

Viscosity and Glass Transition Temperature of Aqueous Mixtures of Trehalose with Borax and Sodium Chloride

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The glass transition temperature (T_g) and viscosity of aqueous trehalose and its mixtures with either NaCl or $\text{Na}_2\text{B}_4\text{O}_7$ (borax) have been measured over a wide range of concentration. Borax and sodium chloride increase the viscosity and T_g of aqueous trehalose. Although the presence of ionic charges may partly explain some of the observed effects, it is clear that ionic solutes that form complexes with trehalose lead to a greater increase in T_g . The viscosity of supercooled aqueous mixtures was measured at several temperatures, most of them above the so-called crossover temperature, T_g' , where a change in the dynamics of relaxation processes is expected. The viscosities of all the binary and ternary mixtures exhibit the same temperature dependence when the temperature is scaled according to the T_g values; a change in the slope of the linearized WLF representation is observed at a temperature close to $1.2T_g$. Above this temperature, a power law expression provides a similar description of the temperature dependence of viscosity as the WLF equation, the former having a physical justification in the mode coupling theory.

Introduction

Trehalose (α -D-glucopyranosyl- α -D-glucopyranoside), a naturally occurring disaccharide of glucose, is well-known for its ability to preserve life in cells,^{1,2} organisms,³ and biomolecules^{4–9} under conditions of extreme drought or low temperatures. Some studies indicate that, compared to other disaccharides, trehalose is a more effective protective agent.¹⁰ Although it has received considerable attention in recent years, its protective mechanisms are not well understood. On one hand, Green and Angell¹¹ suggested that trehalose's protective efficacy is a result of its elevated glass transition temperature (T_g) which is higher than that of other disaccharides.^{11,12} According to this view, the glassy state maintains biological structures in their native state and arrests dynamic processes that lead to a loss of activity. On the other hand, Crowe et al.¹³ proposed a "water replacement hypothesis", by which, in the absence of water, the hydroxyl groups of trehalose (and other saccharides) form hydrogen bonds with polar residues of biological structures.

Evidence in the literature indicates that trehalose acts through a combination of the aforementioned glass-forming and water replacement mechanisms;^{14,15} our experience is consistent with this view. In this work, we make no attempt to elucidate such a mechanism. However, we do investigate the viscosity of aqueous trehalose mixtures, which is relevant to the formation of trehalose glasses. Furthermore, since nearly all biological systems contain ionic solutes, we are interested in their influence on the protective properties of trehalose. This view has led us to examine the mixtures studied in this work.

Recent theoretical work predicted that the presence of ionic solutes in polar solvents dramatically increases T_g .¹⁶ Although experimental results indicate that the effect is weaker than predicted,^{17,18} it is clear that ionic solutes increase T_g . Furthermore, the degree of this increase depends on the nature of the ions involved.

Miller et al.¹⁹ recently demonstrated that aqueous mixtures of trehalose and sodium tetraborate are more effective protective agents than trehalose solutions. That work involved centrifugal vacuum-drying of a labile enzyme, lactate dehydrogenase, in the presence of aqueous protectant mixtures. Formulations that included trehalose and borate were found to maintain the activity of this enzyme during storage under conditions of either high temperature (45 °C) or high relative humidity (from 30% to 100%). While the affinity of sodium tetraborate for water could account for part of the observed effect, it is also worthwhile to investigate the well-known ability of borate to form chemical complexes with polyhydroxy compounds^{20–24} and the effect of ionic solutes on the thermophysical properties of trehalose solutions. A complete understanding of these nonequilibrium formulations requires knowledge of the parameters that govern dynamic processes, viscosity and T_g . These properties have implications for the processing and subsequent storage of biological materials; the temperature dependence of viscosity is of utmost importance to product stability.²⁵ This last issue constitutes the central aim of this work. We present an analysis of the viscosity of aqueous trehalose/borate mixtures at various temperatures, along with the glass transition temperatures of aqueous trehalose/borate and trehalose/NaCl mixtures, and we evaluate the ability of various models to predict the observed temperature dependence.

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Experimental Section

Materials and Methods. D-(+)-Trehalose dihydrate (Pfanstiehl Laboratories, 98.9%), sodium chloride (Aldrich Chemical Co. 99+%, ACS reagent), and sodium tetraborate decahydrate (Aldrich Chemical Co., 99.5+%, ACS reagent) were used for all experimental work. All solutions were prepared gravimetrically on an analytical microbalance; the results of all weighings were adjusted to vacuo by the appropriate buoyancy factor. Most solutions required heating and mixing to dissolve the solutes. Solutions were heated in sealed glass vials in a constant-temperature bath. The pH of the aqueous trehalose/borate mixtures was adjusted by adding drops of concentrated HCl or NaOH.

The absolute water contents of trehalose dihydrate and sodium tetraborate decahydrate were measured using Karl Fischer coulometric titration (Brinkmann, model 737) and thermogravimetry (Netzsch, TG 209), respectively. The water content of all solutes was accounted for in the preparation of aqueous compositions.

