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Nucleation, Growth, and Solvated Behavior of Erythromycin as Monitored in Situ by Using FBRM and PVM

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Abstract:

The application of in situ FBRM and PVM in monitoring the erythromycin dilution crystallization process and the transformation of erythromycin solvates is introduced. Influences of seeding strategies on nucleation and growth were examined in acetone aqueous solutions. It is noted by FBRM monitoring that the dominant chord length of crystal products is dependent on the supersaturation when seed crystals are added. Higher supersaturation leads to a smaller dominant chord length. In the crystallization process, breakage and agglomeration are not determined, and secondary nucleation mainly originates from induced nucleation by local high supersaturation resulting from water addition. The transformation process from metastable erythromycin acetone solvate to stable dihydrate was successfully monitored in pure water by using both PVM and FBRM, and the transformation mechanism can be identified as mediated-solution phase transformation. PVM is proven to be more appropriate to monitor transformation between erythromycin solvates as compared with FBRM.

Introduction

Crystallization from solution is well established as an essential separation and purification technique in pharmaceutical industries. Monitoring and control of the crystallization process are critical to meet special requirements for products such as satisfactory crystal morphology and proper crystal size distribution (CSD). However, these procedures have been based on sampling and off-line analysis by different methods.[1] It is possible that properties of crystal particles are changed because of nonrepresentative sampling, post-sampling,[2] and drying. Especially with respect to polymorphs and solvated crystals, this sample manipulation may have drastic effects on analysis of results, with a metastable state transforming to a stable state, giving erroneous results. Hence, to monitor process variables related to key process information which can aid in improving and optimizing process control, it is necessary to develop sensors in real-time mode. The Process Analytical Technology guidance announced by the U.S. Food and Drug Administration encourages such activities, and Process Analytical Technology is fast becoming an integral part of many active pharmaceutical ingredient production facilities.[3,4] Recently there has been increased interest in using in-line technology to study the crystallization process.[5]

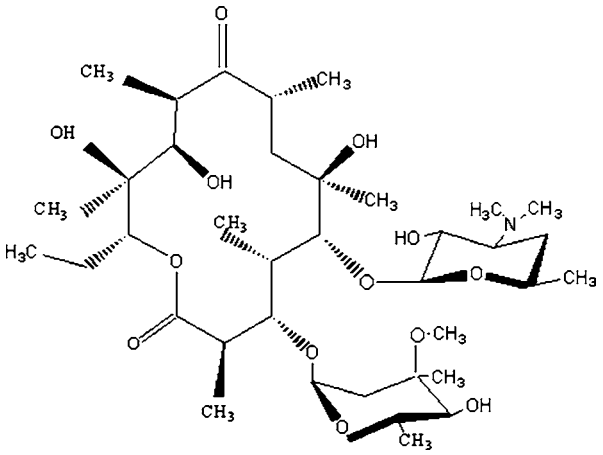

Figure 1. **Molecular structure of erythromycin A.**

Focused beam reflectance measurement (FBRM) and 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. FBRM is a probe-based high solids concentration particle characterization tool, and it is a new method for performing particle size measurements in the range 0.25−1000 μm.[6] 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.[7] The great advantage of this technique is that data are acquired on-line and in real time giving 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] Applying these tools in the crystallization process of polymorphs and solvated crystals is of great benefit in understanding the process dynamics and phase conversion, as well as other associated processes such as agglomeration and breakage.

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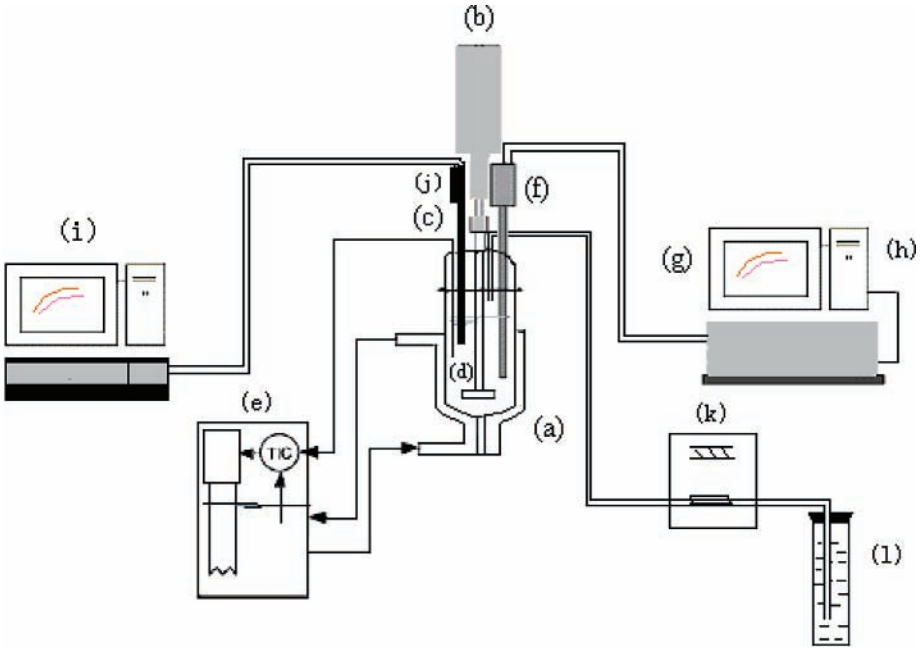

