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Water sorption/desorption—near IR and calorimetric study of crystalline and amorphous raffinose

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Abstract

Mass loss at elevated RH is an established method for determining the occurrence of crystallisation of an amorphous material. Through the combination of near infrared spectroscopy and gravimetric vapour sorption, it has been possible to show the transition of raffinose from its spray-dried amorphous form to a crystalline form without this characteristic mass loss. It has also been possible to observe changes in the crystalline material for a period of 30 h subsequent to exposure to elevated relative humidity by near infrared spectroscopy that are not associated with changes in mass, but are related to repacking of hydrate molecules. Drying of the crystalline pentahydrate in the DVS-NIR was seen to show changes in the NIR peak related to –OH. From this, NIR peaks were tentatively ascribed as relating to a penta-, tetra-, tri- and a di-hydrate form, but the sample returned to the amorphous response by the time the water content fell to the equivalent of the monohydrate, indicating that crystallinity had been lost. These observations would be compatible with the hypothesis that lower hydrates of raffinose exist. Due to the absence of mass loss in association with crystallisation, it was found that the enthalpy of crystallisation of amorphous raffinose, as determined by isothermal microcalorimetry, is similar to the enthalpy of fusion determined by differential scanning calorimetry. Finally, it was observed that the early part of the response in the isothermal microcalorimeter was related to mobility of molecules when Tg was above T. This mobility was able to give the bulk morphology of a crystal before the sample developed long range order and crystalline properties.

Keywords: Amorphous raffinose; Near infrared spectroscopy; Gravimetric vapour sorption; Isothermal microcalorimetry; Differential scanning calorimetry

1. Introduction

Changes in the physical form of powders are known to be of importance, potentially affecting the stability of the material and also the way in which it behaves during manufacture and use. Recently, a number of methods have been developed by which it is possible to study and quantify the amorphous content of predominantly crystalline powders. These methods include water sorption (Fiebich and Mutz, 1999; Hogan and Buckton, 2001), isothermal microcalorimetry (Buckton et al., 1995), solution calorimetry (Gao

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and Rytting, 1997; Hogan and Buckton, 2000) and spectroscopies (Gustafsson et al., 1998; Taylor and Zografi, 1998; Seyer et al., 2000).

Some materials can exist in many different physical forms, an example being those materials that are known to exist in multiple hydrate levels. Pharmaceutical examples of multiple hydrates include both drugs (e.g. nedocromil sodium, Khankari et al., 1998) and excipients (e.g. carbohydrates, such as raffinose). Raffinose is of interest since recent developments in the ability to manufacture therapeutic macromolecules have led to a need for protein stabilisation in the solid state. Since certain carbohydrates have been shown capable of conferring stability to proteins, many researchers have concentrated on developing a greater understanding of the protein–carbohydrate interaction. Many of these investigations have concentrated on the behaviour of carbohydrates in the presence of elevated temperature and/or humidity (e.g. Green and Angell, 1989; Aldous et al., 1995; Saleki-Gerhardt et al., 1995).

Raffinose (β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1-6)-α-glucopyranoside) is a trisaccharide which, as well as existing in a pentahydrate form, has been shown to form intermediates at lower hydrate levels (Saleki-Gerhardt et al., 1995; Kajiwara et al., 1999). The hydrogen bonding of raffinose is complex, since three of its water molecules are both proton donors and acceptors, whilst the remaining two will act only as donors (Jeffrey and Huang, 1990). The complexity of raffinose as a model is also useful as a way of understanding the value and limitations of analytical techniques, such as gravimetric sorption, near-infrared spectroscopy (NIR) and isothermal microcalorimetry.

The aim of this study was to follow the transitions of raffinose between the amorphous and crystalline states, using primarily the techniques of gravimetric sorption, NIR and batch isothermal microcalorimetry, with a view to improving the understanding of both the methods of analysis and the transitions that take place when complex materials crystallise.

2. Materials and methods

2.1. Preparation of amorphous raffinose

Amorphous raffinose was prepared from raffinose pentahydrate (Pfanstiehl Laboratories Inc., Waukegan, IL) by spray-drying of a 10% w/w aqueous solution in a Büchi 190 mini spray dryer (Büchi, Switzerland). The parameters followed are outlined in Table 1. All yields were confirmed amorphous by X-ray powder diffraction (Philips PW3710 X-ray powder diffractometer, Philips, Cambridge, UK).

2.2. Near infrared spectroscopy/gravimetric vapour sorption

Gravimetric studies of amorphous raffinose samples were carried out in a humidity and temperature controlled microbalance dynamic vapour sorption (DVS) apparatus (Surface Measurement Systems, London, UK). Samples of $\approx 40–70$ mg were dried at 0% RH at 25 °C. A total of 6–10 h was generally sufficient for complete drying to be achieved. NIR spectra of the samples were recorded (Foss NIRSystems, UK) using a fibre optic probe situated ≈ 4 mm below the flat-bottomed quartz glass DVS sample pan. NIR absorbance spectra for all samples were recorded as log $(1/R)$, where R is reflectance. The NIR instrument recorded the mean spectrum of 32 scans (taking ≈ 40 s) over the wavelength region 1100–2500 nm. NIR data processing and analysis was carried out using the Vision® software (Version 2.21) (©Foss NIRSystems, UK). After drying, the raffinose

Table 1
Spray drying parameters used to produce 100% amorphous raffinose from a 10% w/v aqueous solution

Parameters	Controls
Inlet temperature (°C)	120–130
Outlet temperature (°C)	60–65
Air flow rate (dial setting)	5
Atomiser air flow rate (norm l/h)	700

samples were exposed to elevated RH values at 25 °C, in order to induce crystallisation. NIR spectra were recorded throughout the sorption/desorption processes.

