

Effect of freezing rate on the thermal, mechanical and physical aging properties of the glassy state in frozen sucrose solutions

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Received 3 November 1993; accepted 20 April 1994

Abstract

The freezing of foods containing significant quantities of sugar and other carbohydrate-based systems involves the removal of water (plasticizer) as ice crystals leading to the complex formation of a metastable amorphous (unfrozen) phase. A study was undertaken using differential scanning calorimetry (DSC) and thermal mechanical analysis (TMA) to characterize the influence of freezing rate on the formation and stability of the glassy state in freeze-concentrated sucrose solutions. Two thermal transitions were evident upon warming these solutions, denoted T_{Tr1} and T_{Tr2} in this study. Rapidly frozen systems exhibited concentration-dependent thermal behaviour (T_{Tr1on} and T_{Tr2} values and level of devitrification) and reduced mechanical resistance to structural flow (TMA). The physical aging behaviour was freezing-rate-dependent (greater excess enthalpy for rapid freezing) and showed maximum excess enthalpy values in good agreement with T_{Tr1} DSC measurements under the various treatments. Annealing at -35°C for 60 min following slow freezing resulted in the greatest increase in T_{Tr1} , up to -40°C (DSC), and an increase in specific volume below T_{Tr2} suggesting that the T'_g occurred in this temperature range. The freezing rate had a substantial influence on the resulting stability and formation of frozen systems which attest to the importance of kinetic variables in understanding food freezing.

Keywords: Ageing; DSC; Freezing rate; Glass; Sucrose; TMA

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1. Introduction

In recent years substantial research has focused on the non-equilibrium behaviour of frozen systems. It is becoming generally accepted that many food materials are inherently far from thermodynamic equilibrium and their properties and relative stability are dominated by kinetics rather than thermodynamics [1]. An understanding of the dynamics of the amorphous phase and the associated time-temperature-dependent influence on mechanical properties will provide a better framework for predicting food stability. In particular, a clear understanding of the kinetics of the unfrozen phase (UFP) above the glass transition representing maximal freeze-concentration, T'_g , is necessary because it dictates the molecular diffusion properties and reaction rates which facilitate product degradation.

Carbohydrate solutions subjected to sub-zero temperature undergo a freeze concentration process, consequently inducing the formation of an amorphous metastable phase. Removal of plasticizer in the form of pure ice leads to a dramatic increase in the effective glass transition temperature T_g , and in the viscosity, which greatly decreases the diffusion kinetics of the unfrozen component. As the temperature is lowered the crystallization process becomes dominated by kinetics; hence partial freeze-concentrated amorphous domains of lower T_g and solute concentration C_g can exist depending on the magnitude of deviation from equilibrium freezing [2]. Increased solute concentration coupled with an increase in viscosity leads to this effect. Because maximum ice formation during dynamic cooling is most likely impossible to achieve, annealing must be employed within a narrow temperature range between the glass transition and melting where time-dependent crystallization can occur [1,3–7]. Consequently, controversy exists about the true position of the T'_g and the origin of other sub-zero thermal events.

Partial freeze-concentrated sucrose solutions under controlled warming conditions typically show two thermal transitions with different magnitudes in ΔC_p . This behaviour was first reported by Luyet and Rasmussen [8] more than 20 years ago in systems where a large amorphous and a crystalline phase co-exist. Levine and Slade [9,10] brought to light the kinetic similarity between carbohydrate systems and amorphous polymers and the importance of T'_g as a physicochemical parameter of food stability. They have reported for non-annealed frozen sucrose system a T'_g (midpoint) at -32°C . Other researchers have criticized this interpretation and have shown that this endothermic step change is the onset of ice melting (T_m) or dissolution, and contains no amorphous component [3–5,7,11,12]. These researchers have concluded that the concentration-independent T'_g occurs at the first transition and is dependent on thermal history, i.e. freezing rate and annealing protocols are necessary to obtain accurate T'_g values. T'_g values of -32°C midpoint [13], -35°C midpoint [3], -46°C onset and -40°C midpoint [4,5,11], and -40°C onset [1], have been reported. Other evidence which supports the standing hypothesis that the T'_g defined by Levine and Slade is not a second-order transition was presented by Ablett et al. [1]. They concluded that annealing above -40°C caused a significant reduction in T_g which resulted from a dilution and concurrent plasticization of the glass due to ice melting. Izzard et al. [3] have pointed out that

the second transition is much sharper and larger in ΔC_p than would be expected for a glass transition. However, Blond and Simatos [14] have shown, using heat capacity data, that both transitions are associated with the glass transition of the amorphous phase while the second transition may overlap the incipient ice melting relaxation.