Figure 2. **Experimental set up: (a) 1-L jacketed glass vessel; (b) overhead stirrer; (c) thermometer; (d) impeller; (e) thermostat bath; (f) PVM probe; (g) PVM images; (h) computer; (i) FBRM curves; (j) FBRM probe; (k) pump; (l) cylinder.**

Table 1. **Experiment configuration for the first stage: A, B, C, D, E**

expt	acetone solvate seed (g)	dihydrate seed (g)	water addition (g)	supersaturation value $(c_s - c_e)^a$
A			40.36	0.34
B		0.1000	22.50	0.05
C	0.1000		22.50	0.05
D	0.1000		25.50	0.10
E	0.1000		25.50	0.10

[a] c_s, the ratio of water to acetone when seeding; c_e, the ratio of water to acetone at equilibrium saturation.

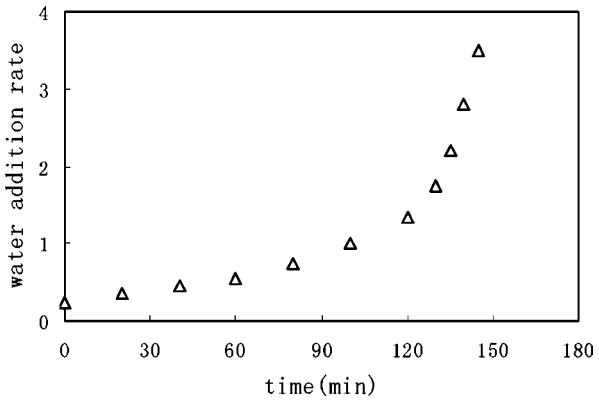

Figure 3. **Curve of water addition rate in experiments.**

Erythromycin is a mixture of macrolide antibiotics produced by fermentation of strains of Saccharopolyspo-raerythrea. Treatment with this antibiotic drug in human and veterinary practice is still very common, because of the high activity against gram-positive and a few gram-negative strains.[8] Moreover, erythromycin is useful as an intermediate for the preparation of roxithromycin, azithromycin, and clarithromycin. Erythromycin can form solvated crystals with water or various organic solvents such as acetone and ethanol. Erythromycin A (Figure 1) is the main and active component in erythromycin mixtures, and commercial erythromycin is usually available as the dihydrate.[9,10] Although much investigation on erythromycin, including properties and degradation, has been done, solvated behavior and the challenge in controlled crystallization processes are essentially not well understood and solved, especially phase transition and the transition mechanism.

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In commercial manufacture, acetone + water are often chosen as the solvent system of erythromycin dilution crystallization. In the present work, nucleation, growth, and the phase transformation process of erythromycin solvates were monitored in situ using FBRM and PVM. The purpose of this study is to obtain some critical information to aid in improvement and optimization of the crystallization process.

