Hydrogen bond dynamics and water structure in glucose-water solutions by depolarized Rayleigh scattering and low-frequency Raman spectroscopy

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The effect of glucose on the relaxation process of water at picosecond time scales has been investigated by depolarized Rayleigh scattering (DRS) experiments. The process is assigned to the fast hydrogen bonding dynamics of the water network. In DRS spectra this contribution can be safely separated from the slower relaxation process due to the sugar. The detected relaxation time is studied at different glucose concentrations and modeled considering bulk and hydrating water contributions. As a result, it is found that in diluted conditions the hydrogen bond lifetime of proximal water molecules becomes about three times slower than that of the bulk. The effect of the sugar on the hydrogen bond water structure is investigated by analyzing the low-frequency Raman (LFR) spectrum sensitive to intermolecular modes. The addition of glucose strongly reduces the intensity of the band at 170 cm^{-1} assigned to a collective stretching mode of water molecules arranged in cooperative tetrahedral domains. These findings indicate that proximal water molecules partially lose the tetrahedral ordering typical of the bulk leading to the formation of high density environments around the sugar. Thus the glucose imposes a new local order among water molecules localized in its hydration shell in which the hydrogen bond breaking dynamics is sensitively retarded. This work provides new experimental evidences that support recent molecular dynamics simulation and thermodynamics results. © *2007 American Institute of Physics*.
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I. INTRODUCTION

Carbohydrates are widely employed in biological and pharmaceutical technologies as stabilizers of biological systems in aqueous solutions, during freezing and drying processes.[1–4] Their protective properties are accounted for considering the different mechanisms arising from the physical conditions employed for their use. For amorphous samples in dehydrated states both the effectiveness in substituting water molecules in the protein hydration shell[3,4] and the ability to easily form glassy matrices have been considered.[5,6] Among other sugars, the particular conservative character of trehalose has been related to its capability to form stronger solute-solvent interactions and larger hydration shells.[7] Furthermore, its greater efficiency in breaking the tetrahedral H-bond network of water has been evidenced.[8] Conversely, preferential solute exclusion from the protein surface seems responsible for the stabilizing effect in aqueous solutions.[9,10] In this context the characterization on a molecular basis of the sugar-water mixtures is a fundamental first step towards the comprehension of the overall phenomenon. More generically, the properties of carbohydrates in aqueous solutions are of considerable significance due to the multiple roles played by this class of materials in diverse biological processes. Moreover, small sugars are relatively simple hydrophilic molecules that may be regarded as a convenient model of more complex biological systems for the study of basic structural and dynamical properties of aqueous media. A current topic of wide interest concerns the modifications induced by a hydrogen bonding solute on the intermolecular organization and mobility of water.[11]

Recently, in our laboratory the effect of the simple monosaccharide glucose on the hydrogen bond (H-bond) network of water has been analyzed by means of Raman and infrared (IR) spectroscopy. The main conclusions included the following:[12] (i) the addition of glucose reduces the number of water free OH groups (not involved in H bonds); (ii) glucose molecules strongly interact with water and the strength of water-glucose and water-water interactions are of similar extent; (iii) the glucose acts as a weak structure breaker on the tetrahedral network of water. This point is in line with Raman findings on other disaccharides[8] and is in qualitative agreement with recent molecular dynamics (MD) simulation results.[13] Conversely, the capability of glucose in enhancing the tetrahedral H-bond structure of water has been discussed by other researchers.[14] (iv) Yet, as previously evidenced,[15,16] the action of glucose on the organization of water is rather modest. The destructive effect on the tetrahedral water is partially compensated by the formation of new H bonds within the sugar hydration shell.[12] (v) Temperature induced spectral changes of the IR profiles reduce with increasing glucose concentration.[12] In terms of pseudochemical equilibrium between H-bonded and non-H-bonded species, this corresponds to a decrease of the average H-bond enthalpy within the system and may be connected to the reduction of energetic H bonds of cooperative tetrahedral water environments.

Considerable experimental efforts in the study of dy-

namical properties of glucose-water solutions involve depolarized light scattering,[17,18] NMR,[19] neutron scattering,[20,21] and dielectric[15,22–28] and acoustic investigations.[27–29] The main problem in drawing a univocal picture at a molecular level is that the dynamics occurs on several time scales involving numerous possible relaxation mechanisms. In addition it is challenging to compare the results obtained from various techniques intrinsically sensitive to different physical quantities,[30,31] and only few works have been devoted to the characterization of the short-time dynamics of the H-bond water rearrangements in these solutions.[17,18] This dynamics typically occurs on the picosecond time scales and suitable experiments are required to access these times.[11] Moreover, it is often not trivial to discriminate between solute and solvent relaxation processes.[22,23,26] Recently, using the depolarized light scattering technique, a distinct relaxation process due to the glucose reorientation has been clearly separated from the fast relaxation mode of water,[32] demonstrating the suitability of these experiments in separating solute and solvent dynamics.

On the other hands, a detailed microscopic description of H-bond features in glucose-water mixtures originates from computational works.[13,16,33–37] In particular, the effect of different sugars on the water dynamics has been reported in a recent MD study by Lee *et al.*[13] The authors showed that sugars restrict the translational and rotational mobilities of water molecules in the solute hydration layer. These hydrating water molecules partially lose the typical tetrahedral arrangement and form stable H bonds with retarded breaking dynamics. This latter point has been substantiated in a successive work on the hydration properties of lactose.[11]

In the present work we extend our previous structural investigations[12] to the study of relaxation properties in water-glucose solutions by means of depolarized light scattering experiments in the 0–1000 cm^{-1} range. Particular attention is addressed to the characterization of both structure and dynamics of the hydrogen bonding network of water in solution. The measured depolarized light scattering spectrum, which constitutes the frequency-domain counterpart of the time-resolved optical Kerr effect (OKE) signal, includes a water relaxation mode plus two resonant contributions at 50 and 170 cm^{-1}. The relaxation process is related to the fast H-bond rearrangement at picosecond time scales and may be described in terms of H-bond breaking mechanism.[38–43] The two resonant modes are usually assigned to damped bending (at 50 cm^{-1}) and stretching (at 170 cm^{-1}) intermolecular modes of H-bonded species and are sensitive to the overall water structure.[17,31,44–49] Thus, in principle, the depolarized light scattering spectroscopy may be used to analyze the influence of glucose on the H-bond dynamics of water and on its supramolecular organization. The main goal of the present study is to investigate this influence and to ultimately compare our achieved results with the latest theoretical findings.

