

An Investigation into the Crystallization of α,α -Trehalose from the Amorphous State

Orla S. McGarvey, Vicky L. Kett, and Duncan Q. M. Craig*

School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, U.K.

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The nonisothermal behavior of amorphous α,α -trehalose has been studied in order to identify the factors influencing the recrystallization process. Particular emphasis has been placed on examining the effect of differential scanning calorimetry (DSC) pan type and initial water content on the thermal response. Samples of freeze-dried trehalose with water levels up to 6% w/w were sealed in pinholed and hermetically sealed pans and analyzed using DSC at a scanning rate of 10 °C/min in conjunction with thermogravimetric analysis (TGA), with the dihydrate studied in pinholed pans for comparison. The dihydrate was found to exhibit two endotherms at ca. 100 °C and 122 °C, followed by a further endotherm at 211 °C that was attributed to melting of the anhydrate. Isolation of the material following the first endotherm indicated that the material generated at this temperature was the T_γ form. Amorphous material heated in pinholed pans exhibited an exotherm at ca. 100–150 °C (the value depending on water content) when the initial water levels were 4.1% or below. This response was accompanied by dehydration and was attributed to the formation of the crystalline anhydrate. Higher water content samples, however, showed an exotherm followed immediately by an endotherm, with the water loss taking place in two distinct stages. XRD studies indicated that these samples initially crystallized into the T_γ form, followed by subsequent dehydration to the anhydrate. Samples analyzed in hermetically sealed pans showed a single exotherm between 100 and 150 °C followed by a broad endotherm between 150 and 200 °C, with higher water content samples having lower values for both events. XRD indicated that the exotherm and endotherm corresponded to the formation and fusion of the T_γ form, respectively. The study has therefore shown that the recrystallization behavior of amorphous trehalose is dependent on both the initial water content and the environment in which recrystallization takes place.

Introduction

Trehalose (α -D-glucopyranosyl, α -D-glucopyranoside) is a nonreducing disaccharide that has been investigated in recent years for use as a cryoprotectant for proteinaceous and other drugs that may be susceptible to degradation during freeze-drying.¹ Trehalose occurs naturally as the α,α -isomer in yeasts, certain varieties of insects, algae, and higher plants such as *Rhizobium leguminosarum*, although other isomeric forms (α,β -trehalose and β,β -trehalose) have been prepared synthetically. The organisms in which trehalose occurs naturally have been shown to induce production of the disaccharide on desiccation, resulting in cell preservation until rehydration takes place; such behavior has been extensively studied as a means of preserving cells and biological membranes via exogenous addition of the cryoprotectant.^{2,3}

In common with the majority of cryoprotectants, trehalose exists in the amorphous form following freeze-drying. An understanding of the properties and stability of this amorphous material is essential in order to optimize processing and storage conditions, both because there may be an association between the physical form of the disaccharide and the corresponding protective activity and also because any products based on this material must be physically stable over the shelf life of the product.⁴ More specifically, it has been suggested that the tendency of amorphous trehalose to partially or completely crystallize into hydrate forms may lead to the material acting

as a “sink” for excess water in the frozen or freeze-dried product, thereby protecting the protein from hydrolysis while also serving to maintain a high glass transition (T_g) within the remaining amorphous matrix via removal of plasticizing water.¹ However, it should be noted the generation of hydrate forms on storage of an amorphous freeze-dried product is unacceptable from a pharmaceutical regulatory viewpoint, irrespective of the effect on protein stability, as it is essential that the physical structure of a formulation remains essentially unchanged over the shelf life of a product. On this basis, it is essential to have an understanding of the interconversion of physical forms of the trehalose, both to assist understanding of the mechanisms of cryoprotection and to enable prediction of solid state changes that are likely to occur on product storage.

Given the importance of understanding the recrystallization of trehalose from the amorphous state, it is perhaps surprising that comparatively few studies have addressed this as a specific issue. The crystalline state of trehalose and associated interconversions have been discussed by numerous authors,^{5–12} although the precise number of polymorphic and pseudopolymorphic modifications that can exist is still uncertain. A significant proportion of work published on the crystalline forms of trehalose has concentrated on transitions occurring during the heating of the dihydrate, leading to dehydration and possibly recrystallization into one of a number of possible crystalline forms, depending on the experimental and sample conditions used. The literature is somewhat contradictory in terms of the number of crystalline forms that may be generated, almost certainly because the behavior of the system appears to be highly

* Corresponding author. Tel/Fax: 44 (0)28 90 272129. E-mail: duncan.craig@qub.ac.uk.