Materials and Methods

Erythromycin A dihydrate (supplied from Xi'an Rejoy Co.Ltd, China, more than 99.0% in purity, Mw 769.94, molecular formula $C_{37}H_{67}NO_{13}\cdot 2H_2O$) was regarded as materials to carry out investigation. Based on erythromycin A dihydrate as materials, preparation of erythromycin acetone solvate was by means of recrystallization from aqueous solutions containing 25% (v/v) acetone under 45 °C. Acetone used for the experiments was of analytical reagent grade. Distilled deionized water of HPLC grade was used. Both crystalline forms obtained by sampling before and after transition were also subjected to TG-DTA (NETZSCH TG 209) analysis. Measurements were carried out at a rate of

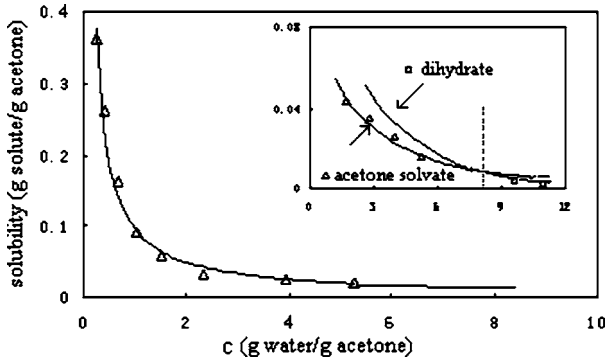

Figure 4. **Solubility curve of erythromycin in acetone + water at 45 °C.**

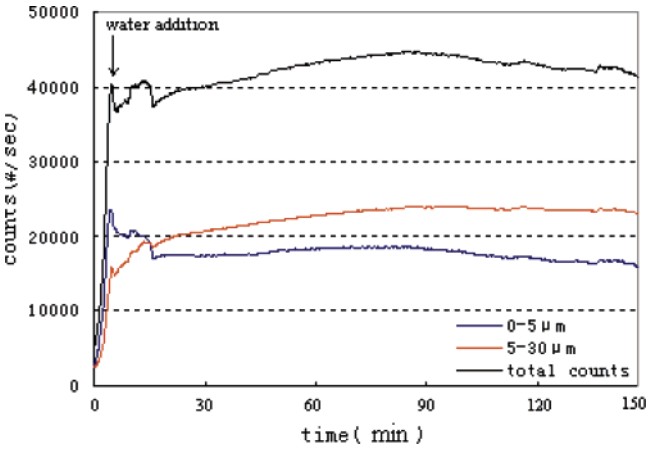

Figure 5. **FBRM counts of crystallization process in experiment A.**

85 mL/min^{-1} under a dynamic atmosphere of dry nitrogen. TG-DTA was performed on samples 4.4 mg in an open aluminum pan from 25 °C to 400 °C.

The solubility of erythromycin was measured in acetone + water at a temperature of 45 °C. Saturated solutions of the pure solid forms were prepared in a well agitated 100 mL jacketed glass crystallizer. The temperature of the crystallizer was controlled by a heating and refrigeration circulator to within ±0.02 °C, and the solution was stirred for at least 6 h at this temperature. After the equilibration, excess solid solute was allowed to settle at no agitation for 6 h. Then, samples of the saturated solution were transferred by a syringe and filtered through a 0.2 μm membrane filter into a previously weighed sample vial. The sample weight was determined and the solvent was allowed to evaporate in a vacuum oven at 20 °C, after which the weight of the constant "dry residue" mass of erythromycin was determined. Suspension samples withdrawn from the crystallizer were analyzed for the solid phase to verify the solid form present at equilibrium by microscope and XRPD (X-ray powder diffraction).

The crystal structure of each solvate was distinguished off-line using XRPD (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 of 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 25 ± 1 °C. The results determined are in agreement with the literature.[11]

For the crystallization process of erythromycin, there are two stages in industry. The one is the nucleation and growth process of acetone solvate in acetone aqueous solutions (I) and the other is the transformation process of acetone solvate to dihydrate in pure water (II). Experimental setup was shown as Figure 2. A 400 mL glass cylindrical crystallizer with a jacket to circulate the thermostated water was used. The erythromycin dihydrate (20.00 g) was dissolved at 45 °C in 80 mL (63.20 g) of pure acetone. The crystallization was carried out by adding water by a pump while stirring with an impeller speed of 150−210 rpm. When the amount of water addition with a rate of 1.5 mL/min reached the water addition value in Table 1, seed was added in experiments B, C, D, and E. After seeding, water addition was followed and the rate of water addition as an operational condition was changed from 0.25 to 3.50 mL/min (Figure 3). The final mass ratio of water to acetone was around 2.53. After water addition was finished, erythromycin acetone solvated crystals obtained were quickly isolated by filtration.

The transformation process (II) was carried out as follows: the wet crystals (composed of 18.00 ± 1.00 g of erythromycin and 7 ± 1.00 g mixed solvents) obtained by filtration were mixed at 45 °C in 150 g pure water and slurred at a speed of 150 rpm for 30 min. After filtration and air-drying, erythromycin dihydrate was obtained.

