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In Situ Monitoring of Polymorphic Transitions

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Abstract:

An investigation of the feasibility of in situ monitoring of polymorphic transitions is presented. Focused beam reflectance measurement (FBRM) and particle vision and measurement (PVM) are used to study changes in crystal population and morphology during the transition of the δ-polymorph of D-mannitol to the thermodynamically stable β-form. The transformation is confirmed via off-line X-ray powder diffraction (XRPD). Nucleation and growth of β-form D-mannitol was detected and measured by FBRM/PVM when δ-form was added to a saturated solution of β-form D-mannitol. Raman spectroscopy is also introduced as an invaluable in situ identification technique for polymorphic crystallizations. The effect of particle size on the intensity of the Raman spectrum is investigated using slurries of D-mannitol and sucrose in toluene. Raman spectroscopy successfully distinguished a mixture of sucrose and D-mannitol in toluene, and a linear correlation between Raman peak intensity and solids content was observed for both. However, this linear relationship was not observed when the particle size distribution of sucrose was changed. It is clear that, especially in the case of many polymorphic conversions, information on the particle size distribution, along with additional structurally sensitive information such as Raman spectra, is essential in gaining a true understanding of the behaviour of the system.

Introduction

Crystallization from solution is well established as an essential separation and purification technique throughout the pharmaceutical, chemical and food industries. However, its extensive application has led to a realisation that there are many aspects of its behaviour that are still not fully understood, particularly with respect to polymorphism.

A polymorph is defined as a substance or compound that has the ability to crystallize into different, yet chemically identical, crystalline forms.[1] Although it has been known to exist since the discovery of the three polymorphic forms of calcium carbonate in the late 18th century,[2] the true significance of polymorphism, especially to the pharmaceutical industry, has only been realized in the past number of years through some relatively high profile cases. In particular, the appearance, in early 1998, of a thermodynamically more stable form of Ritonavir[3] (Abbott Laboratories' protease inhibitor for the treatment of HIV), with different dissolution and absorption characteristics, resulted in manufacturing lots with 50% failure rates, before eventually leading to a complete halt on production until a modified process was found. Such occurrences have led to an increased awareness of the importance of early-stage polymorph identification and characterization, resulting in significant industrial and academic investment in the area. For example, the Crystallographic Cambridge Database recorded 250,000 known polymorphs in 2002 in comparison to 1500 in 1987.[4] This increase in polymorph research has also given rise to improvements in, and modifications of, available analytical instrumentation for their detection and characterization, typically identified as either off-line or in-line analytics.

It is possible to subdivide off-line analytical instrumentation into four techniques:[5,6] crystallographic, spectroscopic, microscopic, and thermal. Crystallographic techniques comprise single crystal X-ray crystallography and X-ray powder diffraction (XRPD). Spectroscopic techniques include infrared spectroscopy (IR), Raman spectroscopy, and solid-state nuclear magnetic resonance (SSNMR). Microscopic analysis involves examining the optical properties of the crystal polymorphs by techniques such as light (LM) and electron microscopy (EM). Finally, some commonly used thermal techniques are differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) for the identification of pseudopolymorphs such as hydrates or solvates. Of the techniques mentioned above, XRPD, while one of the oldest, remains one of the most important and widely used.

A common trait of all of these off-line techniques is that sampling is required, and in many instances, the samples must be manipulated before the final measurement can be taken. Sampling in general, and especially for particulate systems, can account for a significant proportion of the error associated with a measurement, primarily through nonrepresentative sampling and postsampling changes in sample

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(1) Mullin, J. W. *Crystallization*, 3rd ed.; Butterworth-Heinemann: Oxford, 1993.

(2) Blagden, N.; Davey, R. *Chem.Br.* **1999**, *March edition*, 44.

(3) Chemburkar, S. R.; Bauer, J.; Deming, K.; Spiwek, H.; Patel, K.; Morris, J.; Henry, R.; Spanton, S.; Dziki, W.; Porter, W.; Quick, J.; Bauer, P.; Donaubauer, J.; Narayanan, B. A.; Soldani, M.; Riley, D.; McFarland, K. *Org. Process Res. Dev.* **2002**, *4*, 413.

