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REPORT

Concentration dependence of vibrational properties of bioprotectant/water mixtures by inelastic neutron scattering

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Neutron scattering has been demonstrated to be a powerful tool for characterizing the structure and dynamics of biological molecules and for investigating the physical and chemical mechanisms of biophysical processes. The aim of the present work is to investigate by inelastic neutron scattering (INS) the vibrational behaviour of a class of bioprotectant systems, such as homologous disaccharides, trehalose, maltose and sucrose, in water mixtures. INS measurements have been performed on trehalose/H₂O, maltose/H₂O and sucrose/H₂O mixtures at very low temperature as a function of concentration by using the thermal original spectrometer with cylindrical analyzers (TOSCA) spectrometer at the ISIS Facility (DRAL, UK).

The findings allow the analyses of the vibrational features of the INS spectra in order to study the effect of disaccharides on the H₂O hydrogen-bonded tetrahedral network. The obtained neutron scattering findings point out that disaccharides, and in particular trehalose, have a destructuring effect on the water tetrahedral network, as emphasized by the analysis of the librational modes region from 50 to 130 meV energy transfer. On the other hand, the analysis of the bending modes region (130–225 meV) shows a locally ordered structure in the disaccharide/H₂O mixtures.

Finally, the observed experimental evidences are linked to the different bioprotective effectiveness of disaccharides as a function of concentration.

Keywords: disaccharides; bioprotection; water mixtures; vibrational properties; neutron scattering

1. INTRODUCTION

Biotechnology is a field aimed to understand the cellular and biochemical mechanisms in order to improve the quality of life. One of the most important topics for biotechnology is cryobiology, which studies the underlying physical and biological factors affecting the survival of cells at low-temperature values (during the cooling and warming processes) (Leibo *et al.* 1970).

It is well known that water, the most fundamental building block of life, assumes in this frame an essential importance, because the nature of water transformations during freezing determines the ability to preserve or destroy (Leibo *et al.* 1970; Mazur 1984, 1990).

The different role of solutes depends on their interaction with water molecules. Solute molecules interacting more strongly with water than among themselves have a strong effect on the tetrahedral coordination network of water, i.e. they affect the structural properties of water by imposing a new order. This important change has, from a biological point of view, a remarkable consequence, because the stabilization effect on a biomolecule is correlated to the environment in which the biostructures are located. In the case of solutes destroying the natural network of water, the biostructures are stabilized and denaturation processes can be inhibited (Moelbert & Normand 2004).

Cryoprotectants are classified as stabilizing solutes, since they reduce the damage caused by both solution injury and intracellular ice injury, decreasing the intracellular water content without dehydrating the cell. Among cryoprotectants, trehalose is one of the most effective in avoiding cellular damage by low-temperature values.

From a molecular point of view, many experimental studies (Branca *et al.* 1999*a,b*, 2001; Magazù *et al.* 2004, 2005, 2006) have been addressed to understand the bioprotective mechanisms of trehalose. Raman spectroscopy (Branca *et al.* 1999*a*) and neutron diffraction results (Branca *et al.* 1999*b*), together with ultrasonic findings (Branca *et al.* 1999*b*), show that the water hydrogen-bonded tetrahedral network is destroyed by trehalose by means of a strong interaction with water molecules, as indicated by the highest values of the solute–solvent interaction strength and hydration number. For example, at $T=25^{\circ}\text{C}$ the hydration number (n_{H})=15.2 for trehalose, whereas $n_{\text{H}}=14.7$ and 14.1 for maltose and sucrose, respectively. Furthermore, the amount of water for each trehalose molecule decreases by increasing temperature, approaching an almost constant value at the highest temperature values. This trend can be ascribed to the enhanced thermal motions at higher temperature values which lead to lower residence times of water molecules in the disaccharide hydration shells. Therefore, the average number of water molecules moving together with trehalose will be lower, decreasing the hydration number. However, it should be also pointed out that, by increasing temperature, owing to the rupture of a certain fraction of hydrogen bonds in water, the number of water molecules available for bonding with trehalose also increases. As a consequence, the hydration number is determined by these two opposing effects. At temperature values higher than about 70°C , these

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effects compensate and the hydration number tends to a constant value (Branca *et al.* 1999b).

These results indicate that trehalose strongly binds a greater number of water molecules in comparison with the other disaccharides. This trehalose capability implies a marked slowing down of water dynamics, as emphasized by quasi-elastic neutron scattering (Magazù *et al.* 2006). Furthermore, elastic neutron scattering (ENS; Magazù *et al.* 2004) and viscosity measurements (Branca *et al.* 1999b) also allowed the identification of the 'strongest' character in Angell's classification scheme of trehalose in comparison with maltose and sucrose.

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 *et al.* 2003), aimed at comparing the diffusive behaviour of trehalose and sucrose in aqueous solutions. They have found that sucrose and trehalose exhibit different mobilities when dissolved in water. The higher mobility observed in the 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 (Ekdawi *et al.* 2003).

