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Rotational dynamics of trehalose in aqueous solutions studied by depolarized light scattering

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High resolution depolarized light scattering spectra, extended from 0.5 to 2×10^4 GHz by the combined used of a dispersive and an interferometric setup, give evidence of separated solute and solvent dynamics in diluted trehalose aqueous solutions. The slow relaxation process, located in the gigahertz frequency region, is analyzed as a function of temperature and concentration and assigned to the rotational diffusion of the sugar molecule. The results are discussed in comparison with the data obtained on glucose solutions and they are used to clarify the molecular origin of some among the several relaxation processes reported in literature for oligosaccharides solutions. The concentration dependence of relaxation time and of shear viscosity are also discussed, suggesting that the main effect of carbohydrate molecules on the structural relaxation of diluted aqueous solutions is the perturbation induced on the dynamics of the first hydration shell of each solute molecule. © *2010 American Institute of Physics*. [doi:10.1063/1.3430555]

I. INTRODUCTION

Carbohydrates constitute the main part of the organic substance on Earth and play a fundamental role in many processes of biological relevance, such as the organization and function of living organisms. Nowadays, the properties of carbohydrates in aqueous solutions are the object of increasing attention. In particular, simple sugars are considered as a useful model to study the interplay between solute and solvent dynamics and to provide important insight into the behavior of more complex biological molecules. Moreover, the structural and dynamical characteristics of sugars-water solutions are themselves very intriguing, being strongly connected with their exceptional bioprotective properties. Among sugars, the disaccharide trehalose, constituted of two glucose rings linked trough an $\alpha,\alpha-(1\rightarrow1)$-glycosidic bond, possesses the most effective conservative capability toward a variety of biomolecules.[1] This disaccharide is also an effective stabilizer of biological components in aqueous solutions, such as proteins and membranes, and is widely employed in the pharmaceutical industries for the long time storage of labile biochemicals.[2]

Even if extensive studies have been devoted to reach a molecular-level understanding of the origin of trehalose bioprotective capabilities, none of the formulated hypothesis is per *se* adequate to account for its superior effectiveness. However, generally speaking, it is no doubt that the stabilizing effect of carbohydrates toward biomolecules is strongly connected with the dynamical properties of the sugar matrix and the perturbation induced by sugars on the surrounding water molecules. In this respect, it was proven that trehalose can form long-lived hydrogen bonds with water, yielding to an appreciable slowing down of the dynamics of the water molecules located in its first solvation shell.[3–5] Therefore, the characterization of both solute and solvent relaxation processes is fundamental to draw an exhaustive description of the solution dynamics. Nonetheless, the possibility to distinguish the sugar relaxation modes from the water ones is not a trivial task.

It is known that carbohydrates in the aqueous medium originate a number of different relaxation processes which span a very wide temporal window. For example, conformational rearrangements take place over the microseconds time domain,[6–9] while single molecule reorientational motions occur in hundreds of picoseconds.[5,10,11] Concerning the trehalose aqueous solutions, such complex dynamical behavior was investigated using different techniques. In particular, ultrasonic and acoustical measurements were employed to study the dynamics of intramolecular processes[6–9,12] and of the structural relaxation,[13] while the translational diffusion of the sugar molecule was observed by means of photon correlation spectroscopy,[14] quasielastic neutron scattering (QENS),[15–17] and nuclear magnetic resonance (NMR).[18] Analogously to the case of glucose,[19–23] the dielectric spectra of trehalose solutions merely show the presence of a single relaxation term, which occurs in tens of picoseconds, and no evidence of the sugar rotational diffusion was found.[24] The observed relaxation was assigned to a local process related to the hydration dynamics.[24] Summarizing to the best of our knowledge, the diffusive rotational motion of trehalose molecules was detected by means of NMR (Ref. 25) and QENS experiments,[17] the two techniques giving discordant results.

