

Parameters Determining the Agglomeration Behavior of Anhydrous L-ornithine-L-aspartate (LOLA) Crystals Prepared by Drowning Out Crystallization

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Abstract—Crystallization of L-ornithine-L-aspartate (LOLA) by drowning out was performed for the production of the anhydrous form of LOLA. The needle-like LOLA crystals were formed and spherically agglomerated during precipitation in a semibatch crystallizer. The primary crystal size in the agglomerate remains unchanged after completion of the crystallization. Therefore, the agglomeration process of primary crystals played an important role for controlling LOLA crystal size. The agglomeration of LOLA crystals was governed by not only the physico-chemical parameters such as the temperature and feed concentration, but also the hydrodynamic parameters such as agitation speed and feeding rate. The crystal size and the shape have been shown to be important factors in product impurity and flowability. Thus, the optimum condition of LOLA crystallization process by drowning-out could be obtained.

Key words: Drowning-out, L-ornithine-L-aspartate, Pseudopolymorph, Agglomeration, Impurity

INTRODUCTION

Crystallization by drowning out is widely used for the separation of pharmaceutical products and biomaterials in industrial processes [Myerson, 1993; Wolfgang, 1999]. In drowning out crystallization, the supersaturation is generated by the addition of an antisolvent which reduces the solubility of a solute. This method is preferred rather than the evaporative or cooling crystallization for high soluble material which has weak temperature dependence on solubility [David et al., 1997]. In particular, it is also suitable for separation of heat labile material because crystallization can occur at low temperature. However, large quantities of antisolvent need to be handled in order to obtain the desirable yield and to reduce impurities incorporated into the crystal, which are the main disadvantages of this method. In the pharmaceutical industries, both the crystal size and the shape have an effect on the solid-liquid separation and so purity, and on downstream processes, such as capsule-filling and tablet-making for solid dosage forms [Chulia et al., 1994]. According to the study of drowning-out crystallization, the addition rate of antisolvent and agitation rate were found to be significant factors in determining the crystal size and shape [Charmolue et al., 1991; Han et al., 1993]. It was suggested that the purity may be related to size and shape of product crystals. The crystal size and the shape have been also shown to be important factors in flowability and compressibility [Szabó et al., 2001]. Therefore, it is essential to investigate the operational variables for the effective crystallization by drowning out.

Small sized crystals ($<10\ \mu\text{m}$) are often needed in biomaterial or pharmaceutical industries due to their high bioavailability and dissolution kinetics. However, their downstream processing may be difficult such as filtration, handling and storage. Therefore, the production of spherical agglomerates of pharmaceutical compound crystals

has gained great attention, because the modification of the crystal habit can change the bulk density, flowability, compactibility and stability [Kawashima et al., 1982; Brittain, 1999]. Moreover, the formation of the spherical crystal agglomerates is important for preparing the solid dosage forms by capsule filling and tablet making. Agglomeration, which is widely used for enlarging crystals by drowning out crystallization, can be defined as a two step process. One is a step of encounter between particles due to fluid hydrodynamics and the other is a coalescence step [Mulline, 1993]. Two major effects on agglomeration are physico-chemical parameters for probability of sticking and hydrodynamic parameter for collision frequency.

In our work, agglomeration of L-Ornithine-L-Aspartate (LOLA) was investigated for controlling the size and crystal size distribution. LOLA is a complex of the basic amino acid (L-ornithine) with acidic amino acid (L-aspartic acid). L-ornithine ($\text{C}_5\text{H}_{12}\text{N}_3\text{O}_2$), an antidote to ammonia in the blood, is known to play an important role in the urea production of the ornithine cycle in the living body. L-aspartic acid ($\text{C}_4\text{H}_7\text{NO}_4$) also plays a role as an amine-donor in the ornithine cycle and thus is used for preventing hepatic disturbances. Their simultaneous administration in the form of LOLA has proven to be more effective in reducing blood ammonia concentration in patients with acute liver failure [Vogels et al., 1997; Rose et al., 1998, 1999; Rees et al., 2000]. In our previous study, we reported that the agglomerates of needle-shaped crystals of LOLA could be obtained by adding aqueous solution of LOLA to antisolvent, methanol [Kim et al., 2003]. It is important in this method to control the speed of addition of the aqueous solution and the temperature in order to avoid undesired polymorphs, hydrates. The hydrate of LOLA is not suitable for commercial purposes because of coloration and swelling during a long period of storage time, while the anhydrous form does not have such problems. When methanol was added into the LOLA aqueous solution, the anhydrous form was only obtained at temperature above 60°C . On the other hand, when the LOLA aqueous solution was added into methanol, the

