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An investigation into the thermal behaviour of a model drug mixture with amorphous trehalose

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Abstract

The thermal and structural properties of amorphous trehalose mixed with a model drug, paracetamol, have been studied with a view to developing understanding of the thermal events undergone by such binary systems. A physical mixture of paracetamol and spray dried trehalose (1:9 weight ratio) was studied using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot stage microscopy (HSM), and variable temperature powder X-ray diffraction (VTPXRD). The presence of the drug resulted in a lower temperature recrystallisation exotherm for the trehalose compared to the disaccharide alone. Evidence is presented for the trehalose recrystallisation being triggered by the melting rather than simply the presence of the paracetamol particles. HSM studies indicated that the trehalose recrystallised around the drug on heating, with the recrystallisation temperature again corresponding to the melting of the drug. VTPXRD indicated that the presence of the drug again lowered the recrystallisation temperature of the trehalose, although the trehalose anhydrate diffraction peaks were discernible at a lower temperature for both the pure trehalose and the mixed systems than was observed for the DSC studies, suggesting that the association between recrystallisation and drug melting was not apparent when using this approach. It is suggested that while the trehalose recrystallisation process is not significantly influenced by the presence of the drug when studied over relatively long time periods such as those used for the VTPXRD studies, the process is triggered by the melting of the paracetamol when short experimental times and scanning conditions are used such as those relevant to DSC studies. These data have implications for the quality control of trehalose products using DSC, the characterisation of the physical structure of the binary systems and the prediction of the corresponding physical stability.

Keywords: Trehalose; Calorimetry; Amorphous; Spray dried; X-ray diffraction

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1. Introduction

α,α-Trehalose (α-D-glucopyranosyl-(1-1) α-D-glucopyranoside) is a non-reducing disaccharide synthesised by living organisms as a natural protectant against damage due to freezing or dehydration (Crowe et al., 1987; Chen et al., 2001). The molecule derives its name from trehala manna, obtained from the shell of insects from the family *Curculionides* or *Rhynchopores* that contain 25–30% α,α-trehalose. The disaccharide is composed of two α-D-glucosyl units linked by a glycosidic oxygen bridge between two anomeric carbon atoms and is amongst the most chemically unreactive natural sugars, with dissociation into reducing sugars only occurring under extreme hydrolysis conditions or in the presence of the specific hydrolysing enzyme trehalase. This material has attracted considerable interest within the pharmaceutical arena as a means of stabilising protein and peptide drugs during the freeze-drying process (Aldous et al., 1995; Crowe et al., 1996). In addition, trehalose has been used as a spray drying excipient, again to reduce damage to delicate molecules during processing (Webb et al., 2002; Costantino et al., 2002).

Studies into the behaviour of trehalose have been complicated by the multitude of physical forms that this molecule may adopt. As a raw material, trehalose is usually presented as the dihydrate (T_h), the crystallographic structure of which has been determined by Brown et al. (1972). In addition, the material may be presented in the anhydrous form (T_β) (Sussich et al., 1998, 1999) and as an amorphous system, this state being particularly relevant to the production of freeze dried dosage forms (Franks, 1990; Craig et al., 1999). It has also been suggested that a further polymorph of the anhydrous form (T_α), with a melting point of circa 403 K, may be generated (Sussich et al., 1999). A further form (T_γ) has also been described that is now thought to comprise a mixture of the anhydrous and dihydrate materials (Sussich et al., 2001). Other forms such as T_κ and form II have been described but their distinction from T_γ remains controversial.

In addition to considerations regarding the number of forms in which trehalose may exist, there is also ongoing debate with regard to the inter-conversion of these forms, particularly as a function of temperature, time and water content. The understanding of such inter-conversions is essential, primarily in order to understand the stability of trehalose products but also in order to facilitate interpretation of thermoanalytical data. The latter is a non-trivial consideration as DSC remains one of the primary means by which the structure of the material after processing may be ascertained. On heating the dihydrate either the amorphous or anhydrous crystalline form may be generated, depending on the experimental conditions used. In particular, the particle size appears to be a critical consideration as large particles (>circa 400 μm) tend to convert directly into the anhydrous form while smaller diameter particles (<circa 50 μm) convert to the amorphous form (Taylor and York, 1998a,b). Recent studies (McGarvey et al., 2003) have suggested that amorphous trehalose may crystallise into a range of structures depending on the experimental conditions used and the water content of the sample. In particular it was noted that heating the amorphous material in pinholed DSC pans, whereby residual water could be lost, resulted in the formation of the anhydrate, while heating in hermetically sealed pans resulted in the formation of a range of partial hydrates (the T_γ form) depending on the initial water content.