To visualize the processes occurring in situ, both stages were monitored using PVM and FBRM. The PVM probe

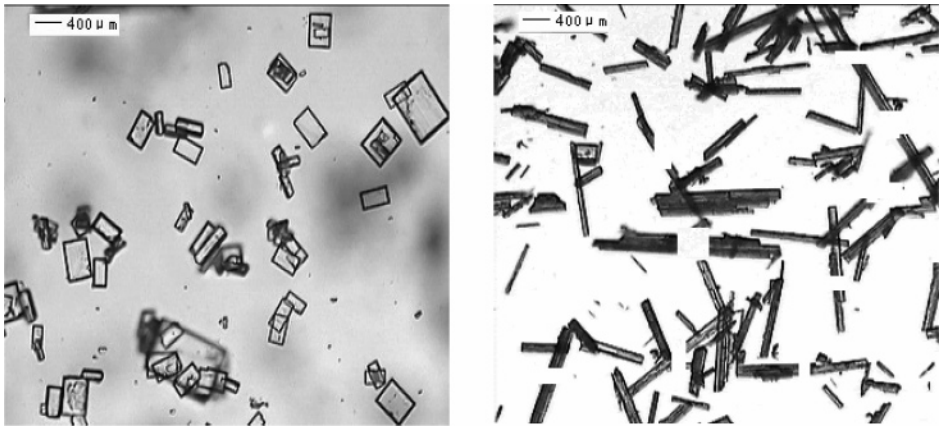

Figure 6. **Microscope images of experiment B seed crystals (the left) and experiments C, D, and E seed crystals (the right).**

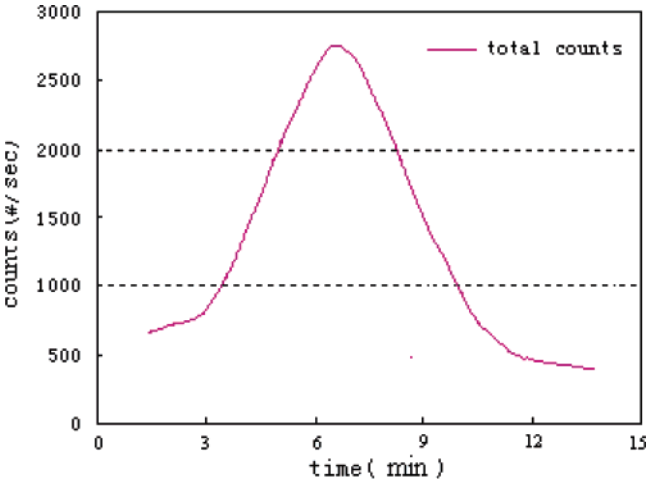

Figure 7. **FBRM counts during experiment B.**

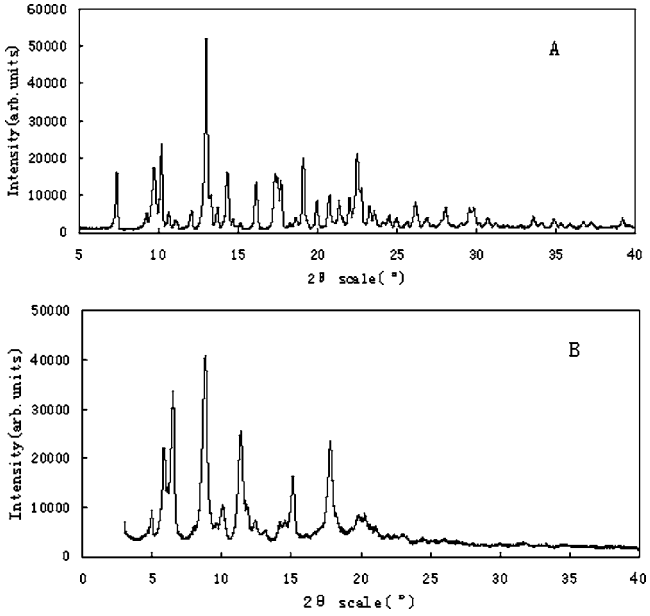

Figure 8. **X-ray diffraction pattern: (A) erythromycin dihydrate; (B) erythromycin acetone solvate.**

(model 800L) 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 three population ranges set that were 0−5 μm, 5−30 μm, and 0−100 μm, respectively. The probe measurement duration was set at 5 s. Off-line 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.

