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Mean-Square Displacement Relationship in Bioprotectant Systems by Elastic Neutron Scattering

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ABSTRACT Neutron intensity elastic scans on trehalose, maltose, and sucrose/H_2O mixtures as a function of concentration, temperature, and exchanged wave vector are presented. The experimental findings show a crossover in molecular fluctuations between harmonic and anharmonic dynamical regimes. A new operative definition for the degree of fragility of glass-forming systems is furnished by using explicitly the connection between viscosity and mean-square displacement. The procedure is tested for the investigated mixtures and for a set of glass-forming systems. In this frame, the stronger character of trehalose/H_2O mixture indicates a better attitude in respect to maltose and sucrose/H_2O mixtures to encapsulate biostructures in a more rigid matrix.

INTRODUCTION

In recent years a lot of attention has been addressed to the understanding of the mechanisms present in organisms able to survive under environmental stress conditions (Crowe and Crowe, 1984).

Cryptobiosis, from the Greek κριπτοσ, meaning ''hidden'' or ''coated'', and βιοσ, meaning ''life'', refers to a particular state of organisms inactivating when prohibitive environmental conditions occur. During cryptobiosis, undetectable (hidden) levels of metabolic functions are maintained, these levels reaching normal values when external conditions become again favorable to life (Crowe and Cooper, 1971; Hirsh, 1987; Storey and Storey, 1992). These extraordinary cryptobiotic organisms (the first one was documented by Anton van Leeuwenhoek in 1702) belong to all the natural kingdoms (Lee et al., 1992; Miller, 1978; Zentella et al., 1999).

Cryptobiotic-activating substances are certainly homologous disaccharides ($C_{12}H_{22}O_{11}$, e.g., trehalose, maltose, and sucrose, with particular reference to trehalose) but, despite the several bioprotection hypotheses formulated (Green and Angell, 1989; Crowe et al., 1998; Donnamaria et al., 1994), the effectiveness mechanisms remain still cryptic.

Although many studies have been focusing on ternary systems such as biostructure/water/disaccharide (Cordone et al., 1998, 1999; Cottone et al., 2001), some researchers retain that the protein dynamics as a whole is slave to the environment properties (Paciaroni et al., 2002; Frauenfelder et al., 1991; Frauenfelder and McMahon, 1998).

Within this framework, Green and Angell (1989) suggest that the higher value of the glass transition temperature of trehalose and its mixtures with water, in comparison with the other disaccharides, is the only reason for its superior bioprotectant effectiveness. In fact the higher T_g values of the trehalose/H_2O mixtures, in respect to those of the other disaccharides/H_2O mixtures, implies that at a given temperature the glass transition for trehalose mixtures always occurs at a higher water content. As a matter of fact such a hypothesis alone is not entirely satisfactory if one keeps in mind that other similar systems, such as, for example, dextran (($C_6H_{10}O_5$)x) (Oliver et al., 1998), a linear polysaccharide with α(1–6) glycosidic links, present even a higher T_g value, but do not show comparable bioprotective action.

Crowe et al. (1998) formulated the hypothesis that a direct interaction between the sugars and the object of protection occurs. More specifically, their water-replacement hypothesis justifies the trehalose protective function with the existence of direct hydrogen bonding of trehalose with the polar headgroups of the lipids as water does. This hypothesis is supported by a simulation of Grigera and co-workers (Donnamaria et al., 1994), which argue that the structure of trehalose is perfectly adaptable to the tetrahedral coordination of pure water, whose structural and dynamical properties are not significantly affected by trehalose.

As a matter of fact experimental findings obtained by several spectroscopic techniques (Branca et al., 2002a,b, 2001a,b, 1999a,b; Magazù et al., 1997, 2001) indicate that the structural and the dynamical properties of water are drastically perturbed by disaccharides, and in particular by trehalose. More specifically, neutron diffraction results (Branca et al., 2002a,b) show for all disaccharides, and for trehalose to a large extent, a strong distortion of the peaks linked to the hydrogen-bonded network in the partial radial distribution functions that can be attributed to the destroying of the tetrahedral coordination of pure water. Raman scattering findings (Branca et al., 1999b) analogously 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.

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These results clearly indicate that disaccharides show a noticeable kosmotropic character—namely that the disaccharide-water molecule interaction strength is much higher in respect to that between the water molecules.

Furthermore, ultrasonic velocity measurements (Magazù et al., 1997; Branca et al., 1999a) demonstrate 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 the hydration number.

As far as dynamics is concerned, quasielastic neutron scattering results on disaccharides solutions (Magazù et al., 2001) indicate that the water dynamics is also strongly affected by the presence of disaccharides and by trehalose in particular, contrary to the predictions of Grigera's simulation (Donnamaria et al., 1994).

