Measurement of Glass Transition Temperatures of Freeze-Concentrated Solutes by Differential Scanning Calorimetry

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Thermal analysis of aqueous solutions in which the solute does not crystallize immediately upon freezing was carried out to define the effects of experimental parameters on thermograms in the glass transition region. The intensity of enthalpy relaxations in the glass transition region is related to both the rate of cooling and the rate of heating through the glass transition region—slow cooling or slow heating increases the extent of structural relaxation in the glassy state and increases the intensity of the endotherm. Plots of the logarithm of heating rate versus $1/T_g'$ are linear, and activation enthalpies for structural relaxation are in the range of 210–350 kJ/mol. For polymeric solutes, both the activation enthalpies for structural relaxation and the heat capacity change accompanying the glass transition increase with increasing molecular weight of the solute. Molecular weight dependence of the observed midpoint of the glass transition agrees with the Fox–Flory relationship. Results are compared and contrasted with glass transitions in solid polymers and with the glass transition of hyperquenched water. Practical implications for characterization of formulations intended for freeze-drying are discussed.

KEY WORDS: thermal analysis; vitrification; polymers; freeze-drying.

INTRODUCTION

Development of pharmaceutically acceptable freeze-dried dosage forms requires that formulation and processing conditions be identified that result in full recovery of activity upon reconstitution, rapid and complete reconstitution, acceptable cake appearance, and adequate shelf life. Processing conditions should also be chosen such that the freeze-drying process is as efficient as possible. This requires that the formulation be characterized to determine the temperature above which the desirable properties of a freeze dried product are lost. The nature of this critical temperature depends on the state of the solute, or solutes, when the aqueous solution is frozen. If the solute crystallizes from a frozen aqueous solution, the maximum allowable product temperature is just below the eutectic point of the formulation. However, it is more common for a solute(s) to crystallize incompletely or not at all with freezing of the system. In this case, the freeze-drying characteristics of the formulation are determined by the viscoelastic properties of the amorphous phase in the interstitial region between ice crystals.

Eutectic-forming systems are generally described by a temperature/composition phase diagram. Systems in which the solute remains amorphous after freezing, however, cannot be described by equilibrium phase diagrams. Franks has described such systems by means of a "state" diagram (1), whereas MacKenzie (2) uses the term "supplemented phase diagram" to present similar information. A generalized state diagram is illustrated in Fig. 1, where the line *ab* represents the freezing-point depression of water in the presence of increasing solute concentration. This line also represents the composition of the freeze concentrate as ice crystals grow in the freezing system. The eutectic point would be observed at point *b* if the solute were to crystallize during freezing; instead, the composition of the freeze concentrate follows the supercooled liquidus curve, becoming more concentrated and more viscous as the temperature decreases. The dashed lines in Fig. 1 represent isoviscosity curves—combinations of temperature and composition which give a constant viscosity. The glass transition curve is an isoviscosity curve representing a viscosity of the order of 10^{14} P. The intersection of the supercooled liquidus curve with the glass transition curve is designated T_g' and is the glass transition of the maximally freeze-concentrated solution. The composition of this freeze concentrate is given by W_g'.

The relevance is T_g' to freeze-drying is that freeze-drying an amorphous system above this value may result in loss of microstructure by viscous flow of solute after the supporting ice structure is removed by sublimation. While it is not identical to the collapse temperature (3), the two quantities are closely related. T_g' is therefore an important characteristic of a formulation intended for freeze-drying, since drying below T_g' should result in retention of the microstructure formed by freezing.

Thermal analysis is useful for the characterization of formulations intended for freeze-drying. We examined with differential scanning calorimetry (DSC) the glass transitions in frozen systems for pharmaceutically relevant solutes which do not crystallize immediately upon freezing the solution, in order to optimize experimental conditions for the detection of T_g'.

MATERIALS AND METHODS

The materials examined in this study are listed in Table I. All materials were either analytical or reagent grade and were used as received. Dextran, polyvinylpyrrolidone (PVP), Ficoll, and lactose were obtained from Sigma Chemical Co. Mannitol, sucrose, glucose, and sorbitol were from Mallinckrodt, and trehalose was obtained from Aldrich Chemical Co. The gelatin used in this study was porcine-derived and was a gift from The Upjohn Company.