Given the complexity of the thermal behaviour of amorphous trehalose alone it is essential to have an understanding of whether the presence of a second component will influence the thermal transitions undergone by the material, particularly in the context of characterising dosage forms, which will invariably be multicomponent. However, to date no information is available as to whether the presence of the drug may alter the thermoanalytical profile of this material. To this effect a simple model drug (paracetamol) was chosen in order to provide an insight into whether the presence of a second component should be considered when characterising products based on trehalose.

2. Materials and methods

2.1. Materials

α-D-glucopyranosyl α-D-glucopyranoside dihydrate (trehalose dihydrate) and 4-acetamidophenol (paracetamol) were obtained from Pfanstiehl (Pfanstiehl Laboratories Inc., Waukegan, IL, USA) and Sigma (Sigma-Aldrich Chemie GmbH, Germany), respectively, and were used without further

purification. The 90–180 μm paracetamol sieve fraction was used for the study, with preliminary SEM studies showing the particles to be a mixture of plate-like and needle-shaped crystals. Amorphous trehalose was prepared by spray drying a 10% w/v aqueous solution of trehalose using a Büchi 190 mini spray drier set with an inlet temperature of 433 K, an outlet temperature of 353 K and a pump speed of approximately 6 ml/min. The spray-dried material was stored in a desiccator containing P_2O_5. Physical mixtures were prepared by gentle mechanical trituration of spray dried trehalose with 10% w/w of paracetamol. Preliminary SEM studies indicated that the spray dried trehalose particles showed the expected spherical morphology (particle size circa 5–30 μm).

2.2. Thermal analysis

DSC experiments were run using a DSC 2920 (TA Instruments, New Castle, DE, USA) with attached refrigerated cooling system (RCS). The DSC was calibrated for temperature using *n*-octadecane, indium and tin as calibrants (onset melting point). Samples were analysed in open aluminium pans under a dry nitrogen atmosphere (35 ml/min) and heated between 298 and 523 K at a heating rate of 2–10 K/min. Open pans were chosen so as to facilitate comparison with both HSM and TGA studies. Data were analysed using Universal Analysis, version 2.6D. Thermogravimetric analysis was performed on a Hi-Res TGA 2950 (TA Instruments). Measurements were run in open pans at a scan rate of 2 or 10 K/min in a dry nitrogen atmosphere from room temperature to 573 K. Data were analysed using Universal Analysis, version 2.6D. Hot stage microscopy was undertaken at a heating rate of 2 or 10 K/min using a Linkam hot-stage and an Olympus BX50 microscope.

2.3. X-ray powder diffraction

The X-ray powder diffraction patterns were recorded using a Philips X'pert PRO powder diffractometer at 40 mA, 45 kV and with monochromatised Cu Kα radiation (λ = 1.54056 Å). The samples were scanned at 293 K in continuous scan mode in the diffraction angle 2θ increasing 0.01°/s over the range 3–40° with a 1 s counting time. Pattern calculation from single crystal structure data was obtained with X'Pert Plus 1.0 software package. Variable temperature powder X-ray diffraction studies were run on a Philips X'pert PRO powder diffractometer at 40 mA, 45 kV with a Cu Kα radiation (λ = 1.54056 Å). Powder samples were packed into the cavity of an Anton Parr TTK-450 holder. A TCU 100 controller was used to heat the sample to the desired temperature and maintain this temperature over the analysis period. X'Celerator was used as a detector and a single measurement required 6 min. The temperature was ramped between 303 and 453 K at a heating rate of 10 K/min.

