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The Effect of Temperature and Solvent Composition on Transformation of β- to α-Glycine As Monitored in Situ by FBRM and PVM

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Abstract:

The application of in situ focused beam reflectance measurement (FBRM) and particle vision measurement (PVM) in monitoring transformation of glycine polymorphs is introduced. The effect of solvent composition and temperature on the transformation from β- to α-glycine was investigated. It is noted that the transformation kinetics are highly sensitive to both the solvent composition and temperature and the transformation rate is a function of ethanol content in aqueous ethanol mixtures. At 303 K, high initial ethanol concentration accounts for a steady transformation. At the same ethanol content, the transformation rate decreases with decrease in temperature. A smoother transformation was observed at 293 K. The results are consistent with the solvent-mediated transformation mechanism in which β-glycine dissolves and α-glycine nucleates and grows. The thermodynamically stable γ-glycine was not observed. Understanding these effects can aid optimization and improve process control.

1. Introduction

Crystallization from solution is well established as an essential separation and purification technique in the pharmaceutical industry. Monitoring and control of the crystallization process is critical to meet special requirements for products such as desired crystal morphology and proper crystal size distribution (CSD). During polymorphic transformation, a desired polymorphic form may be obtained. This is due to the different experimental conditions (stirring, temperature, solvent composition, nucleation mechanism, etc.) that influence the transformation process. Hence, it is essential to learn which experimental factors affect the transformation kinetics. However, these procedures have been based on sampling and offline analysis by different methods.[1] It is possible that properties of crystal particles are changed because of nonrepresentative sampling, postsampling,[2] and drying. Especially with respect to polymorphs, this sample manipulation may have drastic effects on analysis of the results, with the metastable state transforming to the stable state, giving erroneous results.

Glycine exists in three distinct polymorphic forms: α, β, and γ. The β form is a metastable noncentric form. The single crystal structure of this form was first published by Iitaka[3] in 1960 and reported recently by Ferrari.[4] β-Glycine has been found to transform rapidly into the α form in air or water, but the crystals remained unchanged if kept in a dry environment.[3] α-Glycine and γ-glycine have received great attention from researchers,[5,6] but little work has been done regarding β-glycine.

Focused beam reflectance measurement (FBRM) and particle vision measurement (PVM) instruments are powerful tools developed by Lasentec as in situ particle monitoring techniques for inline real-time measurement of particle size and morphology, which have attracted considerable attention in the particulate process industry because of their simplicity and capability for online measurement.[7,8] FBRM is a probe-based high solids concentration particle characterization tool, and it is a new method to perform particle size measurements in the range of 0.25−1000 μm.[9] This system is based on a high-speed scanning laser beam which, when it hits a particle, reflects a light pulse which is directly proportional to the chord length it transcribes over the particle surface.[10] The great advantage of this technique is that data are acquired online and in real time give particle size data and population trends of particles in suspension. PVM is a high-resolution video microscope, which is typically used for in-process high-resolution imaging of particles within the process environment.[2]

As known, crystal forms transition may be accompanied with the dissolution of the metastable form and subsequent nucleation and growth of the stable form, leading to a change in the particle counts and dimensions, which can be monitored by FBRM. The crystal habit changes can be monitored by PVM. Applying these tools in the crystallization process of polymorphs, in which many aspects of their behavior are still not fully understood, is of great benefit in understanding the process dynamics and phase conversion, as well as other associated processes such as nucleation mechanism and secondary processes.

This work demonstrates the use of the FBRM and PVM in monitoring transformation process of β- to α-glycine. In the work reported here, a study of the effects of solvent composition

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and temperature on the polymorphic transformation of β- to α-glycine has been carried out by in situ FBRM and PVM.

2. Materials and Methods

α-Glycine(Supplied from Kewei Co.Ltd., China, more than 99.0% in purity) was used to carry out these investigations. Ethanol used for the experiments was of analytical reagent grade. Distilled deionized water of HPLC grade was used. β-Glycine was obtained from the method promoted by Drebushchak.[11] First, an aqueous acetic acid solution (initial volume ratio of acetic acid to water is 5:1) was saturated with glycine. Then an equal volume of ethanol was added quickly to this solution, and masses of needlelike β-glycine crystals precipitated. The crystals were isolated rapidly by filtration. The purity of β-glycine obtained was more than 99.0% (confirmed by XRD patterns and microscopic images).