Glass transition temperatures were measured in frozen solutions using a computer-aided Perkin-Elmer DSC 7 differential scanning calorimeter. A mechanical cooling accessory was used for cooling the sample chamber to temperatures as low as −75°C. Helium was used as the purge gas at a flow rate of 20 mL/min. This allowed a constant cooling rate of as much as 40°C/min from room temperature to −65°C. Indium and mercury were used for calibration, and the calibration was further verified using double-distilled water and the sodium chloride/water eutectic. Heat flow was

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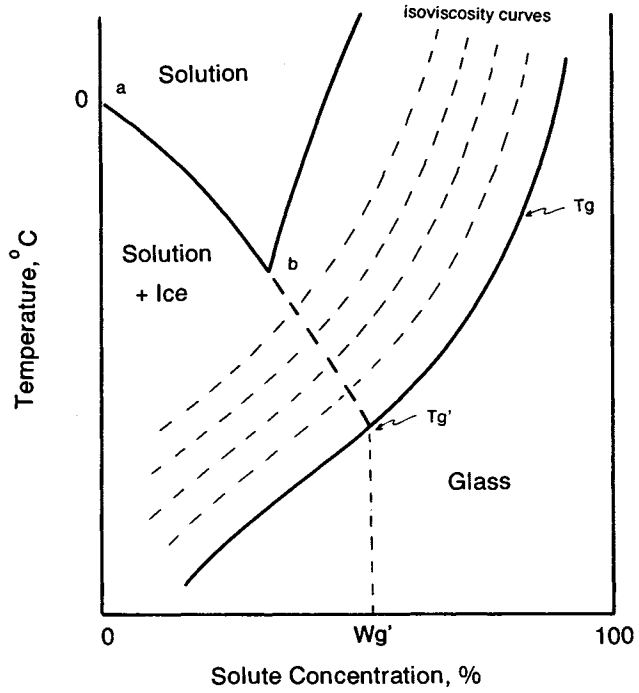

Fig. 1. State diagram for a noncrystallizing solute (redrawn from Ref. 1).

calibrated by using double-distilled water and measuring the area under the ice melting endotherm.

The sample chamber was housed in a glove box to minimize frost formation in the sample cell. The glove box was continuously purged with dry nitrogen.

For the thermal analysis experiment, approximately 25 μL of solution was placed in an aluminum sample pan with a capacity of about 50 μL. An aluminum top was placed on the sample and crimped in place (not hermetically sealed). An empty sample container was used as the reference. The sample was cooled at a controlled rate and held at the lowest temperature for 5 min prior to heating to help ensure thermal equilibration. The thermogram was recorded during heating of the sample at a controlled rate to avoid anomalous results caused by supercooling.

All glass transition values are reported as the midpoint of the transition. Initial studies were carried out to determine the effect of repeated scans through the glass transition region on the thermogram. Reproducibility of results was established by analysis of five separate samples each from single 10% stock solutions of lactose, sorbitol, and dextran. A separate study was carried out to determine the effect of the sample volume on the reproducibility of the results.

Sodium chloride solution was used for comparison of the effects of experimental parameters on the observed eutectic temperature relative to the effects of the same parameters on glass transitions.

RESULTS AND DISCUSSION

A representative thermogram of a "frozen" solution is illustrated in Fig. 2. The inset is the segment of interest in this report. Subsequent plots have been rescaled from the original thermogram showing only the T_g' region.

The standard deviation of the midpoint of the glass transition for replicate samples analyzed under the same conditions of cooling rate and heating rate was 0.5°C or lower for all solutes studied. The observed T_g' values were independent of sample size in the range 10–30 mg. There was more variability in the midpoint of the transition when the sample size was less than 15 mg, probably because of inconsistent wetting of the bottom of the sample pan by the sample.