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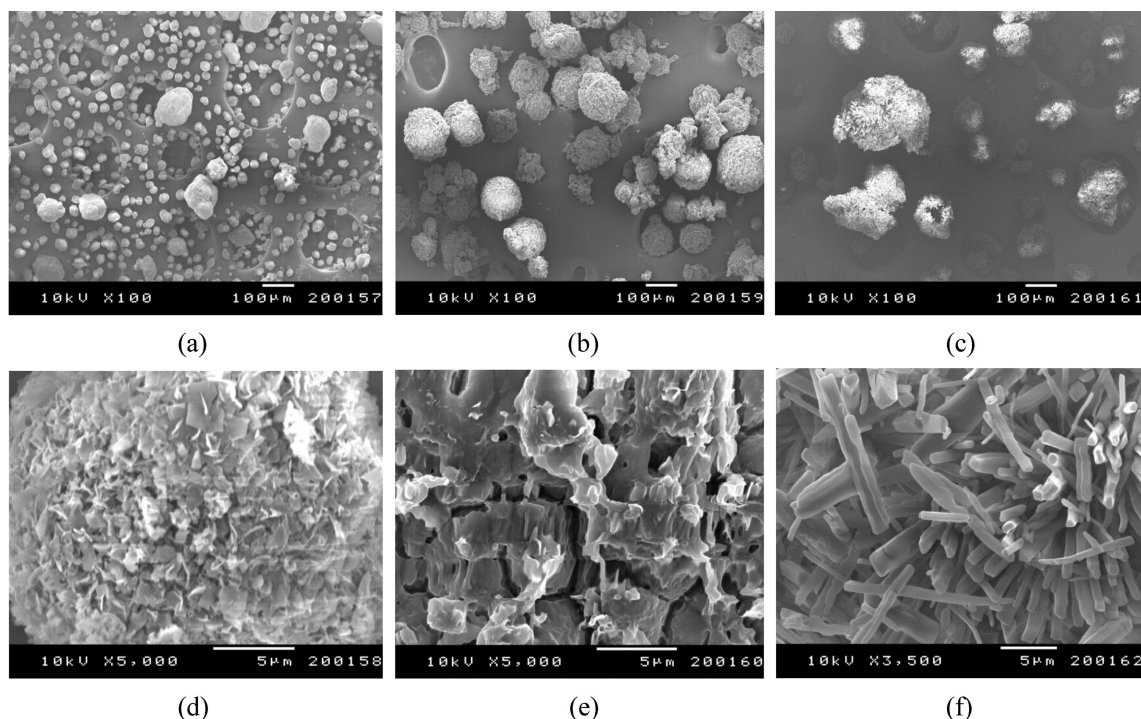


Fig. 1. Polymorphs of LOLA (a) hydrate, (b) amorphous, (c) anhydrate, (d) surface of hydrate, (e) surface of amorphous form, (f) surface of anhydrate.

crystal form depended not only on the operating temperature but also on the feeding rate of the LOLA aqueous and methanol concentration. As such, our previous study has focused on controlling the polymorphs in crystallization, and two agglomerated polymorphs are shown in Fig. 1. The morphology of LOLA crystals was the spherical shape of crystal agglomerates. The overall appearances of the three forms look alike, but the surface features are very different. The hydrate of LOLA is shown to be agglomerates of flake-like crystals (Fig. 1d) and the anhydrate appears to be agglomerates of needle-like crystal (Fig. 1f). When the feeding rate is fast at low temperature (25 °C), the excess supersaturation of the solution becomes large and the amorphous form is obtained (Fig. 1e).

In the present work, the influence of the process parameters including operating temperature, feed concentration, agitation speed, and feeding rate on agglomerations is investigated by using a semi-batch crystallizer. The experimental conditions were limited to the range of parameters in which only anhydrous form was obtained.

EXPERIMENTAL

1. Materials and Apparatus

LOLA ($C_5H_{13}N_3O_2^+ \cdot C_4H_6NO_4^-$, MW 265.3) was supplied by Sigma Chemical Co. and deionized water (conductance less than 4 $\mu S/cm$) and methanol (Merck, ± 99.8 mol%) were used without further purification. The experiments were conducted in a semi-batch crystallizer. A jacketed cylindrical glass vessel with 100 mm inner diameter was used. Four stainless steel baffles with 2 mm thickness and 10 mm width are attached to the walls. The temperature of the crystallizer during the experiments was controlled by a heating medium supplied by a thermostat (PolyScience, model 9510).

Materials were prepared by mass using a balance with an uncertainty of ± 0.0001 g. Weighed quantities of materials were charged to the crystallizer, and agitation was provided with a pitched-blade stainless steel impeller. A peristaltic pump was used to transfer the feed from a stock vessel to the crystallizer at the desired feeding rate. The feeding line was a silicon tube with 3 mm inner diameter.