The solubility of the α form of glycine in pure water was measured by a synthetic method which is described in the literature.[12−14] During the measurement, predetermined excess amounts of solute and solvent of known masses were transferred to the equilibrium vessel. The contents of the vessel were stirred continuously at a set temperature for 30 min. Then, additional solvent of known mass was introduced into the cell. When the last portion of solute had just disappeared, the intensity of the laser beam penetrating the vessel reached a maximum, and the solubility of α-glycine was calculated from the known masses of solvent and solute.

The α and β polymorphs of glycine were distinguished using X-ray powder diffraction (D/MAX 2500 Japan) with Cu Kα radiation at 40 mA and 45 kV. The sample was packed into a plastic holder and was scanned from 2° to 40° 2θ at a step size 0.02° with a dwell time of 1 s. Divergence slits and receiving slits were 1° and 0.15 mm, respectively. Measurement temperature was kept at the range of 298 ± 1 K. The results determined are in agreement with the literature reports.[3,4]

The transformation process of β to α form glycine was carried out as follows: the undried crystals prepared as above were mixed in aqueous ethanol solution in a 400 mL glass cylindrical crystallizer with a jacket to circulate the thermostatted water and were slurried with a speed of 200 rpm for 30 min. To visualize the processes occurring in situ, transformation from the β to α form glycine was monitored using PVM and FBRM. PVM probe (model 800 L) was operated with an image update rate of 6 images per minute. The FBRM probe (model M400LF) has a measurement range of 0.25−1000 μm. In this study, there were six population ranges set that were 0−10 μm, 10−20 μm, 20−60 μm, 60−120 μm, 120−240 μm, 0−300 μm, respectively. The probe measurement duration was set at 5 s. Offline digital images analysis was performed using a Panasonic Lumix DMC-FZ20 system operating the Panasonic image analysis connected to a 3CCD color vision camera mounted on an Olympus BH2 optical microscope.

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Table 1. **Experimental conditions**

		aqueous ethanol solution (mass fraction)	
expt.	temperature/K	ethanol content/%	water content/%
A	303	100	0
B	303	90	10
C	303	50	50
D	303	0	100
E	293	50	50

A series of trials as outlined in Table 1 was performed to examine the effect of temperature and ethanol concentration on the transformation of β to α form of glycine. Experimental setup is shown in Figure 1.

3. Results and Discussion

3.1. Solubility of α-Glycine in Ethanol + Water Mixture and Transformation of *β*- to α-Glycine. The solubility of α glycine at 293 and 303 K is graphically presented in Figure 2. The experimental error is within 5% of measured value. From Figure 2, it can be seen that the solubility of α-glycine is the function of ethanol content in water + ethanol mixtures. The solubility values decrease with the increasing ethanol content in binary solvents.

Microscopic images of β-glycine and α-glycine are shown in Figure 3. β-Glycine is needlelike and α-glycine is platelike. This difference is obvious, suggesting that the PVM and FBRM in situ techniques will readily distinguish between these two polymorphs. The platelike crystal was different from the obtained needle one not only in shape but also in crystal structure. The XRD patterns of crystals before (β form) and after (α form) are shown in Figure 4. As Figure 4 shows, it is

