

### $\alpha,\alpha$ -Trehalose—Water Solutions. 3. Vibrational Dynamics Studies by Inelastic Light Scattering

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Results of Raman Scattering measurements performed on aqueous solutions of  $\alpha,\alpha$ -trehalose, an effective bioprotector against dehydration and freezing, are reported. To acquire some insight into the effects of trehalose on the hydrogen-bond network of water, we investigate the intramolecular O—H and intermolecular OH $\cdots$ O stretching and bending modes, which are particularly sensitive to environmental modifications. We will show that the shape of the O—H stretching band can be reproduced by a suitable superposition of the spectra of bulk and hydration water. The relative amount of the two contributions changes with concentration and temperature, furnishing information on the number of the water molecules bonded to trehalose. In addition, two different approaches are employed for the O—H stretching region analysis, namely a decomposition of the isotropic spectra into an “open” and a “closed” contribution and a spectral stripping procedure to extract the “collective” contribution from the polarized spectra. Both the procedures agree in suggesting that trehalose promotes a destructuring effect on the tetrahedral H-bond network of pure water. These conclusions are also supported by the analysis of the intermolecular spectral contributions. Considered together, these results make plausible the hypothesis that trehalose obstructs the crystallization process, reducing the amount of freezable water, namely destroying the network of water compatible with that of ice.

#### General Considerations

Water is usually thought to be required for the living state, but many organisms can withstand anhydrobiosis when essentially all of their body water has been removed.<sup>1–9</sup> Indeed, numerous restrictions, such as those addressing the phase transition from liquid water to solid ice, have led many organisms,<sup>1–3</sup> in particular some species of soil-dwelling organisms,<sup>4</sup> to some ingenious adaptations to survive, passing during dry times into a state of suspended animation. The mechanism for survival under this kind of stress is connected with the accumulation of trehalose ( $\alpha$ -D-glucopyranosyl  $\alpha$ -D-glucopyranoside), a nonreducing disaccharide of glucose.<sup>5–9</sup>

One of trehalose's principal claims to fame is its effectiveness as an antifreezing agent.<sup>5</sup> It is well-known that the phase transition from liquid water to ice is equated to death because of the damage of the cellular microarchitecture by mechanical stress. One of the most important ways with which many organisms, subjected to temperatures at which water would normally be frozen, survive is to lower the fraction of water remaining in the cells as temperature is lowered. The remaining unfreezable water rests within the hydration shell and cannot crystallize. For other organisms the survival is connected with the role of certain substances that are able to protect the organism to a substantial degree by inhibiting the ice formation. Even at low concentrations, such substances are capable of reducing the amount of freezable water, giving rise to considerable freeze tolerance.

Trehalose is nonhygroscopic over a wide humidity range, remains dry to the touch, and yet is freely soluble in water. Trehalose does not caramelize at normal emulsion processing temperatures, and on heating, unlike other sugars, it does not undergo reactions with proteins or other reactive molecules.

Leading applications<sup>1</sup> are in moisturizing creams and lotions, in food preservation, and in the conservation of transplanted organs, where its properties help to maintain integrity over time. Other sugars, such as maltose, sucrose, and glucose, have shown similar properties to protect against dehydration and freezing,<sup>5,10</sup> but trehalose is significantly the most effective.

The molecular origin of this effectiveness has been the source of considerable speculation,<sup>5–16</sup> but it is far from resolved. Indeed, some peculiarities in the microscopic mechanisms governed by the hydrogen-bond interactions have been noted and remain to be fully interpreted.

Green and Angell<sup>5,6</sup> have related the trehalose effectiveness to the fact that the trehalose—water system possesses the highest glass transition temperature. This implies an exceptional water content at the transition. The high viscosity and the attendant suppression of crystallization of the solid phase would play an important role in maintaining for decades the structural and functional integrity of organisms which reach an anhydrobiotic state by passing into the vitreous state. Crowe et al.,<sup>8</sup> in their extensive work on this subject, have suggested that the removal of water molecules from the head group region of cell bilayer membranes increases the head group packing, thus forcing the dry bilayer from the liquid crystalline to the gel phase. Upon rehydration, dry membranes, which are in the gel phase at room temperature, undergo a phase transition from the gel back to the liquid crystalline phase, during which leakage may occur because of packing defect regions. Trehalose would protect membrane integrity during drying and rehydration since it lowers the transition temperature ( $T_{\text{mel}}$ ) of the dry membranes by replacing the water between the lipid head groups (water replacement hypothesis). In such a way, in the opinions of these authors, it prevents the phase transition and the accompanying leakage upon rehydration.

Grigera et al.<sup>7</sup> have performed a molecular dynamics (MD) simulation of aqueous solutions of  $\alpha,\alpha$ -trehalose at room temperature by using the SPC/E model. It consists of a negative charge in the oxygen location and repulsion–attraction potential of a Lennard-Jones 12–6 type. Two positive charges are located on the hydrogen atoms. The OH distance is kept at 0.1 nm and HOH angle at 109.47°. They found that the disaccharide tends to bond up to 10 water molecules, creating a hydrophilic surface with typical hexagonally coordinated units.<sup>13</sup>

Recently Brady and co-workers<sup>14</sup> proposed a new MD simulation that may be of relevance in providing some insight into the mechanism underlying the unusual behavior revealed. They found that trehalose imposes extensive structuring effects on the adjacent solvent, with structuring extending out to at least the third solvation shell. The details of the structuring are determined both by the specific stereochemical topology of the molecule and by its conformation, with considerable interplay between conformation and solvent structure.