II. EXPERIMENTAL SECTION

D-(+)-glucose, with a purity greater than 99.5%, was purchased from Fluka Sigma-Aldrich. Glucose aqueous solutions were prepared dissolving the sugar in doubly distilled and de-ionized water, at different sugar molar fractions (X_G) in the range $0.005 \leqslant X_G \leqslant 0.107$. All solutions were freshly prepared, filtered through millipore filters of 0.20 μm diameter pores to remove any dust and let sit for approximately 1 h prior to use, in order to permit the anomeric equilibrium to be established.

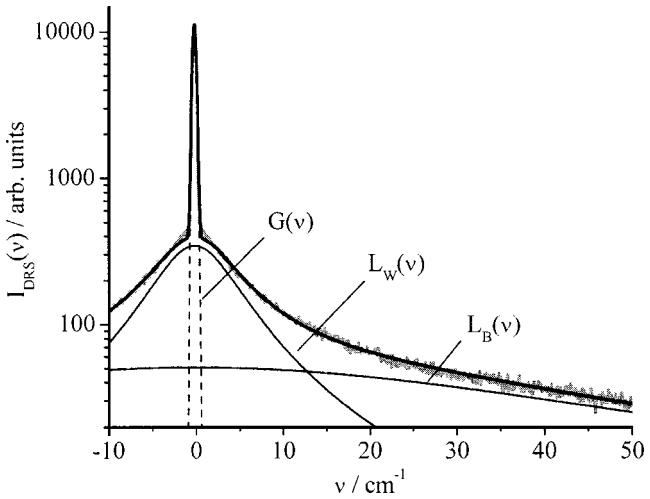

FIG. 1. DRS spectrum of water at 10 °C (gray line) together with the best fit result (thick black line) with the instrumental function $G(\nu)$ (dashed line) and the two Lorentzian components (thin black lines).

Depolarized light scattering measurements were performed using a Coherent-Innova Ar^+ laser operating in single-line excitation mode at λ_0=514.5 nm vertically polarized line as light source (typical power of ~500 mW). The horizontally polarized scattered light in 90° scattering geometry was dispersed by a U1000 ISA Jobin-Yvon double monochromator having 1 m focal length equipped holographic gratings and detected using a thermoelectrically cooled (−30 °C) Hamamatsu photomultiplier (model 943XX). The spectra were recorded in two different frequency regions: from −20 to 80 cm^{-1} with a resolution of 0.4 cm^{-1} and from 1 to 1000 cm^{-1} with a resolution of 2 cm^{-1}. For convenience the spectra obtained in the first region were referred as depolarized Rayleigh scattering (DRS) spectra and classically modeled[38–42] to describe water and glucose relaxation processes. The spectra obtained at higher frequencies, referred as low-frequency Raman (LFR) spectra, were reduced into the imaginary part of the dynamical susceptibility $\chi''(\nu)$ to enhance the intensity of the intermolecular resonant modes.[48] The temperature was controlled by circulating water from a Haake F6 ultrathermostat with a precision of 0.1 °C.

III. RESULTS AND DISCUSSION

A. DRS in neat water and comparison with literature data

The DRS spectral distribution in the −20 to 80 cm^{-1} frequency range can be reproduced considering the sum of one sharp Gaussian [$G(\nu)$] plus two Lorentzian [$L_W(\nu)$ and $L_B(\nu)$] functions. An example of DRS spectrum of neat water from −10 to 50 cm^{-1} is reported in Fig. 1 together with the

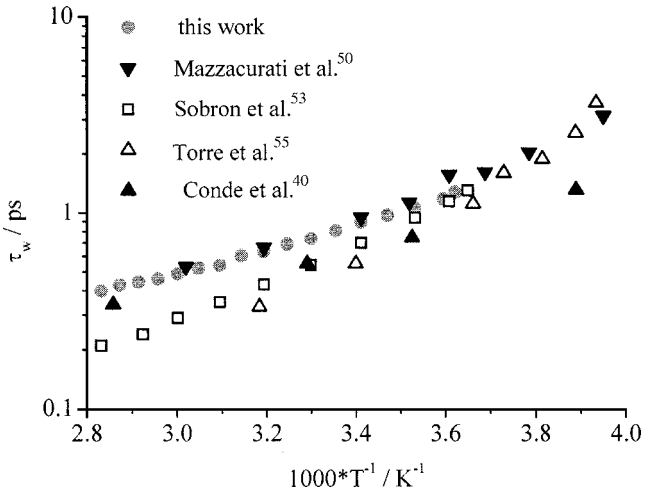

FIG. 2. Comparison of the relaxation time characterizing the main relaxation process detected in different DRS experiments. The average relaxation time $\langle\tau_W\rangle$ obtained by the OKE measurements of Torre *et al.* (Ref. 55) is also reported. A mean relaxation time value is calculated as $\langle\tau_W\rangle = (\tau/\beta)\Gamma(1/\beta)$ considering the τ and β parameters obtained by the authors using a stretched exponential function to reproduce the nuclear Kerr response (Ref. 55).

fitted components. The measured DRS spectrum $I_{\mathrm{DRS}}(\nu)$ is the convolution of the instrumental function $G(\nu)$ and the true spectrum,

$$I_{\mathrm{DRS}}(\nu) = G(\nu) * [K_P\delta(\nu) + K_W L_W(\nu) + K_B L_B(\nu)], \qquad (1)$$

where $\delta(\nu)$ is the spurious polarized contribution and K_P, K_W, and K_B are the intensities of the components. The width of the $G(\nu)$ function coincides to the width of the instrumental function $\Gamma_{\mathrm{ins}}=0.40\ \mathrm{cm}^{-1}$ measured using a latex suspension. Γ_{ins} is small enough to neglect the broadening effects on $L_W(\nu)$ and $L_B(\nu)$. As a result the measured $I_{\mathrm{DRS}}(\nu)$ may be expressed as

$$I_{\mathrm{DRS}}(\nu) \approx K_P G(\nu) + K_W L_W(\nu) + K_B L_B(\nu), \qquad (2)$$

and from the half-width at half-height $(\Gamma/\mathrm{cm}^{-1})$ of the two Lorentzian components the corresponding relaxation times may be obtained, $\tau = 1/(2\pi c\Gamma)$.

