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# Adjusting and understanding the properties and crystallisation behaviour of amorphous trehalose as a function of spray drying feed concentration

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**Abstract**

Trehalose is known to stabilise proteins and peptides in the amorphous state. It is believed that crystallisation to a dihydrate is an important aspect of this protection, with trehalose acting as a desiccant to keep water away from, and prevent damage to, the biomolecules. The structure of amorphous materials has an impact on their ability to crystallise (possibly due to the presence of different amounts of short range order). In this study, repeat batches of trehalose were spray dried from different solution concentrations in water (0.5, 5.0 and 10.0% w/v). Each feed concentration resulted in amorphous spheres. Those from the highest feed concentration were found to be the largest particle size (due to more rapid onset of drying) whereas the lowest feed concentration resulted in the smallest size. The high feed concentration resulted in material that reproducibly and readily crystallised when exposed to water vapour, however the excess water (that over and above the amount needed to form the crystalline dihydrate) was not readily desorbed, presumably as it was entrapped between the recently formed crystals. The most dilute feed concentration resulted in samples that exhibited great variability between batches, often requiring a larger mass of water to be sorbed before the crystallisation would begin than was needed for samples from higher feed concentrations. The exception to this was one batch that was observed by NIR to have the aspects of crystalline structure in its amorphous state (presumably due to longer drying times allowing some short range order). Whilst these samples allowed desorbed water to clear the crystallised material more readily than was observed from higher feed concentration samples, the variability was a perceived disadvantage. A reasonable compromise was the material formed from intermediate feed concentration which showed less variability than seen for low feed materials and greater dissipation of desorbed water than for high feed material. It is concluded that the properties of amorphous trehalose are altered as a consequence of processing and care must be taken to optimise this when attempting to stabilise macromolecular drugs.


*Keywords:* Amorphous; Trehalose; Crystallisation; Processing; Spray drying; Protein stabilisation

## 1. Introduction

Trehalose is a disaccharide that has been found to be an excellent material to use to stabilise proteins in the amorphous state (e.g. Colaco et al., 1994). Indeed, trehalose has been shown to be present in desert plants where it is thought to provide protection against drought. It has been argued (Aldous et al., 1995) that trehalose has excellent ability to stabilise proteins and peptides due to the fact that it can sorb water and then crystallise and phase separate from the protein/peptide, thus acting as a desiccant removing large amounts of water that may otherwise result in detrimental crystallisation of the protein/peptide itself. Raffinose (which forms a pentahydrate in plentiful water vapour) is equally known to have excellent stabilising properties (Saleki-Gerhardt et al., 1995). Crowe et al. (1996) found that increasing the water content of amorphous trehalose resulted in the glass transition temperature (*T*g) remaining high, whereas amorphous sucrose was seen to have the *T*g reduced by the sorbed water, the conclusion being that the trehalose partially crystallised to the dihydrate and the remaining material was essentially dry rather than plasticised by water sorption. The formation of the higher hydrate crystalline forms by some sugars, and the desiccation value of extracting large amounts of water into the higher hydrate structure, would therefore seem to be vital in relation to the biostabilising function of these materials. As such it is necessary to understand factors that impact on the ability of amorphous trehalose to crystallise.

Previously, it has been shown that changes in spray drier conditions can give rise to amorphous materials with different physical stability (Ohta and Buckton, 2005). This is believed to

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be due to the changes in local order within the amorphous material, given that the definition of the amorphous state is an absence of long range order, but very clearly there can be presence of short range order to different extents depending upon processing and storage conditions. It would seem vital therefore to understand whether changes in processing can give rise to changes in properties of amorphous trehalose, which in turn could impact on their tendency to crystallise and thus their functionality as biostabilisers.

Water vapour sorption is a clear mechanism by which amorphous hydrophilic materials can be made to crystallise. It is well understood that the absorption of water will plasticise the amorphous material (lower the glass transition temperature) and give sufficient mobility for the sample to crystallise more rapidly. The rate of crystallisation will be linked to the extent of water sorption, and it is hypothesised that it will also relate to the existence of short range order in the amorphous state (those samples with a longer drying time having a better chance to become ordered even though not crystalline).

The simultaneous use of gravimetric water sorption and NIR spectroscopy was introduced as a method by which the water sorption and its impact on physical form can be studied simultaneously (Lane and Buckton, 2000). This hyphenated approach has been used by Hogan and Buckton (2001) to study the water sorption to amorphous raffinose and the subsequent formation of different hydrate levels.