Previous work<sup>15,16</sup> revealed that trehalose, with respect to the other disaccharides, presents the highest fragile character at high dilution and the lowest fragility at high concentration.<sup>17</sup> The findings so far have been such as greatly to enhance the reputation of a greater ability to conform to the irregular surfaces of biostructures at high dilution and of a greater criptobiotic attitude at high concentration. In addition, the hydration number of trehalose molecules does not vary appreciably in the temperature range between 20 and 85 °C, thus supporting the idea that the water molecules in the neighborhood of trehalose are strongly bound to it. A series of quasi-elastic neutron scattering (QENS) experiments<sup>12,18</sup> suggested that trehalose significantly affects the dynamics of water molecules in its neighborhood and are consistent with the presence of a series of layers or shells each of which is characterized by a relatively slow diffusion.

The main purpose of the present work is to observe the changes in the Raman spectra of water in the presence of trehalose. In the past, Raman spectroscopy has proven valuable in the study of water and its hydrogen-bonded structures. To analyze the effects of trehalose on the hydrogen-bond network of water, we performed Raman scattering measurements on trehalose aqueous solutions as a function of concentration and temperature. The analysis of the intramolecular and intermolecular O–H stretching and bending contributions unambiguously shows that trehalose induces extensive destructuring effects on the adjacent water molecules, playing the role of *structure-breaker*. These results make plausible the hypothesis that the crystallization process, in the presence of trehalose, is obstructed by the reduction of the amount of freezable water.

## Experimental Section

Aqueous solutions of ultrapure  $\alpha,\alpha$ -trehalose, purchased from Aldrich-Chemie, were investigated at different concentrations and temperatures. The solutions were prepared using doubly distilled deionized water. Before measurements, the samples were stored in the dark and left for long times for equilibration; they were filtered through an Amicon filter of 0.45  $\mu\text{m}$  diameter pores to obtain dust-free solutions.

The samples for the Raman light-scattering measurements were sealed in optical quartz cells of 5 mm inner diameter and then mounted in an optical thermostat especially built to avoid any unwanted stray-light contributions. The measurements were performed with a temperature stability better than 0.1 °C. The samples of high purity, as well as the optical purity of the sample holder, ensured the collection of data with a good signal-to-

noise ratio and with high reproducibility.  $I_{VV}$  and  $I_{VH}$  Raman spectra were obtained by a high-resolution fully computerized Spex-Ramalog 5 triple monochromator in a 90° scattering geometry. Vertically polarized radiation of an INNOVA 70 Series Ar–Kr gas mixed laser operating in the 4579–6764 Å range was used as an excitation source. To reduce fluorescence, we chose the 6471 Å laser line with 1 W output. The detection apparatus consisted of a photon-counting system whose outputs were processed on line by a computer. The scattered photons were automatically normalized for the incoming beam intensity to ensure good data reproducibility. To separate the scattering due to the solution from the background, which was almost entirely due to either the Raman scattering or fluorescence of the quartz cell, it was assumed in the removal procedure that the intensity in some distinct spectral regions was due entirely to quartz and other types of noise. This is only approximately correct since a detectable sample intensity at all frequency values exists. However, in comparison to the OH stretching features, the intensity of these spurious contributions is negligible and the assumption is useful. The uncertainty in this correction forms the principal uncertainty in the spectra and was estimated to be less than  $\pm 2\%$  of peak intensity for an  $I_{VH}$  spectrum and less than  $\pm 0.5\%$  of peak intensity for an  $I_{VV}$  spectrum.

Each reported spectrum is the average of different scans. For each isotherm, the individual  $I_{VV}$  and  $I_{VH}$  scans were taken in an alternating sequence to ensure that a definite intensity relationship existed between the final  $I_{VV}$  and  $I_{VH}$  spectra of the same isotherm. Isotropic scattering intensities were calculated from the parallel and perpendicular components of the scattered light by  $I_{\text{iso}} = I_{VV} - \frac{4}{3}I_{VH}$ .

Figure 1 shows, as an example, the polarized  $I_{VV}$  spectra of trehalose aqueous solutions at various water contents at  $T = 293$  K. All the spectral contributions were normalized on the C–H stretching band; finally, the latter was removed from the O–H stretching contour.

## Results and Discussion

It is well-known<sup>19</sup> that the Raman effect results from the interaction of vibrational and/or rotational motions of molecules with electromagnetic radiation. The observed spectrum of the scattered light contains information about both the structure and the dynamics of the molecular system. The dynamical structure factor,  $S(\mathbf{Q}, \omega)$ , measured in a Raman experiment is essentially connected with the Fourier transform of the autocorrelation function of the polarizability tensor

$$J_{\nu\nu}(\mathbf{Q}, t) \propto \left\langle \sum_{i,j,\nu,\nu'} (\epsilon_S \tilde{\alpha}_i^{\nu}(0) \epsilon_I) (\epsilon_S \tilde{\alpha}_j^{\nu'}(t) \epsilon_I) q_i^{\nu}(0) q_j^{\nu'}(t) \times \exp[i\mathbf{Q}(\mathbf{r}_i(t) - \mathbf{r}_j(0))] \right\rangle \quad (1)$$

where  $\epsilon_S$  and  $\epsilon_I$  are the scattered and incoming polarization vectors, respectively,  $\tilde{\alpha}^{\nu} = (\partial \tilde{\alpha} / \partial q)_{q=q_{\nu}}$ ,  $q_{\nu}$  being the vibrational normal coordinate, and  $\mathbf{r}_i$  is the position of the  $i$ th scattering particle. Under the hypothesis of decoupled rotational and vibrational motions, it can be shown that  $S(\mathbf{Q}, \omega)$  can be written as

$$S(\mathbf{Q}, \omega) = \sum_{\nu} (q^{\nu})^2 G_{\nu}(\omega + \Omega_{\nu}) \otimes F_{2\nu}(\omega + \Omega_{\nu}) \quad (2)$$

This equation states that the Raman spectrum is constituted by vibrational peaks  $G_{\nu}(\omega + \Omega_{\nu})$  of the assigned symmetry, convoluted with the rotational second-order Legendre polynomial  $F_{2\nu}(\omega + \Omega_{\nu})$ .