2.3. Isothermal microcalorimetry

Heat flow consequent to water vapour interacting with amorphous raffinose was recorded using a 2227 thermal activity monitor (TAM) (Thermometric AB, Sweden) at 25 °C. Samples were accurately weighed into 3 ml glass ampoules, to which was added a hygrostat containing a saturated salt solution to produce a predetermined relative humidity (RH). Sodium chloride (Sigma) was used to produce 75% RH and potassium carbonate (Aldrich) to produce 43% RH. The influence of sample mass was investigated, between 15 and 150 mg. The ampoules were sealed in order to be air-tight and lowered into the equilibration position in the TAM until dQ/dt (the rate of change of heat flow with time) was zero, then lowered into the measuring site of the channel. Due to the nature of the responses obtained, integration of peak areas was carried out from the baseline immediately prior to ampoule lowering. In order to correct for the disturbance due to the lowering of ampoules, ampoules containing hygrostats but no powdered sample were lowered and the average heat flow from four experiments determined. This blank response was subtracted from all enthalpies calculated.

2.4. Differential scanning calorimetry

Thermographs of raffinose samples (amorphous, pentahydrate (as purchased) and samples removed from the TAM) were obtained from a differential scanning calorimeter (Perkin–Elmer DSC 7 or Perkin–Elmer Pyris 1). Samples of 1.5–3 mg were loaded into non-hermetically sealed aluminium pans and measurements were taken under a nitrogen atmosphere from 25 to 200 °C at a heating rate of 10 °C per minute using an empty pan as reference. Prior to measurements, calibrations using indium and tin were carried out under the same conditions. Samples were run in triplicate unless otherwise indicated.

2.5. Scanning electron microscopy

Samples were mounted onto adhesive carbon discs attached to scanning electron microscopy (SEM) stubs and coated with gold by sputtering (Emitech K550 sputter coater, Emitech, Kent, UK) for 4 min at 30 mA. Scanning electron micrographs were obtained using a Philips XL20 SEM (Philips, Eindhoven, Netherlands) (voltages displayed on micrographs).

3. Results and discussion

Many amorphous materials are plasticised by absorbed water vapour. The glass transition temperature (Tg) then drops below the experimental temperature, thus giving the molecules of the samples sufficient mobility to allow rapid crystallisation. As the sample crystallises, it is usual for the absorbed water to be displaced. Consequently, it now seems to have become accepted that a clear sign of the presence of amorphous material in a sample would be a loss of mass on exposure to increasing relative humidity (RH). Conversely, the absence of this mass loss would be interpreted as the absence of a crystallisation event. The absence of a crystallisation event would not mean that there is no amorphous content, but only that the sample did not crystallise, for example, some materials do not crystallise rapidly in the presence of humidity at ambient temperature (Ahmed et al., 1996); others may be protected from crystallisation (e.g. the interaction between a protein and a carbohydrate).

3.1. Crystallisation without mass change

Despite the growing view that mass loss must occur as a consequence of crystallisation, raffinose provides a good example of a crystallising material which does not give rise to such behaviour. The mass change following the exposure of spray-dried amorphous raffinose to 75% RH at 25 °C is shown in Fig. 1. There is substantial water sorption, giving rise to a mass increase of 13.8% w/w, which is sufficient to lower Tg below the experimental temperature (25 °C) (Saleki-Gerhardt et

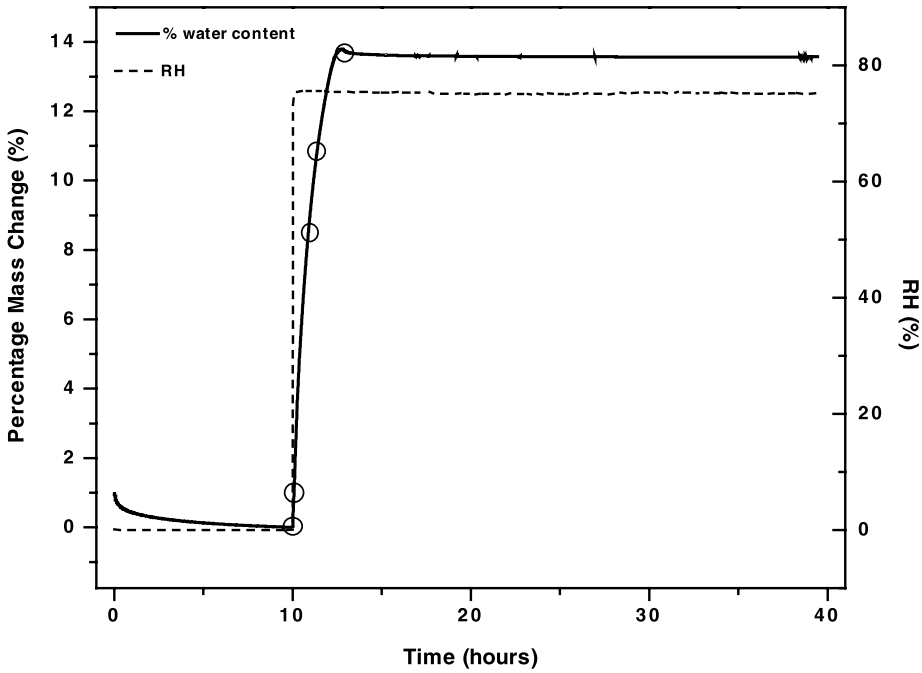
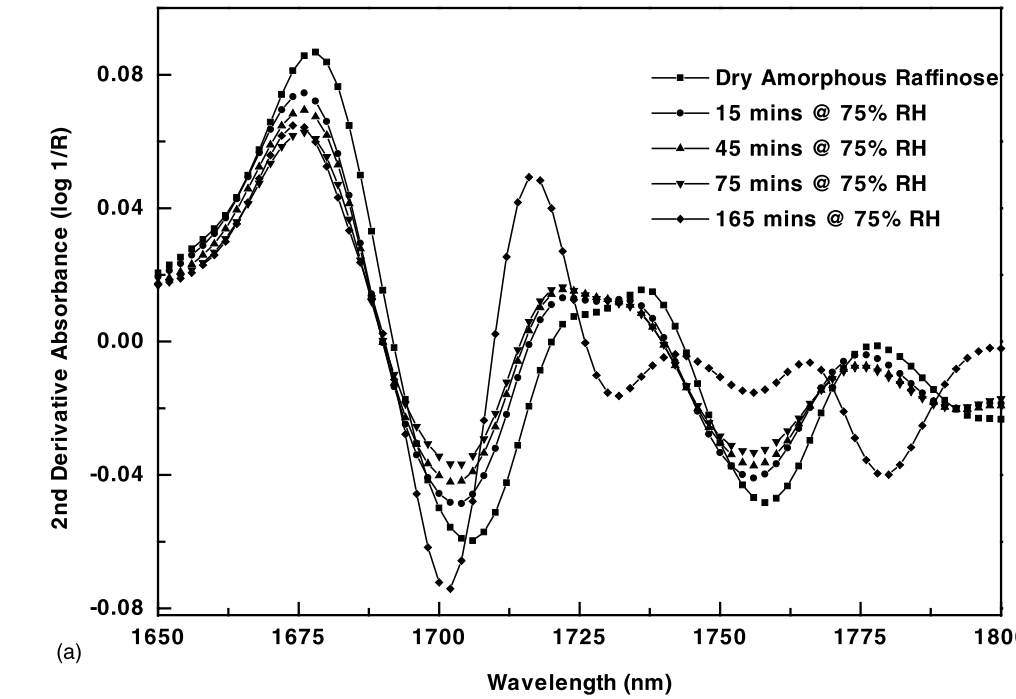