Recently, depolarized light scattering (DLS) spectra of glucose aqueous solutions were performed in our laboratories. This study pointed out the presence of a low frequency

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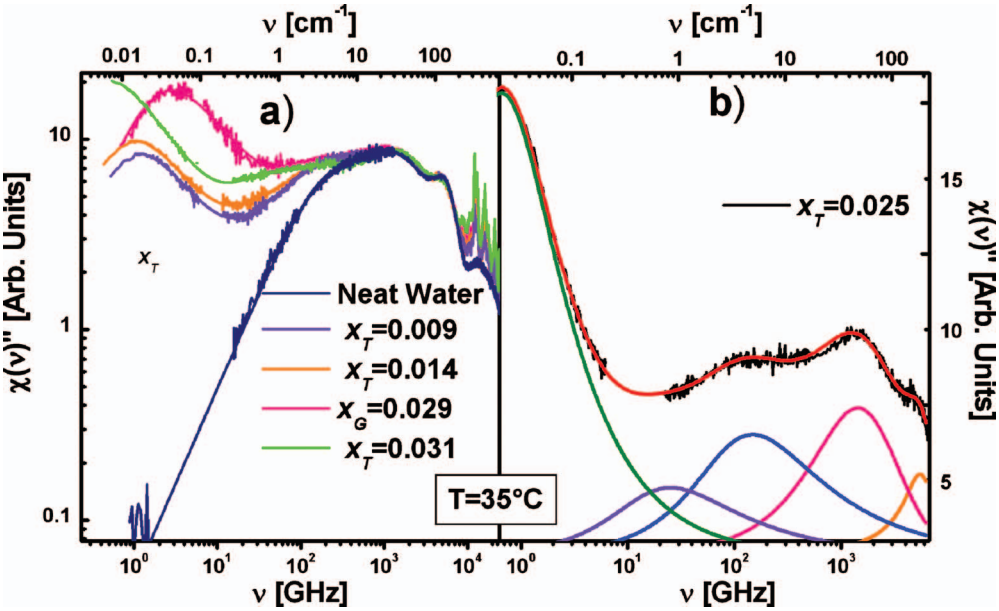

FIG. 1. (a) Imaginary part of the susceptibility $\chi''(\nu)$ for neat water and aqueous solutions of glucose and trehalose at different molar fractions, recorded at 35 °C. Experimental data, reported together with the corresponding fitting curves, have been arbitrary normalized on the maximum of the band located at ca. 5100 GHz, which is assigned to the H-bond bending mode of water molecules. (b) Imaginary part of the susceptibility $\chi''(\nu)$ of a trehalose-water solution with $x_T=0.025$ at 35 °C. Experimental data (black line) are reported together with the fitting curve (red line) and the single components: trehalose relaxation mode (green line), water slow relaxation mode (purple line), water fast relaxation mode (blue line), water bending (pink line), and stretching (orange line) resonant modes.

contribution, which is clearly distinguished from the characteristic signals due to the solvent relaxation modes.[26] Afterwards, the achievement of high resolution DLS spectra, extended in a frequency range that goes from 0.5 to 2×10^4 GHz by the combined used of a dispersive and an interferometric setup, gave the first experimental evidence of separate solute and solvent dynamics.[11] High resolution DLS spectra were performed also on trehalose-water solutions,[5] where the contribution related to the solute reorientational motions could be clearly envisaged and discriminated from the solvent typical relaxation modes.

Here, high resolution DLS spectra of trehalose-water solutions are examined at different temperatures and concentrations, focusing on the reorientational motion of the sugar molecule. The results are discussed in comparison with the findings we previously obtained on glucose-water solutions,[11] and compared with several relaxation processes reported in literature for oligosaccharides solutions.

II. EXPERIMENTAL SETUP

D-(+) -trehalose dihydrate was purchased from Fluka Sigma-Aldrich with a purity higher than 99.5%. The solutions were prepared by weight adding trehalose to doubly distilled and de-ionized water prepared in our laboratory; the complete dissolution of the sugar was assured by heating and stirring the samples. All the solutions, whose sugar mole fraction x_T is comprised in the range $0\leq x_T\leq0.031$, were freshly prepared and conditioned for more than 1 h before recording the measurements. Moreover, light scattering from dust particles was avoided purifying the samples through a cellulose 0.22 μm Millipore filter.

