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Correlation between bioprotective effectiveness and dynamic properties of trehalose–water, maltose–water and sucrose–water mixtures

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Abstract—The aim of the present work is to link the bioprotective effectiveness to the dynamic properties of a class of homologous disaccharides, that is, trehalose, maltose and sucrose, and their mixtures in water. The findings obtained by elastic neutron scattering point out a harmonic–anharmonic transition for all the three disaccharide mixtures. Using a new operative definition of 'fragility', the different degrees of 'strength' of the investigated systems are determined. The links existing between the degree of fragility and the cryptoprotective action are also discussed.

Keywords: Elastic neutron scattering; Disaccharides; Glass transition; Bioprotection

1. Introduction

Neutron scattering is an exceptional tool to investigate structural and dynamic properties of systems of biophysical interest, such as proteins, enzymes, lipids and sugars. Moreover, elastic neutron scattering enhances the investigation of atomic motions in hydrated biosystems in a wide temperature range and on the picosecond timescale.[1]

Among disaccharides, trehalose, a glass-forming disaccharide, is widely distributed in many organisms that show cryptobiotic capabilities. It is particularly effective in terms of its ability to preserve and maintain activity and leavening capacity of many desiccation-resistant organisms. These organisms synthesize a large amount of trehalose that encases its cells like sheaths of rock candy, holding the proteins nearly immobile. Under these conditions, the molecules that make up the proteins exist somewhere between the disordered and mobile state of a fluid and the ordered immobility of a crystal.[2] The bioprotective effects of trehalose have various potential applications, such as in the preservation of food and seeds, in the preservation of blood and vaccines, as well as in the process of, switching off, of cancers and viruses (HIV).[3] There are several hypotheses formulated to clarify the bioprotective action of trehalose. Green and Angell[4] suggest that the bioprotectant effectiveness of trehalose could be ascribed to the higher value of its glass transition and mixtures with water, while Crowe et al.[5] agree to a direct interaction between sugar and biostructure, strengthening their hypothesis with the simulation reported by Grigera et al.[6]

On the other hand, experimental findings obtained by different spectroscopic techniques[7–11] demonstrate that the structural and dynamic properties of water, even at low concentration, are significantly upset by the action of disaccharides and show also that the interaction between solute and solvent is stronger than the interaction between the solvent itself (H_2O–H_2O molecules), for all disaccharide mixtures, and in particular for trehalose mixtures.

In more detail, neutron diffraction results[11] show for all disaccharides, and for trehalose to a large extent, a

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strong distortion of the peaks linked to the hydrogen-bonded network in the partial radial distribution functions that can be attributed to the destruction of the tetrahedral coordination of pure water. Analogously Raman scattering findings[8] show that the addition of trehalose, in respect to the other disaccharides, more rapidly destroys the tetrahedral intermolecular network of water, which by lowering temperature would give rise to ice. These results clearly indicate that disaccharides show a noticeable 'kosmotrope' character, namely the disaccharide–water molecular interaction strength is much higher in respect to that between the water molecules themselves.

Furthermore, ultrasonic velocity measurements[7] highlight that, in respect to the other disaccharides, the trehalose–water system is characterized, in all the investigated concentration range, by both the highest value of the solute–solvent interaction strength and of hydration number.

Viscosity measurements on aqueous solutions[9] of trehalose, maltose and sucrose highlight that trehalose shows in respect to the other disaccharides, a 'stronger' kinetic character in the Green and Angell's classification scheme. QENS and inelastic neutron scattering (INS) were also employed to investigate the low-frequency dynamics across the glass transition of water mixtures of trehalose, maltose and sucrose.[10] The experimental findings, through the relaxational to the vibrational contribution ratio, confirm that the trehalose–H_2O mixture shows a stronger character and furnish for it a higher force pseudo-constant (resilience) value in comparison to that of the other disaccharide–H_2O mixtures.

