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The Use of in Situ Tools To Monitor the Enantiotropic Transformation of *p*-Aminobenzoic Acid Polymorphs

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ABSTRACT: The use of in situ tools to monitor the transformation of a polymorphic material has the potential to provide unique information about the mechanism and rate of transformation of the polymorphs. In this paper, the solution mediated transformation between α and β form p-aminobenzoic acid (PABA) was investigated in detail. Solubility of α and β form PABA in pure ethanol was also reported for the first time, allowing the accurate determination of the transition temperature of 13.8 °C. For the transformation experiments, Raman spectroscopy and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy were used to in situ monitor the solid phase concentration and liquid concentration, respectively; Focused Beam Reflectance Measurement (FBRM) was used to in situ track the changes in the size and morphology of the particles. The observed changes were confirmed using PVM in-process imaging. It was proved by solubility data and transformation experiments that the relationship between α and β form is enantiotropic.

1. INTRODUCTION

Polymorphism is defined as the ability of a compound to exist in more than one crystalline form, each of which has the same chemical structure but different arrangements of the molecules in the crystal lattice.[1] The relationship between polymorphs can be either monotropic or enantiotropic. For monotropic systems, one form has a lower Gibbs free energy than the others at all temperatures below melting point and is, therefore, thermodynamically the most stable form. For enantiotropic systems, the Gibbs free energies of two forms are equal at a certain transition temperature, corresponding to a change in the most stable form. The transformation of the metastable to the stable form is typically described by either a solid-state polymorphic transformation (SST) or a solvent-mediated polymorphic transformation (SMT).[2] The former takes place via the rearrangement of the ions or molecules in solid state, while the latter occurs by dissolution of the metastable form and crystallization of the stable form. The development of a robust isolation method for different polymorphs, through either crystallization or transformation, is important for industry. To develop and design a reliable transformation process, it is crucial to understand the transformation mechanism under different conditions.[3]

Several tools are available for in situ monitoring of the isolation and polymorphic transformation during a crystallization process, including Raman spectroscopy, FRBM (focused beam reflectance measurement), PVM (particle vision measurement) and ATR-FTIR (attenuated total reflectance Fourier transform infrared) spectroscopy.[4] Raman spectroscopy is a light-scattering technique wherein a monochromatic laser source illuminates the sample, and the resulting scattered light is collected and analyzed. Raman spectroscopy has been demonstrated to have good capability to monitor both the liquid phase and the solid phase in studies of the polymorphic transformation of mannitol,[5,6] L-glutamic acid,[7,8] carbamazepine[9] and *p*-aminobenzoic acid.[10] FBRM and PVM are probe-based high solids concentration particle characterization tools and can provide information on the particle size and morphology. Dang et al[11] reported the use of FBRM and PVM to monitor the polymorphic transformation of glycine from β to α form, while O'Sullivan et al[12] additionally employed FTIR spectroscopy to investigate the transformation process of mannitol. ATR-FTIR spectroscopy has emerged as the primary instrument for the assessment of bulk supersaturation during both cooling and antisolvent crystallization processes. Early work presented techniques for supersaturation assessment which linked temperature and spectra values through basic polynomial expressions, to the correlation of solute and solvent peak heights. Recently, a novel calibration-free method for in situ monitoring of supersaturation during crystallization process was described by Barrett et al.[13] This method was successfully used to monitor the crystallization process of benzoic acid and an active pharmaceutical ingredient (API). In this contribution, the use of each of these in situ tools was investigated to provide a better understanding of the polymorphic transformation of PABA. Raman spectroscopy was used to monitor the solid-phase concentration; the ATR-FTIR was used to monitor the liquid-phase concentration; FBRM and PVM will be used to monitor the particle size and morphology.

p-Aminobenzoic acid (PABA), is an essential nutrient for several microorganisms and an important active ingredient in the manufacture of sunscreens, esters, and folic acid.[14,15] PABA is known to have two polymorphs identified as α form

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(a = 18.551 Å, b = 3.860 Å, c = 18.642 Å, Z = 8, space group $P21/n$)[16] and β form (a = 6.275 Å, b = 8.55 Å, c = 12.80 Å, Z = 4, space group $P21/c$).[17] The needle-shaped α form is commercially available and easy to prepare, while pure β is more difficult to obtain by solution crystallization. Gracin and Rasmuson[18] studied the crystallization of PABA in water and ethyl acetate. The solubility data of PABA in water and ethyl acetate and the morphology difference of different forms were reported in this literature. Yang et al.[10] investigated the solid-state transition of PABA at elevated temperatures using in situ Raman spectroscopy. No study of the solvent-mediated polymorphic transformation of PABA has been published. In this report, the successful application of in situ tools to understand the mechanism of transformation between the two polymorphs and to design a process to produce β form PABA by polymorphic transformation was discussed. Solubility data for both forms in ethanol were also determined experimentally and presented for the first time, and the measured transition temperature was compared with previously published data.