3. Results

3.1. DSC and TGA studies

In order to facilitate examination of the data corresponding to the physical mixes at different scanning speeds, it was necessary to examine the responses for the spray-dried trehalose alone. Given the range of transitions that may occur during the heating process, the effect of DSC scanning speed was investigated as a variable. Fig. 1ashows the response for the amorphous material alone at 2 K and 10 K/min, run in conjunction with TGA studies. On heating the material at 2 K/min a small diffuse endotherm is seen (300–350 K) almost certainly corresponding to the loss of sorbed water, followed by a small discontinuity at circa 398 K that, based on previous studies, may be ascribed to the glass transition (McGarvey et al., 2003). An exotherm (peak temperature 465.7 K) is seen followed immediately by an endotherm that, again based on previous studies (McGarvey et al., 2003), may be ascribed to the recrystallisation and subsequent (or simultaneous) melting of the anhydrate. The 10 K/min studies showed a more clearly defined water loss peak and a larger discontinuity at the glass transition, as may be expected due to the greater sensitivity of the instrument when run at higher scanning speeds. On the basis that the glass transition is a second order reaction (or at least has certain characteristics of a second order reaction) the change in heating rate may also be expected to alter the measured value of Tg, with the higher rate resulting in a higher value due to the temperature overshoot effect (Craig et al., 1999). However, the effect appears to be small for the present system and is in any case difficult to identify reliably due to both the limited fragility of the system