Here, we present the results of elastic scans of the intensity of the aqueous solutions of the homologous disaccharides, trehalose, maltose and sucrose, as a function of temperature. The main task of this work is to investigate the behaviour of disaccharide–H_2O mixtures, focusing above all on their degree of 'fragility'. From a biological approach, the aspect dealt with has notable consequences on a large scale, since biological activity is directly linked with conformational changes on a physiological time scale.

In the present study we characterize the degree of rigidity of the investigated systems, suggesting a new definition of fragility. The results agree with the findings previously obtained by viscosity measurements[9] and by QENS[10] and fortify the idea that, in respect to the other disaccharides, the trehalose–water system is characterized by the 'strongest' character in Green and Angell's classification scheme.

2. Experimental section

The experiments have been performed using an IN13 spectrometer at the Institut Laue Langevin (ILL, Grenoble, France). Measurements were carried out on trehalose–, maltose– and sucrose–H_2O and D_2O mixtures in a temperature range of 20–450 K, at different concentration values. The incident wavelength was 2.23 Å, and the Q range was 0.28–4.27 $Å^{-1}$; the elastic energy resolution (FWHM) was 8 μeV and the resolution time was 0.1 ns. In all the figures the error bars are $\sim 5\%$. The samples were prepared starting from ultrapure samples purchased from Aldrich-Chemie, and care was taken to obtain clear, dust-free samples.

3. Results and discussion

In incoherent neutron scattering experiments, the measured function is the incoherent dynamic structure factor $S_{\mathrm{inc}}(Q,\omega)$, namely the Fourier transform of the incoherent intermediate scattering function $I(Q,t)$:

$$I(Q,t) = \sum_{\alpha=1}^{N} x_\alpha \langle \exp[\mathrm{i}Q \cdot r_\alpha(t)] \exp[-\mathrm{i}Q \cdot r_\alpha(0)] \rangle \qquad (1)$$

In this formula, x_α is the fraction of particles following the same dynamics in the potential $V_\alpha(r)$, and $r_\alpha(t)$ is the position vector of the particle α at time t. For low Q values, in the Gaussian scattering approximation, angular coordinates can be eliminated, providing:

$$I(Q,t) \cong \sum_{\alpha=1}^{N} x_\alpha \exp\left[-\frac{Q^2}{6}\langle [r_\alpha(t) - r_\alpha(0)]^2 \rangle\right] \qquad (2)$$

The intensity is a combination of several Gaussian functions:

$$I(Q,t) \cong \sum_{\alpha=1}^{N} x_\alpha \exp\left[-\frac{Q^2}{3}\langle r_\alpha^2 \rangle [1 - C_\alpha(t)]\right] \qquad (3)$$

where $\langle r_\alpha^2 \rangle$ is the equilibrium mean-square displacement and $C_\alpha(t)$ is the stationary position relaxation function. The incoherent dynamic structure factor $S_{\mathrm{inc}}(Q,\omega)$ is composed of two contributions: an elastic contribution $S_{\mathrm{inc}}^{\mathrm{el}}(Q) = S_{\mathrm{inc}}(Q,\omega=0) = I(Q,\infty)\delta(\omega) \cong I(Q,\tau)$ (τ being the experimental resolution time), and a quasielastic contribution that involves energies $\hbar\omega > 0$.[1,12,13]

The mean-square displacement, $\langle u^2 \rangle$, which takes into account the fluctuations of all the particles of investigated systems, is given by

$$\langle u^2 \rangle = -3 \left.\frac{\mathrm{d}\{\ln[S_{\mathrm{inc}}^{\mathrm{el}}(Q)]\}}{\mathrm{d}Q^2}\right|_{Q=0} = \sum_{\alpha=1}^{N} x_\alpha \langle r_\alpha^2 \rangle [1 - C_\alpha(\tau)] \qquad (4)$$

Nevertheless, $C_\alpha(\tau)$ is a constant that rescales the observed mean-square displacement, and therefore we can assume $C_\alpha(\tau) = 0$.[13] Considering that all the particles are dynamically equivalent, in the previous equations x_α has been set equal to 1.

