Organic Process Research & Development **2008,** *12,* 1223–1228

Polymorphic Transformation of Pravastatin Sodium Monitored Using Combined Online FBRM and PVM

Chun-Yan Jia, Qiu-Xiang Yin,* Mei-Jing Zhang, Jing-Kang Wang, and Zhong-Hua Shen

School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, People's Republic of China

Abstract:

Polymorphic transformation of pravastatin sodium in a mixture of isopropanol and water was studied by use of online focused beam reflectance measurement (FBRM) and particle vision measurement (PVM). It is shown that the form A polymorph transformed to the stable polymorph, form B. It was speculated that in the transformation process there was an agglomeration and breakage phenomenon. The transformation mechanism was identified as solution-mediated phase transformation. Influences of temperature, solvent composition, and stirrer speed on the transformation process were examined. It can be seen from the FBRM monitoring results that higher temperature, larger ratio of water to isopropanol, and higher stirrer speed can increase the transformation process.

1. Introduction

Many organic compounds, especially pharmaceuticals, exist in two or more polymorphs,[1] which can impact on downstream processes in terms of, for example, morphology, bioavailability, and purity. Thus, control of crystallisation processes involving polymorphic materials is of paramount importance. According to Ostwald's rule of stages, the crystallization process for materials prone to polymorphism often manifests competitive nucleation followed by growth of a metastable phase, which subsequently transforms to the stable phase.[2] To study systems undergoing polymorphic phase transformation, procedures based on sampling and off-line analysis by different methods made the properties of the crystal particles changed because of nonrepresentative sampling, postsampling, and drying.[3] To avoid this, a number of in situ analytical techniques, for example IR spectroscopy,[4] Raman spectroscopy,[5] optical microscopy,[6] and powder-X-ray diffraction (XRPD),[7] have been applied.

Pravastatin ([1*S*-[1α(*β**,*γ**)2α,6α,8*β*(*R**),8aα]]-1,2,6,7,8,8a-hexahydro,*β*,*γ*,6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-1-naphthalene-heptanoic acid), whose chemical structure is shown in Figure 1, is a member of the statin class of pharmaceutical. Statins currently are the most effective medicaments for lowering serum cholesterol levels in patients with atherosclerosis and hypercholesteremia. It has been found that pravastatin sodium, the monosodium salt of pravastatin, can exist in 16 different polymorphs,[8−11] among which form A is the most common one. In the manufacturing process of pravastatin sodium, it was found that form A can easily transform to form B with almost 100% yield. To monitor the realtime transformation process, and to obtain some critical information to aid in improvement and optimization of the transformation process, combined PVM and FBRM were used in situ in the present work.

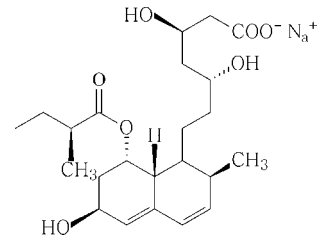

Figure 1. **Chemical structure of pravastatin sodium.**

The focused beam reflectance measurement (FBRM) method is a relatively new method developed by Lasentec to perform particle size measurements in the range of 0.25−1000 μm. The great advantage of this technique is that data is acquired online and in real time to give particle size data and population trends of particles in suspension. The FBRM technique has been used widely in monitoring of crystallization process, for example, to measure the chord size distribution (CSD) for the online measurement of crystallization kinetics,[12] to measure the solubility and metastable zone width,[13] and to control the CSD.[14] Also, the particle vision measurement (PVM) instruments are powerful tools developed by Lasentec as an in situ particle monitoring technique for in-line real-time measurement of particle size and morphology[12,15,16,17]

* Corresponding author. Fax: +86-22-27374971. E-mail: qxyin@tju.edu.cn.