(4) Cambridge Crystallographic Data Centre: http://www.ccdc.cam.ac.uk/.

(5) Yu, L.; Reutzel, S. M.; Stephenson, G. A. *PSTT* **1998**, *1*, 3, 118.

(6) Threlfall, T. *Analyst* **1995**, *120*, 2435.

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characteristics. This sample manipulation may have drastic effects on polymorph analysis, with metastable samples transforming to stable forms, giving erroneous results. For example, in the case of XRPD, this polymophic transformation may be catalyzed by crushing of the crystalline sample to a fine powder.[7] For IR spectroscopy, mixing of the sample with potassium bromide can have the same outcome.[6]

The dynamics of polymorphic crystallizations are often important to characterize, particularly when there is a transition between polymorphs. Therefore, sampling can be avoided completely if an in situ technique can be used for polymorph identification. In-line analytical probes such as Raman spectroscopy have great potential in this area. Raman spectroscopy is based on the detection of changes in the energy spectrum of incident radiation (so-called Raman shift) associated with inelastic scattering of incident photons. While few photons are subject to such inelastic collisions ($\sim$1 in 10^7), this shift, when detected, is indicative of changes in the molecular orientation within the crystal structure.

Advances in the instrumentation used for Raman spectroscopy, especially in lasers and detectors, have greatly facilitated its increasingly wider application since its discovery in 1928.[8] Raman spectroscopy is now widely used in the chemical industry and, more recently, in the pharmaceutical industry, for monitoring such diverse systems as Grignard reactions and catalytic hydrogenations.[9] Another important pharmaceutical application is the detection of polymorphs. Different polymorphs will have different intermolecular bonding, producing differences in electron distribution in the molecular and lattice environment, resulting in unique Raman spectra for different polymorphs.

Other in situ tools widely used for particle analysis in crystallization and, more recently, for monitoring polymorphic transitions are focused beam reflectance measurement (FBRM) and particle vision and measurement (PVM). FBRM is a probe-based high solids concentration particle characterization tool. Briefly, 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.[10] Because many thousands of these measurements are taken every second, a statistically robust measure is rapidly obtained. Of particular importance is the fact that the measurement is made within the actual process environment. The data are typically represented as a chord length distribution (CLD) between 1 and 1000 μm. The PVM is a high-resolution video microscope,[10] which is typically used for in-process high-resolution imaging of particles within the process environment. FBRM and PVM are well-established in situ techniques for characterizing and quantifying changes in crystallization processes.[11−13] Applying these tools to polymorphic crystallizations is of particular use in developing an understanding of the process dynamics. For instance, a polymorphic transformation may be associated with the dissolution of the metastable form of a crystal and the nucleation and subsequent growth of the stable form,[14] resulting in a change in the particle number and dimension which can be successfully monitored with FBRM. A polymorph transformation is often accompanied by a change in the crystal habit (although not always), which can cause dramatic changes to the shape of the chord length distribution measured by the FBRM. Habit shifts can also be successfully tracked using the PVM, and quantified using image analysis. Additionally, since polymorphs often nucleate at different temperatures, FBRM can isolate these additional nucleation events. Therefore, if these probes are used in conjunction with the in situ Raman spectrometer, a significant insight into the fundamentals and dynamics of polymorphic transitions can be gained.

This contribution aims to demonstrate that use of some of these in situ analytical techniques compliments the more traditional off-line instruments such as XRPD. The molecule chosen for investigation is D-mannitol, an acyclic sugar that is extensively used in pharmaceutical formulations as an excipient and diluent for solids and liquids.[7] It has several known polymorphic forms[15] with the β-form the most thermodynamically stable. The transformation in solution from the metastable δ-form to the stable β-form was monitored in situ using FBRM and PVM. The crystalline forms were identified and characterized off-line using XRPD. Additionally, some of the key variables influencing Raman spectra were examined, and suggestions made on how to extract quantitative information correctly from Raman spectra in slurries.