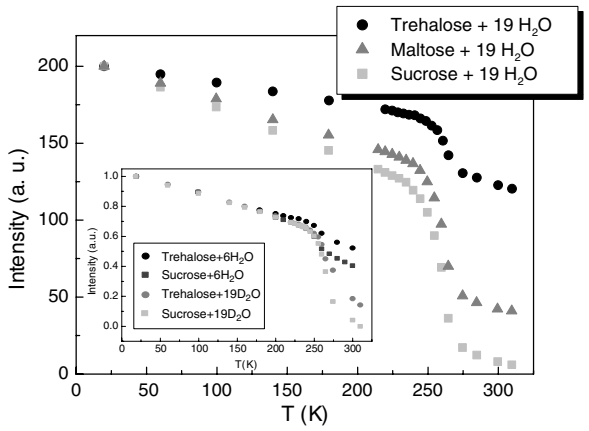

Figure 1. Comparison of elastic intensity of trehalose–, maltose– and sucrose–H_2O mixtures as a function of temperature for different concentration values.

In Figure 1, we can observe the elastically scattered intensity profiles of the three different mixtures (trehalose–, maltose– and sucrose–H_2O) versus temperature for different concentration values.

It appears immediately clear that in correspondence to the glass-transition temperature T_g of the systems analyzed, which is $T \approx 238$ K for the trehalose mixture, $T \approx 235$ K for the maltose mixture and $T \approx 233$ K for the sucrose mixture, as previously experimentally determined,[14] a dynamic transition is present.[1,12] It is important to notice that the glass-transition temperature values and the onset temperature values at which the dynamic transition occurs do not perfectly correspond in all the cases. For the three systems investigated, this correspondence seems to be present.

For temperatures lower than the onset temperature, the elastic spectra follow a Gaussian law and have the form expected for a harmonic solid (the solid of Einstein).

Above the dynamic transition temperature, we observe a decrease in the elastic intensity that can be attributed to the excitation of new degrees of freedom, especially at low Q. An important feature of this fact is that the intensity change of the dynamic transition, as suggested by Figure 1, is very less distinct in the case of the trehalose mixture in respect to the other mixtures, suggesting that trehalose has a larger structural resistance to temperature changes, which is different from the other disaccharide mixtures (maltose–H_2O and sucrose–H_2O) that show a 'softer' character.

As observed, the deviation from Gaussian behaviour increases with temperature, and, since the motion involves jumps of hydrogen atoms to sites of different energies, the process can be described by the well-known double-well potential model.[1,12]

Figure 2 shows the elastic intensity as a function of Q^2 obtained for the disaccharide–D_2O and –H_2O mixtures, together with results of the fitting procedure according

