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Application of in Situ FBRM and ATR-FTIR to the Monitoring of the Polymorphic Transformation of D-Mannitol

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Abstract:

The application of in situ FBRM and ATR-FTIR in monitoring a polymorphic transformation in a solution of D-mannitol is introduced. The isothermal growth of the stable β-form in a supersaturated solution is initially monitored. This β-form has a needlelike habit, and it is noted that the dominant FBRM chord length is a function of the width of these needles. This enables a better understanding of the change in the chord length distribution over time for the β-form growth. It also provides a means to successfully track the dynamics of the β-form during a polymorphic transformation from the metastable δ-form. Monitoring the change in the chord length distribution and, more importantly, the change in the crystal count data reveals a solution-mediated transformation which proceeds via the dissolution of the metastable δ-form and the subsequent nucleation and growth of the stable β- form. ATR-FTIR data also provides evidence to suggest such a transformation.

Introduction

The impact of polymorphism on the food, speciality chemical, and pharmaceutical industries cannot be underestimated. The ability of a single molecule to crystallize into various crystalline structures that share identical chemical properties, but different physical properties, can be either advantageous or disastrous. In such industries, where products are specified, not only by their chemical composition, but also by their performance, a change in the physical properties of a crystal such as solubility, density, hardness, morphology, and dissolution rate can potentially cause numerous problems.[1]

The unexpected appearance of a second polymorphic form of an active pharmaceutical ingredient used for the treatment of HIV, with substantially different dissolution and absorption characteristics, was one of the first high profile cases of the impact of polymorphism in the pharmaceutical industry.[2] Cases such as this highlighted the importance of controlling polymorphic crystallizations and fuelled extensive research into the phenomenon, especially in the pharmaceutical industry.

Polymorphs have been known to exist for over a century,[1] yet the successful identification and control of polymorphic compounds is still primarily based on empirical approaches, with polymorphic forms capable of disappearing and new ones appearing in unpredictable ways. The crystallization of polymorphs as currently understood is governed by Ostwald's Law of Stages,[3] which states that upon crystallization a system will initially adopt the crystal structure which leads to the smallest loss in free energy and that these crystals will subsequently transform stepwise to other more stable forms until the most stable form has been achieved. Therefore, the reason polymorphs disappear and new ones appear is that, for any given compound, only one polymorph is thermodynamically stable for a specific temperature and pressure range. This means that a metastable polymorph can always theoretically transform to its more stable structure.

The relationship between metastable polymorphs and their more stable counterparts can be either monotropic or enantiotropic. If two polymorphs are related monotropically, then the transformation from the metastable form to the stable form is irreversible for all temperatures and pressures, and once the stable polymorph has been formed, it cannot retransform to the metastable crystal structure. On the other hand if the polymorphs are related enantiotropically, the transformation is reversible. At temperatures above the transition point (where the free energies of both crystal forms are equal), one polymorph is the thermodynamically stable polymorph while at temperatures below the transition point the other polymorph becomes the thermodynamically stable form.

Transformation, however, is not necessarily predictable even when a system enters a condition that will theoretically allow it. Transformation can typically only be ensured if a more stable solid phase is already present.[4] This more stable phase can be introduced either through seeding or it can make its appearance through nucleation. There are two proposed mechanisms for polymorphic transformations, i.e., solid-state transitions (SST) or solution-mediated transformations (SMT). SST is thought to occur via the positional rearrangement of the ions or molecules in a metastable crystal structure to achieve a new, more stable structure.[5] These transitions take place without passing through liquid or vapour phases, and in general, the kinetics of such transitions are influenced by the environment (temperature, pressure, relative humidity, etc.) and the presence of crystalline defects, particle size and distribution, and impurities.[6] Solution-mediated transformation occurs in the presence of a solvent and is usually driven