Another confirmation has been furnished by molecular dynamics simulation studies performed by Descamps and co-workers (Bordat *et al.* 2004). In this work, several analysis tools such as the size of hydrogen-bonded water clusters, the Voronoi tessellation, the orientational order parameter and the dynamical structure factor have been combined to differentiate the actions of trehalose, sucrose and maltose. In particular, the distributions of Voronoi volumes, which provide useful information about the local molecular environment or the local free volume, emphasize a dilation and a distortion of the hydrogen-bonded network of water from its tetrahedrality. Concerning with water dynamics, the relaxation times of water in the presence of disaccharides is 1.2–10 times longer than those of pure water, depending on temperature, and trehalose/water solution shows the longest relaxation times, revealing that the dynamics of trehalose molecules are imposed to a larger number of water molecules (Bordat *et al.* 2004).

All these results contribute to clarify the physical processes underlying the biological action of trehalose, which is not directly interacting with the biostructures, as hypothesized by Crowe (Crowe & Crowe 1984).

From an applicative point of view, cryoprotective agents are used not only for preserving foods, but also for storing micro-organisms and biological materials such as enzymes, vaccines, blood and organs. As an example, trehalose has been shown to provide a significant protection against freeze-associated stresses in cryopreservation of human oocytes (Eroglu *et al.* 2002).

The aim of this work is to describe the effects of disaccharide concentration in water mixtures on the vibrational properties of water. The present findings

furnish useful information on the trehalose, maltose and sucrose amounts affecting the water tetrahedral network, giving both confirmation and explanation to previous neutron scattering results (Branca *et al.* 1999a,b, 2001; Magazù *et al.* 2004, 2005, 2006). The obtained physical picture is linked to the biological role of disaccharides, and in particular trehalose, as cryptoprotectants.

2. EXPERIMENTAL SECTION

Inelastic neutron scattering (INS) has considerable advantages, when compared with its optical counterparts, owing to several reasons: (i) no selection rule is involved; (ii) the neutron thermal energy and wavelength are comparable to phonon energies and interatomic distances in condensed phases, respectively; (iii) owing to the hydrogen large incoherent neutron cross-section, hydrogen-bonded systems can be investigated; and (iv) the increased neutron flux and improved resolution of the INS instruments allow measurement of spectra with an accuracy (1–2%) comparable with IR and Raman techniques (Li 1996; Colognesi & Parker 1999; Kolesnikov *et al.* 1999a,b).

INS measurements have been performed by using the thermal original spectrometer with cylindrical analyzers (TOSCA) indirect geometry time-of-flight spectrometer at the ISIS Pulse Neutron Facility (DRAL, UK) (Colognesi & Parker 1999). The high energy resolution of TOSCA ($\Delta E/E \approx 1.5\text{--}2\%$ for energy transfers up to several hundred milli-electron-volt) coupled with the high intensity of the ISIS source makes TOSCA ideal for studying the dynamics of water and aqueous mixtures below 2000 cm^{-1} (250 meV) (Colognesi & Parker 1999).

Ultra-pure powdered trehalose, maltose and sucrose and H₂O, purchased by Aldrich Chemie (Milan, Italy), were used for the experiment. Measurements were performed at a temperature of 27 K on pure H₂O and disaccharides (C₁₂H₂₂O₁₁)/H₂O mixtures at different weight fraction values corresponding to 7, 10 and 14 H₂O molecules for each disaccharide molecule. Since the samples are hydrogenated, the observed intensities are largely owing to the incoherent cross-section of H atoms.

α,α -Trehalose (α -D-glucopyranosyl α -D-glucopyranoside) is a disaccharide of glucose constituted by two pyranose rings in α configuration, linked by a glycosidic bond between the chiral carbon atoms C1 of the two rings (Branca *et al.* 1999b). Figure 1 shows the trehalose molecule in comparison with its homologous (maltose and sucrose). α,α -Trehalose is the only isomer of trehalose found in nature; α,β -trehalose (neotrehalose) and β,β -trehalose (isotrehalose), the other known trehalose isomers, have not yet been discovered. α,α -Trehalose is found naturally linked to two H₂O molecules, owing to the trehalose tendency to capture water. Since the reducing end of a glucosyl residue is connected with the other, trehalose has no reducing power and therefore it avoids Maillard's reactions.

The trehalose homologous disaccharides have the same formula (C₁₂H₂₂O₁₁, $M_w = 342.3$), but show a different structure. Maltose (4-O- α -D-glucopyranosyl-D-glucose) is also constituted by two pyranose rings of glucose in the α configuration, but the oxygen bridge