The effect of repeated scans through the glass transition region on the shape of the thermogram and the midpoint of the transition was studied for 10% lactose and 10% sorbitol. After heating through the glass transition region at a rate of 10°C/min, samples were recooled at a rate of 0.5°C/min, then reheated at 10°C/min. This process was repeated five times. The results indicate no apparent effect of repeated scans on the shape of the thermogram. However, the entire thermogram shifts toward slightly higher temperatures with repeated scans, the magnitude of the shift decreasing with each scan. The total increase in the midpoint temperature from the first through the sixth scan is only about 0.25°C for both lactose and sorbitol.

Effect of Cooling Rate on T_g'

Figure 3 illustrates the effect of cooling rate on the thermogram of 10% lactose solution. The thermogram was recorded during heating the sample at a rate of 10°C/min. The thermogram is characterized by an apparent endotherm superimposed on the glass transition. The relative intensity of this endotherm becomes greater at slower cooling rates and essentially disappears at higher cooling rates. Results of systematic variation of the cooling rate for other solutes gave the same qualitative result; i.e., an endotherm superimposed on the glass transition, the relative intensity of which increases as the cooling rate becomes slower. The relative magnitude of the endotherm differs significantly between solutes, however. For example, the thermogram of 10% dextran (data not shown) has a much more intense enthalpy relaxation peak than that of 10% lactose. For 10% sorbitol (data not shown) the effect is less pronounced than for 10% lactose. The data are consistent with the idea that larger solute molecules are able to undergo more structural relaxation than smaller and less structurally complex solutes. The shape of the thermogram at temperatures just below the glass transition is also affected by the cooling rate. As illustrated in Fig. 3, the slope in the range of −35 to about −30°C increases as the cooling rate decreases.

The effects of cooling rate on the thermogram of the glass transition region are consistent with results reported for polystyrene by Wunderlich and co-workers (4). This effect was interpreted using the free volume theory of the glass transition. At slow cooling rates, there is more time for molecules to relax to lower-energy conformations and minimize the "free volume" in the system. The concept of "frozen-in" states is introduced, where for fast freezing of a material followed by relatively slow heating, some molecules frozen in to a high-enthalpy state by fast freezing will have time to relax back to a lower-energy state prior to the glass transition. Structural relaxation in the glass reduces the enthalpy of the system. This causes an endotherm to be observed at the glass transition because, in the transition from glass to

Table I. Effect of Heating Rate on T_g'

10% solution	Measured T_g' (°C) q = 2	q = 5	q = 10	q = 20	Predicted T_g' (°C) q = 0.1	q = 0.05	Δh* (kJ/mol)	ΔC_p (J/K/g solute)
Trehalose	−31.8	−30.5	−29.2	−27.6	−37.2	−38.4	270	4.7
Lactose	−30.8	−29.5	−28.3	−26.7	−36.1	−37.2	279	5.1
Sucrose	−35.7	−34.3	−32.9	−31.0	−41.8	−43.1	233	3.9
Sorbitol	−49.3	−47.9	−46.1	−44.8	−55.2	−56.5	211	2.3
Dextrose	−46.8	−45.1	−43.8	−42.3	−52.4	−53.7	223	2.8
Mannitol	−31.1	−29.3	−28.0	−26.4	−36.9	−38.3	246	6.8
Dextran								
8.8K	−13.7	−12.7	−11.5	−9.6	−19.2	−20.4	315	9.7
39.1K	−12.6	−11.5	−10.5	−8.5	−18.1	−19.3	319	10.5
70K	−12.6	−11.7	−10.8	−8.8	−18.0	−19.2	331	10.5
503K	−12.3	−11.4	−10.3	−8.5	−17.6	−18.7	337	10.8
PVP								
10K	−28.8	−27.4	−26.0	−23.5	−35.9	−37.3	217	2.6
40K	−23.2	−22.0	−20.5	−18.3	−29.9	−31.3	241	4.5
Gelatin	−11.8	−10.1	−9.1	−7.7	−16.9	−18.1	326	—
Ficoll								
70K	−21.4	−20.4	−19.3	−17.7	−26.4	−27.5	324	4.1
400K	−21.7	−20.6	−19.8	−18.2	−26.3	−27.2	353	4.1

fluid, the system must suddenly "make up" the energy of relaxation at the glass transition and absorb energy in a fashion analogous to melting.