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by the difference in solubility between the two forms. The mechanism of transformation typically involves three steps. First, there is dissolution of the metastable phase into the solution to reach and exceed the solubility of the stable form. This results in the nucleation of the stable form, which is then followed by the crystal growth of the new stable nuclei coupled with the continuous dissolution of the metastable crystals. Typically in solution-mediated transformations the nucleation or crystal growth of the stable phase is often the rate-determining step.[6] This provides a potential method for selective polymorph crystallization through the addition of additives, which may inhibit the nucleation or growth of one form thus promoting the crystallization of the other form. Kitamura et al.[7] discovered that the fraction of the stable A polymorph of L-histidine which crystallized from aqueous solution decreased with increasing ethanol concentration, until eventually a concentration was reached that preferentially crystallized the metastable B polymorph only. It was suggested that the crystallization behaviour was controlled mainly by the relative growth rates of the polymorphs, and a change in this growth mechanism with increasing ethanol concentration was proposed. Attempts have been made[8] attempted to crystallize each of the four polymorphs of the drug sulphathiazole from different solvent systems. These experiments revealed that some solvents selectively favoured the crystallization of a particular form or forms. The researchers proposed that the solvent acted by selective adsorption to certain faces of some of the polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of other forms. The concept of using particle size analysis to examine the mechanism of transformation for polymorphs of ammonium nitrate in aqueous solution has been reported.[5] It was noted that if crystals of a metastable phase are suspended in their saturated solution, then as the transformation proceeded, the crystal size distribution would either remain essentially unchanged if it proceeded via an SST or show a significant change if it underwent an SMT. Therefore, by measuring the change in the crystal size distribution over time, the mechanism of transformation could be identified. The feasibility of using Focused Beam Reflectance Measurement (FBRM) to monitor changes in the morphology of polymorphs, in conjunction with Raman spectroscopy, has been reported,[9] while the use of IR spectroscopy is increasingly used as a standard technique for in situ monitoring of solute concentration in crystallisation implemented through use of a probe-based Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscope.[10]

In this paper, a Lasentec FBRM probe is used to establish the transformation mechanism for the δ to β polymorph transformation in aqueous slurries of D-mannitol by monitoring the dynamics of the crystal size distribution. In addition, an ATR-FTIR probe is used to simultaneously monitor the change in the solution concentration in real time.

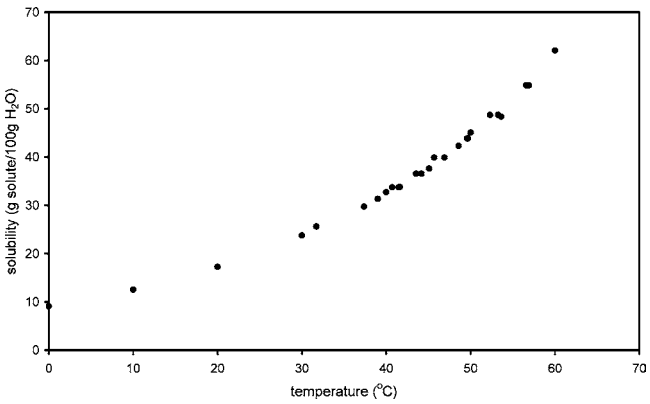

Figure 1. **Aqueous solubility of stable β-form mannitol.[11]**

Experimental Methods

Commerically available D-mannitol (Sigma-Aldrich; ≥98% purity) in deionized water was used for all experiments, which were performed in a 500 mL jacketed glass vessel, agitated at 250 rpm using a pitched blade impeller. D-Mannitol [$CH_2OH(CHOH)_4CH_2OH$] is a well characterized organic molecule belonging to a family of compounds called polyols or sugar alcohols in which there is significant interest as sugar replacers. D-mannitol has three known polymorphic forms, α, β, and δ, of which the β-form is the thermodynamically stable form at room temperature and pressure. The solubility of the β-form in water is known[11] (see Figure 1); the aqueous solubility of the δ-form has not been reported.[11] The β-form is commonly used as a diluent and excipient in drug formulations, although there is some investigation into the use of the δ-polymorph as an excipient in the wet granulation of poorly compactable active pharmaceutical ingredients (APIs) to produce improved tablets.[12] It is also an interesting compound from the point of view that it has a difficult morphology in terms of sizing it as the β-form grows into needle/rod like crystals, while the δ-form typically consists of bundles of very fine needles also known as spherulites. Where required, pure δ-form mannitol was isolated experimentally, according to a procedure outlined elsewhere.[11] An FBRM probe (Lasentec model S400A), an ATR-FTIR probe (Mettler-Toledo ReactIR 4000), and a thermocouple were inserted in the vessel. The ATR-FTIR system was calibrated using standard solutions. A challenge with the mannitol−water system is presented by the apparent influence of the solid phase on the measured spectra, just after nucleation. This effect is rarely reported, but required incorporation into the calibration model developed for the system.[11] In situ images were collected using a Lasentec Particle Vision and Measurement (PVM) probe which is a high resolution, video microscope, which is used for the imaging of particles as they exist in the process environment.[13] Off-line digital image analysis was performed using

