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Secondary nucleation of the β-polymorph of L-glutamic acid on the surface of α-form crystals

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Evidence is presented for the secondary nucleation of β-L-glutamic acid on the surface of the α-polymorph, using a combination of Scanning Electron Microscopy and Raman spectroscopy.

The polymorphic behaviour of organic solids is of crucial importance to the pharmaceutical industry,[1–4] due to differences in properties such as stability, crystal habit, compressibility, density, dissolution rate[5] and bioavailability.[6] L-Glutamic acid (L-Glu) has two known polymorphs, the metastable granular α-form and the stable plate-like β-form.[7,8] Both forms are orthorhombic with space group $P2_12_12_1$,[1,9] and are related monotropically.[1] In agreement with Ostwald's law of stages, the α-form crystallises from solution first, and by a continuous process of dissolution and crystallisation, undergoes solution-mediated transformation to the β-form.[10,11] The transformation does not occur in the solid-state,[11] and the mechanism of transformation is yet unknown. It was speculated that nucleation of the β-form occurs at the surface of the solution, since β-form crystals were observed growing on the crystallising solution surface.[12] More recently it has been suggested that the nucleation process is heterogeneous, whereby α-crystals in solution provide the surface on which β-form nucleates, but no evidence of this has been reported to date.[10] In this paper, we provide evidence for the secondary nucleation of the β-form at the surface of the existing α-form crystals. Secondary nucleation of a stable polymorph on the surface of its metastable form has only been observed on one other occasion; that of 2,6-dihydroxybenzoic acid.[13] The question arises here as to whether this was a single isolated observation, or whether the phenomenon occurs in a widespread manner.

The α-form of L-Glu is not commercially available, and so was synthesised by recrystallisation from 98% pure L-Glu monosodium salt monohydrate (Sigma-Aldrich) using 37% HCl (BDH, Analar), as previously reported.[10] To produce the β-form, the α-crystals were dried, ground in a mortar and pestle and recrystallised by cooling supersaturated aqueous solutions (0.3 M)[10] from 80 °C to 45 °C,[10,12] and maintaining the solutions unstirred at 45 °C for specified time intervals. Scanning Electron Microscopy (SEM) was performed using a JEOL JSM-840 electron microscope subsequent to sample coating with gold in an Edwards S150B sputter coater. Raman spectroscopy was performed using a LABRAM laser Raman spectrometer, utilising excitation radiation at 514.5 nm generated by a green laser working at 50–100 mW. Dried, ground crystals were placed on a glass slide, and were coarse and fine focused at magnifications of ×10 and ×50 respectively using an Olympus microscope. The laser was introduced to the sample unfiltered, the confocal hole was 300 μm and the slit was 150 μm. This enables the laser to focus on 2–3 μm of sample. Five 75 s scans were taken and aggregated over a range of 100–1700 cm^{-1}.

Fig. 1A and B† show electron micrographs of α- and β-L-Glu respectively, which display distinctive morphologies. The α-polymorph grew as well formed individual granular crystals after short crystallisation times, while the β-polymorph, which crystallised later, was plate-like in appearance. At intermediate crystallisation times both forms were present, and it was observed using SEM that the β-form crystals of L-Glu grow out of the surface of the granular α-crystals. The images in Fig. 2 illustrate this observation, which was encountered on many occasions, typically for crystallisation times of 1–6 h, corresponding to β-form nucleation and growth. This report provides the best evidence to date for secondary nucleation of a stable polymorph on the metastable form, using L-Glu, a compound for which the transformation mechanism was previously unknown.

The identities of the α- and β- constituents of such structures were confirmed using Raman spectroscopy and the corresponding individual spectra are presented in Fig. 3. Raman spectroscopy has been used to study L-Glu,[14,15] and only the β-L-Glu spectrum was obtained. This is the first report which distinguishes the polymorphs of L-Glu using Raman spectroscopy; the main α-peaks occur at 869 and 1314 cm^{-1} and the β-peaks at 95, 863 and 1408 cm^{-1}.

The addition of L-α-amino acids during crystallisation of L-Glu is known to stabilise the α-polymorph, by suppressing the growth of the β-form.[16] The addition of 1×10^{-2} M L-tyrosine (Sigma-Aldrich) to saturated solutions of L-Glu prevented transformation from the α- to the β-polymorph over the 24 h test period. This additive was found to be more effective at lower concentrations than either L-phenylalanine or trimesic acid for stabilisation of the α-polymorph, as shown in Fig. 4. Increasing the additive concentration not only decreased the quantity of the

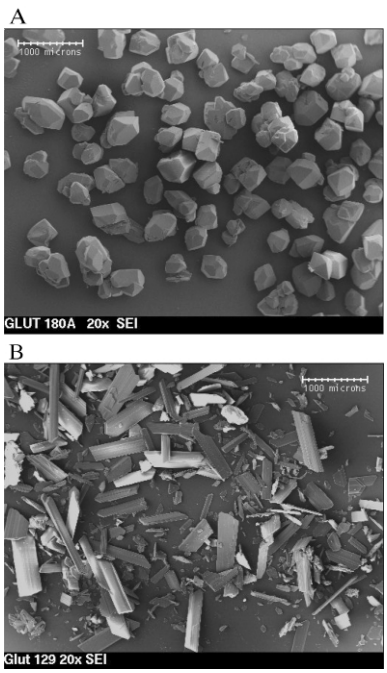

Fig. 1 SEM images of L-glutamic acid (A) α-form: $[Glu]_0$ = 0.3 M, T_{sat} = 80 °C, T_{crys} = 45 °C, t_{crys} = 1 h; (B) β-form: $[Glu]_0$ = 0.3 M, T_{sat} = 80 °C, T_{crys} = 45 °C, t_{crys} = 24 h.