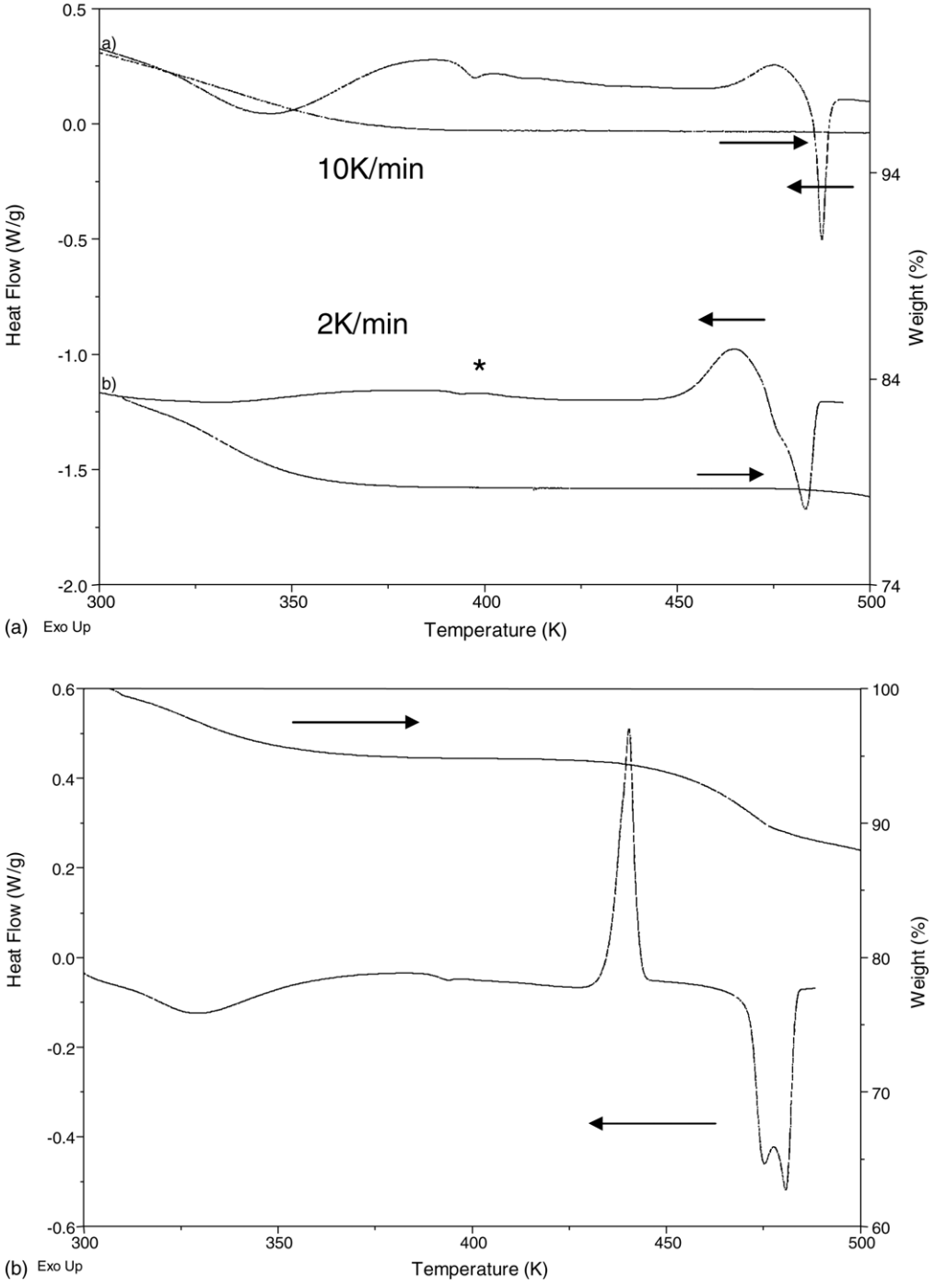

Fig. 1. Differential scanning calorimetry and thermogravimetric analysis responses (weight loss axes not scaled for clarity) for (a) spray dried trehalose alone heated at 10 and 2 K/min (* represents glass transition) (b) binary mixtures with paracetamol at 2 K/min and (c) 10 K/min. TGA data scaled for clarity.

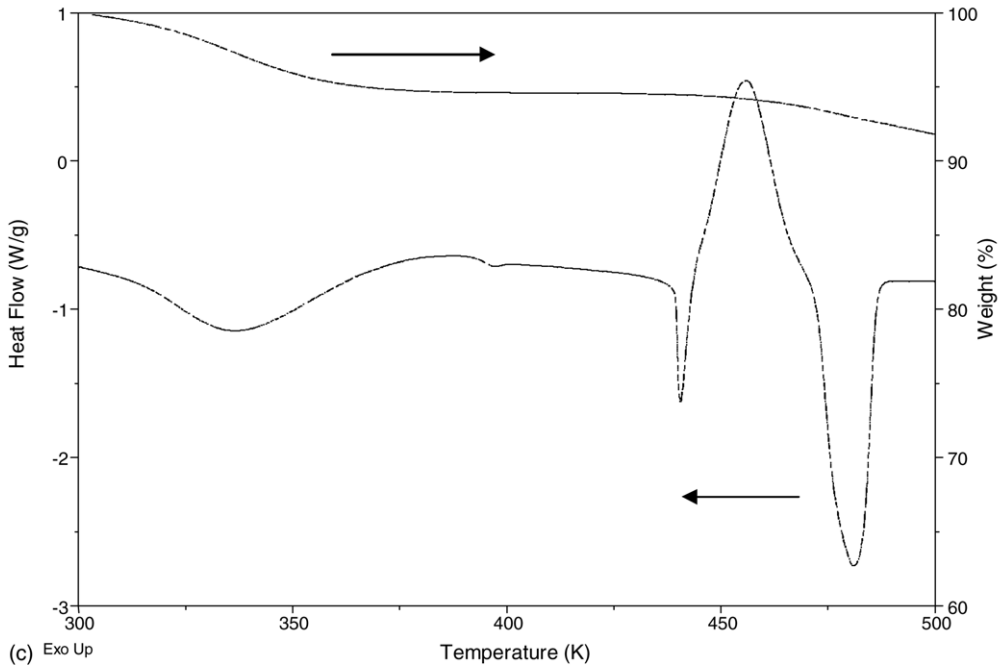

Fig. 1. (*Continued*.)

(rendering the change in heat capacity through the glass small) and the relaxation endotherm superimposed on the glass transition at the higher heating rate. In contrast, the peak recrystallisation temperature increased from 465.7 to 475.9 K on increasing the scanning rate, this being ascribed to the temperature dependence of the growth/nucleation process above Tg. A sharp melting peak for paracetamol at 443.1 K (onset temperature 441.9 K) was noted (data not shown) with a heat of fusion of 176.1 J/g at 2 K/min; this is in good agreement with earlier studies (Lloyd et al., 1997).

The mixed systems run at the equivalent scanning speeds are shown in Fig. 1b and c. It is clear that a markedly different pattern is seen for the binary systems that do not simply correspond to a summation of the thermal responses of the individual components. For samples run at 2 K/min (Fig. 1b), an exotherm is seen circa 30 K lower than for the trehalose alone (peak temperature 439.9 K, note the similarity to the melting onset of paracetamol 441 K), followed by a distinct endotherm (peak temperatures 480.3 K), with no melting peak seen for the paracetamol. In contrast, the 10 K/min samples (Fig. 1c) show an endotherm (peak temperature 440.5 K, which corresponds well to the melting peak of paracetamol alone) truncated by a large exotherm 455.9 K (enthalpy 106.7 J/g), followed by a further endotherm at (peak temperature) 481.3 K. It should be noted that no evidence was obtained for a change in Tg for either scanning rate. However, it should also be noted that there appears to be a correlation at both scanning speeds between the recrystallisation temperature of the trehalose and the melting of the paracetamol. Indeed, the scanning rate dependence of the recrystallisation onset seen for the trehalose alone is lost, with both speeds yielding similar values. Given that melting is a first order process and hence not dependent on rate, this observation is consistent with the suggestion of the recrystallisation process being associated with the paracetamol melting.

