

## **THE RELATIONSHIP BETWEEN THE TMDSC CURVE OF FROZEN SUCROSE SOLUTIONS AND COLLAPSE DURING FREEZE-DRYING**

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### **Abstract**

This study compares measurements of the collapse temperature of sucrose solutions by freeze drying microscopy with features of TMDSC curves both in the scanning and quasi-isothermal modes. The objective was to determine which feature of the TMDSC curve is predictive of collapse and to provide additional evidence for recent interpretations of the physical significance of the low temperature transitions for sucrose solutions. Interpretations based on the heat capacity signal and the kinetic heat flow using TMDSC are consistent with previous reports based on total heat flow measurement, where the lower temperature transition is the glass transition and the higher temperature transition is associated with the onset of ice melting. Quasi-isothermal experiments further support these conclusions, since additional crystallization of ice is observed only in the region of the lower temperature transition. Collapse of sucrose solutions during freeze-drying begins at the approximate midpoint between the end of the glass transition region and the onset of ice melting.

**Keywords:** frozen sucrose solutions, TMDSC

### **Introduction**

Freeze-drying is often the method of choice to stabilize microorganisms, pharmaceuticals, and many food and biological products. Given the relatively

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long duration of freeze-drying relative to other drying processes, it is important to optimize processing conditions, that is, to minimize the drying time without measurable adverse effects on critical product quality attributes. This requires measurement of the maximum allowable product temperature during primary drying and adjustment of process variables such that product temperature is controlled at a temperature just below this maximum temperature which is dependent on the nature of the material being freeze-dried.

If a solute crystallizes from solution during freezing, it is well known that the maximum allowable product temperature during primary drying is the eutectic temperature. A more common type of freezing behavior, however, is where the solute remains amorphous during the freezing process, and the freeze-concentrated phase becomes more viscous as freezing proceeds. This continues until the temperature reaches the glass transition ( $T'_g$ ) temperature of the freeze-concentrate, below which the amorphous material becomes glassy. With primary drying below this transition temperature, the product will retain the microstructure which was established by freezing. At some temperature above this transition, called the collapse temperature, the amorphous material will undergo viscous flow during the time scale of freeze-drying, resulting in a collapsed product and general loss of the desirable properties of a freeze-dried solid. In this case, it is the collapse temperature which represents the maximum allowable product temperature during primary drying.

The two most common methods for characterization of frozen systems intended for freeze-drying are differential scanning calorimetry (DSC) and freeze-drying microscopy. It is generally accepted that the glass transition temperature of the maximally freeze-concentrated solute is the physical event determining collapse in freeze-drying. The glass transition temperature is not identical to the collapse temperature, however, since  $T'_g$  is measured on a closed system and collapse is a dynamic event that takes place during drying. It has been reported by Pikal and co-workers that collapse usually takes place 2–3°C above  $T'_g$  as a result of deplasticization of the partially dried solids [1]. In order to maximize the utility of low temperature thermal analysis in freeze-drying, a better understanding of the relationship between the low temperature thermal behavior of frozen systems and collapse during freeze-drying is needed.

The model system for the data reported here is sucrose-water because of the pharmaceutical relevance of sucrose as a stabilizer of protein formulations and because of the uncertainty over the physical significance of thermal transitions in frozen sucrose solutions. At sucrose concentrations relevant to freeze-dried pharmaceutical formulations, i.e., about 10% (w/v) and below, there is only one transition observed below the ice melting endotherm. Two transitions are observed in DSC curves of higher concentrations of sucrose, and there has been disagreement in the literature about which transition represents the glass transition.

Recent evidence strongly supports the conclusion that the lower temperature transition is the glass transition. The higher temperature transition has been interpreted as arising from the delayed dissolution of ice by some researchers [2, 3] and as possessing some characteristics of a glass transition by others [4, 5]. The main objective of this study is to determine which feature of the TMDSC curve is predictive of collapse of sucrose solutions as measured by freeze-drying microscopy. In addition, further evidence that supports recent interpretations of the physical significance of the low-temperature transitions in DSC curves of sucrose solutions is presented.

## Materials and methods

### *Preparation of sucrose solutions*

Sucrose (analytical grade) was obtained from Mallinckrodt Baker (Paris, KY). Solutions were prepared by stirring weighed amounts of sucrose and double distilled water until clear solutions were obtained. For the concentrated solutions, i.e., >60% (w/w), gentle heating over a hot plate was used to dissolve the sucrose and remove entrained air bubbles. Water lost by evaporation was added back after dissolution.