Furthermore, viscosity measurements on trehalose, maltose, and sucrose aqueous solutions (Branca et al., 2001b) demonstrate that trehalose has, in respect to the other disaccharides, a stronger kinetic character in the Angell's classification scheme. Quasielastic neutron scattering and inelastic neutron scattering (INS) were also employed to investigate the low frequency dynamics across the glass transition of trehalose, maltose, and sucrose water mixtures (Branca et al., 2001a, 2002). The obtained 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 pseudoconstant (resilience) value in comparison to that of the other disaccharides/H_2O mixtures.

Neutrons with a 1 Å wavelength and an energy close to 1 kcal/mol represent excellent probe to characterize thermal molecular motions and conformational changes in biological systems (Zaccai, 2000; Doster et al., 1989; Smith, 1991; Bicout and Zaccai, 2001). In particular they furnish information on mean-square fluctuations in a given timescale by elastic scattering (Zaccai, 2000); on correlation times of diffusion motions by quasielastic scattering (Doster et al., 1989); and on vibrational modes by inelastic scattering (Smith, 1991).

The elastic experiments are the most efficient to perform, having the best signal/noise ratio (Smith, 1991; Bicout and Zaccai, 2001; Réat et al., 2000). The mean-square displacements obtained by an analysis as a function of Q are dominated by hydrogen motions due to its large incoherent cross-section value (Smith, 1991). In complex biological structures hydrogens move together with larger chemical groups and therefore their motion corresponds to the global thermal behavior of the system (Bicout and Zaccai, 2001; Réat et al., 2000).

In this work elastic neutron intensity results on homologous disaccharides (trehalose, maltose, and sucrose)/H_2O mixtures as a function of concentration and temperature are presented. As we shall see the experimental findings allow us to characterize the system's flexibility and fragility, which justify the better cryptoprotectant effectiveness of trehalose.

EXPERIMENTAL SECTION

Elastic incoherent neutron scattering experiments were performed by using the IN13 spectrometer at the Institute Laue Langevin in Grenoble, France. The relatively high energy of the incident neutrons (16 meV) makes it possible to span a wide range of momentum transfer Q (≤ 5.5 Å^{-1}) with a very good energy resolution (full width half maximum (FWHM)) (~ 8 μeV). In the used IN13 configuration the incident wavelength was 2.23 Å and the Q-range was $0.28 \div 4.27$ Å^{-1}. Raw data were corrected for cell scattering and detector response and normalized to unity at $Q = 0$ Å^{-1}.

Ultrapure powdered trehalose, maltose, and sucrose, and H_2O, purchased by Aldrich-Chemie, (Milan, Italy), were used for the experiments. Measurements were performed in a temperature range of $20 \div 310$ K on hydrogenated trehalose, maltose, and sucrose in H_2O and on partially deuterated trehalose, maltose, and sucrose in D_2O at weight-fraction values corresponding to 19 and 6 water (H_2O and D_2O) molecules for each disaccharide molecule.

RESULTS AND DISCUSSION

In Fig. 1, a comparison among elastic incoherent neutron scattering spectra of trehalose + 19 H_2O, maltose + 19 H_2O, and sucrose + 19 H_2O mixtures is shown. It is evident that a dynamical transition occurs for the trehalose/H_2O mixture at $T \sim 238$ K, and for maltose/H_2O and sucrose/H_2O mixtures at $T \sim 235$ K and $T \sim 233$ K, respectively. In Fig. 1 (*inset*) the elastic intensity versus temperature of trehalose + 6 H_2O, sucrose + 6 H_2O, trehalose + 19 D_2O, and sucrose + 19 D_2O mixtures are reported.

The elastically scattered intensity surfaces of trehalose + 19 H_2O, maltose + 19 H_2O, and sucrose + 19 H_2O mixtures as a function of temperature and wave vector Q are reported in Fig. 2. For all the samples below the onset temperature, the elastic intensity has the Gaussian form expected for a harmonic solid (Doster et al., 1989; Smith, 1991; Bicout and Zaccai, 2001). The decrease in the elastic intensity above the dynamical transition temperature can be attributed to the excitation of new degrees of freedom (Doster et al., 1989), especially at low Q, and is very less marked in the case of trehalose/water mixture than for the other disaccharide/water mixtures. This circumstance clearly indicates that trehalose shows a larger structural resistance to temperature changes and a higher rigidity in comparison with the maltose/H_2O mixture and the sucrose/H_2O mixture, this latter showing the softest character.

