Hydrophobic molecules slow down the hydrogen-bond dynamics of liquid water

Artem A. Bakulin

Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4 9747 AG Groningen, The Netherlands

Huib J. Bakker

FOM-institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands

Christian Petersen

FOM-institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands

Maxim S. Pshenichnikov

Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4 9747 AG Groningen, The Netherlands

Abstract

We study the spectral and orientational dynamics of HDO molecules in solutions of tertiary-butyl-alcohol (TBA), trimethyl-amine-oxide (TMAO), and tetramethylurea (TMU) in isotopically diluted water (HDO:D_2O and HDO:H_2O). The spectral dynamics are studied with femtosecond two-dimensional infrared spectroscopy and the orientational dynamics with femtosecond polarization-resolved vibrational pump-probe spectroscopy. We ob-

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serve a strong slowing down of the spectral diffusion around the central part of the absorption line that increases with increasing solute concentration. At low concentrations, the slowing down is observed to scale with the number of methyl groups, indicating that this effect is due to slow hydrogen-bond dynamics in the hydration shell of the methyl groups of the solute molecules. The slowing down of the vibrational frequency dynamics is strongly correlated with the slowing down of the orientational mobility of the water molecules. This correlation indicates that the slowing down of the spectral diffusion and the molecular reorientation find a common origin in the effect of hydrophobic molecular groups on the hydrogen-bond dynamics of water.

I. INTRODUCTION

The attractive interaction of hydrophobic (=water fearing) molecular groups in aqueous environments plays an important role in the structuring and chemical reaction dynamics of biological systems. Examples are the folding of proteins, the formation of membrane bilayers and the binding of substrates to enzymes. Thermodynamic measurements showed that the introduction of a hydrophobic solute in water is associated with a negative excess entropy and a strong increase in heat capacity, thus suggesting a structuring of the liquid and an increase of the strength of the hydrogen-bond interaction. These thermodynamics effects have been explained by Frank and Evans with a model that is often referred to as the "iceberg model" [1]. According to this model the water surrounding the hydrophobic solute acquires a well-ordered (clathrate) structure (explaining the negative excess entropy) that melts when the temperature is increased (explaining the large heat capacity). More recently, Pratt and Chandler developed models for hydrophobic hydration in which the temperature dependence of the entropy and enthalpy changes associated with hydrophobic hydration could be well accounted for using macroscopic parameters of liquid water [2–4].

In contrast to the thermodynamic findings, molecular scale studies did not find evidence

that the structure of water surrounding hydrophobic groups would be different from bulk liquid water. For instance, in neutron scattering studies the oxygen-oxygen distances of the solvating water molecules were observed to be similar as in bulk liquid water [5–8]. Interestingly, the *dynamics* of water molecules surrounding hydrophobic groups were observed to be different from bulk liquid water. With nuclear magnetic resonance (NMR) [9–13], dielectric relaxation [14,15], and femtosecond mid-infrared spectroscopy [16–18] it was observed that solvating water molecules show slower orientational dynamics than the molecules in bulk liquid water. The origin and extent of this slowing down of the orientational dynamics are still under debate [13–21]. For instance, the results of classical molecular dynamics (MD) simulations suggest that the hydrophobic solute slows down the reorientation only as a result of a steric shielding effect, and not because of a change in the local hydrogen-bond dynamics [19]. In a recent quantum MD simulation study by Silvestrelli et al. [20] it was also found that the methyl group of methanol has very little effect on the dynamics of the surrounding water molecules.