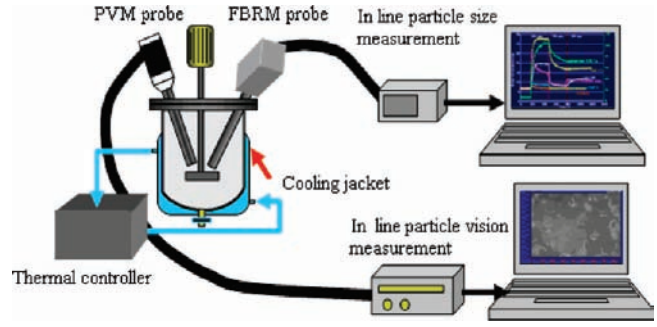

Figure 1. **Equipment of experiments for transformation of *β*- to α-glycine.**

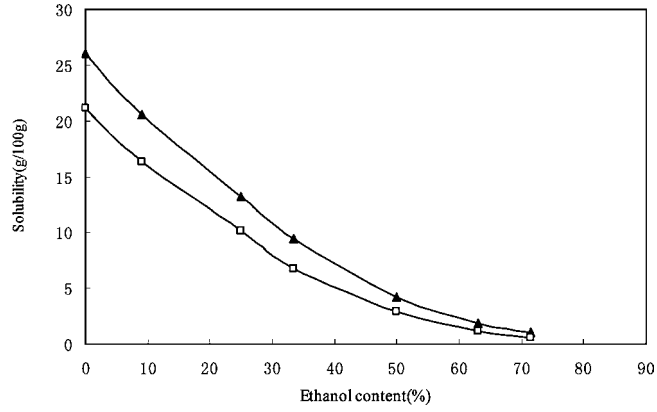

Figure 2. **Solubility curve of α-glycine in water + ethanol at □, 293 K and ▲, 303 K.**

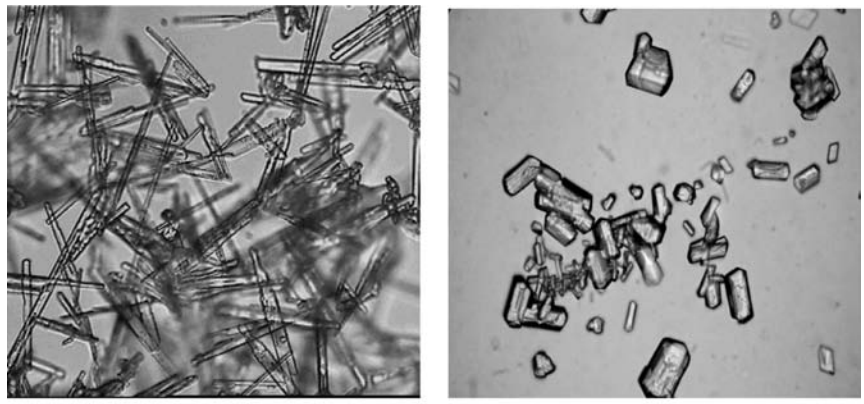

Figure 3. **Microscopic images of glycine crystals: (a) *β*-glycine and (b) α-glycine.**

