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Precipitation Mechanism of Stable and Metastable Polymorphs of L-Glutamic Acid

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Precipitation of the polymorphic compound L-glutamic acid was initiated by premixing aqueous solutions of sodium L-glutamate and sulfuric acid. Samples of the supersaturated solution were either subjected to vigorous post-stirring or left under quiescent conditions. For low supersaturation ($S \leq 13$) without post-stirring aggregated platelet-shaped crystals of the stable beta polymorph formed while post-stirring generated large prismatic crystals of the metastable alpha polymorph. For high supersaturation ($S \geq 17$) first smooth spheres were observed which transformed into rough spherulitic crystals of the beta phase. For high supersaturation it is proposed that the spheres are a metastable phase consisting of droplets formed by liquid-liquid phase separation. Subsequently from these spheres crystals of the stable beta phase nucleate. For low supersaturation without post-stirring aggregated beta platelets form according to the same mechanism, while with post-stirring concentration fluctuations are equalized and metastable alpha crystals nucleate from the homogenized solution. *AIChE J,* 53: 354–362, 2007
Keywords: precipitation, polymorphism, metastable phases, liquid-liquid phase separation, L-Glutamic acid

Introduction

Polymorphism is of great interest to industry because polymorphs have different physical properties, for example, the solubility of pharmaceuticals. Control of the formation of the different polymorphic structures during production is desired, especially in the case of concomitant polymorphs. The relative stability of the polymorphs is defined by thermodynamics, but the structure that will actually form, depends also on the kinetics, that is, competitive nucleation and growth rates. In precipitation the supersaturation is high, and, therefore, formation of a metastable phase is likely to occur, followed by transformation into a more stable phase.

L-Glutamic acid (L-Glu) was chosen because it is a relatively well-studied model compound that has two polymorphs with contrasting morphologies, the metastable alpha form having a rather compact prismatic shape and the stable beta form producing elongated plate-like crystallites. Both polymorphs crystallize in the orthorhombic space group $P2_12_12_1$ structure with $Z = 4$ molecules in the unit cell, but with different axis lengths.[1,2] In the crystal lattices the L-Glu molecules are in the zwitter-ionic form and adopt different conformations with different torsion angles.[3] The metastable alpha form has a 20% higher solubility compared to that of the stable beta form.[4] With increasing (but still low) supersaturation, the growth rate of single crystals of the metastable-alpha phase increased more compared to that of the stable-beta phase while in both cases a birth-and-spread growth mechanism was indicated.[5]

When cooling crystallization is applied in a stirred crystallizer, generally first the metastable alpha phase forms that subsequently transforms to the stable beta phase, according to Ostwald's rule of stages.[6,7,8,9] Without agitation and for low supersaturation the stable beta phase was observed to form directly.[6] There is some evidence that the surface of the stirrer acts as the main source of nuclei by a heterogeneous nuclea-

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tion mechanism but the polymorphic form is not reported.[10] The transformation rate of the alpha form to the beta form increased with crystallization temperature,[6,9] while additives inhibit the transformation.[7,11,12,13,14]

Nucleation of beta phase needles on the surface of the alpha phase prisms was observed.[8,15] Ferrari and Davey[8] assumed that increased attrition of the metastable alpha polymorph due to intensified mixing would result in more surface area available for nucleation of the stable-beta polymorph. Interestingly, the opposite was found by Cashell[16]: stirring a suspension of metastable-alpha crystals would inhibit the transformation process due to disruption of the alpha surfaces.

In cooling crystallization the maximum driving force that can be created is limited by the solubility of L-Glutamic acid in water. The starting point of nucleation during cooling from 80°C to the desired end temperature (usually between 25 and 45°C) is not exactly determined. The formation of L-glutamic acid by pH-shift precipitation from a solution of sodium L-glutamate and diluted acid may have the advantage of a well-defined high-initial supersaturation, provided the solutions are instantaneously mixed.

Industrially L-Glutamic acid is generally produced by fermentation. When the broth is acidified precipitation of prismatic alpha crystals is preferred because these are easier to filter compared to the beta crystals.[11] In agitated pH-shift precipitation a decrease in the purity of alpha crystals from 98 tot 92%, with increasing concentration of the Na-L-glutamate solution from 0.3 to 0.4 M was observed,[11] while pure alpha crystals were obtained from a Na-L-glutamate solution of 0.6 M with Hydrochloric acid.[7] Cashell[16] observed beta-phase crystals inside alpha crystals, when following this procedure and concluded that beta crystals had nucleated in an earlier stage from the surface of alpha crystals, and that these were later overgrown by the faster growing alpha crystals. By slowly (>1 h) acidifying a solution of sodium L-glutamate in a stirred vessel using concentrated hydrochloric acid alpha crystals were obtained again.[17] Precipitation in a stopped-flow cell without further agitation using synchrotron X-ray diffraction resulted in a mixture of alpha and beta crystals when an aqueous solution of 1.8 M sodium L-glutamate was mixed with 99.7% acetic acid in a 1:1 ratio.[18]