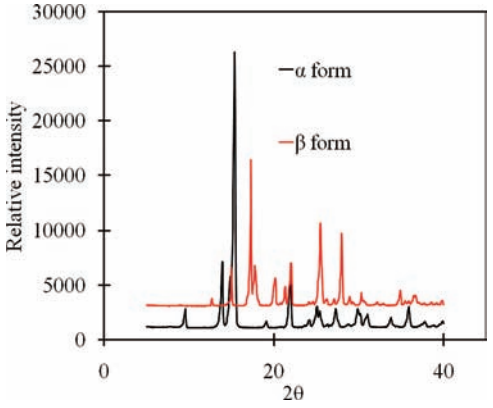

Figure 1. XRPD patterns of α and β form PABA.

2. EXPERIMENTAL SECTION

2.1. Materials. The α form of PABA was prepared by recrystallization of commercial products from Sigma Aldrich Co. Ltd. of the U.K. The β form of PABA was prepared by controlled seeded cooling crystallization in ethanol. The β form seed was prepared by very slow evaporation of PABA ethanol solution. The mass fraction purity of both forms is higher than 99%. The identification of both forms was confirmed by XPRD (X-ray powder diffractometry) and Raman spectroscopy. Analytical-grade ethanol with mass fraction purity higher than 99.5% was purchased from Sigma Aldrich Co. Ltd. of U.K. Deionized water was used throughout.

2.2. Solubility Experiments. The solubility of both polymorphs was measured using FBRM.[19] All solubility experiments were performed in a 100-mL EasyMax vessel (from Mettler Toledo, U.K) in conjunction with the iControl Easymax software. The accuracy of temperature control of this system is 0.01 °C. The mixtures of solute and solvent in the vessel were stirred with an overhead stirrer. A condenser was used to prevent evaporation of solvent. The masses of the solvent and solute were weighed using an analytical balance with an accuracy of ±0.0001 g. In experiments, excess PABA solid and solvent of known masses were transferred into the equilibrium vessel. The solid and liquid mixture was maintained at a fixed temperature for 1 h. Then the solution was heated up at a very slow heating rate, typically of 0.05 °C/min. This procedure was repeated until the last crystal disappeared completely as indicated by FBRM. The temperature at this point was taken to be equivalent to the saturation temperature. The experiment was performed in triplicate with the average value taken as the solubility temperature of PABA. The confidence of the experimental solubility values is ∼99%.

2.3. Transformation Experiments. All transformation experiments were performed in the 100-mL vessel described above. In the first set of experiments, 3 g of β form PABA was added to 80 mL of saturated (to α form) PABA ethanol solution at 35 °C.