An analogous spectral analysis has been accomplished in previous DRS studies.[39,42,50] The wider $L_B(\nu)$ component is almost temperature independent and is representative of a smooth background which accounts for the low-frequency contribution of the intermolecular modes. The $L_W(\nu)$ line describes the principal relaxation process at picoseconds.[51]

A comparative fitting procedure has been performed in which the central region of the spectrum (approximately -1.0 to $1.0\ \mathrm{cm}^{-1}$), due to the instrumental function $G(\nu)$, has been excluded. Both methods give very similar parameters. The temperature dependence of the resulting relaxation time τ_W is reported in Fig. 2 together with further literature values.

Sokolov *et al.*[52] performed a detailed analysis of the DRS spectrum of water in both normal and supercooled domains. These authors adopted quite a complex iterative fitting procedure to model the dynamical susceptibility $\chi''(\nu)$ in the framework of the mode coupling theory (MCT). In this case the main relaxation process is reproduced by a Cole-Davison distribution. The relaxation time $\tau(T)$ is found to follow a power law of the type $\tau(T) = a(T-T_s)^{-\gamma}$. The close connection existing between $\tau(T)$ and the viscosity $[\tau(T) \propto \eta/T]$, previously evidenced by Sobron *et al.*,[53] has been also confirmed. In recent OKE experiments, the long time portion of the time correlation function (TCF) is modeled using either two distinct exponentials[54] or a single stretched exponential form.[55] In particular, Torre *et al.*[55] measured the TCF at very long delays, unambiguously showing that the relaxation is intrinsically not exponential. In agreement with the MCT predictions the TCF is well reproduced by a stretched exponential function $(C(t) \propto \exp(-t/\tau)^{\beta}$; $\beta \approx 0.6$ in the 254–341 K temperature range). The relaxation time follows the $\tau(T) = a(T-T_s)^{-\gamma}$ power law with $T_s = 221$ K and $\gamma = 2.2$. This scenario has been also confirmed by MD simulation studies.[56] With respect to these results, our fitting procedure is somewhat simplified considering that the water relaxation is modeled with a single Lorentzian function (i.e., exponential decay of the relevant TCF). Nevertheless, making use of additional parameters is found to be redundant. This limitation, which seems common in frequency-domain experiments,[39,42,50] can be ascribed to the effect of spurious central contributions and/or to the less favorable signal to noise ratio with respect to time domain data.[54,55] In any case, our simple spectral analysis leads to reliable values of τ_W, in reasonable agreement with widely accepted estimates (Fig. 2). Moreover, the use of a limited number of parameters is essential for a safer parameterization of the aqueous mixtures.

Several interpretations concerning the physical origin of the main DRS relaxation process in water can be found in literature. A clear molecular description is not obvious considering that the relaxation of the polarizability anisotropy includes rotational and translational molecular motions as well as their cross contributions.[56] In many studies it is remarked that the polarizability of the water molecule is nearly isotropic such that DRS and OKE signals must be dominated by intermolecular induced contributions.[50,54,55] The relaxation process should describe the rearrangement of locally organized environments (i.e., a structural relaxation process[55]) in which the polarizability anisotropy is essentially modulated by changes in intermolecular distances. Several studies also suggest a direct connection of this process with the typical H-bond dynamics at picosecond time scales.[38–43] In this respect, even if the presence of H bonds is not a necessary requisite to explain the scattering activity,[50] the H-bond dynamics will certainly affect the molecular mobility and the temporal behavior of the relevant TCF, $C(t)$. Molecular dynamics simulations are suitable tools for the study of the H-bonding dynamics in water. Both energetic and geometric H-bond definitions may be used and different types of H-bond TCFs may be calculated.[57–61] The experimental τ_W is comparable to the fast relaxation time τ_{HB} of the so-defined *continuous* H-bond TCF.[60,61] In Fig. 3, the temperature dependence of the τ_W is compared with some MD results. To note that different calculation methods (i.e., H-bond criteria or H-bond lifetime definitions) may lead to different estimates of τ_{HB}. In any case calculated τ_{HB} values at 300 K are of the order of 0.3–1.6 ps,[43,58–61] in agreement

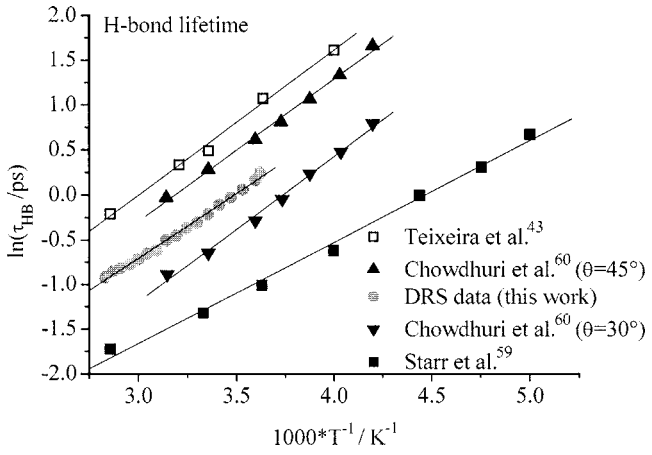

FIG. 3. Arrhenius plot of the experimental H-bond time constant τ_W (circles) together with theoretical values reported in different MD studies: Teixeira *et al.* (Ref. 43) (empty squares), Chowdhuri and Chandra (Ref. 60) with an angle definition threshold $\theta=45°$ (up triangles) and $\theta=30°$ (down triangles), Starr *et al.* (Ref. 59) (filled squares). Corresponding activation energies are between 9.3 and 13.5 kJ/mol.