The presence of an endotherm superimposed on the glass transition has been attributed to a stress relaxation in the material when heated through the glass transition. Roy *et al.* (5) studied the glass transition of an amorphous freeze-dried monoclonal antibody–vinca conjugate and reported that the endotherm disappears on scanning through the glass transition, then recooling, followed by reheating through the glass transition region (see Fig. 5 in Ref. 4). The rate of cooling after first heating through the glass transition is not specified; however, the shape of the second thermogram may arise from the difference between cooling and reheating rates rather than relieving stress within the solid sample when warmed to a temperature just above the glass transition. While the results reported here may not be applicable to analysis of freeze-dried amorphous solids, they seem consistent with the literature on thermal analysis of polymers in the solid state.

The effect of cooling rate on the thermogram of an aqueous solution of mannitol, which forms a metastable amorphous phase initially upon freezing, is illustrated in Fig. 4. At a cooling rate of 0.5°C/min, the thermogram is flat in the temperature range of about −35 to −5°C. At faster cooling rates, there is a glass transition followed by an endotherm, then a crystallization exotherm. The shape and intensity of the exotherm are also affected by the cooling rate. At the faster cooling rate of 40°C/min, the peak of the exotherm appears at about −18°C, whereas at a cooling rate of 5°C/min the exothermic peak is at about −14°C. The reason for

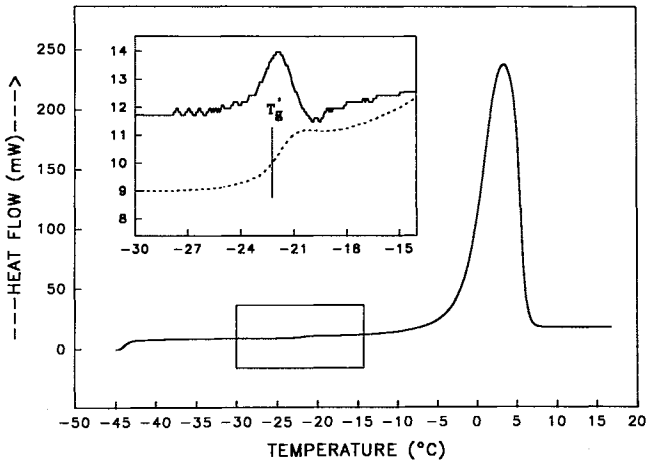

Fig. 2. DSC thermogram of 10% polyvinylpyrrolidone, illustrating the magnitude of the T_g' transition relative to the melting endotherm of ice.

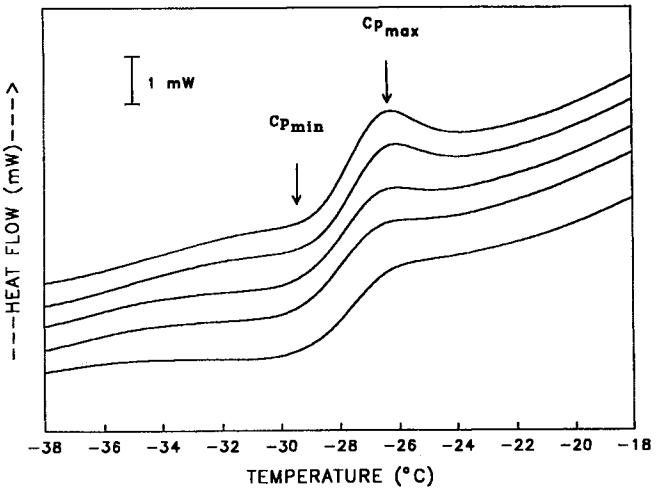

Fig. 3. The effect of cooling rate on the DSC thermogram of 10% lactose. Cooling rate, 10°C/min. Cooling rates are, from top to bottom, 0.5, 5, 10, 20, and 40°C/min.