### *Freeze-drying microscopy*

Collapse temperatures of 5 and 10% sucrose solutions were measured using a cryostage suitable for vacuum operation (Model BCS 196, Linkham Scientific Instruments, Tadworth, Surrey, UK) with a polarizing microscope (Olympus Model BH-2, McCrone Accessories and Components, Westmont, IL). The stage was calibrated for temperature measurement using both *n*-octane ( $-56.76^{\circ}\text{C}$ ) and *n*-decane ( $-29.66^{\circ}\text{C}$ ) at atmospheric pressure and at 100 mT. The measured melting temperatures for the standards were  $-56.8$  and  $-29.7^{\circ}\text{C}$ , respectively. For improved accuracy of the temperature measurement, 13 mm quartz cover slips (Linkham Scientific Instruments, Tadworth, Surrey, UK) and a thermal conductivity compound (EG and G Wakefield Engineering, Wakefield, MA) were used. Quartz cover slips were used because of their much higher thermal conductivity compared to glass cover slips [6]. A five microliter sample was placed between two cover slips, and the temperature was decreased to  $-45^{\circ}\text{C}$  at a rate of  $1^{\circ}\text{C min}^{-1}$ . The system was evacuated to a pressure of 100 mTorr in most experiments, although some experiments were carried out at 25 mTorr in order to examine the pressure dependence of collapse. Freeze-drying was allowed to proceed at constant temperature until the sublimation front was observed in the field of view. Sample temperature was then increased at a constant rate of  $0.3^{\circ}\text{C min}^{-1}$  until the dried material adjacent to the freeze-drying front was completely collapsed. The sample was photographed in  $0.2^{\circ}\text{C}$  intervals at a magnification of 100 $\times$ .

### *TMDSC measurement of heat flow and heat capacity*

DSC measurements were made with a modulated DSC (Model 2920, TA Instruments, New Castle, DE) equipped with a refrigerated cooling system (RCS). The temperature and cell constant were calibrated with aluminum hermetic pans at  $0.75^{\circ}\text{C min}^{-1}$  using the melting temperature of mercury ( $-38.83^{\circ}\text{C}$ ) and a helium cell purge of  $25\text{ ml min}^{-1}$ . These conditions were chosen to allow for more than 6 heat-only modulation cycles in the temperature range of interest as recommended by TA Instruments. The heat capacity measurement was not calibrated due to uncertainties in accurate calibration using aluminum hermetic pans at low temperature [7].

Sucrose solutions ranged in concentration from 5 to 80% (w/w). Samples were first cooled to  $-25^{\circ}\text{C}$  to allow for ice nucleation, held at  $-10^{\circ}\text{C}$  for 20 min, and then cooled at  $0.75^{\circ}\text{C min}^{-1}$  to  $-65^{\circ}\text{C}$ . Samples were then heated at  $0.75^{\circ}\text{C min}^{-1}$  and modulated at  $0.2^{\circ}\text{C}$  every 80 s after a 20 min isothermal hold to allow for steady state. The curves were recorded during the heating cycle. Transition temperatures in the heat capacity signal and the heat capacity change at both the lower and higher transitions were measured with the Universal Analysis software provided by TA Instruments.

### *Quasi-isothermal heat capacity of 40 and 80% sucrose (w/w)*

Samples were first equilibrated at  $-26^{\circ}\text{C}$ . This temperature was chosen because it is below the heterogeneous ice nucleation temperature typically observed at about  $-20^{\circ}\text{C}$ , and above the region of the higher temperature transition. The sample was then cooled (more than  $5^{\circ}\text{C min}^{-1}$ ) to the quasi-isothermal temperature and modulated at  $0.2^{\circ}\text{C}$  every 80 s for up to five hours. The first thirty minutes of data at each temperature was discarded to allow for the steady state of the modulation conditions.

## **Results and discussion**

The progression of collapse of a 5% (w/w) solution of sucrose in water is illustrated in Fig. 1. Freeze-drying at  $-38.6^{\circ}\text{C}$  resulted in complete retention of the microstructure that was established during freezing. The first visual indication of collapse was observed at  $-37.7^{\circ}\text{C}$  as small pockets adjacent to the freeze-drying front. These pockets of collapse became larger as the temperature was increased to  $-36.2^{\circ}\text{C}$ , until total collapse was observed at temperatures greater than  $-34.2^{\circ}\text{C}$ . Collapse temperatures were the same at 100 and 25 mT for both 5 and 10% (w/w) sucrose solutions. This implies that the drying rate was low enough for these samples to be in a region of little dependence of collapse temperature on drying rate [1].