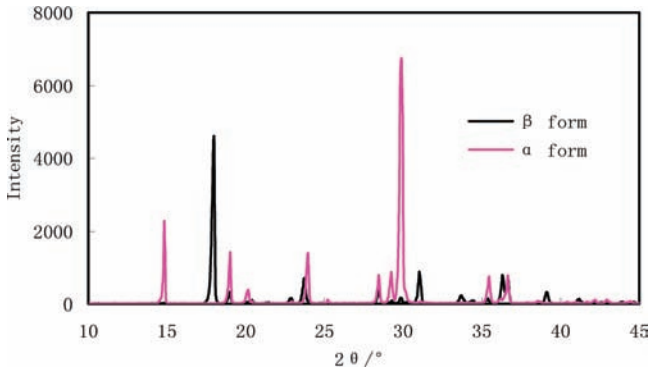

Figure 4. **XRD patterns of *β*- and α-glycine.**

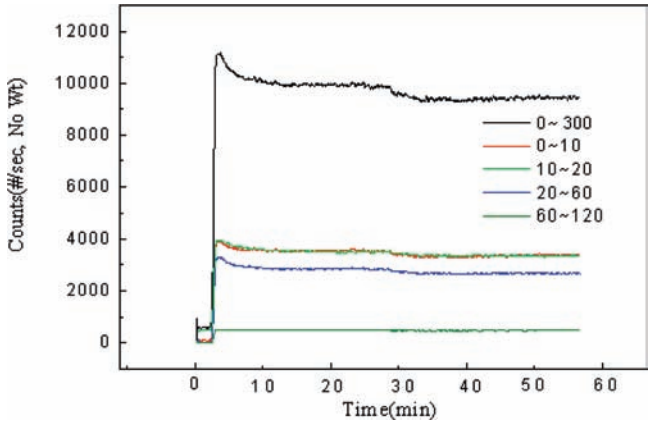

Figure 5. **FBRM counts in experiment A.**

clear that the XRD pattern of the *β* form of glycine is different from the α form of glycine, consistent with the different crystal structures of *β*- and α-glycine. In preliminary experiments, *β*-glycine was found to transform rapidly into the α form in water, and the speed of the transformation was faster at higher temperatures. In order to investigate the transformation process of *β*- to α-glycine, a series of trials was performed to examine the effect of ethanol concentration and temperature on the transformation of the *β* to the α form of glycine.

3.2. Transformation of *β*- to α-Glycine in Aqueous Ethanol Solution at 30 °C. In experiment A, transformation of *β*- to α-glycine was investigated in pure ethanol. As observed in Figure 5, the curves of the FBRM particle counts have a minute drop within the initial 2 min. Subsequently, the counts in all ranges measured by FBRM keep basically constant. It

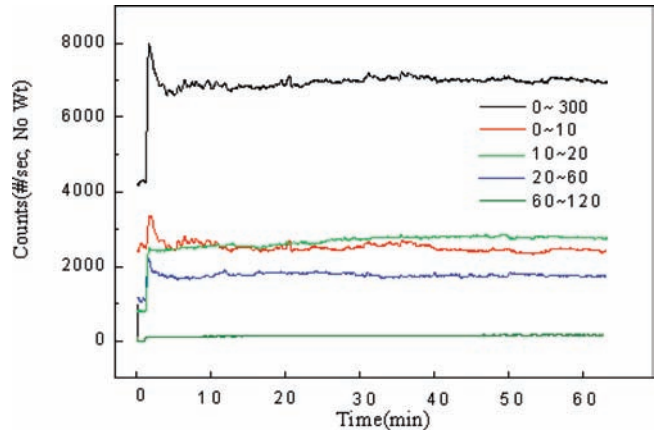

Figure 6. **FBRM counts in experiment B.**

can be shown by PVM that images taken at different times are nearly the same. There are many needlelike crystals suspended in solution, which is also consistent with curves analysis of FBRM counts change. The XRD pattern of crystals was performed after the transformation. The characteristic peaks basically agree with that of *β*-glycine, which confirms that no transformation has occurred.

In experiment B, transformation of *β*- to α-glycine was investigated in 90% ethanol. The FBRM counts change in all particle ranges measured resemble that of experiment A. As observed in Figure 6, the curves of the FBRM particle counts have a relatively obvious drop within the initial 2 min compared with experiment A. Subsequently, the counts in all ranges measured by FBRM remain basically constant. From PVM, images taken at different times show no obvious transformation occurring within 1 h, which is confirmed by XRD.

In order to further analyze the effect of ethanol concentration on transformation of *β*- to α-glycine, experiments C and D were performed. Compared with experiments A and B, remarkable differences in the curves of the FBRM counts change were generated in experiment C.

The FBRM counts change of *β*- to α-glycine in 50% aqueous ethanol solution at 303 K during transformation is graphically presented in Figure 7. From Figure 7, it can be seen that there is relatively quick transformation process within 20 min. The counts in all ranges measured by FBRM decrease, but an immediate drop in FBRM counts present in the first 20 min or so. In addition, it is obvious that 0−10 *μ*m particle counts