Fig. 1. DVS plot for 100% amorphous spray-dried raffinose dried at 0% RH for 10 h and then exposed to 75% RH for 30 h at 25 °C in order to induce crystallisation. Circles indicate times when spectra in Fig. 2(a) were taken.

al., 1995) and consequently, would give the material sufficient mobility to allow crystallisation to occur. Despite this lowering of Tg, there is no obvious weight loss. The absence of a weight loss would usually be interpreted as an absence of crystallisation, however, if the sample is studied during this process with NIR it can be seen that the sample starts to crystallise after ≈ 165 min at 75% RH. Furthermore, it can be seen from the spectra that the solid state properties of the sample continue to change for ≈ 1800 min (30 h) (Fig. 2a,b). The reason why there is a difference between this sample, showing no significant mass change throughout this long crystallisation process and lactose which shows substantial water loss, is that lactose is able to form a monohydrate ($\approx 5\%$ w/w water), however the amount of water sorption needed to cause rapid crystallisation is far greater than 5% w/w, hence, there is free water to be desorbed. In fact, lactose often retains $< 5\%$ w/w water, due to the presence of some anhydrous crystalline form in the sample. Raffinose is able to form different hydrates depending upon the conditions of temperature and humidity (Saleki-Gerhardt et al., 1995). At 75% RH and 25 °C, the sample seems to crystallise to the tetrahydrate (see further discussion below). The tetrahydrate would show a 14.3% w/w mass gain over the anhydrous form, thus the mass required to form the hydrate is equivalent to that which was absorbed. If mass change alone were relied upon to detect crystallisation of samples for which multiple hydrates can form, then it would be possible to miss the crystallisation event. It follows that the monitoring of the material using mass change alone would be an inadequate method of following changes within the sample. Although the absence of a mass change for the crystallisation of raffinose under these conditions is not surprising, these data do show the clear value of using more than just gravimetry as a method to assess onset of crystallisation.