DLS measurements were performed on trehalose-water solutions at 25, 35, and 45 °C; furthermore, the solution with $x_T=0.0198$ was analyzed from 7 to 47 °C. All the samples were placed in a 10 mm path quartz cuvette. The DLS technique, usually limited at low frequency by the finite resolution of commercial monochromators which is rarely better than 30 GHz, is here considerably extended at lower frequencies, down to 0.5 GHz, by use of an interferometer. In fact, the low frequency region from 0.01 to 7 cm^{-1} (0.5–200 GHz) was analyzed by a Sandercock-type (3+3)-pass tandem Fabry–Pèrot interferometer, characterized by a finesse of about 100 and a contrast $>5\times10^{10}$. High frequency measurements were recorded in the $1\div1000$ cm^{-1} frequency range using an ISA Jobin-Yvon model U1000 double monochromator having 1 m focal length with holographic gratings. Low and high frequency spectra were spliced once dark count was subtracted, taking advantage of an overlap of about half a decade in frequency. Further, the susceptibility spectra were calculated, as the ratio between depolarized spectra $I_{VH}(\nu)$ and the Bose–Einstein occupation $n(\nu)=[\exp(\beta)-1]^{-1}$, with $\beta=h\nu/k_BT$. More details on the experimental technique can be found in Ref. 27.

III. RESULTS AND DISCUSSION

Figure 1(a) shows typical DLS spectra of neat water, of a glucose aqueous solution, and of three trehalose solutions at different concentrations (hereafter, the sugar mole fraction is indicated as x_G and x_T in the case of glucose and trehalose, respectively). It can be observed that, in the presence of sugar, a new spectral feature appears in the low gigahertz domain and it is well separated from the region where the characteristic solvent relaxation processes occur. Analogously to the findings reported in the case of glucose,[11] the intensity of this relaxation term increases with the trehalose content, while its position progressively shifts to lower fre-

quencies. Such a concentration dependence suggests that this contribution is originated by the sugar molecules and, owing to the sensitivity of DLS measurements toward the reorientational degrees of freedom of optically anisotropic molecules, it can be reasonably attributed to the rotational motion of the solute. Also notice that in trehalose-water solutions, this component is located at lower frequencies with respect to glucose-water solutions,[11] as expected for the rotation of a larger molecule.

In order to quantify the observed results, a full spectrum analysis was applied, modeling the susceptibility $\chi''(\omega)$ as the sum of five contributions. As shown in the example of Fig. 1(b), three Cole–Davidson functions were used, the one located at lower frequencies describes the sugar dynamics, while the components centered at about 25 and 140 GHz are associated to the relaxation process of hydration and bulk water molecules,[5] respectively. Finally, two damped harmonic oscillator functions were used to represent the typical resonant modes of water molecules, located around 1800 and 5100 GHz (i.e., 60 and 170 cm^{-1}).[28–30] Following this model, one can write

$$\chi''(\omega) = \mathrm{Im}\left\{ -\frac{\Delta_T}{[1+i\omega\tau_T]^{\beta_T}} - \frac{\Delta_{\mathrm{slow}}}{[1+i\omega\tau_{\mathrm{slow}}]^{\beta_{\mathrm{slow}}}} - \frac{\Delta_{\mathrm{fast}}}{[1+i\omega\tau_{\mathrm{fast}}]^{\beta_{\mathrm{fast}}}} + \frac{\Delta_b\omega_b^2}{\omega^2-\omega_b^2-i\omega\Gamma_b} + \frac{\Delta_s\omega_s^2}{\omega^2-\omega_s^2-i\omega\Gamma_s} \right\}, \quad (1)$$

where Δ is the relative amplitude of each term; τ_T, β_T, τ_{slow} and β_{slow}, and τ_{fast}, β_{fast} are the characteristic times and stretching parameters of the sugar relaxation term and the two relaxation processes of water, respectively. ω_b, ω_s, Γ_b, and Γ_s are the frequencies and the widths of the intermolecular bending and stretching modes of water, respectively. In Fig. 1(b), a typical spectrum is reported together with the five components resulting from the fitting procedure. Thanks to the negligible overlapping between solute and solvent relaxation bands, which lie more than two decades apart one from the other, the fit of the sugar contribution was proved to be independent from the number and shape of the fitting functions used to reproduce the spectrum at higher frequencies. The results obtained for the high frequency part of DLS spectra, relative to the solvent relaxation modes, were discussed in a previous work.[5]