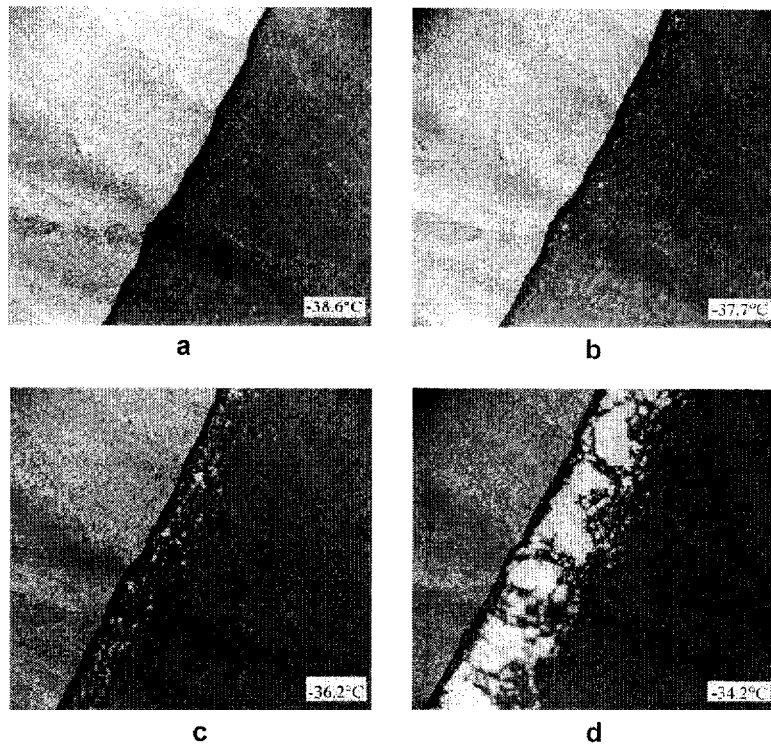


Fig. 1 Photomicrographs of freeze-drying of 5% (w/w) sucrose solution

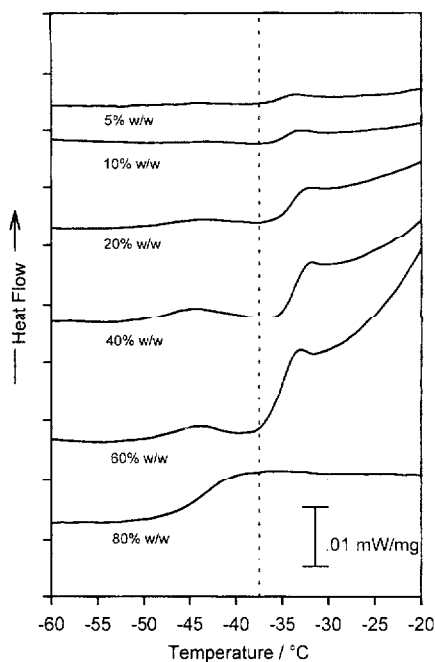
The average value of  $-37.5^{\circ}\text{C}$  for the onset of collapse of sucrose solutions is significantly lower than previously published values (Table 1). Better accuracy is expected for the value reported here. With the cryostage used in these studies, precise control of the temperature is possible along with a very small horizontal temperature gradient ( $\pm 0.1^{\circ}\text{C}$ ) across a small viewing aperture (1 mm). For a stage used in previous studies with a larger viewing aperture (7 mm), larger hori-

Table 1 Collapse temperature of sucrose solutions

Collapse temperature/ $^{\circ}\text{C}$	Sucrose concentration/%	Source
-32	10 (w/v)	Mackenzie
-34		Nail
-37.7	5 (w/w)	measured
-36.2 (end)		
-37.7 (on), -35.5 (end)	10 (w/w)	measured

zontal temperature gradients ( $\pm 0.5^\circ\text{C}$ ) have been observed [8]. In addition, these measurements were made with a calibration of the temperature under vacuum. This calibration issue has largely been overlooked in the past. For example, as much as a  $2^\circ\text{C}$  discrepancy in the temperature of melting of hydrocarbons and NaCl/ice eutectic mixtures has been observed for stages previously used in our laboratory when calibration is checked under vacuum. The use of a thermal conductivity compound has proven essential to the accurate measurement of sample temperatures under vacuum. A combination of the added resistances of poor thermal contact between the stage and quartz cover slip and a wider bore viewing aperture has probably contributed to inaccurate sample temperature measurement under vacuum.