Materials and Methods

The D-mannitol (Sigma-Aldrich) was obtained in the thermodynamically stable β-form. It was recrystallized from deionized water before use. The metastable δ-form was crystallized by rapid cooling of a saturated solution of D-mannitol in dilute aqueous ethanol to below 0 °C. The solution was filtered approximately 30 min after the start of nucleation and dried overnight in a vacuum oven at 40 °C under 800 mbar vacuum. The δ-form is relatively stable in air at room temperature, and no transformation to the β-form was observed by XRPD. Both crystalline forms were also subjected to DSC and TGA to check for any hydrates or solvates. Commercial grade sucrose was also used for some of the Raman experimentation.

XRPD patterns were obtained on a Huber G642 Guinier diffractometer using monochromatic Cu K_α radiation, in the range $2\theta = 5-40°$. Huber G60 (v5.13) control software was used for data acquisition. The samples were prepared by crushing with a pestle and mortar and then applying to a clear film using petroleum jelly. Standard silicon powder was also added to the samples to observe and correct slight shifts in peaks that may occur.

(7) Yoshinari, T.; Forbes, R. T.; York, P.; Kawashikma, Y. *Int. J. Pharm.* **2002**, *247*, 69.
(8) Raman, C. V.; Krishna, K. *Nature* **1928**, *501*, 3048.
(9) Pais da Silva, M. I.; Nery, M. P.; Tellez S.; C. A. *Mater. Lett.* **2000**, *45*, 197.
(10) Barrett, P. In-situ Monitoring of Crystallization Processes, PhD Thesis, University College Dublin, 2002.
(11) Andrews, A.; Osifchin, R. American Institute of Chemical Engineers Annual Meeting, Reno, November 4−9, 2001.
(12) Barrett, P.; Glennon, B. *Part. Part. Syst. Charact.* **1999**, *16*, 207.
(13) Alvarez, M.; Brown, M. American Institute of Chemical Engineers Annual Meeting, Reno, November 4−9, 2001.
(14) Cardew, P. T.; Davey, R. J. *Proc. R. Soc. London* **1985**, *A398*, 415.
(15) Burger, A.; Henck, J.-O.; Hetz, S.; Rollinger, J. M.; Weissnicht, A. A.; Stottner, H. *J. Pharm. Sci.* **2000**, *89*, 457.