Solutions of sodium L-glutamate and sulfuric acid over a range of supersaturation 4–22 were rapidly pre-mixed and the mixed solution was collected in a vessel were precipitation took place. When the vessel was post-stirred, prismatic crystals of the metastable alpha phase were observed while spherulitic crystals of the stable beta phase were obtained when the vessel was not post-stirred.[19] A preliminary mechanism was proposed, assuming both polymorphs to nucleate and to grow concomitantly having different, opposite rates. However, opposite rates for the nucleation and growth rates of the two polymorphs are unlikely when a birth-and-spread (2-D nucleation) growth mechanism is assumed. Furthermore, to explain the formation of the beta spherulites the transformation of previously formed alpha crystals could not be excluded.

The objective of this work is to investigate pH-shift precipitation of L-glutamic acid as a function of high supersaturation. The formation of the polymorphs of L-glutamic acid appears to depend on the initial concentration, but experimental conditions like agitation may as well play a role and will be taken into account. The formation mechanism of the polymorphs is to be elucidated.

Experimental Procedure

In this study the experimental procedure for pH-shift precipitation was followed.[19] An aqueous solution of sodium L-glutamate was mixed with diluted sulfuric acid. First L-glutamic acid is formed in solution by the fast shift in the speciation equilibrium followed by relatively slow precipitation

$$\begin{aligned}\text{Na-L-Glu}\cdot H_2O\,(\text{aq}) + 1/2\,H_2SO_4\,(\text{aq}) &\\ \Leftrightarrow \text{H-L-Glu}\,(\text{aq}) + 1/2\,Na_2SO_4\,(\text{aq}) &\\ \Leftrightarrow \text{H-L-Glu}\,(\text{s}) + 1/2\,Na_2SO_4\,(\text{aq}) &\end{aligned}$$

Solutions were prepared from sodium L-glutamate monohydrate (Orsan-Ajinomoto), from sulfuric acid (Baker), and from ultra pure water made by reverse osmosis. The solutions were filtered over a 0.22 μm filter when they were introduced in the setup. In this work the actual concentration after mixing the flows varied from $c_0 = 0.25$, 0.375, 0.5, 0.75, 1.0 tot 1.25 molal, with corresponding supersaturation ratio with respect to the beta polymorph $S = 4$, 6, 8, 13, 17 to 22. Most experiments were carried out in duplo, except for the experiments at $S = 8$ and 22. All experiments were carried out at room-temperature.

The solutions were premixed in a setup that was built to achieve complete mixing within milliseconds in order to create instantaneously a high supersaturation.[20] The two flows premixed in a Y-shaped mixing-tee, with a static mixer inserted in the outflow tube. After the system had reached steady-state samples of about 100 mL of the mixture were collected at the end of the tube in a beaker. The samples were treated in two ways: either the beaker was covered with foil and left without agitation, or a magnetic stirrer or an overhead impeller was used for post-stirring. In Figure 1, the experimental setup is depicted.

Suspensions were filtered to collect the crystals and washed with ultra-pure water. Raman spectra of the filtered wet cake were acquired using a Kaiser instrument for dispersive Raman with a 732 nm laser. For each cake three different samples

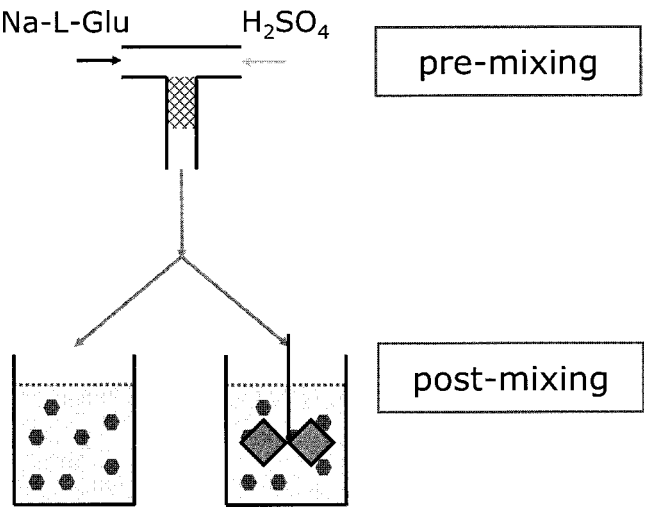

Figure 1. Depiction of the experimental setup.

were measured. The percentage of each polymorph present in the samples was determined using a method for quantitative analysis.[9] Furthermore, microscopic images of the crystals in the suspension were obtained, and some samples were dried for scanning electron microscopy (SEM), focussed ion beam (FIB) imaging and powder diffraction (pXRD).

In a few cases the suspension of alpha crystals made by pH-shift precipitation with post-stirring was kept overnight, while being stirred in order to follow the transformation of the alpha crystals to beta crystals by a solution mediated mechanism.