A series of trials as outlined in Table 1 were performed to examine the effect of seeding strategies on the nucleation and growth process of erythromycin in acetone solutions. Experiment E was the same as experiment D to examine reproducibility. The phase transformation process of erythromycin solvates in pure water was also investigated. All experiments were conducted under isothermal conditions at 45 °C.

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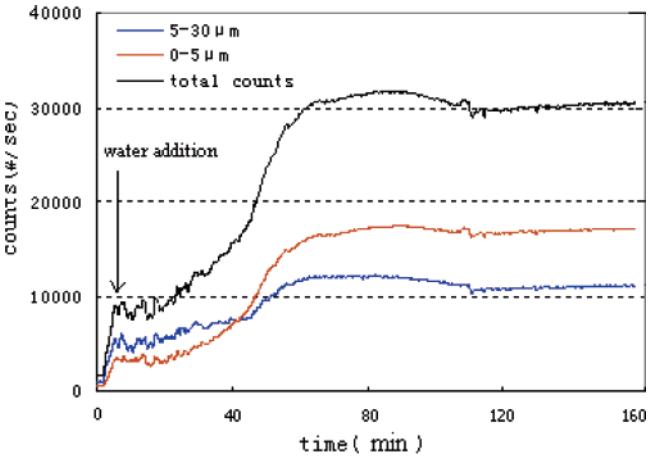

Figure 9. **FBRM counts of crystallization process in experiment C.**

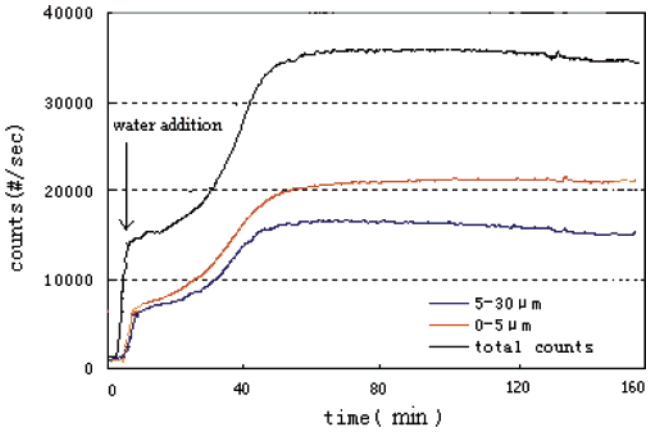

Figure 10. **FBRM counts of crystallization process in experiment D.**

Results and Discussion

The solubility of erythromycin in acetone + water is graphically presented in Figure 4. The experimental error was within 5% of the measured value. From Figure 4, the solubility curve shows an enantiotropic system, namely, the solubility curves of both forms crossing at a certain composition. Approximately, when the ratio of water to acetone is less than 8.0, erythromycin acetone solvate has a lower solubility than dihydrate and is the thermodynamic stable form, while when the ratio is more than 8.0, erythromycin dihydrate has a lower solubility and is the stable form at this temperature.

To illustrate the influence of the seeding strategy on the crystallization process and CSD, experiments A, B, C, and D were performed. In experiment A, no seed crystals were added. Particle counts of different ranges measured by the FBRM are shown Figure 5. On adding 40.36 g of water, there is a steep increase in all population ranges, which accounts for the spontaneous nucleation phenomenon in the solution. Then, as can be seen, counts measured of 0−5 μm crystals decrease, while those of 5−30 μm increase. However, since drastic nucleation greatly consumes supersaturation, particle counts of all ranges reach equilibrium after only approximately 15 min. It can be speculated from this phenomenon that erythromycin in acetone aqueous solution has poor nucleation ability, namely, a wide crystallization

