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The Impact of Operating Parameters on the Polymorphic Transformation of D-Mannitol Characterized in Situ with Raman Spectroscopy, FBRM, and PVM

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Abstract:

D-Mannitol is a typical polymorphic crystalline compound. In this paper, this polymorphic transformation from the α to the β form of mannitol is monitored by in situ Raman spectroscopy, focused beam reflectance measurement (FBRM) and particle vision measurement (PVM). The standard Raman spectra of the two polymorphs of mannitol were determined and the characteristic peaks for the different polymorphs were chosen to track the transformation process. By combining Raman with FBRM and PVM, relationships between fine particles and metastable form dissolution and also between coarse particles and stable form crystallization could be defined. The solution-mediated polymorphic transformation mechanism was confirmed by these in situ tools. The effect of temperature, solvent and substrate mass on the transformation time was also investigated. It was noted that operating temperature and solvent composition have a significant influence on the transformation time.

1. Introduction

As an important purification operation for solid products, crystallization has widely been used in the chemical and pharmaceutical industries. Although polymorphism in crystals has been known for over 200 years, it is only in the last 30 years that its importance in pharmaceutical compounds has fully been realised, with increasing efforts paid to establish the thermodynamic and kinetic conditions required to isolate both metastable and stable forms of solutes.

Polymorphism is defined as the ability of a compound to exist as more than one crystalline form, each of which has the same chemical structure but different arrangements of the molecules in the crystal lattice.[1] As polymorphs may have different functionalities and physical properties, such as bioavailability, solubility and stability, it is essential to investigate the appropriate existing conditions for each polymorph and the probability of transformation between them. The relationship between polymorphs can be either monotropic, where one form has a lower Gibbs free energy than the others at all temperatures below the melting point, or enantiotropic, whereby the Gibbs free energy of the two forms is equal at a certain transition temperature. In different regions of temperature, the metastable polymorph tends to transform to the stable form. Two types of transformation mechanisms have been proposed in the literature,[2] solid-state polymorphic transformation (SST) and solution-mediated polymorphic transformation (SMT). The former takes place via the positional rearrangement of the ions or molecules in solid state while the latter occurs by dissolution of the metastable phase and crystallization of the stable phase. It is crucial to understand the transformation mechanism under different conditions in industry.[3] This paper investigates these mechanisms through in situ monitoring of the change of polymorphic form (Raman), morphology (PVM), and crystal size (FBRM).

Several online methods have been reported in the study of polymorphs and transformations, such as Raman spectroscopy, FBRM, PVM, and FTIR (Fourier transform infrared spectroscopy). Raman spectroscopy is a light scattering technique with good sensitivity to solids, which has been successfully applied to identify different polymorphs[4] and also infer the relative solid content profile during the crystallization process.[5] In the research of the polymorphic transformation of L-glutamic acid,[6−8] carbamazepine[9] and *p*-aminobenzoic acid,[10] Raman showed good applicability as an in situ tool. Both FBRM and PVM are probe-based high solids concentration particle characterization tools.[11] While FBRM can obtain the particle dimension measurements in a large range, PVM is often employed to get particle images. Dang et al[12] have used FBRM and PVM to monitor the polymorphic transformation from the β to the α form of glycine, while O'Sullivan et al[13] combined them with FTIR in the research of transformation process from δ- to β-mannitol. The application of these in situ apparatus and the combination of them can give instant details of the process. In this paper, online Raman Spectroscopy, associated with FBRM and PVM was first used to track the polymorphic transformation from the α to the β form of mannitol.