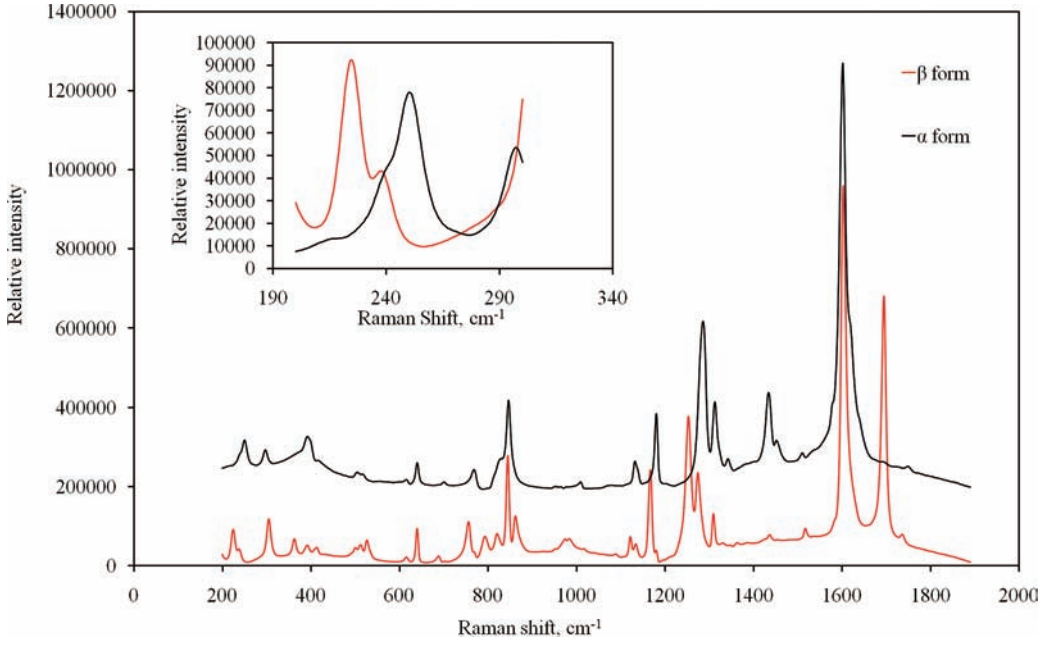

Figure 2. Raman spectra of α and β form PABA. The inset displays the enlarged view of Raman spectra in spectral range of 200−290 cm^{-1}.

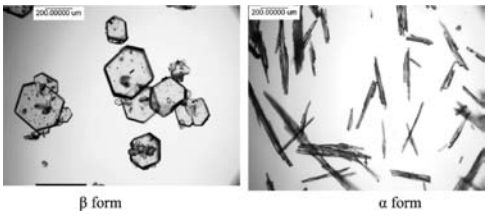

Figure 3. Microscope images of α and β forms of PABA.

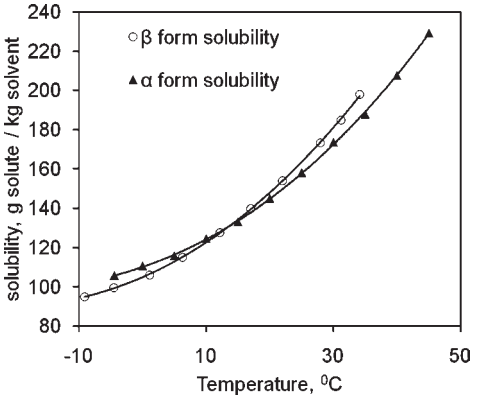

Figure 4. Solubility of α and β forms of PABA.

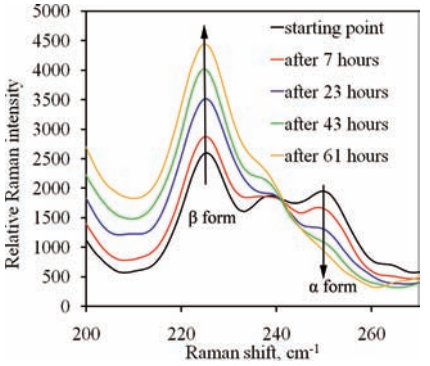

Figure 5. Changing trend of Raman intensity during the transformation test at 13 °C.

In the second set of experiments, 3 g of α form PABA was added to 80 mL of saturated (to β form) PABA ethanol solution at 0 °C.

FBRM, PVM, Raman, and ATR-FTIR spectroscopy were employed to monitor the change of liquid phase and solid phase. After the transformation was complete, the solution was filtered. The obtained solid product was dried and analyzed using XRPD.

2.4. Raman Spectroscopy. The Kaiser Raman RXN2 system was used to measure the Raman spectra of different samples and to in situ monitor the polymorphic transformation in solution. This system is equipped with both a MR probe head and a PhAT probe head. This system is fitted with a Kaiser Invictus laser emitting deep red and nearly invisible (785 nm) emission of 450 mW. The spectral resolution is 5 cm^{-1} average. The IC Raman software was used with this system for instrument configuration, data acquisition, and reaction analysis. In this contribution, the MR probe head with immersion optics was used to in situ monitor the polymorphic transformation of PABA. The PhAT probe head

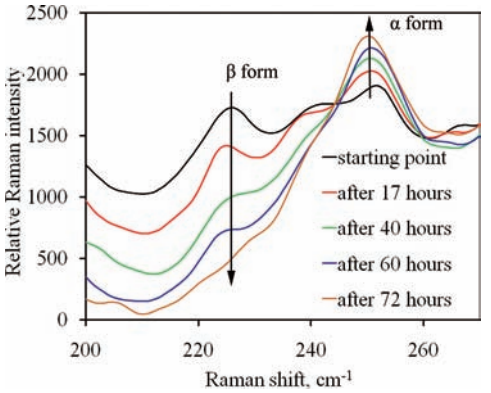

Figure 6. Changing trend of Raman intensity during the transformation test at 15 °C.

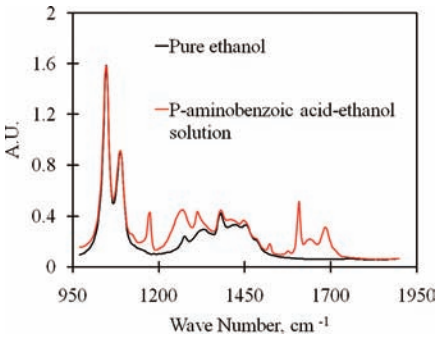

Figure 7. IR spectra of pure ethanol and PABA−ethanol solution.

