Crystallization of L-Ornithine-L-Aspartate from Water/Methanol Mixture

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Abstract

L-Ornithine-L-Aspartate (LOLA), a pharmaceutical material, has two crystal forms: a hydrate and an anhydrous form. The influence of the feeding rate, solvent concentration and temperature on the form of crystal was investigated in drowning-out crystallization that was carried out by two semi-batch methods. In this study, we attempted to unify results for the formation of polymorphs and LOLA with the desired anhydrous form could be produced with controlling temperature and methanol concentration.

Introduction

LOLA is a complex of the basic amino acid (L-ornithine) with acidic amino acid (L-aspartic acid) and Figure 1 depicts the chemical structure of LOLA. L-aspartic acid is a well-known drug used for preventing fatigue and hepatic disturbance. L-ornithine is also known to be an effective antidote to ammonia in the blood. Their simultaneous administration in the form of LOLA has been appeared to have a synergetic effect [1]. LOLA can exist in two different crystal forms: an anhydrous form and a hydrate. Hydrate of LOLA is not desirable because of coloration and swelling during storage for a long period of time. On the other hand, anhydrous form does not show such problems and thus it is advantageous to produce anhydrous form of LOLA for commercial purpose. Therefore, it is essential to understand crystallization behavior of LOLA for desired product. During crystal growth, incorporation of LOLA molecules to the crystal lattice requires that hydration water molecules be disassociated. However, LOLA crystal cannot be fully disassociated with water molecule according to
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operating condition and forms hydrate. From patent [2], it was known that the critical point of crystallization was temperature (−60°C). Moreover, Salunke and Vijayan [3], using X-ray investigations, have shown the existence of water molecule between two aspartates.

Before investigation of the dehydration mechanism during crystal growth of LOLA, we first study the influence of the operating conditions including the feeding rate, solvent concentration and temperature on the form of crystal and attempt to unify results in terms of the dielectric constant.

**Experiments**

LOLA (C{$_6$}H{$_7$}N$_3$O$_5$·2C$_2$H$_6$NO$_2$, MW 265.3) was supplied by Sigma chemical Co. and used after drying for 12hrs in a vacuum oven at 50°C. Deionized water (conductance less than 4 μs/cm) and methanol (Merck, ±99.8 mol%) was used without further purification. The experiments were conducted in a semi-batch crystallizer. A 1liter-jacketed cylindrical glass vessel was used and agitation was provided with a pitched blade impeller of stainless steel. The temperature of crystallizer during experiments was controlled by a heating medium supplied by a thermostat (Polyscience, model 9510). A peristaltic pump was used to transfer the feed from a stock vessel to the crystallizer at desirable feeding rate. The feeding line was a silicon tube with 3mm inner diameter.

In this study, experiments on LOLA crystallization were conducted by two methods and termed the M and W processes. For the M process, solution of LOLA was added to methanol that was used as an antisolvent. For the W process for crystallization, the methanol was slowly added to aqueous solution of LOLA. In this study, 50g LOLA / 100g water solution was selected for recovery of LOLA by drowning-out. After the crystallization was finished, the solutions were filtered and the crystal was dried. Each polymorph was identified using a FT infrared spectrometer (Genesis, ATI-Mattson) and NMR (JNM ECP-300, JEOL).

**Results and Discussion**

From the infra-red absorption spectrum (KBr disc) it is observed that hydrate has an absorption band near 1500cm$^{-1}$, but not in anhydrous form [2]. Results of solid-state C$^{13}$ NMR spectroscopy also clearly exhibit characteristic peaks with crystal structure. Figure 2 shows the values of ν CO chemical shifts for two crystal forms. In hydrate, new chemical shift of ν CO was appeared at 175 ppm. It may be explained the complexes between LOLA and water by hydrogen bond. The crystal structure of hydrate is stabilized by ionic interactions and hydrogen bonds. Each amino group is involved as a
donor in three N-H-O hydrogen bonds. The α-carboxylate oxygens accept three hydrogen bonds. But side chain carboxylate oxygens of L-aspartate accept four hydrogen bonds, including one from the lone water molecule in the structure. Water molecule exists between two aspartic acids [3].

Solubility of anhydrous LOLA in pure water is considerably high as 95g – 129g/100g H₂O in the temperature range from 298.15K to 333.15K[4]. Solubility measurements of anhydrous LOLA were carried out in the methanol/water mixture with methanol content up to 90 mass %. Notably, over the whole range of methanol content studied, the LOLA solution has a drowning out effect by methanol content. In the W process, hydrate is formed below 60°C regardless of controlling methanol content as similar to the previous results [2]. However, anhydrous LOLA was found to form below 60°C in the M process. Figure 3 is typical results of the M process represented on the plot of dielectric constant with methanol content at two feeding rates of LOLA aqueous solution. Solid lines in Figures 3 present dielectric constants measured as a function of methanol content of methanol/water solution without LOLA. In this figure, the starting point of experimental runs is the pure methanol side and thus fraction of methanol in the solution decreases with time. At the initial stage of the M process at 1ml/min, the anhydrous crystals are produced at all temperature range of the present experiments as can be seen from Figure 3. Then the hydrate appears as increasing the addition of LOLA/water solution. These results indicate that bulk methanol takes immediately away water molecules with LO and LA molecules and eventually anhydrous LOLA that is insoluble in methanol are precipitated. As the water content increases, attraction force of methanol with water is weakened and thus hydrates of LOLA are crystallized. It should be noted that the all hydrate forms seem to be generated when the dielectric constant is higher than about 42 independent of temperature.

It was also observed that anhydrous LOLA formed above 60°C in the W process. The nucleation in the W process was occurred at 60wt% and dielectric constant is only below 42 at 60°C. Therefore the dielectric constant of 42 may be a criterion for nucleation of hydrate and anhydrate of LOLA in this system. The extended result of LOLA crystal structure initially formed at 4ml/min is shown in Figure 3. As feeding rate was increased from 1ml/min to 4ml/min, critical temperature for the formation of anhydrate was increased to 40°C. As a result, the mixing of solvents, that is, the transfer of water molecules from the interface crystal and dielectric property of solution, that is, dehydration energy barrier, are important for understanding the dehydration mechanism. Disassociation of hydration water from the crystal surface to the bulk solution is very likely the controlling step in LOLA crystal growth.
ACKNOWLEDGEMENTS

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REFERENCES


![Chemical Structure and SEM photograph of LOLA](image)

Fig. 1. Chemical Structure and SEM photograph of LOLA

![C13 NMR spectra of LOLA](image)

Fig. 2. C^{13} NMR spectra of LOLA
(a) hydrate and (b) anhydrate

![Effect of temperature and methanol concentration on crystallization of LOLA](image)

Fig. 3. Effect of temperature and methanol concentration on crystallization of LOLA
(in M process) (O: 1ml/min, △: 4ml/min; solid: hydrate, open: anhydrate)