(1) Brittain, H. G. *Polymorphism in Pharmaceutical Solids*; Marcel Dekker Press: New York, 1999.
(2) Džidić, I.; Kebarle, P. *J. Phys. Chem.* **1970**, *74*, 1466–1474.
(3) O'Sullivan, B.; Barrett, P.; Hsiao, G.; Carr, A.; Glennon, B. *Org. Process Res. Dev.* **2003**, *7*, 977–982.
(4) Ataka, K.-i.; Osawa, M. *Langmuir* **1998**, *14*, 951–959.
(5) Ono, T; Horst, J. H.; Jansens, P. J. *J. Cryst. Growth* **2004**, *4*, 465–469.
(6) Calderon de Anda, J.; Wang, X. Z.; Lai, X; Roberts, K. J.; Jennings, K. H.; Wilkinson, M. J.; Watson, D; Roberts, D. *AIChE J.* **2005**, *51* (5), 1406–1414.
(7) Hammond, R. B.; Lai, X; Roberts, K. J.; Thomas, A; White, G. *Cryst. Growth Des.* **2004**, *4*, 943–948.
(8) Keri, V. S.; Szabo, C.; Nagyne Arvai, E.; Aronhime, J. Forms of pravastatin sodium. U.S. Patent 7,262,218, 2007.
(9) Pflaum, Z. Crystalline sodium salt of pravastatin. U.S. Patent 6,740,775, 2004.
(10) Keri, V.; Nagyne Arvai, E.; Czovek, Z.; Kovacsne-Mezei, A.; Katai, I. V.; Nemethne Racz, C. Methods of Making Pravastatin Sodium. Pub. U.S. Pat. Appl. 20060194984, 2007.
(11) Martín-Islán, A. P.; Cruzado, M. C.; Asensio, R.; Ignacio Sainz-Díaz, C. *J. Phys. Chem. B* **2006**, *110*, 26148–26159.
(12) Al Nasser, W. N.; Shaikh, A.; Morriss, C.; Hounslow, M. J.; Salman, A. D. *Chem. Eng. Sci.* **2008**, *63*, 1381–1389.
(13) Barrett, P.;Glennon, B.; O'Sullivan, B. Lasentec Technical Papers. **2002**.
(14) Carreta, M. K.; Liebel, J. M. Lasentec Technical Papers. **2002**.
(15) Dharmayat, S.; Calderon De Anda, J.; Hammond, R. B.; Lai, X. J.; Roberts, K. J.; Wang, X. Z. *J. Cryst. Growth* **2006**, *294*, 35–40.

10.1021/op8001257 CCC: $40.75
Published on Web 10/31/2008

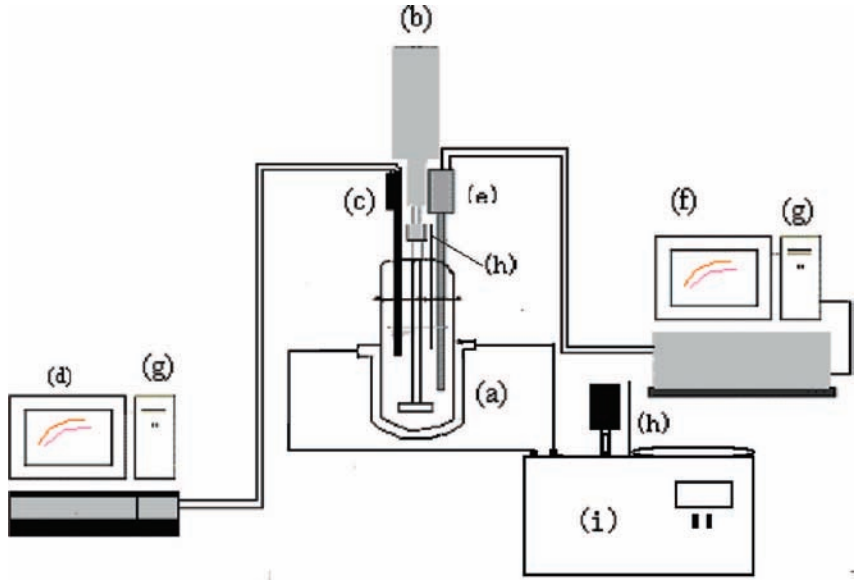

Figure 2. **Experimental setup: (a) 1-L crystallizer; (b) overhead stirrer; (c) FBRM probe; (d) FBRM curves; (e) PVM probe; (f) PVM images; (g) computer; (h) thermometer; (i) thermostat bath.**

