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A Calibration-Free Application of Raman Spectroscopy to the Monitoring of Mannitol Crystallization and Its Polymorphic Transformation

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Abstract:

Raman spectroscopy is one of a number of potential in situ process analysis technologies (PATs) for application in crystallization processes. In this paper, the use of Raman spectroscopy in the cooling crystallization of mannitol is investigated. Mannitol has three known polymorphs (α, *β*, and *δ*) that can be assessed using Raman spectroscopy. Although several multivariate spectroscopic methods for measuring both the dissolved concentration and the polymorphic form using Raman exist, their application requires significant calibration and/or that several assumptions be made about the relationship between the concentration and the Raman spectra. In this paper, a novel and simple calibration-free univariate method for monitoring supersaturation during the cooling crystallization of mannitol is demonstrated. The use of the method to successfully monitor the transformation process of the metastable α form to the stable *β* form in aqueous solution is also presented.

1. Introduction

Several methods are available for in situ analysis of crystallization processes, including focused beam reflectance measurement (FRBM) for monitoring of the solid phase and Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) probes for measurement of the dissolved concentration.[1,2]

Raman spectroscopy is another in situ method that, uniquely for a single-measurement system, has the ability to monitor both the liquid phase and the solid phase at the same time. As with XRD, DSC, and IR, Raman spectroscopy offers a fingerprint of different compositions and solid-state forms. Raman spectroscopy is a light scattering technique in which a monochromatic laser source illuminates the sample and the resulting scattered light is collected and analyzed. Interaction of photons with the molecular vibrations of a sample causes the light to be shifted to a wavelength away from the wavelength of the incident laser.[3,4] This inelastic scattering of light gives rise to the characteristic Raman spectra.[5] Thus, the Raman technique can be used to confirm the chemical structure by the detection of molecular vibrations of a characteristic frequency.

Mannitol is a polymorphic hexahydric alcohol excipient that is regularly used in the manufacture of chewable tablet formulations, granulated powders for oral use, and lyophilized products such as injections. Mannitol commonly exists in three polymorphic forms (α, *β*, and *δ*), depending on the isolation method. The *β* form is the thermodynamically stable form.[6−8] The α and *β* forms exhibit the same space group (orthorhombic *P*212121). The *δ* form exhibits a monoclinic *P*21 space group.[9] The freeze-drying of mannitol solutions has been investigated using a combined approach of differential scanning calorimetry (DSC), cold stage microscopy (CSM), and subambient XRD,[10] with temperature-controlled Raman microscopy at −30 °C[11] and using in situ XRD and DSC.[12] The freeze-drying process of the mannitol solution was also investigated by De Beer et al.[13,14] using process analytical technology (PAT) tools, including on line Raman and NIR spectroscopy. O'Sullivan and co-workers[15,16] investigated the polymorphic transformation of the metastable *δ* form to the stable *β* form using in situ FBRM and ATR-FTIR. Yoshinari and Forbes[17,18] investigated the effects of moisture on the polymorphic transition of the *δ* form to the *β* form using XRD, DSC, and solid-state NMR. Bruni et al.[19] studied the physicochemical characterization of anhydrous D-mannitol and the melt crystallization process using XRD,

