

Using modulated DSC to investigate the origin of multiple thermal transitions in frozen 10% sucrose solutions

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Abstract

Modulated differential scanning calorimetry (MDSC) was used to investigate the effect of annealing on multiple thermal transitions in frozen aqueous sucrose solutions. Two thermal transitions were detected from the reversing heat flow. Here, to maintain consistency with terminology used in the practice of freeze-drying [1], the higher temperature is denoted, T_g' , and the lower transition is given the symbol, T_g'' . The transition at low temperature is usually believed to be a true glass transition. However, the origin of the transition at high temperature is still highly controversial. Based upon a study of 10% sucrose solutions with different cooling histories and annealing conditions, we suggest that the transition at high temperature is also a glass transition. We conclude that the lower transition is a glass transition of a phase plasticized by a higher than equilibrium amount of unfrozen water, and the higher transition, T_g' , corresponds to a glass transition in a maximally freeze-concentrated solute phase.

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1. Introduction

Freeze-drying has become the method of choice for stabilization of labile materials, and is widely used in the pharmaceutical industry for manufacture of protein drugs and other biotechnology products. Minimizing the drying time, which means drying at a temperature as high as possible without damaging product quality, is an important economic issue. However, drying significantly above T_g' leads to structural collapse at the collapse temperature (T_c), normally resulting in an unacceptable product. Therefore, knowing the collapse temperature of different freeze-drying formulations is very important in optimization of a freeze-drying cycle. In practice, there are two methods normally used to measure the collapse temperature (T_c). Freeze-drying microscopy is commonly used, but differential scanning

calorimetry (DSC) is also used to measure the sub-zero transition temperature (T_g'). The concentration of the solute in this freeze-concentrated solution is usually denoted C_g' (weight fraction of solute) [2,3]. The collapse temperature and T_g' are closely related, with the collapse temperature normally being several degrees higher than T_g' [4]. For a single-phase solute system, one would expect that only one glass transition for this freeze-concentrated solution would be detected in DSC experiments. However, two transitions are commonly observed for frozen sucrose and trehalose solutions [5–8]. One transition appears at about -33 °C [6,9], denoted T_g' , while the other (weaker) transition, denoted T_g'' , appears at about -53 °C (quench cool) or -44 °C (slow cool). Unless indicated otherwise, T_g values are mid-points of the transition. Evidence of devitrification of the solution and subsequent ice crystallization between T_g'' and T_g' during DSC scans is also well established [5,10]. Since observations indicate that T_c and T_g' normally differ by only a few degrees (i.e., T_c is slightly higher), T_g' is the transition of relevance to structural collapse. However, it is not clear whether both or only one of these transition are glass transitions.

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While most workers agree that the lower transition, here denoted T_g'' , is a true glass transition, the origin of the higher transition (T_g') is highly controversial [5,7,11,12]. The more common modern view states that T_g' is not a glass transition but rather represents the temperature at which diffusion of water in the amorphous phase becomes significant on the time scale of a DSC experiment. The change in apparent heat capacity at T_g' , $\Delta C_p'$, arises from rapid non-equilibrium ice melting [7,11]. The key observation supporting this viewpoint comes from measurements of ΔC_p for the glass transition in single-phase sucrose systems that have compositions close to the freeze concentrate in a frozen system. It is found that ΔC_p in these single-phase systems is essentially the same as the heat capacity change at T_g'' , $\Delta C_p''$. By contrast, $\Delta C_p'$ is roughly twice ΔC_p which suggests that T_g' is not a glass transition. However, recent “isothermal” modulated DSC studies have established that while equilibrium melting occurs around T_g' , essentially no non-equilibrium melting occurs here. The latent heat associated with this reversible melting adds to the amplitude of the modulated heat flow, which makes the apparent heat capacity at T_g' larger [5].

Recently, Goff et al. [12] used modulated DSC and microscopy to examine the glass transition behavior of frozen 40% sucrose solutions. They concluded that the transition at high temperature, T_g' , is a glass transition that results from the formation of a concentrated, un-equilibrated sucrose phase around or within the rapidly nucleated ice, and/or solute inclusions within the crystals themselves. Curiously, while the two thermal events, corresponding to T_g'' and T_g' in our notation, are apparent after one annealing step at -35°C , a second annealing process at -35°C seems to result in the disappearance of the higher temperature transition [12].