In recent years, it was demonstrated that valuable information on the hydrogen-bond dynamics of liquid water can be obtained with third-order vibrational spectroscopic techniques like spectral hole burning, echo-peak shift spectroscopy and two-dimensional (2D) vibrational spectroscopy [22–26]. With these methods it was found that the hydrogen-bond network of water possesses dynamics on different time scales, ranging from tens of femtoseconds up to picoseconds. Here we use 2D vibrational spectroscopy and polarization-resolved pump-probe spectroscopy to study the hydrogen-bond and orientational dynamics of water molecules interacting with hydrophobic groups of three amphiphilic solutes, tertiary-butyl-alcohol (TBA), trimethyl-amine-oxide (TMAO) and tetramethylurea (TMU). The comparison of these solutes allows for a determination of the effects of the hydrophobic and hydrophylic parts of these molecules on the hydrogen-bond dynamics of liquid water. We find that the hydrophobic solutes lead to a strong slowing down of both the spectral dynamics and the orientational dynamics of the water molecules.

II. EXPERIMENTAL

We perform two-dimensional vibrational spectroscopy and polarization-resolved pump-probe experiments on HDO molecules of solutions of tertiary-butyl-alcohol (TBA), trimethyl-amine-oxide (TMAO), tetramethylurea (TMU) (Aldrich, 99% pure), in isotopically diluted water. These amphiphilic solutes have 3 (TBA, TMAO) or 4 (TMU) hydrophobic methyl groups and one hydrophilic group arranged in a similar geometry (Figure 1). The hydrophilic groups of these solutes are quite different: TMAO possesses a very polar N^+O^- group, while TBA possesses an hydroxyl group that is very similar to water hydroxyl groups. The solute concentrations are defined with a parameter w that is equal to the ratio w of the number of TBA/TMAO/TMU and water; w was ranging from 0 to 0.2 for TBA and TMAO and from 0 to 1 for TMU. In all experiments the concentration of HDO molecules is kept sufficiently low (less then 1:20) to avoid resonant (Förster) energy transfer between the O–H/O–D groups [27–29]. The experiments are performed at a temperature of 295 K.

The probing of the OH/OD stretch modes of $HDO:D_2O/HDO:H_2O$ has several advantages and disadvantages. A disadvantage of probing the OH stretch mode is that its vibrational lifetime of 0.7 ps is $\sim$2 times shorter than the vibrational lifetime of the O–D stretch mode. As a result, the dynamics can be followed over a longer time interval for the O–D vibration. An advantage of probing the OH stretch vibration is that the spectral dynamics of the OH stretch vibration are slower than those of the OD stretch vibration (owing to a slower D_2O bath), and that the absorption band of the OH stretch vibration is less motionally narrowed than that of the OD stretch vibration [30], making the spectral diffusion dynamics of the OH clearer and easier to measure with the 2D IR technique. Therefore, for the polarization-resolved studies the $HDO:H_2O$ combination was chosen while the 2D IR experiments were performed on $HDO:D_2O$.

The 2D spectroscopic technique is a heterodyne detected three-pulse photon echo experiment [25,26,31,32]. In this experiment a pair of optical pulses creates a frequency-modulated population grating in the sample with a finesse determined by the pulse separation τ_{12}. This

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grating evolves during a waiting time T during which hydrogen-bond fluctuations wash out the frequency fringes. The third pulse is scattered off the remaining population grating, thus producing the signal. The signal amplitude is determined by the extent of memory loss for the initial frequencies of fringes and dissipation during the waiting time T. The dependence of the signal on the excitation frequency ω_1 is obtained by performing many experiments in which τ_{12} is varied. Fourier transform of all these signals gives the dependence of the signal on ω_1. The probing frequency axis is acquired by interferometric superposition of the echo signal with a fourth laser pulse that acts as a local oscillator. The frequency (ω_3) dependence of the heterodyned echo signal is obtained by dispersing the signal and the fourth pulse with a polychromator. The light is detected with a 64 elements mercury-cadmium-telluride (MCT) array.