Such a method is very well suited to investigate the crystallisation behaviour of trehalose that has been prepared using different spray drying feed solutions.

## 2. Materials and methods

Trehalose α-(D) dihydrate was spray dried from 0.5, 5 or 10% solutions in water (250 ml volume) using a Büchi 191 Mini Spray Dryer (Büchi, Switzerland). Parameters used are outlined in Table 1. Spray-dried products were stored in a desiccator over phosphorus pentoxide (0% RH) until use. Samples were confirmed as amorphous using a Philips PW3710 X-Ray Powder Diffractometer (Philips, Cambridge, UK). Sample (<1 g) was loosely filled into the shallow well of the XRPD sample holder. A small purpose made block of Perspex (made in-house) was used to press carefully the sample into the sample holder cavity to create a smooth, level, finished powder surface. Any excess sample surrounding the circular sample holder cavity was carefully removed. The powder bed achieved by this method measured a depth of ∼2 mm. The sample holder was loaded into the diffractometer and scanned between 5–50° 2θ. Samples were measured at 45 kV and 30 mA.

Table 1
Operating parameters for the preparation of spray-dried trehalose powders for the feed concentration variability study using the Buchi 191 Mini Spray Dryer

| Operating Parameters | Settings |
|---|---|
| Inlet temperature (°C) | 128–130 |
| Outlet temperature (°C) | 75–77 |
| Feed rate (ml/min) | 2 |
| Pressure (bar) | 3 |
| Atomiser flow rate (normliter/h) | 600 |
| Machine settings (%) | |
| Aspirator | 80 |
| Feed rate | 18 |

Anhydrous crystalline trehalose was produced by holding the dihydrate under vacuum for 4 h at 85 °C, and for 4 h at 130 °C; this yielded alpha and beta anhydrous trehalose respectively (Reisener et al., 1962). For each form, the X-ray diffraction data matched published values (Sussich et al., 1997, 1998; Nagase et al., 2002).

Gravimetric studies of spray-dried trehalose samples produced from various spray dryer feed solution concentrations were carried out using a humidity and temperature controlled microbalance (dynamic vapor sorption (DVS) apparatus, Surface Measurement Systems, London, UK). Samples were loaded onto the flat-bottomed quartz glass sample pan of the DVS apparatus, were dried at 0% RH and subsequently exposed to 75% RH for 10 h to induce crystallisation of amorphous material before a second drying stage. The NIR spectrometer recorded a mean of 32 scans over the wavelength region 1100–2500 nm every 2.5 min or every 15 min of the DVS experiment, via a fibre optic probe (Foss NIRSystems, UK), which was housed immediately below the DVS sample pan (in the RH and T controlled environment). Samples were checked for crystallinity using X-ray powder diffraction as described above.

Scanning electron microscopy was undertaken on sputter coated samples using a Philips XL20 (Eindhoven, Netherlands) microscopy.

## 3. Results and discussion

The particles produced from spray drying 0.5, 5 and 10% w/w/ solution of trehalose were all found to be spherical and all yielded "amorphous halo" patterns when studied using X-ray diffraction, with no signs of peaks, indicating absence of crystallinity (within detection limits). The size of the particles produced did differ, as can be seen in Fig. 1, with the size increasing with the higher feed concentrations. The size of the particles is determined by the stage during drop evaporation when solids start to form. Whilst all particles will be smaller than the droplet from which they precipitate, the droplets with lower solids content will be formed at a later stage, that is, following a longer process of evaporation prior to precipitation onset.

Hydrophilic materials will sorb substantial quantities of water into their amorphous form(s), which in turn will plasticise the solid and increase molecular mobility, which in turn will accelerate the process of crystallisation. This behaviour is readily observed using a temperature and RH controlled microbalance as a rise in mass followed by mass loss (for example Buckton and Darcy, 1995).