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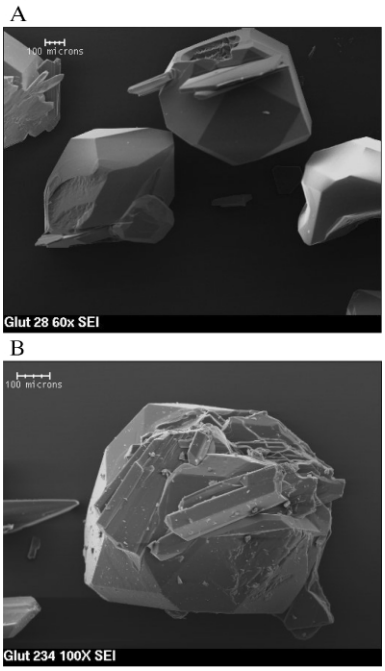

Fig. 2 SEM images of β-crystals of L-glutamic acid growing *out of* the surface of the α-form: $[Glu]_0$ = 0.3 M, T_{sat} = 80 °C, T_{crys} = 45 °C.

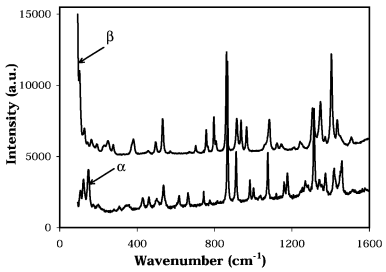

Fig. 3 Raman spectra of α- and β-L-glutamic acid: $[Glu]_0$ = 0.3 M, T_{sat} = 80 °C, T_{crys} = 45 °C.

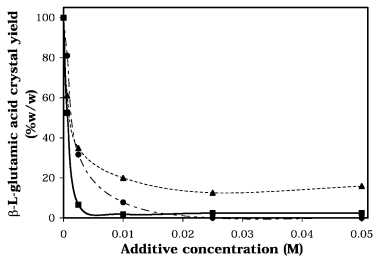

Fig. 4 Kinetic stabilisation of α-L-glutamic acid using L-tyrosine (■), L-phenylalanine (●) and trimesic acid (▲): $[Glu]_0$ = 0.3 M, T_{sat} = 80 °C, T_{crys} = 45 °C, t_{crys} = 24 h.

β-polymorph, but had a profound effect on the morphology of the α-crystals that formed. Fig. 5C displays an α-crystal which has become elongated due to the presence of L-tyrosine.

This observed change in morphology is caused by the attachment of L-tyrosine to specific faces of the developing α-crystals, preventing further growth of these faces. A similar effect was observed using L-phenylalanine,[17] with the hydroxyl group of L-tyrosine being the only difference between these two compounds. The unmodified α-crystals feature predominantly the {001}, {111} and {011} facets of the crystal (Fig. 5B). The modified crystals still possess {001} and {111}, but the new {110} facet appears while the {011} facet disappears (Fig. 5C). The β-crystals are unable to nucleate on the facets of the modified α-crystal due to absence of suitable nucleation sites. It

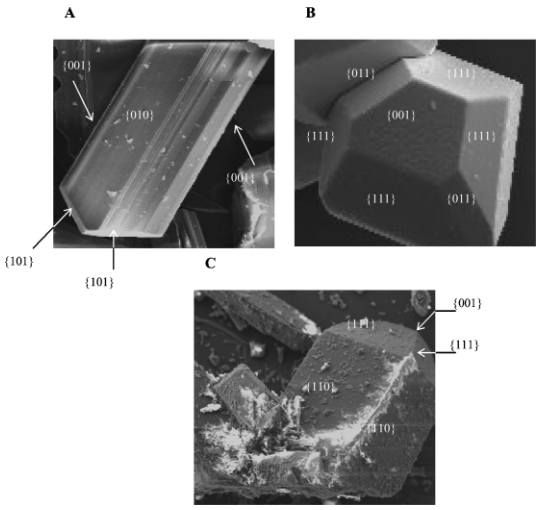

Fig. 5 Facets of (A) β-L-glutamic acid, (B) unmodified α-L-glutamic acid and (C) α-L-glutamic acid modified using 1×10^{-2}M L-tyrosine.

is probable that the {011} facet of α-crystals is necessary for nucleation of β-crystals, and the new {110} face of the α-crystals cannot perform this function. The β-crystals appear to attach to α *via* their {101} facets (facets shown in Fig. 5A), but a more detailed study of the crystallographic fit between the {101} facets of the β-crystal and the {001}, {111}, {011} and {110} facets of the α-crystal is currently underway.

We propose that the polymorphic transformation of L-Glu proceeds by secondary nucleation of the β-polymorph at the surface of the α-polymorph. Additives which have been reported to stabilise the α-form, do so by attachment to specific facets which are necessary for and facilitate the nucleation of the β-polymorph, thereby disrupting nucleation. This work supports the report by Davey *et al.*,[13] and given that it deals with an entirely different organic structure, raised the possibility that this phenomenon may be of very widespread occurrence in polymorphic transformations involving organic compounds.

Notes and references

† $[Glu]_0$ = Initial L-glutamic acid concentration, T_{sat} = saturation temperature, T_{crys} = crystallisation temperature, t_{crys} = crystallisation time.

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