Detailed information on the spectral dynamics can be obtained by plotting the frequency $\omega_{3,\text{max}}$ of the maximum probe response at a given excitation frequency ω_1 and by calculating the slope of the $\omega_{3,\text{max}}(\omega_1)$ function [31]:

$$S(T) = \frac{\partial \omega_{3,\text{max}}}{\partial \omega_1}|_T. \tag{1}$$

When $S(T)$ has its maximum value of 1, the excitation and probing frequency are fully correlated, meaning that there has been no spectral diffusion; when $S(T)$ becomes equal to zero, the correlation has completely decayed, indicating that the spectral equilibration is complete. The slope analysis was initially developed for the case of Gaussian frequency dynamics. However, even if the dynamics are not Gaussian, the slope value can still serve as a measure for spectral equilibration, but it can no longer be directly associated with a single frequency-frequency correlation function [33].

The light source for 2D IR experiments is an optical parametric amplifier (OPA) [34] providing 70 fs pulses centered around the OH stretching mode of the HDO molecule at 3420 cm^{-1}. The procedure of data acquisition and processing was similar to the procedure described before [35]. The sample is a thin ~ 40 μm film in a wire-guided jet [36]. The 2D-IR experiment on the O–H stretch mode of HDO:D$_2$O provides information about the

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spectral dynamics up to waiting times of 2 ps. At later times the 2D spectrum is too much dominated by the thermal response that results from the relaxation and equilibration of the excitation energy over the focal volume.

In the polarization-resolved pump-probe experiments, the O–D vibration of HDO:H_2O with the lifetime of 1.8 ps is probed, which allows the measurement of the anisotropy over an 8-ps long time interval. The O–D vibration of HDO:H_2O has a strong absorption around 4 μm (2500 cm^{-1}). The mid-infrared light pulses are generated via parametric amplification processes in β-barium borate (BBO) and $KNbO_3$ crystals that are pumped with the pulses from a commercial Ti:Sapphire amplifier system (Hurricane, Spectraphysics: 800 nm, 100 fs, 1 mJ, 1 kHz). We obtain pulses at 4 μm with a duration of 150 fs and a pulse energy of $\sim$3 μJ.

Probe and reference pulses are generated by reflecting of small fractions of the generated 4 μm pulses by a wedged CaF_2 window. The main transmitted light beam forms the pump beam. The polarization of the pump is rotated using a $\lambda/2$ plate to 45° with respect to that of the probe beam. The pump excites a few percent of the O–D stretch vibration to the first excited vibrational state. This excitation results in an increased transmission at the frequency of the fundamental $v = 0 \rightarrow 1$ transition and an induced absorption at the $v = 1 \rightarrow 2$ transition. These transmission changes are monitored with the probe pulse that is delayed by a time T with respect to the pump. After the sample the probe and reference beams are focused onto the entrance slit of a spectrometer, which disperses the beams onto a 2x32 liquid-nitrogen-cooled mercury-cadmium-telluride (MCT) array. Before entering the spectrometer, the probe beam passes through a polarizer allowing the selection of either its parallel or perpendicular polarization component with respect to the pump polarization. This selection results in transient absorptions $\Delta\alpha_{\parallel}(\omega, T)$ and $\Delta\alpha_{\perp}(\omega, T)$, respectively. The signals $\Delta\alpha_{\parallel}(T)$ and $\Delta\alpha_{\perp}(T)$ are used to construct the so-called rotational anisotropy parameter:

$$R(T) = \frac{\Delta\alpha_{\parallel}(T) - \Delta\alpha_{\perp}(T)}{\Delta\alpha_{\parallel}(T) + 2\,\Delta\alpha_{\perp}(T)}, \tag{2}$$

which is a quantity whose decay reflects the reorientation of the observed vibration.

III. RESULTS

Figure 1 presents linear absorption spectra of the O–H band of pure HDO:D$_2$O and solutions of TBA, TMAO and TMU in HDO:D$_2$O with w=0.1. It is seen that the O–H absorption spectra look quite similar, in spite of the high concentration of solute. The OH absorption spectrum of the TMAO solution possesses a small red wing, indicating that the average hydrogen-bond is stronger in this solution as a result of the interactions between water and the polar N$^+$O$^-$ group.