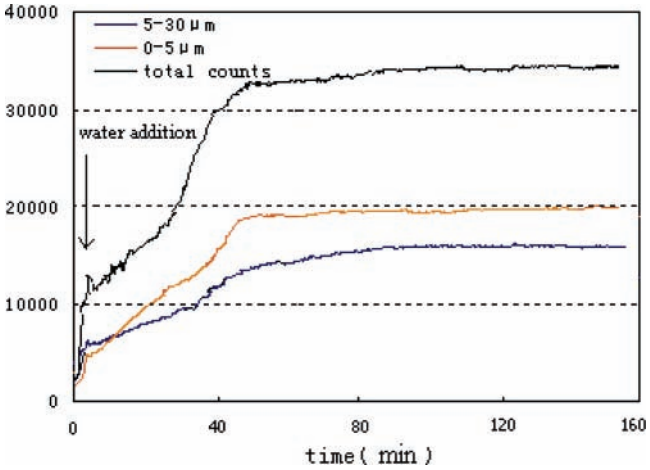

Figure 11. **FBRM counts of crystallization process in experiment E.**

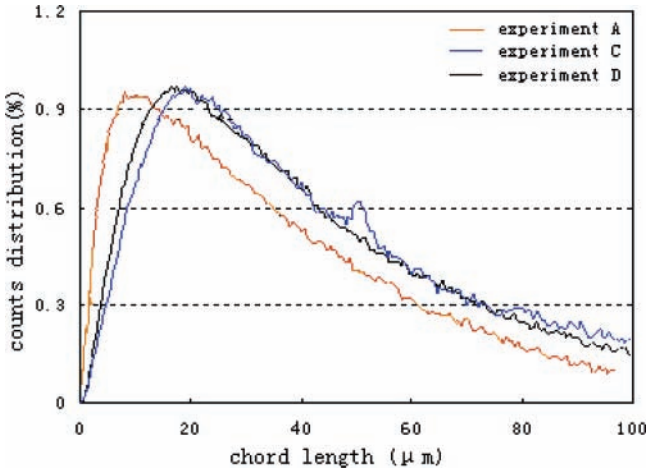

Figure 12. **FBRM chord length distribution of experiments A, C, and D.**

metastable zone, which gives us a cue of addition of seed crystals to improve the nucleation and crystallization process.

In experiment B, on adding 22.50 g of water, seed crystals of erythromycin dihydrate (confirmed by using XRPD, Figure 8A) as shown in Figure 6 were added. This operation leads to an initial rapid rise in the FBRM counts, which is followed by a drop in measured counts as shown Figure 7. This decrease in counts is a result of the dissolution of the erythromycin dihydrate. However, the case is different in experiment C. On adding 22.50 g of water, the acetone solvated seed crystals (conformed by using XRPD, Figure 8B) were added, and there is an immediate increase in FBRM counts, which is brought about by seed crystals and the new induced crystal nucleus. Subsequently the crystals start to grow, and there is a gradual rise in crystal counts of all ranges with the addition of water. Comparing experiment B with experiment C, it is assumed that erythromycin dihydrate has higher solubility than acetone solvate, which agrees with the result of solubility measurements. In experiment C, addition of acetone solvated seed crystals in the metastable zone not only provided growth units but also avoided spontaneous nucleation. It can also be observed from Figures 5 and 9 that crystal counts in experiment C are obviously less than those observed in experiment A. Adding seed crystals in the

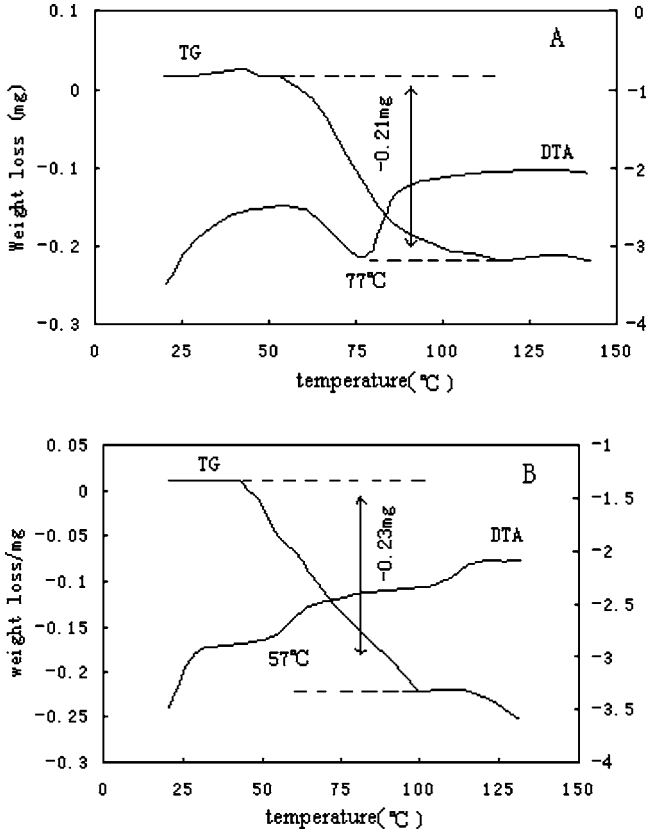

Figure 13. **TG-DTA curves of both erythromycin solvates: (A) erythromycin dihydrate; (B) erythromycin acetone solvate.**