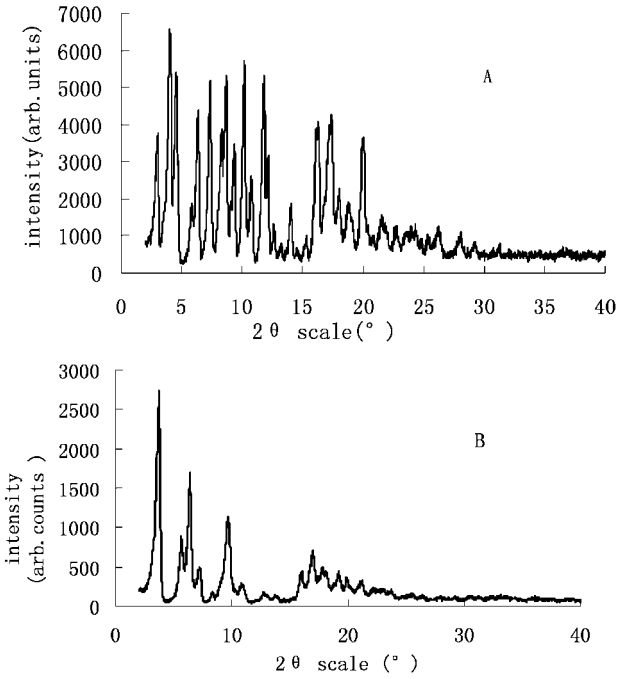

Figure 3. **Powder X-ray diffraction pattern of pravastatin sodium in form A (A) and form B (B).**

2. Experimental Section

Crystalline powder pravastatin sodium in form A (supplied from Shanghai Tianwei Pharmaceutical Co. Ltd., China, more than 99.5% in purity, MW 446.52, molecular formula $C_{23}H_{35}NaO_7$) was used to carry out the investigation. Isopropanol (purchased from Tianjin Chemical Reagent Co., China) used for experiments was of analytical reagent grade, was dehydrated with molecular sieves before use; the mass fraction was >99.5%. Distilled deionized water was used.

Both crystalline forms obtained by sampling before and after transition were also subjected to off-line XRPD and DSC analysis (Figure 3 and Figure 4) to verify the solid form. The XRPD (D/MAX 2500 Japan) analysed was 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 of 0.02° with a dwell time of 1 s. The DSC analysis was carried out on samples of 4.4 mg in an open aluminum pan from 20 to 250 °C at a rate of 5 °C /min. The results determined are in agreement with the literature.[8] From Figure 4 and the literature[11] we can see that form A and form B are both anhydrate polymorphic forms. Off-line digital image analysis was performed using a Hitachi X650 SEM under a voltage of 15 kV, protected by argon (Figure 5). Figure 5 shows that the form A

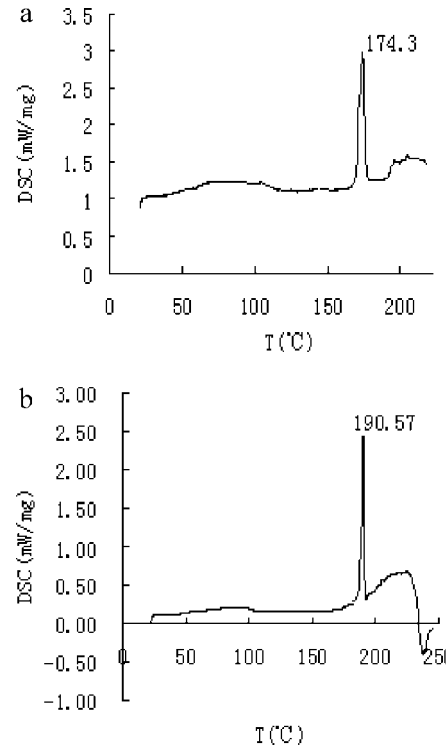

Figure 4. **DSC of form A (a) and form B (b).**

(16) Wang, Z.; Wang, J.; Dang, L. *Org. Process Res. Dev.* **2006**, *10*, 450–456.
(17) Kougoulos, E.; Jones, A. G.; Jennings, K. H.; Wood-Kaczmar, M. W. *J. Cryst. Growth* **2005**, *273*, 529–534.