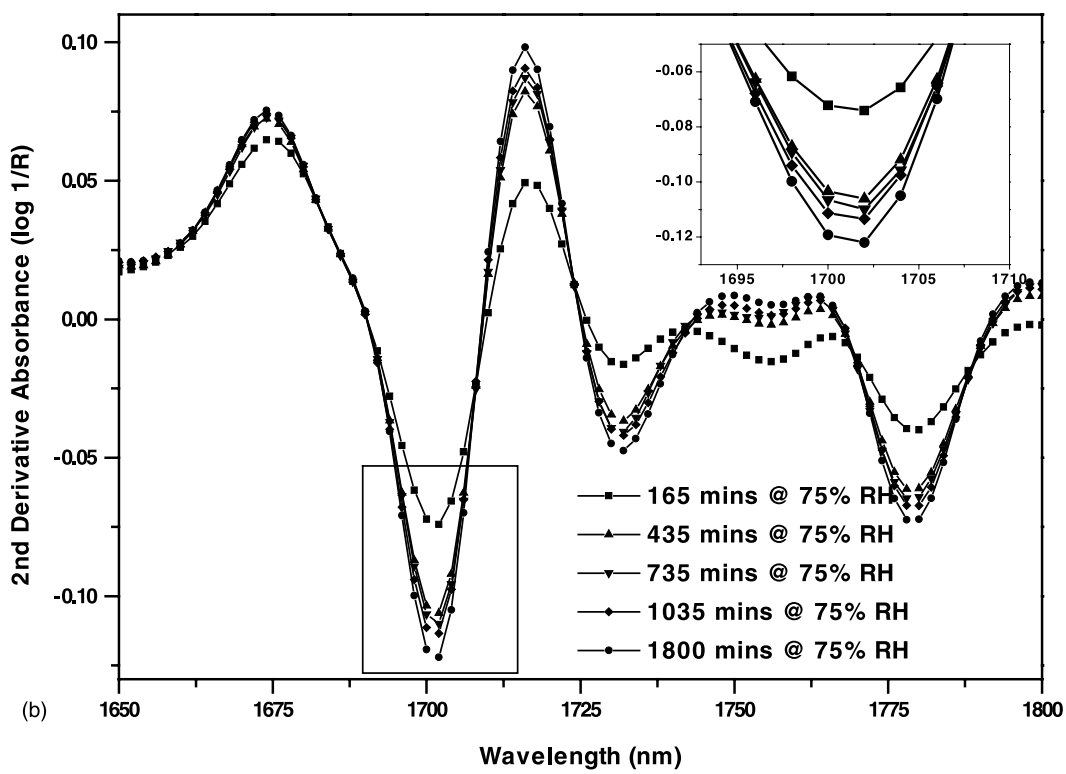


Fig. 2. (a) NIR second derivative spectra for raffinose corresponding to different stages of the DVS plot shown in this figure. Spectra represent the amorphous form and changes up to 165 min exposed to 75% RH at 25 °C, at which point it would seem that a tetrahydrate has formed. (b) NIR second derivative spectra representing the changes observed in the raffinose sample throughout $\approx$30 h exposed to 75% RH at 25 °C.

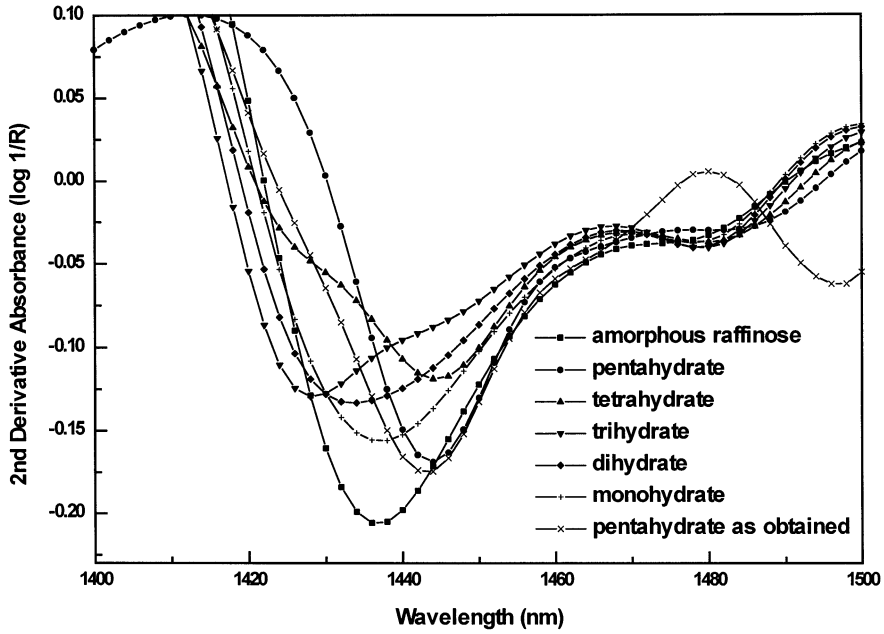


Fig. 3. NIR second derivative spectra corresponding to different hydrate levels by mass (calculated from DVS data). Pentahydrate spectra were taken whilst the sample was still exposed to 75% RH. The spectra for mass equivalent to tetra-, tri-, di- and monohydrates were taken at 0% RH. (Note a mass equivalent to a certain hydrate level does not prove that the structure is that hydrate, as it is possible that mixed hydrates could exist and give the same mass of material.)

3.2. Changes in hydrate level

Exposing the spray-dried amorphous material to 75% at 25 °C gave rise to significant variability in the extent of water sorption. Replicate experiments give peak weight gains within the range 13.7–16.0% w/w (replicate data not shown). The higher end of these mass changes is in excess of that required to form the tetrahydrate, but somewhat less than that required to form a pentahydrate (17.9% w/w). Equally the lower end of these mass changes was less than that needed for complete formation of a tetrahydrate (14.3% w/w) but more than that required for the formation of a trihydrate (10.7% w/w mass gain). It follows that the samples crystallise to mixed hydrates (for example, some regions of trihydrate and some of tetrahydrate, clearly this is an example and other combinations are possible). This gives rise to the question of whether the formation of these mixed hydrate systems can be followed by use of NIR spectroscopy during the crystallisation process.

In Fig. 3, the NIR spectra are shown for a sample that has been dried until the measured mass equates to that of the different stoichiometric hydrates. Other workers (Saleki-Gerhardt et al., 1995; Kajiwara et al., 1999) have used this approach in order to isolate and test different stoichiometric hydrates of raffinose. Kajiwara et al. (1999) have studied the properties of raffinose during dehydration and commented on gradual amorphisation, but also showed evidence of minor changes in X-ray diffraction patterns that indicated the possibility that lower hydrates existed, this being confirmed by DSC such that sequential loss of water atoms was seen at least down to the trihydrate. In common with the work of other authors (Saleki-Gerhardt et al., 1995; Kajiwara et al., 1999), there can be no guarantee that arriving at a sample that has a mass equivalent to a certain hydrate level equates to the existence of that hydrate form, for example a mass equivalent to a tetrahydrate could be achieved by having a mixture of tri- and penta-

hydrates present. Consequently, the data are used in the same spirit as in previous reports using this methodology and do not prove the structures are present.