Figure 2 presents 2D spectral contour plots at different waiting times of pure HDO:D$_2$O and of solutions of TBA, TMAO, and TMU in HDO:D$_2$O, with w=0.1. At a waiting time of 0 ps, the 2D contour is elongated along the diagonal, which means that there is a strong correlation between the frequency at which the O–H stretch vibration is excited and the frequency at which the vibration is probed. This observation demonstrates that the O–H absorption line contains significant inhomogeneous broadening. For the TMU solution the 2D spectrum also shows a small negative feature at frequencies above the fundamental transition. This signal is due to an induced phase modulation effect (Kerr effect) that is only present when the laser pulses overlap ($T \approx 0$). For pure HDO:D$_2$O, the spectral contour acquires an asymmetric shape at short waiting times (T= 0.1, 0.25 ps). It is seen that the response in ω_3 is very broad in case the O–H band is excited in the blue wing (high ω_1), and that the response remains relatively narrow if the absorption band is excited in the center or in the red wing (low ω_1). This phenomenon has been observed before and has been explained from rapid hydrogen-bond dynamics [37,38]. At a longer waiting time of ~ 1 ps, the spectral contour measured for pure HDO:D$_2$O acquires a broad, symmetrical circular shape, which shows that after this time the spectral equilibration between strongly and weakly hydrogen-bonded water molecules is nearly complete.

For the solutions containing TBA, TMAO or TMU, the spectral contour possesses a

similar asymmetry at early waiting times as was observed for pure HDO:D_2O. However, in contrast to pure HD:D_2O, the asymmetry is still present at a waiting time of 1 ps, demonstrating that the spectral equilibration of the different water molecules in this solution is much slower. For all waiting times, pumping in the blue wing results in a much narrower response in ω_3 than observed for pure HDO:D_2O, indicating that the hydrogen-bond dynamics have slowed down significantly.

Figure 3a presents the average slope of the 2D spectra around the position of the absorption maximum as a function of waiting time for different solutions of TMU in HDO:D_2O. We used the slope analysis without correcting for the excited state transition dynamics of the excited state transitions. This approach is justified because of the large anharmonicity of the O–H stretch vibration. For all solutions the slope shows an initial rapid decay and then appears to saturate at a non-zero level. This level increases with increasing concentration of TMU. We cannot measure the slope values at waiting times >2 ps because of the limited lifetime of the O–H stretch vibration and the rise of a thermal signal. The increase of the slope values at 2 ps with concentration TMU shows that for a significant fraction of the water molecules that solvate TMU the spectral diffusion is much slower than for bulk liquid water.

To quantize the slowing down effect of the solutes on the water spectral dynamics, we fit the slope $S(T)$ for all measured solutes and concentrations with the following expression:

$$S(T) = A_1 e^{-T/\tau_1} + A_2 e^{-T/\tau_2} + A_0, \tag{3}$$

where A_1 and A_2 represent the fast and the slow spectral dynamics that are observed for pure liquid water. These time constants have been assigned to local librational motions of the water molecules (τ_1) and more collective diffusional hydrogen-bond dynamics (τ_2) while A_0 represents the fraction of slow water. As we can measure the slope value only with good quality up to a waiting time of 2 ps, we cannot determine the time constant of the slow spectral diffusion process. Therefore, we model the contribution of this water to the spectral dynamics as an offset. We estimate the time constant of this process to be >5 ps.

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We perform a global fit of equation (3), with the restriction that the parameters A_1, τ_1 and τ_2 are the same for all solutions (see Support Materials for details). We find time constants $\tau_1 = 0.15$ ps and $\tau_2 = 0.78$ ps, which agree very well with literature data for HDO:D_2O [24]. The fit results for TMU are represented by the solid curves in Fig. 3a. With increasing solute concentration the amplitude A_2 decreases while the amplitude A_0 associated with the slow water molecules increases.

