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Structural and dynamical properties of water in sugar mixtures

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Abstract

Trehalose, a bioprotective sugar, has very important applications in different industrial fields, such as food, pharmaceutical and cosmetic industry. Trehalose can be used as a new multi-functional ingredient with considerable potential for the industry. The characterization of molecular mechanisms underlying the manifold functions of trehalose is very important for the understanding and exploitation of the potentialities of the disaccharide.

This work deals with experimental findings obtained by light and neutron scattering on structural and dynamical properties of trehalose, maltose and sucrose water solutions. The results provide an explanation about the bioprotective effectiveness of trehalose which is capable to strongly interact with water molecules forming a protective shell preserving biostructures under stress conditions.

Keywords: Water; Trehalose; Structure; Dynamics; Neutron Scattering

1. Introduction

The research on trehalose moves from the observation that many organisms, such as, for example, locusts (*Schistocerca gregaria*), artemie saline (Brine shrimps), cryptobiontes (Phylum Tardigrada), bacteria (*Escherichia coli*) and plants (*Myrothamnus flabellifolia*), show extraordinary surviving capabilities to environmental stress conditions, such as dehydration and freezing, thanks to the synthesis of the disaccharide, which allows them to undergo in a cryptobiotic ("hidden life") state and to reactivate the vital functions when the external conditions come back favourable to the life (Crowe & Crowe, 1984; Hirsh, 1987; Lee, Costanzo, Davidson, & Layne, 1992; Miller, 1997).

Researchers have very different ideas about how sugars exert their protective action. Green and Angell (1989) have concluded that the bioprotection mechanisms are related to the high glass transition temperature of trehalose. In fact the higher T_g values of the trehalose/H_2O mixtures, in respect to those of the other disaccharides/H_2O mixtures, imply that at a given temperature the glass transition for trehalose mixtures always occurs at a higher water content. Crowe, Clegg, and Crowe (1998) however, have noted that vitrification is not sufficient for preservation. Vitrification alone does not explain, for example, why another carbohydrate, dextran, which has a significantly higher glass transition temperature than trehalose, is a much less effective cryoprotectant than trehalose. The water replacement hypothesis, proposed by Crowe, asserting the existence of direct hydrogen bonding of trehalose with the polar head groups of the lipids that constitute biomembranes, accounts for how the non-reducing trehalose preserves the integrity of biological structures. As the systems are dried or frozen, these interactions replace those of the hydration water at the membrane-fluid interface. In such a way, in the opinions of these authors, it prevents the phase transition and the accompanying leakage upon rehydration (Crowe et al., 1998). Recently some experimental findings obtained by several spectroscopic techniques indicate that the structural and the dynamical properties of

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water are significantly affected by disaccharides, and in particular by trehalose (Branca, Magazù, Maisano, & Migliardo, 1999a, 1999b; Branca, Magazù, Maisano, Migliardo, & Romeo, 2001; Branca, Magazù, Maisano, & Migliardo, 2002; Magazù, Maisano, Migliardo, & Mondelli, 2004).

In the present work the results of a study performed by means of the integrated employment of complementary techniques, such as quasi elastic neutron scattering, neutron spin echo, elastic neutron scattering, Raman scattering and neutron diffraction, on homologues disaccharides (trehalose, maltose and sucrose)/H_2O mixtures as a function of temperature are presented with the aim to explain the superior bioprotective effectiveness of trehalose in comparison to maltose and sucrose.

2. Experimental

Ultra pure powdered trehalose, maltose and sucrose, D_2O and H_2O, purchased by Aldrich-Chemie, were used for both the experiments. Measurements were performed in a temperature range of 273–353 K on trehalose, maltose and sucrose ($C_{12}H_{22}O_{11}$) in H_2O and on partially deuterated trehalose, maltose and sucrose ($C_{12}H_{14}D_8O_{11}$) in D_2O at a weight fraction values corresponding to 19 water (H_2O and D_2O) molecules for each disaccharide molecule. All the three disaccharides possess hydrogen atoms, belonging to the OH groups, which exchange easily with the deuterium atoms of heavy water. To focus our attention on the disaccharide dynamics in D_2O, the exchangeable atoms were substituted with deuterium before preparing the solutions. We estimated that in the deuterated solutions (at the investigated concentration) the coherent contribution to the total scattering cross section is ~5%. In order to obtain partially deuterated samples, the disaccharides were first dissolved in pure D_2O to exchange the eight labile hydrogen atoms of the disaccharides and subsequently the solutions were lyophilised.