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DSC, and FTIR. A monotropic relationship was reported between the α and β forms and an enantiotropic relationship between the α and δ forms. Recently, Burger et al.[20] addressed the confusion in the literature concerning the nomenclature of the mannitol polymorphs in addition to investigating their compression behavior. Cornel et al.[21] addressed the thermodynamic and kinetic aspects of mannitol's polymorphs and their transformation monitored using in situ Raman spectroscopy. Little work on the use of in situ Raman spectroscopy to investigate the polymorphic transformation of mannitol or its use to monitor its supersaturation during crystallization has been reported. Cornell and Mazzotti[22] presented a quantitative method for estimating analyte concentrations without the need for full calibration. However, the method employs significant data analysis, including nonlinear optimization regression and simulated annealing algorithms, and is based on several important assumptions. In particular, it is assumed that the analyte Raman spectrum is independent of all other species present and is a linear function of the analyte concentration under all conditions. In addition, the Raman spectra require correction for offset and normalization. In this paper, a univariate calibration-free method for monitoring the mannitol polymorphic transformation is presented. No assumptions are made with regard to the relationship between the measured species and the Raman spectra. Barrett et al.[23] have published a univariate calibration-free method for the monitoring of supersaturation using mid-IR. Briefly, this qualitative method requires the identification of an IR wavelength (or a Raman shift for the work reported here) that uniquely responds to changes in the concentration of the solute in solution. Slow heating of a suspension of the solute in a saturated solution will allow measurement of the peak intensity at the desired wavelength corresponding to saturation at any given temperature. Subsequent monitoring of the batch at the same wavelength (or Raman shift) will give the peak intensity corresponding to the actual dissolved concentration. As the peak intensities for both the saturated and supersaturated solutions at any given point are measured at the same temperature, then the difference between the two intensities is proportional to the supersaturation at that temperature. Because the supersaturation (reported in terms of the difference in peak height) is reported at a given temperature, the effect of temperature on the saturated and supersaturated values is negligible. Here, this method is extended to Raman spectroscopy to provide a simple univariate method for monitoring the supersaturation of the solution in situ. The feasibility of the method for in situ qualitative monitoring of the transformation process of different polymorphs is also investigated. It is shown that Raman spectroscopy can be successfully used to monitor in situ the supersaturation of a solution and the transformation of polymorphs after carefully choosing appropriate peaks that can represent the liquid phase mannitol and its solid phase, respectively.

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2. Experiments

2.1. Materials. β form mannitol (1,2,3,4,5,6-hexanehexol, 99%) was supplied by Sigma Aldrich Co. Its mass fraction purity is greater than 99%. The α form was crystallized by fast cooling of a saturated solution of β form mannitol in mixed solvents of water (30 wt %) and ethanol (70 wt %). The solution was saturated at 50 °C and then put into freezer (−5 °C) without being mixed. After 3 days, the suspension was taken out and filtered. The cake was dried under atmospheric conditions at ∼45 °C for 24 h. The δ form was prepared by a new antisolvent crystallization method; 100 mL of a saturated solution of mannitol in water (20 °C) was slowly added to 230 mL of pure ethanol at −5 °C. After addition of the saturated solution, the resulting suspension was filtered and washed using pure cold ethanol. The resultant cake was dried in vacuo at 45 °C for 24 h. Analytical-grade organic solvents (ethanol and acetone), with a mass fraction purity of >99.5%, were purchased from Sigma Aldrich Co. Deionized water was used throughout.

2.2. Equipment. A Kaiser Raman RXN2 system was used to measure the Raman spectra of different samples. The RXN2 analyzer utilizes fiber-coupled probe optic technology for in situ monitoring. This system was equipped with both an MR probe head and a PhAT probe head for direct insertion or noncontact sampling. This system contains a Kaiser Invictus laser emitting deep red and nearly invisible (785 nm) emission of 450 mW. The spectral range of this system is from 100 to 1890 cm^{-1}, and the spectral resolution is 5 cm^{-1} on average. The iC Raman software (Mettler-Toledo) is used in combination with this system. This software is used for instrument configuration, data acquisition, and data analysis. In this paper, the MR probe head with immersion optics was used to monitor in situ the supersaturation and polymorph transformation of mannitol. The PhAT probe head with noncontact optics was used to measure the powder Raman spectra of different samples off-line. An EasyMax automated 100 mL agitated reactor (Mettler-Toledo) was used for all the transformation experiments and dissolving and cooling crystallization experiments.

To confirm the identity of the different polymorphs of mannitol, X-ray powder diffraction patterns (XRD) of three polymorphs were obtained using a Rigaku MiniflexPlus II powder diffractometer with Cu Kα radiation at a voltage of 40 kV and a current of 20 mA. We prepared samples by placing powders on a low-background silicon powder mount and scanning them from 5 to 40° 2θ at a rate of 0.05°/s. The step size was set to 0.05° 2θ.

Differential scanning calorimetry (DSC) measurements were taken using a Mettler-Toledo 821e series DSC thermal analysis system. Mannitol samples (3−5 mg) were sealed into aluminum DSC pans with vented lids that were placed in sample cells under a dry nitrogen flow. Samples were scanned from 25 to 200 °C at a heating rate of 5 °C/min.