The glassy state relaxes towards thermodynamic and volumetric equilibrium during storage at constant temperature. This process is called physical aging in polymer science and effects several material properties such as relaxation times, mechanical properties, and the overall energy state of the glass [15–17]. Physical aging studies on dehydrated and aqueous sorbitol and glycerol solutions have shown that relaxation behaviour is similar to polymer glasses in terms of T_g shifts and enthalpy overshoot [12,18–20]. Roos [21] has noted that aging effects on sucrose glasses, e.g. candies, may alter their storage stability and physical properties. However, the effects of physical aging on sucrose glasses with respect to excess enthalpy change (energy-state stability) and mechanical properties has yet to be reported. In particular, information on the enthalpy relaxation process in frozen sucrose glasses may provide insight into the effect of freezing rate on overall mechanical and enthalpic stability. Also, isochronal annealing may prove useful in determining T_g .

The purpose of this study was to examine the effect of freezing rate and isochronal annealing conditions on the enthalpy relaxation process (T_g overshoot), and the thermodynamic (DSC) and mechanical (TMA) phase behaviour of 20%, 40%, and 60% sucrose solutions. The overall objective was to characterize the non-equilibrium behaviour of these systems and to identify experimental conditions which favour T_g' formation.

2. Methods and materials

2.1. Preparation of solutions

Sucrose (Fisher Scientific Co. Ltd., Whitby, Ont., Canada) solutions consisting of 20%, 40%, and 60% (w/w) were prepared by slowly adding the appropriate quantity of solute into vigorously mixing 65°C double-distilled water. The solutions were warmed to 75°C and held for 10 min, then rapidly cooled to 4°C and aged for 12 h before being analysed. Moisture driven off during preparation was added back.

2.2. Thermal analysis

2.2.1. Thermal behaviour — differential scanning calorimetry (DSC)

The thermal phase behaviour of the various sucrose concentrations subjected to rapid and slow freezing conditions was measured using a Du Pont Thermal Analyst 2000 System (TA Instruments, New Castle, DE, USA) equipped with a 2910 cell base. The cell constant and temperature calibration were determined using gallium, indium and ethylene glycol as standards. Samples of approximately 7.0 mg were hermetically sealed in standard DSC pans and either rapidly frozen in a liquid nitrogen bath or slowly frozen at a rate of 2°C min⁻¹ to -100°C in the DSC.

Rapidly frozen samples (approximate freezing rate $1.6 \times 10^3 \text{ }^\circ\text{C s}^{-1}$) were quickly inserted into a pre-chilled (-100°C) DSC after 5 s of freezing. Each system was stabilized at -100°C or -80°C and scanned to 15°C at a warming rate of 5°C min^{-1} . The apparent increase in heat capacity (ΔC_p) associated with each transition was determined from the change in heat flow, scanning rate (5°C min^{-1}), sample weight and initial concentration using the following relationship

$$\Delta C_p = \frac{\text{heat flow (W g}^{-1}\text{)}}{\text{heating rate (}^\circ\text{C s}^{-1}\text{)} \times \text{sucrose ratio (w/w)}} \quad (1)$$

ΔC_p ($\text{J g}^{-1} \text{ }^\circ\text{C}^{-1}$) values are presented in terms of the grams of sucrose present.

2.2.2. Mechanical behaviour — thermal mechanical analysis (TMA)

Volumetric changes as a function of temperature were determined on a Du Pont 2940 TMA module (TA Instruments, New Castle, DE, USA) using an expansion probe and the parallel plate rheometer attachment. Sucrose solutions were frozen into $500 \pm 10 \text{ } \mu\text{m}$ disk, 1 cm in diameter, between two circular parallel plates using a brass machined plate holder. Solutions were rapidly frozen by placing the loaded plate holder into a liquid nitrogen bath for 1 min or by slow freezing in a plate freezer set at -20°C . Samples slowly frozen were held for 15 min in the plate freezer to facilitate maximum freeze-concentration. After freezing, the samples were placed into a pre-chilled cage and quickly inserted into the TMA stage cooled to -80°C . Samples were allowed to equilibrate and subsequently warmed to 5°C at a rate of 2°C min^{-1} under a constant stress of 140 Pa.