Concerning the trehalose relaxation process, the stretching parameter was found to be almost constant (β_T=0.8) in the whole temperature and concentration range under investigation. The concentration dependence of the average relaxation time $\langle\tau_T\rangle = \tau_T \cdot \beta_T$ is reported in Fig. 2 at three different temperatures. The exponential raise in $\langle\tau_T\rangle$ as a function of x_T recalls our previous findings on glucose-water solutions[11]

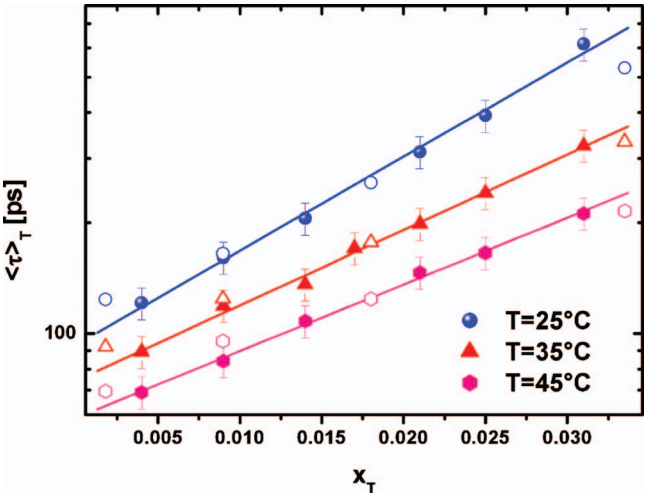

FIG. 2. Trehalose average relaxation time $\langle\tau\rangle_T$ vs the sugar molar fraction x_T at 25 (blue spheres), 35 (red triangles), and 45 °C (pink hexagons) is reported together with sucrose rotational correlation times extrapolated from NMR data (Ref. 35) at 25 (empty circles), 35 (empty triangles), and 45 °C (empty hexagons). Continuous lines are guides for the eyes.

and, analogously, it can be related to the exponential growth of the viscosity observed with increasing the sugar content.[15] The Arrhenius behavior is a very common feature in the dynamics of solutions and is usually associated to the Brownian diffusion of the solute into the solvent. Consistently, the low frequency relaxation in our spectra can be interpreted in terms of the rotational diffusion of the trehalose molecules in a medium of increasing viscosity. In this framework, it must be recalled that the DLS technique detects the collective reorientational time τ, which is related to the single particle correlation time by the relation $\tau = (g_2/j_2)\tau_s$, being g_2 and j_2 the static and dynamic correlation parameter, respectively. Considering that in the high dilution limit, the ratio g_2/j_2 tends to unit, DLS can directly measure the single particle correlation time. As a result, at least in the low concentration range, the detected relaxation time can be described in terms of the Stokes–Einstein–Debye equation, which relates the single particle correlation time to the viscosity of the solution

$$\tau_s = V_h\eta/(k_BT), \quad (2)$$

where $V_h = Vf$ is the hydrodynamic volume, V is the volume of the rotating molecule, f is a rotational friction coefficient dependent on the molecular shape and on the hydrodynamic boundary conditions, and η is the viscosity of the solution.[31]

On the basis of Eq. (2), the trehalose hydrodynamic volumes were calculated for the most diluted sugar molar fraction (x_T=0.004) and V_h values of 460 ± 20, 440 ± 20, and 430 ± 20 Å^3 were obtained at 25, 35, and 45 °C, respectively. In order to better verify the validity of Eq. (2), the temperature dependence of $\langle\tau_T\rangle$ was investigated for the solution x_T=0.0198. The corresponding Stokes–Einstein–Debye plot is reported in Fig. 3, where one can observe that the expected linear relation is verified, giving a hydrodynamic volume $V_h = 390 \pm 20$ Å^3. On the whole, these results suggest the hydrodynamic volume of trehalose to be around V_h=430 Å^3 with an uncertainty of about 30 Å^3.