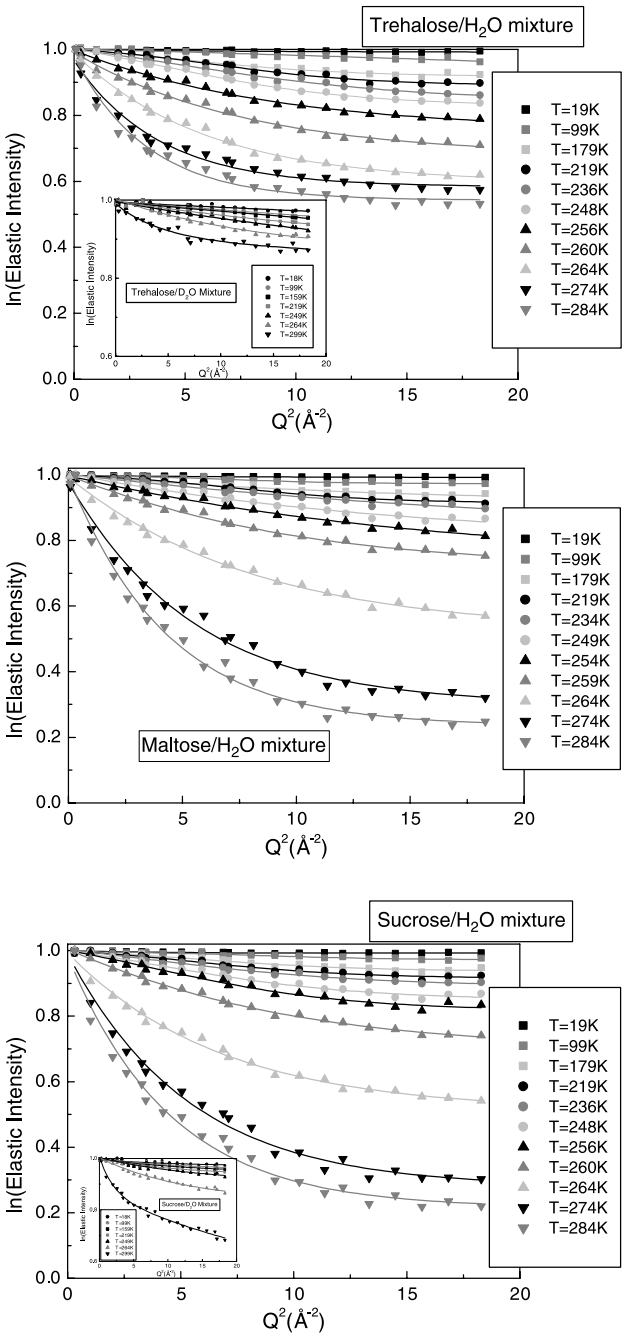

Figure 2. Comparison of the elastic intensity logarithm of trehalose–, maltose– and sucrose–H_2O and –D_2O mixtures as a function of Q^2 for different temperature values.

to the double-well potential model. The values of probability p_1 to find the scattering particles in the ground state [the probability p_2 to find the particle on the excited state is given by $(1 - p_1)$] are shown in Table 1. The values obtained for the occupation probabilities ($p_{1\_sucrose} < p_{1\_maltose} < p_{1\_trehalose}$), together with the value of the distance d between the two potential minima ($d \approx 1$ Å for all investigated temperature values), point out that for the trehalose mixture we have a more

Table 1. Probability of occupation p_1 for disaccharide–H_2O mixtures for different temperature values

Disaccharide–H_2O mixture	T (K)	p_1
Trehalose/H_2O	20	0.90
	180	0.89
	225	0.84
	240	0.83
	265	0.70
	285	0.62
	300	0.55
Maltose/H_2O	20	0.89
	180	0.87
	225	0.83
	240	0.81
	265	0.68
	285	0.56
	300	0.51
Sucrose/H_2O	20	0.88
	180	0.86
	225	0.82
	240	0.80
	265	0.67
	285	0.55
	300	0.51

stalled dynamics in respect to the case of the maltose and sucrose mixtures.

In Figure 3, we observe the temperature dependence of the mean-square displacements obtained for the disaccharide–D_2O and –H_2O mixtures.

The mean-square displacement $\langle u^2\rangle$ of a set of quantized harmonic oscillators for $T < \frac{h\langle\nu\rangle}{2K_B}$ (where $\frac{h\langle\nu\rangle}{K_B}$ is the Debye temperature, K_B the Boltzman constant and $\langle\nu\rangle$ the average frequency of a set of oscillators) is approximately a constant equal to the zero-point fluctuations $\frac{h\langle\nu\rangle}{2K}$ (K being the force constant, which has the dimension of force per unit length, of a set of oscillators considered as an Einstein solid), whereas it linearly increases with the temperature for $T > \frac{h\langle\nu\rangle}{2K_B}$.[1,12,13]