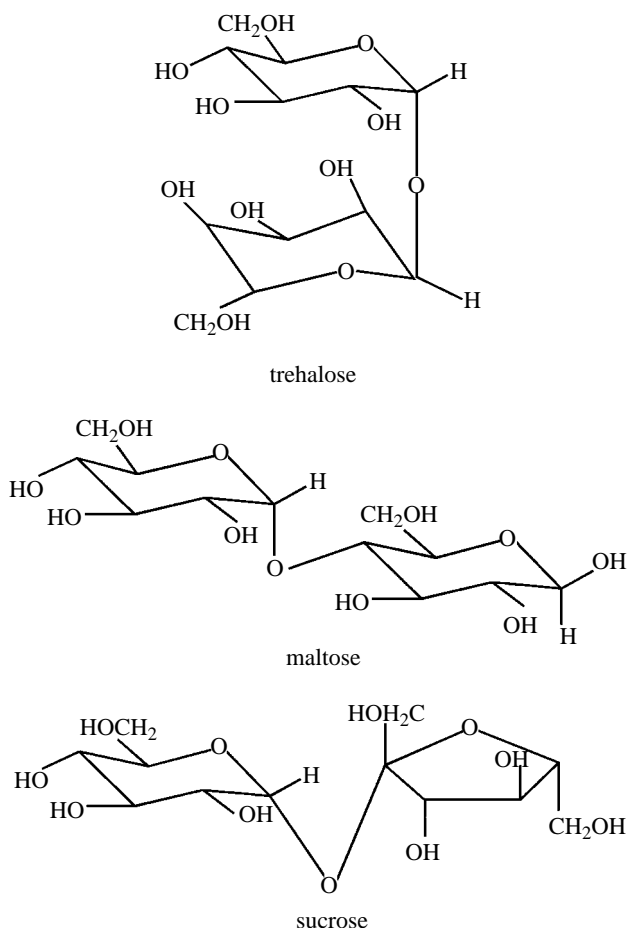


Figure 1. Trehalose, maltose and sucrose molecule.

links the two carbon atoms C1 and C4 of the two rings, and it is found naturally linked to one H₂O molecule. Finally, sucrose (α -D-glucopyranosyl β -D-fructofuranoside) is constituted by a glucose ring (pyranose) in α configuration and a fructose ring (furanose) in β configuration (Branca *et al.* 1999b).

The samples, contained in thin-walled aluminium cells, were cooled to 27 K by a liquid helium cryostat. For all the investigated samples, the measurement time was 12 h for each run. Concerning the sample preparation, homogeneous thin layers were obtained starting from saturated solutions by vaporization under controlled environmental conditions; within the optically isotropic obtained solution, the remaining water content has been checked by thermogravimetry and micro-IR measurements. Finally, ultrasonic measurements (Branca *et al.* 1999b) indicate that, at the investigated concentration values, no bulk water is present.

For the data treatment, the standard GENIE programme has been used (Colognesi & Parker 1999). The multiple scattering contribution has been minimized by using a thin sample in order to obtain a scattering transmission from the sample greater than or equal to 90%. The multiphonon neutron scattering contribution (MPNS), which can be significant at high temperature and large momentum transfer, has been calculated directly from the measured spectra by using a method of sequential iterations. Since measurements were performed at low temperature, the MPNS

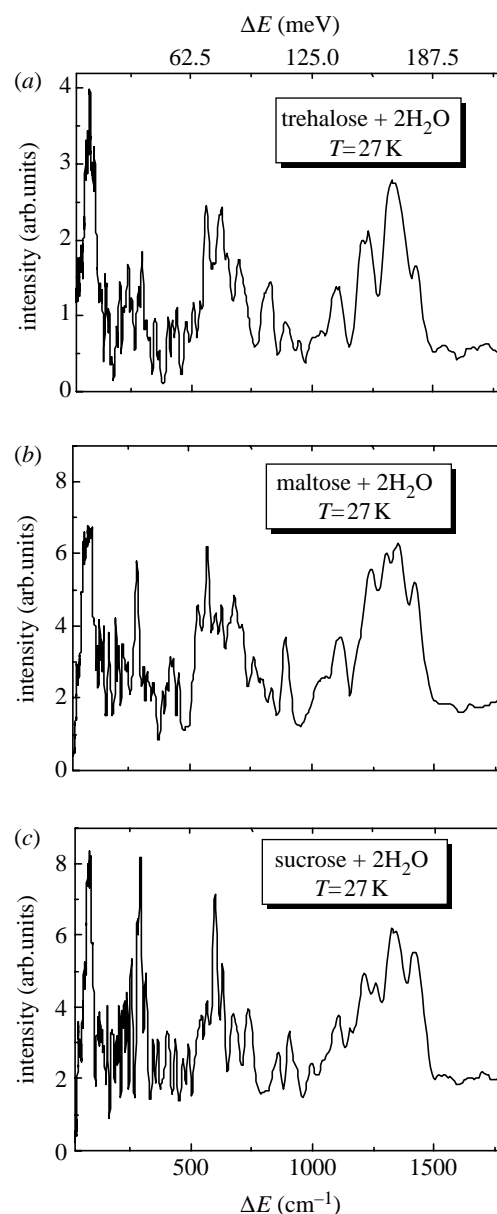


Figure 2. INS spectrum of (a) trehalose/2 H₂O, (b) maltose/2 H₂O and (c) sucrose/2 H₂O mixture at $T=27$ K in the 0–1800 cm⁻¹ region. The MPNS contribution correction has been performed for all the spectra. The disaccharides mixtures profiles are shifted for clarity.

contribution is not large at the translational modes region (i.e. at low Q region) (Li 1996; Kolesnikov *et al.* 1999a,b).

