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SHORT COMMUNICATION

Separation and nucleation control of α and β polymorphs of L-glutamic acid by swift cooling crystallization process

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Abstract Separation of crystal nucleation of the two known polymorphs of L-glutamic acid, the metastable α and the stable β, from pure aqueous solution is attained by following a swift cooling crystallization process. Results elucidate a clear distinction of the preferred nucleation regions of α, β and combinations of α and β in the temperature range between 1 and 40°C. Also, the type of nucleation is supersaturation dependent: higher supersaturation favours α and lower supersaturation favours β. Morphology and structure of the polymorphs confirm their form of crystallization.

Keywords Nucleation · Separation · Crystallization · Morphology · Optical microscopy · X-ray diffraction

Introduction

Polymorphism plays a significant role in the pharmaceutical industry because polymorphs of a specified material have different physical and chemical properties with varied crystallographic internal arrangements such as density, solubility, colour, crystal morphology, etc. which affect the performance of the end products such as bioavailability, shelf life, compactness, etc. (Lu et al. 2010; Purohit and Venugopalan 2009; Hilfiker 2006; Shekunov and York 2000; Vrcelj et al. 2001; Prasad et al. 2001; Slavin et al. 2002). A consistent and reliable production process is required for the targeted polymorph of a material for feasible economic yield as well as for compliance of regulatory aspects. In some cases, it is often easy to obtain the thermodynamically stable form of a material without much complication under suitable crystallization conditions, but very difficult to get crystals of the metastable form in a controlled and repeated manner. In some other cases, the reverse is also true. The main challenge exists in the prevention of the cross nucleation of the stable form in the system (Kitamura 2002). Of the several methods developed, the incorporation of selective additives to stabilize the metastable form is a frequent one, but it is system specific in many cases (Sano and Nagashima 1996; Prasad et al. 2001; Slavin et al. 2002; Srinivasan 2008).

L-glutamic acid ($C_5H_9NO_4$), a well-known amino acid, has two known polymorphs: metastable α (Lehmann and Nunes 1980) and stable β (Hirokawa 1955). Both the polymorphs belong to the orthorhombic crystal system with space group $P2_12_12_1$ and point group 222 and differ in their lattice parameters and also in the structure accordingly. The β form crystallizes easily from aqueous solutions at normal growth conditions with needle-like morphology. But, the α form is rather difficult to crystallize under normal growth circumstances. Crystallization of α always requires some special measures. There have been previous reports on the crystallization of α with different experimental methods, but most of these either involve huge sophisticated instrumentation or the presence of selective additives (Mougin et al. 2002; Schöll et al. 2006; Ono et al. 2004; Kitamura and Ishizu 1998). In the present investigation, a simple and elegant method has been adopted to stabilize the metastable α form from aqueous solution by adopting a swift cooling crystallization process. Repeated results obtained from 40 cooling experiments revealed a clear-cut distinction of the region of preferred nucleation of α and β and combinations of α and β polymorphs of

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L-glutamic acid. The morphology of the grown polymorphs examined with optical microscope and X-ray powder diffraction (XRD) analyses confirm their form of crystallization.

Swift cooling process

The aqueous solution used for this study was prepared from commercially available high pure L-glutamic acid salt (Himedia, purity 99.8%) and double distilled water. Weighed salt for saturation at 40°C in 300 ml of distilled water according to the solubility (1.5 g/100 ml) was taken in an airtight round-bottom flask with ground sleeve stirrer glands attachment for effective stirring. This setup was kept inside a digitally controlled full-visibility constant temperature bath (CTB) with in-built cryostat facility and having temperature controlling accuracy of ±0.01°C. The temperature of the CTB was maintained at 80°C. The mixture was stirred with PTFE stirrer shaft and blades at a speed of 32 rpm constantly through a digitally controlled stepper motor drive. After 4 h of continuous stirring, the solution was filtered with a Duran pipeline filter having porosity in the range of 10–16 μm with the help of a peristaltic pump. The filtrate was collected into another similar airtight round-bottom flask fixed with similar stirring assembly and maintained for about 2 h at 80°C with continuous stirring. Then, the solution with the entire assembly and continuous stirring was swiftly transferred to another similar CTB, which was maintained at the experimental nucleation temperature; at the initial stage of the investigation it was maintained at 1°C. The solution was carefully monitored through the full-visibility window of the bath under bright light illumination and the events were recorded with time. The solution was transparent initially for a short period of time (~10 min), known as induction period τ, i.e. the time interval between the attainment of supersaturation and nucleation, after which it became milky white in colour indicating possible nucleation in the solution. The induction period τ is measured by noting down the difference between the time at which CTB attains the experimental temperature and the time at which the nucleation occurs, i.e. the white colour change is observed in the solution. Within the next 2 min, a precipitate of tiny white crystalline particles was formed in the solution at the bottom of the flask. A small portion of the solution containing these nucleated crystalline particles was taken out using a micropipette and examined under an Olympus BX-51 microscope. These particles were identified as one of the polymorphs of L-glutamic acid and photographed. The experiment was then repeated for every 1°C in the temperature range of 1–40°C and the nucleated crystals were photographed. Sampling of the solution was continued at different time intervals, such as immediately after precipitation (~0 min), and after 15, 45 and 105 min, and the corresponding nucleated crystals were photographed. The remaining precipitated crystalline particles were filtered and dried out in a hot air oven maintained at around room temperature and preserved. The time taken for nucleation, i.e. induction period τ and the time taken for precipitation of the crystalline particles were recorded simultaneously for all the experimental temperatures.