with our experimental findings. The calculated τ_{HB} is directly correlated to the inverse of the forward rate constant k_{HB} for H-bond breaking.[57–60] In this sense τ_{HB} expresses short H-bond lifetimes describing a thermally activated local restructuring process.[43,59,60] The temperature dependence of τ_{HB} is Arrhenian with an activation energy E_A related to the H-bond energy $[E_A(\mathrm{HB})\sim 9-14$ kJ/mol$]$.[43,59,60] The experimental activation energy calculated from our τ_W values is 12 kJ/mol in good agreement with the computed values (see Fig. 3). More cooperative relaxation dynamics are better described by *intermittent* H-bond TCFs characterized by slower decays and more complex non-Arrhenius behavior.[59] In this case the relaxation involves both H-bond breaking and reformation processes coupled with molecular diffusion.[57,58,60]

On the whole, the data support the idea that in the temperature range investigated here the experimental τ_W may be considered a reasonable measure of the average H-bond lifetime in water.[43] Likely, at lower temperatures the relaxation time obtained by DRS and OKE experiments describes a more cooperative structural relaxation process with non-Arrhenius temperature dependence that follows the MCT predictions.[43,55,56]

B. DRS of glucose aqueous solutions

In Fig. 4 DRS profiles measured at different glucose molar fractions (X_G) and $T=35$ °C, are reported in the -10 to 30 cm^{-1} spectral region. These spectra are rescaled to the higher frequency tail of the DRS signal (around 80 cm^{-1}) obtaining a congruent overlaying of the intensities in the 5–80 cm^{-1} spectral region, especially for more diluted samples. Conversely, in the 1–4 cm^{-1} frequency range the DRS intensity increases with X_G. This situation can be rationalized considering the appearance of new sharp spectral component directly related to the solute. In fact, to reproduce the DRS data an additional Lorentzian function $L_G(\nu)$ must be included,

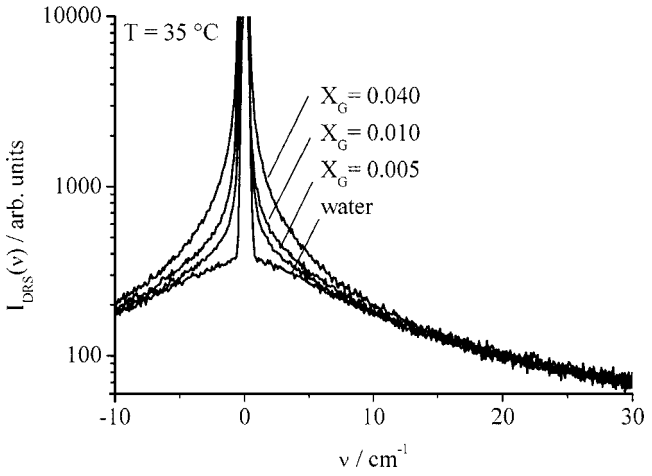

FIG. 4. DRS spectra of glucose-water solutions measured at different solute molar fractions X_G and $T=35$ °C. The spectra are rescaled to the higher frequency tail of the DRS signal around 80 cm^{-1}.

$$I_{\mathrm{DRS}}(\nu)=G(\nu)*[K_P\delta(\nu)+K_GL_G(\nu)+K_WL_W(\nu)+K_BL_B(\nu)]. \quad (3)$$

An example of spectral decomposition is reported in Fig. 5 performed after exclusion of the -1.0 to 1.0 cm^{-1} spectral region. In this case Eq. (3) becomes

$$I_{\mathrm{DRS}}(\nu)\approx K'_GL'_G(\nu)+K_WL_W(\nu)+K_BL_B(\nu). \quad (4)$$

The $L'_G(\nu)$ component is assigned to the glucose relaxation dynamics and is simply modeled as a Lorentzian contribution. Since the resulting FWHH (Γ'_G) is comparable to the spectral resolution, the use of a Lorentzian form is clearly oversimplified [i.e., $L'_G(\nu)=G(\nu)*L_G(\nu)$] and the true Γ_G cannot be accurately determined. In any case the employment of the $L'_G(\nu)$ line is necessary to reproduce the experimental data, leading to reliable values for the $L_W(\nu)$ component. This has been verified for the most diluted sample when the $L_W(\nu)$ and $L_B(\nu)$ components of the water are expected to be only marginally perturbed by the solute.

The Γ'_G value is around 0.7–1.2 cm^{-1} and does not show any specific temperature or concentration dependence owing

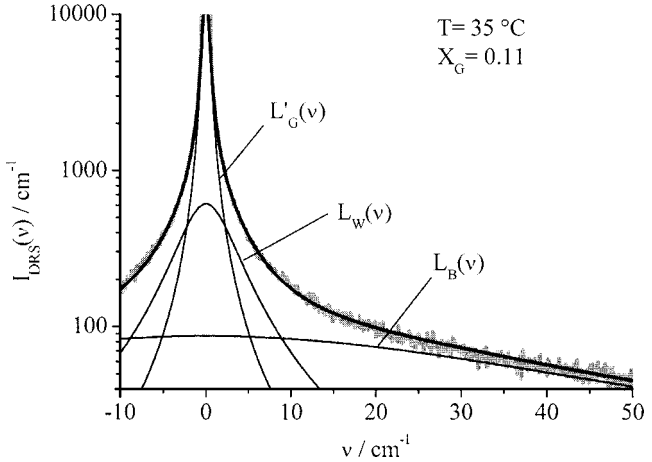

FIG. 5. DRS spectrum of a glucose-water solution, $X_G=0.11$ and $T=35$ °C (gray line) together with the best fit result (thick black line) with the three Lorentzian components (thin black lines).