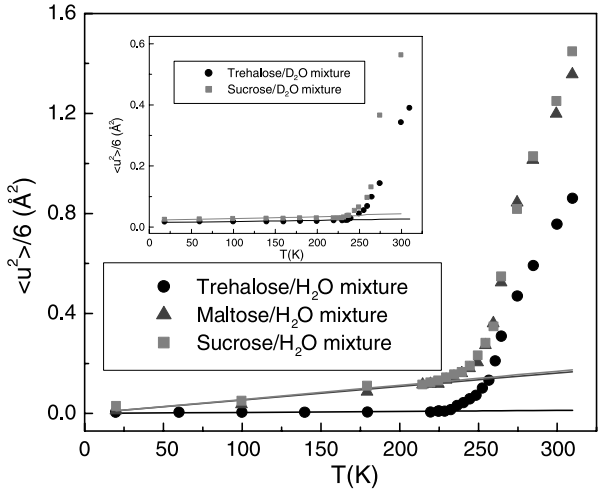

Figure 3. Temperature dependence of the derived mean-square displacements for trehalose–, maltose– and sucrose–H_2O and –D_2O mixtures.

The mean-square displacement can be related to the harmonic vibrational motion by $\langle\Delta u^2(T)\rangle = \left[\frac{h\langle\nu\rangle}{2K}\right] \times \coth\left(\frac{h\langle\nu\rangle}{2K_BT} - 1\right)$. Therefore, below the dynamic transition temperature, i.e., in the harmonic regime, the slope (i.e., the derivative of the mean-square displacement with respect to temperature) of the elastic scan has the dimension of k_B/k.

In the harmonic region, the system can be considered as composed by an Einstein solid, and the best fit performed in the Q range in which the Gaussian model is valid gives for the force constant the values of $K = 0.40$ N/m, $K = 0.25$ N/m and $K = 0.22$ N/m for trehalose–, maltose– and sucrose–H_2O mixtures, respectively, underlining that among the disaccharides the trehalose–H_2O system is the strongest system in the Green–Angell classification of fragile and strong liquids.

Because a force constant is not defined for anharmonic motions, an operational approach in which the 'resilience' of an anharmonic environment is quantified by a pseudo-force constant $\langle k\rangle$ calculated from the derivative of the scan at T according to $\langle k\rangle = 0.00138/(\mathrm{d}\langle u^2\rangle/\mathrm{d}T)$ has been suggested.[1]

In the following we describe a procedure to analyze the anharmonic region, which, in contrast to the other methods that are phenomenological,[1] can be collocated in the framework of an interpretative theoretical model. With the purpose of connecting the bioprotectant effectiveness of disaccharide–H_2O mixtures to the 'fragility degree' of these systems, a new operative definition for fragility, based on the evaluation by neutron scattering of the temperature dependence of the mean-square displacement has been proposed.[15] The procedure is based on the relation between a macroscopic transport quantity, viscosity and an atomic quantity, the nanoscopic mean-square displacement.

Defining $\langle u^2\rangle_{loc}$ as the difference between the mean-square displacements of the disordered phases (amorphous and liquid) and the ordered phase (crystalline) is defined:

$$\langle u^2\rangle_{loc} = \langle u^2\rangle_{anharm} - \langle u^2\rangle_{harm} \quad (5)$$

On the basis of the linear dependence of $\log\eta$ versus $(\langle u^2\rangle_{loc})^{-1}$, the following interpretative model for the elementary flow process (α-relaxation) can be proposed. A given particle is jumping back and forth in the fast processes (β-relaxation) with a Gaussian probability distribution of mean-square amplitude $\langle u^2\rangle_{loc}$. When the amplitude of that fast motion exceeds a critical displacement u_0, a local structural reconfiguration (α-relaxation) takes place. Under the assumption of temperature independence of the time scale of the fast motion, the waiting time for the occurrence of a α process at a given particle is proportional to the probability to find the particle outside the sphere with radius u_0.