In Fig. 3, as an example, the derived mean-square displacements for trehalose, maltose, and sucrose + 19 H_2O mixtures as a function of temperature are shown. In the Q-range in which the Gaussian model is valid and results $S^{\mathrm{el}}_{\mathrm{inc}}(Q) \propto \exp[-\langle \Delta u^2/6 \rangle Q^2]$ (Elastic Incoherent Structure Factor), the mean-square displacement behavior can be fitted within the framework of the harmonic approximation (Doster et al., 1989; Smith, 1991; Bicout and Zaccai, 2001):

$$\langle \Delta u^2(T) \rangle = \frac{h\langle \nu \rangle}{2K} \coth\left(\frac{h\langle \nu \rangle}{2K_{\mathrm{B}}T} - 1 \right), \tag{1}$$

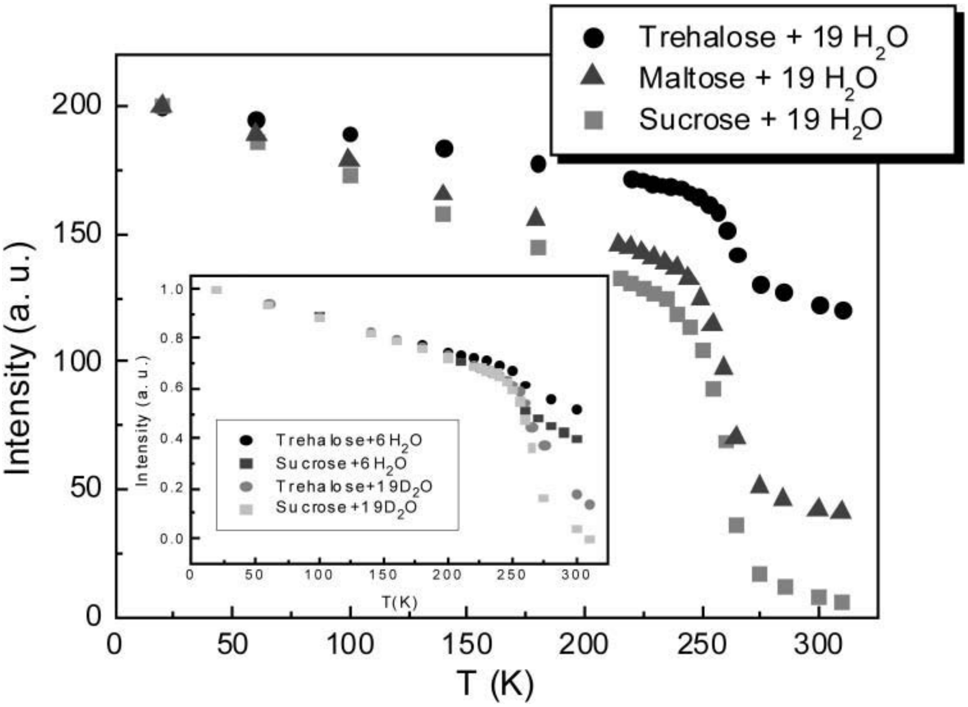

FIGURE 1 Elastic incoherent neutron scattering spectra $S(Q,\omega = 0)$ of trehalose + 19 H_2O mixture (●), maltose + 19 H_2O mixture (▲), and sucrose + 19 H_2O mixture (*shaded squares*) as a function of temperature. (*Inset*) The elastic intensity versus temperature of trehalose + 6 H_2O mixture (●), sucrose + 6 H_2O mixture (■), trehalose + 19D_2O mixture (*shaded circles*), and sucrose + 19 D_2O mixture (*shaded squares*) are reported.

where K_B is the Boltzmann constant, K and $\langle\nu\rangle$ are the average force-field constant and the average frequency of a set of oscillators considered as an Einstein solid, respectively, and the term $h\langle\nu\rangle/2K$ the zero-point mean-square displacement. The fitting procedure according to Eq. 1 furnishes for K the values of $K = 0.40$ N/m, $K = 0.25$ N/m, and $K = 0.22$ N/m for trehalose, maltose, and sucrose + 19 H_2O mixtures, respectively. With the purpose of connecting the bioprotectant effectiveness of disaccharide/H_2O mixtures to the degree of fragility of these systems, we introduce a new operative definition for fragility, based on the evaluation by neutron scattering of the temperature dependence of the mean-square displacement. The procedure is based on the relation between a macroscopic transport quantity, viscosity, and an atomic quantity, the nanoscopic mean-square displacement. As it is well known, the behavioral properties of a glass-forming system can be described in terms of the ($3N$ + 1)-dimensional potential energy hypersurface in the configurational space (Angell et al., 1994, 1997). The complexity of the energy landscape, explored by the system, can be correlated with the density of the minima of the hypersurface (degeneracy $\propto \Delta C_p(T_g)$) and with the distribution of the barrier heights between them $\Delta\mu$. These topological features of potential energy hypersurface determine the structural sensitivity of a system to temperature changes in approaching the glass transition, namely its fragility $\left(\propto \frac{\Delta C_p(T_g)}{\Delta\mu}\right)$, which is operatively defined as