Annealed systems were prepared and frozen in the same manner although during warming were held isothermally at -35°C for 30 min. During warming from -80°C and aging, the stress applied to the frozen system was negligible. Once complete, the systems were rapidly cooled to -80°C , allowed to equilibrate and rewarmed at a rate of 2°C min^{-1} to 10°C (140 Pa). Onset of the first transition was determined as the inflection point of the increase in specific volume (discontinuity in expansion coefficient) while the temperature of the second transition was determined at the point of mechanical collapse (decrease in specific volume). The thermal expansion coefficient above the second transition was determined from the slope of the dimensional change profile using the equation

$$\alpha_1 = \frac{(dH)}{(dT)} \left(\frac{1}{H} \right) \quad (2)$$

where α_1 is the thermal expansion coefficient, H the initial sample height, and dH/dT the change in sample height with temperature T (slope). The expansion of the plates was subtracted from the warming profile prior to analysis. Warming curves were normalized for small variations in sample height.

2.3. Physical aging of freeze-concentrated sucrose solutions — isochronal annealing

Annealing was performed on rapidly and slowly frozen sucrose solutions (40% and 60% sucrose) at various temperatures (-55 to -20°C) to study the tempera-

ture and freezing rate dependency of physical aging. Samples of approximately 7.0 mg were frozen as previously described, warmed at $5^{\circ}\text{C min}^{-1}$ to a specific annealing temperature and held isothermally for 60 min in the DSC. Once complete, the sample was recooled ($30^{\circ}\text{C min}^{-1}$) to -80°C , stabilized and rewarmed at $5^{\circ}\text{C min}^{-1}$ to 20°C . Excess enthalpy (ΔH_{xen}), shown as a T_g overshoot, was determined by integrating the area of the endothermic peak after the second transition temperature.

3. Results and discussion

3.1. Effect of freezing rate on thermodynamic behaviour

The thermal behaviour of rapidly frozen 20%, 40%, 60% (w/w) sucrose solutions is shown in Fig. 1. Freezing characteristics under this condition were found to be dependent on concentration. Two thermal events involving changes in heat capacity were observed during warming of the 20% solution (Fig. 1). For the purpose of this paper, the two transitions will be identified as $T_{\text{Tr}1}$ and $T_{\text{Tr}2}$. The first transition has been labelled the glass transition of the freeze-concentrated phase [5]. However, the second transition is the subject of much controversy [1,3,5,22,23]. This biphasic behaviour is common in systems in which a large crystalline and amorphous phase co-exist [12]. Glass transition temperatures ($T_{\text{Tr}1}$) identified as a second-order step change in the heat flow profile were found to be -54.6 , -49.2 and -99°C for rapidly frozen 20%, 40%, 60% sucrose, respectively (Fig. 1). A large reduction in the apparent T_g ($T_{\text{Tr}1}$) of the 60% sucrose solution suggests the glass was substantially plasticized, most likely due to excess unfrozen water (plasticizer) within the

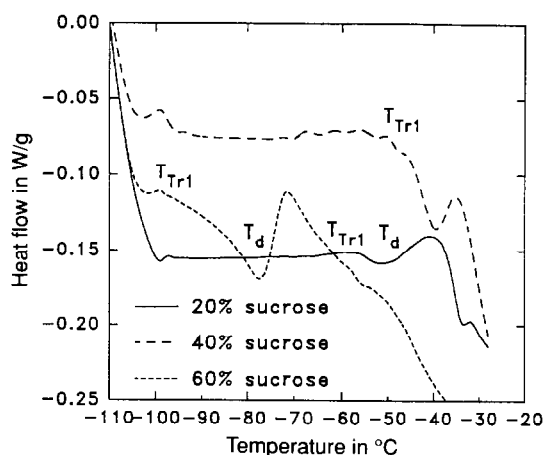


Fig. 1. Thermal behaviour of 20%, 40% and 60% (w/w) sucrose solutions rapidly frozen in liquid nitrogen and warmed at a rate of $5^{\circ}\text{C min}^{-1}$. $T_{\text{Tr}1}$ represents the partial freeze-concentrated glass and T_d is the onset temperature of devitrification during warming.

sucrose matrix. The excess water would result from non-equilibrium freezing leading to a partially freeze-concentrated glass [1,4,5]. This interpretation was supported by the occurrence of a relatively large detectable devitrification exotherm after the first transition. Ablett et al. [1] have shown that the exotherm in a vitrified sucrose solution is the result of a delayed crystallization due to quench-freezing.