Results Precipitation Experiments

L-glutamic acid was precipitated from a solution at a supersaturation ratio of $S = 4$, 6, 8, 13, 17 and 22. The flow that left the tube of the premixing device was visibly clear. The induction time to observe crystals in the beaker decreased with increasing supersaturation. In the nonagitated samples crystals could be observed first at the bottom of the beaker. The induction time period varied from a few seconds for $S =$ 22 to 2 h for $S = 4$. It also depended on sample post-stirring: samples agitated with the magnetic stirrer became turbid first, next the sample agitated with the overhead impeller. Even for low-supersaturation, with post-stirring the solutions became turbid within minutes.

In Figure 2 the fraction of the alpha polymorph in the wet filter cake of the precipitate is shown as function of supersaturation ratio. Furthermore, it is shown whether or not the mixture was post-stirred. Without post-stirring always the beta form was observed. With post-stirring for $S \leq 13$ the alpha form was obtained, while for $S \geq 17$ predominantly the beta form was obtained for both agitated and nonagitated vessels.

To illustrate the effect of post-stirring on the polymorphic form that was obtained microscope and SEM images of the samples are shown for increasing supersaturation. For low-supersaturation $S = 6$ with post-stirring only prismatic-alpha crystals were obtained, while without post-stirring only aggregated beta platelets could be observed (Figures 3a). For high-supersaturation $S = 17$ both with and without post-stirring beta spherulites were observed, while with post-stirring also a number of alpha prisms was found (Figure 3b).

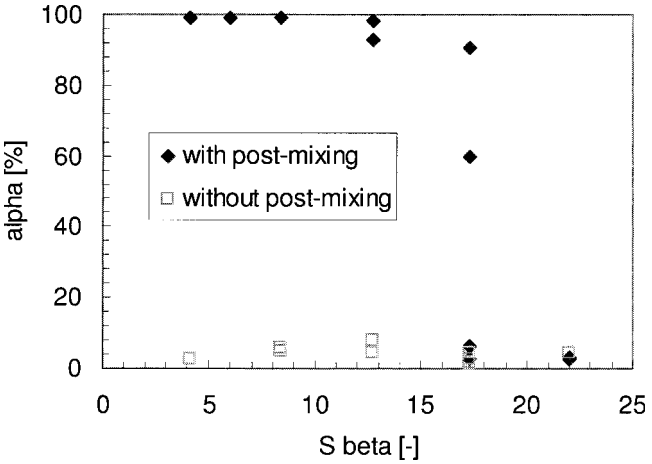

Figure 2. Alpha-polymorph fraction in the wet filter cake as a function of supersaturation *S*, and of post-stirring conditions.

Quantitative analysis based on Raman measurements.

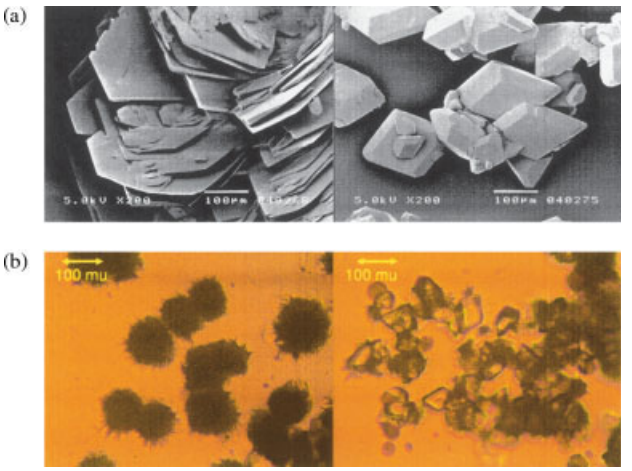

Figure 3. (a) SEM images of L-Glutamic acid crystals precipitated at $S = 6$.

Left: without post-stirring, beta platelets. Right: with post-stirring, alpha prisms.

(b) Microscopic images of L-Glutamic acid crystals precipitated at $S = 17$.

Left: without post-stirring, beta spherulites. Right: with post-stirring, beta spherulites and alpha prisms. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

For the beta crystals obtained without post-stirring it was found that with increasing supersaturation ratio $S = 6$, 13, 17 and 22 the mean size of the platelets decreased, while the aggregates turned into a more spherulitic shape. A detailed SEM image of a spherulite obtained without post-stirring for high-supersaturation $S = 22$ is shown in Figure 4. The surface of the spherulitic crystal consists solely of small beta platelets.