$$m = \left.\frac{d\log\eta}{d(T_g/T)}\right|_{T=T_g^+}. \tag{2}$$

In the Angell's classification of glass-forming systems (Angell et al., 1994, 1997), based on the choice of an invariant viscosity at the scaling temperature T_g ($\eta(T_g) = 10^{13}$ poise), the departure from the Arrhenius law is taken as a signature of the degree of fragility of the system (Angell et al., 1994, 1997). An Arrhenius behavior of viscosity in the T_g-scaled plot and a small heat capacity variation $\Delta C_p(T_g)$ characterize the strongest systems, whereas a large departure from Arrhenius law and a large heat capacity variation $\Delta C_p(T_g)$ characterize the most fragile ones. Between these two limiting cases, intermediate behaviors can be interpreted in terms of different kinetic (η) and thermodynamic contributions ($\Delta C_p(T_g)$): thermodynamically strong (small $\Delta C_p(T_g)$) and kinetically fragile systems (non-Arrhenius η behavior) are characterized by a low minima density of the potential energy hypersurface and low energy barriers. Vice versa, thermodynamically fragile (large $\Delta C_p(T_g)$) and kinetically strong systems (Arrhenius η behavior) are characterized by a high hypersurface configurational degeneracy and high barrier heights.

On the other hand, Sokolov et al. (1993, 1994), taking into account low-frequency Raman data, to characterize the fragility degree of glass-forming systems introduced the ratio of the normalized Raman intensity $I_n = I/\{\omega[n(\omega)+1]\}$ at the minimum, $(I_n)_{min}$, to the intensity of the boson peak maximum, $(I_n)_{max}$, $R_1 = (I_{min})/(I_{max})$. Through the evaluation of the $R_1(T)$ parameter at the glass transition, these authors have found a close correlation between the ratio of the relaxational to the vibrational contribution and the degree of fragility (Sokolov et al., 1994).

Starting from the works on selenium by Migliardo and co-workers (Galli et al., 1986) and Magazù and co-workers