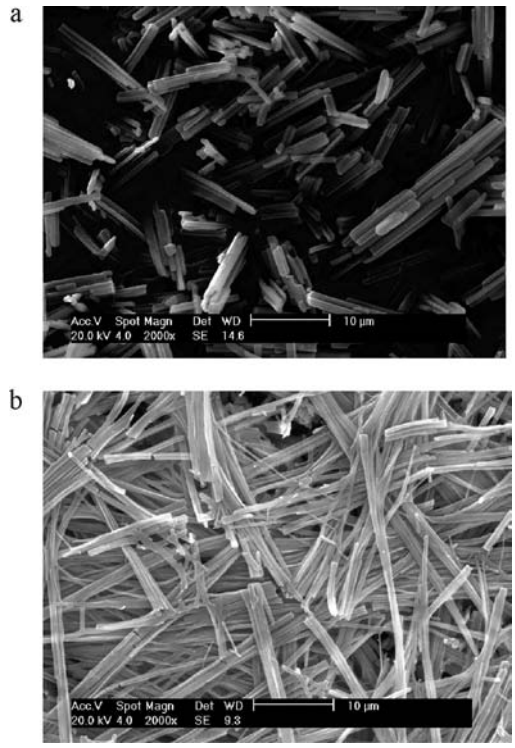

Figure 5. **SEM images of pravastatin sodium (a) form A; (b) form B.**

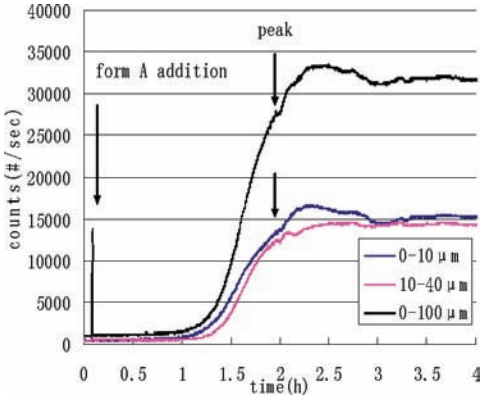

Figure 6. **FBRM counts of polymorphic transformation process in experiment 1.**

Table 1. **Design of experiments for the transformation process**

run	isopropanol/water (w:w)	temperature (°C)	stirrer speed (RPM)
1	20:1	20	150
2	20:1	30	150
3	20:1	35	150
4	30:1	30	150
5	40:1	30	150
6	30:1	30	250

and form B polymorphic both are needlelike. The different is that the form B polymorph is longer than form A.

The transformation process was carried out as follows: 600 mL of distilled deionized water and isopropanol was transferred to the crystallizer. The stirrer was set to a certain rotation speed and the thermostat bath to a certain temperature. When the temperature of the solvent mixture was steady, preweighed pravastatin sodium powder in form A was added to the crystallizer to make the solution exactly saturated (solubility

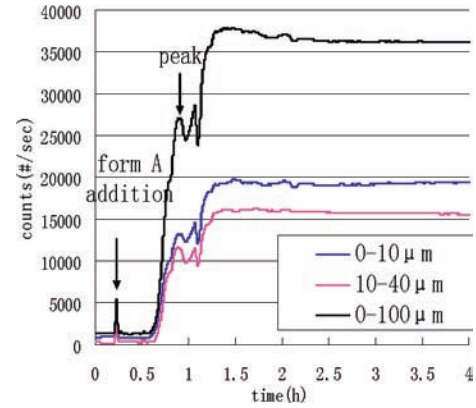

Figure 7. **FBRM counts of polymorphic transformation process in experiment 2.**

of form A pravastatin sodium in the solvent mixture had been measured previously; it was found that if the solution was not saturated, the transformation process would need a much longer time, or even not happen). The powder dissolved instantaneously, and after a certain time, another crystal nucleus would appear and then grew.

To visualize the processes occurring in situ, both stages were monitored using PVM and FBRM. The PVM probe (model 800 L) was operated with an image update rate of 10 images per minute, and the FBRM probe (model M400LF) has a measurement range of 0.25−1000 μm. In this study, there were three population ranges set that were 0−10, 10−30, and 0−100 μm, respectively. The probe measurement duration was set at 5 s. The experiment setup is shown in Figure 2.

A series of trials as outlined in Table 1 were designed to examine the effects of different temperatures, solvent compositions, and stirrer speeds on the transformation process.