Since a number of studies have shown that degradation often increases sharply above the glass transition temperature [13,14], the interpretation of both T_g' and T_g'' has stability consequences. If T_g' is a glass transition temperature, it would be expected that the protein would not be able to unfold on the time scale of primary drying much below T_g' because of the limited mobility. This interpretation suggests that a thermodynamic stabilization mechanism during drying cannot be valid since thermodynamic stabilization demands that the system remains in thermodynamic equilibrium (i.e., folding and unfolding are fast) during the part of the process where potential unfolding occurs. However, if T_g'' is the glass transition, the fact that T_g' is about 10–20 °C higher, depending on thermal history, means that the primary drying is normally carried out well above the glass transition region. Here, a thermodynamic stabilization mechanism like the “water substitute” hypothesis might be a reasonable mechanism since the system could be in conformational equilibrium. Therefore, the interpretation of T_g' and T_g'' is important in understanding the mechanisms of stabilization and in proper process design. That is, does one need to control product temperature below T_g'' during freeze-drying to obtain optimal stability?

Here, we report modulated DSC studies on frozen 10% aqueous sucrose systems which have been allowed to “anneal” at temperatures between T_g'' and T_g' before beginning the DSC scan. This particular concentration was chosen because in a freeze-drying process, the concentration of sucrose usually does not

exceed 10% when it is employed as a stabilizer, and the weak lower transition temperature is difficult to study at lower concentration. Modulated DSC is used since this technique can separate the total heat flow into “reversing” or heat capacity response and a “non-reversing” or pure kinetic component, thereby facilitating the separation of the glass transition from enthalpy recovery and crystallization events.

2. Materials and methods

2.1. Preparation of sucrose solutions

Sucrose solutions were prepared by weight from analytical grade sucrose obtained from Sigma. Double distilled water was added to the weighed sucrose. For highly concentrated sucrose solutions, stirring and gentle heating over a hot plate was used to dissolve the sucrose until clear solutions were obtained.

2.2. Thermal analysis of sucrose solutions

Samples were hermetically sealed in aluminum pans and then run in a TA Instruments Modulated DSC (Model 2920) equipped with a Refrigerated Cooling System. The purge gas used was helium with a flow rate at 50 ml min⁻¹. Calibrations were performed using the melting point of indium and ice as the standards.

Sealed solution samples were run in two different ways: (1) Quench cool the sealed sample pan in liquid nitrogen and then place immediately into the pre-cooled DSC cell. Bring the sample to a selected temperature between T_g'' and T_g' , ranging from -48.5 to -40.5°C , and hold the sample for a given time (i.e., usually 1 h) at this temperature to “anneal”. For the MDSC scan, modulate with amplitude of 0.5 °C and a period of 100 s for 10 min to establish steady state, and then begin the linear scan at 1 °C min⁻¹. (2) First “slow cool” at 1 °C min⁻¹ to the annealing temperature. After annealing for the selected time, begin the modulated DSC scan as described above in (1).

Both T_g' and T_g'' are reported as the mid-point of transition steps.

3. Results and discussion

3.1. Non-annealed solutions

Fig. 1 shows the reversing heat flow curve for the quench-cooled and slow-cooled 10% sucrose solution. The low transition temperatures (T_g'') of quench-cooled samples are much lower than those of slow-cooled samples. The high transition temperatures (T_g') are almost the same, -33 and -35°C for slow-cooled and quench-cooled samples, respectively.