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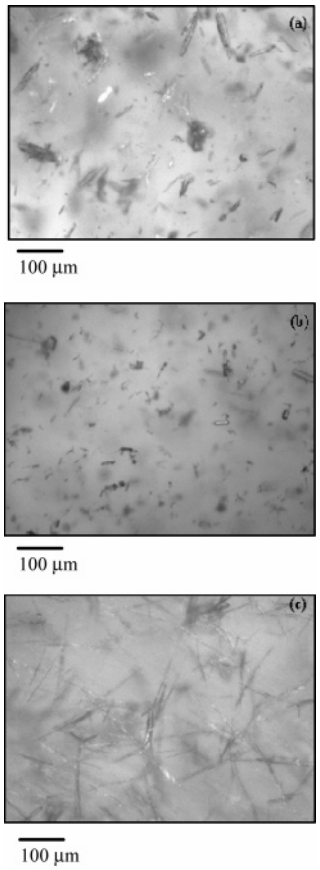

Figure 2. **PVM images of the polymorphic transition of D-mannitol in water at 5 °C taken at (a) 30 s, (b) 30 min, and (c) 5 h.**

Table 1. **Experiment configuration for polymorph transition trials**

experiment	g of β seed	g of δ seed	g of mannitol/ 150 g of water
A	0.2		21
B	0.2	2	19
C	0.2		25.5
D	0.2	1	25.5

a Leica Q500IW system operating the Leica QWin image analysis software connected to a 3CCD color vision camera mounted on an Olympus BH2 optical microscope.

Results and Discussion

A series of trials as outlined in Table 1 were performed to examine the polymorphic transformation of mannitol. All experiments were conducted under isothermal conditions at 5 °C. At this temperature, it is known[11] that the transition from the δ- to β-form is sufficiently slow to permit accurate measurement of the transient system behaviour. PVM images of a typical polymorphic transformation of mannitol[11] are shown in Figure 2.

In experiment A, on addition of the β-form seed crystals there is an immediate increase in the counts measured by the FBRM, and the seed bed then proceeds to grow, as can be seen from the gradual increase in measured counts (see Figure 3). However, in experiment B, while addition of the mixture of β- and δ-form mannitol seeds leads to an initial steep rise in the FBRM counts, this is followed by a drop in measured counts. It is speculated that this decrease in counts is a result of the dissolution of the δ-form mannitol, which dissolves because of its higher solubility compared to the β-form. After dissolution of the δ-form, a bed of β-form seed crystals is left behind to consume the supersaturation and grow. This seed bed is similar to that of experiment A as expected (compare chord length distributions at 0.2 h in Figure 4), and because the seed is now in an similar supersaturated solution to experiment A, due to the dissolution of the δ-form, the crystals from both experiments grow in a similar manner (see Figure 4).