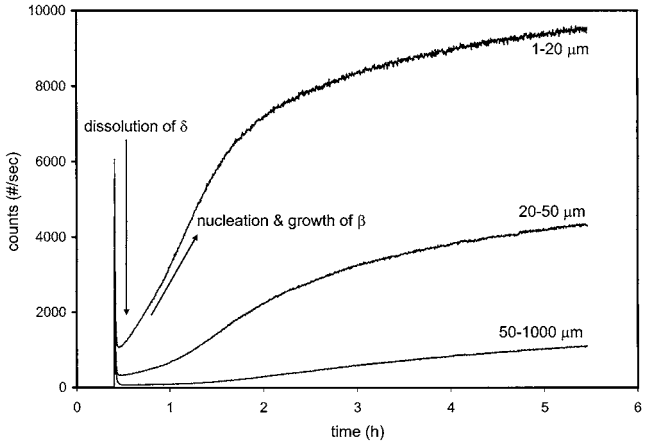

Figure 1. **FBRM population statistics for the δ-form transition.**

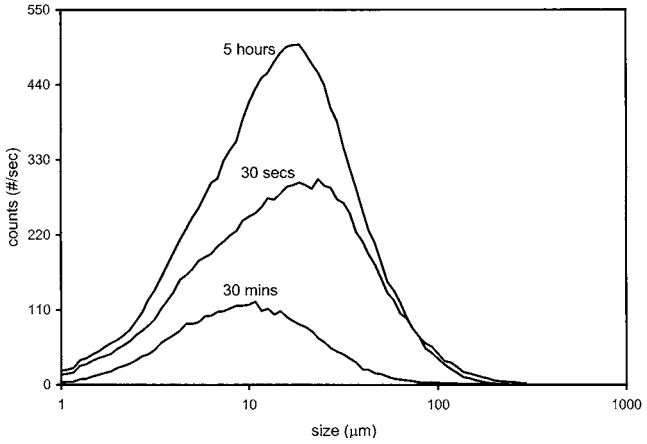

Figure 2. **FBRM chord length distributions for δ-form transition.**

Both the FBRM and PVM particle characterization tools were manufactured by Lasentec Inc., U.S.A. The FBRM probe (model M400LF) has a measurement range of 1−1000 μm. The probe measurement duration was set at 15 s. The PVM probe (model 800L) was operated with an image update rate of 3 images per second.

Raman spectroscopy was mostly performed using a Mettler-Toledo Raman 4000 model probe with a typical laser power of 200 mW at 785 nm. Some experiments were performed using a combined FBRM/Raman probe (Lasentec) with the capabilities of both techniques combined into a single probe.

The DSC was a Mettler-Toledo DSC 822e, and the TGA was performed on a Mettler-Toledo TGA/SDTA 851e.

Experimental Results and Discussion

Tracking a Polymorphic Transition using FBRM and PVM. deionized water (150 g) was cooled to 5 °C in a jacketed glass beaker. Commercial D-mannitol (β-form) was added in excess of its known solubility and stirred constantly until steady state was reached, as indicated by the FBRM (i.e. no change in the particle system chord length distribution). The solution was filtered and the saturated filtrate recooled to the working temperature. Crystallized δ-form (4 g) (confirmed using XRPD) was then added and monitored in-line using the FBRM and PVM for approximately 6 h. Figure 1 summarizes the changes in the measured chord length data. The data are divided into three population ranges: fine (1−20 μm), medium (20−50 μm), and coarse (50−1000 μm) counts. On addition of the δ-form to the saturated solution, a steep and sudden rise is seen in all population ranges. A typical chord length distribution for the δ-form particles measured 30 s after addition is shown in Figure 2. However, because of the higher solubility of the δ-form compared to that of the β-form, it begins to dissolve rapidly, indicated by the subsequent rapid decrease in the FBRM counts. As the δ-form dissolves, it steadily increases the solution supersaturation with respect to the β-form, inducing an associated increase in the β-form crystal population. This increase is likely to occur due to growth in β-form crystals formed by a solid-phase water-mediated transition of the δ-form to the more stable β-form, in addition to the growth of spontaneously nucleated stable β-form crystals. The growth of these β-form crystals can be clearly seen in Figure 1 through an initially rapid increase in counts as the supersaturation resulting from dissolution of δ-form crystals is consumed. The counts slowly reach an equilibrium level as the remaining supersaturation is gradually reduced.

The change in the dynamics of the particle system, due to the polymorphic transition, is also emphasized by the FBRM chord length distributions in Figure 2. The metastable δ-form which is initially added (CLD at 30 s is shown) rapidly dissolves and is followed by subsequent nucleation and growth of the stable β-form. The increase in counts from 30 min to 5 h is indicative of a population growth due to nucleation, while the shift in the mode of the distribution is due to crystal growth. The crystal habit changes are also clear from the PVM images, taken at regular intervals during the transition (see Figure 3).

After 6 h the solution was filtered and the cake dried before confirming the polymorphic form using XRPD (see Figure 4). Samples taken at the beginning (Figure 4a) and the end (Figure 4b) of the experiment show significant differences in the X-ray patterns, consistent with a change in crystal structure. The XRPD patterns also agreed well with other literature results.[7] Single samples were also examined with DSC and TGA. The DSC curves in Figure 5 support the XRPD patterns, showing differences in the crystalline structures of both forms. The δ-form shows a small endothermic and exothermic peak before the large endothermic peak beginning at 167 °C. The β-form has only one large endothermic peak, also beginning at 167 °C. Again, these results are consistent with literature data.[15] The TGA showed no change in mass for either sample, eliminating the possibility of hydrate or solvate formation.