2. Experimental Procedure

The crystallization experiments of LOLA were conducted by adding LOLA aqueous solution into an antisolvent, methanol. As the LOLA aqueous solution with a constant flow rate was added into the bulk methanol phase, a high supersaturated state of LOLA complex was immediately created and the anhydrous form of LOLA crystals tended to be nucleated. As the amount of LOLA aqueous solution increased, the agglomeration of crystals occurred. During the experiment, the supersaturation was determined by monitoring the initial feed concentration of LOLA.

The crystallization temperature was controlled at constant 40, 50 and 60 °C. The feeding rate and feed concentration of LOLA solution were varied from 0.1 to 1.0 g/min and from 0.5 to 2 mol/l, respectively. The total amount of LOLA aqueous solution used was 30 g. The agitation speed was varied from 300 to 700 rpm. At these conditions, no particle breakage was observed. After feeding LOLA aqueous solution, 1 ml of suspension was sampled at a suitable interval from the crystallizer by using a micropipette. The size distribution of the crystals dispersed in a methanol solution was measured by particle size analyzer (HIAC 9703). To investigate the crystal structure and morphology, the slurry was sampled from the solution at a desired crystallization time. Then the crystals were filtered and dried for 24 hr at 50 °C for the analysis. The surface morphology of agglomerated crystals was observed under a scanning

electron microscope (SEM).

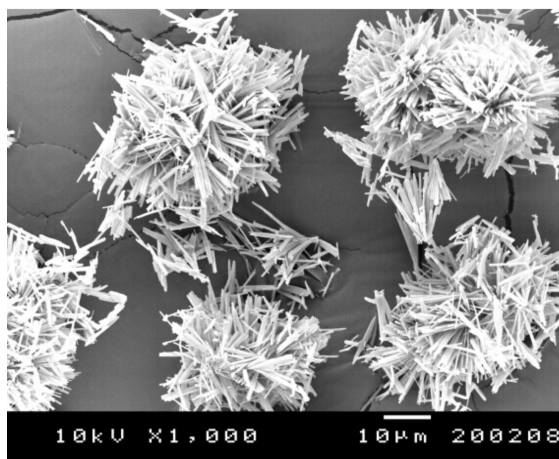
RESULTS AND DISCUSSION

1. Evolution of LOLA Crystal Agglomerate

Crystal agglomeration has been investigated not only from analysis of crystal size distribution but also from microscopic observation. In our previous study, the solubility data of anhydrous LOLA was obtained [Kim et al., 2001]. Solubility of anhydrous LOLA in pure water is considerably high as 95-129 g/100 g H₂O in the temperature range from 25 to 60 °C. However, methanol rapidly reduced the LOLA solubility in water over the whole range of the methanol content studied in the present experiment. Since the solubility of LOLA in 90 mass% of methanol content was below 0.1 g/100 g H₂O, the nucleation was occurring immediately as soon as LOLA aqueous solution was added to the pure methanol. Then, the agglomerates of LOLA crystals could be obtained in the agitated suspension during the feeding period. As shown in Fig. 2, the morphology of the primary crystals was needle type, showing the characteristic morphology of the anhydrous form of LOLA. The needle type primary crystals were agglomerated and then the size



(a)



(b)

Fig. 2. SEM photographs of (a) primary crystal, (b) agglomerates produced after completion of the crystallization.

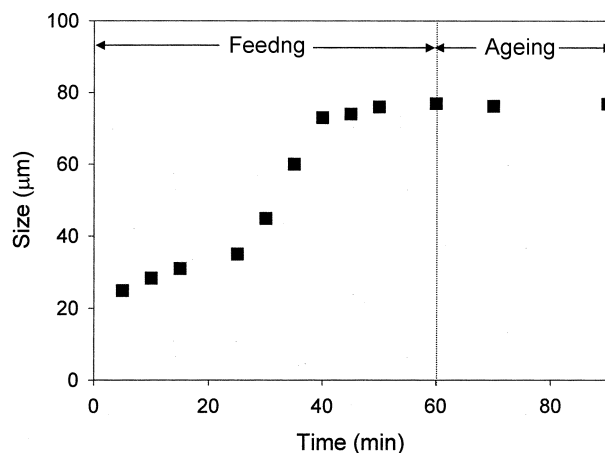


Fig. 3. Effect of feeding time on the mean size of the agglomerates.