Differential Scanning Calorimetry. A Netzsch DSC-200 differential scanning calorimeter was used for calorimetric measurements. An 11 point calibration of both temperature and enthalpy was done for each set of conditions. Approximately 20 mg of each solution was weighed in a Netzsch TG 209 thermogravimetric analyzer at 20 °C, and sealed in an aluminum pan with a crimping press. An empty aluminum pan, identical to that used for the sample, was used as the reference. The sample was immediately quenched by plunging the sealed pan directly into liquid nitrogen. All DSC data were collected during heating. Nitrogen vapor, produced by heating liquid nitrogen in a Dewar, was used to precool the sample cell to temperatures about 40 °C below the expected T_g . The sample pan was then quickly transferred from the liquid nitrogen quench bath to the sample cell. The sample was thermally equilibrated for 5 min before beginning the experiment at a scan rate of 5 °C/min. The sample cell was continually purged with dry N₂ gas (99.9995% pure) at 25 mL/min. Nitrogen from the same source was also used as a protective gas to prevent condensation of water on the DSC cell (300 mL/min).

Sample preparation and experimental conditions were chosen according to ASTM standard test method E 1356-91. The glass transition temperature was determined by constructing tangents to the DSC curve baselines before and after the glass transition. The intersection of these lines to the tangent at the inflection point gives the extrapolated onset and end-point temperatures. As is customary, T_g is reported as the midpoint of these temperatures.

Viscometry. Viscosities of solutions were measured using a Bohlin CVO controlled-stress rheometer with a coaxial cylinder ("cup and bob") measurement geometry. A 14 mm diameter bob was used for highly viscous samples, while a 25 mm bob was used for the less viscous solutions ($\eta < 0.5$ Pa s). The minimum temperature allowed by the cooling system was -20 °C. The shear stress was adjusted between 0.15 and 7000 Pa to provide a shear rate between 1 s⁻¹ and 40 s⁻¹. For measurements at temperatures above 10 °C, a drop of low-viscosity silicone oil (5 mPa s) was placed on the liquid surface to reduce evaporation of water.

Results and Discussion

Effect of Salts on the Glass Transition Temperature. Table 1 summarizes the T_g of aqueous trehalose/borax solutions at nominal trehalose mole fractions, x_T , of 0.075 and boron mole fractions, x_B , between zero and 0.1515. The boron mole fraction

TABLE 1: Glass Transition Temperatures of Aqueous Trehalose/Borax Solutions

x_T	x_B	m_T^a	m_B^b	T_g (K) ^c	T_g (K)	
					pH = 7.0	pH = 9.0
0.0749	0.0000	4.4947	0	199.1		
0.0749	0.0224	4.6064	1.3776	204.5	202.0	205.2
0.0749	0.0597	4.8033	3.8323	213.3	205.5	218.0
0.0747	0.0748	4.8760	4.8825	216.9	211.2	220.2
0.0750	0.1123	5.1187	7.6677	224.7	212.6	229.2
0.0751	0.1515	5.3909	10.8754	233.8	218.3	242.2

^a mol trehalose/kg H₂O. ^b mol boron/kg H₂O. ^c pH not adjusted.

has been calculated from the borax concentration of the solutions by assuming that borax (Na₂B₄O₇) hydrolyzes according to the reaction



To avoid ambiguity, alternative units of molality (mol/kg H₂O) are given for trehalose and boron. These units are also calculated based on the assumption that borax hydrolyzes according to the above reaction. The results at pH 7.0 and 9.0 correspond to solutions in which the pH was adjusted with concentrated HCl and NaOH, respectively.

The glass transition temperatures of aqueous trehalose/NaCl solutions at several trehalose concentrations and NaCl mole fractions have been reported in the literature.²⁶ The effect of an added salt on the glass transition temperature can be described by a T_g increment, ΔT_g , which is given by the difference between the of the ternary solution and that of the binary trehalose solution at the same trehalose mole fraction, x_T . That is,

$$\Delta T_g = T_g(x_T, x_S) - T_g(x_T, 0) \quad (1)$$

where x_S is the mole fraction of added salt and $T_g(x_T, 0)$, the glass transition temperature of the binary trehalose–water mixture, was measured in this work and is shown in Figure 1. We note that, at trehalose concentrations below $x_T = 0.25$, our

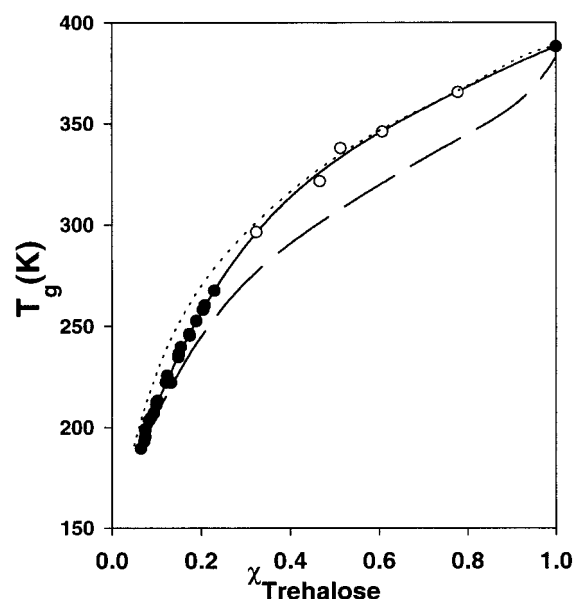


Figure 1. T_g of aqueous trehalose solutions as a function of concentration: (···) Ding et al. (ref 12, based on data from ref 11); (○) Saleki-Gerhardt (ref 27); (●) this work and ref 26; (---) equation of Crowe et al. (ref 28). Solid line represents the best fit of the data reported in this work and the high concentration trehalose data from refs 26 and 27. Dotted line represents a reproduction of the curve reported by Ding et al.