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Mannitol is a natural hexahydric alditol which is widely used in pharmaceutical and food industry.[14] Three polymorphs of mannitol have been identified. Burger et al[15] gave a particular good review of the nomenclature used to describe these polymorphs. The most common notation used in the literature is to refer to the thermodynamically stable form as the β form, and the metastable forms as α and δ.[16−18] The α and β form belong to the same orthorhombic space group $P2_12_12_1$ while δ form exhibits a monoclinic $P2_1$ space group.[19] The β form is commonly the commercial product, and the thermodynamic stability of the three polymorphs is in the order $\beta > \alpha > \delta$.[20] O'Sullivan and Glennon et al[13] investigated the polymorphic transformation from δ to β form of mannitol, and Yoshinari et al[21] paid much attention to the effect of moisture on the same transformation course. There is no particular research on the transformation from the α to the β form. Recently, Cornel et al[22] presented a model for precipitation and transformation of the three polymorphs of mannitol. Somewhat confusingly, Cornel et al. refer to the three forms, in terms of increasing stability, as α, β, and γ, whereas the most common nomenclature is as reported above (δ, α, β, or form III, II, and I according to Burger et al.[15]). In Cornel's work, kinetic expressions were proposed for the transformation processes which fitted well to the available experimental data.

In this study, the specific transformation of the metastable α form to the stable β form is examined in detail, extending the range of available data for this transformation process. The use of in situ Raman spectroscopy, PVM, and FBRM to clarify the solution-mediated transformation is demonstrated. The effect of important process parameters, such as temperature, solvent, and substrate mass on the transformation time are investigated.

2. Experimental Section

2.1. Materials. The stable β form of mannitol (1,2,3,4,5,6-hexanehexol, 99%) was purchased from Sigma Aldrich Co. (UK). The α form was prepared as described in our previous work[20] by fast cooling a saturated mannitol solution in binary ethanol−water (70 wt %) solvent from 50 to −5 °C around in a freezer, and drying the isolated solids under vacuum at 40 °C. The purities of α and β forms were checked by XRD and DSC as described in the former paper.[20] Before using as substrate, α mannitol was ground by a mortar and pestle. Ethanol (99.5%) in analytical grade was also supplied by Sigma Aldrich Co. (UK). Deionized water was used throughout.

2.2. Apparatus. A Kaiser Raman RXN2 system was used in this study. The RXN2 analyzer utilizes fiber-coupled probe optic technology for in situ monitoring. With a large spectral region from 100 to 1890 cm^{-1} and a good resolution of 5 cm^{-1} average, this system could be used flexibly during the transformation process of mannitol. The excitation wavelength was set to 785 nm. In this paper, the MR probe head with immersion optics was used to characterize in situ the polymorphic transformation in solution while the PhAT probe head was used to measure the isolated and dried mannitol solids. IC Raman software (by Mettler-Toledo) was combined with this system.

Both the FBRM and PVM particle characterization tools used in this study were manufactured by Mettler-Toledo. The FBRM probe (model D600L) measurement duration was set at 10 s. Various chord length ranges and the mean FBRM chord length were used to understand the transformation process. IC FBRM software was utilized during the experiment. The PVM probe (model 800 L) was operated with an image update rate of 2 images per second.

2.3. Transformation Experiment. Most experiments were performed in a 100-mL Mettler-Toledo EasyMax system in conjunction with the software iControl Easymax. A 1-L LabMax automatic laboratory reactor (Mettler-Toledo) was used in the experiments where the FBRM and PVM probe heads were simultaneously used to immerse into the system. The transformation experiments started by adding the α form of mannitol as the substrate into the saturated solution of β-mannitol at constant temperature, and at the same time Raman, FBRM, and PVM began to track the process.

3. Results and Discussion

3.1. Identification of Two Polymorphs. Raman Spectroscopy was successfully applied to identify the polymorphs of mannitol as shown in Figure 1. The difference of Raman spectra between α and β forms of mannitol is apparent. For example, in the spectral range of 820−910 cm^{-1}, the β form has only one Raman peak at 876 cm^{-1} while the α form has an additional peak at 887 cm^{-1}. In the spectral range of 1090−1180 cm^{-1}, β form has two main characteristic peaks at 1119 cm^{-1} and 1134 cm^{-1} while the α form only has one main peak at 1130 cm^{-1}.