dependent on the experimental conditions used. There is general agreement on the existence of a stable dihydrate (commonly denoted T_h) and anhydrous form (T_β). However Sussich et al.⁸ have suggested that a second anhydrous form may be generated via gentle dehydration of the dihydrate (i.e., using low heating rates and allowing water to escape). This form (denoted by the authors as T_α) effectively retains the molecular conformation and unit cell characteristics of the dihydrate and may be easily rehydrated on exposure to water due to the comparatively open crystalline structure of this material. On rapid heating the more stable and less hygroscopic T_β anhydrous form may be generated. Sussich et al.⁹ have also suggested that trehalose may also exist in a form designated T_γ , generated by cold crystallization of partially dehydrated dihydrate at ca. 115 °C. Interestingly, these authors suggest that the form is a mixture of the dihydrate and anhydrous (T_β) materials, possibly comprising an anhydrous shell around a dihydrate core on a particulate basis. The authors also stress that the form may only be generated at heating rates between 12 and 40 °C/min. Macdonald and Johari,¹³ however, have suggested that there are only two true crystal forms of trehalose, the T_β anhydrous and a single dihydrate; these authors acknowledge the existence of the T_γ form but do not consider this to be a distinct phase. A further important consideration is the generation of an amorphous phase on heating the dihydrate. Taylor and York⁵ have suggested that the amorphous phase may be an intermediate in the conversion of the dihydrate to the anhydrous material depending on the particle size of the sample, with smaller fractions forming an intermediate amorphous phase followed by recrystallization while larger particles undergo a solid–solid transition to the hydrate. There has been additional debate regarding the existence of a further anhydrous form (designated Form II), prepared by leaving the dihydrate at 323 K for 48 h under vacuum.¹⁴ Nagase et al.¹⁵ have also referred to a further anhydrate as the T_k form, suggesting that this may be equivalent to the T_α form reported by Sussich et al.⁸

As stated earlier, the recrystallization of the amorphous material has not been widely studied, despite the suggestion by Aldous et al.¹ that this process may be intrinsically associated with cryoprotection. In this investigation we have attempted to delineate the recrystallization behavior of amorphous trehalose by focusing on two experimental variables that we believe may have been responsible for some of the confusion in the literature. In the first instance, we have studied the effect of DSC pan type on the thermal response of both trehalose dihydrate and amorphous trehalose. Previous studies in the literature on the effect of pan type have focused on transitions occurring in the crystalline dihydrate form (e.g., ref 5), but to date this has not been studied in the context of amorphous material. Second we have investigated the effects of water content on the recrystallization behavior of the amorphous material. By using DSC, TGA, and powder XRD in conjunction, we intend to both identify the forms adopted by the amorphous trehalose and to understand how different experimental conditions may influence the recrystallization process, with concomitant implications for understanding the stability of freeze-dried trehalose products.

Materials and Methods

Materials. α,α -Trehalose dihydrate (99.9% purity) was obtained from Sigma and used unfractionated and without further purification. The approximate particle size range of the crystals was 50–100 μm .

Preparation of Amorphous Trehalose. Amorphous trehalose was prepared by freeze-drying using an Advantage freeze-dryer. Aqueous trehalose solutions at a concentration of 500

mM were freeze-dried in 7 mL serum vials with a fill volume of 0.5 mL. Vials were cooled to –50 °C and held for 2 h before proceeding with the primary drying at shelf temperatures from –20 °C to +30 °C and the chamber pressure at 500 mTorr for 6 h followed by secondary drying at 60 °C and 800 mTorr for a further 8 h. Vials were stoppered under a dry nitrogen atmosphere. The amorphous nature of the material was confirmed using XRD. After freeze-drying, the samples were found to contain $1.4 \pm 0.7\%$ w/w water. The vials were stored unstoppered at 12% RH for up to 5 days to obtain materials with water contents up to 3% w/w, over 33% RH for up to 7 days to obtain samples with water contents up to 6% w/w. Four vials were prepared under each set of conditions and samples taken from each vial for analysis; tabulated data represent averages of the four samples.

Preparation of T_β and T_γ Forms. The T_β form was prepared by heating a sample of amorphous trehalose at low initial water content (<2.0% w/w) in pinhole pans in the DSC at 10 °C/min and stopping the experiment at just beyond the recrystallization exotherm; the sample was then cooled to room temperature and the material isolated. The T_γ form was prepared using a method based on that of Sussich et al.⁹ whereby trehalose dihydrate was heated in a pinholed DSC pan at a scan rate of 20 °C/min and the experiment terminated at just beyond the first endothermic transition and then allowing the sample to cool to room temperature. Samples of freshly prepared T_β and T_γ were characterized by XRD, and characteristic peaks specific to each form were found to be comparable to existing literature values.