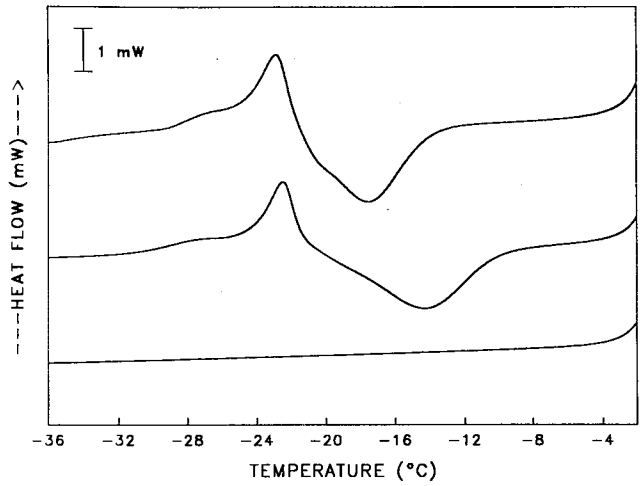

Fig. 4. The effect of cooling rate on the DSC thermogram of 10% mannitol solution. Heating rate, 10°C/min. Cooling rate: bottom curve, 0.5°C/min; middle curve, 5°C/min; top curve, 40°C/min.

the endotherm prior to the crystallization exotherm is not understood at present and is being examined further.

The Effect of Heating Rate on T_g'

The effect of heating rate on the thermogram of 10% dextran (70 kd) cooled at a constant rate is illustrated in Fig. 5. The effect of heating rate is to increase the magnitude of the shift in the baseline due to the heat capacity change accompanying the glass transition. In addition, the midpoint of the transition occurs at a significantly higher temperature at faster heating rates.

It is important to recognize that the heating rates used during the freeze-drying process are ordinarily much lower than the heating rates used in a thermal analysis experiment. The marked temperature dependence of the glass transition temperature on the heating rate indicates that the glass transition occurs at a significantly lower temperature than commonly assumed based on thermal analysis. Table I includes

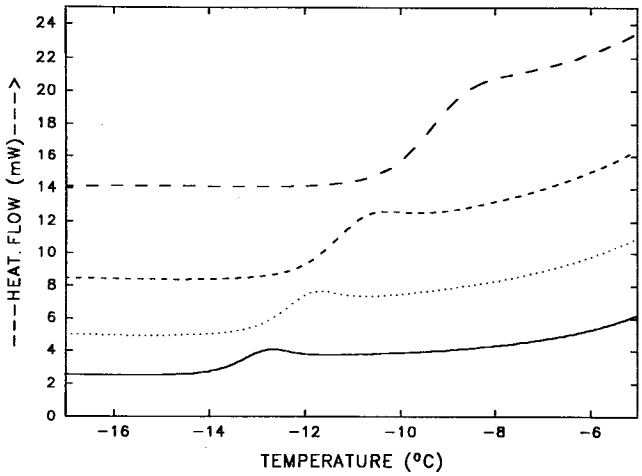

Fig. 5. The effect of heating rate on the DSC thermogram of a 10% dextran solution. Cooling rate, 40°C/min. Heating rate: solid line, 2°C/min; dotted line, 5°C/min; short-dashed line, 10°C/min; long-dashed line, 20°C/min.

calculated values of T_g' assuming heating rates of 0.1 and 0.05°C min^{-1}. These values are significantly lower than those reported by other investigators, where the effect of heating rate on T_g' was not considered (8–10).

The logarithm of the heating rate, q (see Table I), was plotted against $1/T_g'$ at the midpoint of the transition for each material. Linear plots were obtained, with r^2 values ranging from 0.93 to 0.99. The slope of the line is equal to $-\Delta h^*/R$, where Δh^* is the enthalpy of activation for structural relaxation of the glass. For nonpolymeric materials, the quantity Δh^* is equivalent to the activation energy for shear viscosity above T_g (6,7).

The activation enthalpies for structural relaxation of the freeze-concentrated solutes studied here are in the range of 210–350 kJ/mol. Johari *et al.* (11) studied the glass transition of a series of poly(propylene glycols) (PPGs) and the PG monomer and reported values ranging from 200 kJ/mol for the monomer to 350 kJ/mol for PPG 4000. Although the cited study was for a one-component system rather than an aqueous solution, the range of activation enthalpy values is in good agreement with the values reported here. In either case, it seems reasonable that the structural relaxation must involve breaking hydrogen bonds, whether they are between molecules of polymer or between solute and water molecules in the freeze concentrate.