The quasi elastic neutron scattering (QENS) experiment was carried out using the IRIS high resolution spectrometer at ISIS, the world's leading pulsed neutron and muon source located at the Rutherford Appleton Laboratory (RAL, UK). The high resolution combined with the most powerful pulsed neutron source of the world make IRIS the best instrument to study the diffusive dynamics (Adams, Howells, & Telling, 2001). We measured sets of QENS spectra covering a Q, ω-domain extending from $\hbar\omega = -0.3$–0.6 meV (energy transfer) and $Q = 0.3$–1.8 Å^{-1} (momentum transfer) with a mean energy resolution of $\Gamma = 8$ μeV (HWHM). The raw spectra were corrected and normalised using the standard GENIE procedures and the IRIS data analysis package (Telling & Howells, 2003).

We used the V5-SPAN spectrometer at the Berliner Neutron Scattering Centre (BENSC, HMI, Berlin, Germany) to collect neutron spin echo (NSE) data on trehalose and sucrose water solutions as a function of temperature. In the V5 configuration used for the experiment the incident wavelength was $\lambda_o = 6.5$ Å, scanning a Q range of 0.4–1.5 Å^{-1}.

Elastic neutron scattering (ENS) experiments were performed on pure disaccharides and trehalose, maltose and sucrose/H_2O mixtures by using the IN13 spectrometer at the Institute Laue Langevin (ILL) in Grenoble (France) with a Q range of 0.28–4.27 Å^{-1}and an energy resolution of ~8 μeV (FWHM).

3. Results and discussion

QENS measurements, performed both in partially deuterated disaccharides in D_2O and in disaccharides in H_2O, allow separating the solute dynamics from that of the solvent. On the other hand NSE measurements on the same samples make possible to characterise the dynamics of the entire solute–solvent. The techniques show different aspects of the dynamical properties of the investigated solutions.

We performed the fitting procedure on deuterated and non deuterated aqueous solutions of disaccharides according to the fitting function:

$$S_{\text{inc}}(Q,\omega) = A(Q)\left\{ f_{\text{Disaccharide}}\left[F(Q)\frac{1}{\pi}\frac{\Gamma_1(Q)}{\Gamma_1^2(Q)+\omega^2} + (1-F(Q))\frac{1}{\pi}\frac{\Gamma_2(Q)}{\Gamma_2^2(Q)+\omega^2}\right] + f_{\text{hydr}}\frac{1}{\pi}\frac{\Gamma_3(Q)}{\Gamma_3^2(Q)+\omega^2}\right\} \tag{1}$$

where the first two terms refer to the translational and rotational contribution of hydrated disaccharide and the third one refers to hydration water. The random jump diffusion (RJD) model (Bee, 1988), expressed by $\Gamma_1(Q) = D_sQ^2/(1+D_sQ^2\tau)$ well describes the behaviour of the translational linewidth for the investigated solutions, as it is evident in Fig. 1, furnishing the values of the self diffusion coefficient D_s of the molecule and for the residence time τ. For trehalose as a function of temperature the RJD model furnishes for the diffusion coefficient D_s and the residence time τ the values of $D_s = 2.83 \times 10^{-7}$ cm^2/s and $\tau = 24.7$ ps, $D_s = 3.82 \times 10^{-7}$ cm^2/s and $\tau = 20.6$ ps, $D_s = 5.35 \times 10^{-7}$ cm^2/s and $\tau = 19.1$ ps and $D_s = 8.50 \times 10^{-7}$ cm^2/s and $\tau = 18.3$ ps for $T = 283$, 295, 308, 320 K, respectively. From the relation $\langle l^2\rangle = 6D\tau$ we obtain the values $\langle l^2\rangle^{1/2} = 0.64$ Å, 0.68 Å, 0.78 Å, and 1.00 Å $T = 283$, 295, 308, and 320 K, respectively.