Thermal Analysis. Differential scanning calorimetry (DSC) measurements were carried out using a TA Instruments DSC 2920 with a refrigerated cooling system attached. Dry nitrogen was used as the purge gas at a flow rate of 30 mL/min through the DSC head and 150 mL/min through the RCS unit. The DSC was temperature calibrated using three high-purity temperature calibrants, namely, *n*-octadecane ($T_m = 28.24$ °C), indium ($T_m = 156.60$ °C), and tin ($T_m = 231.93$ °C). The cell constant was calibrated using indium. Experiments were run in either pinhole or hermetic aluminum pans (Perkin-Elmer Part No: BO14-3021 and 0219-0062) at a scan rate of 10 °C/min from 0 °C to 240 °C. Between 2 and 4 mg of sample was weighed out into each pan and sample and reference pans were weighed out to within ± 0.1 mg of each other. Pans were sealed using a Perkin-Elmer universal crimping press. To eliminate water uptake during sample preparation, hydrated amorphous samples were handled in a dry nitrogen atmosphere glovebox.

Thermogravimetric analysis (TGA) was used to measure water content of all samples, and experiments were carried out on a TA Instruments Hi-Res 2950 instrument. Samples were analyzed at a heating rate of 10 °C/min from 0 °C to 230 °C in pinholed pans.

Samples were analyzed by X-ray powder diffraction on a Siemens D-5000 X-ray diffractometer. Data were collected between 5° and 40° 2θ in a stepwise scanning mode using a step size of 0.04° and a step interval of 10 s.

Results

Analysis of Trehalose Dihydrate. As mentioned previously, the main thrust of the study was to examine the nonisothermal recrystallization of amorphous trehalose, with particular emphasis on delineating the effects of water content and DSC pan type. However, to provide a comparison with the established literature, the as received dihydrate was analyzed using (pinholed) experimental conditions identical to those used later for the amorphous samples. There is still considerable debate

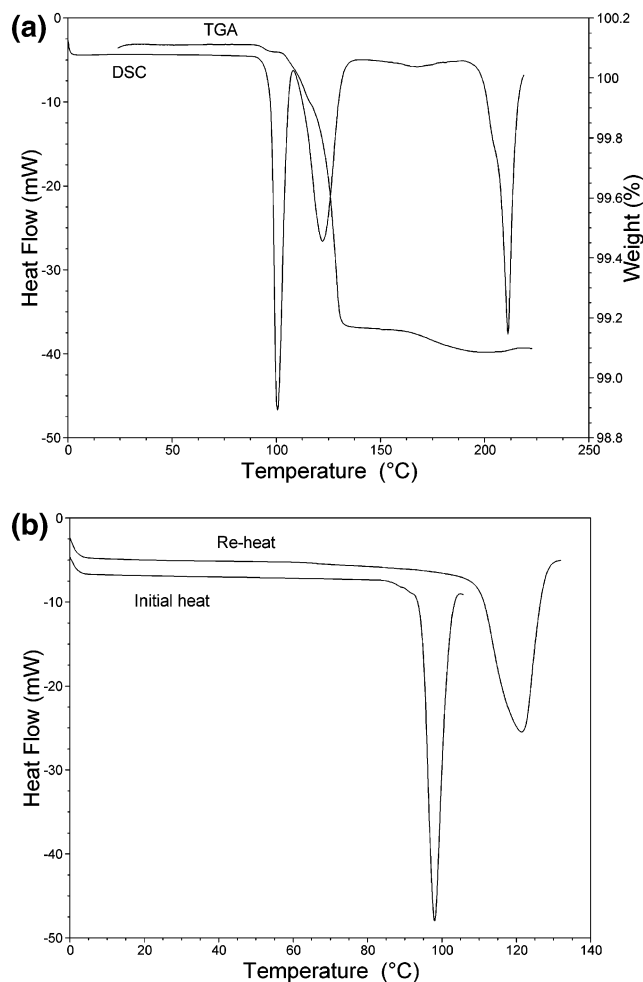


Figure 1. (a) DSC and TGA response for α,α -trehalose dihydrate in pinholed pans (10 °C/min), and (b) DSC response showing cessation of run and subsequent reheating of isolated material.

regarding the interpretation of the DSC data for the dihydrate; hence, in order to facilitate clarification of this behavior, the samples were subjected to temperature cycling and subsequent XRD analysis in order to isolate and identify the species formed at different temperatures.

A typical data set for the dihydrate is given in Figure 1a, showing a sharp endotherm at 100.4 °C (113.4 J/g), a broader endotherm at 121.9 °C (123.3 J/g), a broad discontinuity in the baseline between 160 °C and 190 °C, and a sharp, shouldered endotherm at 210.9 °C (128.1 J/g) (all nonrange temperatures refer to peak maxima). By using pinholed pans for the TGA studies it was possible to compare the data from the two techniques. It was found that while there was a small decrease in weight over the temperature corresponding to the first endotherm, the weight loss appeared to be almost exclusively associated with the second. This corresponds to a loss of 9.39%, which is close to the theoretical value of 9.50% for loss of two water molecules per molecule of trehalose. However, the gas flow conditions and heating arrangements in the two instruments inevitably differ; hence, it is not appropriate to assign the water loss process to one or the other DSC peak from these data alone.