In this study, the peak 1233 cm^{-1} and 1365 cm^{-1} are chosen as the characteristic peaks of β mannitol, while the peak 1355 cm^{-1} is used to track α-mannitol. From the partially magnified Figure 2, it is clear that the peaks are detached and have similar Raman intensity. The Raman intensity in this work is defined as the peak height to the two-point baseline.

3.2. Tracking the Polymorphic Transformation Process by Raman, FBRM and PVM. The transformation process from the α to the β form of mannitol was carried out in water or in a binary ethanol−water solvent mixture. Kaiser Raman system has been successfully applied to monitor the transition of mannitol in water.[20,22] In this paper, the Raman system also shows good applicability and stability in binary ethanol−water system. The polymorphic transformation of mannitol in the 17 wt % ethanol−water solvent at 27 °C was monitored in situ, and the typical Raman waterfall plots in the region of 1338−1377 cm^{-1} over the course are illustrated in Figure 3. It can be seen that the characteristic peak of α form at 1355 cm^{-1} gradually disappears from a strong signal after the substrate adding, while that of the β form at 1365 cm^{-1} presents and increases in peak intensity over time, which

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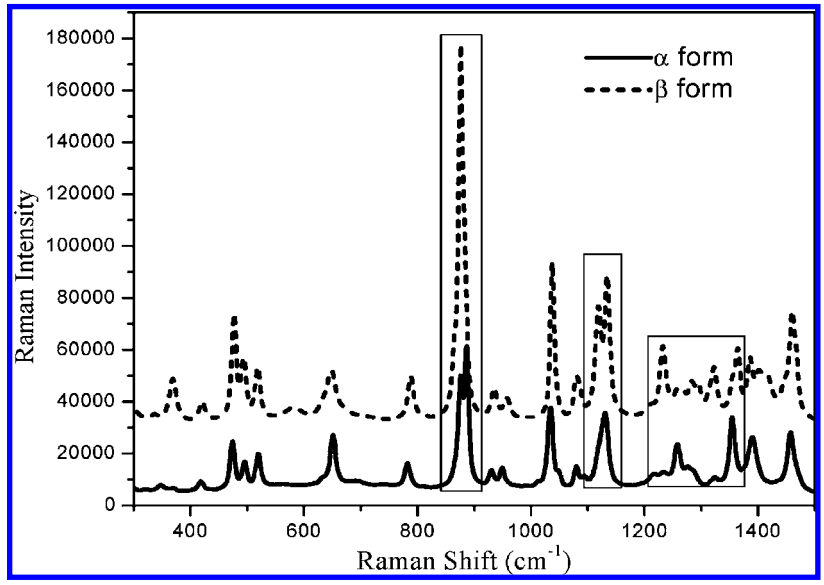

Figure 1. **Raman spectra of α and *β* forms of mannitol.**

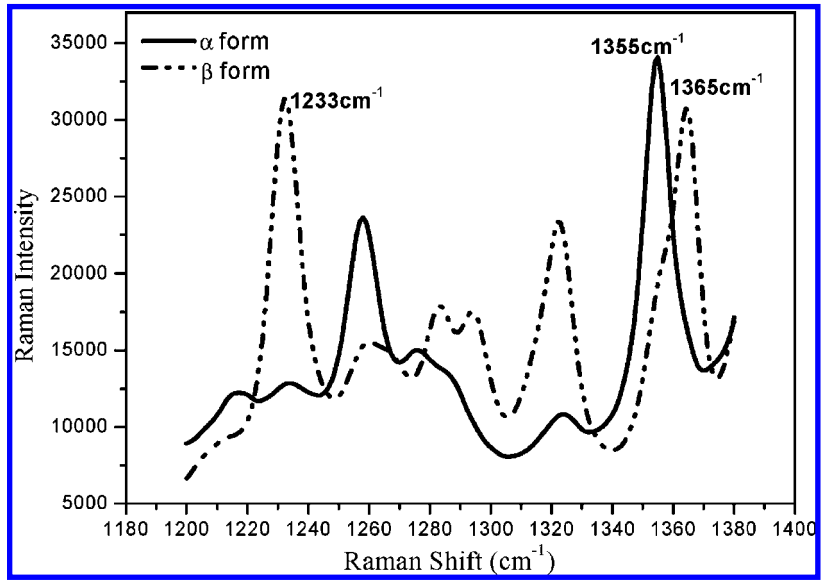

Figure 2. **Characteristic peaks of α and *β* forms of mannitol used to monitor the transformation process.**