By the behaviour of the translational linewidth of water, we conclude that the water dynamics in trehalose solutions for $T = 283$, 295, 308, and 320 K resembles that of water at ~256 K, ~261 K, ~263 K, and ~268 K, indicating that the water has a diffusive behaviour strongly triggered by the trehalose molecules and suffers of a noticeable frozen effect, whereas it is similar to that of water at ~271 K in the case of maltose solution and at ~277 K in the case of sucrose solution. Analogously to the trehalose aqueous solutions, all the disaccharides show a slowing down effect on the

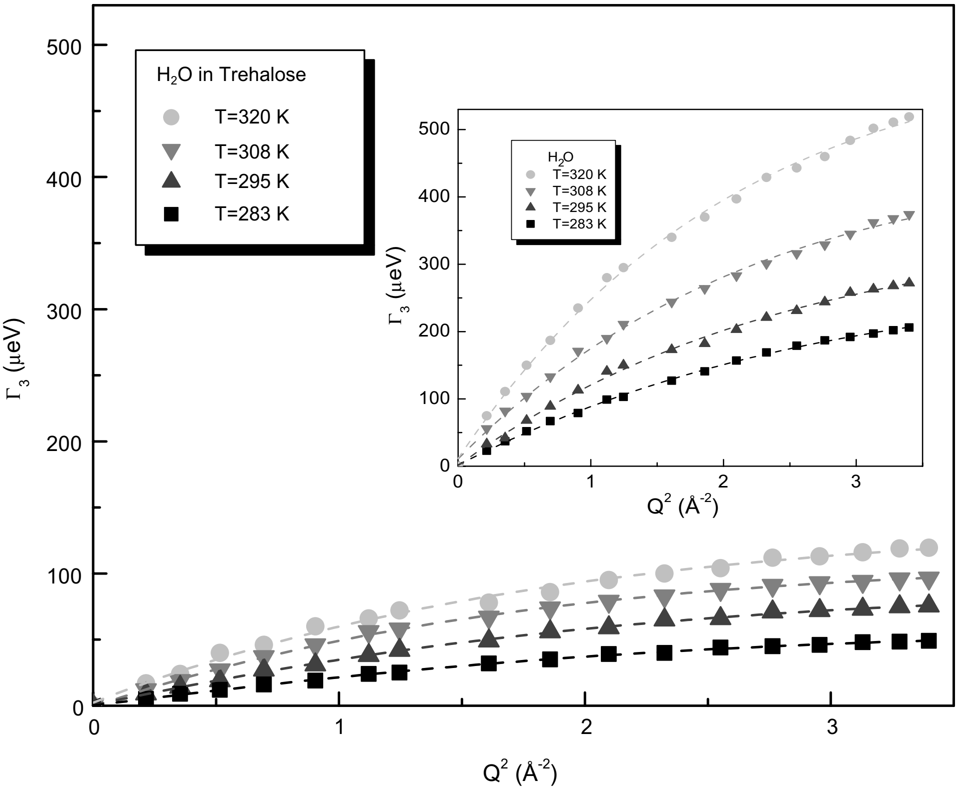

Fig. 1. Linewidth of the translational contribution for water in trehalose aqueous solutions at different temperature values. In the insert the linewidth of the translational contribution for pure water is shown.

water dynamics, which is stronger for trehalose than the other disaccharides.

In Fig. 2, the NSE intensity of aqueous solutions of trehalose for three temperature values is shown. The fitting procedure, carried out according to the Kohlrausch–Williams–Watts function, furnishes for the relaxation time τ_w, the values of $\tau_w = 0.16$ ns and $\tau_w = 0.14$ ns for trehalose/D_2O and sucrose/D_2O solutions, respectively, and for the stretching parameter β the values of $\beta = 0.72$ and $\beta = 0.70$ for trehalose/D_2O and sucrose/D_2O solutions, respectively, whereas for trehalose/H_2O solution we obtain the values of $\tau_w = 0.11$ ns and $\beta = 0.80$ and for sucrose/H_2O solution the values of $\tau_w = 0.10$ ns and $\beta = 0.78$ at $T = 293$ K. These results confirm a slower dynamics in the trehalose/H_2O solutions.