The relative magnitude of the apparent ice crystallization event was found to be dependent on initial solute concentration as were the T_{Tr1} onset values. The amount of delayed crystallization was approximated by integration of the exotherm peak between T_{Tr1} and T_{Tr2} . The enthalpy change associated with the exotherm was found to be as large as 20 J g^{-1} (solution) for the 60% solution demonstrating the kinetic constraint imposed on the crystallization process in concentrated systems, even at low temperatures. Even at the lower concentration, non-equilibrium ice formation dominated the thermal behaviour and the resulting T_{Tr1} . In rapidly frozen 40% and 60% sucrose systems, there was an incomplete resolution of T_{Tr2} , making it almost impossible to detect. Recent studies have shown that T_{Tr2} is a result of initial ice melting and does not involve an amorphous component [1,4,5,11]. Rasmussen and Luyet [2] proposed that the transition involved a relaxation between the ice crystals and the amorphous phase. It appears from the thermograms that the origin of this phenomenon, in solutions where the ice phase is reduced, is dependent on freezing rate, and therefore, on time. This would imply that kinetic variables may have influenced the development of the structure or phase undergoing relaxation. The fact that these systems are unstable and probably not maximally freeze-concentrated suggests that T_{Tr2} is associated with some type of ordering of water molecules or formation of a critical ice content, which requires time.

After slow freezing at 2°C min^{-1} in the DSC, T_{Tr2} was resolved and the apparent exotherm (ΔH_{ex}) was substantially reduced. A small but measurable devitrification peak was observed in sucrose solutions containing 20% and 40% sucrose (Table 1). The ΔH_{ex} was approximately 2 and 2.5 times smaller than values obtained under

Table 1

Thermodynamic data for 20%, 40%, and 60% (w/w) sucrose solutions slowly frozen at 2°C min^{-1} and warmed at 5°C min^{-1} measured by DSC ^a

Sample	T_{Tr1on} ^b in $^\circ\text{C}$	$\Delta C_p(T_{Tr1})$ ^c in $\text{J g}^{-1} \text{ }^\circ\text{C}^{-1}$	ΔH_{ex} ^d in J g^{-1}	T_{Tr2on} ^e in $^\circ\text{C}$	$T_{Tr2,mid}$ ^f in $^\circ\text{C}$	$\Delta C_p(T_{Tr2})$ in $\text{J g}^{-1} \text{ }^\circ\text{C}^{-1}$
20% sucrose	-46.7 (3.63)	1.1 (0.14)	0.25 (0.035)	-33.7 (2.21)	-30.6 (2.14)	3.2 (0.27)
40% sucrose	-48.0 (4.31)	1.2 (0.18)	0.35 (0.048)	-33.8 (2.21)	-30.9 (1.79)	3.5 (0.39)
60% sucrose	-97.3 (9.91)	2.1 (0.24)	11.6 (1.18)	-42.9 (3.84)	-40.4 (5.63)	1.45 (0.27)
81% sucrose	-39.2 (2.55)	0.91 (0.11)	- -	- -	- -	- -

^a Each value represents the mean and standard deviation (in parentheses) of 7 determinations. ^b Onset of T_{Tr1} . ^c Per gram of sucrose. ^d Enthalpy of devitrification after T_{Tr1} . ^e Onset of T_{Tr2} . ^f Midpoint of T_{Tr2} .

rapid freezing for 20% and 40% sucrose, respectively. These results suggest that the rate of freezing has a dramatic effect on the equilibrium crystallization process; however, even during slow freezing, maximum freeze-concentration did not occur, and therefore, a true T'_g was not formed. The occurrence of this exotherm with a measured T_g of -46.7°C for 20% sucrose suggests that the proposed T'_g of -46°C [5] may be a little low. Ice crystallization of the more concentrated 60% solution was also significantly delayed. A ΔH_{ex} of approximately 11.6 J g^{-1} was determined, approximately half the value measured from the rapidly frozen system. As expected, the $T_{\text{Tr}1}$ of this solution was significantly lower than the determined onset value of -47°C and -48°C for 20% and 40% sucrose, respectively. These $T_{\text{Tr}1}$ values correlate well with the T'_g values of properly annealed samples reported by Roos and Karel [4,5,22]. The $T_{\text{Tr}2}$ midpoint values were found to be independent of concentration except for the unstable 60% system which exhibited a substantially lower $T_{\text{Tr}2}$ (Table 1). Keeping the warming rate constant, ΔC_p values are expected to increase slightly with increasing plasticizer [3,5,14]. The ΔC_p values for $T_{\text{Tr}1}$ were concentration dependent, increasing with initial sucrose concentration. The same measurement for the higher transition ($T_{\text{Tr}2}$) was inconsistent and difficult to determine with the 60% sucrose solution because of poor resolution.