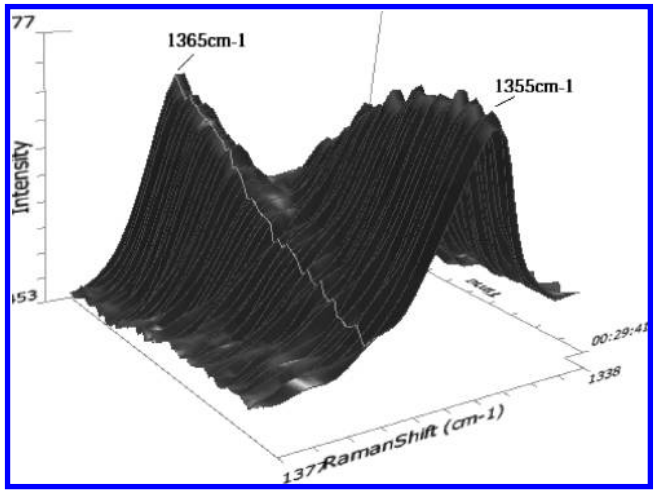

Figure 3. **Changes of Raman intensity at 1355 cm^{-1} and 1365 cm^{-1} during the transformation process.**

distinctly indicates that the α form was gradually transforming into the *β* form.

PVM images taken at key intervals during the transformation course are shown in Figure 4. From Figure 4, it is clear that the high number of fine particles evident in image I (2 min) are the α form of mannitol. As the transformation process progresses, an associated reduction in the fine particle counts and an increase in the coarse particle number (see PVM image II) appear, which identifies this transformation with the presence of larger rods evidently in a sea of fine material. Image III shows the obvious large crystal size of the *β* form at the end of the transformation. The distinct size difference between the α and *β* forms is consistent with the scanning electron microscopy results in the literature.[22]

The dimension change of particles can also be reflected by the unweighted and the square-weighted chord length distributions (CLD) from FBRM as shown in Figure 5. It is obvious in the unweighted CLD that the initial α-mannitol (time = 2 min) has a large number of fine particles present between 1−20 *μ*m. From this point, the fine side of the distribution starts to reduce in number with a corresponding increase in the crystal