In conclusion, the QENS and NSE findings reported in the present paper furnish a clear response about the dynamics slowing down of disaccharide/water solutions. The diffusive dynamics of both disaccharide and water in the solutions has been characterised by QENS measurements, whereas NSE allow to characterise the dynamical properties of the disaccharide/water solution as a whole. The diffusion coefficients extracted by the analysis procedure are in good agreement with those obtained by NMR measurements (Branca et al., 1999b) as well as the general trend; i.e., trehalose diffusion was slower than maltose and sucrose. The performed study clearly pointed out that the dynamics of disaccharide and water is strongly coupled, with a higher coupling strength in the case of trehalose water solution, which supports the hypothesis of a privileged water–disaccharide interaction.

A confirmation to these results can be found by the systematic computational work performed on a series of 13 disaccharides by Choi, Won Cho, Jeong, and Jung (2006) to provide an atomic-level insight of unique biochemical role of the α,α-(1 → 1)-linked glucopyranoside dimer over the other glycosidically linked sugars. Analyses of the hydration number and radial distribution function of solvent water molecules showed that there was very little hydration adjacent to the glycosidic oxygen of trehalose and that the dynamic conformation of trehalose was less flexible than any of the other sugars due to this anisotropic hydration. The trehalose stable hydrogen bond network is derived from the formation of long-lived water bridges at the expense of decreasing the dynamics of the water molecules, which was evident by both the lowest translational diffusion coefficients and the lowest intermolecular coulomb energy of the water molecules around trehalose. Furthermore these findings have been confirmed by a combined pulsed-gradient spin-echo NMR and molecular dynamics simulation study performed by De Pablo and co-workers (Ekdawi, De Pablo, Feick, & von Meerwall, 2003), aimed to compare the diffusive behaviour of trehalose and sucrose in aqueous solutions. They have found that sucrose and trehalose exhibit different mobility when dissolved in water. The higher mobility observed in the

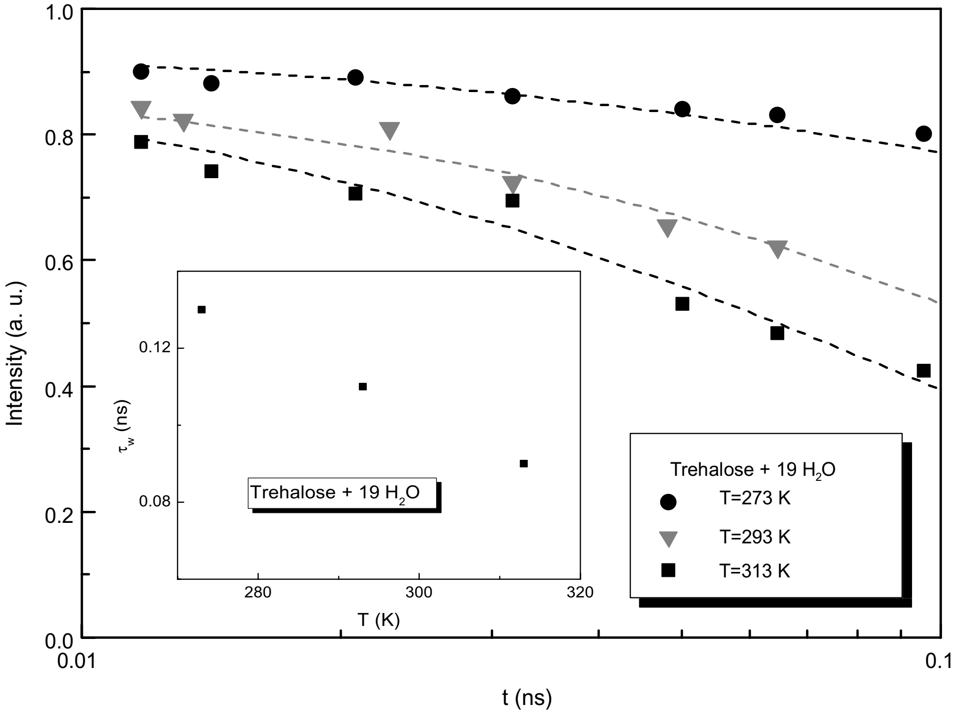

Fig. 2. NSE intensity of aqueous solutions of trehalose at different temperature values. In the insert the temperature dependence of the relaxation time τ_w is shown.