As a control to this experiment, the same procedure was repeated, but with recrystallized β-form added to the saturated solution rather than the δ-form. The FBRM data in Figure 6 show no change in the particle system, a trend which continued for up to 48 h (data not shown). These results are consistent with the assertion that no polymorphic transition occurs, as was indicated with the associated XRPD data. Other researchers have used this concept to compliment their traditional polymorphic screening work, to determine the

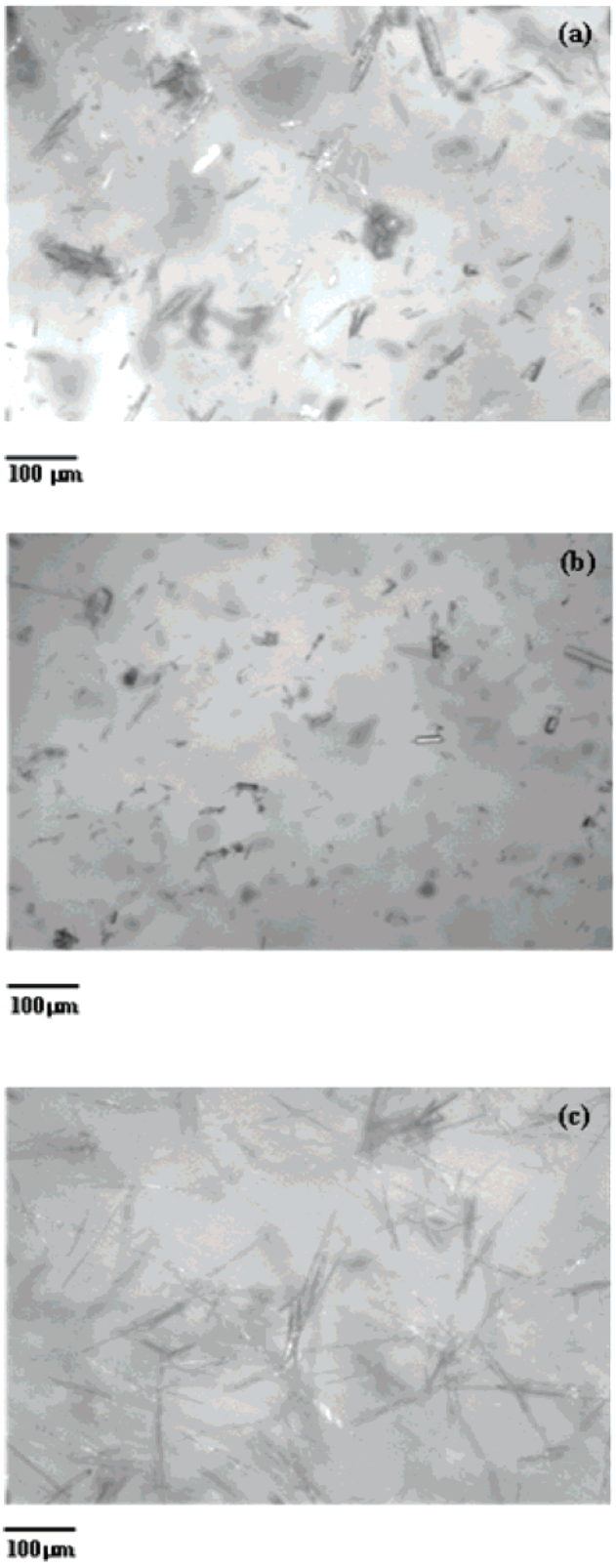

Figure 3. **PVM images at (a) 30 s, (b) 30 min, and (c) 5 h.**

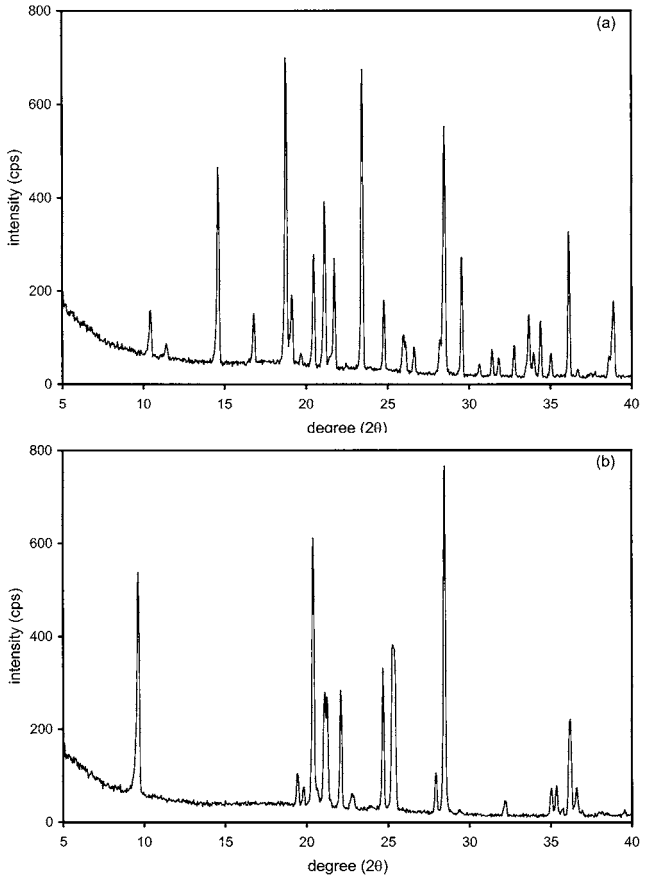

Figure 4. **XRPD of (a) β-form and (b) δ-form.**

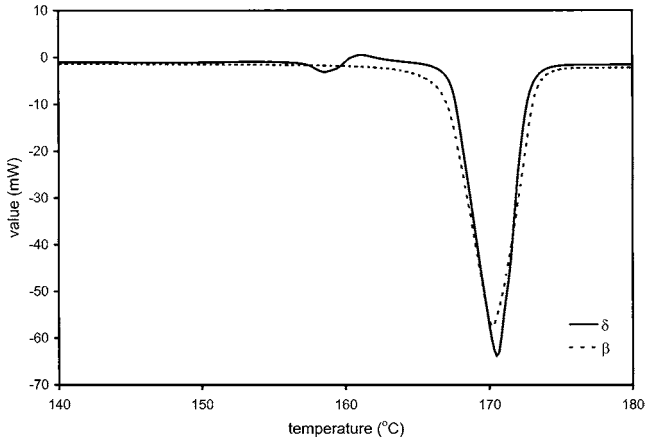

Figure 5. **DSC curves of β-form (- - -) and δ-form (—).**

presence of a polymorphic transition along with the associated kinetics.[16]

The sensitivity of FBRM and PVM to changes in particle size, shape, and number is central to their use as in situ techniques for the monitoring of polymorphic transitions in solution. However, use in conjunction with structural identification techniques such as XRPD is advised. Through a combination of these several techniques, not only is it feasible to identify a crystal structure change, but it is also possible to monitor the effect on the behaviour of the particles, ultimately leading to improved control of the crystallization process.

The experiments discussed here focus on only one of the parameters affecting the rate of a polymorph transition, namely, the solvent composition. Other parameters that influence polymorphic transitions include particle dimension, operating temperature, mixing regime, solvent selection, and

(16) Karpinski, P. 4th International Symposium on Polymorphism and Crystallization, Chester, UK, April 28−29, 2003.