3. Results and Discussion

3.1. Identification of Polymorphs Using Raman Spectroscopy. The XRD patterns of the three polymorphs are shown in Figure 1. These compare very well to XRD patterns of

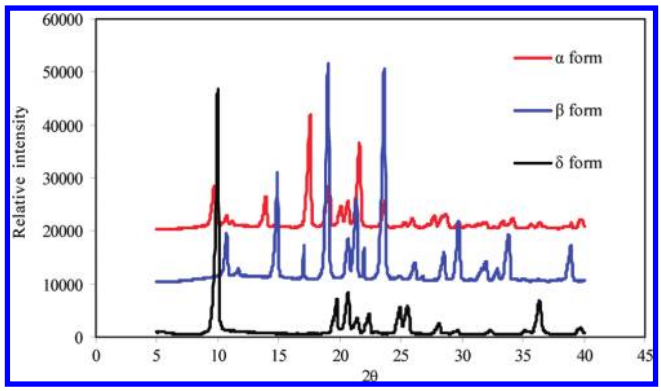

Figure 1. **XRD patterns of mannitol prepared by different methods described in this paper.**

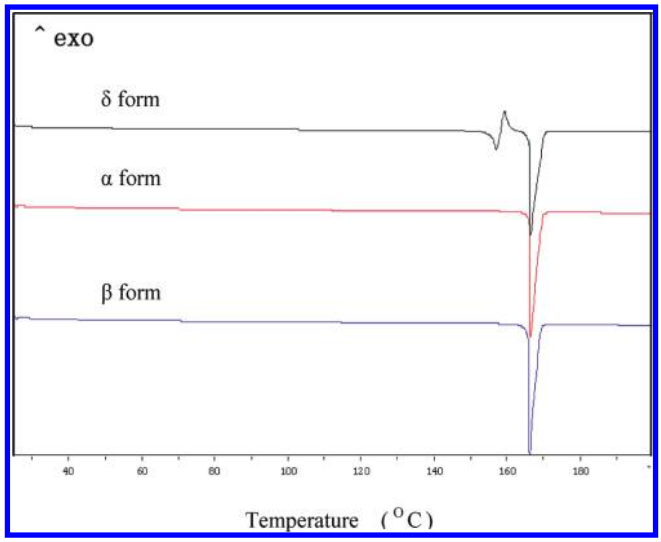

Figure 2. **DSC curves of mannitol prepared by different methods described in this paper.**

different polymorphs published in the literature.[24] The corresponding DSC curves are shown in Figure 2. From the DSC curves of the α, β, and δ forms, the α form is seen to melt at ~166 °C and the β form at 166.5 °C, indicating small energetic differences between these two forms. This result is consistent with the literature. The δ form has one exothermic peak at 159.2 °C and two endothermic peaks at 156.8 and 166.2 °C. It was suggested that the first endothermic peak is the melting point of the δ form. When the temperature increases to ~159.2 °C, the δ form begins to transform into a stable β form (transform enthalpy of 3.67 J/g). After the δ form totally transforms into the β form, the temperature continues to increase to the melting point of the β form (~166.5 °C). From the melting point of these three forms, the order of thermodynamic stability is as follows: $\beta > \alpha > \delta$.

After identifying these three different polymorphs, we recorded their Raman spectra. The results are shown in Figure 3. From Figure 3, many key differences between the spectra are apparent. For example, in the spectral range of 1100−1200 cm^{-1}, the β form has two main characteristic peaks at 1119 and 1134 cm^{-1}, the α form has only one main peak at 1130 cm^{-1}, and the δ form has one peak at 1147 cm^{-1}. In the spectral range of 820−910 cm^{-1}, the β form has only one Raman peak at 876 cm^{-1} while the α and δ forms have an additional peak at 887 cm^{-1}. Using these differences, Raman spectroscopy can be used to identify different polymorphs of mannitol.