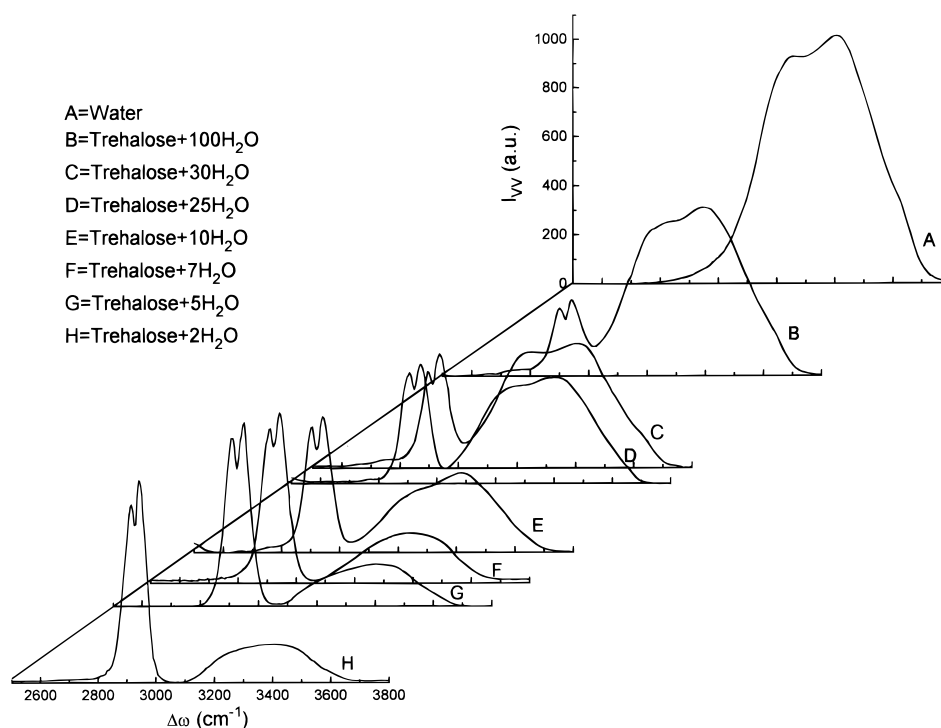


Figure 1. Polarized  $I_{VV}(\omega)$  spectra of trehalose aqueous solutions with various water contents at  $T = 293$  K.

The polarizability tensor is usually split into its isotropic part  $\tilde{\alpha}$  and anisotropic part  $\tilde{\beta}$ ,  $\tilde{\alpha}^v = \tilde{\alpha}^v \tilde{I} + \tilde{\beta}^v$ , with  $\tilde{\alpha} = 1/3 \text{Tr}(\tilde{\alpha})$  and  $\tilde{\beta} = \tilde{\alpha} - 1/3 \text{Tr}(\tilde{\alpha}) \cdot \tilde{I}$ ,  $\tilde{I}$  being the unit tensor. According to the geometry of the experiment, one can select different combinations of the isotropic and anisotropic parts, and the corresponding spectra will reflect different aspects of the (same) motion.

The Raman spectra of water in its condensed phases have been the focus of many studies. From a general point of view, one can distinguish<sup>20</sup> (i) an *intramolecular* region, occurring for  $\omega > 1000 \text{ cm}^{-1}$ , which encompasses the O—H stretching ( $2800 \text{ cm}^{-1} < \omega < 3800 \text{ cm}^{-1}$ ) and bending (centered at  $\omega \approx 1645 \text{ cm}^{-1}$ ) spectral contributions, and (ii) an *intermolecular* region, for  $\omega < 1000 \text{ cm}^{-1}$ , which includes the weak bands below  $300 \text{ cm}^{-1}$ , corresponding to the restricted translational motions of  $\text{H}_2\text{O}$  molecules involved in direct hydrogen-bond interactions (bending  $\omega \approx 60 \text{ cm}^{-1}$  and stretching  $\omega \approx 175 \text{ cm}^{-1}$  for the O—H...O units), and the restricted rotational region  $300\text{--}1000 \text{ cm}^{-1}$ , which reflects the librational motions of  $\text{H}_2\text{O}$  molecules arising from restraints produced by hydrogen bonds.

**(i) The Intramolecular Region.** It is well-known that the early interpretations<sup>20</sup> of the O—H stretching region of the spectrum of water were essentially in terms of normal modes of water molecules engaged in four or fewer hydrogen bonds.