The water sorption behaviour of three repeat batches of amorphous trehalose produced from a 0.5% w/w/ feed solution are shown in Fig. 2. It can be seen that the initial water content was different (5.6, 3.3 and 2.8%), but that all samples had dried to a plateau value after 6 h at 0% RH. Following exposure to 75% RH, the three samples behaved differently. Whilst one sample peaked at a mass gain of *c* 13.5% the other two peaked at just

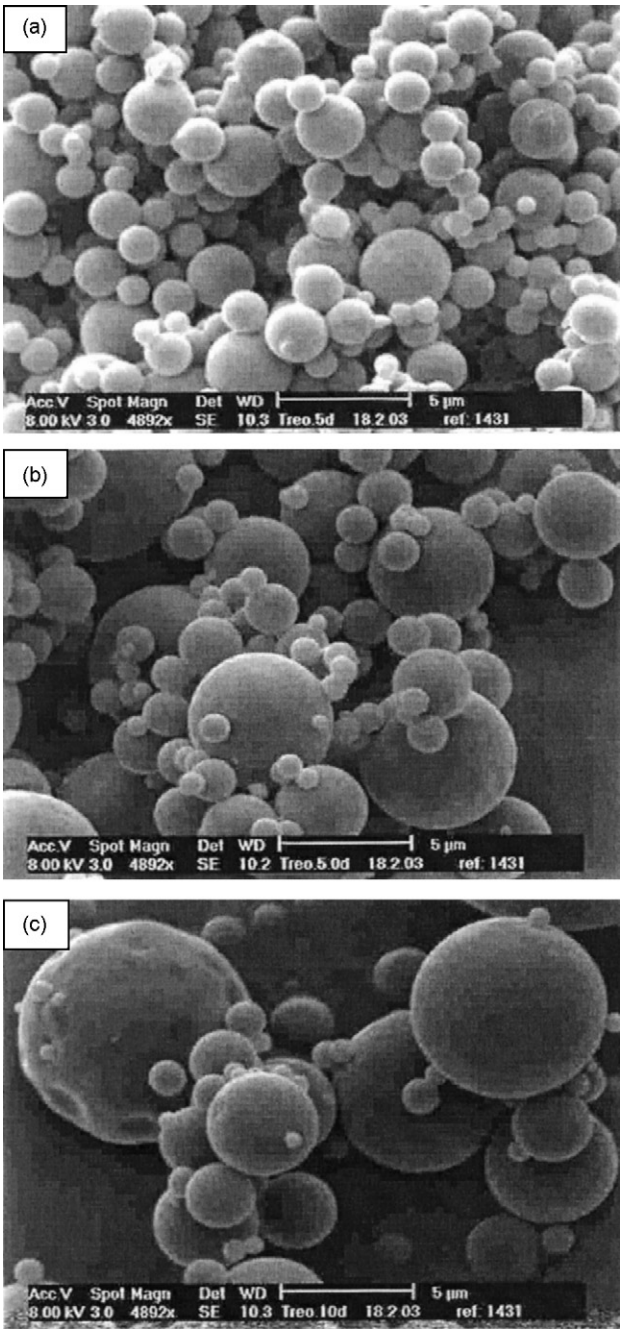


Fig. 1. Spray-dried trehalose from (a) 0.5% w/w (b) 5.0% w/w and (c) 10.0% w/w feed solutions.

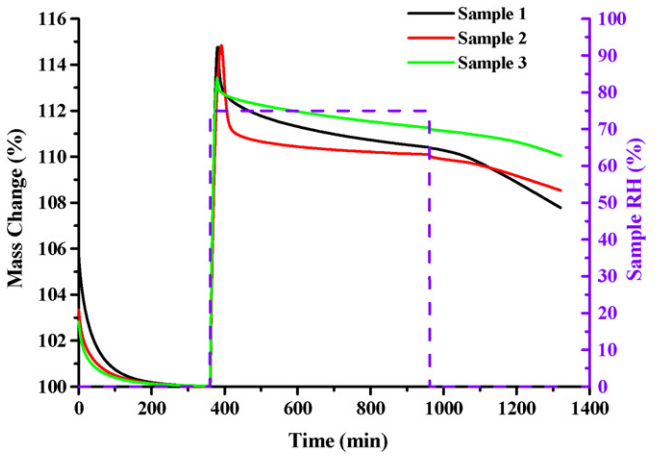


Fig. 2. DVS plots for three spray-dried trehalose samples exposed to 0% RH for 6 h, 75% RH for 10 h and then 0% RH for a further 6 h (at 25 °C). Samples were spray dried from 0.5% w/v trehalose in water solutions. .

