Nucleation control and growth of metastable $\alpha$-L-glutamic acid single crystals in the presence of L-phenylalanine

Dhanasekaran Palanisamy, Srinivasan Karuppannan*

*Department of Physics, Erode Sengunthar Engineering College, Thudupathi, Erode-638 057.

bCrystal growth Laboratory, Department of Physics, Bharathiar University, Coimbatore-641 046.

Abstract

The influence of selective additive L-phenylalanine on the nucleation control and growth of L-glutamic acid polymorphs from aqueous solution was studied. The effect of the selective additive L-phenylalanine on the control of the cross nucleation of polymorphs in the aqueous solution was investigated by slow evaporation method of crystallization. The effect of different additive concentrations on the solubility, pH and induction period of nucleation of the polymorphs was also investigated at room temperature. Nucleation control of the polymorphs was successfully carried out and single crystals of metastable $\alpha$-polymorph were grown. Structural confirmation of the grown polymorph was carried out by powder x-ray diffraction study.

1. Introduction

Several amino acids have polymorphs which are crystallized in more than one crystalline form; likewise L-glutamic acid has two known polymorphs with contrasting morphologies, the metastable $\alpha$-form having prismatic shape and the stable $\beta$-form producing needle like crystallites [1]. The selective crystallization of the metastable $\alpha$-L-glutamic acid is important in industries, because of its attractive properties than that of $\beta$-L-glutamic acid (stable

* Corresponding author. Tel.: +91 422 2428442; fax: +91 422 2422387.
E-mail address: nivas_5@yahoo.com

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Separation of this α-polymorph from the aqueous solution is quite comfortable than the separation of the β-polymorph due to its shape. But the crystallization of the metastable α-polymorph is more difficult than that of the stable β-polymorph at normal growth conditions moreover, the main challenge is the prevention of the cross nucleation of stable β-polymorph along with the already nucleated α-polymorphs. As per some of the previous literatures, the growth parameters such as supersaturation, agitation rate, cooling rate, solvent composition, temperature, additives and impurities at times control this cross nucleation of polymorphs [2-6] and some selective additives are also used to control the growth of L-glutamic acid polymorphs [7-14]. On this account an article has recently been published by us on the nucleation control and crystallization of L-glutamic acid polymorphs in the presence of the selective additive L-tyrosine [15]. In this present work L-phenylalanine was selected as an additive and different experimental works were carried out on the above aspects. The effect of the added L-phenylalanine on the control of the cross nucleation of polymorphs in the aqueous solution was investigated by employing slow evaporation method of crystallization. The effect of L-phenylalanine on the nucleation control and growth of L-glutamic acid polymorphs was investigated. The influence of L-phenylalanine on the solubility, pH and nucleation time of L-glutamic acid polymorphs from aqueous solution was studied. Morphology of the grown α and β-crystals was also studied. The confirmation of the internal structure and the verification of non-incorporation of the additive into the crystal lattice were carried out by powder diffraction X-ray analysis.

2. Experimental Procedure

2.1. Determination of solubility in the absence and presence of L-phenylalanine

To study the variation in the solubility of L-glutamic acid in the absence and presence of various concentrations of L-phenylalanine, a series of solubility study was carried out. The saturated aqueous solution of L-glutamic acid at 32 °C with various concentrations of L-phenylalanine in the range between 0.01 and 0.10 g/100 ml of solution was taken in a 250 ml round bottom flask. The solution was gently stirred continuously for about 6 h and the precipitation of the undissolved salt, if any, was allowed to settle down at the bottom of the flask. Two samples of about 1 ml of the clear solution were pipette out into two similar 5 ml beakers and gently warmed out. After the complete evaporation of the solvent, the solubility of L-glutamic acid salt was determined by averaging the data for the two samples gravimetrically.

2.2. Determination of pH in the absence and presence of L-phenylalanine

It is important to study the variation in the pH of the aqueous solution while the selected concentration of the additive is incorporated into it as this information will throw some light on the effect of pH on the nucleation of different polymorphs. The isoelectric pH point of pure L-glutamic acid is 3.22 and L-phenylalanine is 5.48. In order to study the changes in the pH of the saturated aqueous solution of L-glutamic acid due to the incorporation of various concentrations of L-phenylalanine, the pH of the solution with L-phenylalanine concentration in the range between 0.01-0.10 g/100 ml of solution at 32 °C was determined using EUTECH make pH meter model TUTOR Cyberscan. Accuracy of the measurement was ±0.01pH throughout the measurement.