First, before trying to address the difficult questions concerning the destructuring effect of trehalose on the tetrahedral network of water, we discuss the sort of information that, from a structural point of view, can be useful to obtain information on the trehalose hydration number.<sup>21</sup> Figure 2 shows the isotropic spectra,  $I_{\text{iso}}(\omega)$ , of the O—H stretching band for trehalose aqueous solutions as a function of concentration at  $T = 293$  K. Let us consider a very simple two-state model, already employed in the case of polymeric aqueous solutions.<sup>21</sup> In particular, the water molecules present in solution are classified into two categories: molecules *bonded* to the trehalose and molecules in the *bulk* phase. Obviously, such a model neglects the correlation effects between the two states and assumes that the scatterers act independently, thus preserving a simple relation-

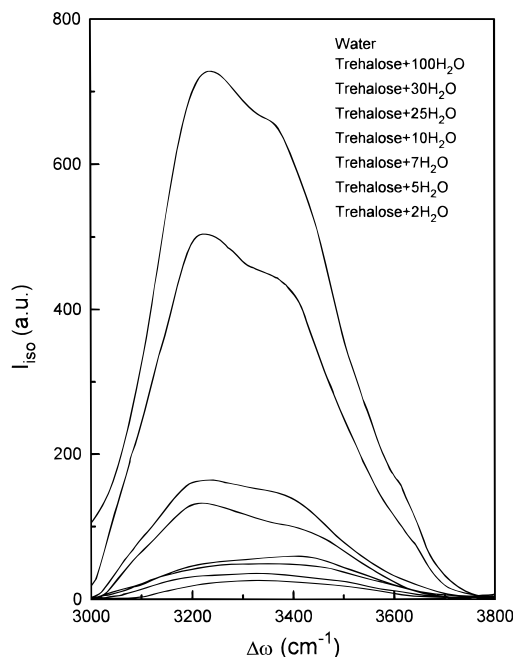
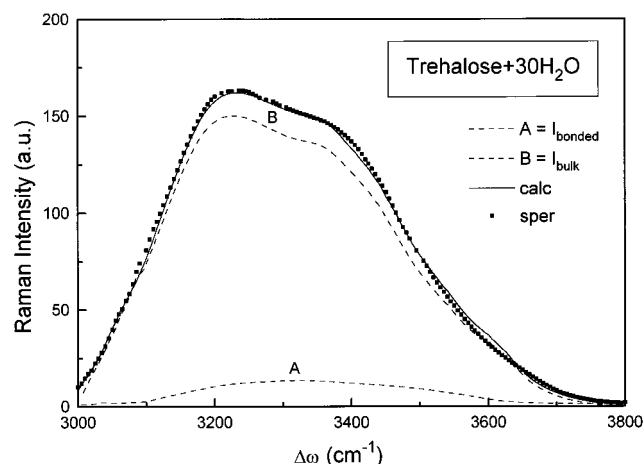
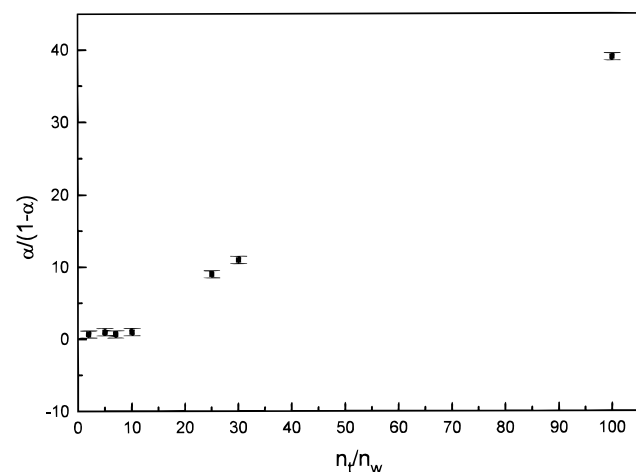


Figure 2. Isotropic spectra,  $I_{\text{iso}}(\omega)$ , of the O—H stretching band of trehalose aqueous solutions with different water contents at  $T = 293$  K.

ship between the number of scatterers and the scattering intensity. This is clearly an assumption of convenience and is in fact inconsistent with the concept of a collective mode. Thus, when examining the results of this analysis, we will be aware of the weakness of this assumption.<sup>22</sup> Hypothesizing that the relative low pure-trehalose contribution to the O—H stretching band does not appreciably change in the presence of water and provided that the pure trehalose stretching band is subtracted from the isotropic spectra of the aqueous solutions, the spectral profiles, at a given temperature, can be reproduced by a suitable combination of two contributions: the spectrum of bulk water,  $I_{\text{bulk}}(\omega, T)$ , and that of the water *bonded* to the trehalose,  $I_{\text{bonded}}$



**Figure 3.** Isotropic Raman spectrum of trehalose + 30 H<sub>2</sub>O at  $T = 293$  K, together with the component contributions,  $I_{\text{bonded}}(\omega)$  and  $I_{\text{bulk}}(\omega)$ , and the resulting spectrum.



**Figure 4.** Ratio of the *free* to *bond* O–H group percentages,  $\alpha(T)/[1 - \alpha(T)]$ , as a function of water content.

( $\omega$ ,  $T$ ), obtained subtracting the spectrum of pure trehalose from the one of trehalose + 2H<sub>2</sub>O. At this concentration, in fact, all the water molecules are supposed to be strongly bonded to the trehalose in entire temperature range, as indicated by adiabatic compressibility measurements.<sup>12,17</sup> This is illustrated by the formula

$$I_{\text{iso}}(\omega, T) = \alpha(T) I_{\text{bonded}}(\omega, T) + [1 - \alpha(T)] I_{\text{bulk}}(\omega, T) \quad (3)$$

where  $\alpha(T)$  and  $[1 - \alpha(T)]$  are the percentages of the bonded and free OH groups, respectively. The fitting of the solution spectral profiles, performed as a function of concentration and temperature by means of eq 3, allows the evaluation of the weights of the component spectra, namely  $\alpha(T)$  and  $[1 - \alpha(T)]$ . In Figure 3, as an example, the spectrum of trehalose + 30 H<sub>2</sub>O is shown at  $T = 293$  K together with the component contributions and the resulting spectrum. From the ratio of the *free* to *bond* O–H group percentages,  $\alpha(T)/[1 - \alpha(T)]$ , reported in Figure 4, the Raman hydration number can be evaluated from the crossover between the two regimes. Despite the simplicity of the model, the trehalose hydration number,  $n_{\text{H}}$ , equals 12, remaining almost constant with temperature, in excellent agreement with the behavior obtained by adiabatic compressibility measurements and by MD simulation.<sup>7,12,17</sup>