In subsequent studies, the run was terminated immediately after the first of the two endotherms, whereupon the sample was allowed to cool to room temperature; in this manner it was intended that the species formed immediately after the first endotherm could be characterized and hence the process associated with the endotherms identified. The material was then

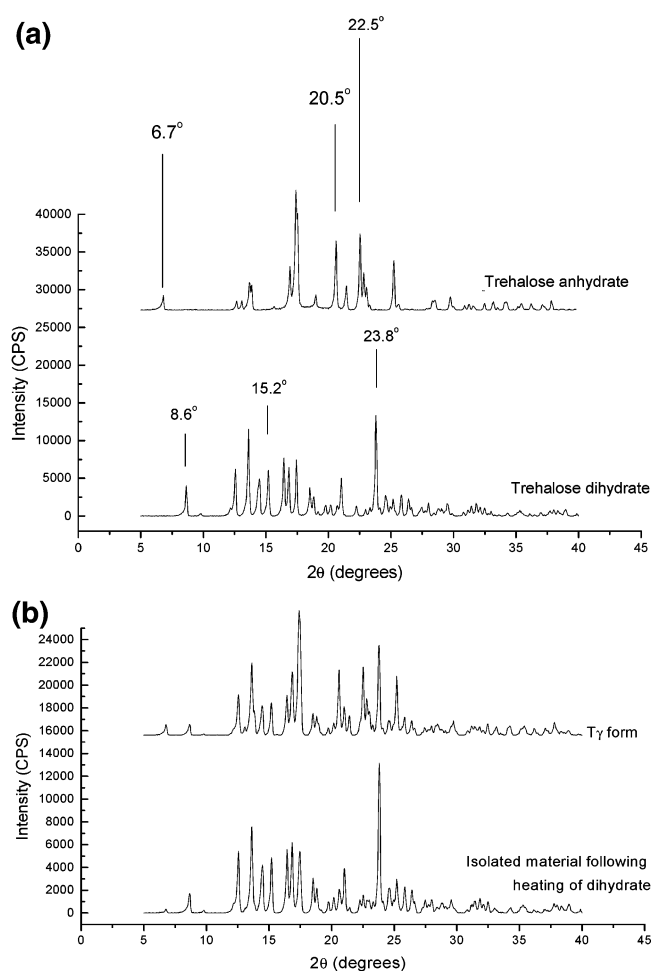


Figure 2. XRD spectra of (a) the T_h (dihydrate) and T_β (anhydrous) forms, prepared as described in methodology (b) the T_γ form of α,α -trehalose prepared using the method of Sussich et al.⁹ and the isolated material following heating in pinholed pans (6.1% initial water content).

analyzed using DSC and TGA. Figure 1b shows these data, indicating that on reheating the sample the lower temperature endotherm was not observed but instead the second peak (121.4 °C) was seen, with TGA data indicating a loss of 8.1% over a corresponding temperature range (the remainder of the data set was identical to that shown in Figure 1a). These data therefore indicate that the second peak does indeed coincide with the major water loss process; while partial dehydration may have occurred during the initial heating process, it is clear that the majority of the water is retained despite the sample undergoing the first enthalpic event.

To allow identification of the isolated material, powder XRD studies were conducted on the anhydrate and dihydrate materials (Figure 2a) and on the isolated material in comparison with the material prepared using the method of Sussich et al. (ref 9, Figure 2b) and designated by these authors as T_γ , this having been established by XRD as being a mixture of dihydrate and the T_β anhydrous forms.^{9,12} There are clearly similarities between the two spectra, although it should be noted that there may not be a "single" T_γ "form" as theoretically the two components could be present in a range of proportions, although this concept has not yet been explored in the published literature. More specifically, characteristic peaks, specific to anhydrous trehalose, are seen to occur at 6.7°, 20.5°, and 22.5°, while characteristic peaks for trehalose dihydrate are observed at 8.6°, 15.2°, and 23.8°. The similarity of peak occurrence between the form isolated in this study and the material prepared using the method

TABLE 1: Thermal Properties of Amorphous α,α -Trehalose, Heated in Pinholed Pans, as a Function of Initial Water Content, Showing the Glass Transition Temperature (T_g), the Crystallization Temperature (T_c) and Enthalpy (ΔH_c), and the Fusion Temperature (T_m) and Enthalpy (ΔH_m) (Numbers in parentheses show \pm SD, $N = 4$)

% w/w water	$T_g/^\circ\text{C}$	$T_c/^\circ\text{C}$	$\Delta H_c/\text{J}\cdot\text{g}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_m/\text{J}\cdot\text{g}^{-1}$
2.0 (1.4)	89 (4.0)	159.8 (9.5)	73.5 (8.1)	210.5 (0.3)	119.6 (8.4)
3.2 (0.2)	77 (1.0)	147.1 (2.4)	50.8 (15.6)	211.4 (0.4)	138.5 (8.2)
4.2 (0.4)	59 (1.0)	121.3 (0.9)	32.4 (4.4)	211.8 (0.3)	127.8 (9.2)
5.4 (0.7)	41 (5.0)	116.6 (4.5)	37.0 (8.2)	211.2 (0.5)	106.5 (10.5)
6.1 (0.2)	39 (2.0)	112.9 (1.7)	24.9 (4.2)	210.3 (0.3)	123.6 (14.4)

of Sussich et al.⁹ would indicate that the former corresponds to this material. On this basis it would appear that the partial dehydration that occurs during the first endotherm leads to the generation of the T_γ form (using the term in the sense of this form being a mixture of the dihydrate and anhydrous forms of unspecified proportionality).