2.3. Determination of nucleation time in the absence and presence of L-phenylalanine

Different polymorphs of a material crystallize from its aqueous solution at different intervals of time. Nucleation time is one of the identification parameters to distinguish the types of polymorphs nucleated in the solution at times. It is the time interval between the subjection of the saturated solution for controlled evaporation of solvent and the observation of the first speck of nucleation in it. The difference in the nucleation time of α and β polymorphs of L-glutamic acid in the present case plays a crucial role in controlling the nucleation and separation of the polymorphs in the crystallization experiments. Hence the nucleation time of the polymorphs in each of the experimental solution with different concentrations of L-phenylalanine in the range 0.01-0.10 g/100 ml subjected to slow evaporation method was determined periodically. Nucleation time for the pure aqueous solution was also determined for comparison. Depending on the concentration of the incorporated additive and the rate of evaporation, different
levels of supersaturation were created in the series of solutions subjected to slow evaporation which in turn have a direct impact on the nucleation time of the polymorphs. This nucleation time of the polymorphs in each of the experimental solution was determined by measuring the time taken by the solution to yield the first speck of visible nucleation after the attainment of necessary supersaturation in the solution upon solvent evaporation.

2.4. Nucleation and growth of α and β L-glutamic acid single crystals in the presence and absence of L-phenylalanine by slow evaporation method

Saturated pure aqueous solution of L-glutamic acid was prepared using the raw material of commercially available L-glutamic acid salt (purity of 99.98% from Himedia, India) and double distilled (DD) water at room temperature. The solution was filtered with Whatmann No. 1 and No. 41 filter sheets by vacuum method and distributed equally in 10 similar crystallizing vessels each of having volume 100 ml. Concentration of the additive L-phenylalanine in incremental steps of 0.01 g in the range between 0.01 and 0.10 g was added in each of the experimental solution in series, stirred well for about 6 h, filtered and kept inside a dust-free evaporation chamber. Pure aqueous solution of L-glutamic acid of similar volume was also kept for evaporation in a similar vessel for reference. Due to the continuous evaporation of solvent the solutions get supersaturated and yield nucleation consecutively. The nucleated micron size crystals were allowed for further growth up to mm size for a period of about 20 days uniformly and then harvested for further investigations.

2.5. X-ray diffraction analysis

Powder X-ray diffraction pattern were recorded for the grown polymorphs by employing Bruker AXS D8 Advance X-ray diffractometer with Cu Ka (λ=1.5406 Å) radiation using a tube voltage of 40 kV and tube current of 30 mA at room temperature. The β-crystals grown by slow evaporation from pure aqueous solution and the α-crystals grown in the presence of the additive of concentration of 0.03 g/100 ml and 0.07 g/100 ml were finely powdered and subjected to powder X-ray diffraction analysis. The respective diffraction pattern were obtained by scanning between 10° and 60° in 2θ with step size of 0.05° in 2θ and scan speed of 0.5° in 2θ per second.

3. Results and Discussion

3.1. Variation of solubility, pH and nucleation time in the presence of L-phenylalanine

The size of the crystal depends on the amount of material available in the solution, which in turn is decided by the solubility of the material in that solvent. The presence of L-phenylalanine at various concentrations in the range of 0.01-0.10 g/100 ml of solution alter the solubility of L-glutamic acid considerably. This variation in the determined solubility of L-glutamic acid in the presence of various concentrations of L-phenylalanine is shown in Fig.1. (a). At 32 °C solubility of L-glutamic acid in DD water is about 0.96 g/100 ml. From the solubility curve, it is clear that, when the additive is added the solubility of L-glutamic acid increases with increasing additive concentration linearly and it is about 1.41 g/100 ml when the additive concentration is 0.1 g/100 ml of solution.

The incorporation of L-phenylalanine at various concentrations also alters the pH of the solution and the variation in the determined pH value of the L-glutamic acid solution with various concentrations of L-phenylalanine is shown in Fig.1. (b). The pH of the pure aqueous solution at room temperature is 3.15 and it increases to a value of about 3.65 when the additive concentration is 0.1 g/100 ml of solution. The increasing interaction between L-phenylalanine and L-glutamic acid molecules through hydrogen bonding in the solution may be the reason for the increase in solubility as well as the pH of the solution.

The effect of various concentration of L-phenylalanine on the solubility of L-glutamic acid has direct impact on the nucleation time. The variation in the nucleation time with respect to various concentrations of the additive in the solution is shown in Fig.1. (c). Time taken for the nucleation of polymorphs increases with the increase in the concentration of L-phenylalanine. Pure aqueous solution yielded only the β-nucleation at a lower nucleation time of about 110 h. In the presence of various concentrations of L-phenylalanine in the solution, the nucleation time
increases gradually with the additive concentration and it is about 320 h when the additive concentration is 0.07 g/100 ml of solution.