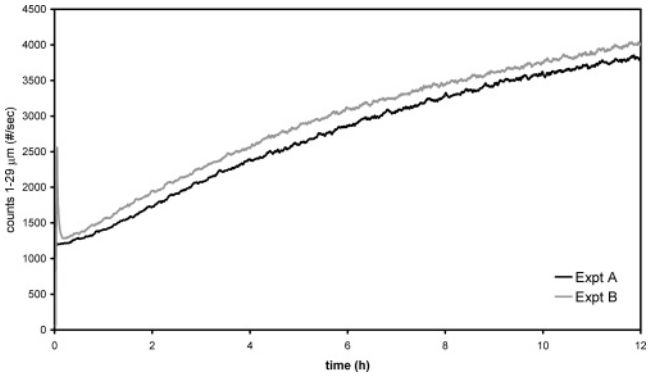

Figure 3. **FBRM counts for the first 3 h of experiments A and B.**

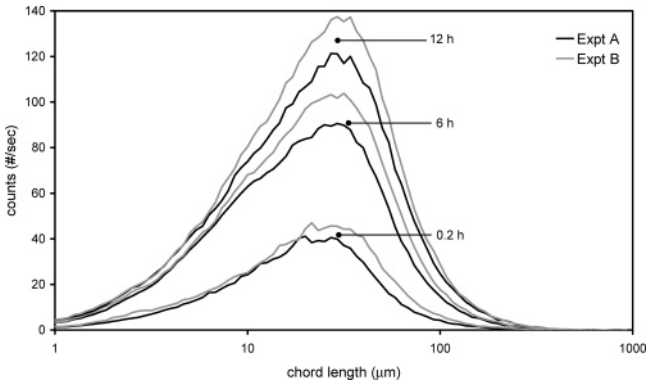

Figure 4. **Development of chord length distributions during experiments A and B.**

Examination of the solute dissolved concentration data in Figure 5 indicates good agreement with the observations of the FBRM solid-phase data. The dissolution of the δ-form seed is clearly visible, with the subsequent desupersaturation profile for both experiments following a trajectory similar to that indicated by the FBRM data.

It is useful at this point to examine more closely the chord length data, so as to correctly understand the particle population trends just highlighted. The measurement method of the FBRM is based on the detected backscatter of laser light, as a laser beam traverses over the surface of a particle. The duration of this backscattered light is converted into a chord length, and this chord length is representative of some characteristic dimension of the particle just measured. The

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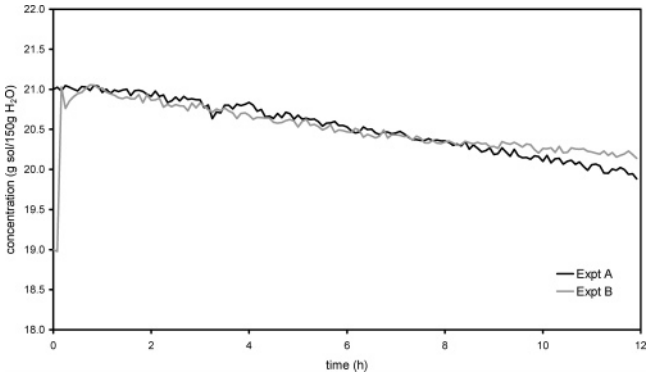

Figure 5. **Dissolved solute desupersaturation curve for experiments A and B.**

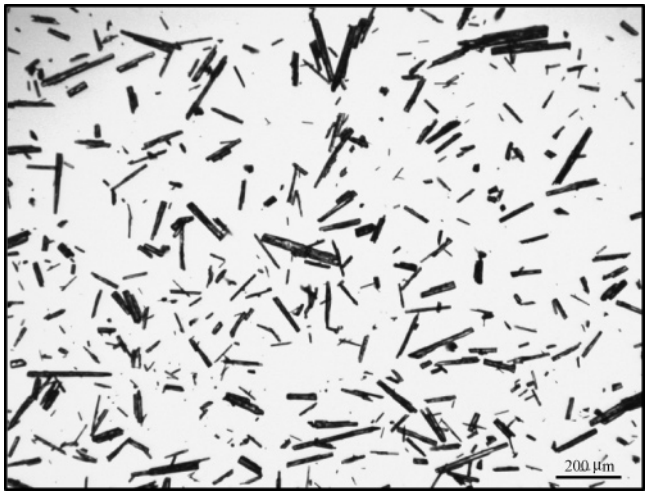

Figure 6. **Microscope images of the β-form seed crystals used in experiment A.**