Thermal Behavior of Amorphous Trehalose—Effect of Water Content on T_g . Amorphous trehalose has been studied by several authors in the past, with the majority of recent papers assigning a value of 120–125 $^\circ\text{C}$ for the glass transition temperature (e.g., ref 16). In this investigation we focus on the effect of water content on the recrystallization behavior, although clearly it is essential to have an understanding of water plasticization effects as the lowering of the T_g will influence the temperature at which recrystallization occurs. To this effect the influence of initial water content on the value of T_g was measured in both hermetically sealed and pinholed pans. As expected, the presence of water lowered the T_g (Table 1,2) although it must be appreciated that when studying the behavior of trehalose in pinholed pans the water content will be continuously changing over the temperature range under study. It was also noted that the samples containing higher water contents showed evidence of having partially recrystallized during the equilibration process (Figure 3). A more detailed study regarding both the modeling of the plasticization effect and the glass transitional behavior will be discussed in a further communication.

Thermal Behavior of Amorphous Trehalose—Effect of Water Content on the Behavior in Pinholed Pans. A series of DSC traces were obtained in pinholed pans using amorphous material with differing initial water contents (Table 1). Interestingly, two sets of behavior were observed, depending on the water levels. This is illustrated for the low water content samples ($<4.5\%$) in Figure 4a, whereby a T_g is seen followed by an exotherm, the size and temperature range of which varied with water content, and an endotherm that occurred at 210.6 $^\circ\text{C}$. The magnitude and temperature range of the exotherm decreased with increasing water content, as indicated in Table 1. Furthermore, the TGA studies indicated that the temperature range over which water loss occurred corresponded well to the exotherm, indicating that water was being expelled from the material as the thermal event took place; it is at this stage reasonable to ascribe the exothermic thermal event to crystallization. Isolation of the material following the exotherm and subsequent study by powder XRD indicated formation of the T_β anhydrate (data not shown).

A very different series of profiles were seen for systems containing higher initial water contents. Figure 4b shows the response for a sample containing 6.1% water (the 5.2% water behaved in a very similar manner). It may be seen that instead of the single exotherm, a dual event is seen comprising an exotherm followed immediately by an endotherm. In addition,

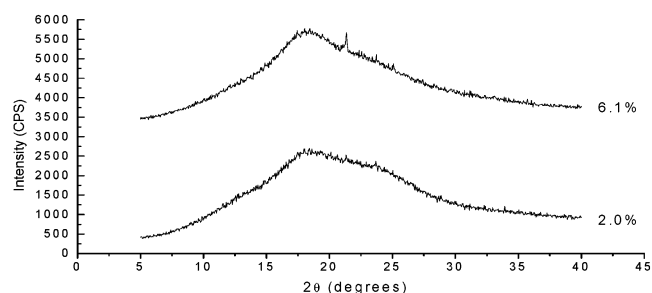


Figure 3. XRD data for amorphous trehalose at initial water contents of 2.0 and 6.1% w/w.