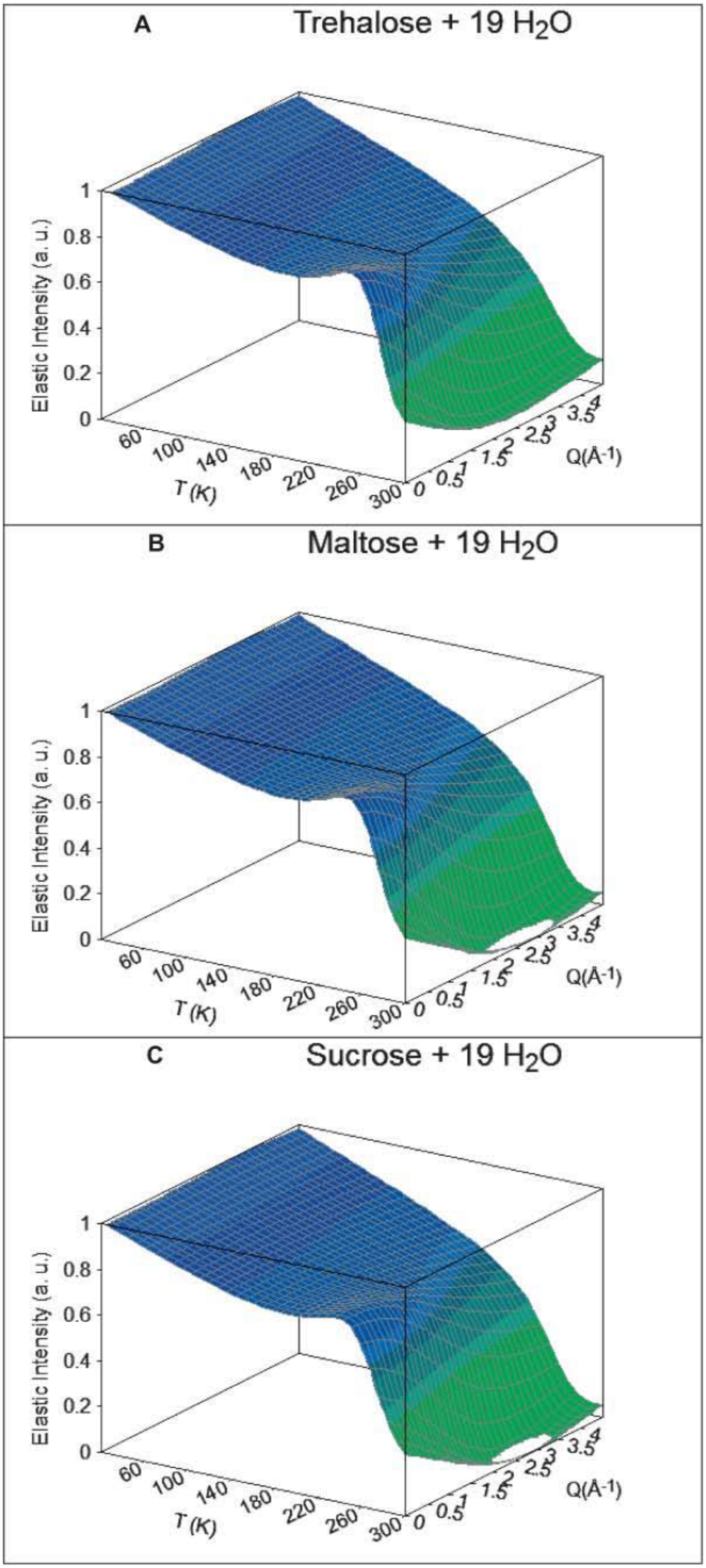

FIGURE 2 Elastic incoherent neutron scattering spectra $S(Q,\omega = 0)$ for (*A*) trehalose/H_2O mixture, (*B*) maltose/H_2O mixture, and (*C*) sucrose/H_2O mixture as a function of temperature and wave vector Q. The solid curves are guides for the eye.

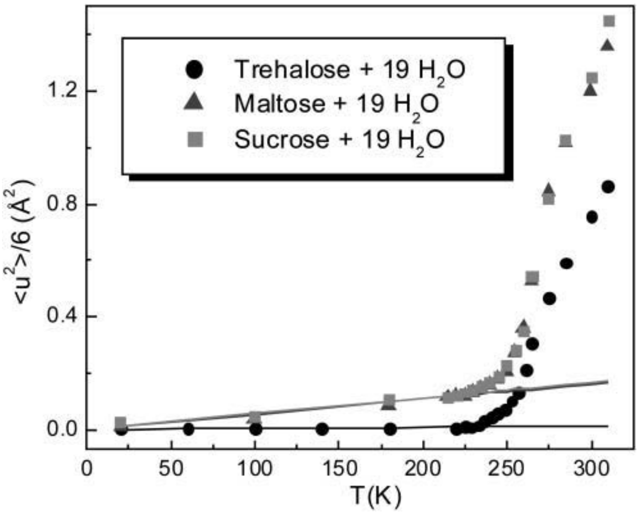

FIGURE 3 Dependence on temperature of the mean-square displacements of trehalose/H_2O mixture (●), maltose/H_2O mixture (▲), and sucrose/H_2O mixture (*shaded squares*). The mean-square displacements are derived by the Elastic Incoherent Structure Factor as described in the text. The solid lines are the harmonic fit results performed according to Eq. 1.

(Burattini et al., 1988), a correlation between viscosity and the atomic mean-square displacement has been firstly proposed by Buchenau and Zorn (1992). More specifically defining $\langle u^2\rangle_{\mathrm{loc}}$ as the difference between the mean-square displacements of the disordered phases (amorphous and liquid) and the ordered phase (crystalline):

$$\langle u^2\rangle_{\mathrm{loc}}=\langle u^2\rangle_{\mathrm{disord}}-\langle u^2\rangle_{\mathrm{ord}} \tag{3}$$

a linear relation is observed between the logarithm of viscosity and the inverse of $\langle u^2\rangle_{\mathrm{loc}}$. Practically the same relation can be obtained from the liquid data on the basis of $\langle u^2\rangle_{\mathrm{loc}}=\langle u^2\rangle_{\mathrm{liq}}-\langle u^2\rangle_{\mathrm{hard}}$ where $\langle u^2\rangle_{\mathrm{liq}}$ is the mean-square displacement for motion faster than the resolution limit and $\langle u^2\rangle_{\mathrm{hard}}$ takes into account only the typical lattice vibrational frequencies. This linear relation includes both the region below the glass transition temperature and above the melting temperature.

On the basis of the linear dependence of log η vs. $(\langle u^2\rangle_{\mathrm{loc}})^{-1}$ 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 $\langle u^2\rangle_{\mathrm{loc}}$. 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 timescale of the fast motion, the waiting time for the occurrence of an α-process at a given particle is proportional to the probability to find the particle outside the sphere with radius u_0.

Within this picture, one can express viscosity with the expression

$$\eta=\eta_0\exp[u_0^2/\langle u^2\rangle_{\mathrm{loc}}]. \tag{4}$$

Equation 4 allows fitting the experimental data for selenium of Magazù and Migliardo and co-workers (Burattini et al., 1988; Galli et al., 1986) better than the Vogel-Fulcher law (Buchenau and Zorn, 1992). The linear fitting procedure of log η vs. $(\langle u^2\rangle_{\mathrm{loc}})^{-1}$ by Eq. 4 furnishes through its slope the u_0 parameter value.