To explain these findings additional measurements were carried out. The spherulites obtained at high supersaturation were examined in detail. In Figure 5 microscopic images are shown of a sample taken from a beaker without post-stirring at high $S = 17$. The first image was made within seconds after premixing, while the second image was obtained approximately one minute later. On the first image smooth spheres

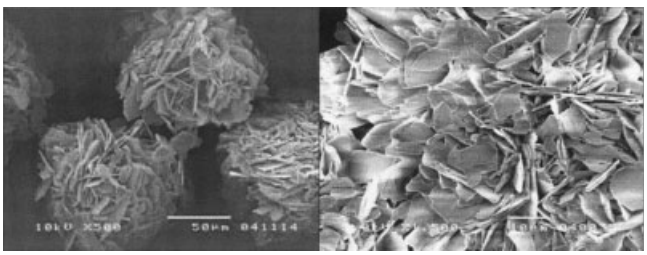

Figure 4. SEM images of details of spherulitic crystals of L-Glutamic acid precipitated at $S = 22$.

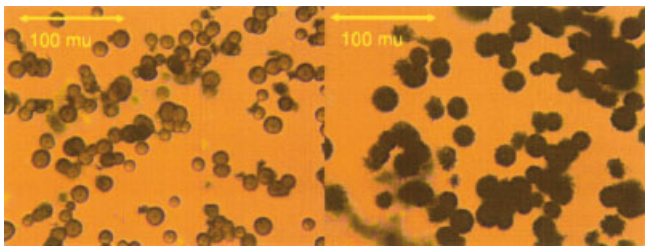

Figure 5. Microscopic images of L-Glutamic acid crystals precipitated at $S = 17$ without post-stirring.

Left: spheres. Right: beta spherulites. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

can be observed, while on the second image the previously shown rough spherulites are visible.

Unfortunately the lifetime of the smooth spheres was short, usually smaller than a minute, and it was not possible to isolate them by filtration due to crystallization on the filter of the beta form. To study the internal structure of the spherulitic crystals, dried samples were further analyzed using FIB and pXRD. It was investigated whether the structure of the spheres could be maintained inside these spherulites. In Figure 6 the powder diffractogram of a spherulitic crystal sample is shown obtained for $S = 17$ without post-stirring. The sample consisted completely of the stable-beta phase, with no indication of peak broadening due to the presence of an amorphous phase inside the spherulites. Furthermore, characteristic peaks can be observed of sodium sulfate, crystallized from adhering solution during drying.

Using FIB single spherulitic crystals were examined. Half of the crystal was cut away to gain access to the interior. In Figure 7 images are shown of the crystal before and after cutting. The structure inside the crystal is difficult to discern but seems to be homogeneous. The platelets at the exterior appear

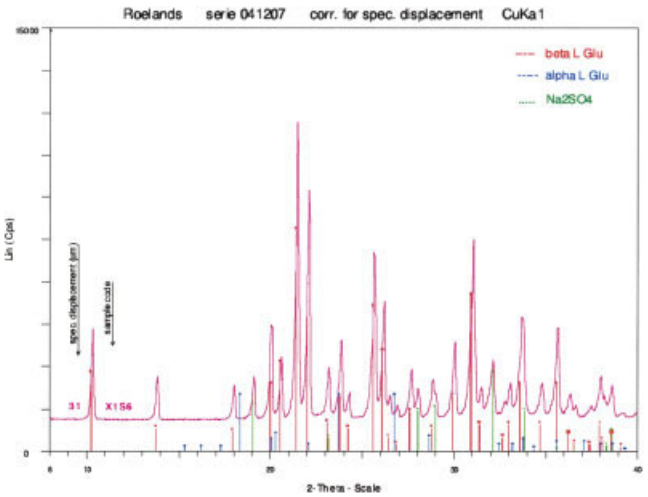

Figure 6. Powder diffractogram of L-Glutamic acid crystals precipitated at $S = 17$ for spherulites obtained without post-stirring.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

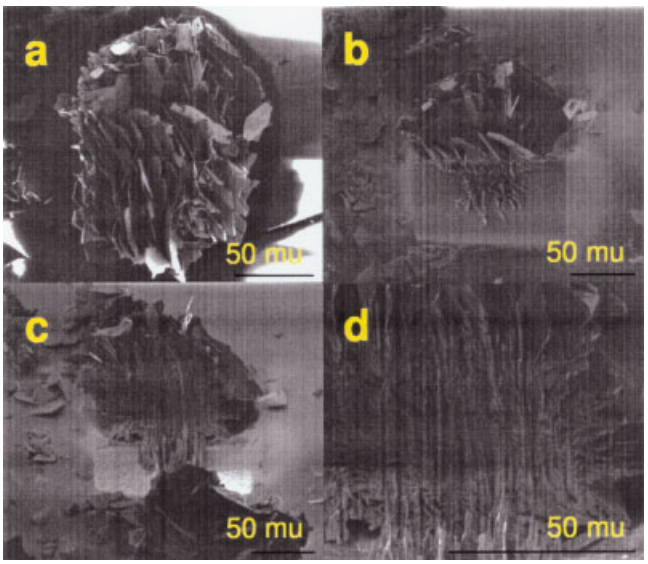

Figure 7a–d. FIB images of a spherulitic L-Glutamic acid crystal precipitated at $S = 17$, (a) before cutting, (b) after cutting, (c) tilted, and (d) in detail.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to be radially oriented, indicating that the spherulites were not formed by random agglomeration.