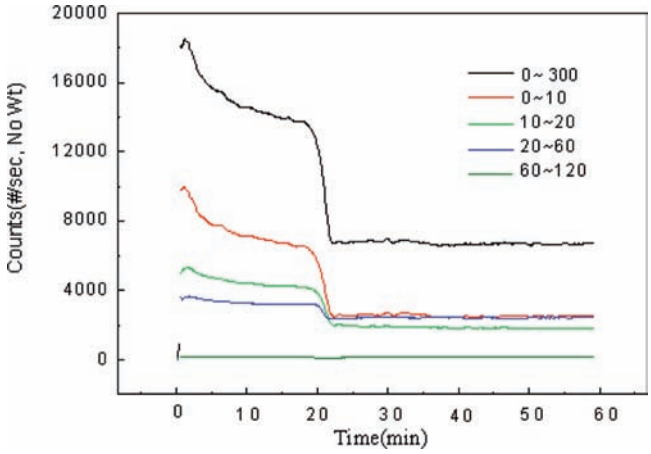

Figure 7. **FBRM counts in experiment C.**

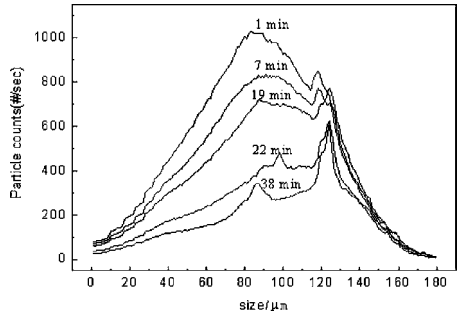

Figure 8. **Chord length distribution during transformation in 50% aqueous ethanol solution at 303 K.**

decrease rapidly, which is followed by 10−20 μm and 20−60 μm particle counts in measured population ranges.

The changes in the FBRM signal are consistent with the change in crystal shape. The needles of β-glycine give rise to many FBRM counts corresponding to the needle width. The blocks of α-glycine give rise to fewer counts corresponding to their 'blocky' shape. This effect is enhanced by the agglomeration of α-glycine, seen clearly in Figure 9f. The agglomerates have a much larger volume than the needles, so there are fewer of them, consistent with a reduction in the total number of counts.

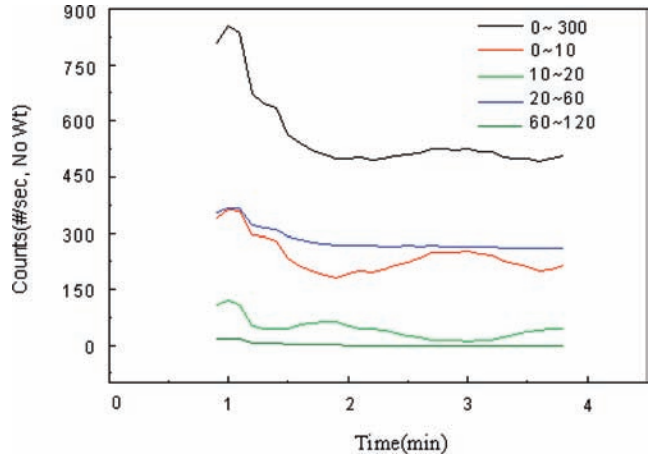

Figure 10. **FBRM counts in experiment D.**

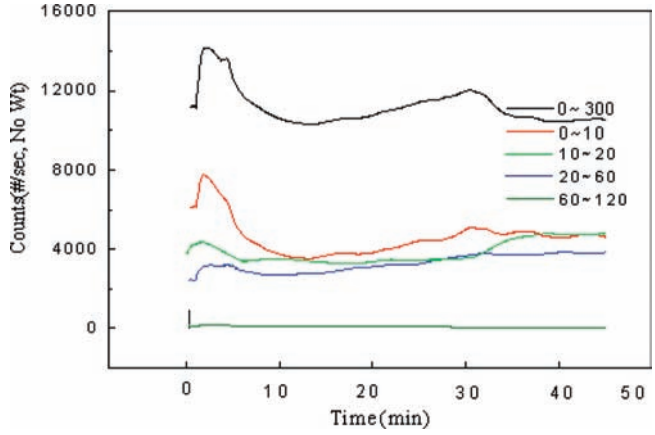

Figure 11. **FBRM counts in experiment E.**

The change in the dynamics of the particle system, due to the polymorphic transition, is also emphasized by the FBRM chord length distributions. Figure 8 shows the trend history of different particle size groups. From Figure 8, with the proceeding of the transformation process, smooth drops in counts and peak shifts in the distribution mode from the FBRM chord length distribution are observed, which accounts for two crystal form transformation processes in the solution. It can also be