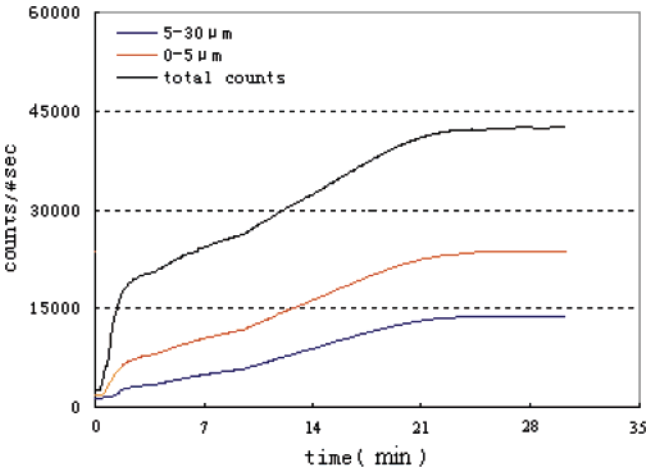

Figure 14. **FBRM counts during transformation.**

system inhibits forming high supersaturation, which benefits crystals growing smoothly.

Because erythromycin is slightly soluble in water, supersaturation is formed by water addition. Experiments C and D aimed to examine the influence of water addition amounts on nucleation under circumstances of adding same seed crystals. In experiment D, on adding 25.50 g of water, higher supersaturation was generated than that in experiment C. As observed in Figure 10, the counts in all ranges measured by FBRM are also more than those of experiment C, which illuminate that the number of nucleations induced by seed crystals has great dependence on the supersaturation. Therefore, it is possible to find proper supersaturation by assistance of FBRM in which seed crystals are added. To check the

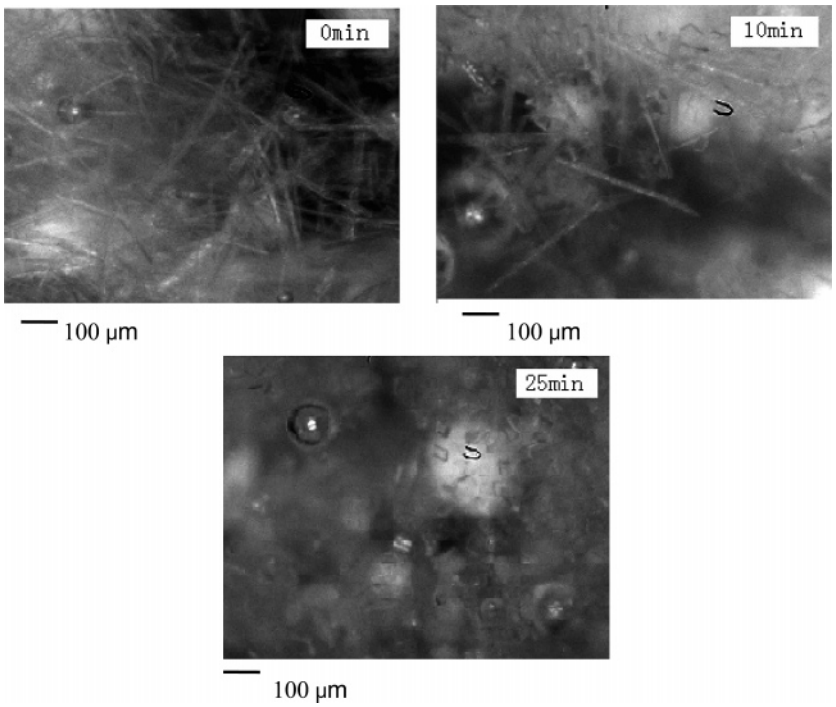

Figure 15. **PVM images during transformation.**

experimental reproducibility, experiment E was performed. As shown in Figures 10 and 11, good reproducibility is obtained for experiments D and E. Figure 12 compares the chord length distribution measured by FBRM for experiments A, C, and D after 2 h. It is noted that the dominant chord length is a function of the supersaturation when seed crystals are added. Higher supersaturation leads to a smaller dominant chord length of crystal products. The trends of three curves are well consistent with the experiment process. The shift in the mode of the distribution is due to crystal growth.