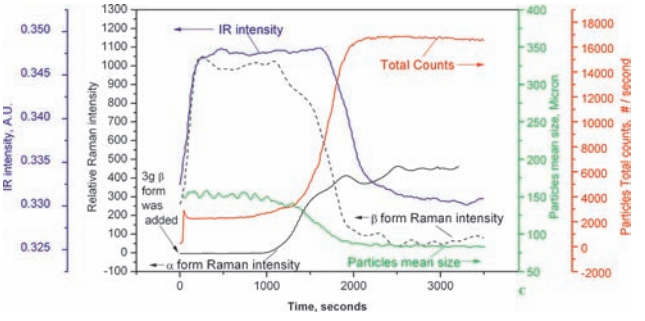

Figure 8. FBRM data, Raman intensity, and IR intensity change during PABA polymorphic transformation process from β to α at 35 °C.

with noncontact optics was used offline to measure the powder Raman spectra of different samples.

2.5. X-ray Powder Diffractometry (XRPD). To confirm the identity of different polymorphs of PABA, X-ray powder diffraction (XRPD) patterns were collected by using a Siemens D500 powder diffractometer which was fitted with a diffracted beam monochromator. Samples were prepared by placing powders on a low background aluminum powder mount. Diffraction patterns were recorded between 5 and 40° (2θ) using Cu Kα radiation with steps of 0.05° 2θ with 2 s counting time per step.

2.6. ATR-FTIR, FBRM, and PVM. The FBRM, PVM, and ATR-FTIR probes used in this paper were manufactured by Mettler-Toledo. The sample measurement duration for both the FBRM

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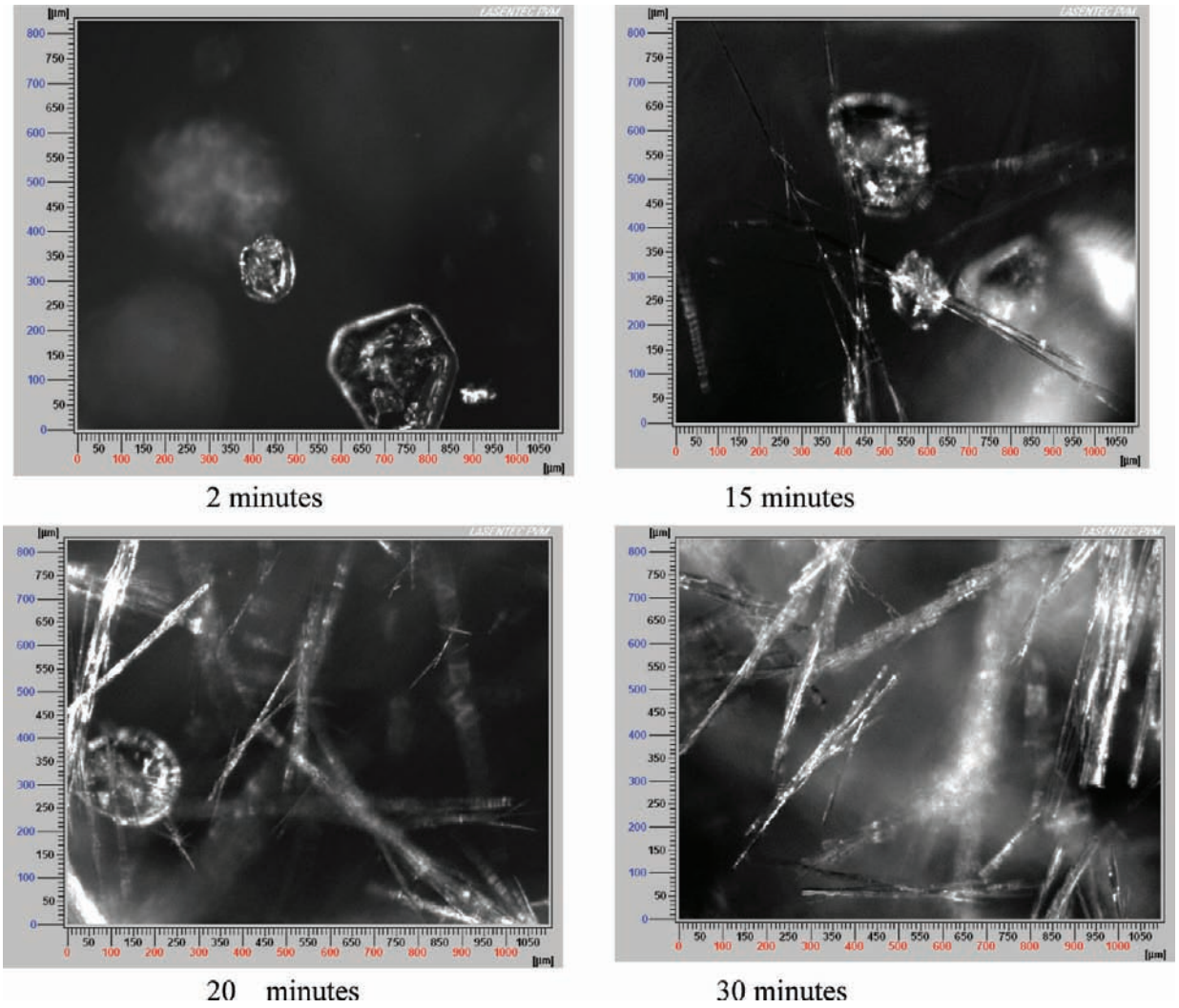