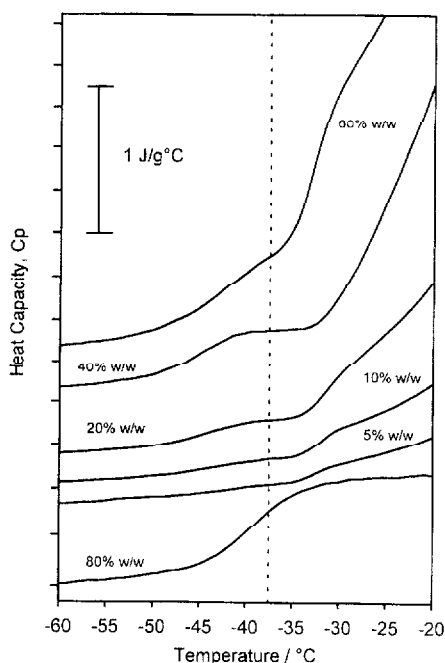
Better reproducibility of the collapse temperature was obtained when sample volumes of 3–5 microliters on a 13 mm cover slip were used. Larger volumes of 8–10 microliters resulted in higher variability between experiments and onset temperatures of 2–3°C higher than those observed with smaller volumes. This may be because of the inability to observe the onset of collapse in the material at the bottom cover slip when the sample is relatively thick. Finally, the use of slow and constant ramping of the sample temperature during freeze drying appears to provide a more standard method of observing the sample compared to step-changes in sample temperature, particularly since collapse is a time-dependent phenomenon.



**Fig. 2** Total heat flow for frozen sucrose solutions. Dotted line indicates the onset of collapse

The total heat flow, heat capacity, and kinetic heat flow curves for sucrose solutions ranging from 5 to 80% (w/w) are shown in Figs 2–4, respectively. The same features are apparent in the heat flow curves that have been reported before [2, 4, 5, 9, 10]. Aside from the ice melting endotherm, only one transition is observed for sucrose concentrations less than 20% (w/w). At higher concentrations, a lower temperature transition is also observed. In addition, an exotherm is apparent between the two transitions for 40 and 60% (w/w) sucrose solutions. This has been most often interpreted as ice crystallization during warming. At a still higher concentration of 80%, approaching the maximum freeze concentration, only one transition is observed. The lower transition is shifted to lower temperatures for frozen sucrose solutions that formed ice compared to the 80% sucrose-water glass. This has been explained as arising from incomplete ice formation and plasticization of the amorphous phase.

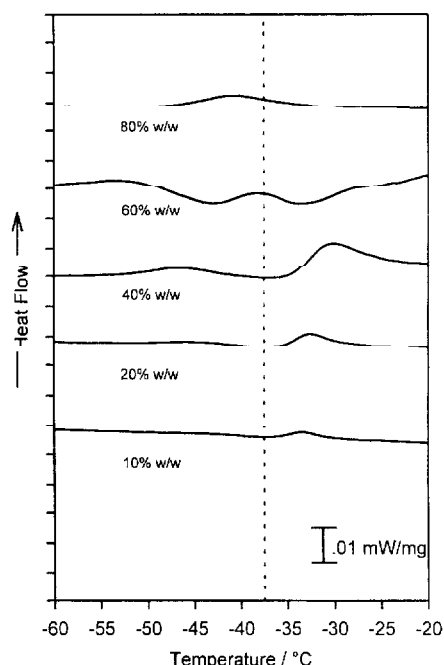
The lower temperature transition appears to be shifted to lower temperatures in the heat flow signals (Fig. 2) relative to the heat capacity signals (Fig. 3). This is likely because of the superposition of the crystallization exotherm on the heat flow signals that is not seen in the heat capacity signals. In Fig. 4, the crystallization exotherm is observed in the kinetic heat flow signals and extends throughout the region of the lower temperature transition for the case of 60% (w/w) sucrose. A superimposed peak in the kinetic heat flow signal is apparent



**Fig. 3** Heat capacity signal for frozen sucrose solutions. Dotted line indicates the onset of collapse

for all of the solutions which indicates that there is most likely an additional frequency effect on the glass transition to account for the shift [11].