In another experiment the two reactant solutions were poured directly in a stirred crystallizer so premixing and post-stirring were not separated. In Figure 8 a series microscopic images are shown of a sample taken within seconds after the solution in the crystallizer became turbid. Under the microscope, under stagnant conditions, spheres, spherulites and prisms can be observed to co-exist, and to grow simultaneously for $S = 17$ with-

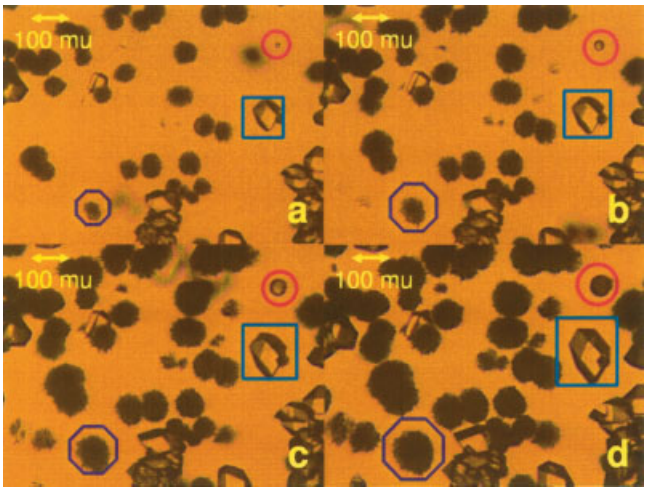

Figure 8a–d. Time series of microscopic images of growing L-Glutamic acid crystals precipitated at $S = 17$.

Framed particles: sphere (circle), alpha prism (square), beta spherulite (octagon). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 9. Clustered beta platelets of L-Glutamic acid precipitated at relatively low supersaturation without post-stirring.

Left: microscopic image crystals precipitated at $S = 6$. Right: SEM image crystals precipitated at $S = 8$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

out transformation taking place. The time period between the images is in the order of seconds.

Furthermore, some images are shown of samples that were precipitated for low-supersaturation $S = 6$ and 8. Figure 9 shows microscopic and SEM images of clusters of beta platelets obtained by precipitation without post-stirring. It can be observed that the centered platelets are very similar to the spherulites shown before, although the number of platelets is smaller and the size of the platelets is larger for the crystals obtained for low-supersaturation. This is an indication that the beta platelets and spherulites that are obtained without post-stirring for both low- and high-supersaturation are formed according to the same mechanism.

For comparison, the microscopic image in Figure 10 shows acicular beta crystals obtained by solution mediated transformation from prismatic alpha crystals in a stirred crystallizer over 24 h. In this figure the prismatic alpha crystals can be observed as well. The shape of the acicular-beta crystals obtained by slow recrystallization differs significantly from the shape of the aggregated beta platelets obtained by precipitation. This indicated that the beta platelets were not obtained by recrystallization from alpha prisms.

In Figure 11, the effect of post-stirring is shown on the alpha prisms for low supersaturation $S = 6$ for samples taken from the suspension at $t = 3$ and 17 mins. With increasing residence time the mean size of the alpha prisms seems to increase, while at the same time especially the larger prisms (>100 μm) become damaged at the corners, probably by attrition. The formation appeared to be faster when a magnetic stirrer was used compared to the use of steel or Teflon-lined overhead stirrers.

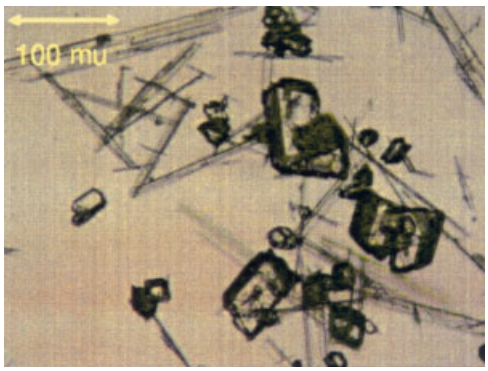

Figure 10. Microscopic image of L-Glutamic acid crystals during transformation in solution with prismatic alpha crystals and acicular beta crystals.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Postulation of a Formation Mechanism

The experimental formation of the metastable alpha phase and of the stable beta phase of L-Glu can be divided between high ($S \geq 17$) and low ($S \leq 13$) supersaturation. A new mechanism will be proposed to explain the experimental results as a function of supersaturation and of post-stirring.

For the experiments carried out for high-supersaturation ($S \geq 17$) the observations can be summarized as follows:

1. Without post-stirring:

- Spherulites consisting of many small beta platelets are formed within seconds.
- Spheres are preceding the spherulites, spheres could not be isolated.
- pXRD: spherulites pure beta form, no amorphous phase.
- FIB: platelets at exterior spherulite are radially oriented, spherulites are not agglomerates.