3. RESULTS AND DISCUSSION

Previous INS results on trehalose and its homologous, i.e. maltose and sucrose (Magazù *et al.* 2005), emphasized the differences among disaccharide vibrational properties starting from a comparison with the H₂O spectrum obtained at the same temperature value. In the previous paper (Magazù *et al.* 2005), the effects on the tetrahedral network of water have been discussed pointing out the strongest interaction of trehalose with water which justifies its stronger bioprotective effectiveness. In order to analyse the changes induced by disaccharides on water, we distinguish the following

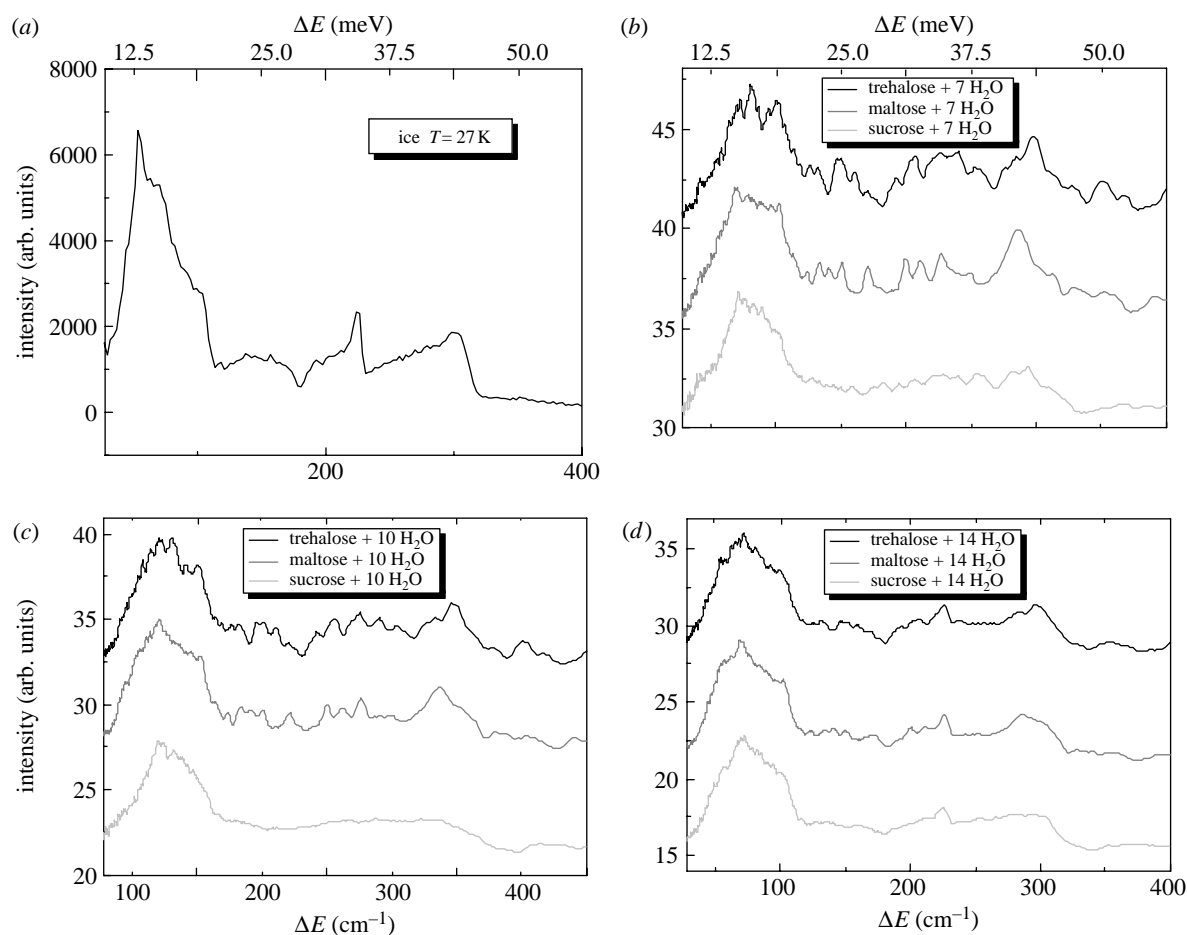


Figure 3. INS spectra of (a) H_2O , (b) disaccharides/ $7\text{H}_2\text{O}$ mixture, (c) disaccharides/ $10\text{H}_2\text{O}$ mixture and (d) disaccharides/ $14\text{H}_2\text{O}$ mixture at $T=27\text{K}$ in the $0\text{--}400\text{ cm}^{-1}$ translational spectral region. The MPNS contribution correction has been performed for all the spectra. The disaccharides mixtures profiles are shifted for clarity.

vibrational modes in the H_2O spectrum: (i) the region of the H_2O intermolecular vibrations up to approximately 1060 cm^{-1} , in which one distinguishes a ‘translational’ part up to approximately 400 cm^{-1} and a ‘librational’ part; and (ii) the region of the H_2O intramolecular vibrations up to approximately 3600 cm^{-1} , which presents the bending modes at approximately 1600 cm^{-1} and the stretching modes at approximately 3360 cm^{-1} (Li 1996; Kolesnikov *et al.* 1999*a,b*).