3.2. Physical aging and annealing of freeze-concentrated sucrose solutions

Excess enthalpy as a function of concentration and annealing temperature (T_a) for rapidly and slowly frozen solutions is shown in Fig. 2. Measurable excess enthalpy was not detected for either rapidly frozen system until the system was annealed at -60°C . This was followed by a curvilinear increase in ΔH_{xen} with T_a

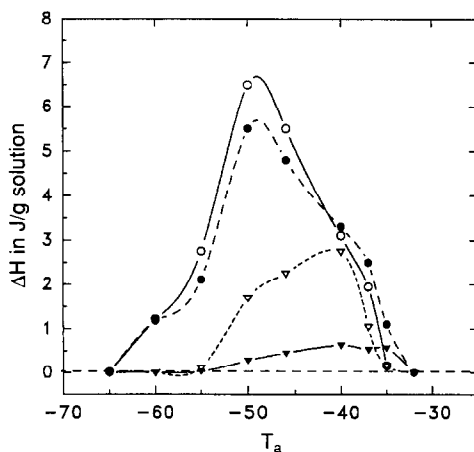


Fig. 2. Excess enthalpy profile defined as ΔH_{xen} (y axis) for 40% and 60% (w/w) sucrose solutions rapidly (60% (○); 40% (●)) and slowly (2°C min^{-1}) (60% (▽); 40% (▼)) frozen as a function of annealing temperature (T_a). Each point represents the mean excess enthalpy of three determinations following isothermal annealing for 60 min.

until a maximum was observed at -48°C . At annealing temperatures past this point, excess enthalpy gradually decreased in a somewhat linear manner up to -35°C , after which no overshoot was measured. According to theory, annealing at various sub- T_g temperatures (T_a) should result in a range of enthalpy compensation overshoots (excess enthalpy) leading to a maximum at the onset of T_g . Because the translational intramolecular mobility necessary for rearrangement and enthalpy loss would be greatest at the onset, greater compensation in the form of an excess enthalpy overshoot would be expected. It is believed that this result indicates that the true T_g onset started at approximately -48°C under rapid freezing. Chang and Baust [20] studied the physical aging behaviour of vitrified glycerol solutions with various annealing conditions. They reported that annealing at sub- T_g temperatures led to an overshoot in heat flow which reached a maximum immediately before the T_g . The gradual decrease in ΔH_{xen} may represent the relative amount of glass which is relaxing at each temperature past the onset. Past the onset, the amorphous component available to undergo relaxation continued to decrease with increasing temperature up to -32°C at which point the UFP existed as a rubbery liquid. This result supports the idea that a second-order transition occurs over a temperature range. With regards to cooling, it appears that the supercooled liquid may start to deviate from equilibrium around -32°C . Similar trends in excess enthalpy were also reported to 60% sorbitol solutions and glycerol solutions [12,19,20].

In systems frozen slowly the degree of physical aging was found to be substantially reduced (Fig. 2). The magnitude of excess enthalpy for the 60% sucrose solution had decreased approximately 43% as a result of slow freezing. This result reflects the relative excess enthalpy of the two systems. A large excess enthalpy reduces the average relaxation time so that physical aging can occur more rapidly as observed with the rapidly frozen system [24]. However, slow freezing apparently promoted a more stable, lower enthalpy amorphous state and, therefore, the relaxation kinetics during aging occurred within a much longer timescale. The evolution of excess enthalpy under other conditions clearly shows that freezing rate not only affects the crystallization kinetics but also the energy state of the amorphous component.

The maximum ΔH_{xen} for both slowly frozen systems increased to approximately -40°C (Fig. 2). Formation of the sucrose glasses under slow conditions apparently shifted the T_{Tr1} onset temperature. During aging, two processes were occurring simultaneously. Ice formation during rewarming and annealing (time-temperature dependent), as well as the reduction of free volume during rearrangement of the amorphous phase, shift the relaxation (T_{Tr1}) to higher temperatures. This accounts for the apparent anomaly between onset temperatures measured from the DSC scan, dynamic measurement (Table 1), and the onset values interpreted from the excess enthalpy profile.