over 14.5% mass gain. The time to peak also varied, with "sample 2" lagging behind the other two samples. Whilst maintaining 75% RH, all three samples continued to lose mass after the peak water uptake, but the mass loss profile was very different in each batch. In each case, the retained mass was higher than the 10.5% water gain that equates to the dihydrate. In order to understand why these three samples showed different behaviour, NIR spectra were recorded at the end of the initial drying sequence (in situ in the DVS instrument) and these are shown in Fig. 3. It is obvious, in each of these regions of the spectra, that these three samples are different. The 1350–1390 nm region is attributed to the first C–H overtone in $CH_2$ moieties, those at 1860–1890 nm are less readily ascribed to any functional grouping.

The spectra in Fig. 3 were compared to NIR traces for the crystalline anhydrous forms of trehalose, which were measured in dry conditions using an identical method. The spectra seen for the spray-dried samples 1 and 3 did not match those observed for either of the anhydrous materials, however sample 2 had peaks at 1860 and 1886 nm which were also present in the alpha anhydrous trehalose. From this, it can be concluded that the internal bonding in the samples differs between batches, with one of these (sample 2) potentially showing some retention of an anhydrous crystalline form arrangement. It should be stressed that this does not mean that the sample was partially crystalline, but rather that short range order was present which was of the form that is in the anhydrous crystalline form. It is interesting that the sample with peaks that correspond to the crystalline form is the one that most readily crystallises (Fig. 2), in that the mass loss (onset of crystallisation) was seen rapidly and after a lower mass gain than was observed for the two samples that did not have NIR peaks corresponding to the crystalline state. Furthermore, this sample (sample 2) also equilibrated rapidly (followed by a small slow drift) to a mass gain that is in keeping with the water content of the dihydrate, whereas the other two samples showed slow water redistribution and loss over the period at 75% RH, with sample 3 not reaching the mass expected for the dihydrate. These data show that for dilute feed solutions there is considerable variability in the ordering of molecules in the amorphous state. Whilst none of these samples were discernibly crystalline (by X-ray), the one with peaks that were common to a crystalline form was found to crystallise more readily and more easily equilibrate to the dihydrate.

It is important to realise that the amorphous state is a lack of long range order rather than a lack of order. The presence of different order in the amorphous state has been identified here using NIR and the differences have significance for the properties of the amorphous form. Given the role that trehalose can play in stabilising amorphous structures, we believe that this is an important finding.