In the NIR region shown in Fig. 3, it is observed that there is a substantial shift in the peak (the region is that associated with –OH group interactions) at masses equivalent to the different hydrate levels. To collect these spectra, an amorphous sample was crystallised in the DVS-NIR at 90% RH and then dried at 0% RH to gradually remove the water. At 90% RH, the sample gained 19.6% w/w mass, which is more than that required to form a pentahydrate. The sample then crystallised, giving a spectrum at 90% RH for which the mass corresponded to an increase of 17.9% w/w (i.e. it had gained the equivalent of that required to form a pentahydrate). The NIR peak moved from 1436 to 1444 nm as a consequence of crystallisation. Progression through the drying step facilitated the further removal of water and the spectra in Fig. 3 follow this process. The most interesting stage of this peak development is that associated with the removal of sufficient water for the sample to have a mass equivalent to that of a trihydrate. In this instance, the peak moves from its previous position at 1444 nm (tetra-) to occupy a wavelength of 1427 nm (tri-hydrate). The peak for water content equivalent to dihydrate moves towards the position of the response for the amorphous form. Further dehydration (to the equivalent of monohydrate water content) results in the peak returning to the exact position which it had originally occupied in the amorphous form (1436 nm) (the size of the peak being different due to the water content and possibly the changed geometry of the sample[1]). This is in keeping with observations made by Kajiwara et al. (1999), who could only find clear evidence of retained crystallinity when drying raffinose to the trihydrate level. Below the trihydrate water content, it was felt that 'sugar–water' hydrogen bonds were being replaced with 'sugar–sugar' bonds which gave disorder in the long range packing (i.e. loss of crystallinity). The current study (Fig. 3) cannot prove the existence of the different hydrate structures, however the data are compatible with the hypothesis that suggests that penta-, tetra-, tri- and di-hydrate forms exist, but that the sample becomes amorphous when the mass equates to the monohydrate.

There is a difference between the spectra for a sample of pentahydrate that was purchased (from Pfanstiehl) and that produced in the DVS (Fig. 3) in the region of 1495 nm. It is believed that this is because the pentahydrate had not been given long enough to come to equilibrium (given that it has been shown above that changes in internal bonding continue for a long time after crystallisation has occurred). However, the shifts seen that have been tentatively ascribed to the various hydrate levels here (Fig. 3) were also seen when the commercially available pentahydrate was dried in the DVS-NIR (data not shown).

From these data, the possibility of following crystallisation of spray-dried raffinose upon exposure to elevated RH was considered. Fig. 4 illustrates spectra taken during the exposure of an amorphous sample to 75% RH in the DVS-NIR. The first four spectra shown represent the amorphous material and its transitions throughout the first hour of exposure to elevated RH, which corresponds to the period of the experiment when the sample is seen to sorb significant quantities of water. The peak starts at 1436 nm is seen to move slowly up to first occupy 1438 nm and to finally settle at 1442 nm when the sample crystallises. The final two spectra represent the remainder of the 11 h exposure to 75% RH and in this region there is no further change in the NIR spectra. These data indicate that the amorphous material crystallises to the tetra-hydrate (by comparison with Fig. 3 and taking into account the mass change).

Although the peak at 1442 nm does not change significantly after crystallisation has occurred, there are other regions of the spectra which do show changes post-crystallisation. The region around 1700 nm is one such region and this has already been discussed for a different sample (Fig.

[1] The NIR will penetrate throughout the entire sample. The basic NIR spectra are affected by sample geometry, however the data are used having been treated with standard normal variate and second derivative treatments, this results in spectra that show very minimal differences due to sample geometry.

2b). Furthermore, there are changes in the region of 1930 nm (relates to the hydrate content, spectra not shown) where a marked deepening of the response is seen with time, despite the fact that the mass recorded by the DVS does not change, further indicating that water movement within the sample continues long after the sample has ceased to take up or expel any further moisture.

3.3. Isothermal microcalorimetry

The enthalpy of crystallisation of many materials has been studied by use of isothermal microcalorimetry (e.g. Briggner et al., 1994; Aso et al., 1995; Ahmed et al., 1996; Lehto and Laine, 1998). It has been noted (Darcy and Buckton, 1998) that for lactose the measured enthalpy of crystallisation is significantly lower than would be expected (for example, it is much smaller than the melting endotherm enthalpies measured by DSC, which should be equal and opposite to the enthalpy of crystallisation). Darcy and Buckton (1998) have argued that the small measured response is due to the fact that substantial water desorption (endotherm) occurs during the crystallisation and that the net response is thus significantly smaller than the exotherm that would be expected for crystallisation alone. For raffinose, however, as discussed above, it has been seen that there is no significant water desorption when the material crystallises (Fig. 1). In this instance then it would be expected that the net enthalpy of crystallisation would be similar to the enthalpy measured during melting.

3.4. Measured enthalpies during crystallisation

The enthalpy associated with crystallisation that was measured during the TAM experiments (75% RH, 25 °C) was found to have two distinct regions. The first exotherm was substantial and protracted, the exact duration depending upon sample mass, being ≈ 8 h for a mass of 100 mg (labelled A in Fig. 5). The long duration of this region means that it cannot simply relate to thermal equilibration of the sample following lower-