To obtain information on the effects of trehalose on the hydrogen-bonded network of water, we now analyze differently

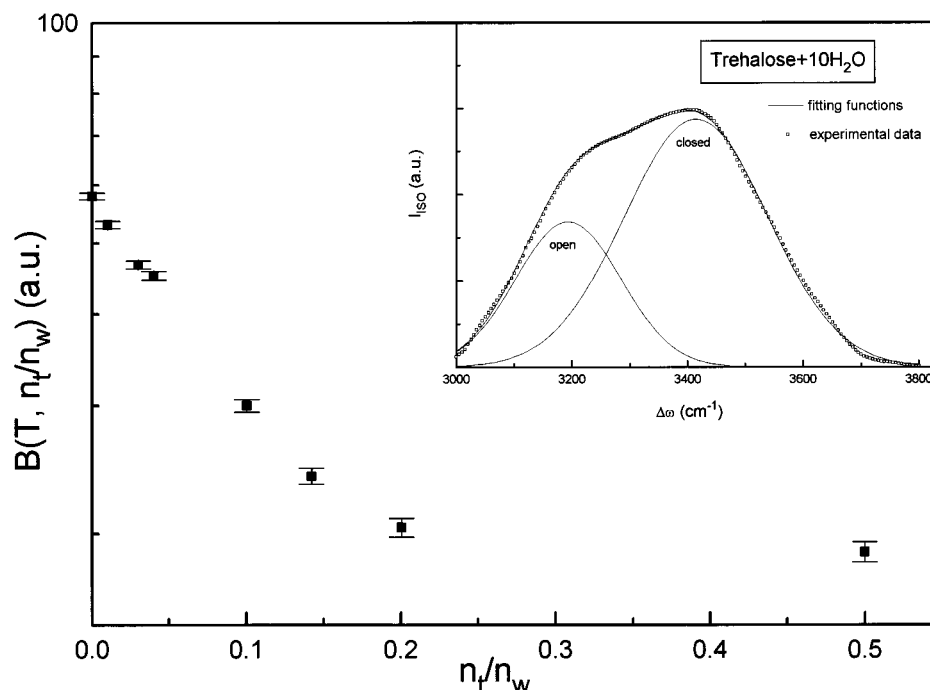
the intramolecular O–H stretching spectral region.<sup>23</sup> It is a fact<sup>20</sup> that the O–H stretching in water, due to the presence of the hydrogen bond, shows a red shift and a large broadening with respect to the gas-phase vibrational band centered at  $\omega \approx 3600$  cm<sup>-1</sup>. Its spectral shape can be related to the distribution of H-bonds and is determined by the distribution of the forces acting on the internal degrees of freedom, causing a shift and an inhomogeneous broadening due to the anharmonicity of the oscillators.

The existence of an isosbestic point in the isotropic spectrum of pure water has been explained in terms of two competitive classes of OH oscillators with opposite temperature dependences, and some authors<sup>23</sup> have suggested the decomposition of each spectrum into an “open” and a “closed” contribution. The first one has been attributed to the O–H vibration in tetrabonded H<sub>2</sub>O molecules that have an “intact bond” and originate low-density patches in the system (stretched water), while the second one would correspond to the O–H vibration of H<sub>2</sub>O molecules that have a not fully developed hydrogen bond (distorted bond). The open Gaussian component is centered near 3210 cm<sup>-1</sup>, while the closed component is centered near 3420 cm<sup>-1</sup>.<sup>24</sup> With declining temperature, one finds that the “open” contribution increases, indicating an enhanced hydrogen bonding, which gives rise to patches of four bonded water molecules. In the supercooled regime, however, a third phase, that of heterogeneous ice, occurs and disturbs the isobestic behavior.<sup>25</sup>

In the case of an aqueous sugar solution,<sup>26</sup> also because of the relatively low pure sugar contribution, such an approach has been demonstrated still valid. A remarkable observation resulting from an inspection of the isotropic Raman spectra of concentrated trehalose aqueous solutions is that no additional Gaussian components at higher frequencies were required to fit the O–H stretching contour;<sup>26</sup> that is, no components, whatever, near 3500 and 3620 cm<sup>-1</sup> were required at high concentration. The higher frequency components arise when hydrogen bonds are broken, and their absence in the spectra indicates that trehalose aqueous solutions are fully hydrogen-bonded. The result of the decomposition of each spectrum into an “open” and a “closed” contribution is reported in Figure 5, at  $T = 293$  K, which shows the fractional open band intensity,  $B(T, n_t/n_w) = \int I_{\text{iso}}^{\text{open}}(\omega) d\omega / \int I_{\text{iso}}^{\text{tot}}(\omega) d\omega$ , as a function of  $n_t/n_w$ ,  $n_t$  and  $n_w$  being the mole numbers of trehalose and water, respectively. In the insert we show, as an example, the decomposition of the spectrum of trehalose + 10 H<sub>2</sub>O. The most noteworthy feature is that, by decreasing the water content, one observes a marked decrease of the integrated intensity  $B(T, n_t/n_w)$ , which tends to a plateau value for  $n_t/n_w \leq 10$ . These findings are consistent with the picture of a trehalose destructuring effect on the H<sub>2</sub>O tetrabonded network that forms low-density conformations similar to that of supercooled water.