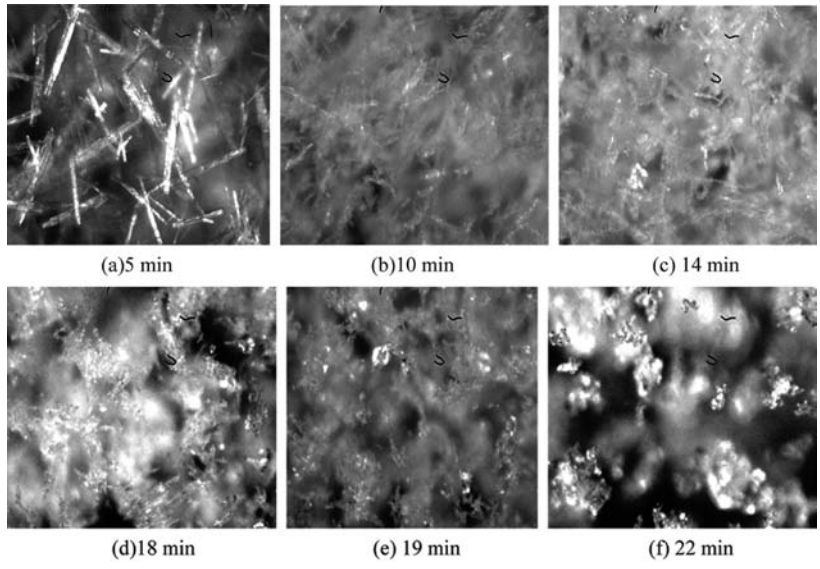

Figure 9. **Images taken during transformation in 50% aqueous ethanol solution at 303 K.**

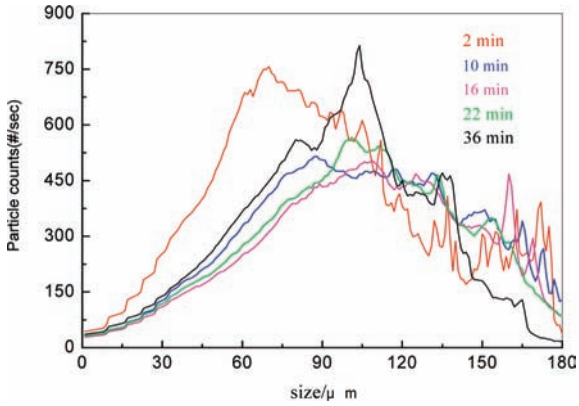

Figure 12. **Chord length distribution during transformation in 50% aqueous ethanol solution at 293 K.**

seen that particle counts of all population ranges decrease during the initial 10 min, which implies that dissolution of needlelike crystals and nucleation of platelike crystals can proceed synchronously. During the time between 19 and 20 min, the area of the peak declines quickly, which accounts for the agglomerate phenomenon in the solution. Then the area of the peak in the smaller particle range becomes very small, while the area of the peak in the larger particle range increases, in agreement with the typical images taken by PVM in Figure 9. At the initial time of transformation, there are only needlelike (β form, confirmed by XRD) crystals suspended in solution (Figure 9a). With the dissolution of needlelike crystals, platelike crystals (α form, confirmed by XRD) appear. At 20 min or so, it is clearly seen that platelike agglomerates without needlelike crystals are suspended in solution, consistent with the observed reduction in 'total counts'.

The transformation of β- to α-glycine in pure water at 303 K was performed in experiment D. Compared with experiment C, an obviously faster transformation process was observed by particle counts change as shown in Figure 10. The counts in all ranges measured by FBRM decrease quickly, which illustrates that quick dissolution of β-glycine occurred. The rise of supersaturation leads to nucleation of α-glycine, which is confirmed by increase of 0−10 μm particle counts after 2 min.