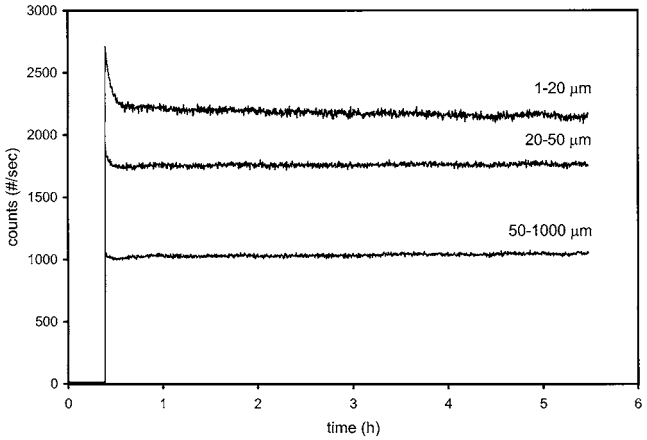

Figure 6. **FBRM population statistics for the β-form.**

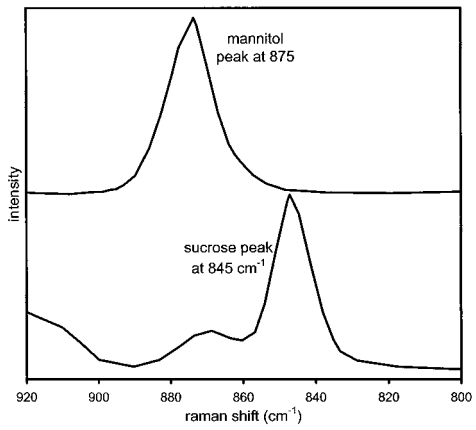

Figure 7. **Raman spectra for sucrose and D-mannitol in toluene.**

supersaturation. For example, by examining the transition at various temperatures one could determine if the polymorph relationship is monotropic or enantiotropic over the working temperature range. Understanding the effects these parameters have on the polymorph transition rate can aid optimization and improve process control.

Effect of Particle Dimension on Raman Spectra. Raman spectroscopy has been widely used as a tool for the in-process characterization of polymorphic crystallizations. The traditional approach to using Raman spectra in a quantitative sense has been to build a "calibration" curve relating slurry samples of known polymorph composition to the intensity of the measured Raman spectra.[17]

To demonstrate this concept, several studies were conducted on D-mannitol (β-form) and sucrose. Both of these solutes have distinct nonoverlapping peaks which are well separated and do not require deconvolution (Figure 7). These materials were chosen to allow a full characterization of some of the variables affecting the Raman spectra, in the absence of a polymorphic transition. Several samples of sucrose and D-mannitol (of varying crystal sizes and solids content) were prepared in toluene, in which both materials have limited solubility.

(17) Wang, F.; Wachter, J. A.; Antosz, F. J.; Berglund, K. A. *Org. Process Res. Dev.* **2000**, *4*, 391.

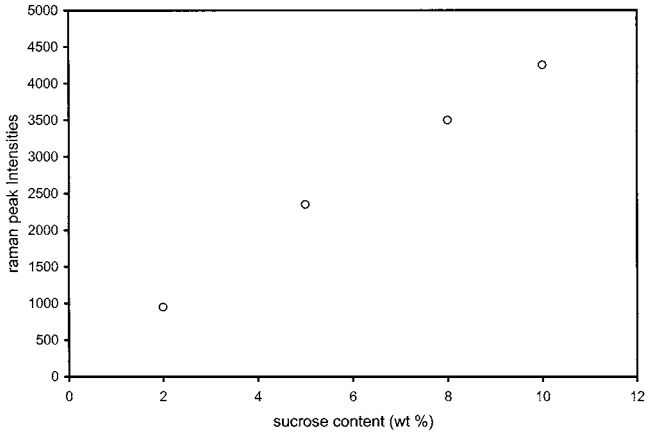

Figure 8. **Raman peak intensity at 845 cm^{-1} as a function of the sucrose content.**

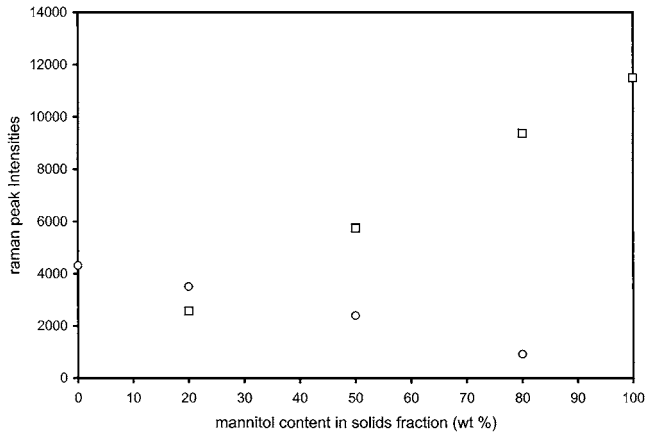

Figure 9. **Peak intensities for sucrose (○) and D-mannitol (□) as a function of D-mannitol content in a slurry of equally sized particles of sucrose and D-mannitol.**