Ablett and co-workers have presented a convincing argument that the lower temperature transition is the glass transition of the freeze-concentrated solution [2]. Perhaps the most convincing evidence has been the agreement of the glass transition temperature assignment to the lower temperature transition with the sucrose-water state diagram [12]. Additional evidence that the higher temperature transition is not a glass transition is provided by Fig. 3, where it appears that the heat capacity signal at the higher temperature transition is merging with the ice melting endotherm for the 40% (w/w) solution of sucrose and water. The curve is similar in shape to the predicted equilibrium heat capacity using a thermodynamic model [2]. For the other sucrose concentrations, the kinetic portion of the total heat flow signal (Fig. 4) in this region can only be enthalpy recovery or the effect of kinetically limited melting. Since the samples were cooled and warmed at the same underlying heating rate, enthalpy recovery is unlikely [13]. More likely is the explanation given by Ablett *et al.* [2] and Franks *et al.* [3] of delayed dissolution of ice crystals that formed at lower temperatures. Since the distribution of the heat flow due to melting between the periodic and kinetic components has a complex frequency dependence, it is not surprising that the chosen modulation conditions were not sufficient to eliminate all of the heat flow



**Fig. 4** Kinetic heat flow for frozen sucrose solutions. Dotted line indicates the onset of collapse



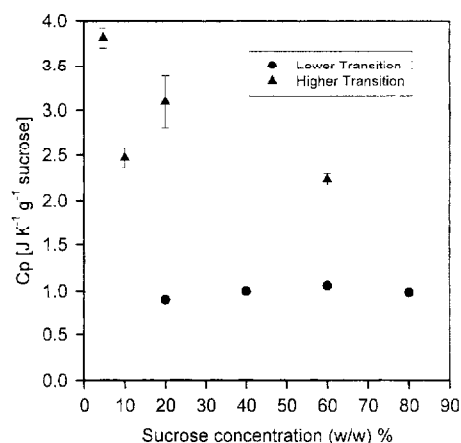
associated with the delayed dissolution of ice from the heat capacity signals. By varying the modulation frequency, resolution of the true heat capacity signals for these solutions as is observed for the 40% (w/w) solution may be obtained by distributing all of the heat flow due to fusion to the kinetic heat flow signal.

It has been widely accepted that the higher temperature transition is the glass transition [5, 14], and that this transition is predictive of collapse in freeze-drying. This has been based at least in part on the fact that at concentrations representative of pharmaceutical formulations, the higher temperature transition is the only transition readily observed. In this study, collapse was observed to begin at the approximate midpoint between the end of the lower temperature transition and the onset of the higher temperature transition (Table 2). At least for sucrose solutions, it appears that an average of these two values ( $-37^{\circ}\text{C}$ ) gives a reasonable estimate of the onset of collapse as measured by freeze-drying microscopy. Similar measurements for other solutions of non-crystallizing solutes will enable testing of the general applicability of this observation.

**Table 2** Transition temperatures for sucrose solutions

Lower transition			
Sucrose/%	$T_o$	$T_m$	$T_c$
5	-37.0	-36.9	-36.7
10	45.1	42.7	-40.3
20	-46.3	-43.4	-40.5
40	-49.3	-45.5	-41.7
60	-47.8	-44.2	-40.6
80	-44.7	-39.4	-34.1
Higher transition			
Sucrose/%	$T_o$	$T_m$	$T_c$
5	-33.9	-32.5	-31.1
10	34.1	32.5	-30.9
20	-34.0	-32.0	-29.9
40			
60	-34.4	-32.8	-31.1

Figure 5 illustrates the relative values of heat capacity changes for the two transitions compared to an 80% sucrose glass. The values are normalized according to the mass fraction of sucrose in each sample. The heat capacity change at the lower transition is close to the heat capacity change of an 80% sucrose glass. The heat capacity change at the higher temperature transition for 10, 20, and 60% sucrose solutions is significantly larger than an 80% sucrose glass. This is con-



**Fig. 5** Relative heat capacity changes for the lower and higher temperature transitions in frozen sucrose solutions

sistent with predicted values for the equilibrium heat capacity change using a thermodynamic model over the temperature range that spans this transition [15]. These results provide added support to the conclusion that the lower temperature transition is the glass transition of the freeze concentrate. The data are similar to measurements made by Sahagian and Goff [16], with a  $\Delta C_p$  of  $\sim 1.2 \text{ J K}^{-1} \text{ g}^{-1}$  sucrose except for the 60% (w/w) sucrose solution. They found that the measured heat capacity change for this case was significantly higher than an 81% sucrose glass that remained amorphous. They attributed the discrepancy to the instability of the 60% solution during warming and uncertainty in measuring the baseline shift. The advantage of TMDSC in measuring heat capacities is very clear for this case where the heat of crystallization interferes with accurate heat capacity measurement by standard DSC.