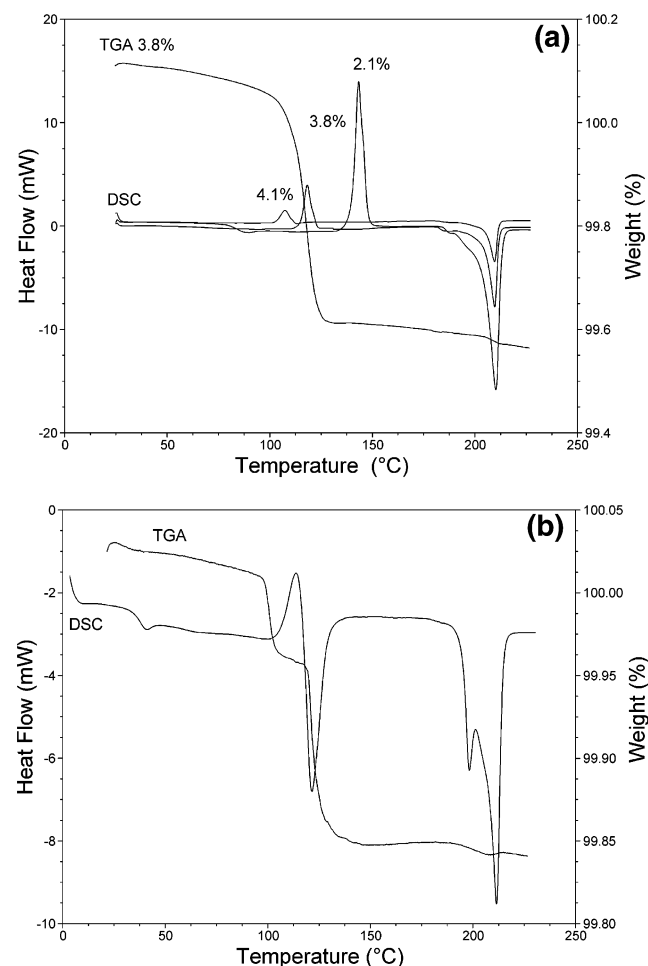


Figure 4. DSC data for amorphous α,α -trehalose in pinholed pans as a function of water content (a) up to 4.1%, and (b) 6.1% w/w.

examination of the TGA data indicates that water loss occurs in two stages, one immediately prior to the recrystallization and a second associated with the endotherm. Examination of the XRD data for the material isolated following the exothermic event indicated that the T_γ form had been generated prior to further dehydration.

Thermal Behavior of Amorphous Trehalose—Effect of Water Content on the Behavior in Hermetically Sealed Pans. Amorphous trehalose was then heated in hermetically sealed pans as a function of water content. In all cases, a single exothermic transition was seen in the 100–160 $^\circ\text{C}$ temperature range, with no evidence for an endothermic event seen in this region (Figure 5a). On further heating the sample, a broad endotherm was observed ca. 180–220 $^\circ\text{C}$. In both cases, lower enthalpy and temperature values were observed for higher water content samples (Figure 5b, Table 2).

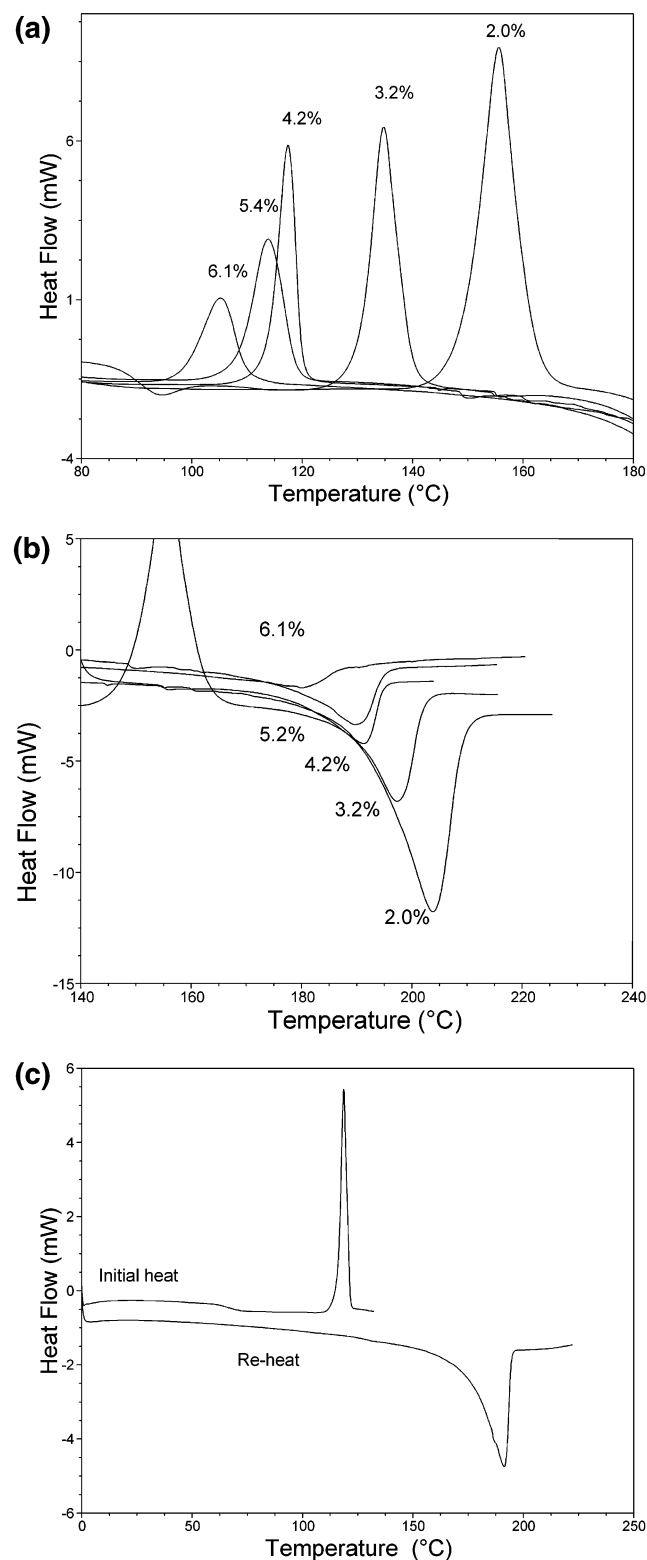


Figure 5. DSC data for amorphous α,α -trehalose in hermetically sealed pans as a function of water content (a) 80–180 °C, (b) 140–220 °C, and (c) reheating following isolation of crystallized material (initial water content 4.2%).