orientation of the scanning laser beam, or of the probe itself, is not changed throughout the experiment, and therefore, the instrument can only measure particles that are presented to it by the flow field in the vessel. The orientation of a particle at the probe window where measurement takes place plays an important role in determining the length of the chord measured, with a single particle giving rise to a range of potential chord lengths. The shape of the particle can also influence the measurement. Figure 6 shows microscopic images of the seed crystals of β-form seeds used in experiments A and B. As can be seen, these crystals have a needlelike habit. When measuring such crystals with FBRM, it would be expected to measure the width of a particle far more often than the length, particularly as the crystals grow. From image analysis data the average seed length is determined to be approximately 74 μm, and the width, approximately 14 μm (based on a sample of 303 crystals). During the 12 h of growth, these crystals will be expected to grow in all dimensions, i.e., get longer and wider. Figure 7 shows typical crystals at the end of experiment A, and as expected, they have grown in both directions with an average length of approximately 200 μm and a width of 23.5 μm (based on a sample of 167 crystals). Figure 8 is a comparison of the cumulative chord length distribution of the crystals as measured by the FBRM probe at the end of the batch with the cumulative distribution of crystal widths as measured

Figure 7. **Microscope images of the β-form mannitol crystals at the end of experiment A.**

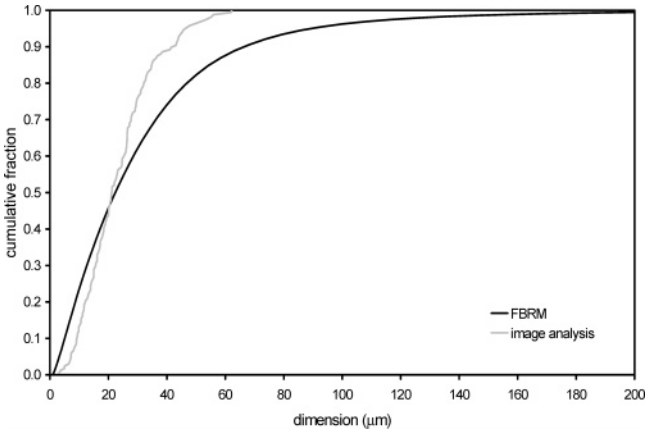

Figure 8. **Cumulative chord length distribution as measured with in situ FBRM, and cumulative crystal width distribution as measured by off-line image analysis at the end of experiment A.**

by image analysis. The good agreement between both sets of data supports the contention that the chord length distribution measured by the FBRM in a suspension of crystals of needlelike habit is a strong function of the needle width.

In the comparative experiments discussed above, experiment B did not provide conclusive mechanistic information for the $\delta - \beta$ transformation since the seed mixture was added into a solution supersaturated in the β-form, but undersaturated in the δ-form, so all the δ-form seed crystals dissolved. Both experiments, however, demonstrated the excellent sensitivity of the in situ analytics to changes in both the solid and solution phases. In an effort to understand the actual polymorphic transformation mechanism, i.e., whether it is a solution mediated or a solid-state transformation, two further experiments (C and D) were conducted (see Table 1). Figures 9−11 show a comparison of the FBRM population statistics for both experiments. As can be seen, when the β-form seed crystals are added in experiment C there is an increase in all population ranges. Because of the high supersaturation, relative to the β-form (saturated concentration at 5^{o}C is 18 g/150 g H_2O), the seed crystals

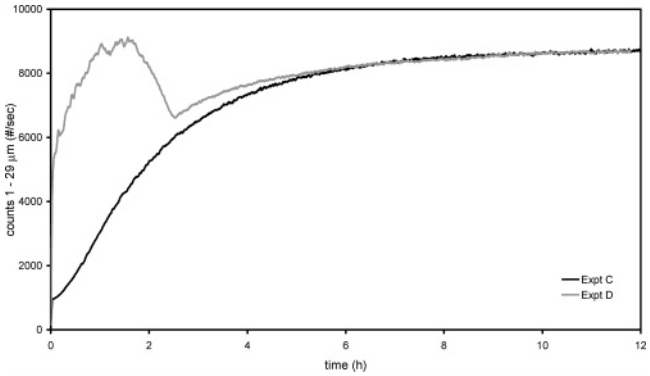

Figure 9. **Trends for counts of fine chords as measured with in situ FBRM during experiments C and D.**