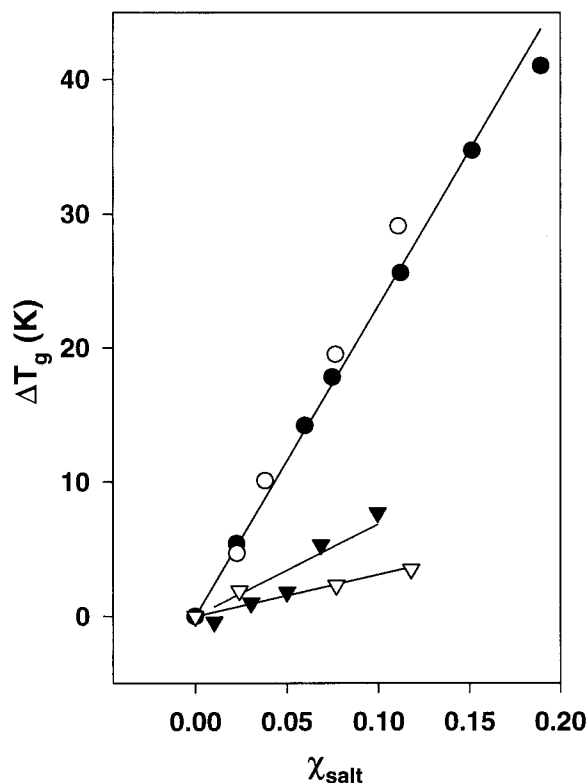


Figure 2. The T_g increment, ΔT_g , as a function of the mole fraction, χ_{salt} , of electrolyte added to aqueous disaccharide solutions: (●) borax to aqueous trehalose; (○) borax to aqueous sucrose; (▽) NaCl to aqueous trehalose; (▼) boric acid to aqueous trehalose. The mole fraction of the disaccharide is $x = 0.075$ in all cases. Lines are drawn to guide the eye.

T_g measurements are nearly 10 K lower than those reported by Ding et al.¹² Figure 1 also shows that the T_g values measured in this work in the water-rich region ($x_T < 0.3$) are consistent with those obtained by Saleki-Gerhardt²⁷ in the trehalose-rich region. Figure 1 also includes values obtained with the equation reported by Crowe et al.²⁸ for aqueous trehalose solutions at concentrations between $x_T = 0.1$ and 1.0. Over the entire range of concentration, those results are systematically lower than those reported by us and other authors.

At trehalose mole fractions greater than 0.17 (80 wt % trehalose), the deviations between the results of Crowe et al. and the other data, including ours, are marked. We suspect that this is a result of the method they used to measure the water content of their glassy samples. In that work, after the measurement of T_g , the wet trehalose samples were placed in an oven (130 °C) for 2–3 h, followed by high vacuum (10 mTorr) for 24 h. The resulting product was assumed to be 100% dry, anhydrous trehalose. In our experience, it is extremely difficult to remove the water from such highly viscous mixtures. It is possible that these samples either retained or readsorbed some residual moisture before the final weighing. This would result in a systematically high trehalose concentration, which could explain the differences between their and our results.

A fit of our data in the range of trehalose mole fraction between 0.06 and 0.25 yields the following equation (Kelvin):

$$T_g(x_T, 0) = 123.22 + 1301.3x_T - 4919.5x_T^2 + 8783.6x_T^3 \quad (2)$$

The standard deviation of this fit is less than 2 K.

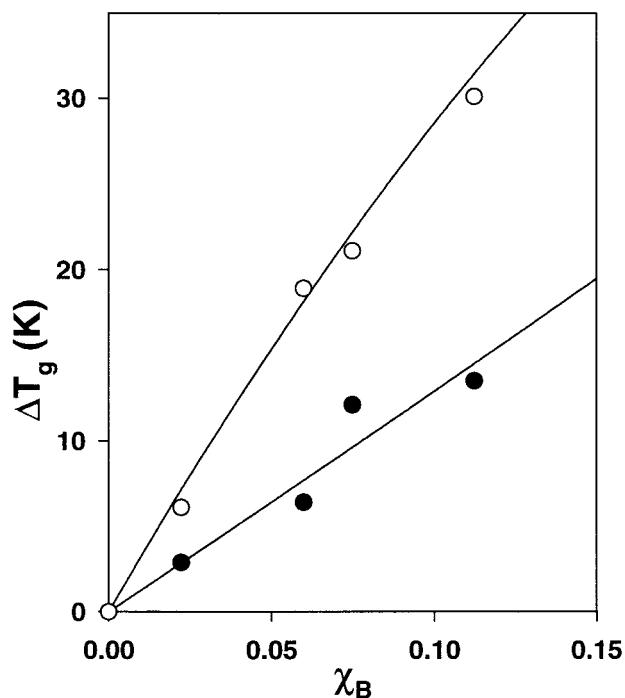


Figure 3. The T_g increment, ΔT_g , of aqueous trehalose solutions ($x_T = 0.075$) of different pH values as a function of boron mole fraction, χ_B : (●) pH = 7.0; (○) pH = 9.0.