sucrose system is attributed to its small hydration number and more compact shape. Interestingly at concentrations below 72 wt%, the diffusion of water appears to be largely independent of the type of sugar. In 80 wt% disaccharide solutions, water diffuses twice as fast in sucrose solutions than in trehalose solutions. In addition, the mechanism of water diffusion changes from a continuous trajectory to a hopping mechanism with increasing disaccharide concentration.

Concluding, the disaccharides investigated in the present work, and trehalose with a greater extent, by the slowing down effect on the dynamics of water, show a high "switching off" capability. This circumstance also implies a trehalose higher ability to hold volatile substances, coherently with its superior bioprotective effectiveness.

Water molecules are arranged in presence of trehalose in a particular configuration which avoids the ice formation, so preserving by the damages due to freezing and cooling. Raman spectroscopy (Branca et al., 1999a), by the analysis of the intramolecular OH stretching vibration band, shows that the addition of trehalose, in respect to the other disaccharides, more deeply destroys the tetrahedral intermolecular network of water, which by lowering temperature would give rise to ice. In the Raman spectrum of liquid water one can distinguish the existence of an isosbestic point in the isotropic spectrum of pure water allowing the decomposition of each spectrum into an "open" contribution, attributed to the O–H vibration in tetrabonded H_2O molecules that have an "intact bond" (see Fig. 3), and a "closed" contribution, corresponding to the O–H vibration of H_2O molecules that have a not fully developed hydrogen bond (distorted bond). In Fig. 3 the decomposition of each isotropic spectrum of the three disaccharide aqueous solutions at $\phi = 0.09$ for $T = 293$ K, into an "open" and a "closed" contribution. One can observe that for the same concentration the integrated area of the "open" band is smaller in the trehalose aqueous solution. This allows us to state that a more marked destructuring effect occurs in the presence of trehalose rather than in the presence of sucrose or maltose. As a confirmation, neutron diffraction results (Branca et al., 2002) confirm the changes induced by disaccharides on water tetrahedral structure. In fact the peak at 4.5 Å in $g_{OO}(r)$ of pure water, which is associated with the "degree of tetrahedrality", in the distribution function of trehalose + $40H_2O$ at $T = 300$ K is absent and the general trend is significantly distorted. Uchida, Nagayama, Shibayama, and Gohara (2007), detecting freeze-fractured replica images of disaccharide (trehalose, sucrose, and maltose) solutions using a field-emission type transmission electron microscope, confirmed that trehalose molecules has a greater inhibitory effect of sucrose on the growth of ice crystals. Furuki (2002) interpreted their different degree of anti-freeze effects in view of molecular structure of the disaccharides, concluding that the aqueous unfrozen behaviour induced by the presence of trehalose depends on the position of the glycosidic linkage between the two constituent units. The results show that in general the presence of an β-type of linkage results in a larger amount of water unfrozen than does the presence of the α-type. The unfrozen water