3.3. Transformation of β- to α-Glycine in Aqueous Ethanol Solution at 20 °C. The temperature effect on the transformation behaviors of β- to α-glycine crystals was examined at 50% ethanol concentration at 293 K in order to make a comparison with in the case of 303 K. The change in FBRM particle counts at 293 K is shown in Figure 11. As observed in Figure 10, in the initial 10 min, particle counts of all population ranges have a decrease, in which particle counts of 0−10 μm range have a very drastic decrease. It is speculated that dissolution rate of small particles is greater than that of large particle for β-glycine. With dissolution of β-glycine particles, the supersaturation of the solution rises. After 10 min, particle counts of 0−10 μm range start to slowly increase, which has resulted from nucleation of α-glycine. In comparison with 0−10 μm, the cases of 10−20 μm and 20−60 μm are different. A trend of increase is shown after 10 min. This can be result of α-glycine crystal growth, which can be confirmed by the history of different chord length distributions at different times from Figure 12. From Figure 12, chord length distributions decrease in the initial 10 min, while a shift of peak in chord length distribution is observed after 10 min, which indicates transformation from β- to α-glycine. After 16 min, the peak in chord length distribution start to rise smoothly, consistent with further growth of α glycine. The PVM images taken are consistent with FBRM particle counts change in Figure 13. This confirms that the transformation can be monitored in situ by FBRM and PVM, and illustrates the temperature effect. So it is possible to monitor the effect on the behavior of the particles by changing operating conditions, ultimately leading to improved control of the crystallization process.

Table 2 shows a summary of the results from these five experiments. Note that the order has been changed from Table 1 to list the experiments in order of solubility.

Nucleation of α-glycine appears to be instantaneous when the water concentration is high, and was not detected here when the water content is low. Once nucleation has occurred, the rate of transformation increases with increasing solubility. This finding could be confirmed by studying seeded transformations. The fact that the thermodynamically stable γ-glycine was not

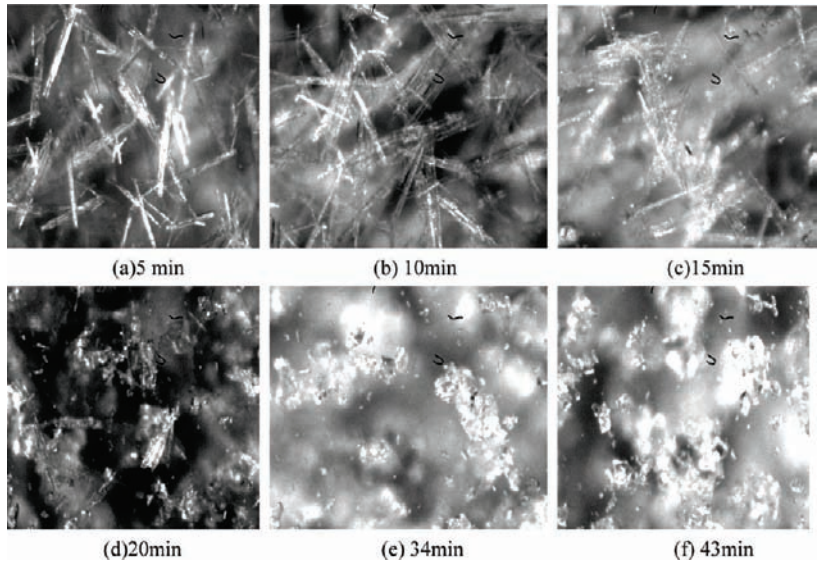

Figure 13. **Images taken during transformation in 50% aqueous ethanol solution at 293 K.**

Table 2. **Summary of transformation experiments**

expt.	*T*/K	ethanol content/%	form α solubility g/100 g	time to start (min)	time from start to finish (min)
A	303	100	≪1	>60	−
B	303	90	<1	>60	−
E	293	50	4	<1	36
C	303	50	5	<1	22
D	303	0	25	<1	3

observed under these conditions is consistent with previous observations about the reluctance of γ-glycine to nucleate and grow.