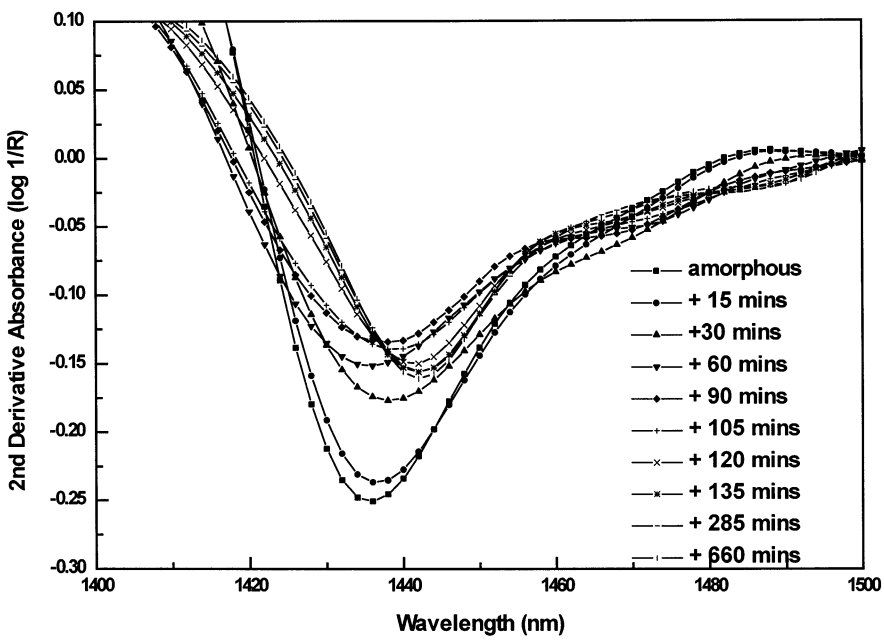


Fig. 4. Second derivative NIR spectra following the spectral progression through wetting and crystallisation of spray-dried raffinose exposed to 75% RH at 25 °C.

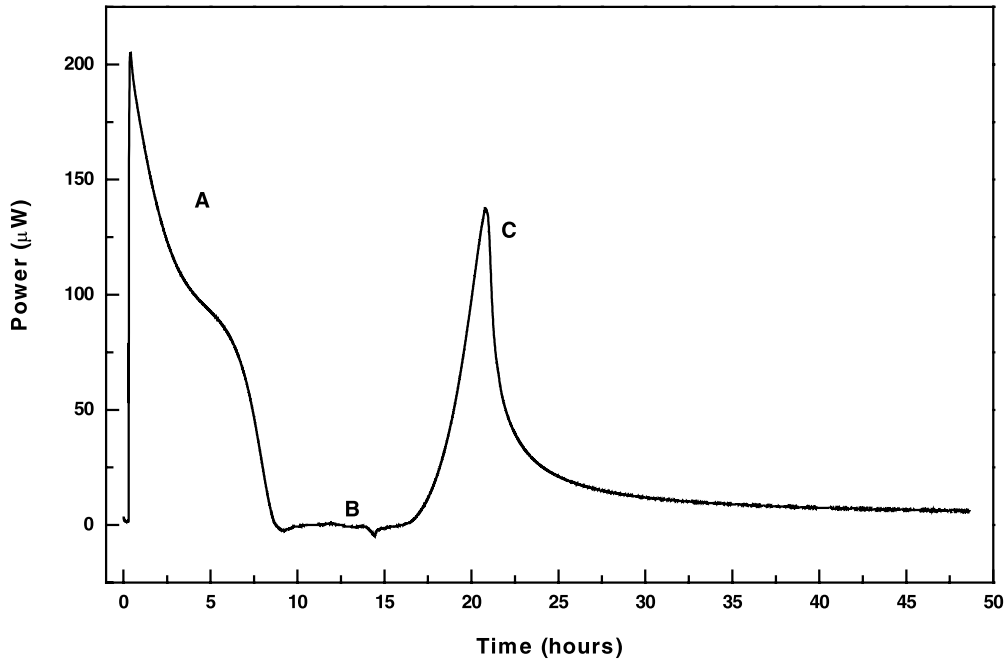


Fig. 5. Crystallisation of spray-dried raffinose in the TAM at 75% RH and 25 °C.

ing into the measuring site. It has been argued that the wetting response would be very small in this type of experiment, as the uptake of water by the sample (exotherm) would be approximately balanced by the generation of that humidity from the saturated salt solution (evaporation being an endotherm). Consequently, the large and protracted initial response is likely to relate to processes other than just wetting, for example a reordering of molecules in the solid as a consequence of water sorption. After the initial exotherm, there is a lag phase that is again sample mass related. This lag phase lasts ≈ 6.5 h for 100 mg sample load (shorter duration with lower mass of sample), during which time the response is close to baseline. The shape of this lag phase response is different if the sample mass is changed and for low loads (15–27 mg) a clear endothermic peak is seen in this region. This peak is less clear but still present for larger sample loads (labelled B on Fig. 5). Following from this there is a further substantial exotherm (labelled C on Fig. 5). It can be seen that this response is very protracted and does not return to baseline even for an experiment of 50 h duration. This prolonged response is in keeping with the changes that are observed in the DVS-NIR experiments (see above) and indicates that the sample has crystallised but continues to undergo solid state transitions post crystallisation. As was discussed above, the solid state transitions relate to transitions from whatever hydrates form during the initial crystallisation to the existence of the most stable form at the experimental conditions. This gives rise to a problem with respect to defining the area under the power-time curve that best describes crystallisation. The lowest enthalpy values were obtained for large sample masses and the high values for low sample mass (15–27 mg). This difference may reflect the fact that the tail has not come close to completion for large sample masses, but the process is almost complete (however, not truly back to baseline) for the small mass samples. Another important factor to consider is the availability of water vapour to the sample. As already discussed, amorphous raffinose requires a 17.9% w/w mass increase in order to form a pentahydrate, therefore a spray-dried sample of 100 mg will require almost 20 mg

of water vapour in order to completely crystallise. It is unlikely that a sealed ampoule/hygrostat experiment would be capable of providing such quantities in the time scales used here. Therefore, it is assumed that the data for the large sample masses will be restricted and they will not be able to form the higher hydrates. Consequently, data for low sample masses were considered further.