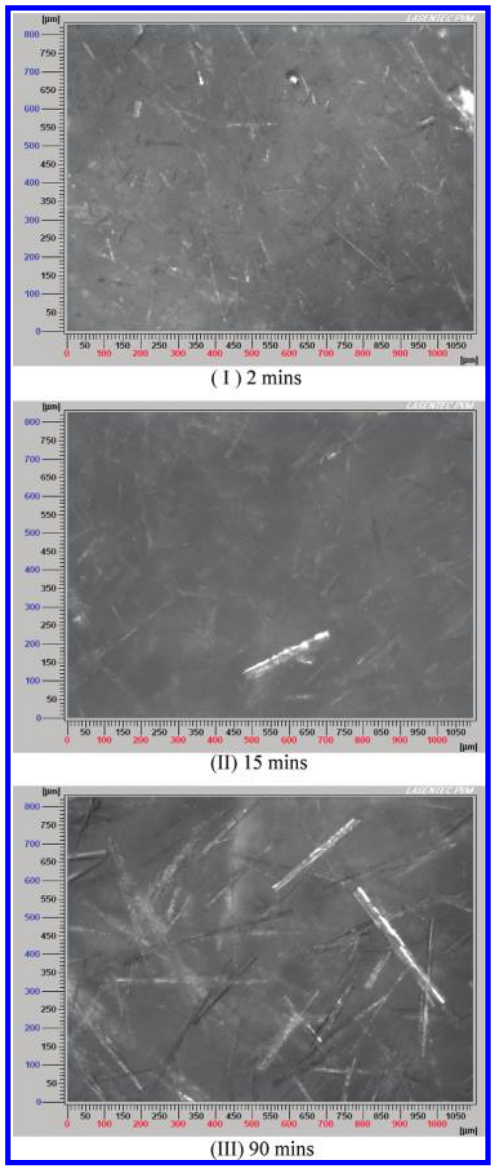

Figure 4. **PVM images taken at different times after the addition of substrate.**

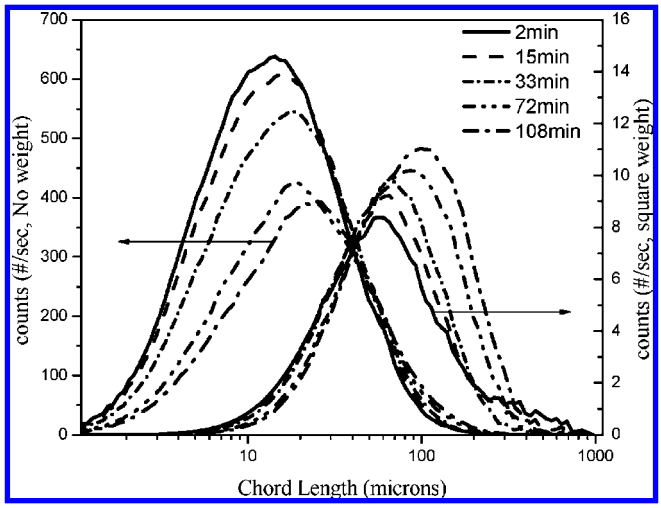

Figure 5. **CLD describing the transformation in crystal number and dimension during the transformation.**

size. At the same time, while analyzing the square-weighted CLD there are a distinct increase in crystal size and a rising number of the coarse particles. Additionally considering the morphology changes shown in Figure 4, it is expected that the number of the fines present between 1−20 μm should track the relative change in the metastable form population while the coarse chord counts between 80 −1000 μm should monitor the subsequent crystallization of the stable form. This is clearly seen in Figure 5 where the unweighted CLD which is dominated by the small chord lengths, shows a gradual decrease in counts over the course of the batch, while the square-weighted CLD, which is dominated by the larger chord lengths gradually increases in counts as the larger, stable crystals are forming and growing at the expense of the metastable form.

These trends are further illustrated in Figure 6 which compares the evolution of the Raman peaks with the associated average chord lengths. It can be seen that the reduction in the presence of the α form, and the associated fine chord counts, (1−20 μm) is clear, while the β form and the associated coarse counts (80−1000 μm) are seen to increase during the transformation. The mean chord length continues to increase during the transformation process, which is consistent with the dimension change emphasized in Figures 4 and 5. In addition, it is worth noting that the same ending point is obtained by Raman and FBRM in Figure 6. When the Raman peaks indicate the end of the transformation, FBRM data remain changing simultaneously and stay constant after that.

It appears that the decrease of α-mannitol is caused by the dissolving of the α form due to the higher solubility of the metastable α form compared to that of the stable β form.[12] Once the dissolution of α form occurs, it steadily increases the solution supersaturation with respect to the β form, which then results in the nucleation and growth of the β form. When the crystallization of the β form consumes the supersaturation, the dissolution can proceed continuously. In this way, the dissolving of α form and crystallizing of β form, which are the two main steps of solution-mediated polymorphic transformation,[23] would last during the entire transformation process until the α form disappears and the solution becomes saturated with respect to the β form again. From Figure 6, the rate of α form dissolution corresponds to the disappearance of the fine particle counts between 1−20 μm. And the coarse particle counts also correspond extremely well to the crystallization of the stable form shown by Raman. The ending points of polymorphic transformation from Raman spectroscopy and FBRM are consistent, which means both Raman spectroscopy and FBRM can give the efficient information about the monotropic SMT process from the α to the β forms of mannitol.