It is well known that the actual manner of ice growth depends on the degree of under-cooling and the nucleation rate in the under-cooled liquid phase [2]. During quench cooling, the ice crystals formed are much smaller than those formed during slow cooling because of the faster nucleation rate. Also, the amorphous sucrose phase can trap more unfrozen water during quench cooling than during slow cooling, and unfrozen water

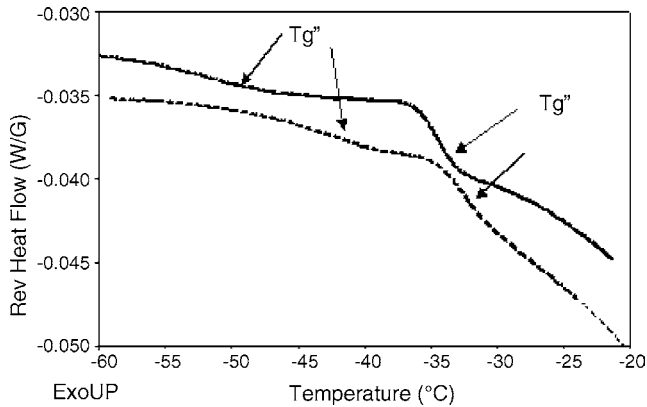


Fig. 1. Reversing heat flow for modulated DSC on quench-cooled and slow-cooled 10% sucrose solution. (—) quench-cooled; (---) slow-cooled.

plasticizes the amorphous sucrose and makes the glass transition lower.

Fig. 2 compares the non-reversing heat flow signal of the quench-cooled and slow-cooled 10% sucrose solutions. Between the two transition temperatures, there appears to be an exothermic shift, which is ascribed to the crystallization of non-equilibrium unfrozen water. This crystallization shift for a quench-cooled sample is much larger than that of a slow-cooled sample, which also suggests that quench-cooled sample traps much more water than does the slow-cooled sample. In short, while the “slow-cooled” sample is apparently not quite maximally freeze-concentrated, it is much more so than the quench-cooled sample.

The width of the glass transition region, $\Delta T_g''$, is another parameter of interest. The width of the low temperature transition, $\Delta T_g''$, is about $8.0 \pm 0.5^\circ\text{C}$ for the quench-cooled sample and $5.3 \pm 0.3^\circ\text{C}$ for the slow cool sample without annealing (reversing heat flow curves). However, the width of the high temperature transition, $\Delta T_g'$, is relatively small at about $3.2 \pm 0.2^\circ\text{C}$. While uncertainties given for the ΔT_g data (standard deviation) are relatively small, it must be recognized that for $\Delta T_g''$, there could be significant systematic errors because the T_g'' transition

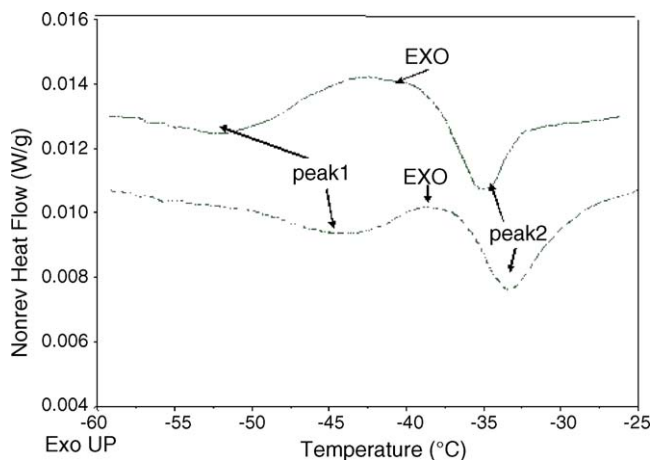


Fig. 2. Non-reversing heat flow for modulated DSC on quench-cooled and slow-cooled 10% sucrose solution. Peaks 1 and 2 are endothermic peaks. (---) slow-cooled; (—) quench-cooled.

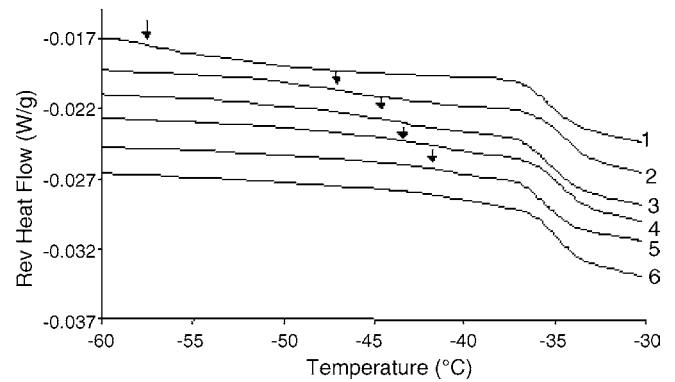


Fig. 3. Reversing heat flow for MDSC on quench-cooled 10% aqueous sucrose annealed for 1 h at selected temperatures. Arrows denote T_g'' . (1) No annealing; (2) annealed at -48.5°C ; (3) annealed at -46.5°C ; (4) annealed at -44.5°C ; (5) annealed at -42.5°C ; (6) annealed at -40.5°C .

is very weak. However, it does appear that the rank order of ΔT_g values given above is correct.