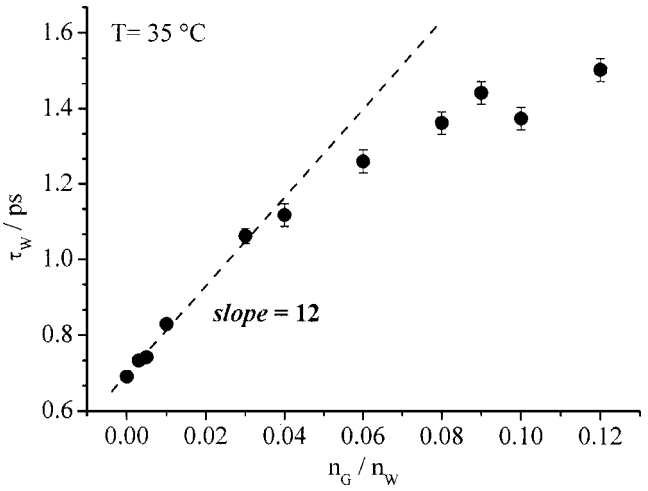

FIG. 6. The water relaxation time τ_W in glucose-water solutions obtained at different glucose molar ratio $f_G=n_G/n_W$ with the corresponding linear fit (dashed line). A linear dependence is found until $f_G \sim 0.04$ and leads to a slope of 12.

to the limited spectral resolution. A Γ'_G value of 0.7 cm^{-1} is obtained for the 900 mg/ml solution at 70 °C. These conditions are more favorable for an estimation of Γ_G because of the high sugar concentration (i.e., large K_G) and high temperature (i.e., large Γ_G). The true Lorentzian width Γ_G, obtained following the numerical deconvolution method proposed by Asthana and Kiefer,[62] is approximately 0.4 cm^{-1} corresponding to a relaxation time τ_G of approximately 25 ps: this roughly represents the upper limiting time that can be detected in the present experiment. The corresponding water relaxation time τ_W is approximately 1.1 ps. Considering the large time scale separation existing between the two processes, only minor contributions will arise from cross correlation terms and the glucose and water dynamics can be selectively analyzed in DRS spectra. This constitutes a noticeable chance to study the relaxation properties of water induced by a solute. The whole picture has been confirmed performing measurements with a higher resolution apparatus to better describe the glucose relaxation dynamics.[32] In the present study only the water relaxation time will be analyzed and discussed.

The effect of the sugar on the τ_W is reported in Fig. 6 at 35 °C. The statistical precision on τ_W values decreases at higher molar ratios ($f_G=n_G/n_W$) due to the increasing of the relative weight of the $L'_G(\nu)$ contribution. The water relaxation dynamics clearly slows down with the increase of glucose content reproducing the trend of other dynamical properties.[13,19,21,23] In a previous DRS study on glucose-water solutions a stronger concentration dependence of the detected relaxation time has been reported by Wang and Tominaga.[17] Our results show that the relative increment $\tau_W(f_G)/\tau_W(0)$ at $f_G=0.04$ is approximately 1.5 while a ratio of 7 was previously measured.[17] Wang and Tominaga[17] reproduced the dynamical susceptibility $\chi''(\nu)$ considering a single Cole-Cole relaxation mode. Thus, the use of an additional low-frequency component [i.e., $L'_G(\nu)$] in our fitting procedure should explain the observed discrepancies. The relaxation time reported by Wang and Tominaga may partially account for the glucose dynamics as also suggested by the decrease of the Cole-Cole β parameter observed at higher concentrations.[17] In this respect our data indicate that the relaxation dynamics, selectively attributed to the H-bond rearrangements of water molecules, decelerates in presence of glucose less than previously reported.

The glucose relaxation dynamics is at least one order of magnitude slower than τ_W; this means that fast H-bond fluctuations of water take place in a quasistatic glucose molecular arrangement. It is expected, in agreement with MD findings,[13] that the mobility restriction mainly involves water molecules in the solute hydration shell. Both IR and Raman anisotropic OH stretching profiles show that the addition of glucose is accompanied by a frequency redistribution toward lower frequencies (approximately 3300 cm^{-1}) due to OH groups stabilized by strong H bonds.[12] This indicates that water-water and water-glucose H bonds are of similar magnitude confirming that very stable hydration shells may be formed around the carbohydrates.[11,13] Likely, the steric constraints imposed by the large solute decrease the degrees of freedom of the surrounding water molecules and their possibilities to find favorable pathways for H-bond rearrangements.

Clearly, the $L_W(\nu)$ component in the DRS spectrum accounts for the whole ensemble of water molecules in solution including molecules not localized in the solute hydration shell. MD results show that, for a diluted sample, sugars essentially affect the water dynamics in a region within 5.5 Å from the carbohydrate; at longer distances the water mobility resembles that of neat water.[13] If the water's rate of exchange between the bulk and the hydration shell is small compared to τ_W then, in principle, the DRS spectrum should consist of two separate contributions. Many experimental and theoretical findings suggest that the exchange dynamics of water molecules around ionic or nonionic groups is considerably longer than the fast hydrogen bonding lifetime. Bakker and co-workers[63,64] measured the spectral diffusion time of the OH stretching vibration in ionic salvation shells by femtosecond IR spectroscopy. The characteristic time constant of this process, that roughly represents the residence time of water molecules within the shell, is found to be 12–25 ps which is 20–50 times longer than τ_W. MD simulations confirm that the residence times around halide ions are within tens of picoseconds and that are longer than the H-bond lifetime within the shell.[60] Moreover, the sugar-water H-bond TCFs, calculated from MD trajectories,[13] relax at temporal scales of tens of picoseconds suggesting that sugar-water correlations survive longer than the relaxation dynamics described by τ_W. These facts confirm that in aqueous solutions the existence of two distinct relaxation processes due to hydrating and bulk water molecules can be envisaged; yet their experimental separation is not easily practicable considering that both contributions span the same temporal domains. Nevertheless, we can roughly assume that the $\tau_W(f_G)$ is an average relaxation time resulting from the relative weight of the relaxation times $\tau_{WH}(f_G)$ and $\tau_{WB}(f_G)$ of proximal and bulk water molecules, respectively. This

means that the relevant TCF $C_W(t)$ is the sum of two exponential contributions $C_{WH}(t)$ and $C_{WB}(t)$ and that $\tau_W(f_G)$ is its average relaxation time.