Figure 3b presents the anisotropy measured with polarization-resolved pump-probe spectroscopy as a function of the delay T for four different concentrations of TBA. The anisotropy shows a non-exponential decay as a function of the delay time T, similar to the observations for the slope $S(T)$. Likewise, the decay $R(T)$ is described as:

$$R(T) = B_1 e^{-T/\tau_{or,f}} + B_0, \tag{4}$$

where B_1 represents the contribution to the observed anisotropy of the water molecules possessing fast orientational dynamics with time constant $\tau_{or,f}$ and B_0 the contribution of water molecules showing a much slower reorientation. For all concentrations we obtain a good fit with $\tau_{or,f} = 2.5$ ps, which is the same reorientation time constant as is observed for pure HDO:H_2O. The reorientation time of the solvation shell water varies from roughly 10 ps at low concentrations to 30 ps for TMU solutions with $w > 0.2$ [18]. In this study we take the reorientation time constant of the water molecules in the hydration shell to be infinitely large, to allow for a quantitative comparison with the hydrogen-bond dynamics measured in the 2D-IR experiments. Hence, we model this contribution as an offset.

Figure 4 presents the value of A_0 (open symbols) as a function of concentration for the solutions of TBA, TMAO, and TMU. For all three solutions $S(T)$ shows a steep linear increase with solute concentration at low concentrations, and starts to saturate for concentrations $w > 0.1$. At low concentrations, the increase is steeper by a factor ~ 1.3 for TMU than for TBA and TMAO, which demonstrates that the slow spectral dynamics is associated with water molecules solvating the hydrophobic part of TMU and TMAO (TMU has four methyl groups while TBA and TMAO have three methyl groups). It should be noted here that the

size of the hydration shell of the hydrophobic part of the molecule does not need to scale rigorously with the number of methyl groups. The hydration shells of individual methyl groups overlap, thus possibly leading to a somewhat slower increase of the hydrophobic hydration shell with the number of methyl groups. The present results also demonstrate that the hydrophilic part of the molecule does not play an important role in the water hydrogen-bond dynamics. The TBA and TMAO solutions strongly differ in their hydrophilic part, but nevertheless show a similar increase of A_0 at low concentrations ($w \leq 0.1$).

In Figure 4 we also plot the fraction B_0 (filled symbols) of the slowly reorienting water molecules as a function of concentration. For TMAO and TMU this fraction is obtained from earlier studies [16,17]. To make a comparison between the concentration dependencies of A_0 and B_0, we use a scaling factor of 0.8 that is the same for all solutes and all concentrations. It should be reminded at this point that in the polarization-resolved pump-probe experiments the OD vibration of HDO:H_2O is probed whereas in the 2D-IR experiments the OH vibration of HDO:D_2O. Previous studies on the spectral and orientational dynamics of the pure liquids (no solute added) demonstrated that the orientational dynamics are strongly determined by the molecular motion of the solvent molecules. Due to the slightly faster solvent molecular motion of H_2O in comparison to D_2O, the time constants are slightly faster ($\sim$10%) for the OD of HDO:H_2O than for the OH vibration of HDO:D_2O. For all three solutes we observe a perfect correlation between the dependencies of A_0 and B_0 on the concentration w. A_0 and B_0 show the same linear increase at low concentrations ($w<0.1$) and the same saturation behavior at high concentrations ($w>0.1$).

IV. DISCUSSION

The large and rapid changes in frequency observed in the blue wing of the O–H stretch vibration of bulk HDO:D_2O have been have been explained from the fast evolution of the molecules absorbing in the blue wing to a weakly hydrogen-bonded structure [37,38]. This weakly hydrogen-bonded structure has been assigned to a configuration in which the H atom