There are also two endothermic peaks in the non-reversing heat flow as shown in Fig. 2. Peak 1 is “probably” the enthalpy recovery for T_g'' . The dispute concerns the origin of the second endothermic peak, denoted peak 2. Izzard et al. [10] ascribed the second endothermic peak in the non-reversing heat flow signal as irreversible ice melting and water dissolving into the softened glass. Knopp et al. who also used modulated DSC in their work explained this peak as simply a complex frequency effect related to the delayed irreversible dissolution of ice [15]. These interpretations regarding “peak 2” are in agreement with the interpretation offered by several other researchers [7,11], who used conventional differential scanning calorimetry in their studies. In another words, the claim is that the peak arises because of the “burst” of irreversible melting, and this “burst” also contributes to the apparent change in heat capacity at T_g' [7]. However, this interpretation is not consistent with the observed spontaneous crystallization of ice between T_g'' and T_g' . Spontaneous crystallization means that the chemical potential of water in the amorphous phase is higher than in ice below T_g' . Yet, if peak 2 is attributed to an irreversible burst of ice melting, the chemical potential of water in ice must be greater than in the amorphous phase for a finite temperature range preceding T_g' . Thus, we have a contradiction, and if we assume ice really does form spontaneously between T_g'' and T_g' , as the DSC data suggest, peak 2 cannot arise from irreversible “burst” melting.

3.2. Annealed samples

Fig. 3 shows the reversing heat flow signal for the quench-cooled 10% sucrose solution sample, which has been annealed for 1 h at different temperatures between the two transition steps T_g'' and T_g' . The data show that the lower transition temperature increases and the change of heat capacity at T_g'' , $\Delta C_p''$, decreases as the annealing temperature increases. However, $\Delta C_p'$, the heat capacity change at T_g' does not change with annealing. At the annealing temperature of -40.5°C , the two transition steps merge and only one transition can be detected, at least within the

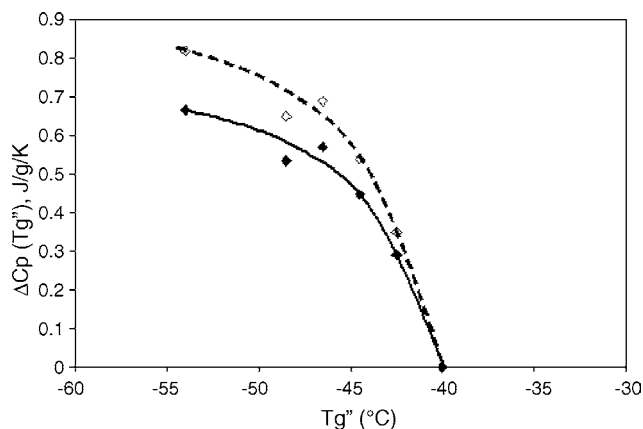


Fig. 4. Variation of ΔC_p at T_g'' with annealing temperature. Open symbols represent the apparent heat capacity change at T_g'' using the mass of sucrose (i.e., without water) to normalize for mass. Solid symbols represent the corrected heat capacity change at T_g'' , $\Delta C_p''$ calculated using total mass of sucrose phase to normalize for mass. The evaluation of the water content of the freeze-concentrated phase at T_g'' , which is need for this correction, is described later.

sensitivity of our equipment. Fig. 4 shows the decreasing trend of $\Delta C_p''$ with increase in T_g'' caused by annealing. In addition, after annealing, $\Delta T_g''$ decreases with increasing annealing temperature or annealing time. For example, when the quench-cooled sample was annealed at -42.5°C for 1 h, the $\Delta T_g''$ decreased from $8.0 \pm 0.5^\circ\text{C}$ (quench-cooled) to $2.4 \pm 0.2^\circ\text{C}$ (annealed).