3.1.1. Hot stage microscopy

The HSM profile of the trehalose alone is shown for the 2 K/min samples in Fig. 2a–d. It may be seen that the particles appear to liquefy above the glass transition temperature (Fig. 2a,b), whereupon recrystallisation is then seen at circa 481 K (Fig. 2c) followed by melting

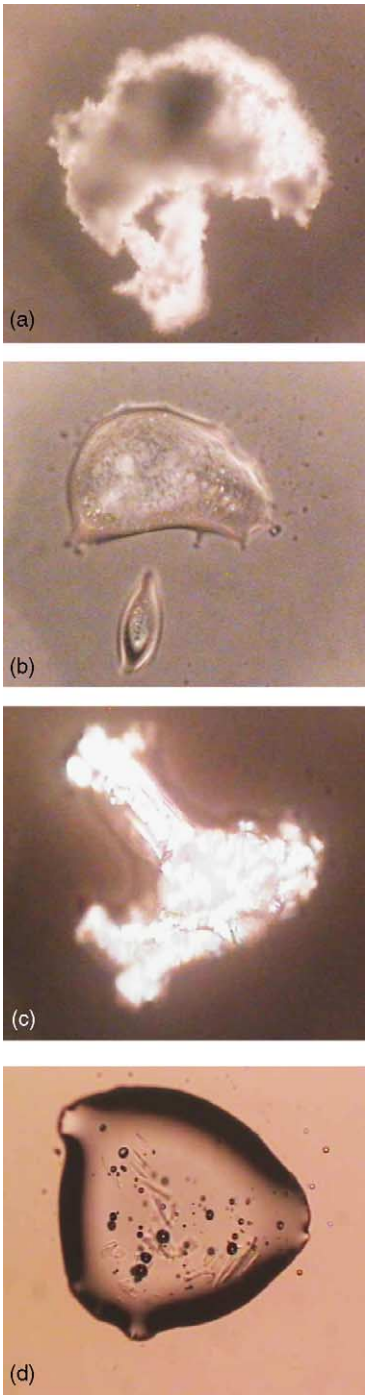

Fig. 2. Hot stage microscopy images for spray dried trehalose alone heated at 2 K/min at (a) 296 K (b) 432 K (c) 481 K (d) 505 K (width of image equivalent to 1 mm).