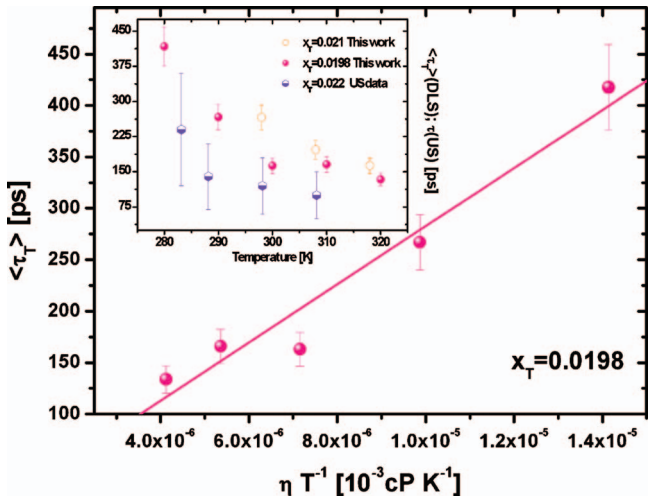

FIG. 3. Stokes–Einstein–Debye plot for a trehalose-water solution with sugar molar fraction x_T=0.0198. Experimental data are reported together with the corresponding linear fit. In the inset, the trehalose average relaxation time $\langle\tau\rangle_T$ is plotted as a function of temperature for x_T=0.0198 and 0.021, together with the relaxation time revealed from the ultrasonic spectra of a solution at x_T=0.022 (Ref. 8).

To estimate the value of the friction coefficient f of trehalose, one has to consider that owing to the complex molecular structure of trehalose and to its conformational flexibility, the disaccharide molecule can be hardly approximated to a symmetric top rotator. Furthermore, the need of a Cole–Davidson function to reproduce the sugar relaxation peak accounts for a distribution of relaxation times, which is possibly related to the asymmetry of the disaccharide molecule. The trehalose moments of inertia were calculated from molecular dynamics simulations taking into account the average conformation of the molecule.[25] Within this description, the molecule can be described as a prolate spheroid with an axial ratio equal to 0.6. If stick boundary conditions are applied, as is usually done for hydrogen bonding liquids,[31] the value f =1.29 is found for the friction coefficient and, from Eq. (2), the trehalose molecular volume is V=330 Å^3. This value is very close to the estimations made by different methods: the van der Waals volume V_V=285 Å^3 was calculated considering groups contribution,[32] V_p=344 Å^3 was found from the partial molar volume,[33] and V_d=345 Å^3 from the crystal density. As a result, the assignment of the observed dynamics to the rotational diffusion of the single sugar molecule is confirmed by the reasonable estimate of the molecular volume. This result points out an interesting difference between the dynamics of sugar molecules in water and that of small charged molecules as, for example, simple ions,[34] where the solute rotational diffusion implies the simultaneous reorientation of the overall solvation shell. Different from this, water molecules in carbohydrate solutions remain in proximity of the solute for units of picoseconds, as suggested by the structural relaxation process also visible in our spectra,[5] so that the rotational motion of trehalose, occurring at longer times, is associated to the reorientation of the sole sugar molecule and its first hydration shell is destroyed and reformed several times during the period of one single rotation.