3.2. In Situ Monitoring of Supersaturation. To monitor the supersaturation of crystallization processes, the solubility of the solute in solution must be known, in addition to the dissolved solute concentration, at any point in the batch. It has been shown[23] that the supersaturation in a crystallization process can be tracked using ATR-FTIR in a univariate, calibration-free method. This approach can also be extended to Raman spectroscopy. By monitoring the solubility and concentration in terms of relative Raman intensity, we can directly track the supersaturation of a cooling crystallization process.

To monitor the supersaturation of the mannitol crystallization process, peaks that are characteristic of mannitol in solution need to be determined. The Raman spectra of water, pure solid-state β mannitol, a clear saturated aqueous mannitol solution, and a suspension of mannitol in water are shown in Figure 4. These spectra were obtained in situ using the immersion MR probe with the exception of the solid β form mannitol for which the PhAT probe was used. Because the MR probe uses a sapphire window to protect the lens, the Raman spectra of sapphire were also recorded when using the MR probe. In Figure 4, the Raman peaks at 378, 418, 433, 451, 578, and 751 cm^{-1} are characteristic peaks of sapphire. From the data in Figure 4, we can conclude that the Raman peaks at 332 and 1022 cm^{-1} are characteristic peaks of mannitol in solution since they do not appear in either the pure water or pure solid-state β mannitol Raman spectra. This can clearly be seen in Figures 5 and 6 which show the spectra around 1022 and 332 cm^{-1}, respectively. We can also conclude that the Raman peaks at 369 and 1037 cm^{-1} for solid-state mannitol shift to 332 and 1022 cm^{-1}, respectively, for mannitol in solution.

Therefore, there are characteristic peaks at 369 and 1037 cm^{-1} for pure solid-state mannitol and characteristic peaks at 332 and 1022 cm^{-1} for a clear saturated solution of mannitol. For a suspension of mannitol in water, there are characteristic peaks not only at 369 and 1037 cm^{-1} but also at 332 and 1022 cm^{-1} because both solid-state and dissolved mannitol exist in the suspension. From Figures 5 and 6, it can also be seen that although either the peak at 331 cm^{-1} or the peak at 1022 cm^{-1} can be chosen to monitor the dissolved concentration, the former is better because it is more separated from the nearby solid-state peak. Therefore, the Raman peak at 332 cm^{-1} was chosen to track the dissolved mannitol in solution.

To reduce the effect of the exposure time, the temperature, the laser power of Raman equipment, and the mixing state of the vessel on the accuracy of the data, the Raman peak at 578 cm^{-1} for sapphire was chosen as the reference peak as its intensity is comparable to the intensity of mannitol in solution. The relative Raman intensity of mannitol at 332 cm^{-1} with respect to the reference peak was used to track in situ the dissolved concentration in solution during a cooling crystallization process. The mannitol solubility in terms of relative Raman intensity was obtained by slowly heating a suspension of mannitol in water.[23] The heating rate (0.07 K/min) was sufficiently slow to ensure the solubility data are accurate.[23] After complete dissolution, the clear solution was cooled at

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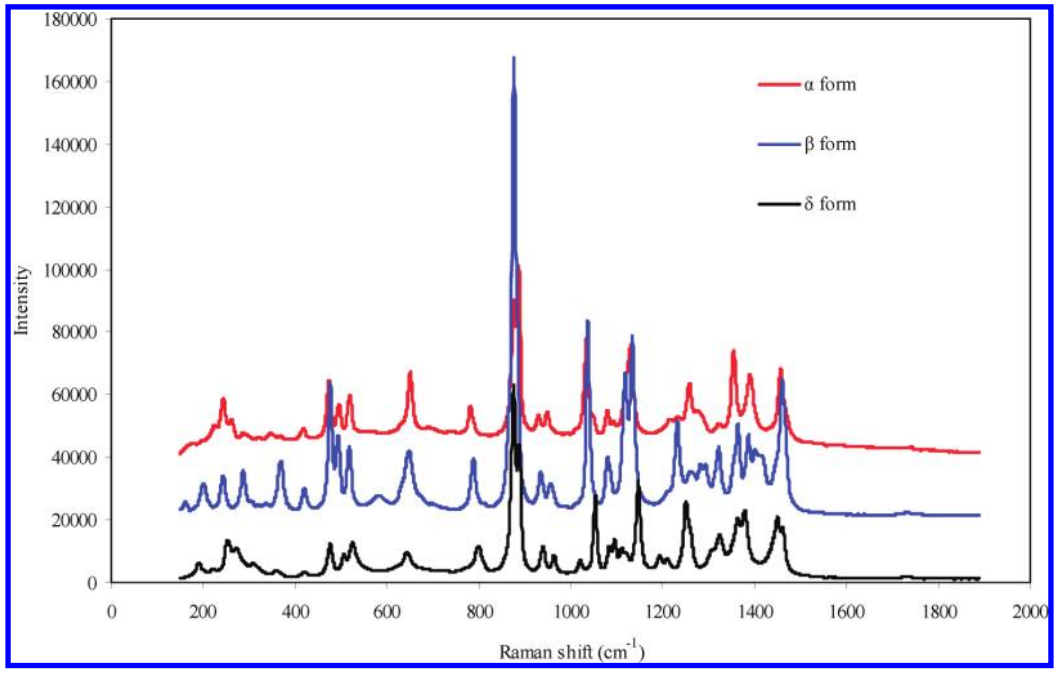