Taking into account Eqs. 2–4, we now introduce a new operative definition to characterize the degree of fragility by elastic neutron scattering as

$$M=\left.\frac{d(u_0^2/\langle u^2\rangle_{\mathrm{loc}})}{d(T_g/T)}\right|_{\mathrm{T=T_g^+}}. \tag{5}$$

Obviously such a definition implies a fragility parameter depending on the instrumental resolution, which determines the observation timescale. However we are interested in a comparison ceteris paribus of the degree of fragility, and such a comparison is meaningful when an identical experimental setup for IN13 is employed. We shall show in fact that coherent results are obtained also for other glass-forming systems, such as B_2O_3 (Engberg et al., 1998), glycerol (Wuttke et al., 1995), PB (Frick and Richter, 1995), o-terphenyl (Tölle et al., 2000), and selenium (Buchenau and Zorn, 1992).

Fig. 4 shows, as an example, logη as a function of $1/\langle u^2\rangle_{\mathrm{loc}}$ for trehalose + 19 H_2O mixtures. As it can be seen the linear behavior is very well observed in a wide temperature range, with u_0 = 1.41 Å. In the inset the logarithm of the viscosity as a function of $1/\langle u^2\rangle_{\mathrm{loc}}$ for trehalose + 6 H_2O mixture is shown.

From Eq. 5 we evaluate a fragility parameter M of 302 for the trehalose + 19 H_2O mixture, of 346 for maltose + 19 H_2O mixture, and of 355 for the sucrose + 19 H_2O mixture, respectively.

Employing a less-wide viscosity data set, for trehalose/D_2O and sucrose/D_2O mixtures at a concentration value corresponding to 19 water molecules for each disaccharide molecule we obtain a fragility parameter M of 272 and of 295, respectively, whereas for the trehalose/H_2O and sucrose/H_2O mixtures at a concentration value corresponding to six water molecules for each disaccharide molecule we obtain for the fragility parameter M the value of 241 and of 244, respectively.

Table 1 reports the value of fragility parameters m, R_1, and M as obtained by viscosity measurements (m) (Sokolov et al., 1994; Branca et al., 2001b), Raman (Sokolov et al., 1994), and neutron scattering by analysis of the relaxational and vibrational contribution (R_1) (Sokolov et al., 1994), by

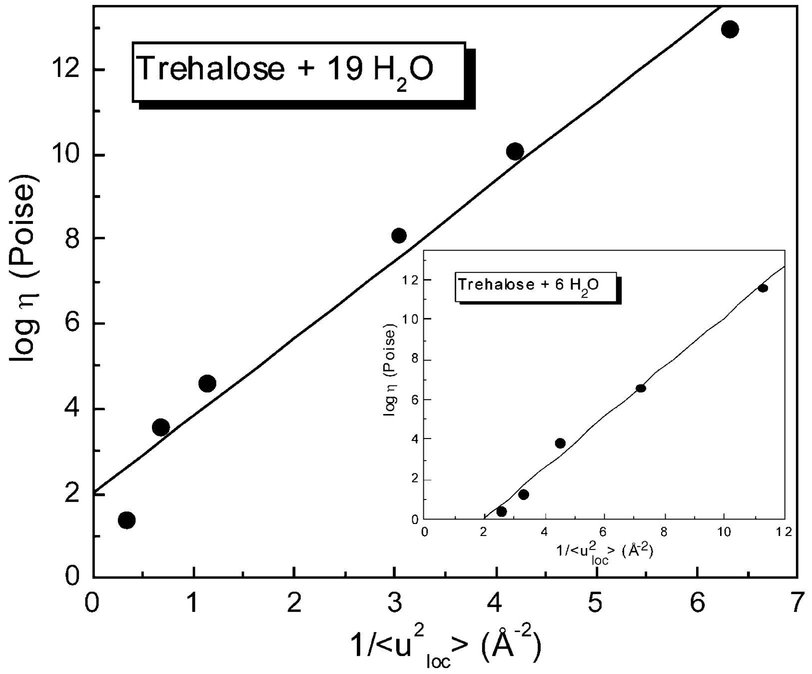

FIGURE 4 Linear dependence of the logarithm of the viscosity on $1/\langle u^2\rangle_{loc}$ for trehalose + 19 H_2O mixture. In the inset the logarithm of the viscosity as a function of $1/\langle u^2\rangle_{loc}$ for trehalose + 6 H_2O mixture is shown. The solid lines indicate the best fit of the experimental data.

elastic neutron scattering (M) by IN13 working with a resolution of 8 μeV (Wuttke et al., 1995; Frick and Richter, 1995; Tölle et al., 2000), and by IN6 working with a resolution of 150–200 μeV (Buchenau and Zorn, 1992; Engberg et al., 1998).