Fig. 1. Variation in (a) Solubility; (b) pH value; (c) Nucleation time of L-glutamic acid in the presence of various concentrations of L-phenylalanine.

3.2. Effect of various concentrations of L-phenylalanine on the nucleation and growth of L-glutamic acid polymorphs

The molecular structure of L-glutamic acid has two carboxylic and one amino group. The hydrogen bond formation between carboxylic and amino groups of the neighboring molecules enables the stacking of L-glutamic acid molecules in the crystalline lattice. Additive selected in the present study, L-phenylalanine has one carboxylic, one amino and the bulky phenyl group. When the L-phenylalanine is added as an additive there are possibilities for the formation of hydrogen bonds between the carboxylic and amino group of the L-glutamic acid and L-phenylalanine molecules. This interaction has significant impact on the growing crystals and this can be realized very well from the crystals grown in the present study by slow evaporation method. The slow evaporation experiment yielded good optical quality single crystals of the only metastable α with habitual external morphology when the concentration of L-phenylalanine was in the range 0.01-0.03 g/100 ml of solution at the nucleation time in the range of 140-180 h. The nucleation of β was completely suppressed because of the inhibitory effect of L-phenylalanine towards the suppression of β-nucleation in the solution. This effect is more obvious even at the comparatively lower concentration of L-phenylalanine i.e. <0.03 g/100 ml in the solution. Increase in the additive concentration increases the possible interactions between the L-phenylalanine and L-glutamic acid molecules through hydrogen bonding. The characteristic hydrogen bond formation of the β-form with the additive molecule is more due to its lower capability of molecular recognition than the α-form and this hydrogen bond formation hinders the next molecule to be adsorbed.

This result clearly explains the preferential inhibition of β-form in the presence of additive. It is reported that the growth rate of the prominent (101) face of β-polymer is generally 50 times greater than that of (010) face of β-polymer [9]. Hence the additives molecules preferentially interact with the (101) face of β-polymer and control their growth. The solution with the L-phenylalanine concentration in the range of 0.04-0.07 g/100 ml of solutions yield the nucleation of metastable α single crystals with extended morphology at the nucleation time in the range of 210-320 h. The morphological difference between the polymorphs grown at two different concentration ranges (i.e.) ≤ 0.03 g/100 ml and ≥ 0.03 g/100 ml shows clearly the inhibitory effect of additive L-phenylalanine on the selected facets of α-L-glutamic acid polymorphs too. Increase in additive concentration also affects the nucleation and growth of α-polymorphs to reasonable amount. Moreover, the dominating {001} and {011} facets of the α-crystals are slowly disappearing or their size becomes reduced with increasing additive concentration. But at the same time {110} and {111} facets are found to be dominated with increase in the additive concentration. Similar observations were also reported previously with L-tyrosine as an additive [15]. The additive molecules preferentially inhibits the growth of {111} and {110} facets of the α-polymer and made them as morphologically important faces. The presence of L-phenylalanine in the solution does not alter the growth rate of {001} faces which is mainly
due to the repulsion of carboxylic acid group [4]. The strength of the inhibition depends on the stability on the crystal faces, i.e., the similarity in the hydrogen bond formation and the way the next molecule adsorbs on the growing face of the crystal. These hydrogen bond formations create more possibilities for the hindrance of further stacking between L-glutamic acid molecules because of the presence of bulky phenyl group of L-phenylalanine. Moreover this bulkier side chain of the additive may occupy more lattice spaces and act as a repellent to the normal stacking process of {110} and {111} facets of α-L-glutamic acid. Similar observations were also reported in the literature previously with L-amino acid as an additive [12].

When the concentration exceeds 0.07 g/100 ml (i.e.) the critical level in the present experimental work, the inhibitory effect of additive is very high on both α- and β-polymorphs. Hence there is no nucleation of either α or β observed in the solution. This result clearly indicates that the incorporation of L-phenylalanine at the concentrations greater than 0.07 g/100 ml completely inhibits the nucleation of both the L-glutamic acid polymorphs in the solution. From the above experimental results it clearly depicts that, the incorporation of L-phenylalanine in the solution favours only the growth of α-L-glutamic acid crystals with habitual prismatic morphology up to the concentration level of 0.03 g/100 ml and with elongated morphology above 0.03 g/100 ml up to the critical concentration level of 0.07 g/100 ml in the present study. Beyond this critical level it suppresses both α- and β-nucleations completely. The incorporation of L-phenylalanine in the solution completely inhibits the β-nucleation throughout the mixing range in the present study. It also makes clear that higher mixing concentrations of L-phenylalanine yield nucleations with lower rate while, the lower mixing concentrations yield nucleations with higher rate. The photographs of the nucleated β-crystals from pure aqueous solution, α-crystals from aqueous solution with L-phenylalanine concentration of 0.03 g/100 ml and elongated α-crystals from aqueous solution with L-phenylalanine concentration of 0.07 g/100 ml are shown in Fig. 2 (a), (b) and (c) respectively.
Fig. 2. Photograph of the grown (a) β-L-glutamic acid single crystals grown from pure aqueous solution, α-L-glutamic acid single crystals grown from aqueous solution in the presence of (b) 0.03 g/100 ml and (c) 0.07 g/100 ml of L-phenylalanine by slow evaporation method.