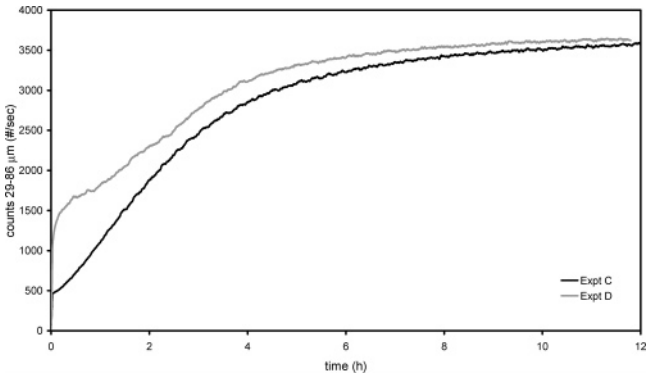

Figure 10. **Trends for counts of medium-sized chords as measured with in situ FBRM during experiments C and D.**

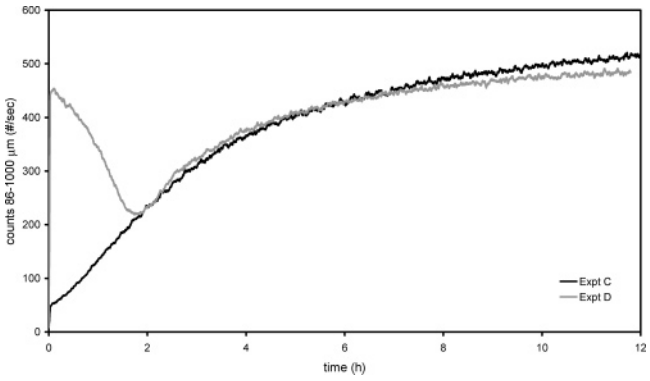

Figure 11. **Trends for counts of coarse chords as measured with in situ FBRM during experiments C and D.**

grow relatively quickly, and this is indicated by the consistent increase in measured counts over the first 4 to 5 h. The increase in counts observed is consistent with growth-dominated kinetics.[13] As the isothermal crystallization proceeds, the FBRM counts slowly level off as the solution approaches equilibrium.

In experiment D, a larger mass of seed is added resulting in a larger number of FBRM counts than observed in experiment C. The solution is supersaturated with respect to the β-form and is at least saturated with respect to the δ-form (solubility of the δ-form is unknown, but because rapid dissolution after seed addition is not seen as was the case with experiment B, it is assumed that the solution is not undersaturated in δ). The β seed crystals begin to grow immediately due the high level of supersaturation. This can

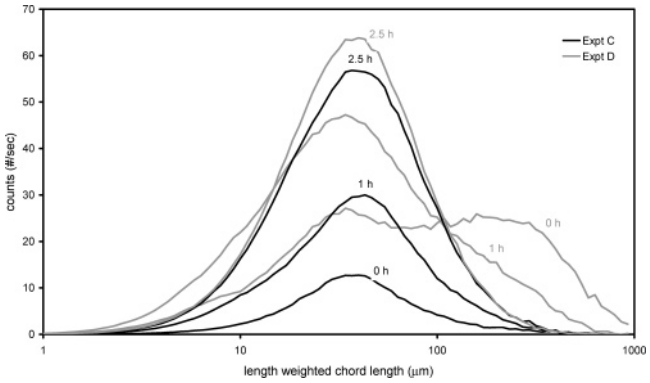

Figure 12. **Comparison of length weighted chord length distributions for experiments C and D.**