2. With post-stirring:

- Many spherulites, also few alpha prisms.
- Quiescent sample taken from a stirred crystallizer:

Spheres, spherulites and prisms grow simultaneously.

From these observations it is proposed that the observed spheres consist of a metastable, short-living L-Glu rich phase created by a liquid-liquid phase separation. It is further proposed that crystals of the stable beta phase of L-Glu nucleate from or within the spheres by a heterogeneous nucleation mechanism, and, subsequently grow into spherulites, while consuming the remaining supersaturation. The observations by FIB and pXRD of the spherulites, and the impossibility to isolate the spheres by filtration suggest that the spheres do not consist of a solid, possibly amorphous, phase.

The liquid-liquid phase separation occurs after supersaturation is created by instantaneous premixing. The two liquid phases are assumed to be thermodynamically metastable with respect to the formation of a crystalline phase from the supersaturated solution but their formation may be kinetically

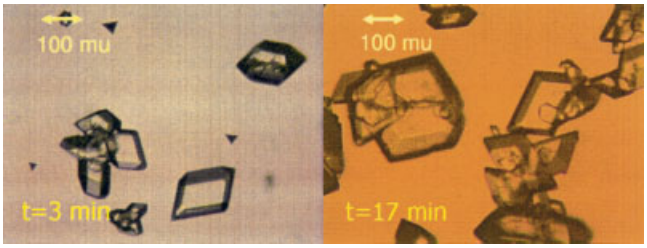

Figure 11. Microscopic image of prismatic alpha crystals of L-Glutamic acid from stirred suspension.

Left: sample taken at $t = 3$ min. Right: sample taken at $t = 17$ min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

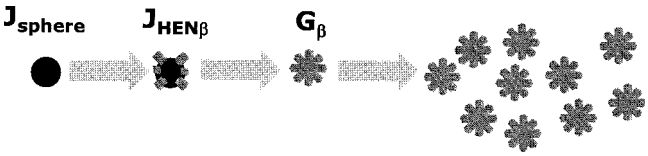

Figure 12. Depiction of the possible precipitation mechanism for high-supersaturation ($S \geq 17$).

favored. The liquid phases, one rich in solute and one poor, coexist temporarily in equilibrium with each other, so the chemical potential, and, hence, the supersaturation of the solute would be equal in each phase. From the droplets the stable beta phase nucleates, either from the bulk or at the interface between droplet and surrounding solution. In Figure 12 the possible mechanism is depicted.

For the experiments carried out for low-supersaturation ($S \leq 13$), the observations can be summarized as follows:

1. Without post-stirring:

- Aggregated beta platelets are formed within minutes (slow).
- Centered platelets resemble spherulites obtained for high *S*.
- Shape distinctively different form needle-like crystals obtained by transformation.

2. With post-stirring:

- Alpha prisms are formed within seconds (fast).
- Prisms grow within minutes to size >50 μm, attrition takes place.
- Prisms form independent of mixer type, magnetic stirrer or overhead impeller.

From the observations without post-stirring it is concluded that the formation of the aggregated and centered beta platelets takes place according to the same mechanism, as was observed for high-supersaturation: beta crystals nucleate heterogeneously from metastable droplets. Because of the lower supersaturation the number of droplets is small and their size remains small as well, so these are not visible.

It is proposed that with post-stirring the liquid-liquid phase separation is disrupted because concentration fluctuations are equalized by mixing. From the homogenized solution the metastable alpha phase nucleates directly, probably by a heterogeneous mechanism. High-shear rates around the stirrer favor dispersion of alpha crystals over the solution. When the alpha crystals have grown to a sufficiently large size to become subject to attrition (>50 μm), this mechanism may additionally contribute to the formation of the alpha phase. In Figure 13 the possible mechanism is depicted.

Discussion

The occurrence of a liquid-liquid phase separation (oiling-out) resulting in the formation of droplets of a solute-rich phase in a continuous solute-poor phase was reported for large molecules,[21,22,23,24,25,26,27] and for relatively small molecules.[28,29,30,31,32,33,34] The occurrence of the liquid phases is thermodynamically metastable compared to the formation of a solid phase from solution. After some time crystals formed from the continuous phase, while the droplets disappeared, suggesting a solution mediated transformation mechanism,[29,30,31] while also crystallization both within the droplets and from the surrounding solution was recently observed.[35] Spherulitic crystals formed according to this mechanism were evaluated for their compression properties in pharmaceutical applications.[36]

The phenomenon of liquid-liquid phase separation generally takes place in highly-supersaturated solutions, while for small molecules these solutions were concentrated as well. The concentration of the solutions used in the experiments described in this work varied from approximately 5–20 wt% for L-Glu with 2–7 wt% sodium sulphate. This relatively high-concentration agrees well with the concentration ranges given in the studies on liquid-liquid phase separation supporting the proposed mechanism.