Slowly frozen systems annealed at temperatures between -55 and -35°C resulted in increased T_{Tr1} onset values (51 to -40°C). However, annealing at -32°C and above leads to a reduction in T_{Tr1} values of about 8°C . Based on these observations, systems annealed at -35°C were considered as close as possible to maximum ice content, and stability and, therefore, thermodynamic and mechanical properties were compiled on these systems.

3.3. Maximally freeze-concentrated T'_g of sucrose solutions

The transition represented by T_{Tr2} in this study has been denoted by Levine and Slade [25] as the T'_g of the maximally freeze-concentrated unfrozen phase (-32°C midpoint). It is now believed that the correct onset of the T'_g is considerably below this temperature and the glass relaxation and subsequent ice melting and dissolution are highly associated, but separated events. However, MacInnes [26] has reported that the two transitions would coincide if measured at low frequency (mHz). Therefore, with regard to DSC, it is possible that separation is partially the result of a finite heating rate and infinitely small heating rates would lead to one transition.

The thermal behaviour of the various systems slowly frozen and annealed at -35°C for 60 min is shown in Table 2. Under these conditions, the transitions directly follow each other, while at annealing temperatures above or below, separation was observed. Separation was assumed to be an indication of incomplete freeze concentration and excess free energy within a system. This protocol was considered optimal and resulted in an average T_{Tr1} of -40.5°C and -41.0°C for 20% and 40% sucrose, respectively. Maximum ice formation appeared to occur only within a specific annealing temperature, which most likely represented the end of the transition. These onset data correlate highly with the T'_g reported by Ablett et al. [1,7] and MacInnes [26] but were much higher than that of Roos and Karel [4,5,22]. The 60% solution may not be maximally freeze-concentrated based on the behaviour of the other two systems under these conditions. The T_{Tr1} and ΔC_p values at the T_{Tr1} are close to the measured T_{Tr1} (-39.2°C) and ΔC_p ($0.91 \text{ J g}^{-1}\text{C}^{-1}$, Table 1) for 81% sucrose solutions. The difference may be a result of uncertainty during measurement of the baseline shift. In any case, the ΔC_p of $1.2\text{--}1.1 \text{ J g}^{-1}$ sucrose strongly supports the current literature that the first transition represents the T'_g of the maximally freeze-concentrated sucrose matrix. As pointed out by Ablett et al. [1] and Izzard et al. [3], the ΔC_p at T_{Tr2} between 3.9 and 3.1 J g^{-1} sucrose is clearly in excess of the value of the total vitrified system (0.91 J g^{-1} sucrose). T_{Tr2} onset,

Table 2

Thermodynamic data for 20%, 40%, and 60% (w/w) sucrose solutions slowly frozen at 2°C min^{-1} , annealed at -35°C for 60 min and warmed at 5°C min^{-1} , measured by DSC^a

Sample	T_{Tr1on} ^b in $^\circ\text{C}$	$\Delta C_p(T_{Tr1})$ ^c in $\text{J g}^{-1}\text{C}^{-1}$	T_{Tr2on} ^d in $^\circ\text{C}$	T_{Tr2mid} ^e in $^\circ\text{C}$	T_{Tr2end} ^f in $^\circ\text{C}$	$\Delta C_p(T_{Tr2})$ in $\text{J g}^{-1}\text{C}^{-1}$
20% sucrose	-40.5 (1.2)	1.2 (0.096)	-32.6 (0.58)	-31.3 (0.58)	-29.5 (0.73)	3.9 (0.20)
40% sucrose	-41.2 (1.3)	1.1 (0.11)	-32.8 (0.52)	-31.1 (0.60)	-29.1 (0.58)	3.2 (0.17)
60% sucrose	-45.6 (2.1)	1.6 (0.15)	-34.5 (0.55)	-32.3 (0.61)	-30.1 (0.72)	3.1 (0.16)

^a Each value represents the mean and standard deviation (in parentheses) of three determinations.