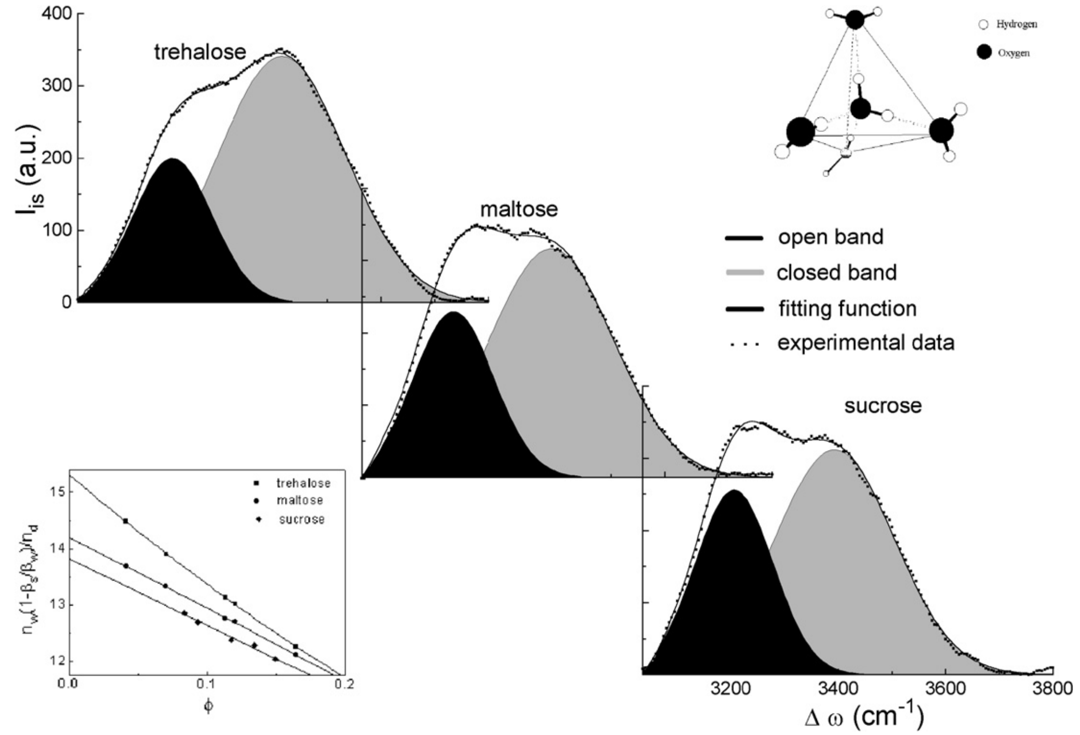

Fig. 3. Overview of results on the "destructuring effect" of trehalose.

content depends not only on the position, but also on the type of linkage between the constituent monosaccharides. It was observed that aqueous trehalose has a larger amount of unfrozen water content in comparison with the other disaccharide mixtures. Furthermore ultrasonic findings (Branca et al., 1999b) show that trehalose reveals the highest values of the solute–solvent interaction strength and hydration number, as shown in Fig. 3. In addition, the amount of water for each trehalose molecule decreases by increasing both disaccharide concentration and