Figure 9. PVM images at different transformation times.

(model D600L) and ATR-FTIR was set at 10 s for the transformation from β to α form and 60 s for transformation from α to β form. Total counts and mean size (square weighted) FBRM data were used to monitor the particle change during the polymorphic transformation process. The chord length distributions (square weighted) of PABA before and after the transformation were recorded and compared. The ATR-FTIR spectra from 900 to 1890 cm^{-1} of solution were collected. IC FBRM software and IC IR software were used during the experiments to collect and analyze the data. The PVM probe (model 800 L) was operated throughout the transformation processes. The image of particles morphology was recorded at an update rate of 2 images per second.

3. RESULTS AND DISCUSSION

3.1. Identification of α and β Forms of PABA. The XRPD data of the α and β forms prepared in this work (see Figure 1) are consistent with published data.[10,18] The Raman spectra of both forms, shown in Figure 2, exhibit several differences which can be used to monitor the polymorphic transformation. For example, in the spectral range of 200−290 cm^{-1}, the β form has one characteristic peak at 224 cm^{-1} while the α form has one characteristic peak at 250 cm^{-1}. This difference can be seen clearly in the magnified spectra in the inset of Figure 2. In this report, this difference was used to monitor the transformation between α and β forms.

Since the morphologies of the α and β forms are quite different (see Figure 3), the transformation can also be easily monitored with both FBRM and PVM.

3.2. Solubility of α and β Forms of PABA. The measured solubility of both α and β forms in ethanol are shown in Figure 4. The temperature dependence of the solubility of α and β forms in ethanol was correlated using polynomial eqs 1 and 2, respectively. The R^2 values for these equations are 0.9997 and 0.9999, respectively.

α form

$$S = 1.187 \times 10^{-4} T^3 + 2.861 \times 10^{-2} T^2 + 1.107T + 110.2 \quad (1)$$

β form

$$S = 7.290 \times 10^{-5} T^3 + 3.524 \times 10^{-2} T^2 + 1.408T + 104.9 \quad (2)$$

where S is the solubility of PABA in the form of g solute per kg solvent; T is the Celsius temperature.

From both Figure 4 and eqs 1 and 2, the transition temperature is seen to be around 13.8 °C. This temperature is less than the value of 25 °C reported by Gracin and Rasmuson in both water and ethyl acetate.[18] However, in this earlier work, solubility was determined from sampling of a filtered saturated solution and subsequent drying of the sample. The reported solubilities of both forms in the region of the transition were typically within 5% of each other, making accurate determination of the transition point extremely difficult.

To verify the transition temperature reported here, transformation experiments at 13 and 15 °C were carried out. In these experiments, 3 g of α form and 3 g of β form were added into saturated solution of PABA. The subsequent polymorphic transformation was observed using inline Raman spectroscopy (Figures 5 and 6). From Figure 5, it can be seen that the α form slowly transformed into β form at 13 °C, suggesting that the β form is the stable form at this temperature. However, at 15 °C, the β form slowly transformed into the α form (see Figure 6) indicating that the α form is stable at this point. On the basis of these observations, the transition temperature should be between 13 and 15 °C.

This observation is consistent with the work of Gracin and Rasmuson[18] who reported an α to β transformation in ethyl acetate at −11 °C, while they also reported the transformation of β to α at 28 °C in ethyl acetate and at 45 °C in water.

The solubility difference between the two forms is 0−6% in the temperature range between −10 to 45 °C in ethanol as a function of temperature. This difference provides the driving force for the solvent-mediated transformation between the α and β forms.