be seen from the large increase in counts in the 1−29 μm region. As the counts in this region increase, the counts in the coarse chord range (86−1000 μm) gradually decrease. This decrease can be accounted for by the gradual dissolution of the δ-form seed crystals as a result of a drop in the solution concentration associated with the growth of the β phase. As δ-form dissolution proceeds, the size of the δ crystals reduces, and this size reduction is reflected in the large increase observed in the small chord length population measured by the FBRM. There now is a very dynamic crystallization with the β-form consuming supersaturation through growth of the original seed, while simultaneously the δ-form restores this consumed supersaturation through dissolution. The coarse counts stop decreasing after approximately 2 h and then begin to increase. This increase is most likely due to the β-form crystals growing into this size range. As the β-form continues to grow, the supersaturation is consumed at an increasing rate and the δ-form continues to dissolve. The relatively sharp and quick decrease in the fine counts can be attributed to dissolution of the last of the δ-polymorph. After this point, the β-form continues to grow for the remainder of the batch. Figure 12 compares the chord length distributions during the first 2.5 h of experiments C and D. The distributions are length weighted to emphasize the trends. It is noticeable that as the δ-form dissolves, the β-form can be seen to grow, and once all the δ-form has dissolved, the chord length distributions for both experiments approach each other, indicating that ultimately the final chord length distribution is a function only of the original charge of β-form seeds. That the trends ultimately approach similar end points suggests a solution-mediated transformation for this transition. A solid-state transition of the initial charge of the δ-form would have led to significantly more β-form crystals present in the system (since the effective β-form seed load would have been substantially higher), leading to substantially different chord length distributions. This, however, was not the case.

Finally, solution concentration data were in good agreement with the proposed transformation predicted from the FBRM data (see Figure 13). For experiment C (the growth of pure β-form only), the rate of desupersaturation is quite rapid immediately after seeding but decreases towards the end of the batch as equilibrium is approached. In experiment D, the concentration profile over the first 2.5 h is markedly

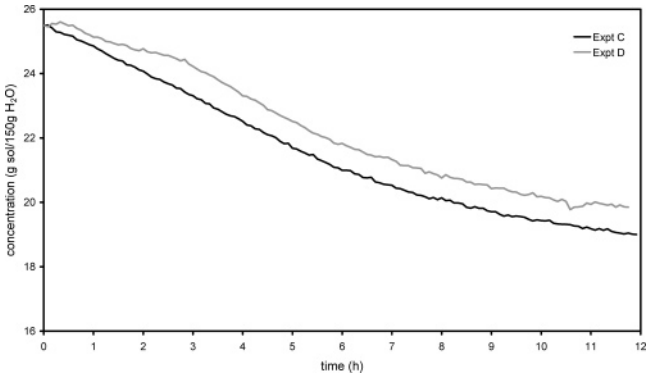

Figure 13. **Comparison of the dissolved solute concentration profiles for experiments C and D.**

slower when compared to experiment C. This is as expected because, as the β-form grows, there is a concomitant dissolution of the δ-form seeds, thereby reducing the rate of desupersaturation. However, once all the δ-form is dissolved, the rate of desupersaturation is almost identical to experiment C indicating similar growth kinetics.

Conclusions

The ability of FBRM combined with in situ ATR-FTIR to successfully monitor the $\delta - \beta$ polymorphic transformation of D-mannitol in aqueous solution was demonstrated. Initially, the growth behaviour of β-form seed crystals in an isothermal supersaturated solution was characterized. It was noted that the dominant chord length measured by the FBRM was a function of the width of the needle-shaped crystal, and the chord length distribution at the end of the batch was in good agreement with the needle width data as estimated from image analysis. This provided the opportunity to successfully monitor the dynamics of the metastable δ-form polymorphic transition. A seed mixture of the β- and δ-forms was added to various supersaturated solutions. Various FBRM population statistics monitored the progress of the transformation. From these trends, the transformation mechanism was readily identified as a solution-mediated transformation as opposed to a solid-state transition. FBRM data also indicated that the rate of this transformation decreased with increasing δ-form seed loading, again as would be anticipated. In situ solution concentration data, as measured by an ATR-FTIR spectroscope, corroborated the FBRM findings.

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