$$\tau_W(f_G) = X_{WH}\tau_{WH}(f_G) + X_{WB}\tau_{WB}(f_G). \quad (5)$$

X_{WH} and X_{WB} are the molar fractions of water molecules localized in the sugar hydration shell (proximal water) and in the bulk, respectively; namely, $X_{WH}+X_{WB}=1$. An analogous description in terms of free and H-bonded molecules has been used to describe the dielectric relaxation process of water.[65] Further, we neglect any concentration dependence of both relaxation times [i.e., $\tau_{WB}=\tau_W$ and $\tau_{WH}(f_G)=\tau_{WH}$]; this is reasonable in diluted conditions. Thus, defining the hydration number n_h as the number of water molecules in the glucose hydration shell we can rearrange Eq. (5) as

$$\tau_W(f_G) = f_G n_h(\tau_{WH} - \tau_{WB}) + \tau_{WB}. \quad (6)$$

For diluted samples n_h can be considered constant and a linear dependence of $\tau_W(f_G)$ vs f_G is expected. As it can be seen in Fig. 6 a linear fitting is appropriate until $f_G \sim 0.04$; the resulting slope is $m=12$. Apparently m decreases at higher concentrations. In any case, because of the large contribution of the sugar component in the DRS spectrum the error on $\tau_W(f_G)$ is relevant and is not clear if the detected dependence has some physical origin. Thus, quantitative information is only deduced for the more diluted samples. Reasonable hydration numbers for glucose are around 8–10 as obtained by computational studies[13,16,66] and ultrasonic measurements.[67] These n_h values properly represent the number of water molecules directly H-bonded with the glucose whose dynamics is more strongly influenced by the solute. In this case, Eq. (6) leads to $\tau_{WH} \sim 2.2-1.9$ ps indicating that the relaxation dynamics of the proximal water is about three times slower than the bulk. The MD study of Lee *et al.*[13] shows that the rotational relaxation time of water molecules, which is at picosecond time scales, increases significantly when the water molecule is located within the first hydration shell of the sugar. In particular, the water rotation slows down by a factor of 2–3 at distances smaller than 3.5 Å in the case of glucose. At the same time the decay of the sugar-water H-bonding TCFs is considerably retarded when compared to the water-water one. As a result the reduced mobility of the vicinal water has been connected to the formation of more stable carbohydrate-water H-bond. Our data confirm this situation; moreover, the retardation effect on the H-bond dynamics deduced by DRS spectra is in reasonable quantitative agreement with MD results. In this respect the presented treatment leads to a direct experimental estimate of the H-bond lifetime that characterizes hydrating water molecules. The slowing down of the water mobility around solutes and the concomitant increase of the hydrogen bond lifetimes are probably a quite general effect induced by hydrophilic molecules.[11,13,61]

C. LFR spectra of neat water

The depolarized low-frequency Raman spectrum $I_{LFR}(\nu)$ was reduced into the imaginary part of the dynamical susceptibility $\chi''(\nu)$ by multiplying for the thermal factor $[1-\exp(-hc\nu/kT)]$ (i.e., the Bose-Einstein correction).[48]

$$\chi''(\nu) = [1 - \exp(-hc\nu/kT)]I_{LFR}(\nu). \quad (7)$$

$\chi''(\nu)$ for pure water at different temperatures is reported in Fig. 7. The spectral distribution shows two main peaks at 50 and 170 cm^{-1} assigned to the H-bond (O···O···O unit) bending B and H-bond (O···O unit) stretching modes, respectively.[44–48] These intermolecular modes correspond to restricted translations of water molecules in a direction perpendicular B or parallel S with respect to the H-bond and are also described as transversal and longitudinal acoustic phonons.[46,48] Hindered motions of reorientational character (librations) originate the broadband in the 400–800 cm^{-1} frequency range.[49] Molecular dynamics simulations give general support to these interpretations.[68]

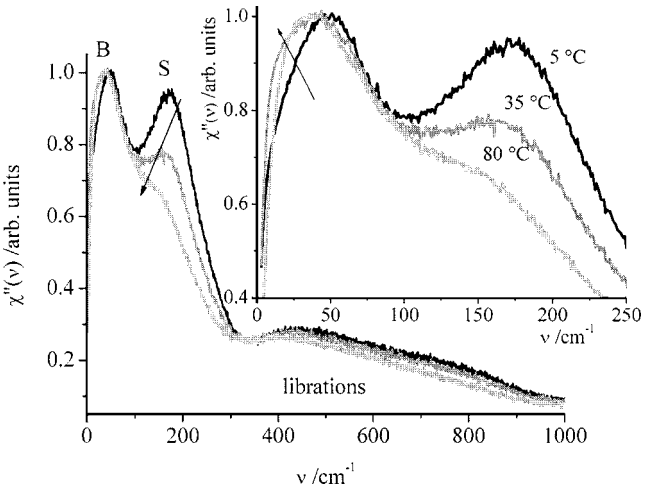

FIG. 7. Temperature dependence of the of the dynamical susceptibility $\chi''(\nu)$ of pure water from 0 to 1000 cm^{-1}. The spectra are normalized on the maximum of the 50 cm^{-1} band. The inset shows the 0–250 cm^{-1} frequency region in more detail. The two main components are assigned to the bending B (~50 cm^{-1}) and stretching S (~170 cm^{-1}) intermolecular modes. Arrows indicate the direction of spectral variations observed with temperature rising. Changes around 30 cm^{-1} essentially depends on the water relaxation process.