In the present framework, the fragility degree by elastic neutron scattering is defined as follows:[15]

$$M = \left.\frac{\mathrm{d}(u_0^2/\langle u^2\rangle_{\mathrm{loc}})}{\mathrm{d}(T_{\mathrm{g}}/T)}\right|_{T=T_{\mathrm{g}}^{+}} \tag{6}$$

Obviously such a definition implies a fragility parameter depending on the instrumental resolution, which determines the observation time scale. However, we are interested in a comparison of the degree of fragility, and such a comparison is meaningful for the same resolution value.

By fitting the parameter $\langle u^2\rangle_{\mathrm{loc}}$ previously obtained as $\langle u^2\rangle_{\mathrm{loc}} = \langle u^2\rangle_{\mathrm{anharm}} - \langle u^2\rangle_{\mathrm{harm}}$, and using Eq. 6, we evaluated a fragility parameter M of 355 for the sucrose–H_2O mixture, 346 for the maltose–H_2O mixture and 302 for the trehalose–H_2O mixture. In disaccharide–D_2O solutions, we obtained a fragility parameter $M = 272$ for trehalose–D_2O and $M = 295$ for sucrose–D_2O.

The new parameter of fragility could be connected with the non-ergodicity factor, $f(Q,T)$, that is, the long time limit of the normalized density–density correlation function. This quantity represents the amount of decorrelation introduced by the vibrational dynamics, and it depends on both the (T-dependent) amplitude of the vibrations and the degree of disorder of the glassy structure.[16] It has been shown that the low temperature dependence of the non-ergodicity factor for several glasses stands in a fashion similar to the one exhibited by the Angell plot.[16] It is indeed possible to define a glass fragility as the derivative of $f(Q,T)$ in the $T = 0$ limit:[16]

$$m_{\alpha} = \left.\frac{\mathrm{d}f(Q,T)}{\mathrm{d}(T/T_{\mathrm{g}})}\right|_{T=0} \tag{7}$$

where $f(Q,T)$ is linked to the mean-square displacement by the relation:

$$f(Q,T) = \left[1 + f_{\mathrm{IS}}(Q,T)\sum_{p}\frac{\left|\sum_i [Q\cdot e_p(i)]\exp(\mathrm{i}Q\langle u^2\rangle_i)\right|^2}{\omega_p^2}\right]^{-1} \tag{8}$$

A study of the correlation existing between the two fragility parameters is in progress (unpublished results). The values of fragility degree obtained following Eq. 6 are in a good agreement with the values previously achieved by viscosity measurements.[9] So it clearly reveals that, among the different solutions investigated, whose behaviour in Angell's strong-fragile classification scheme is considered 'intermediate', the trehalose–H_2O mixture is the strongest.

From this analysis, it clearly emerges that the trehalose–H_2O mixture is characterized in respect to the maltose–H_2O and sucrose–H_2O mixtures by a lower fragility, namely by a higher resistance to local structural changes when temperature decreases towards the glass transition value.

This means that the trehalose–H_2O system is able to protect biological structures in a more rigid environment. This circumstance is relevant because it implies a higher capability of cryptoprotection at high disaccharide concentration. In this frame the 'stronger' character of the trehalose–H_2O mixture can justify its better cryptobiotic effect in respect to maltose– and sucrose–H_2O mixtures.

4. Conclusions

The present elastic neutron scattering experimental findings show a dynamic transition in trehalose–, maltose– and sucrose–water mixtures that can be correlated to the different nature of the involved dynamic processes in bioprotection. These findings are coherent with those previously obtained,[9,10] showing that the trehalose mixture highlights a 'stronger' character in the 'strong-fragile' range of Angell's classification scheme, when compared with maltose–H_2O and sucrose–H_2O mixtures. The higher effectiveness of trehalose 'cryptobiotic' action can be warranted by the lower fragility of the trehalose–H_2O mixture.

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