The reported values indicate that this operative definition for fragility furnishes an excellent direct proportionality between M and m.

In fact if one reports on a plot the R_1 parameter, as defined by Sokolov et al. (1993, 1994), versus m (see Fig. 5 *A*), one realizes that the experimental points are quite spread out along a linear behavior and that, therefore, the quantitative evaluation of the fragility factor R_1 suffers from a certain indeterminacy.

On the other hand, when the M parameters, obtained with a given resolution, are reported as a function of m (see Fig. 5 *B*) the data arrange themselves on a straight line whose slope depends uniquely on the instrumental resolution. In Fig. 5 *B*, the fragility parameter values obtained at two different experimental resolutions, specifically at 8 μeV by IN13 (Wuttke et al., 1995; Frick and Richter, 1995; Tölle et al., 2000) and at 150–200 μeV by IN6 (Buchenau and Zorn, 1992; Engberg et al., 1998), are reported. As it can be seen the first data set follows a strict linear dependence. 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 toward the glass transition value.

To unravel the physical mechanisms that determine such peculiar trehalose behavior we have performed INS experiments on trehalose/H_2O, maltose/H_2O, and sucrose/H_2O mixtures by the thermal original spectrometer with cylindrical analyzers (TOSCA) spectrometer at the ISIS facility (Chilton, UK).

An important feature in the bending modes vibrational spectral region (see Fig. 6) is that the intensity profile of trehalose in this INS region appears more structured, i.e., trehalose shows, in comparison with the other disaccharides, the most crystalline character. In more detail, trehalose shows more distinctly, in respect to the other disaccharides, the characteristic three peaks typical of the crystalline state at ~1118 cm^{-1}, ~1240 cm^{-1}, and ~1365 cm^{-1}, which can be

TABLE 1 Values of fragility

Compound	T_g (K)	m	M	$R_1(T_g)$ Neutron	$R_1(T_g)$ Raman
SiO_2	1446	23		0.50	–
B_2O_3	526	39	29	–	0.41
Glycerol	186	58	96	0.60	0.39
PB	181	64	188	0.95	–
Trehalose + 19 H_2O	238	68	302	0.61	–
Maltose + 19 H_2O	235	71	346	–	–
Sucrose + 19 H_2O	233	73	355	0.73	–
o-terphenyl	243	81	483	1.0	0.70
Selenium	305	87	120	–	–

Values of fragility as obtained by viscosity measurements (Sokolov et al., 1994; Branca et al., 2001b), Raman (Sokolov et al., 1994), and neutron scattering by analysis of the relaxational and vibrational contribution (Sokolov et al., 1994), by elastic neutron scattering by IN13 working with a resolution of 8 μeV (Wuttke et al., 1995; Frick and Richter, 1995; Tölle et al., 2000), and by IN6 working with a resolution of 150–200 μeV (Buchenau and Zorn, 1992; Engberg et al., 1998).