3. Results and Discussion

3.1. Influence of Temperature. To illustrate the influence of temperature on the polymorphic transformation process and CSD, experiments 1, 2, and 3 were performed. In the three experiments, the ratio (w/w) of isopropanol to water was 20 to 1, and the stirrer speed was 150 r/min. In experiment 1, the temperature was set to 20 °C. Particle counts of different ranges measured by the FBRM are shown in Figure 6. The experimental results show that, on adding the crystal powder of pravastatin sodium in form A, there is a steep increase in all population ranges. However, particle counts return to nearly zero instantaneously, which accounts for the dissolution of the powder. After about 1 h, as can be seen, the counts of crystals sized 0−10 μm began to increase, which was because of the nucleation of a new form of crystal. Then counts measured of those sized 10−40 μm began to increase after a very short time, accounting for the growing of the nucleus. Particle counts of all ranges reached equilibrium after approximately 3 h.

For experiments 2 and 3, the settings were the same as for experiment 1 except for the temperature which was 30 and 35 °C, respectively. In the two experiments, which are shown in Figures 7 and 8, respectively, the track of particle counts of all ranges was similar to that of experiment 1. However, in the two experiments, the times from adding crystal powder in form A to the crystal nucleus of the new form appearing both were

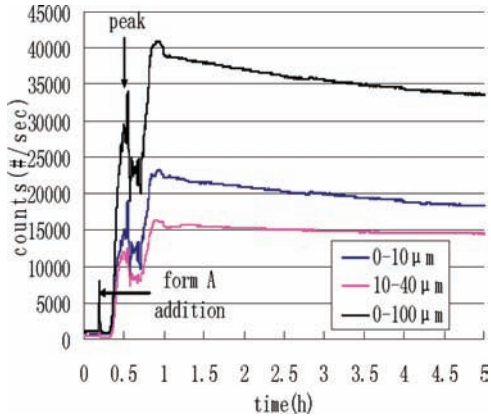

Figure 8. **FBRM counts of polymorphic transformation process in experiment 3.**

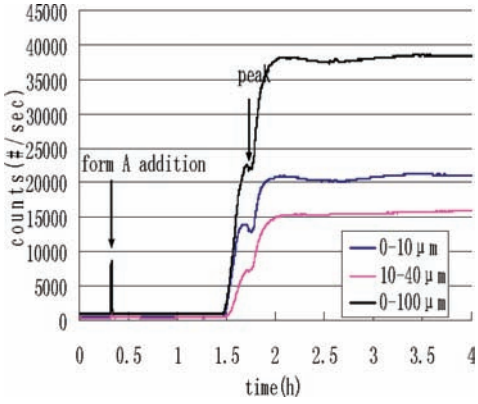

Figure 9. **FBRM counts of polymorphic transformation process in experiment 4.**

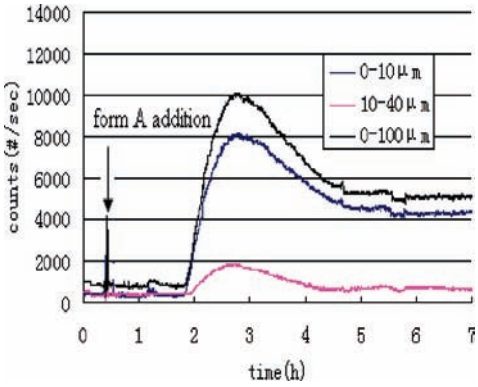

Figure 10. **FBRM counts of polymorphic transformation process in experiment 5.**

shorter compared with that in experiment 1, which were 0.4 and 0.2 h respectively. It can be speculated from this phenomenon that higher temperatures made the polymorphic transformation process quicker.

Besides, as can be seen from the two figures, there is an obvious peak before the particle counts reach the maximum, which was not very clear in experiment 1, but still existed. To find out the reason for this, we repeated the three experiments. Before the peak appeared, the suspension samples were transferred by a syringe, filtered through a 0.2 μm membrane filter, and then dried in a vacuum oven at 40 °C. The dried solid was analyzed by XRPD and DSC. The results were the same as those of form B, showing that the peak was not caused