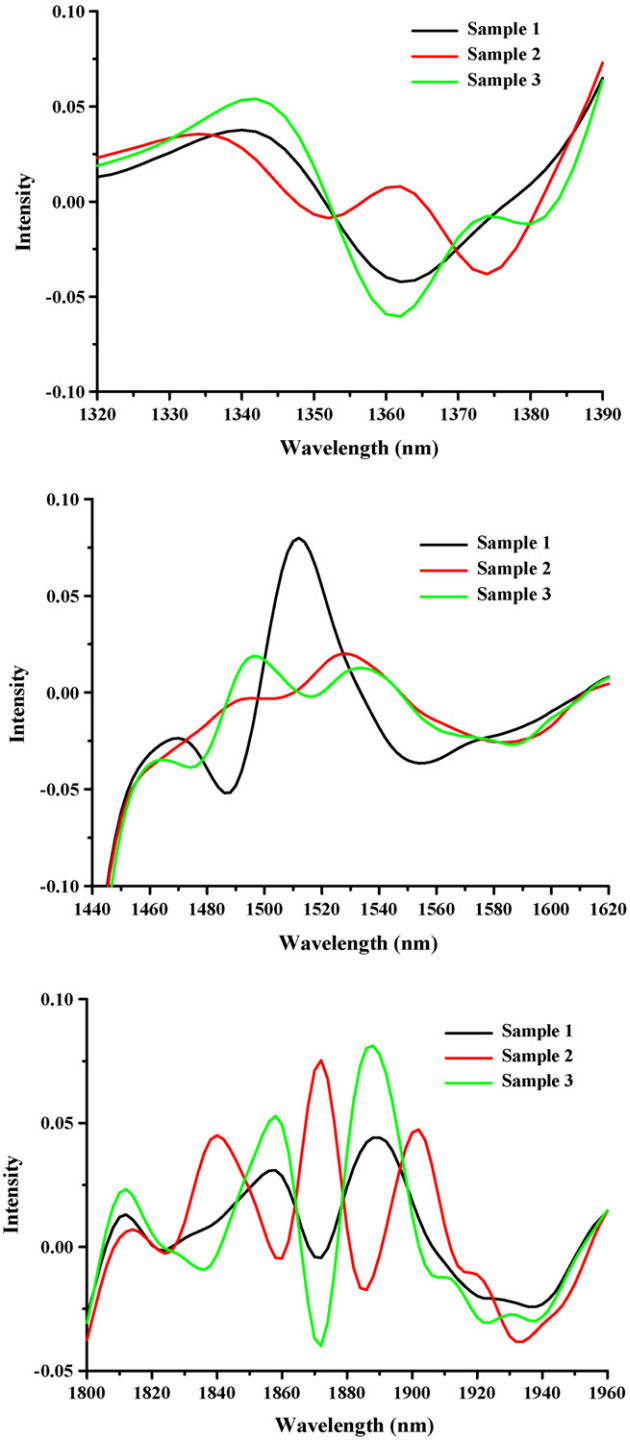


Fig. 3. NIR spectra (between 1320–1390 nm, 1440–1620 nm and 1800–1960 nm) of spray-dried trehalose samples 1, 2 and 3 from the end of the first drying stage of the DVS experiment shown in Fig. 2.

### 3.1. Amorphous trehalose prepared from 5% w/w/ feed solution

The water sorption (0% - 75% - 0% RH) data for three batches of this feed concentration are shown in Fig. 4. It can be seen that the onset of crystallisation was much more consistent than had been observed for the 0.5% w/w/ feed, however this was followed by two samples behaving in an identical fashion but one showing a very different water sorption pattern. All three samples exhibited unexpected behaviour in that the mass continued to fall during the period at 75% RH. Whilst two samples did end up with the mass expected for the dihydrate, one sample (sample 4) was seen to be losing mass even though the uptake was below that expected for the formation of a dihydrate. The NIR traces (not shown) for these three samples were very similar to each other, with two being essentially identical (samples 4 and 5) and one being slightly different in the region 1340–1380 nm (sample 3) (the difference being a peak at 1360 nm for two samples, and at 1373 nm for the other). Surprisingly, the two samples which were the same based on NIR were not the two which showed the same water sorption behaviour. The implication of this would seem to be that these three samples were sufficiently similar in structure that the onset of crystallisation was identical. The difference observed subsequent to onset for sample 4 may well have been unrelated to the initial sample structure.

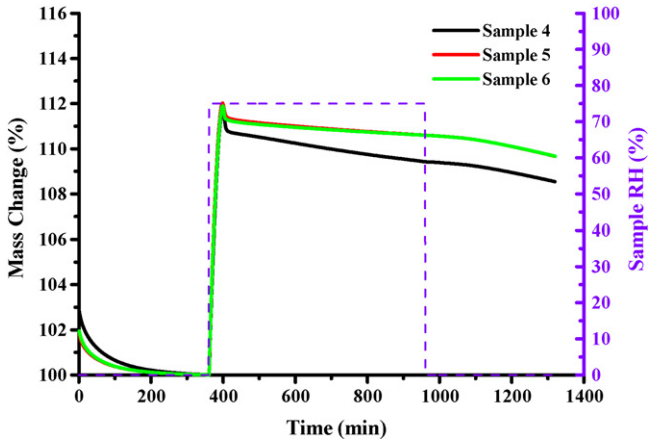


Fig. 4. DVS plots for three spray-dried trehalose samples (runs 4–6) exposed to 0% RH for 6 h, 75% RH for 10 h and then 0% RH for a further 6 h (at 25 °C). Samples were spray dried from 5.0% w/v trehalose in water solutions.