Ice formation has been observed in sucrose solutions with annealing treatments below the higher temperature transition range as measured by increased enthalpy of melting [9], and increased  $T_g$  [2, 9]. Since this process involves heat capacity changes associated with the conversion of water from the sucrose solution phase to ice, isothermal measurements of heat capacity over time are a direct measure of the kinetics of ice formation.

For the 80% sucrose solution the heat capacity did not vary dramatically over the course of one hour for the entire temperature range studied (Fig. 6). This is in direct contrast to measurements of a 40% (w/w) sucrose solution for five hours that formed ice on cooling as shown in Fig. 7. Since no ice forms in an 80% sucrose glass, the only source of change in heat capacity with time can be from structural relaxation of the amorphous phase as the glass approaches equilibrium. This implies that the structural relaxation time is fast for sucrose-water glasses ( $<30$  min) compared to the kinetics of ice crystallization which is seen on

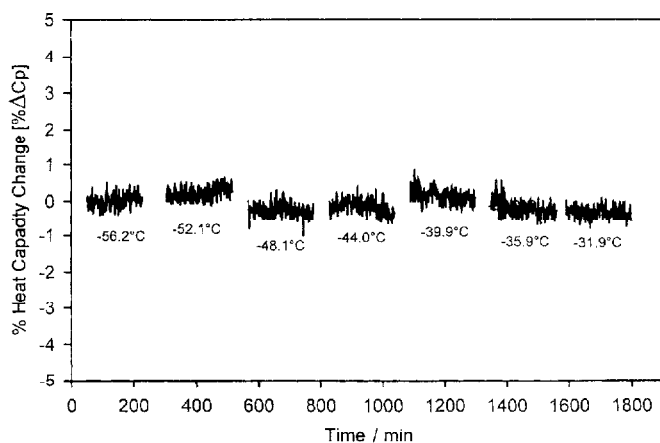


Fig. 6 Quasi-isothermal heat capacity signal for 80% (w/w) sucrose solution

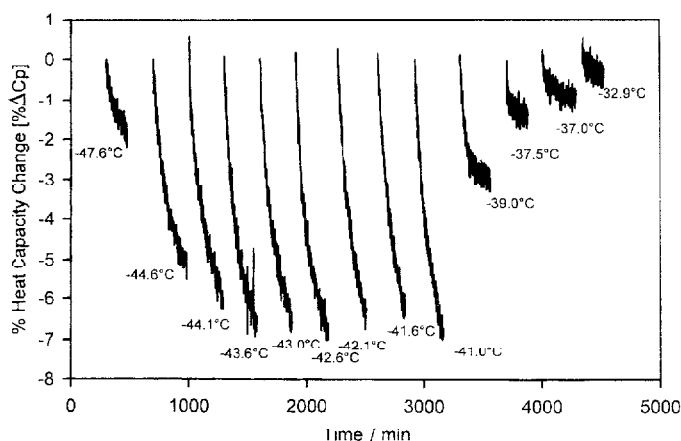


Fig. 7 Quasi-isothermal heat capacity signal for 40% (w/w) sucrose solution

a longer time scale. This is consistent with a general description by Moynihan of structural relaxation times in the glass transition region on the order of seconds to tens of minutes [12]. The changes seen in a 40% (w/w) sucrose solution can therefore be mostly attributed to ice crystallization and concentration of the amorphous phase.