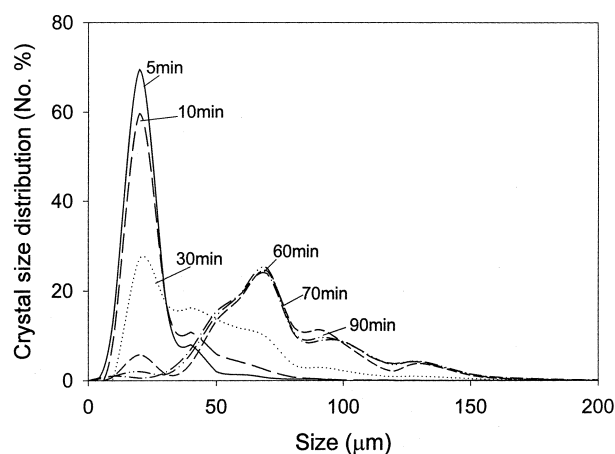


Fig. 4. Effect of feeding time on the crystal size distribution of the agglomerates.

of agglomerates was increased during the feeding time. The size of the primary crystals in the agglomerate was 15-20 µm and remained unchanged after completion of the crystallization. Therefore, these results indicate that the crystal size was dominantly determined by the agglomeration. The mean size of the agglomerates determined by particle size analyzer was plotted as a function of the residence time at constant temperature, 60 °C in Fig. 3. As shown in Fig. 3, the mean crystal size was about 23 µm at 5 min and agglomeration was continued to 75 µm until the end of feeding time, 60 min. The size distributions of the agglomerated crystals are also shown as a function of the residence time in Fig. 4. The primary crystals are well represented by the first population peak within the size range 0-20 µm and the CSD shifted to right due to agglomeration as the feed amount increased. Subsequently, the first population peak disappeared and a broader CSD was obtained as shown in Fig. 4.

After the feeding time was ended, however, the mean crystal size and crystal population of the product suspension were not varied with ageing. After the feeding of desired amount of aqueous LOLA solution into methanol, the supersaturation in the solution was decreased and the agglomeration was terminated. This suggested that supersaturation was necessary to consolidate the agglomerates of

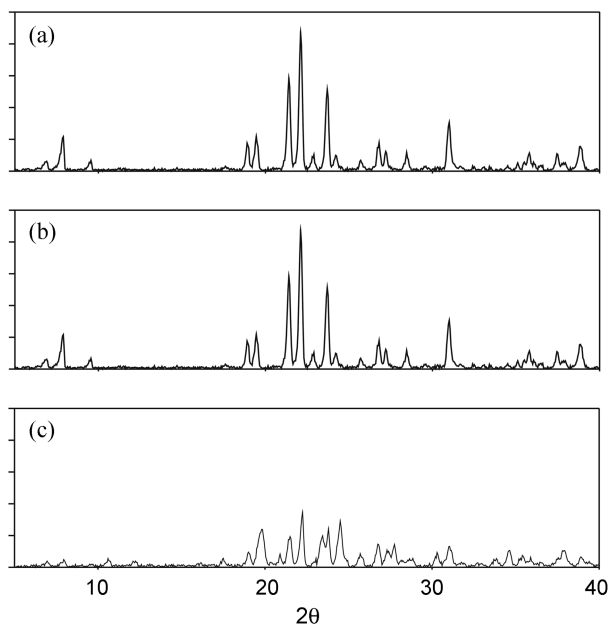


Fig. 5. Powder X-ray diffraction patterns of agglomerates and primary crystals.

(a) anhydrate, (b) anhydrate produced after completion of agglomeration, (c) hydrate

LOLA. When LOLA aqueous solution was fed, the high supersaturation proceeded and hence rapid primary nucleation resulted. Simultaneously, a large number of small crystals present in a highly supersaturated solution may agglomerate easily. This led to the conclusion that feed concentration as well as its feeding rate was important in determining the characteristics of crystal agglomeration.

In this agglomeration process, the recovery rate of the product ranged from 90% to 95%. It was also confirmed that no polymorphic change of the primary crystals was observed in any of the agglomerates by the powder X-ray diffraction patterns of the primary crystals consisting of agglomerates as shown in Fig. 5.

2. Influence of the Temperature and Feed Concentration

The effect of temperature and feed concentration on the agglomeration was investigated at an agitation speed of 500 rpm and feed-

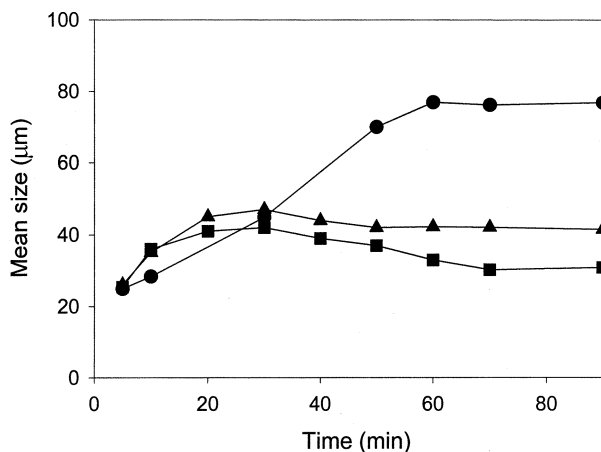


Fig. 6. Effect of temperature on the mean size of the agglomerates (●: 60 °C, ▲: 50 °C, ■: 40 °C).