### 3.2. Samples spray dried from 10% w/w/ feed solution

The water sorption data for three batches of 10% feed amorphous trehalose are shown in Fig. 5. The three traces are superimposed and show a good deal of reproducibility in the behaviour of the spray-dried material. Equally, the NIR traces for the three samples at the end of the first drying stage were indistinguishable (not shown). The water sorption traces do, however, show that even though the results are consistent between samples, the mass uptake is higher than expected, showing retention of water over and above that needed to form the dihydrate, most probably due to the entrapment of water between the newly formed crystals, with limited ability to desorb (as has been reported previously for other materials, Columbano et al., 2002a,b)

## 4. General discussion

The results from the three feed concentrations used during spray drying reveal that, for progressively more dilute solutions,

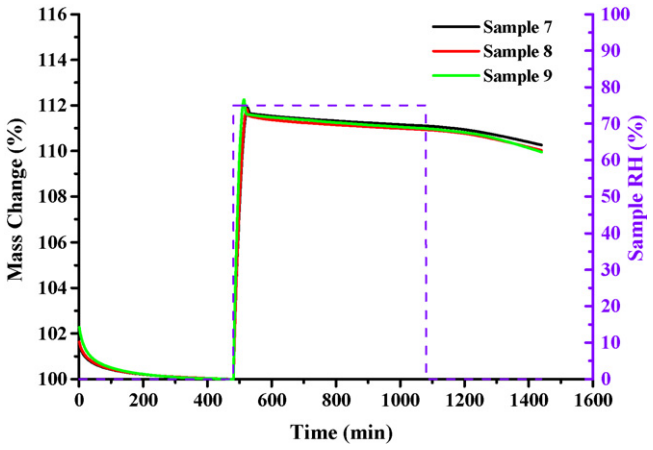


Fig. 5. DVS plots for three spray-dried trehalose samples (runs 7–9) exposed to 0% RH for 8 h, 75% RH for 10 h and then 0% RH for a further 6 h (at 25 °C). Samples were spray dried from 10.0% w/v trehalose in water solution.

there is greater variability in the structures that are formed and this in turn results in variability in the water sorption and crystallisation behaviour of the amorphous material. Spray drying dilute solutions will result in longer drying times, as more water must be removed before any solid material will be formed. The shorter drying time from more concentrated feed solutions result in samples that are more consistent in behaviour, but which are complex in their tendency to crystallise, perhaps due to the fact that the amorphous form has not been able to form structures akin to crystal packing.

It can be seen from Figs. 2, 4 and 5 that the samples made from the most dilute feed resulted in the greatest variability in crystallisation, with two of these samples needing larger water sorption to result in spontaneous onset of crystallisation. The two higher feed concentrations gave more consistent data with respect to the water sorption required for onset of crystallisation. The sample from the most dilute feed that was able to crystallise most readily was the one in which signs of crystalline like bonding was observed with NIR. For the higher feed concentrations, it is possible that the greater sample mass in each particle may have been significant in aiding crystallisation onset. Samples formed from 0.5 and 5.0% w/v feed solutions showed variability with respect to mass loss post onset of crystallisation. Each of these showed the presence of dihydrate by NIR, but each sample had either excess water (presumably physically entrapped between recently formed crystals, and hence slow to desorb), or incomplete hydrate formation. Only the sample from the 10% w/v feed solution had entirely consistent results, here the mass did not return to that expected for the dihydrate, which is believed to be a reflection of crystallisation followed by some desorption of the plasticising water, with that desorbed water being trapped between the newly formed crystals and unable to freely desorb. It is clear that the large size and mass of amorphous particles that are produced from high feed concentrations allows a much more consistent crystallisation and more consistent entrapment of water between those crystals.

Given that trehalose is believed to protect proteins/peptides by its desiccant action during crystallisation, it is reasonable to believe that these products of different feed concentration may well have different properties. It could well be that the high concentration which liberates free water that is unable to diffuse from voids within the crystallised sample may be detrimental to proteins (when present). It may well be that lower feed concentrations (especially the lowest) are better, in that they sorb more water initially and then more readily liberate the desorbed water (that is not in the hydrate) back into the atmosphere. The issue with these dilute samples is, however, great variability between batches. Perhaps the best compromise, therefore, is the intermediate concentration with more consistency, and relatively greater ability of desorbed water to diffuse away than is seen for the 10% feed.

## 5. Conclusion

Spray drying trehalose from different feed concentrations resulted in different packing in the amorphous state, due to different drying rates. This resulted in changes in particle size and also changes in the ability to crystallise. The samples made from dilute feed were found to vary most, they generally required more water to cause crystallisation onset (perhaps due to greater space between the molecules limiting crystallisation onset), but when crystallisation started they more readily allowed water that had been desorbed to escape the crystallised mass. The most consistent data were for the highest feed concentration, however these samples were least able to allow desorbed water to diffuse away from the crystallise mass.

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