Fig. 5 shows the non-reversing heat flow signal for the quenched-cooled 10% sucrose sample which has been annealed for 1 h at different temperatures between the two transitions denoted T_g'' and T_g' . Since there is some overlap of the exothermic shift with endothermic “peak 1”, it is difficult to quantify areas of the exothermic shift. However, the figure still suggests that the exothermic shift between two transitions is decreasing in magnitude as the annealing temperature increases. More water can diffuse out the amorphous sucrose phase and crystallize as the annealing temperature increases, leaving less water for devitrification and crystallization during heating. As a result, the exothermic shift caused by ice re-crystallization during the scan becomes smaller with increasing annealing temperature.

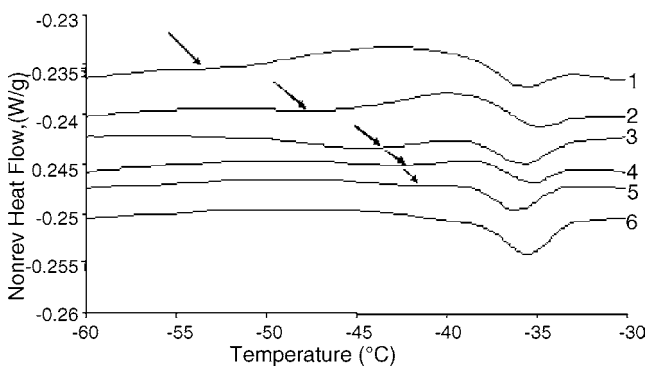


Fig. 5. Non-reversing heat flow for MDSC on quench-cooled 10% aqueous sucrose annealed for 1 h at selected temperatures. Arrows denote T_g'' . (1) No annealing; (2) annealed at -48.5°C ; (3) annealed at -46.5°C ; (4) annealed at -44.5°C ; (5) annealed at -42.5°C ; (6) annealed at -40.5°C .

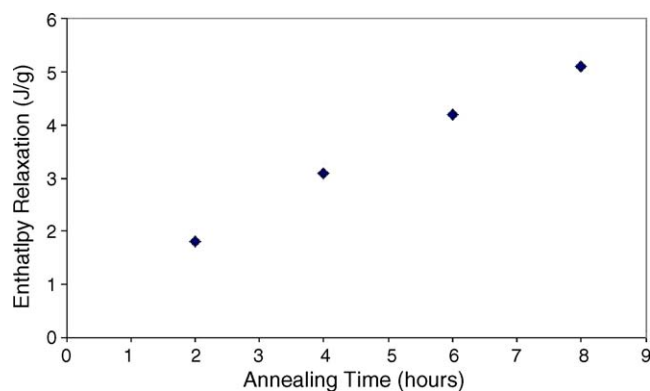


Fig. 6. Area of the endotherm at T_g' in the non-reversing heat flow as a function of annealing time (quench-cooled 10% sucrose solution and annealed at -46°C for different times). Data were corrected for the frequency effect by subtracting the endotherm peak area for the non-reversing DSC curve during cooling from the total peak area. The value at zero annealing time is not available because of excessive overlap of the ice crystallization exothermic shift with the endothermic peak.

If the quenched 10% sucrose solution sample is annealed at a temperature between the end point of T_g'' and the onset of T_g' for different times, there is an increase in the area of the endotherm (peak 2) for the non-reversing heat flow signal as the annealing time increases. This behavior is the classic signature of enthalpy recovery, and enthalpy recovery at T_g' must mean that T_g' is a glass transition temperature [16,17]. However, there is a portion of the non-reversing heat flow endotherm that is not related to annealing, which is called the “frequency effect” [10]. The contribution of the frequency effect is constant for a given material and fixed experimental conditions and is evaluated by integrating the endothermic peak on the DSC curve during cooling. After correction for the frequency effect, the data still show a clear endotherm whose area increases with annealing time (Fig. 6). Although previous workers [10] reported the endothermic peak in the non-reversing heat flow signal, they did not quantify the endothermic peak area and they still attributed T_g' to the onset of irreversible ice melting. Pyne et al. [8], who used conventional DSC to study frozen trehalose solutions, also reported an endothermic peak at T_g' whose area increased with annealing time although they did not take this observation as evidence for T_g' being a glass transition. Thus, the existence of an endothermic peak at T_g' whose area increases with aging time is not in dispute. In our view, the observed increase in area with an increase in aging time is not consistent with identification of this endothermic peak as irreversible melting of ice but rather must mean the peak represents enthalpy recovery, which in turn must mean T_g' is a true glass transition temperature.