Furthermore, owing to the spectral features observed by increasing the water content in the mixtures, in figure 2 the INS spectra of (i) trehalose/ $2\text{H}_2\text{O}$ (the dehydrate form is the natural state of trehalose), (ii) maltose/ $2\text{H}_2\text{O}$, and (iii) sucrose/ $2\text{H}_2\text{O}$ are shown as references.

3.1. $0\text{--}400\text{ cm}^{-1}$ region

In figure 3*a*, two bands in the H_2O spectrum are observed at lower energy: the first one, having a sharp peak at approximately 56 cm^{-1} , is higher than the second one centred at approximately 148 cm^{-1} . They can be assigned to acoustic modes. It is known, in fact, that in the ice spectrum the peak at approximately 56 cm^{-1} denotes the first Van Hove singularity in the dynamics of acoustic phonons. The two peaks present at approximately 224 and 304 cm^{-1} with cut-offs on

their right-hand sides are owing to molecular optical modes (Li 1996; Kolesnikov *et al.* 1999*a,b*).

From figure 3*b–d*, it is observed that in the presence of trehalose, maltose and sucrose, the sharp peak of the low-energy acoustic modes appears significantly lower and broader and shifted at 72 cm^{-1} . The shift at higher energy points out a strong interaction between disaccharide and water molecules. Important changes are also appreciable for the other bands and the second peak of the optical mode is strongly deformed. The water contribution in this region starts to become significant only for the concentration value corresponding to $14\text{H}_2\text{O}$ molecules for each disaccharide molecule, showing a spectrum more similar to that of water. By comparing trehalose to maltose and sucrose spectra, it is evident that, among the investigated disaccharides, sucrose is more strongly influenced by water and for sucrose mixtures the spectral features relative to the disaccharide contribution are more rapidly lost than for trehalose and maltose mixtures.

3.2. $400\text{--}1060\text{ cm}^{-1}$ region

It was found that the position of the librational low-energy cut-off is a characteristic value for the band in this INS spectral region for different ice forms (Li 1996; Kolesnikov *et al.* 1999*a,b*). The observed shifts of the cut-

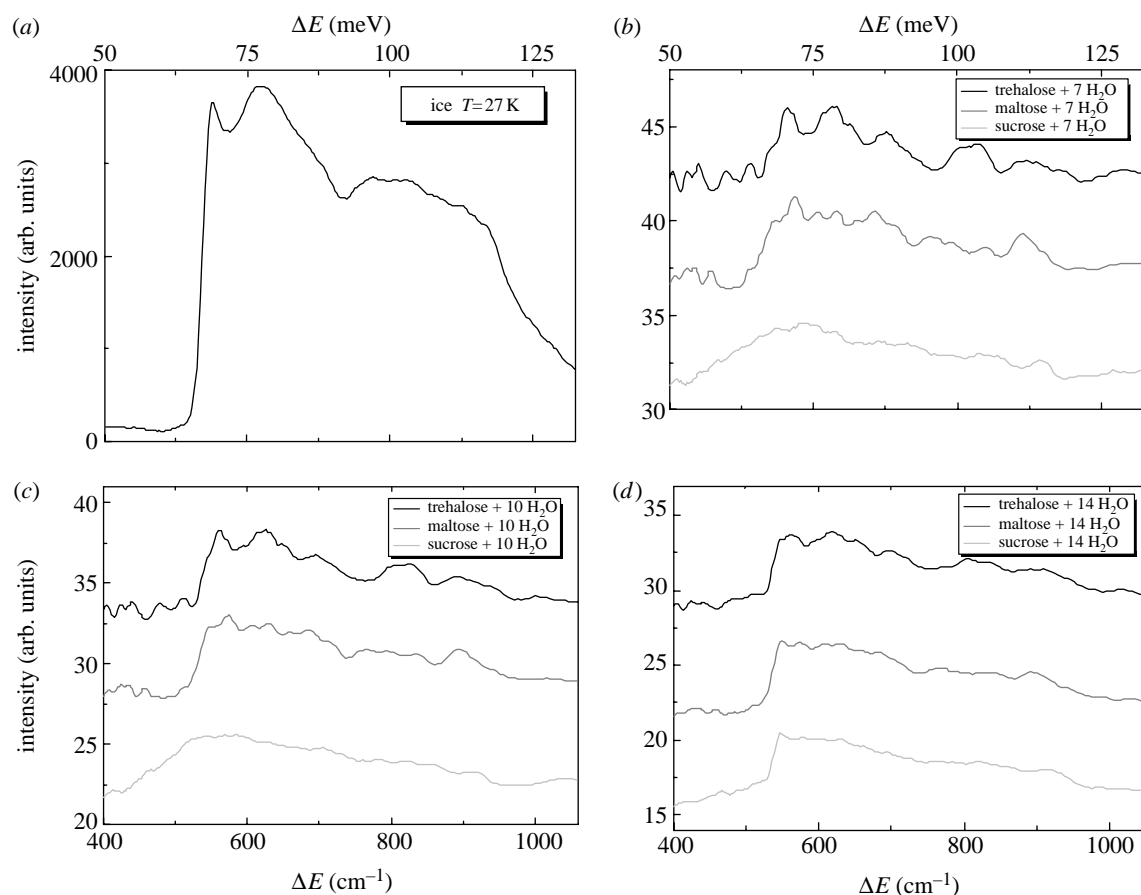


Figure 4. INS spectra of (a) H_2O , (b) disaccharides/ $7 \text{H}_2\text{O}$ mixture, (c) disaccharides/ $10 \text{H}_2\text{O}$ mixture and (d) disaccharides/ $14 \text{H}_2\text{O}$ mixture at $T=27 \text{ K}$ in the $400\text{--}1060 \text{ cm}^{-1}$ librational spectral region. The MPNS contribution correction has been performed for all the spectra. The disaccharides mixtures profiles are shifted for clarity.