Our analysis of the O–H stretching contribution also includes the spectral stripping procedure of the polarized O–H stretching band suggested by Green, Lacey, and Sceats<sup>27</sup> (GLS) for obtaining information on the extension of the bond–bond correlation. More specifically, GLS proposed that the low-frequency shoulder in the  $I_{\text{VV}}$  spectrum of H<sub>2</sub>O arises from collective motions of the O–H bonds. The collective band is essentially that part of the spectrum characterized by a depolarization ratio which differs from that of uncoupled OH oscillators. Coupling of OH oscillators gives rise to a strongly polarized band on the low-frequency edge of the spectrum. To obtain the relative intensity of this mode, it is assumed that the  $I_{\text{VH}}$  spectrum is basically like the scaled-down version of the





**Figure 5.** Behavior of the normalized integrated area of the open contribution  $B(T, n_t/n_w)$  as a function of  $n_t/n_w$ . In the insert, the decomposition of the isotropic spectrum of trehalose + 10 H<sub>2</sub>O into an “open” and a “closed” contribution is reported.

$I_{VV}$  spectrum without the collective band  $I_c$ ; then  $I_c$  can be obtained by subtracting  $I_{VH}$  from  $I_{VV}$ . For this procedure,  $I_{VH}$  has to be multiplied by a factor  $a$  and shifted slightly on the frequency scale by a parameter  $\delta$ :

$$I_c(\omega, T, \delta) = I_{VV}(\omega, T) - aI_{VH}(\omega - \delta, T) \quad (4)$$

Therefore, the  $I_{VH}(\omega)$  spectra are scaled with  $a$  and by a frequency shift  $\delta$  before subtraction from  $I_{VV}(\omega)$ .  $\delta$  was chosen to best symmetrize  $I_c$  prior to its reflection symmetrization. The spectrum was then refined to remove residual structures at high frequency by assuming that the band is symmetrical about the peak and using the low-frequency line shape to define the entire line shape. After this stripping procedure, the fractional collective band intensity,  $C(T, n_t/n_w) = \int I_c(\omega) d\omega / \int I_{VV}(\omega) d\omega$  in pure water, was shown to increase linearly with falling temperature and to approach the value of ice at the singularity temperature of water ( $T_s = 218$  K). GLS have conjectured that this collective band arises from the collective in-phase stretching motion of the water molecules in the fully bonded tetrahedral network, to which liquid water tends as it is supercooled. The band appears as a result of the large net polarizability change of this vibrational mode. The tetrahedral nature of the bonding is such that the appearance of this band is strongly influenced by the intermolecular coupling of OH oscillators rather than the intramolecular coupling. Other studies on H<sub>2</sub>O/D<sub>2</sub>O mixtures<sup>25</sup> and on aqueous solutions<sup>28</sup> have shown that the presence of “defects”, broadly identifiable as broken hydrogen bonds, reduces the collective band intensity.

The result of the decomposition of all the spectra is reported in Figure 6, which shows the integrated intensity  $C(T, n_t/n_w)$  as a function of  $n_t/n_w$ . In the insert is shown the result of such a stripping procedure at  $T = 293$  K for all concentration values investigated. The behavior of the relative strength of the in-phase collective spectrum, closely mimics the one obtained by following the previous spectral decomposition, supporting, once more, the fact that trehalose promotes a destructuring effect on the tetrahedral H-bond network of pure water, typical of H<sub>2</sub>O

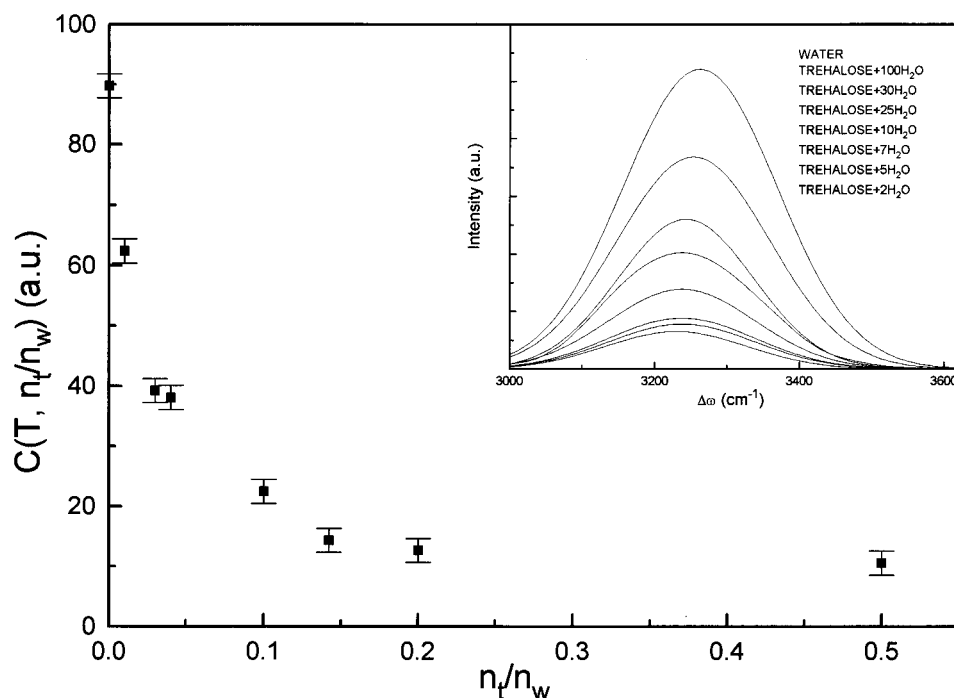
ice. As a result,<sup>28</sup> the amount of freezable water is reduced and the crystallization process is hindered.