It is also noticed that if we annealed the slow-cooled 10% sucrose solution at -40.5°C , the same annealing temperature that causes the T_g'' of a quench-cooled sample to disappear, a 2 h annealing time (instead of 1 h), is required to cause the transition to disappear. The difference in kinetics is consistent with the expected lower specific surface area of ice (and longer diffusion pathway for water) in slow-cooled samples. Since the amorphous phase in the quench-cooled samples reaches equilibrium faster than in the slow-cooled samples, less time is required

for T_g'' to disappear in a quench-cooled sample. The fact that non-equilibrium unfrozen water in the amorphous sucrose phase is a result of kinetic retardation of water diffusion instead of tight equilibrium binding by the solute has been well established [18–20].

The small difference in T_g' between slow-cooled and quench-cooled samples requires some comment. Fig. 1 shows that T_g' for a quench-cooled sample is about 2 °C lower than T_g' for a “slow-cooled” sample. This difference, although small, is reproducible. It was reported by Zhang et al. that the glass transition temperature of several molecular liquids is lowered due to confinement in porous silica glass. The depression of the glass transition temperature has a linear dependence on the inverse of the pore radius ($1/R$). That is, the smaller the pore radius R , the lower the T_g of the confined liquid. For example, ΔT_g of glycerol is about 1.7 °C when the pore radius changes from 1.8 to 15.2 nm [21]. Thus, it appears possible that the glass transition temperature of a quench-cooled sample is lower than that of a slow-cooled samples (as shown in Fig. 1) since the pore size of ice crystals in quench-cooled samples is smaller. However, the pore sizes in an amorphous sucrose systems are about 1–2 μm [22], which seems too large for this pore size effect to be important. It is more likely that this small difference represents the effect of a very slight difference in freeze concentration between the two types of samples. That is, the quench-cooled sample did not fully freeze concentrate during the DSC experiment, perhaps because it originally contained a higher amount of “non-equilibrium” unfrozen water.

3.3. Estimation of C_g' from T_g versus water content for single-phase systems: comparison of $\Delta C_p'$ for frozen systems with ΔC_p for (single-phase) solutions

A modified Gordon–Taylor equation (MGT, Eq. (1)) can be used to estimate the T_g values of single-phase sucrose solutions (i.e. no ice present).

$$T_g = \frac{kT_{g2} - C(kT_{g2} - T_{g1})}{C(1 - kT_g) + kT_g} + \alpha_1 C(1 - C) + \alpha_2 C^2(1 - C) \quad (1)$$

where C is the concentration of water at T_g (weight fraction), T_{g1} and T_{g2} are the mid-points of the glass transitions for pure water and sucrose, while k , α_1 and α_2 are constants. Here we use 348.2 K for T_{g2} , the glass transition temperature of pure sucrose, and T_{g1} for water is taken as 135 K. Glass transition temperature data for sucrose with various water contents were obtained from the literature and were fit by Eq. (1) (Fig. 7). The “best fit values” for the parameters are $k=0.092$, $\alpha_1=481$ and $\alpha_2=-1225$. Thus, from Eq. (1), C_g' can be estimated given the value of T_g' (Fig. 7). As noted earlier, we find that, using the reversing heat flow signal with modulated DSC, the value of T_g' for a slow-cooled sample is -33 °C. It is well known that glass transition temperatures determined from the reversing signal are several degrees higher than the corresponding data determined from the total heat flow curve or from conventional DSC [10,15,23]. Since the data in Fig. 7 were obtained using conventional DSC,