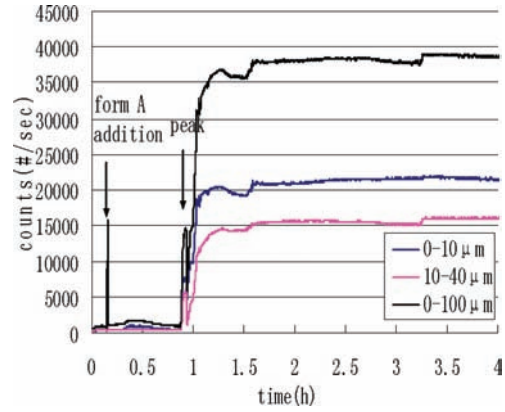

Figure 11. **FBRM counts of polymorphic transformation process in experiment 6.**

by a third polymorph appearing in the transformation process from form A to form B. It was speculated that the spontaneous nucleation made the nucleus conglomeration before growing. When the conglomerations got to a certain bulk, they were broken by the stirrer to smaller particles. Some of these particles that formed were in the range of 0−0.25 μm which cannot be detected by the FBRM probe. The result was that the counts of all ranges decreased, and the peaks appeared. The growing of the small particles made the counts increase again. As the temperature increased, the concentration of the solution was higher, the agglomeration and breakage process became quicker, and the peaks became thinner. The result was they detached from the bulk curves.

3.2. Influence of Solvent Composition. Experiments 4 and 5 (Figure 9 and Figure 10, respectively) are aimed at showing the influence of solvent composition on the transformation process. In the two experiments, the temperature and stirrer speed were the same as those of experiment 2. The difference was that the mass proportion of isopropanol to water was 30:1 and 40:1, respectively, compared with 20:1 in experiment 2. In Figure 9 and Figure 10, as the composition of isopropanol in the solvent mixture increased, the time lapse from addition of the crystal powder of form A to the appearance of the new nucleus became longer (1.2 and 1.6 h, respectively), and the peak corresponding to agglomeration and breakage became more indistinct, and even disappeared in Figure 11. It was caused by the lower concentration of the solution. This is also a proof of the speculation of the "influence of temperature" section that the peak is related with the concentration of the solution.

3.3. Influence of Stirrer Speed. To uncover the effect of the stirrer speed on the polymorphic transformation process, experiment 6 has been carried out. The experiment settings of experiment 6 were the same as those of experiment 4 except that the stirrer speed was 250 r/min. Figure 11 shows the result of experiment 6. As can be seen from it, higher stirrer speed increased the phase transformation process, so that the time elapsed to get the new phase nucleus was much shorter (shorter than 1 h), and the peaks corresponding to the agglomeration and breakage processes completely detached from the bulk curve. This is because higher rotatation speed brought forward the breakage process.