The heat capacity of ice is higher than the heat capacity of sucrose solutions and the limiting heat capacity of frozen sucrose solutions as it undergoes maximum freeze-concentration decreases with increased sucrose concentration [15, 17]. These trends are illustrated in Fig. 8 which is a graphical representation of the limiting heat capacities of frozen sucrose solutions as predicted by Ablett *et al.* using a thermodynamic model [15]. Ice formation therefore contributes to an

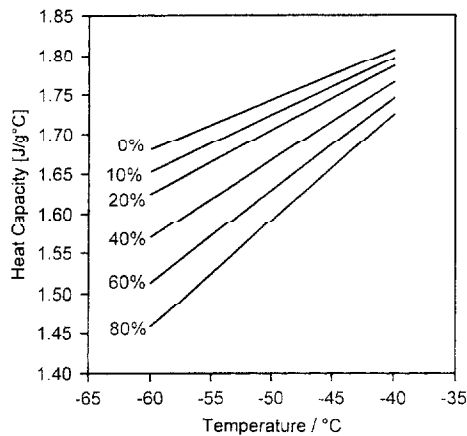


Fig. 8 Limiting heat capacities for maximally freeze concentrated sucrose solutions, plotting from thermodynamic model reported by Ablett *et al.* [15]

increase in the measured heat capacity, whereas freeze concentration contributes to a decrease in the measured heat capacity. While the kinetics of the heat capacity change is expected to be a complex function of the initial concentration, kinetics of ice crystallization, and the heat capacity of the limiting glass, the overall change would be expected to be dominated by the change in heat capacity that accompanies freeze concentration. The reason for this is that the amount of additional ice formed is expected to be small in comparison to the amorphous fraction that undergoes freeze concentration. For example, the  $T_g$  of the 40% (w/w) solution and the sucrose-water glass transition curve [18] were used to estimate a concentration of 76.9% (w/w) for the amorphous fraction compared to a maximum freeze concentration of 80% (w/w). An increase of about 20% in the mass fraction of ice is accomplished by a change in the mass of ice that is only about 2% of the total sample mass. About 88% of the sample participates in freeze concentration.

The decrease of the heat capacity with time in Fig. 7 supports this reasoning. Near the end of the lower temperature transition region ( $-37$  to  $-39^\circ\text{C}$ ), the heat capacity change and additional ice formation occurs over the time period of approximately five hours. This is not the case throughout the region of the lower temperature transition, where an equilibrium value is not attained over the five hour experiments. The kinetics of the heat capacity change appear to be very similar in this temperature range, with a slight decrease in the rate at lower temperatures, which is consistent with the expected change in the rate constant for ice crystallization as a function of temperature. At temperatures less than the onset of the lower temperature transition, the kinetics of ice formation are expected to be very slow. Minimal change would be expected at temperatures below the onset of the glass transition region. Near the onset of the higher temperature transition region ( $-32.9^\circ\text{C}$ ), the heat capacity is essentially constant. This is consis-

tent with the physical process of ice formation from a fluid-like state that progresses rapidly with relatively little kinetic limitation compared to ice crystallization in the glass transition region.

## Conclusions

Measurements of the collapse temperature of frozen sucrose solutions have been made using an improved freeze-drying microscopic method that are several degrees lower than published values. More confidence is placed on this new value based on calibration issues, an improved design, and an improved method that offers good reproducibility.

TMDSC scans of sucrose solutions have revealed evidence to support the conclusion that only the lower temperature transition is a glass transition. Heat capacity changes measured for both transitions have confirmed a reported similarity between the lower temperature transition and an 80% sucrose glass. A portion of the heat flow at the higher temperature transition was found to be non-periodic (kinetic) which supports previous conclusions that this thermal event arises from delayed ice dissolution.

Additional evidence to support the conclusion that only the lower temperature transition is associated with a glass transition is the time dependence of the heat capacity of a 40% (w/w) sucrose glass at quasi-isothermal conditions. A decrease in the heat capacity with time in the region of the lower temperature transition was attributed to kinetically limited ice crystallization and freeze concentration of the amorphous phase. Very little change in the heat capacity with time was observed below temperatures corresponding to the onset of the lower temperature transition and above the higher temperature transition, which is consistent with slow or negligible ice crystallization, respectively, at these temperatures and observation times. In the future, mathematical models of this crystallization and concentration process using both the heat capacity and the kinetic heat flow signals will allow for the prediction of the rate of crystallization of ice in the glass transition region, and an estimate of the true onset of the glass transition range.

The collapse temperature of sucrose solutions was found to be equal to the approximate average of the values for the end of the glass transition region and the onset of ice melting. Further experiments with other non-crystallizing solutes are needed in order to determine whether this is a general rule that can be followed to estimate the collapse temperature by TMDSC.

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