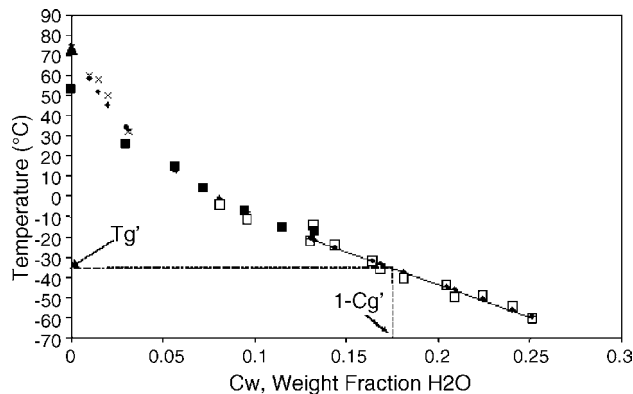


Fig. 7. T_g in aqueous sucrose solution as a function of different water content: literature data ((x) data from [24]; (■) data from [25]; (□) data from [6]; (▲) [26]; (◆) calculated T_g from Eq. (1)).

the appropriate value of T_g' (mid-point) corresponding to these data is then -35 °C, a value in good agreement with numerous studies using conventional DSC. Using -35 °C for T_g' determined by conventional DSC, the value of C_g' found from Fig. 7 is $C_g' = 1 - 0.175 = 0.825$. The value of C_g' can also be determined by noting the concentration at which ice first forms in a cooling solution. Our experimental data (Table 1) indicates that the maximum freeze concentration of sucrose is between 82.13%, where ice was detected, and 82.42%, where the system remained a single-phase (without forming ice). The corresponding T_g' is about -33 °C (Table 1), which is the same as we found in the studies with 10% sucrose solution. Thus, the data in Table 1 and the literature values for T_g shown in Fig. 7 are consistent with the higher temperature transition, T_g' , being the glass transition of the maximally freeze-concentrated solute. It must be noted that this comparison does not fix the value of T_g' with a high degree of accuracy. A variation in T_g of several degrees still gives a value of C_g' that is consistent with literature and Table 1 data. However, a value of T_g in the range of T_g'' for quenched or slow-cooled samples gives values of C_g' which are too low

Table 1

The glass transition temperature (T_g') and the heat capacity change (ΔC_p) at T_g' for the single-phase sucrose solution measured by modulated DSC

Sucrose concentration (%)	T_g (°C)	ΔC_p (J/g·K)
87.44	-14.7	0.67
86.25	-19.8	0.62
85	-23.6	0.68
84.3	-26.4	0.66
84	-28.1	0.66
83.79	-28.3	0.56
83.3	-30.7	0.69
82.95	-30.2	0.63
82.8	-31.2	0.67
82.42	-32.8	0.69
82.13 ^a	-34.6	0.59
81.98 ^a	-35.1	0.54

The average value of T_g width is 8.2 ± 0.8 °C. The linear fitting equation for T_g as a function of sucrose concentration, C_g' : $T_g = 118(1 - C_g')^2 - 711 \times (1 - C_g') + 56$.

^a Ice melting was observed, indicating the system is not a single-phase system.

(i.e., ≈ 0.76 for quench-cooled). Thus, the lower temperature transition, T_g'' , is not consistent with the C_g' value expected for maximally freeze-concentrated solute.

As noted earlier, the open symbols in Fig. 4 represent the apparent $\Delta C_p''$ at T_g'' . That is, here the weight of sample used to normalize for mass was the amount of pure sucrose (without water). In fact, the ΔC_p should be corrected by dividing by the total amount of amorphous sucrose phase (i.e., including the water). This calculation requires a knowledge of the amount of water in the freeze concentrate at T_g'' , here denoted C_g'' . C_g'' can be estimated by substitution of T_g'' into the equation describing the dependence of T_g on solution composition (Table 1), solving for C , and calculating: $C_g'' = 1 - C$. The solid symbols (Fig. 4) represent the heat capacity change, $\Delta C_p''$, at T_g'' based upon total mass of sucrose phase (i.e., sucrose plus water). Without annealing, our data (Fig. 4), indicate the heat capacity change at T_g'' (-53°C) is about 0.66 J/g/K . The data (Table 1) show that the heat capacity changes for the single-phase systems are essentially independent of composition in concentrated sucrose systems and average about 0.66 J/g/K . Therefore, it seems that essentially all of the system has formed a single amorphous sucrose phase which has trapped higher than equilibrium amount of water during cooling. However, Fig. 4 shows that the $\Delta C_p''$ decreases with increasing annealing temperature and could not be detected once the annealing temperature reaches about -40°C . These data suggest that the “high moisture” phase disappears, due to crystallization of excess water, thereby forming the maximally freeze-concentrated phase with a glass transition temperature of T_g' .