Figure 3. **Raman spectra of different mannitol polymorphs.**

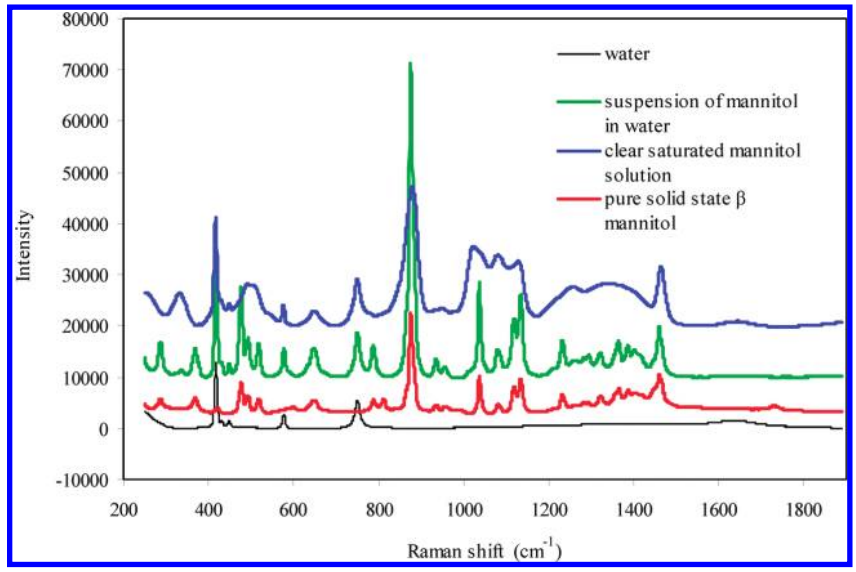

Figure 4. **Raman spectra of different samples.**

different rates. The changes in concentration in terms of Raman intensity during the cooling process were recorded. The experimental results are shown in Figure 7. Both seeded and unseeded experiments were performed to evaluate the effect of operating parameters on the supersaturation. From the solubility line in Figure 7, it can be seen that the relative Raman intensity increased as the mannitol dissolved, but after dissolution, the relative Raman intensity exhibited little or no dependence on temperature.

As expected, the level of supersaturation at a given temperature was higher for the fast cooling rates of 0.5 °C/min (both seeded and unseeded) than for the slow cooling rate of 0.2 °C/min. From Figure 7, it is also clear that the unseeded batch exhibits a very wide metastable zone (>30 °C).

To test the feasibility of this method in other solvents, the cooling crystallization was repeated in 20 wt % ethanol in water. The same dissolved mannitol peak at 332 cm^{-1} and reference

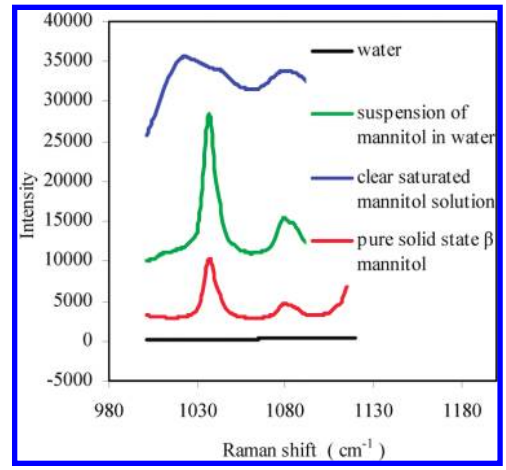

Figure 5. **Raman spectra of different samples in the Raman range of 1000−1120 cm^{-1}.**