In Figure 2, we compare the increase of T_g due to the addition of NaCl, borax, or boric acid to aqueous trehalose solutions. It is clear that borax has a remarkable effect on T_g . Over the range of concentration studied, the measured ΔT_g due to the addition of borax is larger than that of NaCl by a factor of 3. Therefore, we conclude that the dramatic effect of borax on T_g is not only a result of ionic charges in the system. We also note that this effect is not unique to trehalose but is also observed for other disaccharides. For example, Figure 2 shows that the ΔT_g of aqueous solutions of sucrose ($x_{\text{sucrose}} = 0.075$) increases with the boron mole fraction at almost the same rate as that of aqueous trehalose/borax mixtures.

In water, borax hydrolyzes to form a mixture of boric acid and sodium borate. To elucidate which of these species is responsible for the observed increase in T_g , we measured T_g of aqueous mixtures of trehalose and boric acid. Since boric acid is a weak electrolyte in water, we expect it to be undissociated in aqueous trehalose solutions. Figure 2 shows that boric acid has a minor effect on T_g , which leads us to conclude that the borate ion, $\text{B}(\text{OH})_4^-$, plays the dominant role in the increase of T_g of these solutions. It is well-known that the borate ion is involved in mono- and dicomplexation reactions with polyols by splitting two or four water molecules, respectively.²⁹ These complexes are formed in 1,2-diols and 1,3-diols of cis configurations, leading to five- or six-membered ring complexes. Conner and Bulgrin²² measured the stability constant of complexes formed between borate and diols or polyols of different stereochemistry, including glucose. The stability of the 1:1 borate/ α -D-glucose complex was found to increase as the temperature decreased from 35° to 0 °C, while the amount of 1:2 complex did not change with temperature. Lajunen et al.³⁰ showed that the borate ion forms the strongest complexes with disaccharides with vicinal *cis*-hydroxyl groups and reported the stability constant of 1:1 complexes with trehalose at 298 K. Although they did not consider the formation of 1:2 complexes in their analysis of pH/concentration data, their presence cannot be dismissed.

TABLE 2: Viscosity of Several Aqueous Trehalose and Aqueous Trehalose/Borax Mixtures as a Function of Temperature

x_T 0.0316 T (K)	x_B 0.000 η (mPa s)	x_T 0.0465 T (K)	x_B 0.000 η (mPa s)	x_T 0.0601 T (K)	x_B 0.000 η (mPa s)	x_T 0.0749 T (K)	x_B 0.000 η (mPa s)	x_T 0.1339 T (K)	x_B 0.000 η (Pa s)
268.2	19.87	264.1	80.60	263.2	322.0	263.1	1433.	253.2	1480
273.4	15.52	268.2	64.18	268.2	204.5	273.1	564.0	258.2	599
278.2	12.60	273.2	47.40	273.2	147.3	283.1	241.8	263.2	258
283.2	10.14	283.2	27.60	278.2	101.0	293.0	120.8	268.2	104
288.2	8.417	293.2	17.22	283.2	72.72	303.2	64.7	273.2	60.0
293.2	6.887	303.3	11.48	293.2	41.00	313.2	38.9	278.2	37.4
303.3	5.079	313.2	8.130	303.8	25.80	323.2	24.6	283.2	24.5
313.2	3.898	323.2	6.096	313.2	17.99	333.1	16.5	288.2	15.4
323.2	3.143	333.2	4.690	323.2	12.65	343.1	11.6		
333.2	2.574	343.2	3.776	333.2	9.320	353.1	8.51		
343.2	2.266	353.2	3.104	343.2	7.110				
353.2	1.988	358.1	2.915	353.5	5.985				

x_T 0.0749 T (K)	x_B 0.0224 η (mPa s)	x_T 0.0749 T (K)	x_B 0.0597 η (Pa s)	x_T 0.0747 T (K)	x_B 0.0748 η (Pa s)	x_T 0.0750 T (K)	x_B 0.1123 η (Pa s)	x_T 0.0751 T (K)	x_B 0.1515 η (Pa s)
258.2	5890	253.2	61.2	263.2	35.4	253.2	1430	256.5	5400
263.2	2920	258.2	31.0	268.2	18.8	258.2	414	258.2	3410
273.2	1057	263.2	13.6	273.2	9.93	263.2	159	263.2	1080
283.2	446.	268.2	7.05	283.2	3.42	268.2	73.7	268.2	386
293.2	205.	273.2	4.03	288.2	2.00	273.2	35.2	273.2	157
303.2	106.	283.2	1.45	293.2	1.27	278.2	16.5	278.2	66.6
313.2	60.3	293.2	0.614	303.3	0.569	283.2	9.35	283.2	35.1
323.2	37.5	303.2	0.287	313.2	0.278	288.2	5.06	288.2	16.6
333.2	24.5	313.2	0.151	323.2	0.155	293.2	3.01	293.2	9.59
343.2	17.4	323.2	0.0858	333.2	0.0939	303.6	1.12	303.4	3.01
		333.2	0.0575	343.2	0.0623	313.2	0.518	313.2	1.24
		343.2	0.0377	353.2	0.0425	323.2	0.260	323.2	0.565
		353.2	0.0296			333.2	0.144	333.2	0.299
						343.2	0.0867	353.2	0.0977
						353.2	0.0557		