To clarify the processes involved, the runs were terminated immediately after the exotherm and the samples analyzed by DSC and XRD. The DSC traces (Figure 5c) indicated that the reheated material showed a broad endotherm in the high-temperature region as before, with no thermal events seen up to this temperature. XRD studies (Figure 6) showed that the

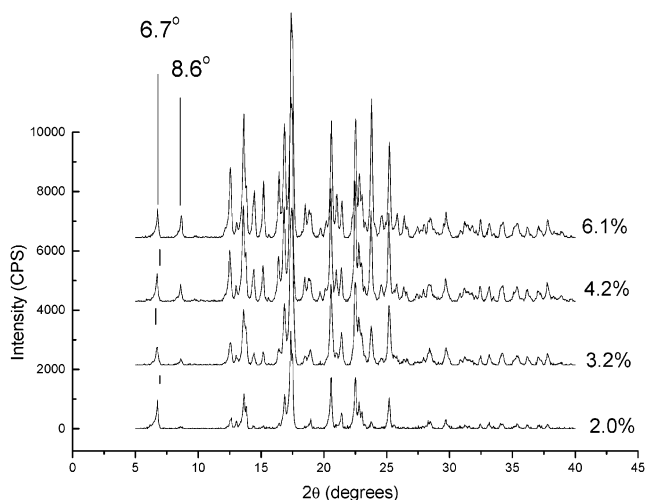


Figure 6. XRD data for α,α -trehalose isolated after heating in hermetically sealed pans.

TABLE 2: Thermal Properties of Amorphous α,α -Trehalose, Heated in Hermetically Sealed Pans, as a Function of Initial Water Content, Showing the Glass Transition Temperature (T_g), the Crystallization Temperature (T_c) and Enthalpy (ΔH_c), and the Fusion Temperature (T_m) and Enthalpy (ΔH_m) (Numbers in parentheses show \pm SD, $N = 4$)

% w/w water	$T_g/^\circ\text{C}$	$T_c/^\circ\text{C}$	$\Delta H_c/\text{J}\cdot\text{g}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_m/\text{J}\cdot\text{g}^{-1}$
2.0 (1.4)	90 (7.0)	155.8 (3.8)	94.6 (12.2)	203.6 (0.2)	114.0 (12.7)
3.2 (0.2)	76 (2.0)	134.9 (4.5)	72.5 (9.8)	197.3 (1.2)	80.8 (18.6)
4.2 (0.4)	59 (3.0)	117.4 (8.4)	70.0 (11.9)	191.2 (2.8)	73.1 (2.4)
5.4 (0.7)	44 (2.0)	113.9 (1.2)	39.4 (6.7)	189.7 (3.4)	64.9 (1.8)
6.1 (0.2)	37 (4.0)	105.2 (2.0)	34.8 (16.4)	180.1 (2.7)	54.4 (2.8)

samples corresponded to a mix of the dihydrate and anhydrous samples (i.e., the T_γ form), with a greater proportion of the dihydrate found for the higher moisture samples, as may be expected. This implies that the ratio of the two forms is correlated to initial water content.

Discussion

In broad terms, the study has attempted to deconvolute the effects of encapsulation and water content on the temperature-dependent behavior of α,α -trehalose. However, it is helpful to consider each facet of the study in isolation in the first instance.

Analysis of Trehalose Dihydrate. The study indicated that two low-temperature (100–120 °C) endotherms are seen for this material when studied in pinholed pans, the first corresponding to partial dehydration to the T_γ form and the second to the dehydration to the anhydrate that subsequently melts at 211 °C. This behavior is in itself not fully reflected in other studies such as that of Macdonald and Johari,¹³ who, using a relatively high scanning speed and open pans, reported only one endotherm over the equivalent temperature range; the differences in these observations from our own may be logically ascribed to the different experimental conditions used. Interestingly, Taylor and York⁵ saw two endotherms at 101 °C and 112–125 °C for the dihydrate, associating these events to water loss but concluding that it was not possible to ascribe the peaks to specific events. Macdonald and Johari¹³ present some interesting arguments with regard to the single endotherm seen during their study over the equivalent temperature range. In brief, they suggest that the peak corresponds to a combination of the evaporation of water and one or more of a number of further processes including decomposition of the dihydrate to

anhydrate and water, dissolution of trehalose in the released water, and any molecular rearrangement such as the exchange of tautomeric forms in any aqueous solution (suggested on the basis of glucose exhibiting six such forms in solution) and crystal rearrangement between different forms of anhydrous trehalose. Our studies indicate that two distinct processes are involved, probably revealed by the greater resolution associated with slower scanning speeds, and that the second (higher temperature) endotherm appears to correspond to the dehydration of the T_γ form to the anhydrate. It is not clear at this stage what the lower temperature endotherm corresponds to, although it is certainly associated with partial dehydration. One may speculate that if the suggestion of Sussich et al.¹² regarding the distribution of hydrated and anhydrous material is correct then the first endotherm may correspond to the formation of an anhydrous outer layer on the particles that acts as a barrier to further dehydration. Full water loss then occurs at a slightly elevated temperature and is seen as a discrete thermal event. However, more work is required to confirm or refute this suggestion.