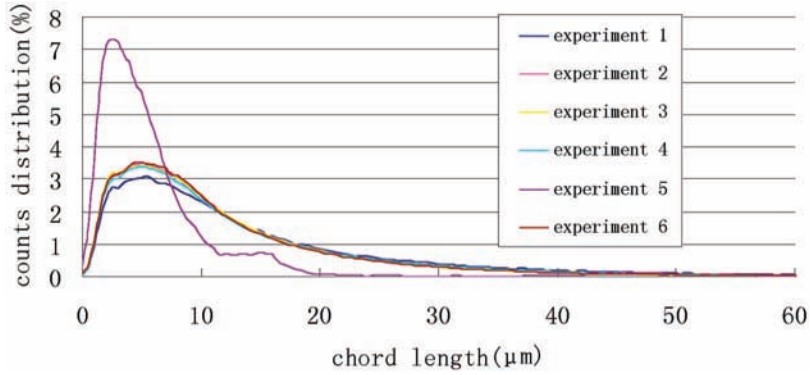

Figure 12. **FBRM chord length distribution of experiments 1−6.**

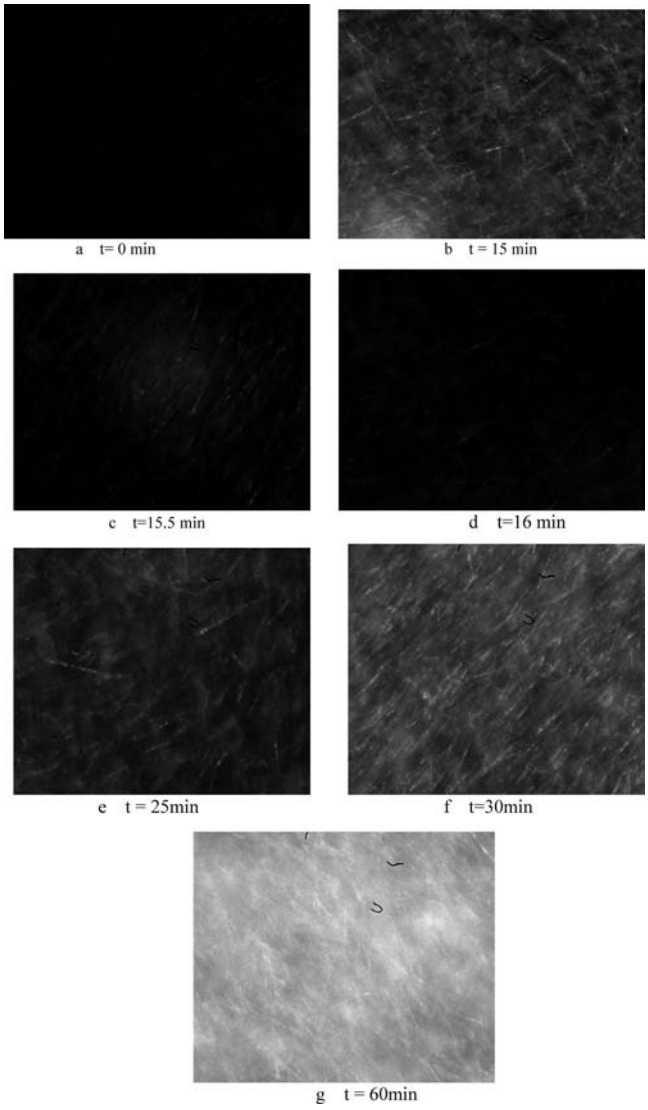

Figure 13. **PVM images during transformation.**

Figure 12 compares the chord length distribution measured by FBRM for experiments 1−6 when the transformation process equilibrized. The figure shows that there are no great differences between the final particle size distributions except for experiment 5, whose mean particle size is small and the particle size distribution is narrow. This is because the solution was too dilute for the particles to grow. To obtain a large particle size, the mass proportion of isopropanol to water should be smaller than 40.

3.4. Results of PVM Detection. FBRM can be used to monitor the change in the particle counts and dimension, and the crystal habit changes can be monitored by PVM. To demonstrate and provide a better understanding of the phase transition process between form A and form B, the transformation process was monitored by in situ PVM. As is known, the form transition process is mainly accompanied with the dissolution of form A and subsequent nucleation and growth of form B. Typical images taken for the transformation process of experiment 2 are summarized in Figure 13. In Figure 9, at t = 0 min, there was nothing in the solvent. At t =15 min, when the form A crystal powder was added to the crystallizer, needlelike crystals appeared in solution. At t = 15.5 min, needlelike crystals in the crystallizer became very small, which meant the dissolution of form A had been started. At t = 16 min, it was hard to see any crystal particles in the crystallizer. At t = 25 min, crystal particles appeared again, which were also needlelike, corresponding to the nucleation of the new polymorphic form. As the nucleation grew, the needlelike crystals increased at t = 30 min. At t = 60 min, the PVM image became very unclear. The reason was that with increasing solids concentration within the slurry, an impenetrable barrier to the light from the external stroboscopic light source was created. This caused the light to be reflected by the solids at high concentration, so that only the few crystals present near the PVM probe were illuminated.

The result showed that PVM is ideally suited for monitoring the transformation process. Since it is difficult to distinguish the agglomeration and breakage process, by combining FBRM and PVM monitoring of the transformation from form A to form B can be successfully developed. From changing both the number of particles and the crystal habit by FBRM and PVM, the conclusion that the transition mechanism is a typical solution-mediated transformation is obtained.

4. Conclusions

In situ FBRM and PVM are very useful process measurement techniques that were successfully used to monitor the polymorphic transformation of pravastatin sodium from form A to form B in the solvent mixture of distilled deionized water and isopropanol.

It was concluded that lower ratio (w/w) of isopropanol to water, higher temperature, and higher stirrer speed can increase the transformation process. The impact of the three factors to the transformation process is the solvent ratio > the temperature

> the stirrer speed. In the transformation experiment there was an agglomeration and breakage process. Higher temperature, higher concentration of solution, and higher stirrer speed would make the process more obvious.

The particle size distributions were the same for the different experiments except for that in which the composition of the solvent was 40:1 isopropanol/water. Because the concentration was dilute, the particles could not grow large. Thus, the particle size distribution was thinner.

The transformation mechanism can be identified as a solution-mediated transformation.

Received for review May 27, 2008.

OP8001257