3.4. Transformation Mechanism of β- to α-Glycine. Transformation of β- to α-glycine was found to be in agreement with solvent-mediated transformation as monitored by in situ FBRM and PVM. This was consistent with analysis of offline microscopic images of the transformation in 50% aqueous ethanol solution at 303 K as shown in Figure 14. On the basis of in situ FBRM, PVM, and offline microscopy analysis, a polymorphic transformation mechanism from β- to α-glycine was suggested.

Agglomerates of needlelike β-glycine crystals first begin to disperse and then break into many small crystals as they dissolve. With dissolution of β-glycine, the supersaturation of the solution increases. Nucleation of stable α-glycine takes place. Crystal growth of α-glycine is driven by further dissolution of β-glycine.

FBRM and PVM in this work provide very valuable information in monitoring glycine phase transition. From images taken by PVM and offline analysis, together with population statistics monitored by FBRM, the transformation process can be successfully monitored. If in situ ATR-FTIR is available, it is also necessary to monitor concentration changes of both glycine polymorphs to gain a truer understanding during transformation.

4. Conclusions

The effect of ethanol concentration and temperature on the polymorphic transformation behavior from β- to α-glycine was investigated. The phase transformation of β- to α-glycine can be investigated using optical microscopy and can also be monitored by in situ PVM and FBRM. It is clear from FBRM and PVM data that the transformation kinetics are highly sensitive to both the composition of the solvent mixture and transformation temperature.

The transformation will be faster in water and at higher temperature. No transformation was observed at high initial ethanol concentration (≥90 wt %) in ethanol + water mixtures at 303 K within 60 min. The nucleation behaviors of glycine polymorphs at 293 K are very different from those at 303 K.

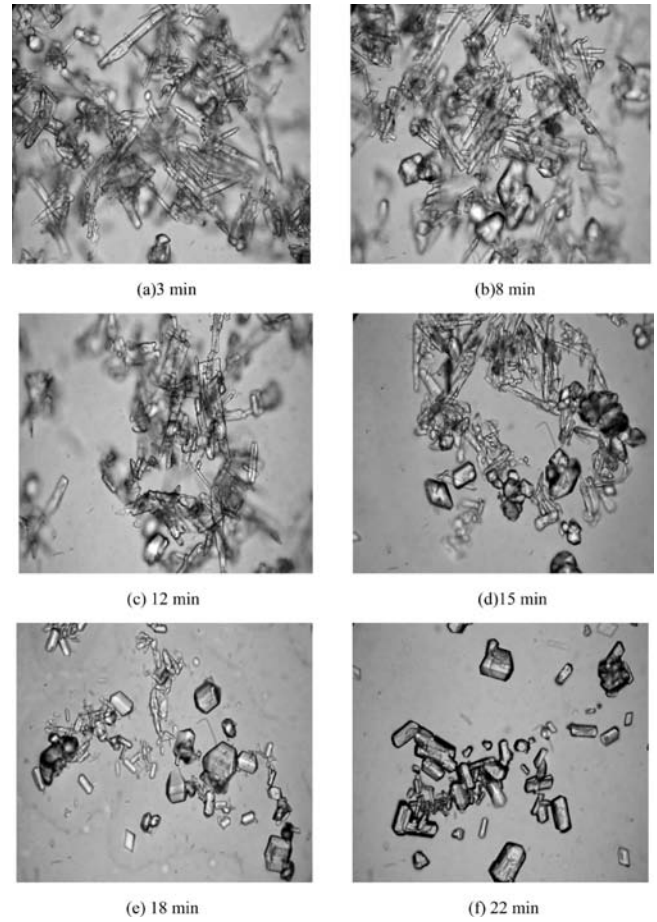

Figure 14. **Progress of polymorphic transformation from β- to α-glycine.**

At the same ethanol content, a slower and smoother transformation from the β form to the α form was observed, and it was found that the transformation rate decreases with decrease of temperature. An exact observation of transformation from β- to α-glycine was obtained. Further studies with varying amounts of α-glycine seed would provide more information about the transformation mechanism. Understanding these effects can aid optimization and improve process control.

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