off position are proportional to the transverse forces between the water molecules whose intensities depend on the different ice forms (Li 1996; Kolesnikov *et al.* 1999*a,b*).

In order to point out the differences owing to the trehalose, maltose and sucrose mixtures in the librational spectral region, in figure 4 the INS spectra for pure H_2O and disaccharides/ $7 \text{H}_2\text{O}$, disaccharides/ $10 \text{H}_2\text{O}$ and disaccharides/ $14 \text{H}_2\text{O}$ in this region are shown. A depression of the intensity of the cut-off is observed and, as can be expected, it is more evident for trehalose, maltose and sucrose/ $7 \text{H}_2\text{O}$, the cut-off becoming sharper by increasing the water content. Concerning the shift of the cut-off position, which for H_2O at $T=27 \text{ K}$ is at approximately 550 cm^{-1} (Li 1996; Kolesnikov *et al.* 1999*a,b*), for all the investigated concentration values in trehalose mixtures we observe the same shift of approximately 16 cm^{-1} , whereas for maltose mixtures the shift is of approximately 3 cm^{-1} , and for sucrose mixtures no shift occurs. This result can provide a confirmation to previous light and neutron scattering findings (Branca *et al.* 1999*a,b*; Magazù *et al.* 2005), which emphasized that trehalose is more capable than maltose and sucrose of modifying the H_2O spectral features by affecting the intermolecular interaction forces and the arrangements of the H_2O molecules.

In the trehalose vibrational spectrum obtained by simulation (Ballone *et al.* 2000), we note that the peak linked to the C–O stretching mode at approximately

1000 cm^{-1} does not appear, or at least it is not detectable, indicating that this kind of mode in the rings is constrained owing to the presence of hydrogen bonds with water. In general terms, however, below 960 cm^{-1} it is not possible to assign a well-defined character to all the bands (Ballone *et al.* 2000). The stretching of the C–O bonds occurs in a range between 970 and $11\,000 \text{ cm}^{-1}$, while the oscillations of the OH groups around the minimum of the C–C–O–H torsional potential are well localized below 950 cm^{-1} . These modes possess energy between 420 and 570 cm^{-1} , with the exception of the OH group giving rise to the intramolecular hydrogen bond, whose torsional mode has energy of 700 cm^{-1} (Ballone *et al.* 2000). From figure 4, it is evident that the peak corresponding to the torsional mode is increasingly depressed by increasing the water content.

3.3. $1060\text{--}1800 \text{ cm}^{-1}$ region

This spectral region corresponds to bending vibrational modes range for ice (Li 1996; Kolesnikov *et al.* 1999*a,b*). As can be observed from figure 5*a*, the H_2O spectrum is characterized by two distinct bands centred at approximately 1224 and 1608 cm^{-1} , respectively. In the spectra of the investigated trehalose, maltose and sucrose/ H_2O mixtures, shown in figure 5*b–d*, these features are totally changed. The band at approximately 1608 cm^{-1} is absent in the disaccharide/ H_2O spectra, confirming that