Results and discussion

The variation of induction period τ with temperature is shown in Fig. 1a. It is clear from the figure that the induction period for α nucleation is short when compared to that of β nucleation. It is about 13 min for a typical α nucleation up to the experimental temperature of 9°C and about 175 min for a typical β nucleation at the experimental temperature of 30°C. Moreover, the change in the induction period with temperature is minimal in the case of α nucleation and is high in the case β nucleation. The induction time of a typical β nucleation increases more than 12 h when the experimental temperature increases

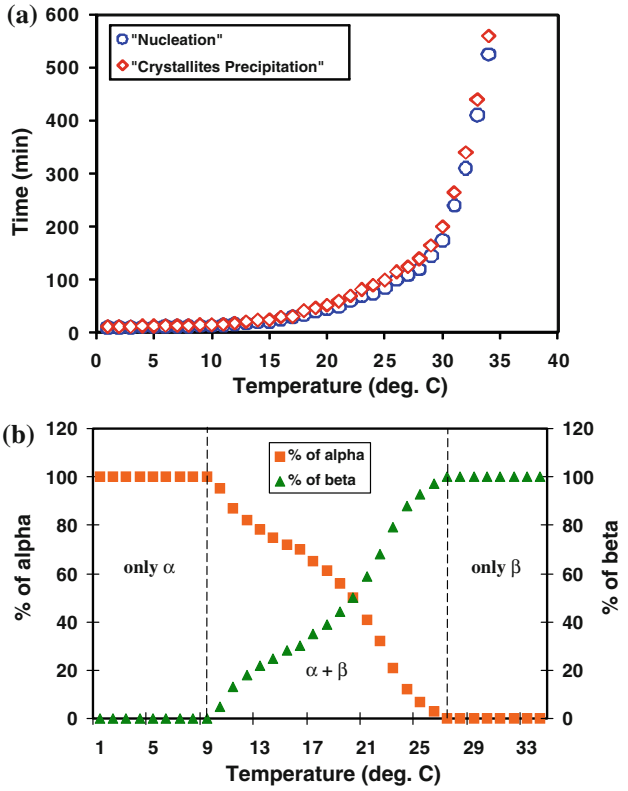

Fig. 1 **a** Variation of induction period τ with experimental temperature of L-glutamic acid solution in the range 1–34°C. **b** Percentage of α and β crystallites nucleated at each experimental temperature in the range 1–34°C

above 34°C. The average induction time for both the α and β nucleations in the intermediate temperature region is also temperature dependent and changes according to the compositions of α and β nucleations in the solution. The percentage of α and β crystallites nucleated at each experimental temperature was determined and its variation with temperature is given in Fig. 1b. It is obvious from the figure that 100% of α crystals nucleated in the temperature range 1–9°C, indicating that this temperature region and the resultant supersaturation in the solution favours only α nucleation. The observations made after 15, 45 and 105 min at all the experimental temperatures also confirm that the nucleated crystallites still retain their α phase in solution. In addition, the harvested small α crystallites from this temperature region preserved at room temperature also retained their phase even after several weeks. Similarly, 100% of β crystals nucleated in the temperature range 27–34°C, indicating that this temperature region and the resultant supersaturation favours only β nucleation. It is obvious that the higher supersaturation region of the experimental temperature range favours α nucleation and the lower supersaturation region favours β nucleation. In the intermediate temperature region between 9 and 27°C, both the α and β nucleations occur and the percentage of α decreases and that of β increases with temperature in this region. Photograph of the nucleated α micro crystals from solution cooled to 2°C and β micro crystals from solution cooled to 28°C are shown in Fig. 2a, b, respectively. Their morphology with indexed growth faces are shown in Fig. 2c, d and their corresponding XRD spectra in Fig. 2e, f, respectively. Both the polymorphs have marked difference in their morphology. The XRD data obtained from the spectra confirm that both the polymorphs belong to the orthorhombic crystal system with space group $P2_12_12_1$ and