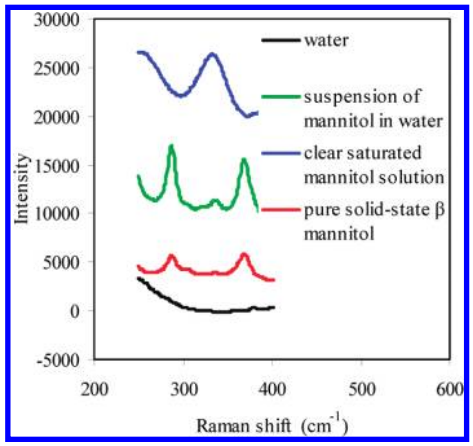

Figure 6. **Raman spectra of different samples in the Raman range of 250−400 cm^{-1}.**

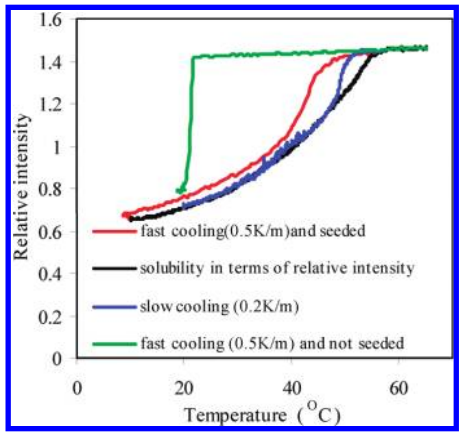

Figure 7. **Dissolved concentration of mannitol in water in terms of relative Raman intensity.**

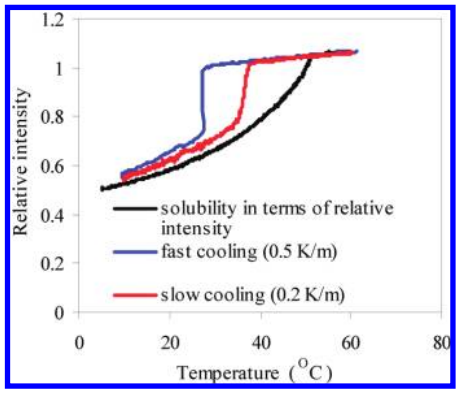

Figure 8. **Dissolved concentration of mannitol in 20 wt % ethanol in water in terms of relative Raman intensity.**

peak of sapphire at 578 cm^{-1} were used. Initially, the solubility in terms of the relative Raman peak was determined by slowly heating the mannitol suspension as described above. The clear solution was then cooled under a variety of conditions. The changes in concentration in terms of Raman intensity during these cooling processes are presented in Figure 8.

From Figure 8, it can be seen the method reliably tracks the dissolved concentration through the batch, indicating, as expected, an increase in the metastable zone width with an increase in the cooling rate. There is a slight dependence of the relative intensity on temperature in this case, but because the effect on the solubility and dissolved concentrations will be the same at any given temperature, this effect is not significant.[23]

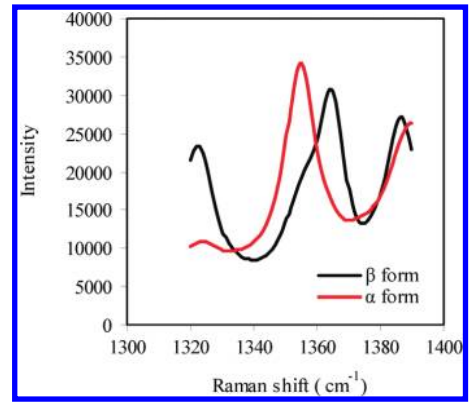

Figure 9. **Raman spectra of α and *β* form mannitol in the spectral range of 1320−1390 cm^{-1}.**