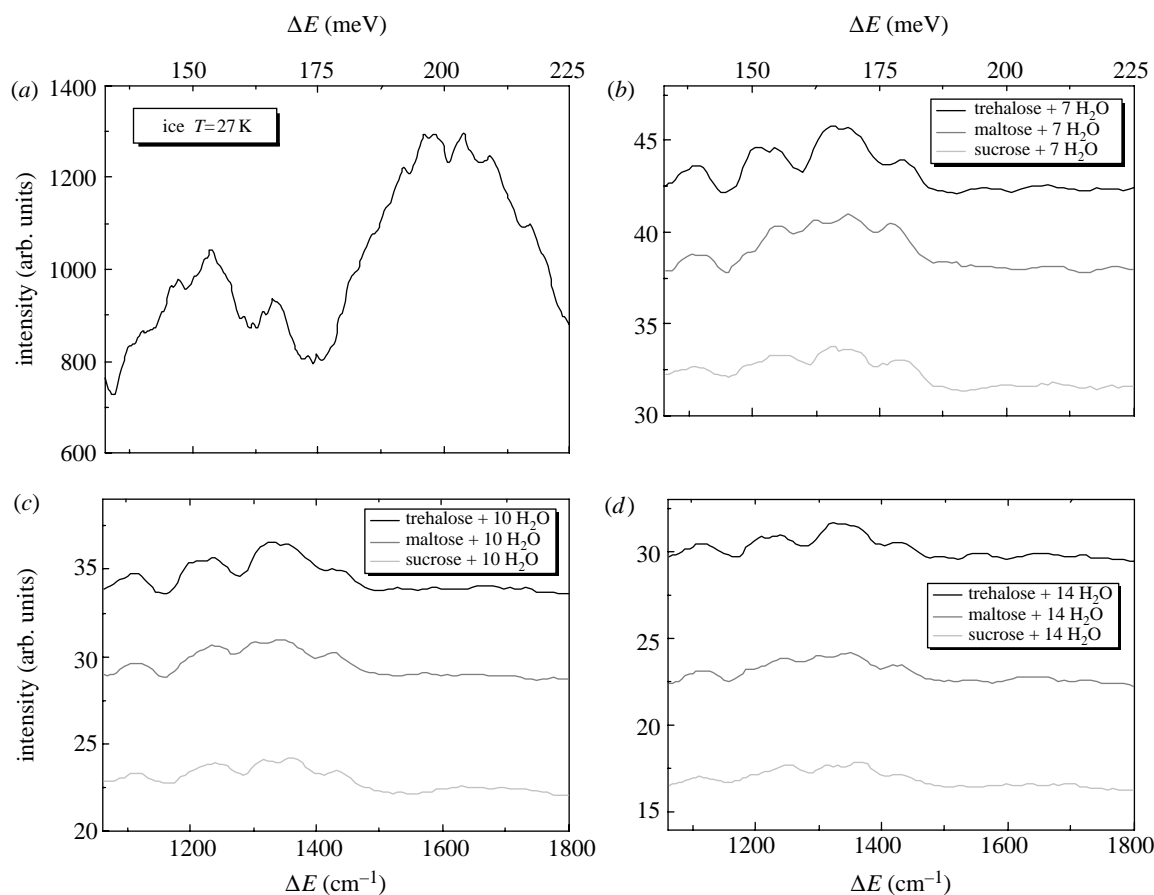


Figure 5. INS spectra of (a) H_2O , (b) disaccharides/7 H_2O mixture, (c) disaccharides/10 H_2O mixture and (d) disaccharides/14 H_2O mixture at $T=27\text{ K}$ in the 1060–1800 cm^{-1} bending spectral region. The MPNS contribution correction has been performed for all the spectra. The disaccharides mixtures profiles are shifted for clarity.

disaccharides are able to affect the water hydrogen bond O–H \cdots O bending modes connected to the strength and tetrahedrality of the hydrogen bonding (Li 1996; Kolesnikov *et al.* 1999*a,b*). In particular, the role played by disaccharides is to impose on water a network which deviates from tetrahedral bonding and for which the hydrogen bonding among water molecules is diminished while that among disaccharides and water molecules is increased.

By the observation of the second band in figure 5, it is evident that the trehalose/ H_2O spectra appear more ‘structured’ in comparison with the other disaccharide mixtures and show more distinctly the three peaks present in the trehalose/2 H_2O spectrum (figure 2). These peaks correspond to the hybridized H–C–H, C–C–H and C–O–H bending modes, as indicated by simulation (Ballone *et al.* 2000). By increasing the water content, the three peaks are still evident even if they are less marked. For sucrose mixtures these peaks appear larger and broader.

This trend finds a correspondence with ENS findings (Magazù *et al.* 2004), which have shown the enhanced rigidity of the disaccharide/ H_2O systems by increasing disaccharide concentration. ENS spectra show a dynamical transition for all the investigated mixtures as a function of temperature with an intensity decrease less marked in the case of trehalose/water mixture than for the other disaccharide/water mixtures. This

circumstance indicates that trehalose shows a larger structural resistance to temperature changes and a lower ‘fragility’ in comparison with the maltose and sucrose/ H_2O mixtures.

4. CONCLUSIONS

In this paper, INS results on trehalose/ H_2O , maltose/ H_2O and sucrose/ H_2O mixtures as a function of concentration are shown. The experimental data point out that trehalose interacts more strongly with water than the other disaccharides, influencing more significantly its vibrational modes.

The physical picture obtained from the performed studies shows that the highest bioprotectant effectiveness of trehalose in comparison with the other disaccharides is owing to the combined effect of different cofactors. What emerges is that the biological action of disaccharides can be explained by the strong interaction with water molecules, as emphasized by the highest value of interaction strength and hydration number of the trehalose/ H_2O system with respect to the other disaccharide mixtures obtained by ultrasonic measurements (Branca *et al.* 1999*b*). This capability implies that disaccharides, and in particular trehalose, promote an extensive layer of structured water around its neighbourhood, which destroys the tetrahedral hydrogen-bond network of pure water, providing a structure

whose spatial positions and orientations are not compatible with those of ice, as pointed out by Raman (Branca *et al.* 1999*a*) and the present INS findings.

By the ENS results, it has been shown that the glass-forming properties of disaccharides play an important role in the bioprotection mechanisms. We concluded that trehalose, besides modifying significantly the structural and dynamical properties of water, forms with H₂O a less fragile entity able to encapsulate biological structures and to protect them in a more rigid environment. The trehalose–water system shows a macroscopic amorphous conformation in which nano-crystallized domains are mixed with remaining liquid (Magazù *et al.* 2004), thus revealing a ‘cryptocrystalline’ character. The present INS findings help to give an explanation to the previous results, because the locally most ordered structure of trehalose, or as it can be conveniently said its ‘cryptocrystallinity’, can justify the highest rigidity of this system (Magazù *et al.* 2004).