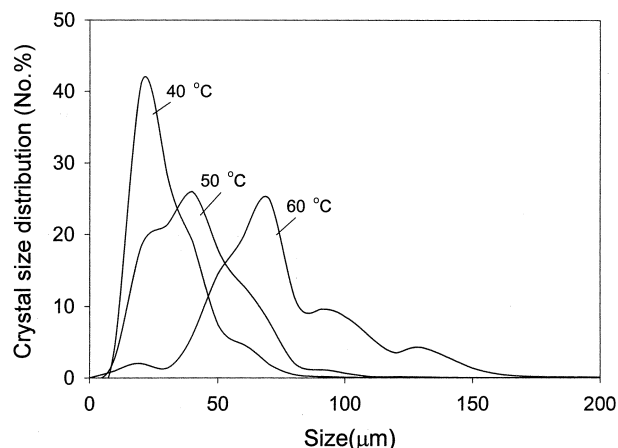


Fig. 7. Effect of temperature on the crystal size distribution of the agglomerates.

ing rate of 0.5 g/min of LOLA aqueous solution. A 30 g of solution of 0.5, 1, 1.5 and 2 LOLA solute mol/kg water was added into 500 ml of methanol at 40, 50 and 60 °C, respectively. The mean size of the agglomerates was plotted as a function of residence time at various crystallization temperatures in Fig. 6. As shown in Fig. 6, the mean crystal size was 35–50 μm in which the LOLA agglomerates formed at the temperatures of 40 and 50 °C. However, at a higher temperature (60 °C), agglomerates larger than 70 μm were observed because of high agglomeration probability for the successful coalescence of the crystals due to the increased diffusion rate of solute at high temperature [Myerson, 1993]. Fig. 7 shows the size distribution of the agglomerates obtained under different temperature conditions. In Fig. 7, the crystal size distribution (CSD) shifted to the right due to agglomeration as the temperature increased. Subsequently, the primary crystalis population peak disappeared and a broader CSD was obtained which was attributed to the strong agglomeration when we compared the CSD at 40 °C with the one at 60 °C.

The cumulative number density was defined as the increasing rate of particle number by nucleation and the decreasing rate of particle number by agglomeration since nucleation and agglomeration occurred simultaneously [Randolph and Larson, 1971].

$$\frac{dN}{dt} = \left(\frac{dN}{dt} \right)_{nuc} + \left(\frac{dN}{dt} \right)_{agg} \quad (1)$$

Fig. 8 shows the variation of cumulative number density against the crystallization temperature and feed concentration. These results better indicated that at higher temperature and feed concentration, more agglomeration was generated. The cumulative number density of the agglomerates was the largest at the crystallization temperature of 40 °C. At temperatures higher than 40 °C, the cumulative number density decreased from 3×10^6 to 10^5 No./ml. As the nucleation rate was increased with increasing the feed concentration at the same temperature and the agglomeration rate was also increased, the change of total cumulative number density was not varied in Eq. (1). Therefore, the difference in the number density at the same temperature was less important for increasing agglomeration rate as shown in Fig. 8.

The mean primary crystal number in agglomerates was plotted

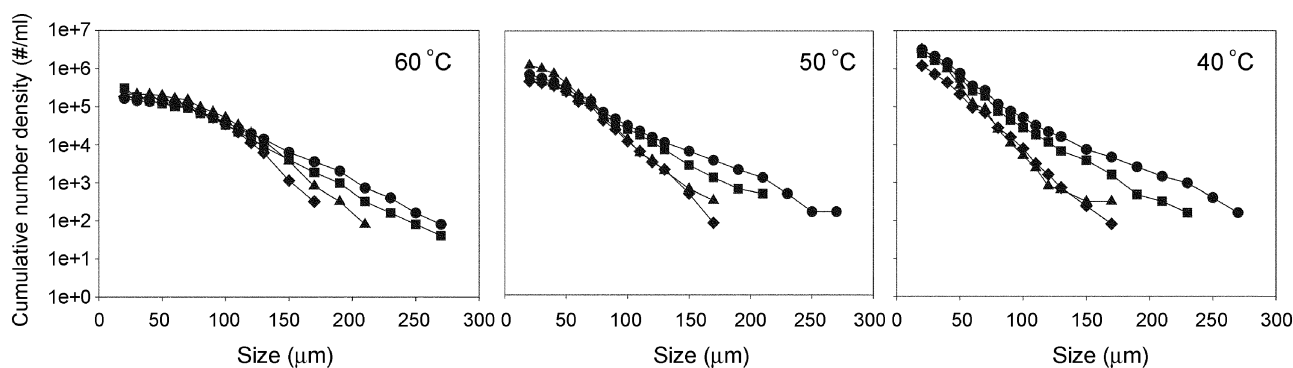


Fig. 8. Cumulative number density of LOLA products (●: 2 mol/kg, ■: 1.5 mol/kg, ▲: 1 mol/kg, ◆: 0.5 mol/kg).