^b Onset of T_{Tr1} or T'_g . ^c Per gram of sucrose. ^d Onset of T_{Tr2} . ^e Midpoint of T_{Tr2} . ^f End point of T_{Tr2} .

midpoint and endpoint values indicated the transition was concentration independent and relatively sharp, occurring, on average, over a 4°C temperature range (Table 2). This in itself suggests that the mechanism of this transition is dominated by something other than a second-order transition.

3.4. Influence of freezing rate and annealing on mechanical behaviour of frozen sucrose solutions

Changes in volumetric structure as a function of temperature for 40% rapidly frozen and slowly frozen annealed systems are shown in Fig. 3. As evident from the thermograms, thermal history had a dramatic effect on mechanical behaviour. For the rapidly frozen system, thermal deformation and viscous flow occurred around -58°C (representing T_{Tr1}) and continued up to -35°C where a second relaxation process was observed (Table 3). Volume expansion due to devitrification during warming was not seen. It appeared that under rapid freezing conditions, mechanical deformation and flow above -58°C was an indication that the glass transition had occurred.

Non-equilibrium freezing resulted in an increase in unfrozen water and a reduction in T_{Tr1} . This suggests that a less than maximum crystalline phase coupled with an unstable amorphous phase of excess volume due to rapid freezing, created a system of reduced mechanical resistance. Systems of this nature exhibited large variation in the temperature at which mechanical deformation was observed, because ice content, size distribution and stability of the amorphous state were probably quite sensitive to experimental conditions and difficult to repeat. Struc-

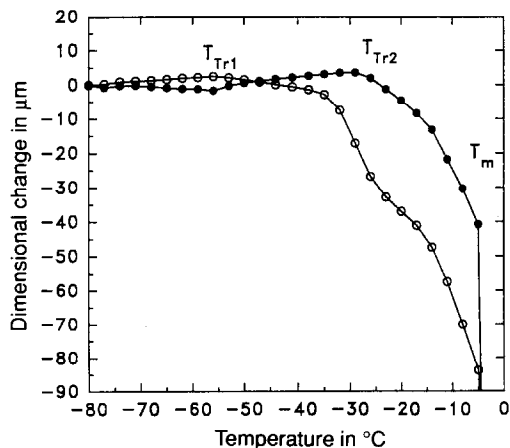


Fig. 3. Thermomechanical behaviour of 40% (w/w) sucrose solutions rapidly frozen (○) and slowly frozen (●) (plate freezer) and annealed at -35°C for 30 min showing T_{Tr1} and T_{Tr2} . After thermal treatment, systems were equilibrated at -80°C for 3 min and warmed at a rate of $2^{\circ}\text{C min}^{-1}$ under a stress of 140 Pa. Data points represent the mean of three determinations.

Table 3

T_{Tr1} , T_{Tr2} and thermal expansion coefficient (α) above T_{Tr2} for 20%, 40%, and 60% (w/w) sucrose solutions slowly frozen ($<2^{\circ}\text{C min}^{-1}$), annealed and rapidly frozen, measured by TMA ^a

Sample % (w/w)	Slowly frozen ^b , annealed -35°C for 30 min			Rapidly frozen ^c
	T_{Tr1}	$\alpha \times 10^{-4}$ ^d	T_{Tr2}	T_{Tr2}
20% sucrose	-47.3 (2.14)	1.4 (0.12)	-30.8 (0.65)	-34.1 (0.99)
40% sucrose	-52.1 (2.22)	1.5 (0.11)	-30.1 (0.71)	-35.5 (1.56)
60% sucrose	-54.1 (3.55)	1.3 (0.12)	-32.1 (0.55)	-40.2 (2.22)

^a Each value represents the mean and standard deviation (in parentheses) of 4 determinations.

^b Frozen in a plate freezer set at -20°C ; approximate rate, $2^{\circ}\text{C min}^{-1}$. ^c Approximate freezing rate $1.6 \pm 103^{\circ}\text{C s}^{-1}$. ^d Thermal expansion coefficient determined by Eq. (2).

tural collapse at -33°C was in good agreement with the mechanical spectrum reported by Maurice et al. [27] and Schenz et al. [28]; however, both groups identified this transition as the T'_g . Structural deformation and flow past T_{Tr2} appeared to proceed in a bi-phasic manner. Rearrangement and flow between -35 and -18°C may have involved ice melting/recrystallization mechanisms inducing a stabilizing effect. Le Meste and Huang [29] observed similar behaviour in more dilute systems and hypothesized that migratory ice recrystallization had a large effect on mechanical properties. Annealing slowly frozen systems at -35°C for 30 min resulted in a dramatic increase in mechanical rigidity (Fig. 3). In contrast to the rapidly frozen systems, slowly frozen and annealed samples showed an increase in thermal expansion coefficient (volume increase) within the temperature range -53°C to -47°C for the three systems investigated (Table 3).