Hallbrucker *et al.* (12) also studied the glass transition of hyperquenched water and reported a value of 55 kJ/mol for the activation enthalpy of glassy water, for which the glass transition temperature is about 136 K. This value was said to reflect the breaking of two hydrogen bonds in order for rotational-translational diffusion of a water molecule to occur.

For the polymeric solutes examined here, the activation enthalpy increases with increasing molecular weight, which is consistent with the data reported by Johari *et al.* (11). Increased activation enthalpy with increased molecular weight implies increased configurational restrictions for segmental motions within polymers with increased molecular weight.

Heat capacity changes accompanying the glass transition are summarized in Table I. With the exception of Ficoll, the change in heat capacity at the glass transition increases with increasing molecular weight of the solute. Johari *et al.* reported that, for PPGs, ΔC_p increases with molecular weight as the molecular weight increases from the monomer to PPG 200. Above PPG 200, ΔC_p decreases with increasing molecular weight.

The observed change in heat capacity was independent of the heating rate, within experimental error. Although the baseline deflection is larger at faster heating rates, the heat capacity change per unit mass is calculated by dividing the power input (mW) by the heating rate.

Effect of Concentration on T_g'

DSC thermograms of lactose solutions at concentrations of 2.5, 5, and 10% are shown in Fig. 6 for a cooling rate of 40°C/min and a heating rate of 10°C/min. As expected, the baseline deflection increases markedly as the concentration increases from 2.5 to 10%. The midpoint of the glass transition does not appear to change significantly as a function of concentration. For sorbitol, sucrose, and lactose, the mid-

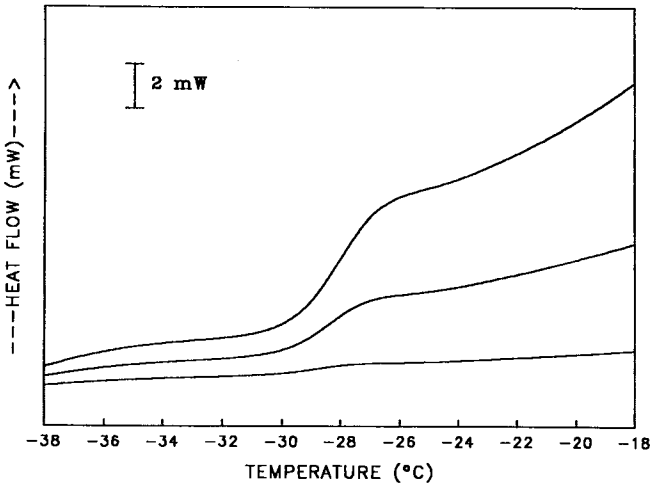

Fig. 6. DSC thermograms of lactose solutions at concentrations of 2.5, 5, and 10%. Cooling rate, 40°C/min; heating rate, 10°C/min.

point of the transition increased by 0.8, 1.4, and 0.4°C, respectively, for an increase in solute concentration from 5 to 20%. For dextran (70 kd), PVP (40 kd), glucose, and trehalose, the increase in T_g' was 0.2, 0.7, 0.6, and 0.8°C, respectively, for the same increase in concentration. Thus, increasing the solute concentration improves sensitivity while causing only a small change in the observed value of T_g'. This observation is consistent with the "state diagram" in Fig. 2, which indicates that the composition of the maximally freeze-concentrated solute should be independent of the starting concentration. The effect of increasing the starting concentration should be only to increase the ratio of freeze-concentrate phase to ice.

Effect of Molecular Weight on T_g'

The relationship between glass transition and molecular weight of polymers is usually described by the Fox–Flory equation:

$$T_g = T_{g\infty} - C/\mathrm{MW}$$

where $T_{g\infty}$ is the limiting glass transition temperature as the molecular weight of the polymer approaches infinity, and C is a constant. Levine and Slade (8), studying frozen solutions of starch hydrolysis products, have shown that the Fox–Flory relationship holds for a series of such compounds.