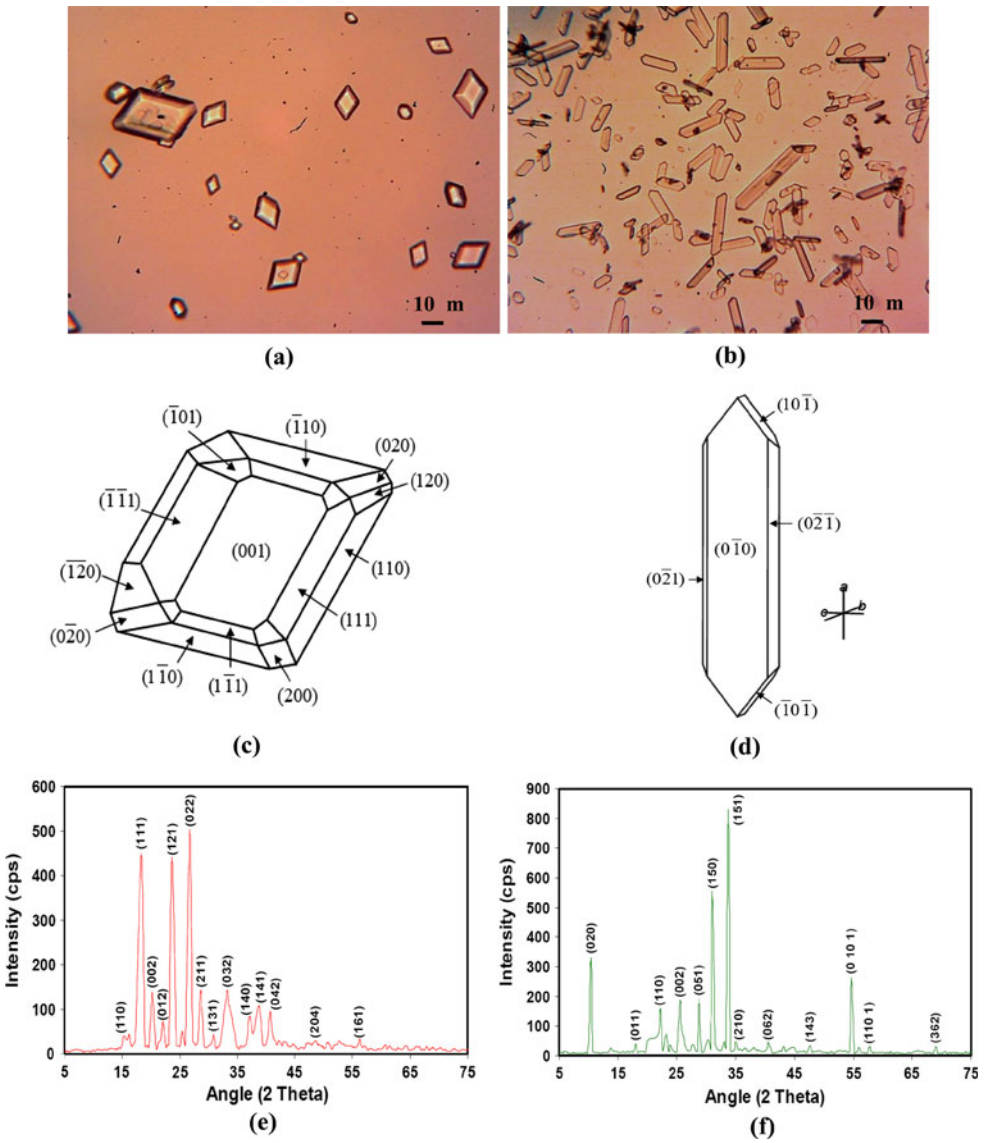

Fig. 2 Photograph of the nucleated microcrystals of L-glutamic acid: **a** α polymorph and **b** β polymorph; their morphologies: **c** α polymorph and **d** β polymorph; and X-ray diffraction patterns: **e** α polymorph and **f** β polymorph

point group 222 and differ in their lattice parameters explicitly and also in their structure accordingly. The determined lattice parameters for α polymorph are $a = 7.051$ Å, $b = 10.285$ Å and $c = 8.766$ Å, and for β polymorph $a = 5.183$ Å, $b = 17.315$ Å and $c = 6.973$ Å, and they are in line with the literature values.

Conclusion

The crystal nucleation of α and β polymorphs of L-glutamic acid from pure aqueous solution in the temperature range 1–40°C was obtained; their separation and control were performed by adopting a laboratory-scale swift cooling crystallization process. The induction time τ for α nucleation is short and that of β nucleation is long. The variation in the induction time τ with temperature is high in the case of β nucleation when compared to α and the induction time τ exceeds more than 12 h above 34°C. Solution cooled down to temperatures in the range 1–9°C favours only α nucleation and that in the range 27–34°C favours only β nucleation. Results indicate that the formation of crystal nucleation of α and β polymorphs of L-glutamic acid from aqueous solution is greatly supersaturation dependent: higher supersaturation favours only α and lower supersaturation favours only β nucleation. Micromorphological analysis and XRD study of grown polymorphs confirm their form of crystallization.

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