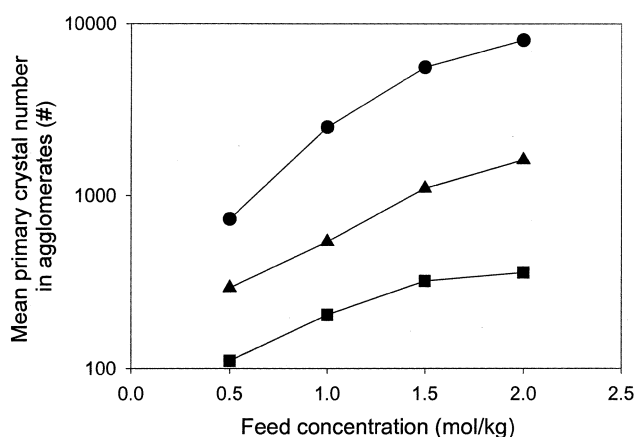


Fig. 9. Effect of feed concentration and temperature on the mean primary crystal number in agglomerates (●: 60 °C, ▲: 50 °C, ■: 40 °C).

against the feed concentration with temperature as shown in Fig. 9. The mean primary crystal number was calculated by

$$N_{mean} = N_{pri} / N_{agg}, \quad N_{pri} = \frac{w_T}{\rho_c V_{pri}} \quad (2)$$

where N_{pri} is the mean primary crystal number in agglomerates, N_{agg} is the number of agglomerates, w_T is solid concentration, ρ_c is the crystal density, and V_{pri} is the primary crystal volume. The number of agglomerates and solid concentration was obtained from experimental data and the mean primary crystal number in agglomerates was calculated by using Eq. (2). As expected, the mean primary crystal number in agglomerates increased with increasing temperature and feed concentration in Fig. 9. The mean primary crystal number in agglomerates was the largest at the crystallization temperature of 60 °C. At temperatures lower than 60 °C, the mean primary crystal number decreased from 10000 to 100 crystal No./agglomerate. The spherical agglomerates of LOLA crystals and their surfaces are shown in Fig. 10. It could be seen that the unagglomerated original crystals were needle type and had a length of 10-15 μm , and their shape and size were not changed. These SEM photographs indicated that temperature and feed concentration were the physicochemical parameters which control the sticking and coalescence of the crystals.

3. Influence of the Agitation Speed and Feeding Rate

The extended results of agglomeration of LOLA with different feeding rates and agitation speeds are shown in Fig. 11. The experiments were performed at 60 °C and the feed concentration of 20 mass% and all the other experimental conditions remained the same as the previous experiments. The feeding rate was varied from 0.1 to 1 g/min corresponding to the residence time, from 30 min to 300 min. The agitation speed was varied from 300 to 700 rpm to change the turbulent intensity, thereby inducing turbulent shear and impact forces on the particles. For particles larger than the Kolmogoroff microscale of turbulence,

$$(D_{agg})_{max} \propto (R)^{-1} \quad (3)$$

where R is the agitation speed and D_{agg} is the size of agglomerates. In Fig. 11, the mean size was found to be in inverse proportion to the feeding rate and the agitation speed. The size of LOLA agglomerates was decreased inversely from 200 μm to 70 μm by increasing agitation speed at constant feeding rate as shown in Eq. (3). If the agglomerates have increased to the maximum size, the stable agglomerates can no longer be ruptured by primary crystals. The higher agitation speed seemed not only to enhance the collision probability, but also to limit the adhesion time among crystals by the shear stress. With increasing agitation speed, the shear force applied to the crystals increased, leading to more dispersed and lower consolidated time, and then resulted in reducing the crystal size of the product. This negative effect of the agitation speed dominated in which the number density of crystals was high and the crystal growth rate was low. From the results, it was concluded that the increase of the collision probability by the agitation speed became negligible. The mean agglomerated crystal size was enhanced at low feeding rate because the residence time was increased. However, when the agitation speed was increased, the effect of the residence time on the mean size became less important.