Figure 3 illustrates the effect of pH on the ΔT_g of trehalose/borax solutions. Although a lack of information on the acid/base equilibria precludes an estimate of the borate/boric acid ratio, it can be argued that the enhancement of ΔT_g is a result of the increase of this ratio with pH. We speculate that this effect is due to the lack of complexation between boric acid and trehalose.³¹

The effect of the borate ion on the glass transition temperature of trehalose aqueous solutions is of significant practical relevance; trehalose/borate mixtures provide excellent storage media for preservation of biomolecules.¹⁹ Such mixtures deserve extensive studies in order to optimize their formulation in the freeze-dried or highly concentrated states. We also note that very concentrated aqueous trehalose solutions (e.g., ca. 80–85%) have T_g values in the range -30 to -5 °C; the addition of borate salts could raise T_g above 0 °C, thereby forming storage media that could work under simple ice-cooled conditions.

Viscosity of Trehalose/borax/Water Mixtures. In a previous work,²⁶ we reported the viscosity of supercooled aqueous solutions of trehalose and an aqueous mixture of trehalose and NaCl at temperatures between 250 and 293 K. In this work, we have measured the viscosity of several other binary solutions of trehalose mole fraction between 0.0316 and 0.1339 from 253 to 358 K.

The viscosities, $\eta(T)$, of aqueous trehalose and aqueous trehalose/borax mixtures are summarized in Table 2; Figure 4 is a graphical representation of these data, which span more than 6 orders of magnitude in viscosity. A generalized Arrhenius plot can be drawn for these data by using T_g/T as a scaling factor (Figure 5). This plot shows that, although borax has a strong influence on the viscosity, its solutions with trehalose behave in a manner similar to that of aqueous trehalose solutions.

The shape of the curve in Figure 5 is characteristic of fragile liquids above their T_g .^{32,33} This non-Arrhenius behavior has been frequently described by the empirically based WLF equation:³⁴

$$\log\left(\frac{\eta}{\eta_g}\right) = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \quad (3)$$

where C_1 and C_2 are constants that have been found to be valid for a variety of polymers ($C_1 = 17.44$ and $C_2 = 51.6$). Several authors^{35,36} have indicated that the WLF equation adequately describes the viscosity of supercooled liquids at temperatures between T_g and $T_g + 100$ K. We have previously shown²⁶ that eq 3, with these values of C_1 and C_2 , fails to fit the $\eta(T)$ data for aqueous trehalose solutions for any chosen values of the glass transition temperature and the corresponding viscosity of the glass, η_g , at T_g .

Champion et al.³⁷ used the WLF equation to describe the temperature dependence of the viscosity of aqueous sucrose solutions by alternately fixing C_1 , C_2 , or η_g . For instance, by fixing $\eta_g = 10^{12}$ Pa s, they found that the best fit to their data was provided by the values $C_1 = 19.2$ and $C_2 = 49.6$ for sucrose concentrations between 43.5% and 65.3% (w/w) at temperatures from 258 to 293 K; that is, $T > T_g + 40$ K.

Kerr and Reid³⁸ used a linearized form of the WLF equation,

$$\frac{(T - T_g)}{\log(\eta/\eta_g)} = -\frac{C_2}{C_1} - \frac{1}{C_1}(T - T_g) \quad (4)$$

to analyze the temperature dependence of the viscosity of glucose, sucrose, and maltodextrin solutions. At temperatures between 50 and 140 K above T_g , they found that all the viscosity data lie on a common line with $C_1 = 16.5$, $C_2 = 37.5$, and $\eta_g = 10^{12}$ Pa s.