As far as the intramolecular bending mode is concerned, Figure 7 shows, as an example, the Raman spectra relative to the spectral region within  $1500 \div 1800$  cm<sup>-1</sup> of trehalose aqueous solutions at different concentration values at  $T = 293$  K. The relative area of this spectral contributions scales with the water content, not evidencing any anomalous behavior of these intramolecular modes due to the presence of trehalose.

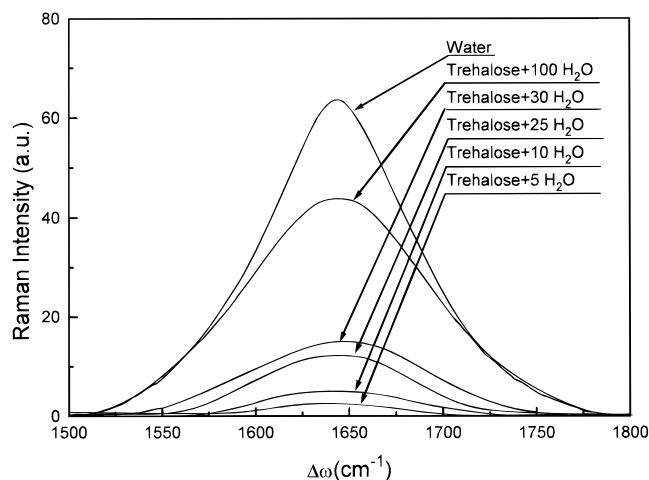
**(ii) The Intermolecular Region.** The intermolecular region, which has a collective character involving second and third nearest-neighbor interactions, reveals strong modifications in the Raman spectral bands of water. It is well-known<sup>26</sup> that the 175 cm<sup>-1</sup> vibration is thought to arise from the restricted translation of two H<sub>2</sub>O molecules against each other along the OH...O parallel direction, whereas the 60 cm<sup>-1</sup> vibration is attributed to the perpendicular restricted translations, e.g., the H-bond intermolecular bending mode. The latter has the advantage over the stretching mode in that it reflects oscillations of the water molecule in the potential well determined by several neighboring bonds, rather than just one, but it is quite difficult to determine accurately the shape of this band contour which, in water, is centered at  $\omega \cong 60$  cm<sup>-1</sup>, even at very low trehalose content. In particular, we observe that the addition of trehalose to water gives rise to the disappearance of the latter spectral contribution.

Figure 8 shows Raman density of states  $g(\omega) = I(\omega)/[1 + n(\omega)]$  at various water contents. It clearly emerges that the addition of trehalose rapidly destroys the intermolecular network of water. In fact, upon an increase in the water content, the area of the intermolecular contribution is almost negligible when the number of water molecules for each trehalose molecule is less than 12 and rapidly increases for  $n_w > 12$ .

The peculiar trend of the intramolecular and intermolecular modes suggests that trehalose promotes an extensive layer of structured water around its neighborhood, which destroys the tetrahedral H-bond network of pure water. Trehalose, in fact,



**Figure 6.** Behavior of the normalized integrated area of the collective contribution  $C(T, n_t/n_w)$  as a function of  $n_t/n_w$ . In the insert, the collective bands at various water contents are reported.



**Figure 7.** Raman spectra of the intramolecular bending mode at various concentration values.

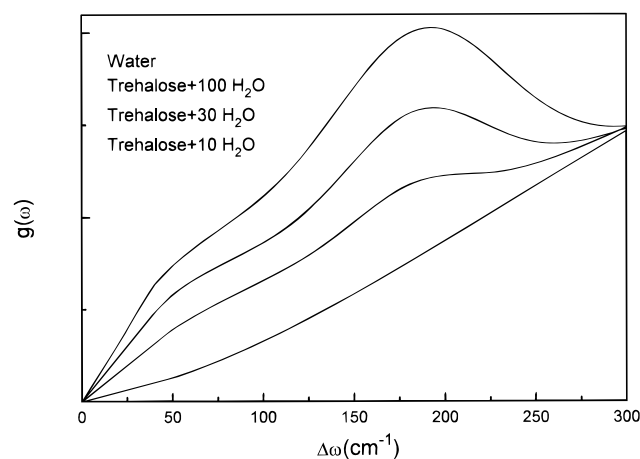
possesses a hydrophilic surface which could give rise to a strongly bound and oriented first layer of water molecules. This could induce orientation of a second layer, which likewise could influence the third, and so on. These results agree with QENS data which indicate that trehalose also affects significantly the dynamics of water molecules in its neighborhood, modifying, at low concentration, the network imposed by H-bonds.

In the context of the previous remarks, it clearly emerges that the trehalose effect on water is to provide a structure which enables spatial and orientation positions that are not compatible with those of ice.

### Concluding Remarks

The main purpose of this work is to show how Raman spectroscopy is a powerful tool to probe water structure in trehalose–H<sub>2</sub>O mixtures.

First, from the analysis of the O–H stretching contribution, we obtain information on the concentration of the bonded and



**Figure 8.** Raman density of states  $g(\omega) = I(\omega)/[1 + n(\omega)]$  at various water contents.

unbonded OH units and, in turn, on the hydration number of trehalose and on its temperature dependence. The extracted values are in excellent agreement with previous determinations by adiabatic compressibility measurements and by MD simulation.<sup>7,12</sup> Second, we compare different analysis criteria of this intramolecular spectral contribution, by hypothesizing a distribution of “open” and “closed” configurational arrangements in the reproduction of the isotropic spectra or a collective contribution similar to that of ice in the polarized spectra. More specifically, the low-frequency shoulder of the O–H stretching Raman spectrum is developed as a probe of in-phase collective motions in water. Both procedures indicate that the addition of trehalose tends to destroy the configurational arrangement of water, which would give rise, by cooling, to ice. In the presence of trehalose, substantial changes take place also in the intermolecular spectral bands characteristic of pure water: intermolecular O–H···O bending and stretching modes tend to disappear in agreement with the collective contribution of the O–H intramolecular stretching mode, thus denoting the destruction of the intermolecular network of pure water.