3.3. Structural analysis

The recorded powder x-ray diffraction patterns of the grown β-polymorph from pure aqueous solution, α-polymorph from solution with L-phenylalanine concentration of 0.03 g/100 ml and α-polymorph from solution with L-phenylalanine concentration of 0.07 g/100 ml by slow evaporation method are given in Fig.3. (a), (b) and (c) respectively. The powder x-ray diffraction study made on the grown α- and β-crystals reveals that both of them belong to orthorhombic crystal system with space group P2_1_2_1 and point group 222 with only difference in their lattice parameters. The determined lattice parameters for the grown stable β-crystals from pure aqueous solution, α-crystals grown from solution with L-phenylalanine concentration of 0.03 g/100 ml and α-crystals grown from solution with L-phenylalanine concentration of 0.07 g/100 ml are presented in Table 1. There is no remarkable difference between the lattice parameters determined for the α-crystals grown with the L-phenylalanine concentration of 0.03 g/100 ml and 0.07 g/100 ml of the solution, these determined lattice parameter values are in line with the JCPDS files (JCPDS No: 30-1740) and also in literature values [15-19]. We could conclude that the increase in additive concentration from 0.03 g to 0.07 g/100 ml does not alter the lattices of the crystals. It means that the incorporated additive does not enter into the lattices whereas it plays its inhibitory role only by just making their presence in the solution.
Fig. 3. PXRD patterns of grown (a) \( \beta\)-L-glutamic acid single crystals grown from pure aqueous solution, \( \alpha\)-L-glutamic acid single crystals grown from aqueous solution in the presence of (b) 0.03g/100ml and (c) 0.07g/100ml of L-phenylalanine.

Table 1 Lattice parameter values of the grown L-glutamic acid polymorphs.

| Lattice parameters | \( \beta\)-crystals grown from pure aqueous solution | \( \alpha\)-crystals grown from solution with L-phenylalanine concentration of 0.03g/100ml | \( \alpha\)-crystals grown from solution with L-phenylalanine concentration of 0.07g/100ml |
|-------------------|---------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| a                 | 6.951 Å                                           | 7.073 Å                                                                          | 7.060 Å                                                                          |
| b                 | 17.329 Å                                          | 10.278 Å                                                                         | 10.268 Å                                                                         |
4. Conclusion

The influence of the selective additive L-phenylalanine on the nucleation control and growth of the polymorphs of L-glutamic acid is investigated by slow evaporation method. The aqueous solution of L-glutamic acid in the absence of L-phenylalanine yields only the β-nucleations and the presence of L-phenylalanine up to the concentration of 0.07 g/100 ml of the solution yields only the α-nucleation. When the concentration of additive exceeds 0.07 g/100 ml there is no nucleation of either the α or β in the solution. This range of additives completely suppresses the nucleation of β-polymorphs. Up to the additive concentration of 0.03 g/100 ml of the solution prismatic α-L-glutamic acid was obtained. Beyond this concentration and up to the critical level of 0.07 g/100 ml of the solution α-L-glutamic acid was observed with elongated morphology along its [001] direction. This quantifies the effect of the incorporated L-phenylalanine on the morphology of the growing crystals. This is mainly because of the possible interaction between L-glutamic acid molecules with L-phenylalanine in the solution medium. When the concentration of additive exceeds the critical level i.e 0.07 g/100 ml the nucleation of both α and β are completely suppressed. The reason for the suppression of β-nucleation throughout the experiment and the α-nucleation beyond the critical level is mainly because of the presence of bulkier side chain of the additive. These molecules block the further stacking of L-glutamic acid molecules by adhering on the lattices thereby resulting in the strong inhibition of the nucleation of β-polymorphs. The adherence of these additive molecules onto the crystal lattice increases the solubility, pH of the solution and the nucleation time significantly.

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