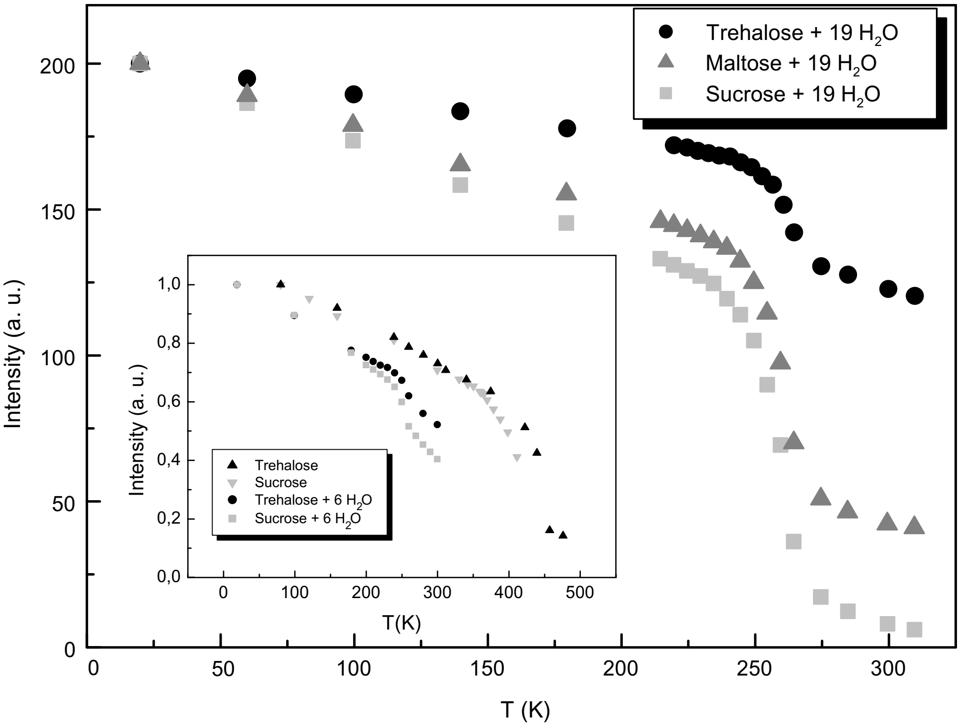

Fig. 4. Elastic intensity of disaccharides and their H_2O mixtures as a function of temperature.

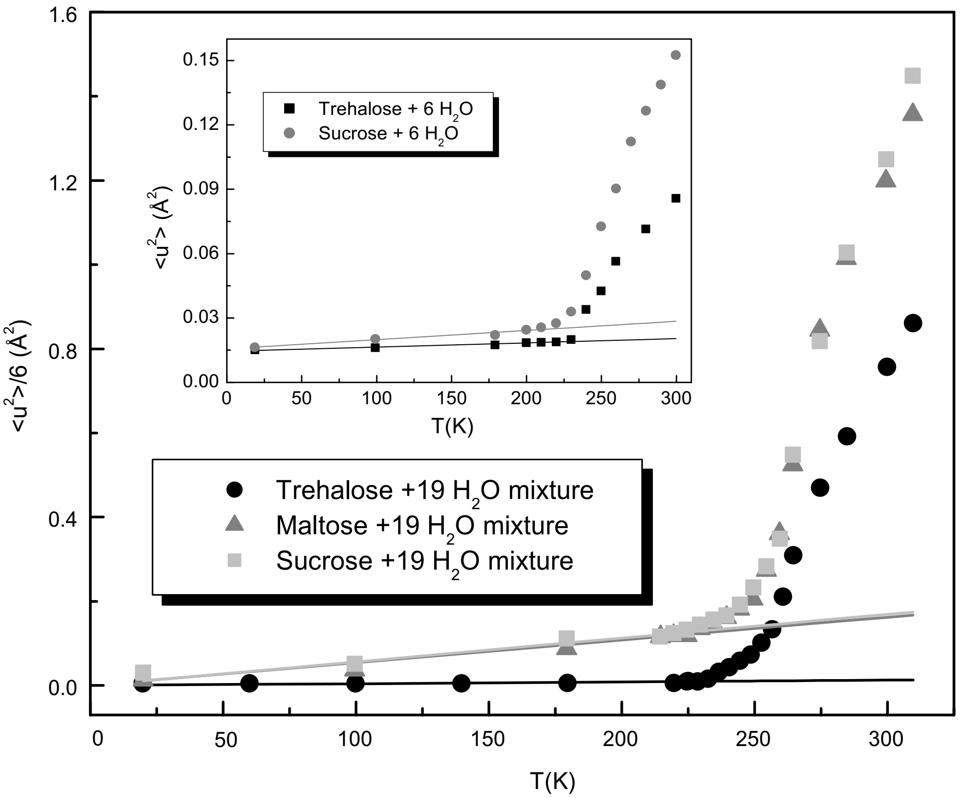

Fig. 5. Mean square displacements of disaccharides/H_2O mixtures at different concentration values as a function of temperature.