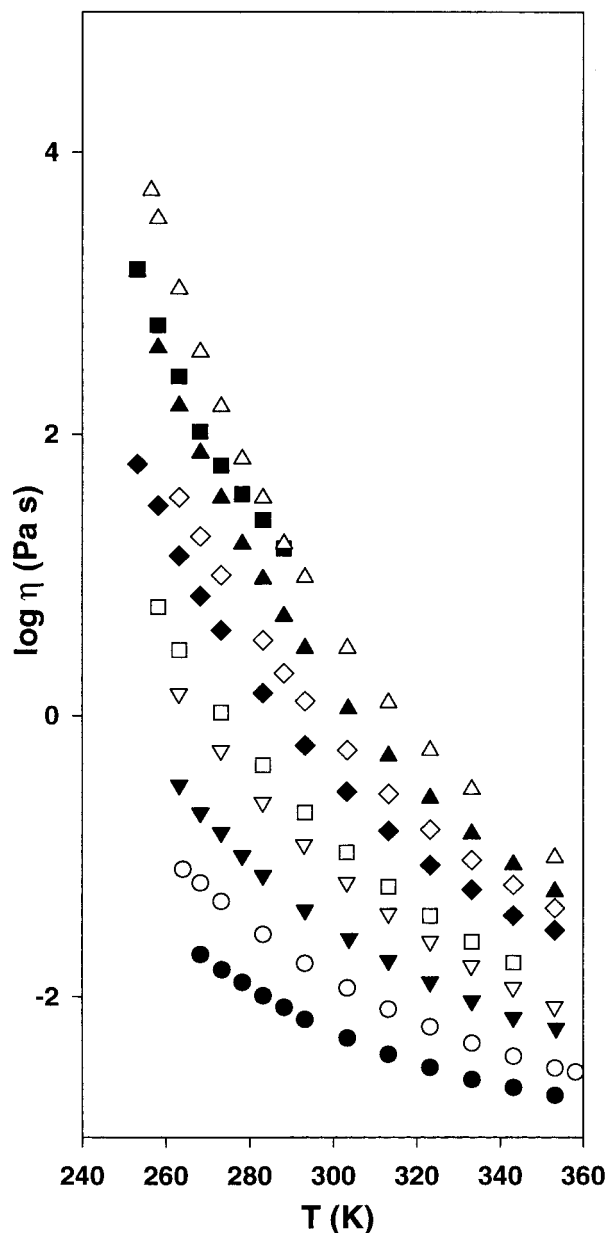


Figure 4. Temperature dependence of the viscosity of aqueous trehalose solutions ((●) $x_T = 0.0316$; (○) $x_T = 0.0465$; (▼) $x_T = 0.0601$; (▽) $x_T = 0.0749$; (■) $x_T = 0.1339$) and that of aqueous trehalose/borax solutions ((□) $x_T = 0.0749$, $x_B = 0.0224$; (◆) $x_T = 0.0749$, $x_B = 0.0597$; (◇) $x_T = 0.0747$, $x_B = 0.0748$; (▲) $x_T = 0.0750$, $x_B = 0.1123$; (△) $x_T = 0.0751$, $x_B = 0.1515$).

We have used a slightly different procedure to fit the viscosity of trehalose and trehalose/borate solutions to the WLF equation. We calculated η_g for each trehalose solution using an equation given by Soesanto and Williams³⁹ for aqueous fructose/sucrose mixtures:

$$\log \eta_g = 1.918x_T + 11.021 \quad (5)$$

where, in this case, x_T , is the mole fraction of trehalose. In the range of concentration studied in this work, η_g varies between 1.2×10^{11} and 1.9×10^{11} Pa s. Although eq 5 has been derived using the "universal" polymer constants for the WLF equation, it provides a less arbitrary approach than using a fixed value of η_g .

The best C_1 and C_2 values are shown in Table 3. A linear regression of these coefficients as a function of x_B shows that

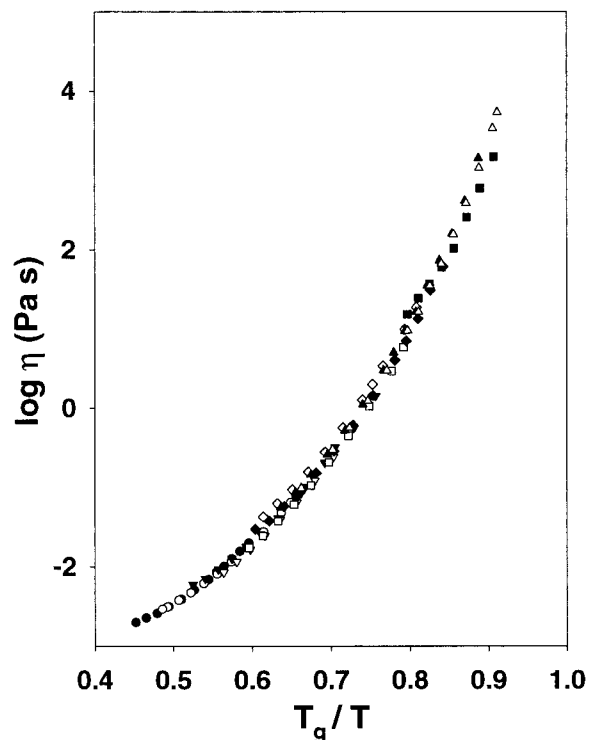


Figure 5. Arrhenius plot of the viscosity of supercooled aqueous trehalose solutions and aqueous trehalose/borax solutions. Symbols are the same as in Figure 4.

TABLE 3: Parameters of the WLF Equation for Aqueous Trehalose and Aqueous Trehalose/Borax Mixtures

x_T	x_B	T_g (K)	$\log \eta_g$ (Pa s)	C_1	C_2
Trehalose/Borax Mixtures					
0.0749	0.0000	199.1	11.165	15.48	26.26
0.0749	0.0224	204.5	11.165	15.27	25.33
0.0749	0.0597	213.3	11.165	14.87	24.10
0.0747	0.0748	216.9	11.164	14.91	26.19
0.0750	0.1123	224.7	11.165	14.70	24.93
0.0751	0.1515	233.8	11.165	14.15	22.01
Aqueous Trehalose					
0.0316	0	159.7 ^a	11.082	15.26	19.40
0.0465	0	174.0 ^a	11.110	15.45	23.99
0.0601	0	185.5	11.136	15.37	24.80
0.0749	0	199.1	11.165	15.48	26.26
0.1339	0	229.6	11.278	12.08	11.85

^a Predicted using eq 2.

both C_1 and C_2 decrease with increasing x_B . In principle, this behavior could be ascribed to the different fragility of the mixtures, as proposed by Angell et al.⁴⁰ However, if we plot all the sets of data using the linearized form of the WLF equation (eq 4), common behavior is observed for all the mixtures studied, as shown in Figure 6. This figure exhibits two distinct linear regimes which intersect near $T_g + 45$ K or a viscosity near 100 Pa s. For all the aqueous mixtures, we found $C_1 = 14.9$ and $C_2 = 27.3$ for $T > T_g + 45$ K, and $C_1 = 12.5$ and $C_2 = 15.8$ for $T_g < T < T_g + 45$ K.