At first sight, the experimental results on the pH-shift precipitation of L-Glutamic acid without post-stirring do not seem to follow Ostwald's rule of stages because the stable beta polymorph is directly precipitated, and not the metastable alpha polymorph. However, according to the proposed mechanism, the temporary formation of two highly metastable liquid phases precedes the formation of the stable beta phase. With post-stirring and low-supersaturation the liquid-liquid phase separation is assumed to be disrupted by vigorous mixing and from the homogenized solution the metastable stable alpha phase nucleates first (compared to the stable beta phase), and, therefore, Ostwald's rule is followed as well. When a suspension of alpha crystals was kept overnight while being stirred, the prismatic-alpha crystals transformed to needle shaped crystals of the more stable beta phase. It is noted that Ostwald's rule of stages is an observation rule and not a physical law.

The following relative stability is proposed for the phases according to the postulated mechanism. Least stable is the supersaturated solution created by instantaneous mixing, followed by the two liquid phases obtained by the liquid-liquid phase separation, next is the metastable alpha phase, and finally the stable beta phase. The polymorphic phase that has the lowest nucleation work, is actually formed.[37] For both low- and high-supersaturation, starting from the supersaturated solution, the nucleation work of the highly metastable droplets is assumed to be smaller than that of the crystalline phases.

Possibly, the subsequent nucleation of a crystalline phase is temporarily arrested because of increased viscosity inside the highly concentrated droplets. The unexpected formation of the stable-beta phase from the droplets may be explained by the interface of the droplets acting as a template or precursor. Another explanation may be the different distribution of L-glutamic acid over its two conformers in solution inside the drop-

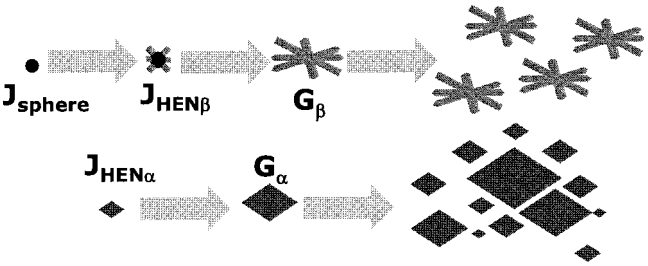

Figure 13. Depiction of the possible precipitation mechanism for low supersaturation ($S \leq 13$), without post-stirring (top), and with post-stirring (bottom).

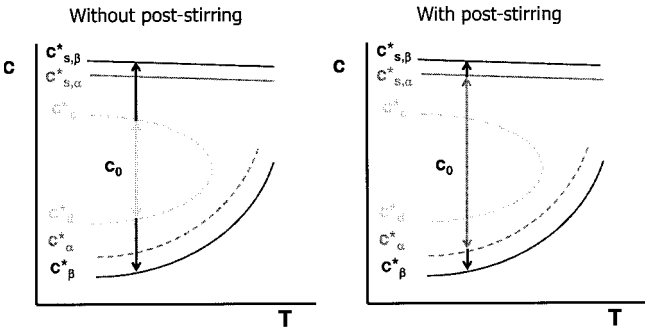

Figure 14. Phase diagram for L-Glutamic acid.

Left: without post-stirring. Right: with post-stirring.

lets. Furthermore, it is noted that the solution contains apart from L-glutamic acid also a considerable amount of sodium sulfate. It may be of interest to test different acids for precipitation (hydrochloric acid, acetic acid) apart from sulfuric acid to study the effect on the liquid-liquid-phase separation.

In Figure 14 the resulting phase diagram is shown. Three phases can be observed in equilibrium with the solution: the highly metastable liquid phase (denoted by *c* for the concentrated phase, and *d* for the dispersed phase), the metastable crystalline alpha phase and the stable crystalline-beta phase. In the case of precipitation without post-stirring, first liquid-liquid-separation occurs, followed by a transformation of the droplets into the stable-beta phase. In the case with post-stirring first the metastable-alpha phase crystallizes, followed by a solution mediated transformation towards the stable beta phase.

Finally, it is noted that in precipitation processes taking place at high-supersaturation levels amorphous metastable phases are commonly observed. These metastable phases often subsequently recrystallize into more stable crystalline phases by a solution-mediated mechanism. Some examples of ionic compounds able to form both amorphous and crystalline phases are calcium carbonate,[38] calcium phosphate[39] and alumina.[40] The number of publications on the formation of amorphous phases of molecular compounds from solution is surprisingly small: the preparation of an amorphous form of iopanoic acid by precipitation from an aqueous solution of sodium iopanate with diluted acid.[41] For L-glutamic acid pXRD of the spherulites did not indicate the formation of an amorphous phase. It might be allowed, however, to view the formation of amorphous phases and of liquid-liquid phase separation as similar processes, while considering the amorphous phase as a liquid of indefinite viscosity.

Conclusions

L-glutamic acid was precipitated from solutions of sodium L-glutamate and diluted sulfuric acid that were continuously premixed in a Y-mixer to create instantaneously a homogeneous supersaturation. Using this setup the formation of the L-Glu crystals was studied as a function of increasing supersaturation ratio by increasing the concentrations in the reacting solutions. Samples of the premixed supersaturated solution were collected in beakers that were either subjected to post-stirring or left under quiescent conditions.