The spectra in Fig. 7 are normalized on the 50 cm^{-1} bending band scarcely affected by temperature changes.[44] As it can be observed all the bands shift to lower frequency at higher temperature, consistently with the intermolecular nature of the these modes. Moreover, a strong relative intensity decrease of the S band may be observed. It should be observed that intensity changes at lower frequencies (around 25 cm^{-1}) may be partially ascribed to the relaxation mode. This, in the $\chi''(\nu)$ spectrum, appears as a Debye relaxation mode with the peak maximum blueshifting (i.e., τ_W shortening) at higher temperatures within the 5–15 cm^{-1} frequency range. This makes the performed normalization somewhat arbitrary. Even so the observed intensity depletion of the S signal is qualitatively reliable and in agreement with previous reports.[44,45] Walrafen emphasized the importance of the tetrahedral order in water and related the intensity of the S band to the fraction of linear or weakly bent H bonds.[45,46] A widely accepted value of ~11 kJ/mol for H-bond breakage is calculated considering the thermal variations of the low-

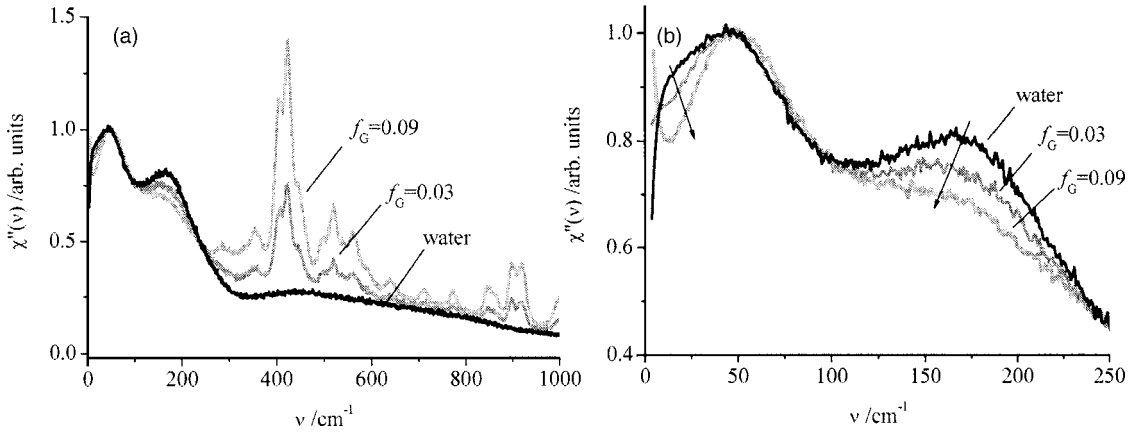

FIG. 8. (A) The dynamical susceptibility $\chi''(\nu)$ of pure water and glucose-water solutions at different molar ratios $f_G=n_G/n_W$ and $T=25$ °C. The spectra are normalized on the maximum of the 50 cm^{-1} band. (B) Detail of the 0–250 cm^{-1} spectral region assigned to the water contributions. Arrows indicate the direction of spectral variations observed at higher glucose concentrations.

frequency Raman intensity, which is essentially caused by the changes of the *S* band.[44–46] The fact that the intensity of the *B* band is only marginally affected by thermal variations may be explained taking into account that besides the *B* mode, which intensity would decrease at high temperature, an additional low-frequency contribution with opposite temperature dependence must be considered at similar frequencies.[45–47] This contribution, which survives even at 340 °C, was attributed to unbounded species and described in terms of a relative kinetic energy distribution.[46] Agmon[47] interpreted more specifically the *S* peak as an asymmetric stretching mode of the water tetrahedron [i.e., $\nu_3(T_2)$ within the T_d symmetry group], which drives the slow diffusion process observed in dielectric experiments. It is proposed that the diffusion involves a water displacement among tightly bound tetrahedral.[47] Within this framework, the *S* band accounts for extended regions dominated by interacting tetracoordinated units. This picture confirms the analysis of Krishnamurty *et al.* who previously invoked the collective nature of the *S* mode.[44] Thus, the intensity reduction depicted in Fig. 7 follows the population decrease of water molecules embedded within extended tetrahedral environments.

D. Low-frequency Raman spectra of glucose aqueous solutions

Figure 8(a) shows the normalized $\chi''(\nu)$ obtained at different glucose molar ratios. The lower frequency band assigned to the glucose appears at ~285 cm^{-1}; we neglect any direct glucose contribution below 250 cm^{-1}. The assumption may be partially justified. In fact the intensity ratio $I_{\mathrm{DRS}}(1125)/I_{\mathrm{DRS}}(50)$ between the intramolecular band at 1125 cm^{-1} and the intensity at approximately 50 cm^{-1} linearly scales with the glucose molar ratio f_G extrapolating at zero when $f_G \rightarrow 0$. This indicates that around 50 cm^{-1} the water contribution is dominating. This is also substantiated by the deconvolution analysis of Wang and Tominaga.[17] These authors reproduced the *B* and *S* bands of the $\chi''(\nu)$ spectrum of water-glucose and water-galactose by using two damped harmonic oscillators with parameter similar to those employed for pure water. In the frame of this assumption the main outcome from the comparison of Fig. 8 is the relative intensity reduction of the *S* band, which reproduces the effect of a temperature increase. This can be interpreted as the signature of the reduction of tetrahedral water environments induced by glucose. In this respect the glucose will create a new orientational and translational organization on the proximal water molecules that will be locally arranged in environments with less tetrahedral degree. This effect has been already evidenced by MD simulations[13] and by Raman experiments on the OH stretching region.[12] We remark that the effect of glucose on the OH stretching signal is very small and that the reduction of the tetrahedral component has been evidenced only after a careful procedure that involves both polarized and depolarized profiles.[12] On the other hand the spectral changes evidenced in Fig. 8 may be easily observed. This gives support to the previous findings and indicates the high sensitivity of the *S* band towards tetrahedral environments. As already pointed out, sugars lead to the formation of stable hydration shells and are classified as kosmotropes: solutes able to impose their own order to the tetrahedral (i.e., *icelike*) H-bond network of water.[69,70] Our findings on glucose confirm this situation. To note that the conservative action of sugars has been related to their capability of disrupting ordered H-bond configurations which at lower temperatures would give rise to ice.[8,69,70]

Interestingly, Batchelor *et al.*[71] based on heat capacity measurements showed that glucose decreases the fraction of less dense species within its hydration shell, thus acting as a "structure breaker." This is consistent with the idea that in liquid water transient low-density regions may be formed in which tetrahedral units interact cooperatively.[47,72] These environments, often described as icelike, give rise to the coupled intermolecular *S* stretching mode[44,47] depleted by the sugar. In a previous low-frequency Raman study sucrose was classified as a "structure maker.[73]" In that case it was proposed that the formation of strong water-sugar H bonds could be responsible for the relative increase of the *S* signal. Actually, our data seem to indicate that the new glucose-water H bonds lead to minor contributions in the 150–200 cm^{-1} region which is very specific to tetrahedral water arrangements. In Fig. 8(b) an apparent intensity reduction below 40 cm^{-1} can be also observed. These variations,