It is worthwhile to note that only the three mixtures having the highest T_g values in Table 3 have been studied at temperatures below $T_g + 45$ K. Therefore, the crossover temperature for these mixtures ($T_g + 45$ K) is within the range $1.18T_g$ to $1.20T_g$.

This behavior has also been observed in other glass-forming liquids. For instance, when the viscosity data for 1,3-bis(1-naphthyl)-5-(2-naphthyl)benzene (or 1,3- α -5 β TNB) reported by Plazek and Magill^{41,42} are represented with the linearized WLF

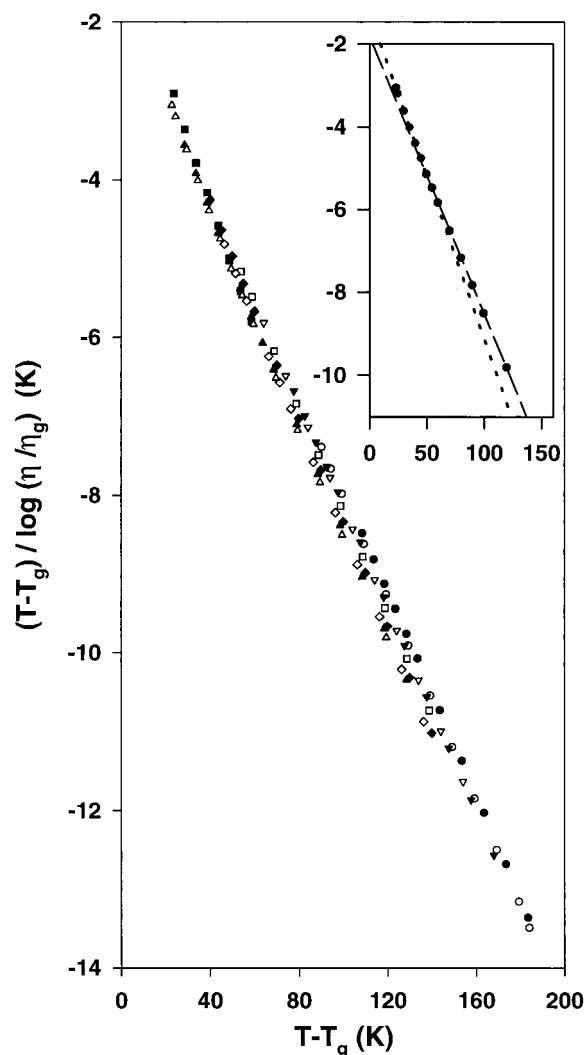


Figure 6. Linearized WLF representation of the viscosity of aqueous trehalose and aqueous trehalose/borax solutions. Symbols of main plot are as in Figure 4. Titles of inset axes are the same as for the main plot; inset symbol, (●) $x_T = 0.0751$, $x_B = 0.1515$.

equation, the delimitation temperature between the two regimes, T_c , is $T_g + 80$ K (that is, $1.23T_g$) with a viscosity close to 100 Pa s.

Rossler,^{43,44} in a corresponding-states analysis of the viscosity of simple organic liquids, proposed a distinction between the two viscosity regimes. Rossler found that for $\eta > 10$ –100 Pa s, the WLF equation gives a good description of the viscosity, while a power law equation, given by

$$\eta = \eta_0(T - T_g')^\gamma \quad (6)$$

where η_0 , γ , and T_g' are constants, describes the region in which $\eta < 10$ –100 Pa s. The temperature delimitation between these two regimes was found to be $T_g'/T_g = 1.18$ for all organic liquids studied. Taborek et al.⁴⁵ also found two distinct regimes of viscosity behavior for aqueous solutions of lithium chloride. They concluded that, for $T_g > T_g'$, the temperature dependence of the viscosity of many liquids can be described more accurately by the power law than the VTF equation, which is equivalent to the WLF equation.

The power law equation arises in the mode-coupling theory of relaxation in supercooled liquids.⁴⁶ Gotze and Sjogren⁴⁶ associated T_g' with a crossover temperature. Above T_g' , they found the α -relaxation scale to be dominated by the overall

TABLE 4: Parameters of the Power Law Equation (Eq 6) to Fit the Temperature Dependence of the Viscosity of Aqueous Trehalose and Aqueous Trehalose/Borax Mixtures

x_T	x_B	T_g' (K)	$\log \eta_0$ (Pa s)	γ
Trehalose/Borax Mixture				
0.0749	0.0000	234.9	5.495	−3.647
0.0749	0.0224	241.3	5.136	−3.420
0.0749	0.0597	251.7	5.034	−3.276
0.0747	0.0748	255.9	5.572	−3.489
0.0750	0.1123	265.1	5.399	−3.404
0.0751	0.1515	275.9	5.282	−3.315
Aqueous Trehalose				
0.0316	0	188.5 ^a	5.029	−3.549
0.0465	0	205.3 ^a	5.158	−3.546
0.0601	0	218.9	5.451	−3.634
0.0749	0	234.9	5.495	−3.647

^a Based on T_g predicted from eq 2.

molecular reorientation in the liquid. Below T_g' , they proposed that an activated hopping mechanism predominates. This results in a decoupling of diffusion and viscous flow, leading to a breakdown of the Stokes–Einstein relationship.