4. Influence of Agglomerates Size on Crystal Purity

The spherical agglomerates have good flowability and compressibility. However, solvent could easily be trapped between the individual crystals which formed agglomerates. The agglomerates were smaller, contained fewer crystals, and were accompanied by less entrapped methanol. The crystal impurity, the methanol content, was determined by using gas chromatography (HP 4890D). Table 1 indicates that the purity of final products correlated with the size of the agglomerates. Agglomerates of the higher impurity were produced when the mean size of agglomerates increased. When the

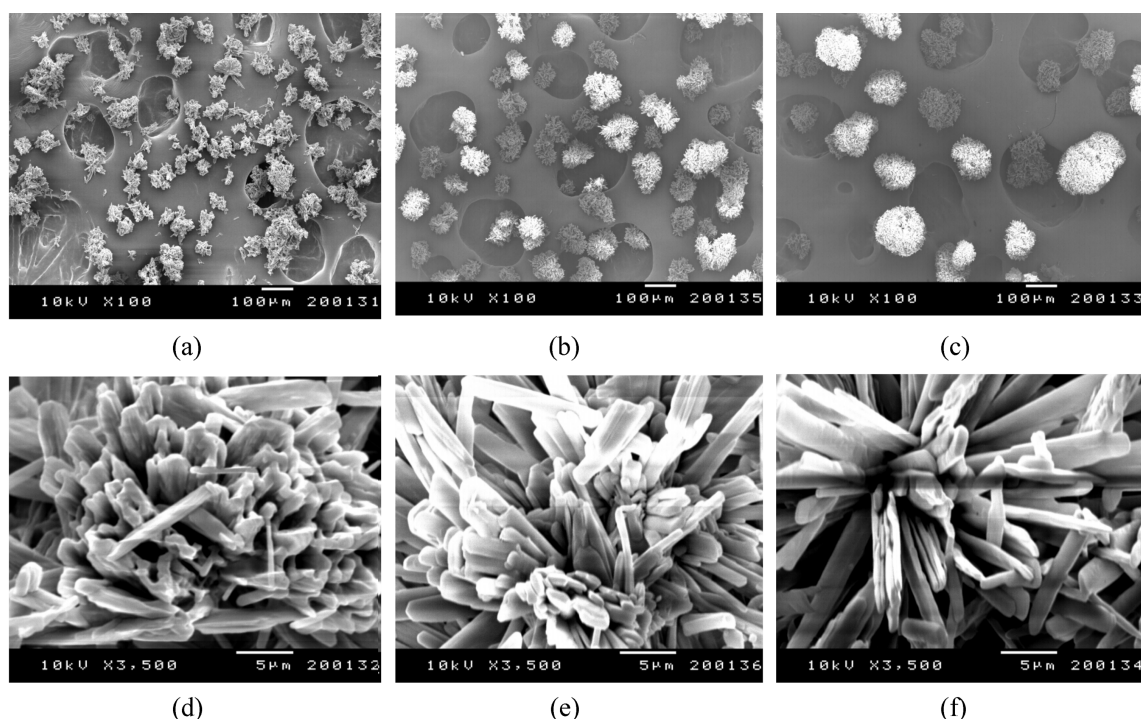


Fig. 10. SEM photographs of LOLA crystals: (a) 40 °C, (b) 50 °C, (c) 60 °C, (d) Surface of (a), (e) Surface of (b), (f) Surface of (c).

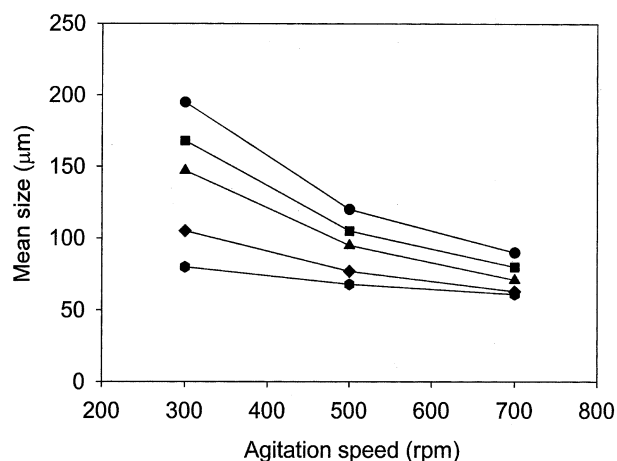


Fig. 11. Effect of feeding rate and agitation speed on the mean size of agglomerates (●: 0.1 g/min, ■: 0.2 g/min, ▲: 0.3 g/min, ◆: 0.5 g/min, ●: 1.0 g/min).

mean size of agglomerates was increased from 30 μm to 147 μm, the methanol content was increased from 163 to 400 ppm. The methanol content in agglomerates having different morphology is also shown in Table 1. The methanol content was two times higher for hydrate than for anhydrate in the same agglomerate size. This in-

dicates that the anhydrate is preferable in terms of purity. Purity, size and morphology appear to be closely related. However, crystal flowability and impurity of agglomerates are restricted conditions with each other. Thus, the optimum condition could be obtained through a compromise between flowability and impurity.