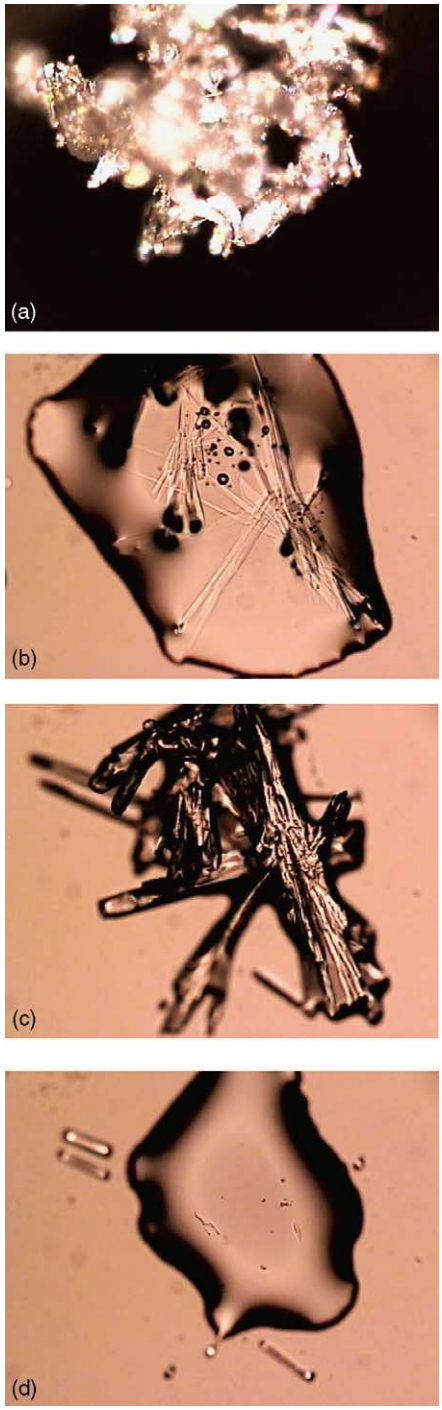

Fig. 3. Hot stage microscopy images for binary mixtures with paracetamol heated at 2 K/min at (a) 419 K (b) 442 K (c) 445 K (d) 488 K (width of image equivalent to 1 mm).

(Fig. 2d). A similar pattern was seen for the samples heated at 10 K/min (data not shown). Fig. 3a–c shows the morphological changes that take place on heating the mixed sample at 2 K/min. It was noted that at temperatures around 420 K the initially solid trehalose (Fig. 3a) softened to the extent of achieving a liquid consistency (Fig. 3b). However at circa 440–445 K the trehalose recrystallised (note again the similarity to the melt onset of paracetamol, 442 K), with the drug clearly acting as focal points for the solidification process (Fig. 3c); melting then took place at circa 480–490 K. Similar patterns were seen for the 10 K/min sample. It should be noted that measurement of the onset temperature of the above processes was inevitably partially subjective, hence differences between the behaviour profiles at the two heating rates was difficult to quantify reliably. Nevertheless it was noted that the paracetamol melted immediately prior to the trehalose recrystallisation and that regions of the sample containing a preponderance of drug were seen to recrystallise first.

3.1.2. Temperature dependent powder X-ray diffraction

The (ambient conditions) X-ray powder diffraction patterns for the trehalose dihydrate, spray dried trehalose and paracetamol are shown in Fig. 4. In addition, the X-ray powder diffraction pattern for the anhydrous crystalline material is shown for comparison, calculated from single crystal data (Jeffrey and Nanni, 1985).

Fig. 5a shows the response of the spray dried trehalose alone (using a heating rate of 2 K/min) showing that the halo pattern typical for an amorphous material was present at room temperature up to 433 K when reflection peaks characteristic for anhydrous trehalose (e.g. 20.5°, 22.5°) started to appear. At 438 K and above the diffraction pattern corresponded to that of anhydrous trehalose up to the melting point of the material. It should be noted that the discrepancy between the recrystallisation behaviour observed using VTPXRD and DSC might be anticipated due to the quasi-isothermal nature of the former studies, leading to differences in the sample thermal history between the two sets of measurements. More specifically, the sample would have been held at each temperature for approximately 6 min between measurements, thus allowing more time for the system to nucleate and the corresponding anhydrous form to be generated. It should also be noted that it was difficult to discern the paracetamol peaks against the amorphous halo at the lower temperatures.