The enthalpy value obtained from the total AUC for the TAM experiment for the lowest sample mass (see discussion below) was still lower than the enthalpy measured for melting and dehydration of the commercially obtained pentahydrate using DSC (143 ± 7 J/g, this value being similar to the 149 J/g reported by Kajiwara and Franks, 1998). This is in keeping with the observation (from DVS) that the sample does not equilibrate to form a pentahydrate at 75% RH. However, the total enthalpy measured in the TAM (sum of all peaks) during crystallisation is in keeping with DSC data for dehydration and melt for samples that have been crystallised in the TAM. For example, the mean DSC melt for ex-TAM high mass samples was 39 J/g, which was in keeping with the enthalpies of formation measured in the TAM for large mass samples. The DSC melt for a low mass sample that had been crystallised in the TAM was 74.2 (± 10.9) J/g, as compared with an enthalpy of crystallisation from the TAM (all peaks) of 80.6 (± 14.5) J/g. It follows that there was a good correlation between enthalpy of crystallisation and the enthalpy of melting, indicating that the hypothesis of Darcy and Buckton (1998) was correct in that the water evaporation was the cause of differences between the DSC and TAM experiments for lactose.

3.5. Transitions before crystallisation

From the discussion above, the entire process recorded in the TAM must be measured as being related to crystallisation, with the first endotherm being due to an increased mobility of molecules as the Tg starts to drop and the final phase being crystallisation followed by solid state transitions to optimise hydrate water distribution. If this division into two distinct regions of activity is true, then exposure of samples to an RH that does not give rise to crystallisation should produce a response similar to the first exotherm seen during exposure to 75% RH. Such a response is shown in Fig. 6 for a sample exposed to 43% RH. The area under the curve in Fig. 6 at 43% RH (25 J/g) is similar to that seen for peak A (28 J/g) in Fig. 5 at 75% RH (both samples being of similar mass). If the experiment at 43% RH is stopped and the RH changed to 75% and the ampoule returned to the TAM, then the second exotherm is seen.

A further check of what occurs during the first exotherm was made by exposing an amorphous spray-dried sample to 43% RH and taking SEM photomicrographs. It can be seen (Fig. 7a) that the original spray-dried material consists of spherical amorphous particles, but exposure to 43% RH for 5 weeks (Fig. 7b) causes major bulk changes to take place. It is obvious that the material is developing the external appearance of the crystalline state, although it does not have the properties of the crystal form (no melting point and amorphous by NIR spectroscopy) even after this period of storage. In principle, the material would be expected to crystallise following very long exposure to low RH, although at present the existence of an anhydrous crystalline form and indeed categorical proof of the existence of lower hydrates of raffinose remains absent. Hancock and Zografi (1997) have shown that materials must be stored 50 °C below their Tg for mobility to be sufficiently limited to prevent crystallisation over a multiple year shelf life that would be required for pharmaceuticals. In this instance, raffinose at 43% RH has a Tg of ≈ 27 °C which is in accord with the Tg of 28 °C reported at this RH by Iglesias et al. (2000), so the softening that occurs here allows the formation of a different external morphology but not packing into the necessary long range order for molecules which would define crystallinity. The observation that we report here, that raffinose develops bulk crystalline appearance (sharp edges etc.) without crystallising (in terms of long range order at the molecular packing level) is an extension of, but in keeping with, the observation by Saleki-Gerhardt et al. (1995) and Kajiwara et al. (1999), who observed that heat dried raffinose hydrates be-