A gradual increase in volume expansion, representing a volume recovery process once adequate thermal energy was acquired [30], occurred from -52 to -30°C at which point flow began (Fig. 3). The onset of the increase in thermal expansion coefficient was considered a linear increase in free volume associated with the glass transition. Calculated expansion coefficients (α) determined from the slope of the volume increase using Eq. (2) are shown in Table 3. Similar values were found for all three systems. Volume reduction through structural relaxation and rearrangement during aging appeared to be recovered at the T'_g similar to the enthalpy recovery process observed in DSC. In the present systems, volume expansion was only observed after annealing. Overall, annealing at -35°C created a structure of increased integrity and reduced free volume, possibly through increased ice formation (delayed crystallization) and/or better overall packing. This result also provides more evidence that an amorphous domain exists that can physically age above -35°C .

The T_{Tr1} of 20% sucrose was found to agree with the T'_g proposed by Roos and Karel [4,5,11] and that of an 80% solution reported by Le Meste and Huang [29].

However, as concentration was increased, T_{Tr1} onset was lowered. This was likely due to the presence of excess plasticizer (unfrozen water) leading to increased free volume. In concentrated systems, it is assumed that the unfrozen sucrose matrix dominates the kinetics of ice formation and the overall mechanical response. These results suggest that the present annealing conditions and sample setup of this study was insufficient in facilitating continued ice formation in concentrated systems. Owing to large differences in sample size, geometry and resulting thermal history, the DSC T_{Tr1} and TMA T_{Tr1} do not agree. However, the TMA results support the DSC in that T_g onset may occur within the -50 to -40°C range, not at -33°C as originally proposed [9].

A shift in the beginning of viscous flow at T_{Tr2} accompanied by a substantial increase in mechanical resistance past this transition was observed after annealing (Fig. 3 and Table 3). Le Meste and Huang [29] observed an increased resistance to deformational flow after annealing and attributed this to a stabilizing effect of devitrification. Although the latter is feasible, it is conceivable that only a minimal amount of unfrozen water crystallizes during aging, enough to cause a T_g shift. However, during annealing at temperatures before melting rearrangement, crystal size and/or structural changes may facilitate favourable packing, reducing free volume and leading to a more dense system. Enthalpy, volume reduction and a more packed system will cause molecular restrictions leading to increased relaxation times, and hence higher temperatures and times are required to initiate flow. Therefore, within a temperature range where ice melting is reversible, the total crystalline phase as well as its structure in terms of free volume voids may control the dynamic flow of the rubbery viscoelastic liquid.

4. Conclusions

Rapid freezing resulted in a low T_{Tr1} , measurable devitrification, and reduced mechanical resistance to structural flow. Slow freezing increased T_{Tr1} ; however, following annealing at -35°C for 60 min, T_{Tr1on} increased to -40°C and the two transitions (T_{Tr1} and T_{Tr2}) appeared to be associated. Volume recovery (expansion coefficient) following annealing suggested that the T_g onset from TMA occurred at or below the T_{Tr1on} determined from DSC. The physical aging profile (excess enthalpy) was freezing-rate-dependent (greater values for rapid freezing) and showed maximum values in agreement with the T_{Tr1} determined by rapid and slow freezing/annealing treatments from DSC. This suggests that the energy state of the amorphous component may contribute to the experimental T_g onset. It can be concluded from the above discussion that the transition identified in this paper as the T_{Tr1} is in fact the T'_{gon} , and the T_{Tr2} appeared to evolve from first-order processes. However, the presence of a small overshoot after T_{Tr2} following annealing at -35°C may suggest some second-order component to this transition in addition to ice melting. Because T_{Tr2} is associated with more than one process, the result is an enormous increase in flow and solute mobility. Therefore, as pointed out by Le Meste and Huang [29], the implications of the second transition are of great

importance in terms of the overall stability of frozen systems. It is also apparent from this study that a rapid freezing rate had a great influence on the stability of frozen systems and therefore may need to be optimized to ensure maximum storage quality.

Acknowledgment

The authors thank the Natural Sciences and Engineering Research Council of Canada for financial support of this project.

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