When samples of sucrose of nominally the same crystal size were measured, the intensities of these peaks were found to be linearly dependent upon the amount of material present (Figure 8). A series of mixed samples, containing the same overall solids content, but differing amounts of D-mannitol, were also analyzed. The sucrose and D-mannitol crystals used were of the same nominal size. The peak intensities measured for both solutes are reported in Figure 9. As is clear, the linear relationship between the peak intensity and solids content for an individual solute is still maintained. This final set of experiments was repeated with larger sucrose crystals, but with the same overall solids content. The results of these experiments are shown in Figure 10. As may be expected, the peak intensities for the sucrose differed from those reported in Figure 9. However, despite the size and number of D-mannitol crystals remaining the same, the reported peak intensities for D-mannitol were appreciably higher. The linear nature of the relationship for D-mannitol previously noted is also not apparent here. This has significant ramifications for polymorphic crystallization work, as crystallization processes are dynamic, with size and number of the crystals changing over time. In the case of a particle system with two components, the Raman signal of one component will be affected by dimensional changes in the other. Studies based upon Raman spectroscopy alone are not quantitative when

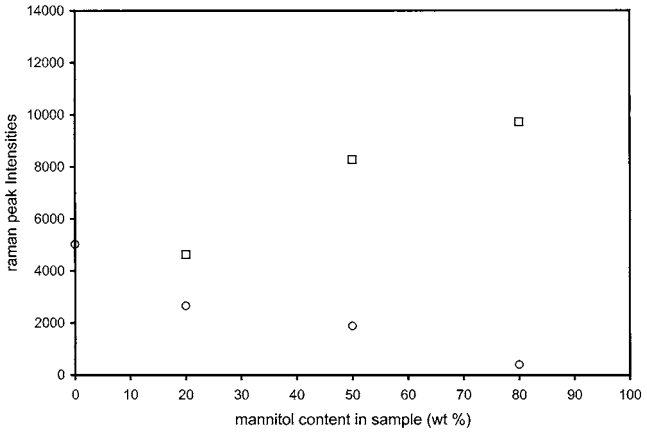

Figure 10. **Peak intensities for sucrose (○) and D-mannitol (□) as a function of D-mannitol content in a slurry of D-mannitol crystals and larger sucrose crystals.**

applied to dynamic particle systems. Recent experiments by other researchers have also shown this to be the case. Zhou et al.[18] at Merck examined two polymorphs by Raman and XRPD in a series of experiments in which the ratio and size of the two polymorphs were varied. It was found that the apparent ratio between the two polymorphs as determined by Raman could vary significantly when compared to XRPD. It was concluded that Raman may only be useful in a qualitative sense unless corrections for particle size effects were considered.

(18) Zhou, G.; Wang, J.; Ge, Z., Sun, Y. *Am. Pharm. Rev.* **2002**, *Winter*.

Conclusions

Experiments conducted with FBRM, PVM, and Raman spectroscopy have shown how these techniques can track changes in particle dimension, shape, solubility, and crystal structure. Nucleation and growth of β-form D-mannitol was detected and measured by FBRM/PVM when δ-form was added to a saturated solution of β-form D-mannitol. This $\delta-\beta$ transformation was corroborated by off-line XRPD and DSC. Raman spectroscopy successfully distinguished a mixture of sucrose and D-mannitol in toluene and a linear correlation between Raman peak intensity and solids content was observed for both. However, this linear relationship was not observed when the particle size distribution of sucrose was changed. Therefore, because particle systems are dynamic, especially in the case of many polymorphic conversions, information on the particle size distribution, along with additional structurally sensitive information such as Raman spectra, is essential in gaining a true understanding of the behaviour of the system.

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