3.3. Enantiotropic Transformation between α and β form PABA. To test the feasibility of using ATR-FTIR spectroscopy to monitor the liquid phase concentration of PABA, the IR spectra of pure ethanol and PABA−ethanol solution were measured

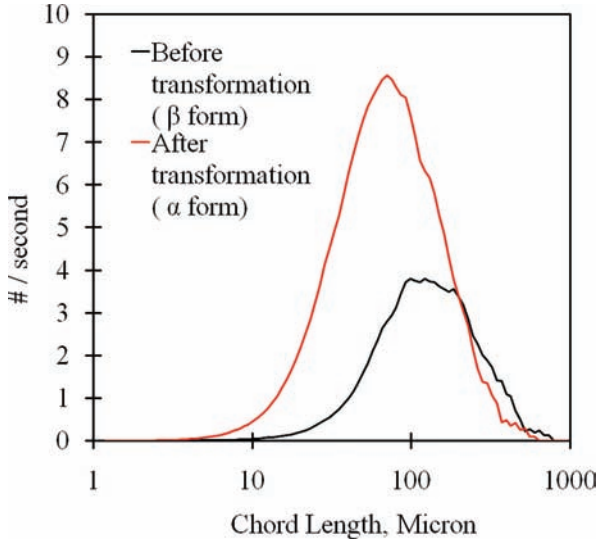

Figure 10. Change of chord length distribution before and after transformation from the β to the α form.

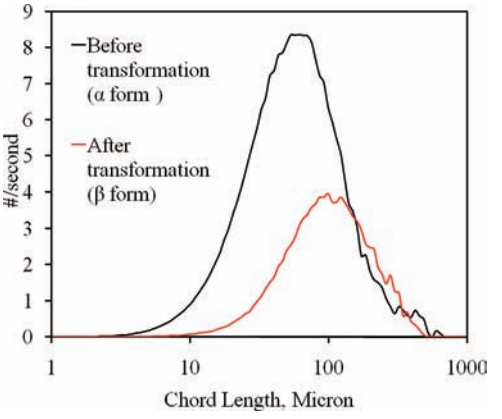

Figure 12. Change of chord length distribution before and after transformation from α to β form.

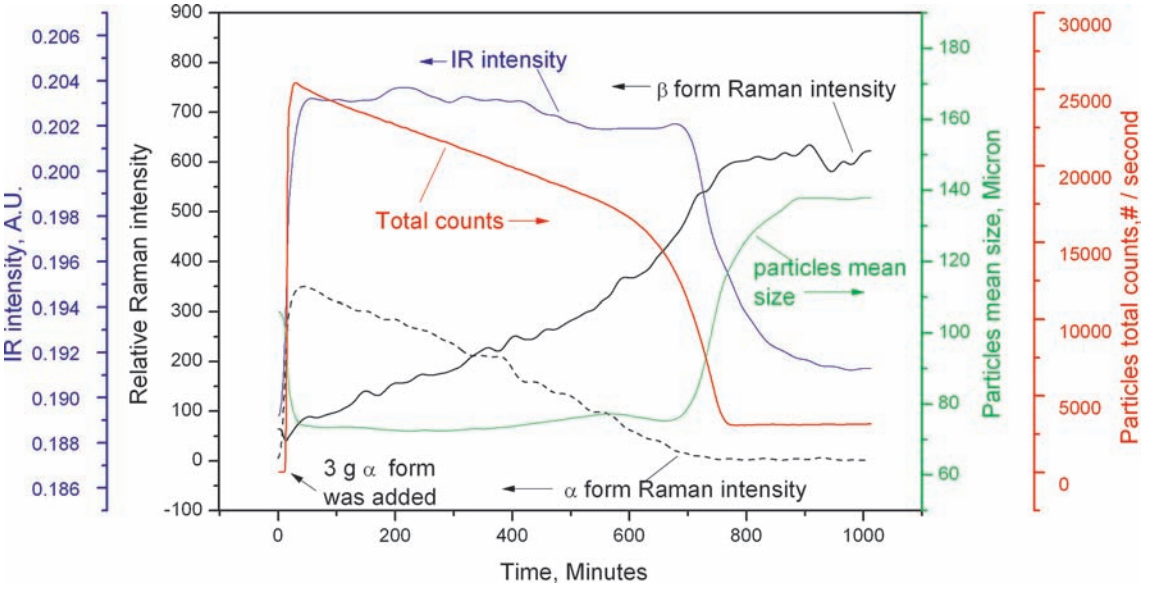

Figure 11. FBRM data, Raman intensity, and an IR intensity change during the polymorphic transformation process from α to β at 0 °C.