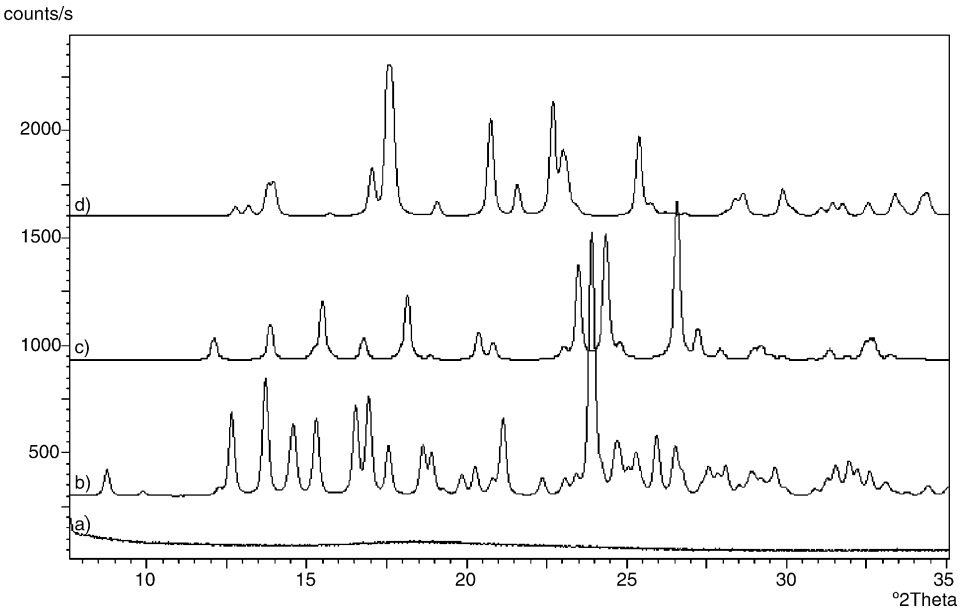

Fig. 4. Powder XRD patterns for (a) spray dried trehalose, (b) trehalose dihydrate, (c) paracetamol and (d) anhydrous trehalose (calculated).

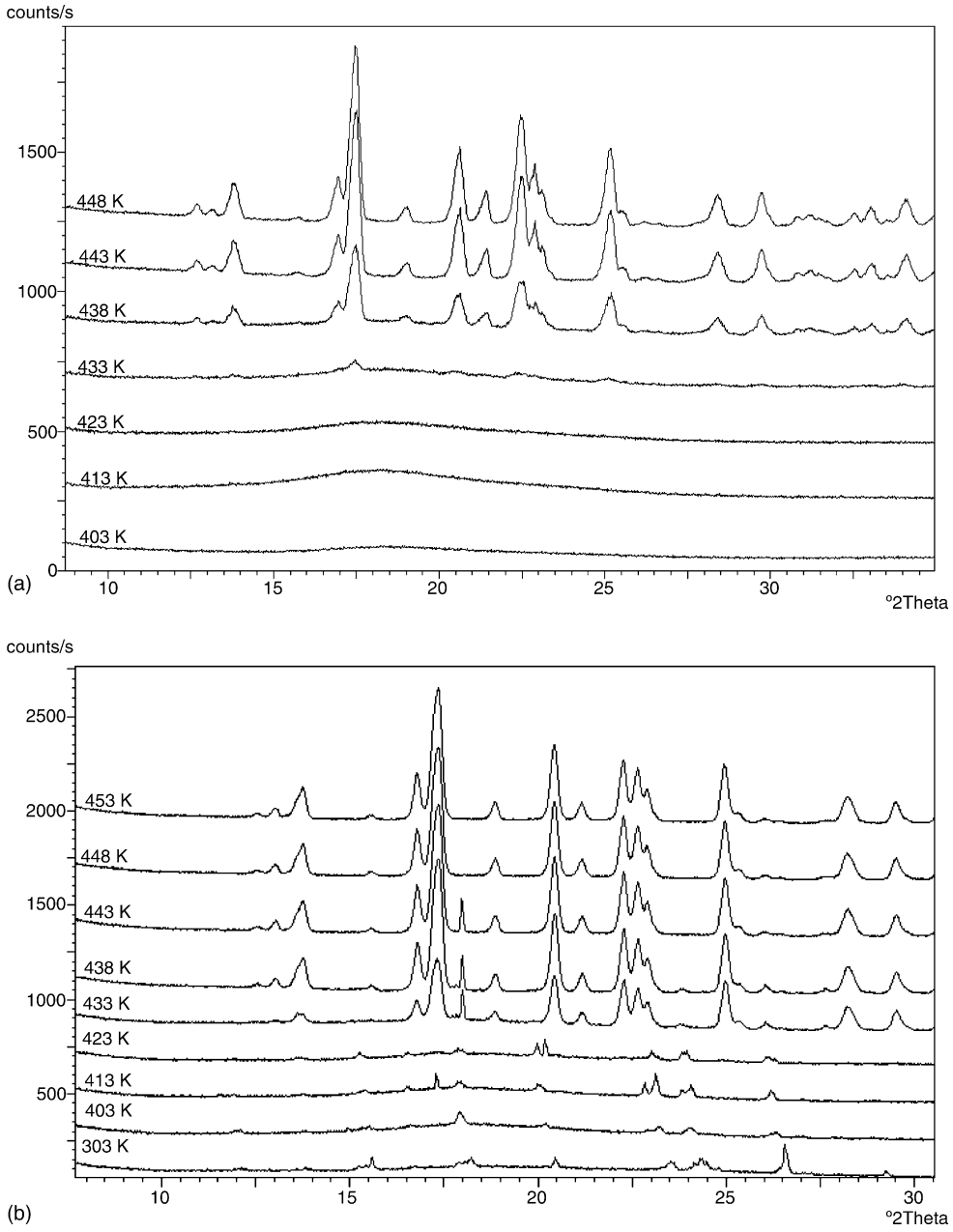

Fig. 5. Variable temperature powder XRD response for (a) spray dried trehalose alone (b) binary mixtures with paracetamol (heating rate between scans 2 K/min).