One is tempted to identify T_g' with the delimitation temperature, T_c , of the aforementioned results of trehalose and 1,3 α -5 β TNB because the ratio T_c/T_g is close to 1.2 in both cases. If we take $T_g' = 1.18T_g$ as the crossover temperature, it is clear that most of our viscosity measurements have been done at temperatures above T_g' and we should therefore use the power law instead of the WLF equation to fit our data.

Table 4 summarizes the values of $\log \eta_0$ and γ obtained from the fit of our viscosity data to eq 6 by fixing $T_g' = 1.18T_g$. We emphasize that the exponential parameter, γ , is a strong function of the value chosen for T_g' . At temperatures more than 20 K above T_g' it is possible to represent the viscosity data by using a single power law line, as shown in Figure 7.

A similar result was found by Kerr and Reid,³⁸ who used the power law to describe the temperature dependence of the viscosity of glucose, sucrose, and maltodextrin solutions. However, since they used a different cross-over temperature (see eq 6) in their analysis, we are unable to compare our results. The standard deviation of our fit is similar to that obtained with the WLF equation. This fact raises a point which is very frequently omitted in the treatment of the temperature dependence of the viscosity of supercooled liquids: whether the WLF equation is applicable at temperatures above T_g' .

Richert and Bassler⁴⁷ analyzed the viscosity of supercooled liquids using the WLF equation and an equation derived from a random-walk model,

$$\frac{\eta}{\eta_0} = \exp\left(\frac{T_0}{T}\right)^2 \quad (7)$$

where η_0 and T_0 are constants. They concluded that the three-parameter WLF equation is quite flexible for characterizing the temperature dependence of viscosity, even in the region where the physical principles leading to its derivation could not be valid. On the basis of their analysis of viscosity data of several supercooled liquids, they proposed that eq 7 should be used in the region $T_g < T < T_g'$, whereas the power law equation (eq 6) should be used at temperatures above T_g' .

Conclusions

The glass transition temperatures of aqueous mixtures of trehalose and NaCl are higher than the corresponding values of the pure trehalose solutions, confirming that the presence of

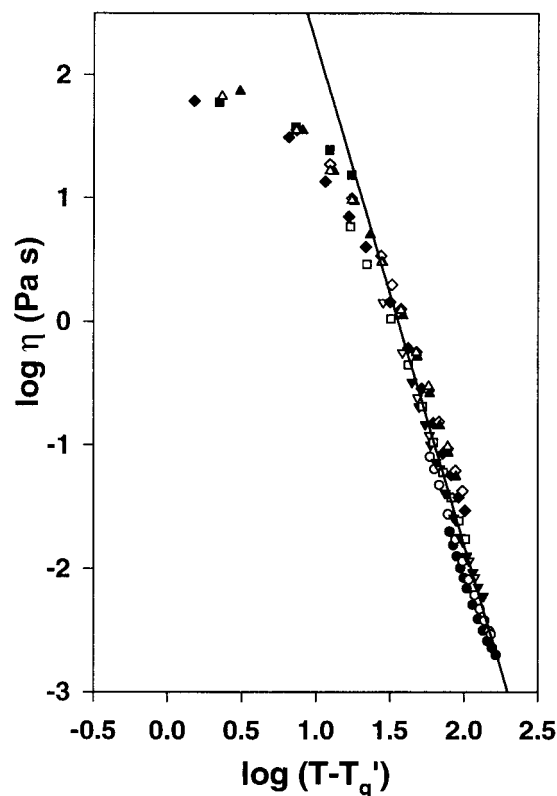


Figure 7. Power law representation of the viscosity of aqueous trehalose and aqueous trehalose/borax mixtures at temperatures above $T_g' = 1.18T_g$. Symbols are the same as in Figure 4. Solid line corresponds to the best fit of the entire data set for $T > T_g' + 20$ K.

ions increases T_g . Mixtures of trehalose and borax have an even greater increase of T_g , which cannot be explained solely on the basis of charge effects. We propose that the formation of chemical complexes between trehalose and borate plays an important role in the elevation of T_g . However, separation of the effects due to charges and to complexation requires precise knowledge of the equilibrium composition of the mixture. We are currently performing experiments to determine the stability constants of the trehalose–borate complexes in aqueous solution as a function of temperature.

On a basis of constant trehalose mole fraction, the viscosities of aqueous trehalose/borax mixtures are significantly higher than those of trehalose solutions. However, a common curve is obtained when the data are plotted with a temperature scaled with the T_g values of the solutions. Two distinct regions with different viscosity behavior can be clearly identified with a temperature delimitation near the so-called crossover temperature, $T_g' = 1.18T_g$. The WLF equation could adequately fit the data in both regions using different values of the C_1 and C_2 parameters. However, from a theoretical point of view, the power law equation is a better description of the viscosity above T_g' since it is based on the mode coupling theory which predicts the asymptotic value of the relaxation time of supercooled liquids in this temperature region.

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