed was added at the non-dissociated form of the zwitterion shown in Fig. 2. The study was then focused on the pH range where the abundance of the non-dissociated reached 100%. Since change of nucleation is small and growth is dominant, nucleation becomes dominant and the amount of fine crystals increases, which is an undesirable outcome. Thus understanding the relationship of the abundance ratio of the non-dissociated form and the solubility curve is important for identifying the pH level where they converge.

Exp.-2 Addition of Seed In general, seed is used to control the amount of nucleus and the nucleation time. The region between the solubility curve and supersolubility is defined as MSZW in Fig. 4. As shown in the La Mer model, since change of nucleation is small and growth is dominant in this region, nucleation can be restricted in this region. Assuming that the most suitable point of seeding would be in the MSZW, this was the target site. As indicated by the solid line in Fig. 4, MSZW was supposed to be around pH 1.2–1.4 when the concentration was defined by $C_0$ (0.034 mol/L). However, nucleation did not occur in the acidic solution of 3-alkenyl cephem when NaOH was added as a reactant.

The reaction of the (+) form and NaOH was thought poor. It was assumed that the dissociation equilibrium inclined to the non-dissociated side when NaOH was added as a reactant.

Exp.-3 Crystallization by pH-Modulation When crystallization naturally occurs in the pH range where the abundance ratio of the non-dissociated form approaches 100% in Fig. 2, the supersaturation will be high and solubility will be low. Therefore, nucleation becomes dominant and the amount of fine crystals increases, which is an undesirable outcome. On the other hand, crystallization does not generally occur in the pH range where the non-dissociated form exists in only small amounts. A desirable outcome could be derived from this method. This is not realistic in the actual manufacturing process of pharmaceutical intermediates that requires greater efficiency. It seems that the reaction of the (+) form and NaOH was affected by the dissociation equilibrium, since nucleation was took long aging time, the reactivity between the (+) form and NaOH was thought poor. It was assumed that the dissociation equilibrium inclined to the non-dissociated side when NaOH was added as a reactant.

Table 2. Result of Powder Tests

<table>
<thead>
<tr>
<th>Entry</th>
<th>$d_{50}$ of primary particles ($\mu$m)</th>
<th>$d_{50}$ of secondary particles ($\mu$m)</th>
<th>$CV$ of secondary particles (%)</th>
<th>Number of primary particle ($\times 10^4$ count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>22</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>26</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>28</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>90</td>
<td>60</td>
<td>81,494</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>73</td>
<td>26</td>
<td>45,634</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>59</td>
<td>20</td>
<td>526</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>50</td>
<td>38</td>
<td>14,367</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>74</td>
<td>30</td>
<td>45,339</td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
<td>96</td>
<td>28</td>
<td>129,502</td>
</tr>
</tbody>
</table>

$CV$: Coefficient of variation of crystalline particles. $N$: Number of primary particles ($\times 10^4$ count).

In order to conduct restricting the nucleation by seed, the study was then focused on the pH range where the abundance ratio of the non-dissociated reached 100%. Seed was added at the $pK_a$ (pH: 1.7) and crystallization occurred slowly in entries 4, 5, and 6. After aging, NaOH was dropped at 0.5 mL/min until pH 5 in each experiment. $Cr_{ini}$ was restricted by seeding and aging as seen in Table 1. And it became possible to increase $Cr_{agi}$ after aging. As a result, the filtration time that had been a problem was improved in entry 6. The longer the aging time was, the bigger the primary particle became. The monodispersity of secondary particles is shown in Fig. 6(a). However, a long period of aging was required in this method. This is not realistic in the actual manufacturing process of pharmaceutical intermediates that requires greater efficiency. It seems that the reaction of the (+) form and NaOH was affected by the dissociation equilibrium, since nucleation was took long aging time, the reactivity between the (+) form and NaOH was thought poor. It was assumed that the dissociation equilibrium inclined to the non-dissociated side when NaOH was added as a reactant.
appropriate range merely by monotonous operation. For this reason intermittent dropping of NaOH was investigated.

NaOH was dropped intermittently in entries 7, 8, and 9. In this section, the following effects were expected by intermittent addition of NaOH: (1) temporary increase of pH, (2) generation of supersaturation associated with this increase of pH, (3) crystallization induced by this supersaturation, (4) decrease of pH by crystallization, and (5) repeat steps (1) to (4). The crystallization would occur while adjusting the pH within a certain range by the pH-modulation operation. When the pH was modulated at 1.7, which is very close to pK_a, Cr_{ini} was restricted and 80% of the crystals were precipitated during pH-modulation as shown in Cr_{mod} of Table 1. Also, the amount of secondary particles was further increased by modulating the pH around pK_{at}. It is presumed that the number of primary particles N influences the creation of the monodispersity in Fig. 6(b). And monodispersed secondary particles were made filtration speed fast. Within this monodispersity, crystallite did not grow largely, but nano-sized primary particles were closely aggregated and finally became large solid secondary particles as shown in Table 2. One possible reason for this could be the fact that around pK_{at}, the (+) form is exsisted as well as the non-dissociated form when the crystal lattice is formed. It is possible that the surface of the primary particles to be easily charged and the secondary particles are dispersed. Thus it was considered that the nano-sized primary particles were aggregated and the surface of secondary particles was charged near pK_{at}, so that the secondary particles were growing in a monodisperse state.

In addition, by operating in the pH range of entry 9 and increasing the modulation operation range and times, it became possible to perform a crystallization operation along the solubility curve as shown in Fig. 7. The filtration time of the slurry was 26 min, and the filtration time improved from entry 6 in which the aging time had been so long. The crystallization ratio for each pH was measured in Fig. 8 and the crystallization curve was compared with entry 2. In entry 2, the crystallization operation in the vicinity of solubility and determining the optimal modulation frequency. In this research, pH-modulation of crystallization is demonstrated to be the method to create beneficial effects both in the crystallization/filtration step and in the zwitterion compound production where process efficiency is of high importance.

**Conclusion**

Traditionally, the crystallization of 3-alkenyl cephem has been produced industrially at low temperatures in order to protect the stability of the substance. However, this process at low temperatures is a contradictory parameter in temperature-keyed mechanisms such as chemical reaction and crystal growth. To address this problem, this study focused on the reaction between NaOH and the (+) form of 3-alkenyl cephem by the crystallization method called pH-modulation operation. It was revealed that the crystallization could be controlled to gradually raise the pH, to incline the dissociation equilibrium toward the non-dissociated form, and to generate the supersaturation. Initial nuclear content could be suppressed and secondary particles increased without an extended aging time. The obtained monodisperse particles were like a pseudogrowth body in which a large number of primary particles N were aggregated and increased, rather than being a crystallite growth. The CV value was maintained while primary particles gathered, and the crystal itself was made large. This method has the possibility to shorten filtration time by repeating the crystallization operation in the vicinity of solubility. Such maneuver is enabled by widening the modulation pH range and determining the optimal modulation frequency. In this research, pH-modulation of crystallization is demonstrated to be the method to create beneficial effects both in the crystallization/filtration step and in the zwitterion compound production where process efficiency is of high importance.

**Acknowledgments** We are grateful to Dr. Pengyu Xu, Dr. Norihisa Hamaguchi, and Kazuhiro Fukae (Otsuka Chemical Co., Ltd.), who cooperated for analytical support and discussion during crystallization experiment and powder test.

**Conflict of Interest** Ms. Sato is employees of Otsuka
Chemical Co., Ltd. She used a research theme and research facility of Otsuka Chemical Co., Ltd. Dr. Kudo has no conflict of interest. Prof. Takiyama has no conflict of interest.

References