The equivalent studies for the mixed systems heated at 2 K/min between spectra (Fig. 5b) show the diffraction halo for the amorphous trehalose from 303 to 423 K. On recrystallisation to the anhydrous form at 433 K, the paracetamol peaks could be discerned at, for example 18.1°. These peaks were not seen above 443 K (which corresponded well to the melting of the paracetamol), with only those corresponding to anhydrous trehalose detected. A similar set of data was obtained for systems heated at 10 K/min between scans.

4. Discussion

The expected DSC response of glass transition, recrystallisation to the anhydrous form and subsequent melting were observed for the trehalose alone; this was supported by HSM and powder XRD studies. However, on physically mixing the system with paracetamol the response differed from that expected from the behaviour of the individual components in a heating-rate dependent manner. Systems heated at 10 K/min showed the expected glass transition of trehalose but also exhibited a sharp endotherm that corresponded well to the melting point of paracetamol; recrystallisation of the trehalose occurred immediately after the melting process. In the case of systems heated at 2 K/min, no melting peak was seen for the paracetamol. This may be due either to the recrystallisation of the trehalose coinciding with the melting of the drug, thereby effectively masking the melting process, or to the lower heating rate resulting in slow dissolution of the drug into the liquefied sugar, as has been observed for paracetamol mixes with polyethylene glycol (Lloyd et al., 1997). Inspection of the HSM and VTPXRD data would support the former explanation, as the drug particles appeared to remain intact up to the melting point. At both heating rates, the recrystallisation took place at considerably lower temperatures than for the trehalose alone. The intuitively obvious explanation for this would be that the paracetamol particles (or asperites thereof) were acting as heterogeneous nuclei for the trehalose. However, this is not supported by the observation that the recrystallisation process took place after or coincident with the melting of paracetemol in both cases rather than below this temperature. Indeed, the similarities of the temperatures of the two processes suggests that the melting of the drug acts as trigger to the recrystallisation of the trehalose. This is supported by the HSM studies which indicated that at both heating rates, recrystallisation of the trehalose took place around the paracetamol at a temperature corresponding closely to the melting point of the drug. One may speculate that this reflects the drug particles, during melting, releasing smaller particles that are more effective nuclei, possibly due to the more intimate contact they may have with the trehalose in this more fluid form.

In contrast, the VTPXRD studies indicated that while the presence of the drug lowered the recrystallisation temperature by circa 10 K, this temperature was lower than that seen during the DSC studies for both the loaded and unloaded systems. This discrepancy can be reasonably ascribed to the quasi-isothermal nature of the VTPXRD measurements. Nevertheless, these data indicate that the association between drug melting and disaccharide recrystallisation is only pertinent to the short timescales and scanning conditions used for DSC studies. This does not by any means render this effect irrelevant, as DSC remains one of the key means by which binary pharmaceutical systems are characterised. It does, however, strongly imply that care must be taken when interpreting DSC data of such systems, both in terms of ascertaining the solid state structure of the binary systems and also extrapolating information regarding product stability from observing the effect of drug inclusion on the recrystallisation kinetics.

5. Conclusion

The study has demonstrated that binary mixes of paracetamol and amorphous trehalose may lead to complex thermal behaviour that is highly dependent on the experimental conditions used. However, comparison of the behaviour at the two scanning speeds, particularly using a range of complimentary techniques, allows specific interpretation of the thermal data and identification of drug-induced changes in the trehalose thermal response. More specifically, the presence of the drug may induce recrystallisation, although it is suggested that at least in temperature scanning mode this effect is associated with the melting of the drug rather than the presence of the crystalline particles per se. The study has indicated that, when designing quality control

protocols for binary systems of drugs and amorphous materials such as the one studied here, a combined instrumental approach is advisable for facilitating accurate interpretation of the corresponding DSC data. Moreover, the investigation has indicated that the presence of a second component such as a drug may profoundly influence the thermal response of amorphous trehalose, even when present as a simple physical mixture.

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