For the crystallization of erythromycin, large particles and fine particle size distribution can greatly improve the isolation process as well as the performance of final products. In the present experiments, dominant chord length ranks as experiment C > experiment D > experiment A. Corresponding to experiments C, D, and A, yields of crystallization are 89.85%, 88.90%, and 87.62%, respectively, which are smaller than the yield (91.22%) predicted from the solubility curve. The yield loss can come from the isolation procedure.

Breakage and agglomeration are called the secondary process during crystallization. FBRM and PVM can also be used to describe and distinguish the breakage and agglomeration phenomenon. From Figures 9 and 10, it is clear that there is a slow rise in crystal counts of each range monitored by FBRM within the initial 40 min. The rise of 0−5 μm crystal counts come from secondary nucleation, including the nucleus induced by local high supersaturation and breakage resulting from attrition. However, there is a very small change in crystal counts after 40 min, which implies that breakage is not determined in the crystallization process. High local supersaturation formed by water addition is the main reason for secondary nucleation. Therefore, to obtain larger particle crystals, some measures should be taken to strictly control the manner and the speed of water addition. Meanwhile, agglomeration was not observed throughout the crystallization process by monitoring of PVM (not shown). Understanding these effects can aid optimization and improve process control.

Water addition was stopped after 2.5 h, and subsequently the solution was filtered. Samples taken at the beginning and the end of the transformation experiment were subjected to TG-DTA analysis. As Figure 13 shows, mass losses of 0.21 mg (Figure 13A) and 0.23 mg (Figure 13B) from the total weight 4.4 mg correspond to two water molecules and two-thirds of the acetone molecules in one erythromycin molecule, respectively.

To demonstrate and provide a better understanding of the phase transition process between erythromycin solvates, the transformation process in pure water was monitored by in situ PVM and FBRM. As known, crystal form transitions may be accompanied with the dissolution of the metastable form and subsequent nucleation and growth of the stable form. FBRM can be used to monitor the change in the particle counts and dimension, and the crystal habit changes can be monitored by PVM. A typical particle size change in each population range and typical images taken for the transformation process were summarized in Figures 14 and 15. In Figure 15, at $t = 0$ min, there are only needlelike crystals suspending in solution. At $t = 10$ min, platelike crystals at the surface of needlelike crystals appear, which means the nucleation stage of erythromycin dihydrate. At $t = 25$ min, it is clearly seen that platelike crystals without needlelike crystals are suspending in the solution. Changes of both solvates in number and in crystal habit are clear from the PVM images, which supports the conclusion that the transition mechanism is a typical solution-mediated transformation. Figure 14 indicates the trend history of different particle size groups. From Figure 14, it can be seen that particle counts of all population ranges increase during the initial 20 min, which implies that the dissolution of needlelike

crystals and nucleation of platelike crystals proceed synchronously. This trend may be because that there are no significant differences in solubility between the two solvates in water at 45 °C. After 20 min, the counts slowly reach equilibrium from FBRM data as the remaining supersaturation is gradually reduced. The result showed that PVM is ideally suited for monitoring the transformation process. In contrast, in the data presented by FBRM, it is difficult to distinguish the transformation from metastable acetone solvate to stable dihydrate. However, by combining FBRM and PVM, monitoring of the transformation between erythromycin solvates can be successfully developed.

Conclusions

In situ FBRM and PVM are very useful process measurement techniques that were successfully used to monitor erythromycin dilution crystallization and the transformation of erythromycin solvates. It is noted that the supersaturation when seed crystals are added is a critical control point to improve crystal products. In the crystallization process, breakage is not determined and secondary nucleation mainly originates from induced nucleation by local high supersaturation resulting from water addition. Hence, the mode of water addition is another important control operation. Besides, agglomeration can be neglected throughout the crystallization process.

Completeness of transformation from erythromycin acetone solvate to dihydrate affects downstream processes such as filtration, drying and, thus, the quality of the final products. FBRM and PVM in this work were proven to be very valuable in monitoring erythromycin phase transition by providing images and population statistics information on line. The transformation mechanism can be identified as a solution-mediated transformation. Compared with the FBRM, in situ PVM was found more suitable to obtain insight into the transformation process of erythromycin solvates. If in situ ATR-FTIR is available, it is also necessary to monitor concentration changes of both solvates to gain a truer understanding during transformation.

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