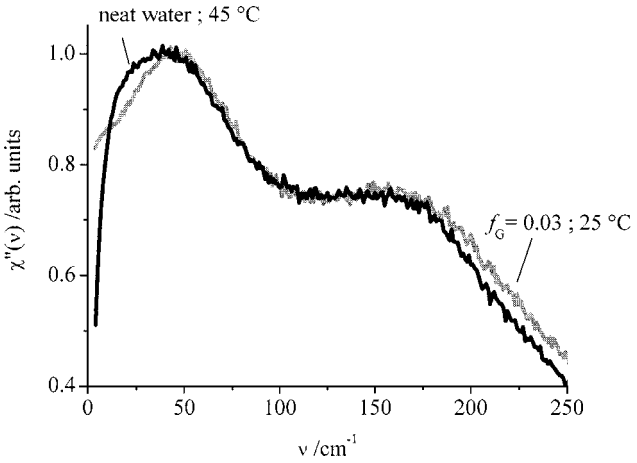

FIG. 9. The dynamical susceptibility $\chi''(\nu)$ of pure water at 45 °C is compared with that of a glucose-water solution at 25 °C corresponding to a glucose molar ratio $f_G=n_G/n_W=0.03$. The spectra are normalized on the maximum of the 50 cm^{-1} band.

essentially related the water relaxation mode, are due to the slowing down of the dynamics previously described and reproduce the effect of a temperature decreasing.

The comparison in Fig. 9 indicates that the $\chi''(\nu)$ of the $f_G=0.03$ solution at room temperature is similar to the $\chi''(\nu)$ of pure water measured at 45 °C in the 40–250 cm^{-1} range. This suggests that the effect of the sugar on the tetrahedral water environments resembles a temperature increment of 20 °C. This supports the view that the two main features of the low-frequency Raman spectra in glucose-water solutions are basically due to water and that the adopted comparison may define a useful method to easily evaluate the effect of a solute on the water network organization.

IV. CONCLUSIONS

In this work we used DRS experiments to quantify the effect of glucose on the water relaxation process at picosecond time scales directly connected to the H-bonding dynamics. The addition of glucose increases the H-bonding lifetime of water τ_W. The DRS spectrum of the mixture is modeled considering distinct contributions arising from the relaxation of glucose and water molecules. The large time scale separation between the two processes allows for a safe analysis of the water relaxation time. We note that our data treatment differs from the analysis previously performed by Wang and Tominaga[17] in which the glucose contribution was not explicitly considered and leads to results that are quantitatively different.

The concentration dependence of the water relaxation time $\tau_W(f_G)$ is discussed taking into account the existence of two water subensembles that interconvert slower than $\tau_W(f_G)$: water molecules localized in the sugar hydration shell (proximal water) with a longer H-bond lifetime τ_{WH} and water molecules localized at larger distances (bulk water) whose dynamics is not perturbed with respect to the pure liquid. For the more diluted samples ($f_G<0.04$) the H-bond lifetime τ_{WH} of the proximal water molecules is found to be ~2.0 ps: about three times larger than the bulk value.

These findings are reasonably supported by recent MD simulations.[13] In fact, the rotational dynamics of water molecules localized within the glucose first hydration shell ($r<3.5$ Å), where water-glucose interactions dominate, is found to increase by a factor of 2–3. This mobility reduction has been ascribed to the retardation of H-bond dynamics between the solute and proximal water molecules evaluated comparing H-bond time correlation functions. In this sense our results represent the experimental evidence of the increasing of the H-bonding lifetime of hydration water molecules and give a quantitative estimate of the effect.

Very recently Heugen *et al.*[11] employed terahertz spectroscopy in combination with MD calculation to investigate changes in the water network and dynamics induced by the disaccharide lactose. Also in this work the spectral profiles have been modeled considering hydrating and bulk water contributions. In this case the hydration layer is determined to extend to approximately 5.1 Å from the solute surface, corresponding to about 123 water molecules affected per lactose molecule. The number of affected molecules seems large when compared with hydration numbers evaluated in previous studies[13,16,66,67] and considered by us. Likely, the terahertz absorbance enhancement of the hydrating layer makes this technique very sensitive even to minor structural changes. Nevertheless, in the same study Heugen *et al.*[11] showed that major changes on the computed H-bond correlation time involve water molecules within 4 Å from the solute: the obtained H-bond lifetime of bulk water is 0.9 ps and becomes approximately 2 ps within 2 Å. These results basically agree with our findings. In this sense the H-bond lifetime of the proximal water obtained in the present work ($\tau_{WH}\sim2.0$ ps) should mainly refer to those water molecules more heavily affected by the solute. In this respect it is reasonable to assume that this inner hydration layer comprises an order of magnitude of 10 water molecules per glucose.

The comparison of low-frequency Raman distributions evidenced the relative intensity reduction of the intermolecular stretching mode (S band) at 170 cm^{-1} when the glucose concentration increases. This mode has a collective nature, similar to the low-frequency component of the polarized Raman OH band at 3200 cm^{-1}; most likely it involves small groups of tetracoordinated molecules whose motion is correlated. In this respect our data clearly evidence that the addition of glucose decreases the tetrahedral ordering of water in agreement with spectroscopic[12] and computational results.[13] The effect reproduces an increase of temperature. The reduction of the S signal may be connected with the formation of more dense water regions around the glucose recently evidenced by heat capacity measurements.[71] Following MD results it can be argued that also in this case the main structural change involve water molecules that more strongly interact with the sugar ($r<4$ Å). Thus, the glucose destroys the tetrahedral water environments forming stable hydration shells; these have higher local density and slower H-bonding breaking dynamics than the bulk. Considering the high sensitivity of the S band the proposed spectral comparison seems promising to evidence eventual differences in the destructuring capability of different sugars.[8,13] This is the object of a future study.

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