From a biological point of view, the superior cryo- and cryptobiotic action of trehalose can therefore find a full elucidation, since trehalose shows a more marked destructuring effect and a more evident cryptocrystalline character, which make it so effective as cryo- and cryptoprotectant, respectively.

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REFERENCES

- Ballone, P., Marchi, N., Branca, C. & Magazù, S. 2000 Structural and vibrational properties of trehalose: a density functional study. *J. Phys. Chem.* **104**, 6313–6317.
- Bordat, P., Lerbret, A., Demaret, J.-P., Affouard, F. & Descamps, M. 2004 Comparative study of trehalose, sucrose and maltose in water solutions by molecular modelling. *Europhys. Lett.* **65**, 41–47. (doi:10.1209/epl/i2003-10052-0)
- Branca, C., Magazù, S., Maisano, G. & Migliardo, P. 1999*a* Anomalous cryoprotective effectiveness of trehalose: Raman scattering evidences. *J. Chem. Phys.* **111**, 281–287. (doi:10.1063/1.479288)
- Branca, C., Faraone, A., Magazù, S., Maisano, G., Migliardo, F., Migliardo, P. & Villari, V. 1999*b* Structural and dynamical properties of trehalose–water solutions. *Rec. Res. Develop. in Phys. Chem.* **3**, 361–403.
- Branca, C., Magazù, S., Maisano, G. & Migliardo, F. 2001 Vibrational and relaxational contributions in disaccharide/H₂O glass formers. *Phys. Rev. B* **64**, 224 204–224 212. (doi:10.1103/PhysRevB.64.224204)
- Colognesi, D. & Parker, S. F. 1999 *The TOSCA manual*. Oxfordshire, UK: Rutherford Appleton Laboratory.
- Crowe, J. H., Crowe, L. M. & Chapman, D. 1984 Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science* **223**, 701–703.
- Ekdawi, N., De Pablo, J. J., Feick, E. & von Meerwall, E. 2003 Diffusion of sucrose and α,α -trehalose in aqueous solutions. *J. Phys. Chem. A* **107**, 936–943. (doi:10.1021/jp020187b)
- Eroglu, A., Toner, M. & Toth, T. L. 2002 Beneficial effect of microinjected trehalose on the cryosurvival of human oocytes. *Fertil. Steril.* **77**, 152–158. (doi:10.1016/S0015-0282(01)02959-4)
- Kolesnikov, A. I., Li, J. C., Ahmad, N. C., Loong, C.-K., Nipko, M., Yocum, L. & Parker, S. F. 1999*a* Neutron spectroscopy of high-density amorphous ice. *Physica B* **263–264**, 650–652. (doi:10.1016/S0921-4526(98)01438-0)
- Kolesnikov, A. I., Li, J. C., Parker, S. F., Eccleston, R. S. & Loong, C.-K. 1999*b* Vibrational dynamics of amorphous ice. *Phys. Rev. B* **59**, 3569–3578. (doi:10.1103/PhysRevB.59.3569)
- Leibo, S. P., Farrant, J., Mazur, P., Hanna Jr, M. G. & Smith, L. H. 1970 Effects of freezing on marrow stem cell suspensions: interactions of cooling and warming rates in the presence of PVP, sucrose, or glycerol. *Cryobiology* **6**, 315–332.
- Li, J. C. 1996 Inelastic neutron scattering studies of hydrogen bonding in ices. *J. Chem. Phys.* **105**, 6733–6755. (doi:10.1063/1.472525)
- Magazù, S., Maisano, G., Migliardo, F. & Mondelli, C. 2004 Mean-square displacement relationship in bioprotectant systems by elastic neutron scattering. *Biophys. J.* **86**, 3241–3249.
- Magazù, S., Migliardo, F. & Ramirez-Cuesta, A. J. 2005 Inelastic neutron scattering study on bioprotectant systems. *J. R. Soc. Interface* **2**, 527–532. (doi: 10.1098/rsif.2005.0059)
- Magazù, S., Migliardo, F. & Telling, M. T. F. 2006 α,α -trehalose–water solutions. VIII. Study of the diffusive dynamics of water by high-resolution quasi elastic neutron scattering. *J. Phys. Chem. B* **110**, 1020–1025. (doi:10.1021/jp0536450)
- Mazur, P. 1984 Freezing of living cells: mechanisms and implications. *Cell Physiol.* **16**, C125–C142.
- Mazur, P. 1990 Equilibrium, quasi-equilibrium, and nonequilibrium freezing of mammalian embryos. *Cell Biophys.* **17**, 53–92.
- Moelbert, S., Normand, B. & De Los Rios, P. 2004 Kosmotropes and chaotropes: modelling preferential exclusion, binding and aggregate stability. *Biophys. Chem.* **112**, 45–57. (doi:10.1016/j.bpc.2004.06.012)