Our result is also in agreement with previous molecular dynamics simulation[10] data, which predicted a relaxation time for the rotational diffusion of trehalose in a high diluted solution comparable to that reported here. The rotational correlation times of the sugar were also obtained experimentally from NMR measurements[25] and, even if these data are affected by considerably high uncertainties, the resulting correlation times are of the same order of magnitude of ours. More recently, NMR experiments were also performed on sucrose-water solutions,[35] probing the disaccharide rotational correlation time at different temperatures and concentrations. In Fig. 2, it is possible to observe the excellent agreement existent between the trehalose average relaxation times and the sucrose rotational correlation times. This surprising accord can be related to the similarity of the molecular volumes for the two homologous disaccharides; in fact, the van der Waals volume of sucrose is only 3% smaller than that of trehalose.[32]

The trehalose rotational relaxation time τ_r was also determined from QENS experiments[17] performed on a dilute solution (x_T=0.005). The rotational time found at 35 °C (τ_r=12.4 ps) is considerably smaller than the relaxation time extrapolated at the same concentration from DLS data $\langle\tau\rangle_T$ =94 ps; this discrepancy, found also in the case of glucose solutions,[11] suggests that different microscopic mechanisms should stand at the origin of the observed relaxation processes.

The intramolecular dynamics of several monosaccharides and disaccharides in water was extensively investigated by means of ultrasound (US) experiments.[8,9] Interestingly, US spectra show the presence of a high frequency relaxation peak, which, in the case of disaccharides, is centered at ca. ~1 GHz. For monosaccharides, this high frequency relaxation process was tentatively assigned to the sugar dimerization mechanism. On the other hand, recent molecular dynamics simulations[36] predict that in the whole temperature and concentration ranges investigated (T=280÷320 K; x_T =0÷0.031), aggregation phenomena, such as dimerization processes, can be reasonably neglected. In fact, the trehalose hydration number N_h does not change appreciably and, also, the average number of intermolecular sugar-sugar hydrogen bonds formed by a molecule of trehalose is smaller than unit. Now, from the comparison with our data in the inset of Fig. 3, it can be seen that DLS and US relaxation times of trehalose-water solutions at similar concentrations as a function of the temperature are very close the one to the others, possibly suggesting that the two techniques may be sensitive to similar microscopic dynamics.

Once we have shown that the rotation of trehalose molecules is governed by the viscosity of the solution, we now focus our attention on the analysis of the relaxation time and viscosity as a function of the solute molar fraction since it gives important information on the hydration process. First of all, we notice that our experimental data in Fig. 2 follow the well established empirical relationship proposed by Arrhenius,[37] with an exponential dependence of viscosity on molecular fraction of the solute x

$$\log \eta/\eta_0 = \theta x, \tag{3}$$

where η_0 is the viscosity of pure water. From a different point of view, a similar equation was recently tested on

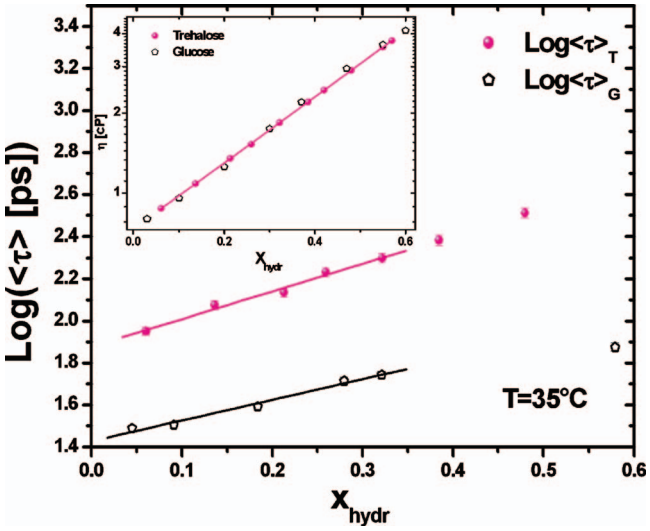

FIG. 4. The logarithm of trehalose (pink spheres) and glucose (empty pentagons) average relaxation times are reported as a function of hydration water mole fraction X_{hydr} together with the corresponding linear fit. In the inset, viscosity of trehalose-water (black spheres) and glucose-water (empty pentagons) solutions as a function of the hydration water mole fraction.