3.4. Rationale for two glass transitions

During cooling a concentrated sucrose solution, a higher than equilibrium amount of water is “trapped” in the freeze concentrate. Upon heating the sample, crystallization of ice begins once the sample temperature passes the glass transition temperature, T_g'' . However, as ice crystallizes, the water content of the sucrose phase decreases thereby causing the glass transition temperature to increase. We suggest that ice crystallization continues until equilibrium is reached at a temperature slightly below T_g' . As the sample temperature continues to increase, passing through T_g' , the system undergoes a second glass transition corresponding to the newly created maximally freeze-concentrated sucrose phase. If a sample is annealed, excess water crystallizes to ice during annealing. Thus, overall water content is decreased, with some maximally freeze-concentrated sucrose being formed, at least at the boundaries of the ice: solution interface, and with some sucrose being present in a system of higher than equilibrium water content but still lower in water content than before annealing. Heating the annealed sample causes the sample to go through a glass transition at T_g'' , which is at higher temperature than before annealing since the water content is less, and since some of the sucrose is now maximally freeze-concentrated, the amount of material that has higher than equilibrium water is less, so $\Delta C_p''$ is less. Eventually, with more annealing, essentially all non-equilibrium freeze concentrate is converted to maximally freeze-concentrated solute, and the lower temperature transition

can no longer be detected. While this interpretation seems to be consistent with all the data on 10% sucrose solutions, we must admit that alternate interpretations might be possible. However, we do believe this interpretation is plausible. If the exothermic event that occurs between T_g'' and T_g' really is an ice crystallization event as we and others have concluded, the “mechanism” we suggest is perhaps even “probable”. However, it must be noted that this mechanism demands that ice crystallization and freeze concentration do occur nearly to completion during heating—at least for the 10% systems we investigate. Most important, the fact that annealing causes the lower temperature transition to disappear, leaving only the higher transition at T_g' must mean that the higher transition is a glass transition, regardless of the details of how it might be formed. The alternative is to conclude that the amorphous sucrose phase has completely disappeared upon annealing. However, in general, sucrose has not crystallized to any significant extent, and if the lower transition is the only “true” glass transition, we would be forced to conclude that annealing causes an amorphous solid to lose its glass transition. The literature observation [12] that the high temperature transition, denoted T_g' in this research, can be lost by a double annealing process does not fit our speculation as given above. However, these observations [12] are also inconsistent with our data. In fact, we have never observed the transition that is close to the collapse temperature to disappear upon annealing for any material relevant to freeze-drying, unless the solute crystallized. It appears that, for unknown reasons, the thermal behavior of highly concentrated solutions is not exactly the same as for solutions of more modest concentration. Perhaps, ice crystallization never occurred during the second annealing and heating scan in this highly concentrated system. We note, however, that it is the interpretation of T_g'' and T_g' in more dilute solutions that is most relevant to freeze-drying applications as freeze-drying with 40% sucrose formulations does not occur in practice.

4. Conclusions

Our data suggests that T_g'' cannot be the only “true” glass transition temperature for a frozen sucrose solution for the following reasons: (1) annealing causes the thermal event denoted T_g'' to disappear; (2) the area of the endotherm at T_g' in the non-reversing heat flow curve increases with annealing time, which indicates that the transition at T_g' must also be a glass transition. We suggest that most of the system forms a single amorphous sucrose phase containing higher than equilibrium amount of water during cooling. However, during heating or annealing, water crystallizes out after passing the glass transition at T_g'' , causing loss of some or all of the non-equilibrium freeze-concentrated phase, leaving the glass transition of the maximally freeze-concentrated phase at T_g' , leading to what looks like a double glass transition.

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