Overall in the present study, by combining the three in-situ tools, Raman spectroscopy, PVM, and FBRM, the solution-mediated polymorphic transformation mechanism from α- to β-mannitol can be clearly confirmed.

3.3. Effect of Temperature on Polymorphic Transformation Time. A series of trials were performed to identify the influence of operating temperature on the transformation time from the α to the β form of mannitol. The transformation time in this study is defined as the time from the addition of the α form substrate to the Raman intensity of the α form peaking

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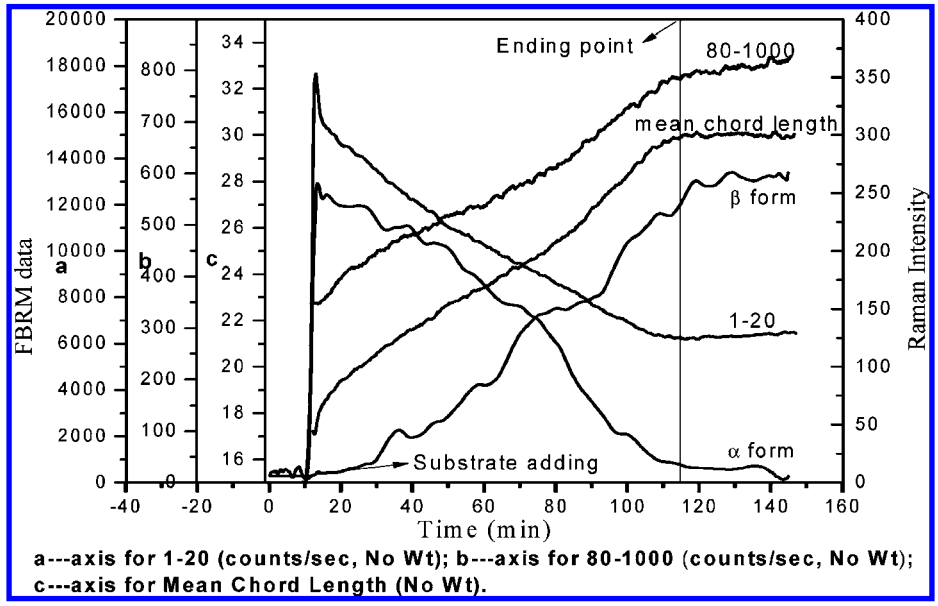

Figure 6. **FBRM data and Raman intensity change during the transformation.**

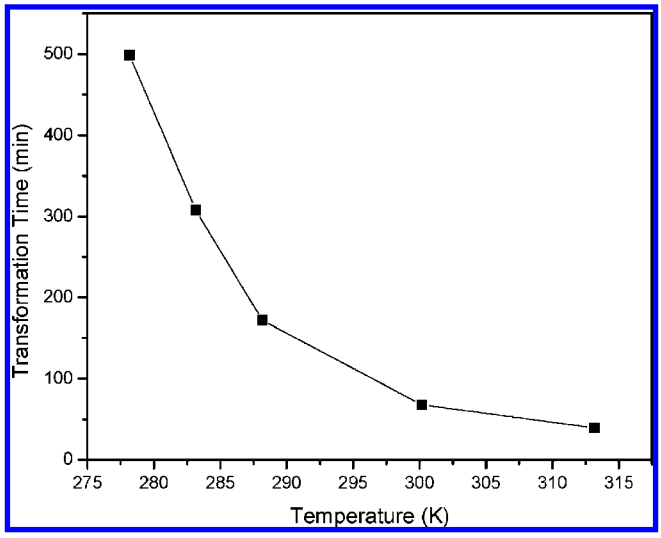

Figure 7. **Effect of operating temperature on transformation time from α- to *β*-mannitol.**

reaching a constant value (see Figure 6). All experiments were performed in 100 mL of water with 4 g of the α form substrate added. Figure 7 shows the variation of transformation time with temperature. It is clear that as the temperature increases, a notable decrease in the transformation time is observed.