CONCLUSIONS

Agglomerates of LOLA with spherical shape were obtained by drowning out in a semibatch crystallizer. The crystal size and size distribution varied with the process parameters. In the experimental range under consideration, agglomeration phenomenon of LOLA crystals appeared to be ruled by not only by the physico-chemical parameters but also the hydrodynamic parameters. However, because of the complexity of the combined processes (nucleation, crystal growth or crystal agglomeration), the mechanism of LOLA agglomeration is still poorly understood. Therefore, future work should study the mechanism of LOLA agglomeration by using the seed batch crystallizer.

ACKNOWLEDGEMENT

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Table 1. Effect of agglomerate size on the purity of LOLA crystals

Form	Hydrate	Anhydrate	Anhydrate	Anhydrate	Anhydrate	Anhydrate
Mean size (μm)	120	30	41	76	95	147
MeOH content (mg/LOLA kg)	818	163	179	221	310	400

NOMENCLATURE

D	: particle diameter [m]
N	: number of particles
R	: agitation speed [s^{-1}]
t	: time [s]
w_T	: mass of solid [kg]

Greek Letters

ρ_c	: density of crystal [$kg\ m^{-3}$]
v	: volume of crystal [m^3]

REFERENCES

- Brittain, H. G., "Polymorphism in Pharmaceutical Solids," Marcel Dekker, New York (1999).
- Charmolue, H. and Rousseau, R. W., "L-serine Obtained by Methanol Addition in Batch Crystallization," *AIChE J.*, **37**, 1121 (1991).
- Chulia, D., Deleuil, M. and Pourcelot, Y., "Powder Technology and Pharmaceutical Processes," Elsevier, Amsterdam (1994).
- David, A. B., Susan, R. D. and Ka, M. N., "Synthesis of Drowning-out Crystallization-based Separations," *AIChE J.*, **43**, 91 (1997).
- Han, H. K., Lee, S. I. and Lee, C. S., "On the Control of Crystal Size in a Semibatch Crystallizer," *Korean J. Chem. Eng.*, **10**, 100 (1993).
- Kawashima, Y., Okumura, M. and Takenaka, H., "Spherical Crystallization: Direct Spherical Agglomeration of Salicylic Acid Crystals during Crystallization," *Science*, **216**, 1127 (1982).
- Kim, Y. H., Haam, S., Koo, K.-K., Shul, Y. G., Son, J. H. and Jung, J. K., "Representation of Solid-Liquid Equilibrium of L-ornithine-L-aspartate+Water+Methanol System Using the Chen Model for Electrolyte Solution," *J. Chem. Eng. Data*, **46**, 1387 (2001).
- Kim, Y. H., Haam, S., Shul, Y. G., Kim, W. S., Jung, J. K., Eun, H.-C. and Koo, K.-K., "Pseudopolymorphic Crystallization of L-ornithine-L-aspartate by Drowning Out," *I&EC*, **42**, 883 (2003).
- Myerson, A. S., "Handbook of Industrial Crystallization," Butterworth-Heinemann, London (1993).
- Mulline, J. W., "Crystallization," Butterworth-Heinemann, London (1993).
- Randolph, A. D. and Larson, M. A., "Theory of Particulate Processes," Academic press (1971).
- Rees, C. J., Oppong, K., Al Mardini, H., Hudson, M. and Record, C. O., "Effect of L-ornithine-L-aspartate on Patients with and Without TIPS Undergoing Glutamine Challenge: A Double Blind, Placebo Controlled Trial," *Gut*, **47**(4), 571 (2000).
- Rose, C., Michalak, A., Pannunzio, P., Thénien, G., Quack, G., Kircheis, G. and Butterworth, R. F., "L-ornithine-L-aspartate in Experimental Portal-systemic Encephalopathy: Therapeutic Efficacy and Mechanism of Action," *Metabolic Brain Disease*, **13**(2), 147 (1998).
- Rose, C., Michalak, A., Rao, K. V., Quack, G., Kircheis, G. and Butterworth, R. F., "L-ornithine-L-aspartate Lowers Plasma and Cerebrospinal Fluid Ammonia and Prevents Brain Edema in Rats with Acute Liver Failure," *Hepatology (Baltimore, Md.)*, **30**(3), 636 (1999).
- Szabó-Révész, P., Göczö, H., Pintye-Hódi, K., Kasa Jr., P., Erős, I., Hasznos-Nezdei, M. and Farkas, B., "Development of Spherical Crystal Agglomerates of an Aspartic Acid Salt for Direct Tablet Making," *Powder technology*, **114**, 118 (2001).
- Vogels, B. A., Karlsen, O. T., Mass, M. A., Boveé, W. M. and Chamuleau, R. A., "L-ornithine vs. L-ornithine-L-aspartate as a Treatment for Hyperammonemia-induced Encephalopathy in Rats," *J. of Hepatology*, **26**(1), 174 (1997).
- Wolfgang, B., "Nucleation Phenomena During the Crystallization and Precipitation of Abecarnil," *J. of Crystal Growth*, **198-199**, 1307 (1999).