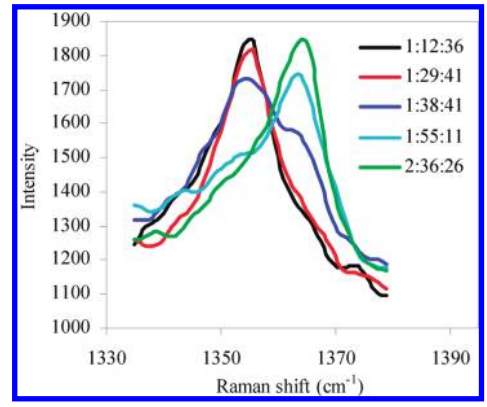

Figure 10. **Change in the Raman spectra in the range of 1330−1390 cm^{-1} in the transformation experiments.**

3.3. In Situ Monitoring of the Transformation from the α Form to the *β* Form. As discussed above, the Raman spectra of the three polymorphs of mannitol exhibit some distinct differences. Using these differences, Raman spectroscopy can potentially be used to monitor the polymorphic transformation in situ. To achieve this, appropriate peaks that can represent different polymorphs must be chosen. Considering, for example, the transformation from the α form to the *β* form, the Raman spectra of both forms in the spectral range of 1320−1390 cm^{-1} are shown in Figure 9. It can be seen that the α form has a characteristic peak at 1355 cm^{-1}, while this peak shifts from 1355 to 1364 cm^{-1} for the *β* form. These two peaks are almost totally detached and have a similar Raman intensity. Therefore, these two peaks can be chosen to represent the α and *β* forms, respectively, and monitor the transformation from the α form to the *β* form. In the transformation experiments, the metastable α form was added to a saturated solution of *β* mannitol in water. The changes in the Raman spectra during the transformation are shown in Figure 10. The corresponding changes in the relative intensity of the Raman peak at 1355 cm^{-1} for the α form and at 1364 cm^{-1} for the *β* form are shown in Figure 11. To evaluate the relative intensity, the peak height to a two-point baseline is used for both polymorphs. From Figures 10 and 11, it can be seen that the magnitude of the Raman peak of the α form increases suddenly after the α form is added to the solution. As the α form begins to transform into the *β* form, the magnitude of the Raman peak of the α form gradually decreases while the magnitude of the peak of the *β* form increases. After approximately 50 min, the α form has totally

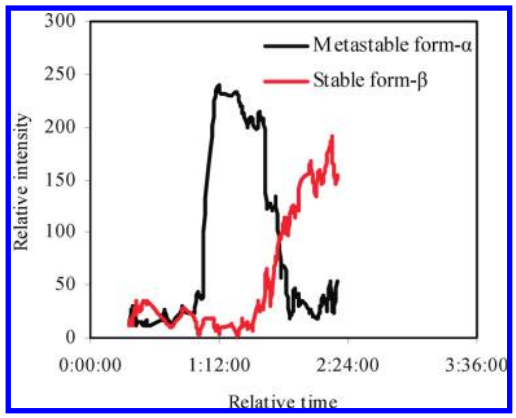

Figure 11. **Relative intensity change trend of the peak at 1355 cm^{-1} for the α form and at 1364 cm^{-1} for the β form in the transformation experiments.**

transformed into the β form. Previously published work[16] using FBRM suggested that the complete transformation of the least stable δ form to the β form took >2 h at a lower temperature (5 °C).

4. Conclusions

Raman spectroscopy was used to monitor the cooling crystallization of mannitol. The Kaiser Raman spectroscopy system was used to record the Raman spectra of three different polymorphs of mannitol. It was found not only that the different Raman spectra can be used to identify the different polymorphs but also that the differences provide the basis for a novel method for monitoring the supersaturation during cooling crystallization processes. The calibration-free univariate method was successfully used to monitor in situ the supersaturation of mannitol in water and in aqueous ethanol solutions. The use of Raman spectroscopy to monitor the polymorphic transformation of mannitol was also successfully demonstrated for the transformation of the metastable α form to the stable β form. As with the recently published calibration-free method for in situ monitoring using IR, this approach provides a robust basis for the application of Raman spectroscopy to crystallization monitoring and control.

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