Solution-mediated polymorphic transformation is a complex process due to the fact that several mechanisms, such as the dissolution of the metastable form, the primary heterogeneous nucleation and secondary nucleation of the stable form, the growth of the stable form crystals, and sometimes secondary processes, such as agglomeration and attrition, are involved in the process.[24] Temperature typically has a positive influence on the kinetics of these chemical processes. According to the classical theory, the molecular motion can be accelerated by higher temperature, and the interfacial energy between the solid and liquid phases can be lower when temperature increases.[2,25] This is crucial in both the dissolving of α particles and the process of small *β* nuclei becoming visible crystals. It is expected, therefore, that high temperature can facilitate the two main processes of SMT (dissolution and crystallization), which directly lead to the drop of transformation time as shown in Figure 7.

3.4. Effect of Solvent on Polymorphic Transformation Time. Solvent composition also has a significant influence on transformation time, especially in a solution-mediated polymorphic transformation process. Figure 8 shows the increasing trend of transformation time with the increase of ethanol content in the binary ethanol−water solvent mixture, as well as the solubility of *β*-mannitol in these different solvents. All of the transformation experiments were performed in 100-mL volume at 27 °C with 4 g of substrate added.

The transformation time is determined by a balance of solubility and the solvent−solute interactions in SMT process.[26] Usually the solvent that gives a high solubility can facilitate polymorphic transformation. From Figure 8, it is clear that the increase in ethanol content reduces the mannitol solubility dramatically, which can generally lead to a decrease in the nucleation and growth rate of the stable form.[26] In the solution-mediated transformation process from the α to the *β* form of mannitol, it appears that the dissolution of the α form is faster than the crystallization of the *β* form, since the induction time for crystallization is between 10−15 min, while quick dissolution occurs in the first 3 min after addition (see Figure 6). Thus, the crystallization of the *β* form may be the rate-determining step. When the lower solubility causes the drop of nucleation and growth rate of the *β* form, the transformation time will significantly rise, especially in the high ethanol content. The decreasing trend of solubility in Figure 8 is also consistent with the fact that more ethanol favors the existence of the metastable form of mannitol. Generally, cooling a saturated solution in 70 wt % ethanol−water will favor formation of α-mannitol, while the *β* form could be often obtained in pure water.[20]

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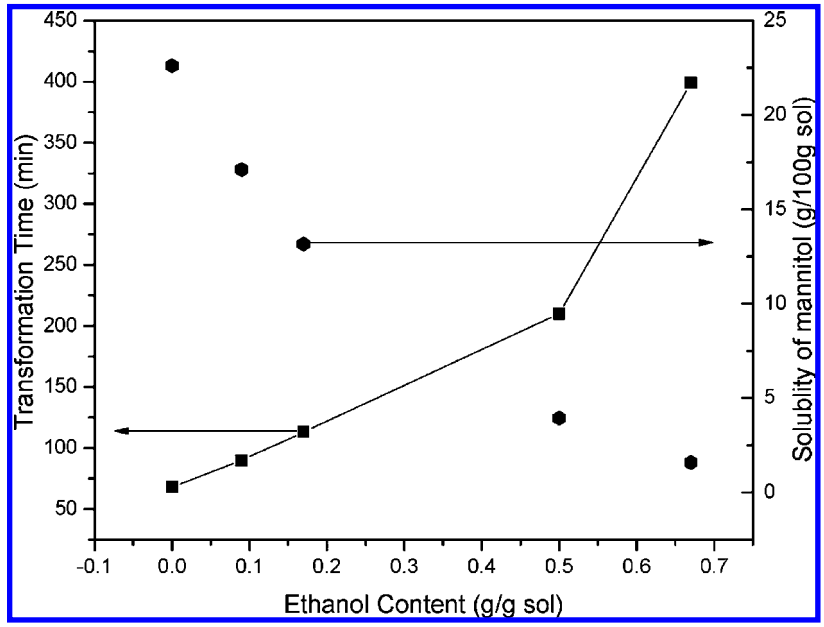