monosaccharides and disaccharides in Ref. 38. In that case, the increase in viscosity with concentration of the solute was attributed to an increase in the activation free energy of the solutions. Interestingly, the analogous of the parameter θ was found to be proportional to the sugar molecular weight. To this respect, we notice that the hydration number of carbohydrates is also proportional to the molecular weight, i.e., it roughly double on going from monosaccharides to disaccharides, so that the viscosity of carbohydrates can be directly related to the amount of hydration water rather than to an increase in activation energy. From a microscopic point of view, we suggest that in diluted solutions, the major effect of the addition of the solute to the viscosity of the solution comes from the perturbation induced on the first hydration layer of each solute molecule. To test this hypothesis, the inset of Fig. 4 shows the viscosities of glucose and trehalose solutions as a function of x_{hydr}, namely, the mole fraction of water molecules located in the solute first hydration shell with respect to the total amount of water. This quantity was calculated on the basis of the following relation: $x_{hydr}=N_h \cdot f_s$, where $f_s=n_s/n_W$ is the sugar mole ratio. The glucose hydration number $N_h=8$ was taken from molecular dynamics simulation data[3,39] while, in the case of trehalose, $N_h=15$ was derived from the analysis of water relaxation processes detected in the higher frequency region of DLS spectra,[5] its value being in agreement with the results of previous molecular dynamics simulation.[40] The perfect coincidence between the behavior of glucose and trehalose solutions strongly supports the idea that the contribution to viscosity of carbohydrates is related to the amount of hydration water, rather than to different activation energies.

In the same (Fig. 4), the glucose and trehalose average relaxation times are reported as a function of the molar fraction of hydration water x_{hydr}, showing an analogous Arrhenius dependence, underlined by the corresponding linear fits. The longer relaxation time of trehalose can be related to its larger hydrodynamic volume and the difference between the intercepts of the reported linear fits ($R=2.96$) matches with the ratio between the hydrodynamic volumes of the two sugars, i.e., 2.62, obtained from $V_h=164\pm8$ Å^3 (Ref. 11) and 440 ± 20 Å^3 at $x_G=0.005$ and $x_T=0.004$, respectively.

IV. CONCLUSIONS

One of the most challenging tasks in the study of the dynamics of aqueous solutions is to distinguish among the various relaxation processes associated with solute and solvent molecules. Here we demonstrate that differently from other techniques, high resolution DLS spectra give direct evidence for solute and solvent separated dynamics in trehalose-water solutions. As a result, the analysis of DLS spectra provides an exhaustive picture of the dynamical processes of the solutions, helpful to clarify the molecular origin of several relaxation processes reported in the literature. In particular, the relaxation term located between 0.5 and 5 GHz was studied as a function of temperature and concentration and attributed to the rotational diffusion of trehalose molecules, consistent with the results previously obtained for glucose solutions.[11] This picture is corroborated by the reasonable value of the molecular volume $V=330$ Å^3, obtained from the relaxation times through the Stokes–Einstein–Debye relation. The rotational diffusion of trehalose, once the effect of the molecular volume is properly taken into account, is comparable to that of the other oligosaccharides, as pointed out by the comparison with the rotational motions detected in glucose[11] and sucrose[25] solutions. To this respect, it is important to clarify how this interpretation can be compared with that more frequently present in literature where a strong influence of disaccharides, especially of trehalose, on the water structure emerges from spectroscopic techniques.[17,41] Our result indicates that the distinctive properties of trehalose as, for example, the strong perturbation of the H-bond network[18,42,43] or the stronger slowing down undergone by water molecules in the first solvation shell,[3–5,40] are expected to emerge only above a threshold concentration,[18,43] which is higher than the most concentrated solution studied here. Conversely, in the diluted condition, only a local perturbation of the water structure restricted to the first solvation shell occurs, as confirmed by recent models of terahertz response in polar liquids.[44] To this respect, here we showed that the viscosity of glucose and trehalose solutions falls on a single master plot if reported as a function of the fraction of proximal water. In other words, we suggest that in the diluted condition, the main effect of the addition of the solute to the viscosity of the solution comes from the perturbation of the dynamic properties of the first hydration layer, independent of the type of carbohydrate molecule.

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