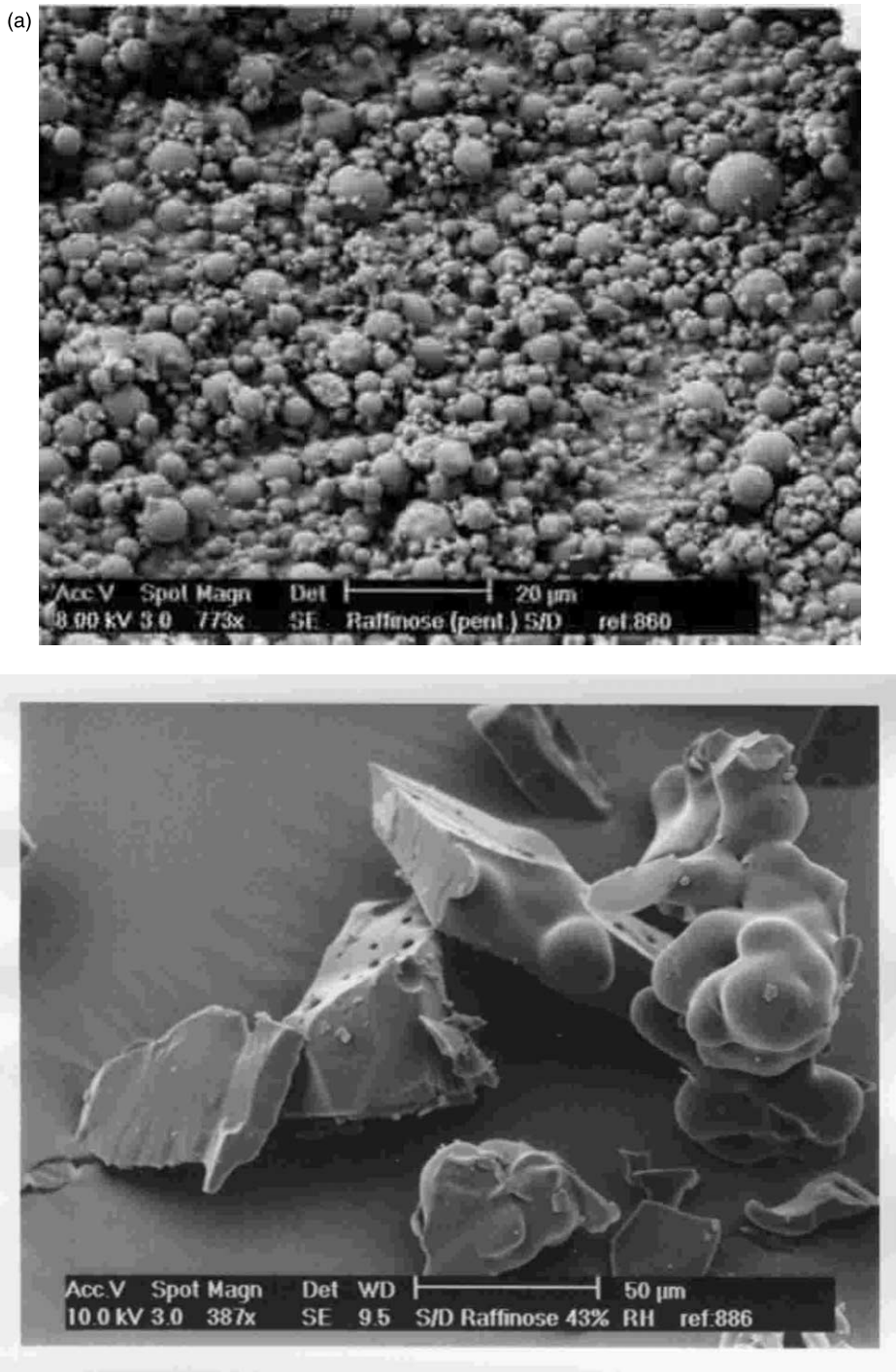


Fig. 7. (a) Scanning electron micrograph of spray-dried amorphous raffinose displaying the characteristic spherical appearance expected. (b) Scanning electron micrograph for spray-dried raffinose following exposure to 43% RH for 5 weeks. Evidence of the previous spherical morphology remains, however, there has been significant agglomeration of morphology similar to that seen with crystals.

came amorphous whilst retaining the external shape of the crystalline form.

4. Conclusions

It has been reinforced that reliance on mass loss and the expulsion of plasticising water is not a reliable method for determining the occurrence of crystallisation. NIR has been found capable of detecting changes in raffinose during prolonged exposure to elevated RHs that are not associated with mass changes, indicating that water movement continues in the sample long after the initial water sorption process. Furthermore, the technique is able to follow changes in hydrate level and onset of amorphisation during drying of crystalline raffinose.

IMC has also indicated that the crystallisation of amorphous raffinose is a prolonged process, and that, depending on the amount of water available to the sample, different hydrates or hydrate mixes are formed, which are characterised by different enthalpies of crystallisation (and fusion, determined by DSC). Since amorphous raffinose does not expel plasticising water during crystallisation, there appears to be much greater agreement between the enthalpies of crystallisation and fusion, compared with those figures in relation to lactose.

Finally, it has been seen that raffinose starts to take on the bulk external morphology of the crystalline form before developing the long range order of the crystalline state. It is argued (e.g. Kajiwara et al., 1999) that the amorphisation of raffinose during drying is due to the disruption of hydrogen bonding causing the formation of new 'sugar–sugar' bonds to replace the 'sugar–water' bonds, giving rise to disruption and amorphous domains. In this study, not only have we observed the conversion to the amorphous form on drying, the crystallisation at high humidity, but also the development of morphology (but not long range molecular order) at low RH.

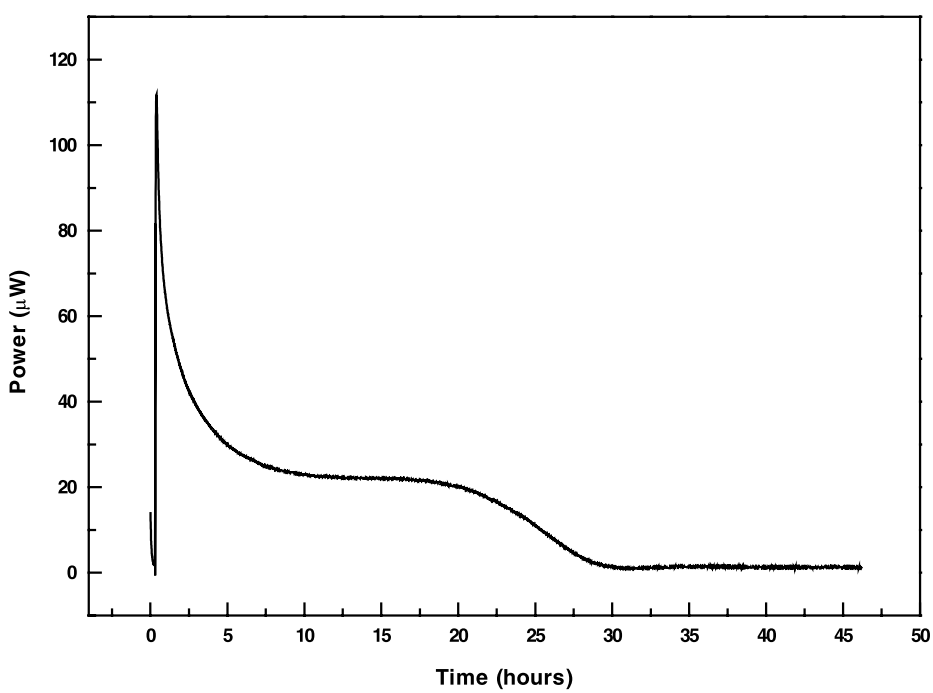


Fig. 6. Spray-dried raffinose exposed to 43% RH and 25 °C in the TAM.

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