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Table 1. Transformation time at different temperatures

from α form to β form			from β form to α form	
T (°C)	transformation time -unseeded	transformation time - seeded (5% mass) (h)	T (°C)	transformation time (min)
−10	no transformation was observed after 5 days	36	35	40
−5	no transformation was observed after 5 days	29	38.5	35
0	no transformation was observed after 5 days	16	40	23

separately (see Figure 7). It can be seen clearly that several IR peaks, such as those at 1607, 1685, and 1174 cm^{-1}, are characteristic peaks of dissolved PABA. In this work, the peak at 1607 cm^{-1} was chosen to track the PABA concentration because this peak is the most distinct. The ethanol IR peak at 1048 cm^{-1} was chosen as the reference peak.

To fully understand the transformation mechanism of PABA, Raman and ATR-FTIR spectroscopy were combined with FBRM and PVM to track the change of liquid- and solid-phases during the transformation process. Typical FBRM, IR, and Raman data during the transformation from the β to α form at 35 °C are shown in Figure 8. Typical PVM images during the transformation are shown in Figure 9.

In Figure 8, the IR intensity represents the liquid phase concentration of PABA. The relative Raman intensities of the α and β forms represent the solid state concentration of α and β forms PABA, respectively. It can be seen from Figure 8 that, while the solid-phase β form concentration increases rapidly after the addition of 3 g of β form to a clear saturated solution of α form PABA, the liquid concentration of PABA also increases. Along with the corresponding FBRM count data, this indicates some rapid dissolution of the added metastable substrate. No solid-state α form is initially detected. For solvent-mediated polymorphic transformations, the rate of transformation is controlled by the dissolution of the metastable form (β form at 35 °C) and the nucleation and growth of the stable form (α form at 35 °C). In this case, the β form PABA is metastable while the α form is stable at this temperature. Although the clear solution is saturated with respect to the α form, it is undersaturated to the β form because the solubility of the β form is higher than that of the α form at 35 °C. The β form will partially dissolve until equilibrium is reached. Since the solubility difference between the two forms is small, only partial dissolution of the β form occurs. After a new thermodynamic equilibrium is established, no further dissolution of the β form, or nucleation of the stable α form, is observed for the subsequent 15 min, as indicated by the FBRM, IR, and Raman trends. This period can be regarded as the induction period for nucleation of the α form. After this induction period, the Raman intensity of the α form starts to increase with a corresponding drop in the Raman intensity of the β form. At the same time, the total FBRM counts increase, while the mean FBRM chord length is seen to decrease. It is clear that α form nucleation and subsequent growth starts at this point and continues for approximately 25 min. The IR intensity does not begin to drop for approximately the first 10 min of this process, until most of the solid β form has dissolved; the liquid-phase concentration then begins to fall with the solubility of the α form. The change in the FBRM data is due to the change in crystal morphology associated with the transformation observed in Figure 9. A comparison of the initial and final chord length distribution is shown in Figure 10. A change from the platelet shape of the β form to the needle shape of the α form would be expected to result in more counts per second since one larger platelet-shaped PABA β form can transform into several smaller, needle-shaped α form crystals. The mean chord length would, however, be expected to drop as the FBRM probe will primarily measure the needle width rather than the longer length dimension.

To summarize, the transformation process from the β to the α form can be divided into three periods: the dissolution period of β form, the induction period of α form, and the crystallization period of α form. In each phase, the in situ tools provide a highly detailed picture of the mechanisms at work.

The transformation from the α form to the β form at 0 °C was also investigated. When pure α form is added to a solution at the saturated concentration of β form, no transformation was observed after up to 5 days, despite its being a metastable form. If 5% of the β form was also added into the saturated solution along with the 3 g of the α form, then the transformation was observed. However, the transformation from α to β takes a much longer time than the transformation from β to α (generally of the order of 10 h). FBRM, IR, and Raman data from a typical seeded transformation experiment at 0 °C are shown in Figure 11. As in Figure 8, the IR intensity tracks the liquid-phase concentration of PABA, while the relative Raman intensity of α and β form represents the solid-state concentration of α and β form PABA, respectively. From Figure 11, it can be seen that the trends are similar to those observed for the transformation from the β to the α form. As the α form dissolves faster than the β form crystallization, it is observed that the liquid-phase PABA concentration increases and then remains constant during most of the transformation process before decreasing back to the solubility level for the β form at the end of transformation. The solid-state concentration of the α form is seen to decrease slowly, while the solid-state concentration of the β form increases slowly during the transformation process. No induction period was observed because β form seed was used. As in the previous experiment, FBRM measures far fewer counts in a population of β form particles due to their size and shape, while the mean particle size is larger. This is reflected in the FBRM trends in Figure 11. This can be further seen from the chord length distribution data in Figure 12.