Considered together, these results support the hypothesis of a great ability of trehalose, at low concentration, to reduce the amount of those H-bond configurations which, on decreasing temperature, promote the formation of ice.

## References and Notes

- (1) Vegis, A. *Annu. Rev. Plant Physiol.* **1964**, *15*, 185.
- (2) Sussman A. S.; Halvorson, H. O. *Spores: Their Dormancy and Germination*; Harper & Row: New York, 1966.
- (3) Clegg, J. S. *Comp. Biochem. Physiol.* **1967**, *20*, 8.
- (4) Crowe J. H.; Crowe, L. M. In *Biological Membranes*; Chapman, D., Ed.; Academic Press: New York, 1984; Vol. 5, p 57.
- (5) Green J. L.; Angell, C. A. *J. Phys. Chem.* **1989**, *93*, 2880.
- (6) Angell, C. A. *Hydrogen-Bonded Liquids*; NATO ASI Series, Vol. 329; Kluwer: Dordrecht, The Netherlands, 1991; p 59.
- (7) Donnamaria, M. C.; Howard, E. I.; Grigera, J. R. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 2731.
- (8) Leslie, S. B.; Israeli, E.; Lighthart, B.; Crowe, J. H.; Crowe, L. M. *Appl. Environ. Microbiol.* **1995**, *61*, 3592.
- (9) Fox, K. C. *Science* **1995**, *267*, 1922.
- (10) Chan, R. K.; Pathmanathan, K.; Johari, G. P. *J. Phys. Chem.* **1986**, *90*, 6358.
- (11) Crowe, L. M.; Reid, D. S.; Crowe, J. H. *Biophys. J.* **1996**, *71*, 2087.
- (12) Magazù, S.; Majolino, D.; Middendorf, H. D.; Migliardo, P.; Musolino, A. M.; Sciortino, M. T.; Wanderlingh, U. *Biol. Macromol. Dyn., Proc. Workshop Inelastic Quasielastic Neutron Scattering Biol.*, 1996 **1997**, 155.
- (13) Geiger, A.; Mausbach, P.; Schnitker, J. *Water and Aqueous Solutions*; Series IV; Adam Hilger: Bristol, U.K., 1986; p 15.
- (14) Liu, Q.; Schmidt, R. K.; Teo, B.; Karplus, P. A.; Brady, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 785.
- (15) Magazù, S.; Migliardo, P.; Musolino, A. M.; Sciortino, M. T. *J. Phys. Chem. B* **1997**, *101*, 2348.
- (16) Magazù, S.; Maisano, G.; Middendorf, H. D.; Migliardo, P.; Musolino, A. M.; Villari, V. *J. Phys. Chem. B* **1998**, *102*, 2060.
- (17) Magazù, S.; Maisano, G.; Middendorf, H. D.; Migliardo, P.; Villari, V. *J. Chem. Phys.* **1998**, 1170.
- (18) Magazù, S.; Maisano, G.; Majolino, D.; Migliardo, P.; Musolino, A. M.; Villari, V. *Prog. Theor. Phys. Suppl.* **1997**, *126*, 195.
- (19) Volino, F. In *Spectroscopy Methods for the Study of Local Dynamics in Polyatomic Fluids*; Dupuy, J., Dianoux, A. J., Eds.; NATO ASI Series B, Vol. 33; Plenum Press: New York, 1978.
- (20) Magazù, S.; Maisano, G.; Majolino, D.; Migliardo, P. *Proceedings of the 12th International Conference on the Properties of Water and Steam. Physical Chemistry of Aqueous Systems: Meeting the Needs of Industry*; Begell House: New York, Wallingford, U.K., 1995; p 317.
- (21) Crupi, V.; Jannelli, M. P.; Magazù, S.; Maisano, G.; Majolino, D.; Migliardo, P.; Ponterio, R. *J. Mol. Struct.* **1996**, *381*, 207.
- (22) Hare, D. E.; Sorensen, C. M. *J. Chem. Phys.* **1990**, *93*, 25.
- (23) D'Arrigo, G.; Maisano, G.; Mallamace, F.; Migliardo, P.; Wanderlingh, F. *J. Chem. Phys.* **1981**, *75*, 4264.
- (24) Walrafen, G. E. *The Physics and Physical Chemistry of Water; Water: A Comprehensive Treatise*, Vol. 1; Plenum: New York, 1972; Chapter 5, p 151.
- (25) Karger, N.; Sceats, M. G.; Lüdemann, H. D. *Proceedings of the 12th International Conference on the Properties of Water and Steam. Physical Chemistry of Aqueous Systems: Meeting the Needs of Industry*; Begell House: New York, Wallingford, U.K., 1995; p 386.
- (26) Walrafen, G. E.; Hokmabadi, M. S.; Chu, Y. C. *Hydrogen Bonded Liquids*; NATO ASI Series, C: Mathematical and Physical Sciences, Vol. 329; Kluwer: Dordrecht, The Netherlands, 1989; p 261.
- (27) Green, J. L.; Lacey, A. R.; Sceats, M. G. *J. Phys. Chem.* **1986**, *90*, 3958.
- (28) Green, J. L.; Lacey, A. R.; Sceats, M. G. *Chem. Phys. Lett.* **1986**, *130*, 67.