If the midpoint of the glass transition is plotted against 1/MW for the solutes examined in this study excluding mannitol, a straight line is obtained with a correlation coefficient of 0.98. The data support the conclusion that the Fox–Flory relationship applies for the more general situation, where there is not necessarily a common monomer unit among solutes of different molecular weight. An exception is mannitol, which is an isomer of sorbitol and has a molecular weight close to that of glucose, yet the mannitol data point is well above the regression line. The reason for this is not well understood, but the more symmetrical structure of mannitol, which makes it crystallize more readily than sorbitol, may also mean that less water is associated with mannitol in the amorphous freeze concentrate than glucose or sorbitol, which causes a higher glass transition temperature.

Free volume theory is generally used to explain the dependence of glass transition temperature on molecular weight, where the free volume associated with chain ends is greater than that associated with the middle because of more disordered packing around chain ends, and a higher free volume causes a lower glass transition temperature. As molecular weight increases, the relative contribution of chain ends becomes negligible, and the glass transition temperature approaches a limiting value.

Comparison with Thermal Analysis of Eutectics

The effects of cooling rate and heating rate on the observed eutectic temperature of a 1% sodium chloride solution were determined in order to compare these effects between crystalline and amorphous systems. Varying the heating rate from 2 to 20°C/min resulted in an increase in the observed onset of eutectic melting from −21.8 to −20.5°C. This contrasts with the heating rate effect on the observed midpoint of the glass transition of a noncrystallizing solute, which may increase by over 5°C for the same range of heating rates. The overshoot observed for sodium chloride and other crystalline systems is caused by thermal lag attributable to the thermal analysis equipment itself. The effect of varying the cooling rate between 0.5 and 40°C/min is to increase the onset of eutectic melting by only about 0.1°C.

Practical Considerations

For solutes which do not crystallize immediately from water when the solution is frozen, the glass transition of the maximally freeze-concentrated solute is an important property of the system but often a very subtle feature of the DSC thermogram. Detection of these transitions can be enhanced by systematically varying the parameters in the thermal analysis experiment; for example, using very fast heating rates to maximize sensitivity for detection of the glass transition, then using a slower rate to obtain a more accurate estimate of the glass transition temperature. Cooling rate is a particularly important parameter when the solute forms a metastable amorphous phase from which the solute crystallizes with subsequent heating. Multiple cooling rates should be used for characterizing formulations.

Using concentrated solutes is a useful method for en-

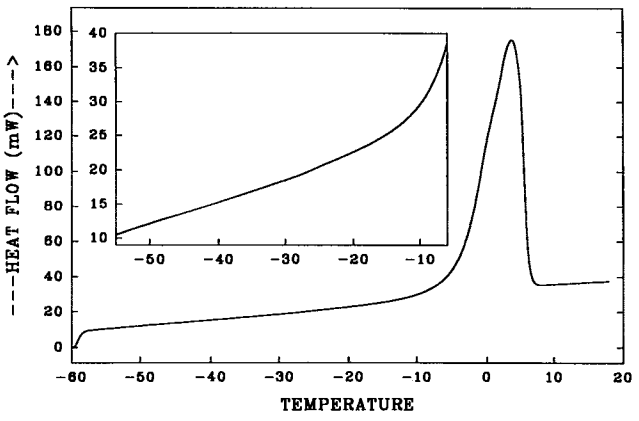

Fig. 7. DSC thermogram of hemoglobin solution at 50 mg/mL. Cooling rate, 40°C/min; heating rate, 10°C/min.

hancing sensitivity without introducing bias in the results. However, analysis of actual formulations in our laboratory has shown that often no transition is observed, regardless of measures taken to enhance sensitivity. An example is the thermogram of hemoglobin, illustrated in Fig. 7. It may be that the heat capacity change is too small to be detected by the instrumentation or that the glass transition occurs over a broad temperature range, and no abrupt shift in the baseline is apparent. It is also possible that the glass transition occurs at a temperature high enough for the glass transition to be obscured by the leading edge of the ice melting endotherm. Current research is directed at studying other methods which are potentially more sensitive for measurement of glass transition temperatures, particularly electrical thermal analysis and dynamic mechanical analysis.

ACKNOWLEDGMENTS

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