For relatively low supersaturation ($S \leq 13$), with post-stirring prismatic crystals of the metastable-alpha phase were formed while without post-stirring aggregated platelets of the stable beta phase were obtained. The induction time in post-stirred samples was smaller, usually less than a minute, compared to up to one hour for nonagitated samples. For high supersaturation ($S \geq 17$), within seconds spherulites of the stable-beta phase were observed both with and without post-stirring. With post-stirring apart from beta spherulites, also alpha prisms were formed.

A mechanism was postulated to explain the formation of the polymorphs of L-Glu as a function of supersaturation and of agitation. It is proposed that temporarily a highly metastable liquid-liquid phase separation can take place preceding nucleation of crystals of the stable-beta phase from the droplets. Subsequent growth of the platelet-shaped beta crystals results in the formation of spherulites. For low-supersaturation, without post-stirring the beta phase formed slowly according to the same mechanism, forming aggregated platelets. With post-stirring the liquid-liquid phase separation was disrupted resulting in the formation of prismatic crystals of the alpha phase nucleated by a heterogeneous mechanism.

On microscopic images of samples taken at high-supersaturation, smooth, droplets-like, spheres were observed that over a time scale of seconds to minutes turned into rough spherulites. However, these spheres could not be isolated for analysis. Powder diffraction of the spherulites revealed that these consisted solely of the beta phase without any indication of peak broadening due to the presence of an amorphous phase. By analysis of a spherulitic crystal by focused ion beam it was observed that the beta platelets at the exterior had a radial orientation, indicating that the spherulites were not formed by agglomeration.

On microscopic images of a quiescent suspension sample from a stirred-crystallizer spheres, alpha prisms and beta spherulites grew simultaneously without transformation.

For the experiments carried out at low-superaturation, the aggregated and centered beta platelets that formed without post-stirring resembled spherulites obtained at high-supersaturation. The shape differed from needle-like crystals obtained by transformation. Large alpha prisms (>50 μm) that were obtained when post-stirring was applied became damaged due to attrition.

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Appendix A: Nucleation Theory

In this study the classical nucleation theory (CNT) was followed as described and adapted by Kashchiev.[35] Solute molecules collide with each other at a certain frequency due to Brownian motion. After the creation of a driving force for precipitation, these collisions lead with certain efficiency to the formation of clusters of solute molecules. This is a dynamic process and molecules will attach and detach successively. The driving force for the creation of clusters is the decrease in chemical potential by molecules leaving the solution, while forming a cluster due to the decrease in Gibbs-free energy between the two bulk phases. However, a penalty has to be paid for creating an interface between the cluster and its surrounding solution.

The work of formation of an n-sized cluster W[J] can be approximated by

$$W(n) = -nkTlnS + \gamma A_c(n) \tag{A.1}$$

With Boltzmann constant k [J K-1], absolute temperature T [K], supersaturation with respect to the stable polymorph beta

S [−], interfacial energy γ [J m^{-2}] and cluster surface area $A_c(n)$ [m^2]. The work of formation of a cluster will at first increase with increasing number of molecules until a maximum is reached. At this point the flux of solute molecules attaching to the cluster f^* equals the flux of molecules detaching from the cluster g^*. Up from this size, the cluster size will increase with every molecule that attaches and the work of formation will decrease with n. The cluster with the maximum work of formation also called nucleation work W^* is called the nucleus n^*.

For spherical clusters the surface area $A_c(n) = (36\pi v_0^2)^{1/3} n^{2/3}$ with $v_0 = M/\rho_c N_a$ [m^3] the molecular volume with M [kg mol^{-1}] molecular weight, ρ_c [kg m^{-3}] crystal density, and N_a [mol^{-1}] Avogadro's number. The interfacial energy γ [J m^{-2}] is a weighed average over all crystal faces. Mersmann[36] derived the following relationship between the interfacial energy and the natural logarithm of the bulk solubility of the compound c_e [mol m^{-3}]

$$\gamma = \beta kT \frac{1}{v_0^{2/3}} \ln \frac{1}{v_0 c_e} \tag{A.2}$$

For spherical clusters the shape factor $\beta = 0.514$.

For spheres the nucleus size n^* and the nucleation work W^* can be derived from the condition $dW/dn = 0$ for $n = n^*$ using Eq A1

$$n^* = \frac{32\pi {v_0}^2 \gamma^3}{3(kT)^3 \ln^3 S}$$
$$W^* = \frac{16\pi {v_0}^2 \gamma^3}{3(kT)^2 \ln^2 S} = \frac{1}{2} n^* kT \ln S \tag{A.3}$$

The nucleus size and the nucleation work depend on two main parameters: The externally controlled supersaturation and the interfacial energy that is controlled by crystal-solution interaction.

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