Thermal Behavior of Amorphous Trehalose—Effect of Water Content on the Behavior in Pinholed Pans. The studies on the low moisture content (up to 4.1%) material analyzed in pinholed pans indicated that the material dehydrates and simultaneously recrystallizes into the anhydrous T_β form, as may be expected from basic theory. However, both the temperature and magnitude of the exotherm decrease with increasing water content. This may be associated with the lowering of the T_g caused by the presence of water that in turn increases the molecular mobility of the system at equivalent temperatures compared to systems with lower water contents, combined with higher endothermic enthalpies associated with water evaporation at higher water contents. It is interesting to note that the higher water content samples showed initial crystallization into the T_γ form prior to dehydration to the T_β conformation. This may be a direct result of the higher water content itself or else may reflect nucleation or prior partial recrystallization into the dihydrate, as indicated by the evidence for peaks seen for the 6.1% w/w water systems shown in Figure 3. In either case, the study has clearly indicated that even though the end product remains unchanged (the anhydrous form), the temperature and route of the recrystallization process may vary markedly depending on initial water content.

Thermal Behavior of Amorphous Trehalose—Effect of Water Content on the Behavior in Hermetically Sealed Pans. For samples sealed in hermetic pans, the data indicated a single exotherm followed by a broad endotherm in all cases, suggesting recrystallization followed by melting. However, the temperature at which both events occurred varied according to the water content. Taken with the powder XRD data that indicated formation of the T_γ form as a result of the exothermic event, it is reasonable to suggest that the material that is formed in each case differs, with varying ratios of dihydrate and anhydrous forms. This may be of some significance as it indicates that the recrystallization process may result in an extremely wide range of different crystal conformations, based on a combination of the two aforementioned phases.

Overall Comments. The study has indicated that the recrystallization behavior of amorphous trehalose may be interpreted in terms of the formation of the dihydrate, the anhydrate, or a combination of the two (the T_γ form), with the behavior being highly dependent on water content and pan type. This in turn implies that great care is required when designing and interpreting quality control and structural characterization studies for

these materials. However, it is also notable that the prevalence of the T_γ form appears to be much greater than anticipated from examination of the literature. Indeed, our data indicate that it may be formed from both amorphous and crystalline materials, either as an intermediate prior to full dehydration or as product of recrystallization. Furthermore, the T_γ “form” may perhaps be more helpfully regarded as a range of compositions rather than as a single phase, as there is evidence for a range of compositions being generated that still correspond to the single descriptor. This is of considerable relevance to the understanding of the stability of freeze-dried trehalose products and implies that the current thinking in terms of simple recrystallization in the presence of water to the dihydrate may be an oversimplification.

Although not a specific objective of the study, the data must also be considered in the light of the debate regarding the mechanism by which trehalose exhibits cryoprotection. Green and Angell¹⁷ have used the phrase “trehalose anomaly” to describe the absence of obvious structural features that may explain the ability of this disaccharide to act as an effective protective agent. To date, a satisfactory explanation for this behavior remains elusive, although it should be noted that there is debate with regard to whether trehalose may be considered to be “anomalous” in its behavior at all.³ The present study has suggested that the generation of the T_γ form may be more commonplace than was originally anticipated. If one reexamines the argument of Aldous et al.¹ that trehalose may maintain desiccation of materials even after recrystallization via formation of the dihydrate, it is interesting to speculate that the ability of this material to form a range of hydrated species at sub-stoichiometric ratios may also play a role in the cryopreservation process. Indeed, one may anticipate that the ability to generate a range of forms comprising the dihydrate and anhydrate (the latter being unstable in the presence of free water) at varying ratios would produce a highly efficient and flexible “water sink” that would be capable of protecting the species in question on storage following freezing or freeze-drying.

Conclusions

The study has indicated that the thermal behavior of amorphous α,α -trehalose is highly dependent on the conditions used to perform thermal analysis experiments, particularly in terms of the initial water content and encapsulation environment under which the studies are performed. The material may recrystallize into a wide range of forms based on the dihydrate, the anhydrate or, as may be more prevalent than previously thought, a combination of these two phases. These findings have implications for the quality control and structural assessment of trehalose, the understanding of the storage behavior of products based on this material, and possibly the mechanism by which trehalose acts as a cryoprotectant.

References and Notes

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