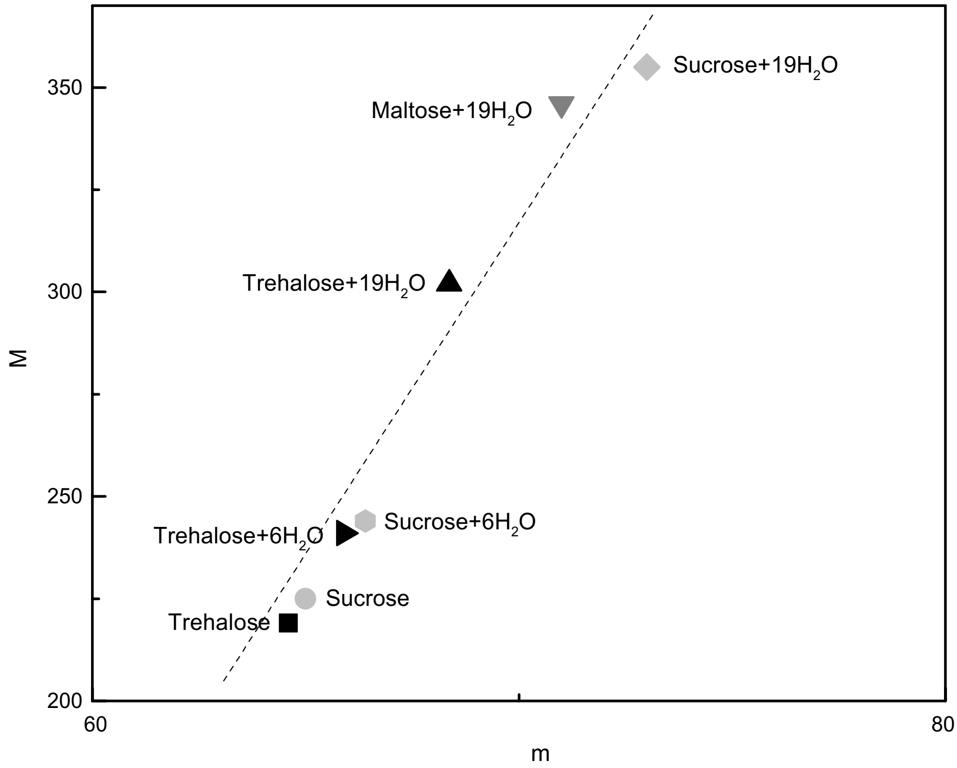

Fig. 6. Linear behaviour of the *M* fragility parameter versus the fragility parameter *m*. The solid line indicates the best fit.

temperature approaching an almost constant value at the highest temperature values and revealing a solute–solvent interaction strength higher for trehalose in respect to the other disaccharides. An overview of the above described results, pointing out the destructuring effect of disaccharides, is shown in Fig. 3.

In Fig. 4 the elastic intensity of disaccharides and their H_2O mixtures as a function of temperature at different concentration values are shown, emphasising that a dynamical transition occurs for all the systems (Doster, Cusack, & Petry, 1989). The mean square displacements of pure trehalose and sucrose and their H_2O mixtures are reported in Fig. 5. It is evident from the analysis of both the intensity gap and the onset temperature values that trehalose is more "rigid" than maltose and sucrose. By using a procedure to analyse the anharmonic region which is based on the relation between a macroscopic transport quantity, viscosity, and an atomic quantity, the nanoscopic mean square displacement, the fragility degree can be expressed by (Magazù et al., 2004):

$$M = \left. \frac{d\left(u_0^2/\langle u^2\rangle_{\mathrm{loc}}\right)}{d(T_{\mathrm{g}}/T)} \right|_{T=T_{\mathrm{g}}^{+}} \tag{2}$$

where $\langle u^2\rangle_{\mathrm{loc}} = \langle u^2\rangle_{\mathrm{anharm}} - \langle u^2\rangle_{\mathrm{harm}}$ is the difference between the mean square displacements of the disordered phases and the ordered phase. Fig. 6 shows the M parameters as evaluated from Eq. (2) as a function of m. The fragility parameter values M are obtained at the experimental resolution of 8 μeV by IN13 (Magazù et al., 2004). The slope of the observed linear dependence depends on the instrumental resolution.

As it is evident form Fig. 6, trehalose show in all the cases the lowest values of fragility. These results find a confirmation by viscosity measurements on trehalose, maltose, and sucrose aqueous solutions (Branca et al., 1999b). It has been pointed out that trehalose is the most viscous disaccharide and shows, in respect to the other disaccharides, a "stronger" kinetic character in the Angell's classification scheme.

From this analysis it clearly emerges that trehalose and its mixtures are characterized in respect to the other disaccharides and their mixtures by a superior structural resistance to thermal stress which allows them to create a more rigid environment to protect biological structures.

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