More transformation experiments at different temperatures were performed. For the transformation from α to β below the transition point, changes in FBRM, Raman, and IR data were similar to the transformation at 0 °C. For the transformation from the β to the α form above the transition point, changes in FBRM, Raman, and IR data were similar to those of the transformation at 35 °C. The transformation time changed with the transformation temperature. The results are listed in Table 1.

From all the transformation experiments, it is seen that the transformation from α to β is much slower compared to the transformation from β to α, which agrees with previously reported observations for PABA in water and ethyl acetate.[18] There are two possible reasons. The first potential reason is the lower temperature for the transformation from the α form to the β form. As we know,

the temperature will affect both the dissolving and crystallization rate of particles. Higher temperature will normally increase both the dissolution rate and crystallization rate which will result in faster transformation. The second possible reason is the lower crystallization kinetics of the β form which has also been proven in former research.[18]

4. CONCLUSIONS

The use of in situ tools to rapidly investigate the transformation of two polymorphs of PABA was shown to provide a detailed insight into the mechanisms and rates of transformation. This is the first time this transformation was successfully monitored throughout the process. The solubility can also be accurately measured in situ, without the need for potentially inaccurate sampling and associated handling. The enantiotropic behavior between α and β forms of PABA was confirmed by both solubility data and transformation experiments. Although the transformation from α to β is slower than the transformation from β to α, it is possible to produce pure β-PABA by a controlled transformation process. It was also found that the transformation from β to α is dominated by dissolution of the β form and nucleation of the α form, while the seeded transformation from α and β was dominated by dissolution of the α form and growth of the β form.

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REFERENCES

(1) Bernstein, J. *Polymorphism in Molecular Crystals*; Oxford University Press: New York, 2002.
(2) Mullin, J. W. *Crystallization*, 4th ed; Butterworth-Heinemann: London, 2001.
(3) Zhang, G. G. Z.; Law, D.; Schmitt, E. A.; Qiu, Y. *Adv. Drug Delivery Rev.* **2004**, *56*, 371–390.
(4) Sarra, N. C.; Adrian, C. W.; Ian, M. G.; Steven, W. B. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1135–1147.
(5) Hao, H. X.; Su, W. Y.; Barrett, M.; Caron, V.; Healy, A. M.; Glennon, B. *Org. Process Res. Dev.* **2010**, *14*, 1209–1214.
(6) Su, W. Y.; Hao, H. X.; Barrett, M; Glennon, B. *Org. Process Res. Dev.* **2010**, *14* (6), 1432–1437.
(7) Qu, H.; Alatalo, H.; Hatakka, H.; Kohonen, J.; Louhi-Kultanen, M.; Reinikainen, S. P.; Kallas, J. *J. Cryst. Growth.* **2009**, *311*, 3466–3475.
(8) Scholl, J.; Bonalumi, D.; Vicum, L.; Mazzotti, M.; Muller, M. *Cryst. Growth Des.* **2006**, *6*, 881–891.
(9) Liu, W.; Wei, H.; Black, S. *Org. Process Res. Dev.* **2009**, *13*, 494–500.
(10) Yang, X.; Wang, X.; Ching, C. *J. Raman Spectrosc.* **2009**, *40*, 870–875.
(11) Dang, L.; Yan, H.; Black, S.; Wei, H. *Org. Process Res. Dev.* **2009**, *13*, 1301–1306.
(12) O'Sullivan, B.; Glennon, B. *Org. Process Res. Dev.* **2005**, *9*, 884–889.
(13) Barrett, M; Macnamara, M; Hao, H. X.; Barrett, P.; Glennon, B. *Chem. Eng. Res. Des.* **2010**, *88*, 1108–1119.
(14) Tripathi, G. N. R.; Su, Y. *J. Am. Chem. Soc.* **1996**, *118*, 2235–2244.
(15) Chignell, C. F.; Kalyanaraman, B.; Mason, R. P.; Sik, R. H. *Photochem. Photobiol.* **1980**, *32*, 565–570.
(16) Lai, T. F.; Marsh, R. E. *Acta Crystallogr.* **1967**, *22*, 885–893.
(17) Gracin, S.; Fischer, A. *Acta Crystallogr.* **2005**, *E61*, o1242–o1244.
(18) Gracin, S.; Rasmuson, A. C. *Cryst. Growth Des.* **2004**, *4* (5), 1013–1023.
(19) Barrett, P.; Glennon, B. *Chem. Eng. Res. Des.* **2002**, *80*, 799–805.