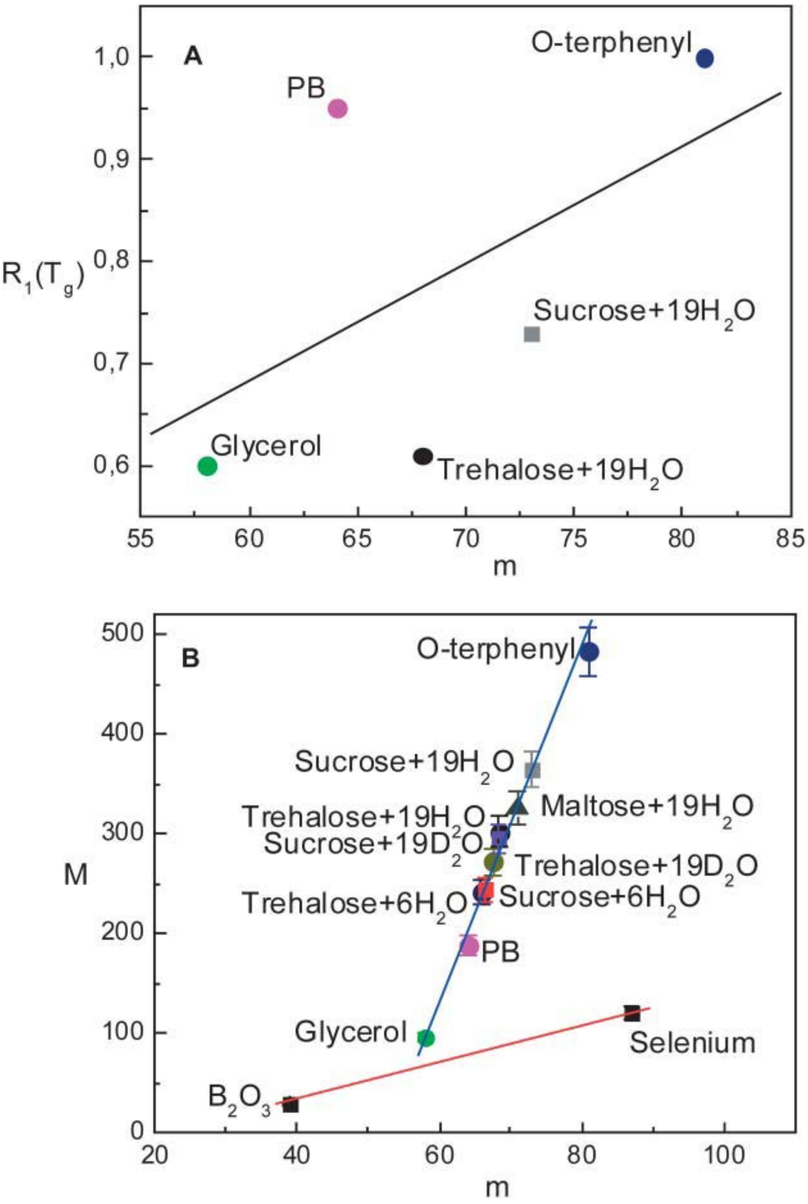

FIGURE 5 (*A*) $R_1(T_g)$ parameter, as defined by Sokolov et al. (1993, 1994), versus the fragility parameter *m*. The solid black line is a guide for eyes. (*B*) Linear behavior of the *M* fragility parameter, as defined in this work, versus the fragility parameter *m*. The variously colored points are experimental data obtained by using the IN13 spectrometer at an instrumental elastic energy resolution of 8 μeV (FWHM) (Wuttke et al., 1995; Frick and Richter, 1995; Tölle et al., 2000); the black points are experimental data obtained by using the IN6 spectrometer at an instrumental elastic energy resolution of 150–200 μeV (FWHM) (Buchenau and Zorn, 1992; Engberg et al., 1998). The solid blue and red lines indicate the best fits.

linked to the hybridized H-C-H, C-C-H, and C-O-H bending modes of the trehalose molecule as suggested by a density functional simulation of Magazù and co-workers (Ballone et al., 2000).

Such a result clearly indicates that trehalose, besides significantly modifying the structural and dynamical properties of water, forms a unique entity with H_2O molecules that shows a more crystalline character, which makes it able to protect biological structures in a more rigid environment. Such a property can be summarized by saying that trehalose maintains a more cryptocrystalline behavior, namely a locally more ordered structure, which justifies its lower fragility. This cryptocrystalline conformation could be linked to a glacial phase (Ha et al., 1996; Hédoux et al., 1998, 2002), an apparently amorphous phase in which nanocrystallized domains of the stable crystalline phase are mixed with remaining liquid. In our opinion this circumstance is relevant because it implies a better cryptobiotic effect and hence a higher capability of cryptoprotection at high disaccharide concentration.

It is curious that some of the cryptic secrets of trehalose lie within the etymologic definition of its most appropriate adjective: κριπτοσ, which contains the reference to the ''hidden'' life of cryptobiosis and to the word ''cover'', that is, the capability of encapsulating the biostructures for protection, creating a protecting shell characterized by ''cryptocrystallinity''; i.e., the capability to give rise to a (hidden) nanoscopic crystalline structure that is responsible for the higher structural resistance.

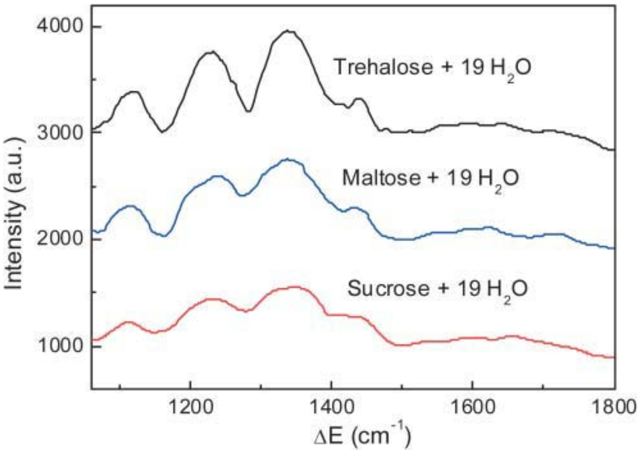

FIGURE 6 Comparison among trehalose/H_2O mixture (*black line*), maltose/H_2O mixture (*blue line*), and sucrose/H_2O mixture (*red line*) in the INS bending (1060 ÷ 2000 cm^{-1}) spectral region. The disaccharide mixture profiles are shifted for clarity.

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