Figure 8. **Effect of solvent composition on transformation time from α- to *β*-mannitol (filled circles = solubility data; filled squares = transformation times).**

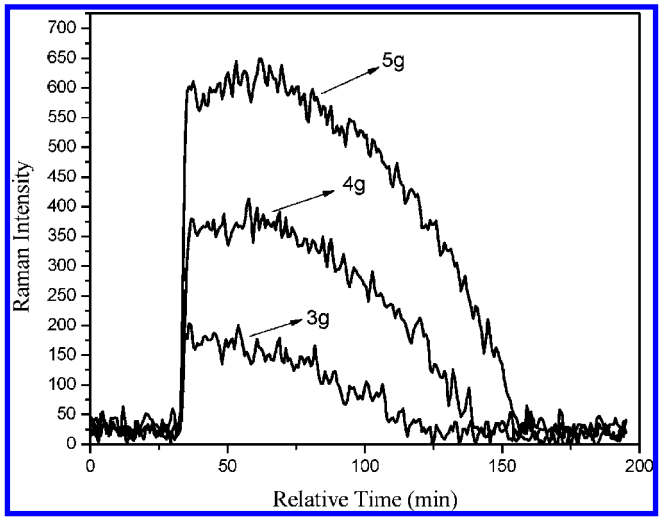

Figure 9. **Effect of substrate mass on transformation time from α- to *β*-mannitol.**

3.5. Effect of Substrate Mass on Polymorphic Transformation. Different amounts of the α form substrate were investigated in the same 100-mL saturated solution in 17%w ethanol−water at 27 °C. As shown in Figure 9, the transformation time is moderately prolonged as the substrate mass increases.

While the supersaturation does not change in the same temperature and solvent, the rate of crystal growth should be constant. The effect of substrate (especially when the chord length distribution is constant) on nucleation is also limited. Considering that the transformation might be controlled by the crystallization (nucleation or growth) of *β*-mannitol, the transformation rates should be similar in the experiments with different amounts of substrate added. In the solution-mediated polymorphic transformation which starts and ends in the same saturated solution of *β*-mannitol, more substrate means that in total more substrate needs to be transformed, and thus, a longer time is required when the substrate mass changes from 3 to 5 g as shown in Figure 9.

4. Conclusions

The transformation process from the metastable α to the stable *β* form of mannitol was studied in this work. Raman spectroscopy was applied to distinguish the spectra of different polymorphs of mannitol, and the obvious difference of Raman spectra made it feasible to monitor the existence of the two forms and thus the transformation process. Experiments conducted with PVM, FBRM, and Raman spectroscopy have shown how these in-situ techniques can track changes in particle morphology, dimension, and crystal structure which indicated the main steps of solution-mediated transformation. While the Raman spectroscopy permitted in-situ real-time monitoring of the disappearance and appearance of the metastable and stable forms respectively, FBRM provided a method for independent verification of the observations. PVM in-situ imaging, in turn, verified the data-interpretation strategy employed for FBRM, without recourse to potentially problematic sampling. In the work, the operating temperature and solvent composition were shown to have a dramatic impact on the transformation time of the polymorphs. The combination of these in-situ tools facilitated a notable increase in process understanding with respect to the mechanism of this transformation process and in turn allowed for the definitions of the conditions necessary for the isolation of the metastable and stable polymorphic forms. Work is currently underway to employ IR spectroscopy to monitor changes in the dissolved concentration to assist in the identification and verification of the available kinetic models for the transformation process.

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