of the O–H group forms a bifurcated hydrogen bond with the oxygen atoms of two other water molecules [38–41]. The bend character make these hydrogen bonds very weak, with the result that the O–H frequency has a high value. By rapid contraction of one of the branches of the bifurcated hydrogen bond while simultaneously breaking the other branch, the bifurcated configuration rapidly evolves to a structure in which there is only one strong hydrogen bond between the H atom of the O–H with one oxygen atom of a neighboring water molecule. This change of the hydrogen-bond structure is accompanied by a large and rapid change of the frequency of the O–H stretch vibration, thus leading to a broad response in ω_3 in the 2D spectrum. These structural dynamics were also proposed to form the mechanism for the reorientation of liquid water [39–42]. The return from the bifurcated state to a single strong hydrogen bond can involve the reformation of the original hydrogen bond, or lead to the formation of a hydrogen bond to the new partner, i.e. a switching or jumping event [38–42]. In case the original hydrogen bond is reformed, the transition through the bifurcated configuration corresponds to a large librational motion of the O–H group. In case a new hydrogen bond is formed, the O–H group has rotated over ∼60 degrees [39,41].

For the spectral dynamics it does not make a difference whether a new hydrogen bond is formed (successful switch) or whether the original hydrogen bond is reformed (unsuccessful switch). In both cases the frequency of the OH stretch strongly changes, giving rise to a broad response in ω_3. Classical MD calculations predict that ∼25% of the molecules in the blue wing of the spectrum will make a successful switch within 125 fs [41]. Most of the water molecules will return to the original hydrogen bond, meaning that they have undergone a large-amplitude libration. For the solutions of TBA, TMAO, and TMU in HDO:D_2O we observe a much narrower and slower response in ω_3 than for pure HDO:D_2O, indicating that for a significant fraction of the water molecules in these solutions the evolution to the bifurcated hydrogen-bond state is strongly hindered. Figure 5 depicts the cross-sections through the 2D spectra at the frequency of $\omega_1 - \omega_0$=100 cm^{-1} for pure HDO:D_2O and a TMAO solution with w=0.2 in HDO:D_2O at different waiting times. For pure HDO:D_2O

(Fig.5a), the spectra are broadened and shifted from the on-diagonal position within 0.1 ps [38] and for a waiting time of T=0.25 ps the spectra already peak at zero detuning. Also, the decrease of the on-diagonal amplitude (i.e. at the frequency of $\omega_3 = \omega_1$) is clearly visible when the waiting times increases to 1 ps. These features are characteristic for the rapid hydrogen-bond dynamics of molecules in the blue wing of the absorption of pure HDO:D$_2$O [37,38]. In sharp contrast to neat water, the TMAO solution demonstrates a completely different behavior (Fig.5b). After some initial broadening (within 0.1 ps), there are very little subsequent dynamics both in terms of spectral shift and broadening. This observation indicates that the evolution to the bifurcated hydrogen-bonded structure has slowed down substantially.

To analyze the spectral dynamics on the blue wing for the water and TMAO solutions more quantitatively, we followed a procedure similar to the one developed by Fayer and coworkers for the chemical exchange analysis [43]. In Figure 6 we present the temporal evolution of the fraction η of high-energy oscillators with respect to high- and average-energy oscillators for different TMAO concentrations after excitation in the blue wing ($\omega_1 - \omega_0 = 100$ cm^{-1}). To this purpose, we integrated the positive part of the response in the regions where the relative weakly hydrogen-bonded water molecules ($50 < \omega_3 - \omega_0 < 150$ cm^{-1}) and the relatively strongly hydrogen-bonded water molecules ($-50 < \omega_3 - \omega_0 < 50$ cm^{-1}) absorb. The parameter η is calculated as the ratio between the first integral and the sum of both integrals. Hence, η represents the relative number of OH oscillators that keep their initial excitation frequency in the blue region after a certain time. For pure HDO:D$_2$O, the fraction η is small already at short time delays and decays quickly with a time constant of ~0.2 ps. Already after 0.5 ps η reaches a value of 0.43 which is close to the value of ~0.4 of the equilibrium spectrum at room temperature. With increasing TMAO concentration, the parameter η acquires a higher initial value and shows an increasingly slower decay.

The results of Figs. 2-6 demonstrate that the addition of TBA, TMAO and TMU to HDO:D$_2$O leads to a strong slowing down of the spectral dynamics, especially for the molecules that absorb in the blue wing of the spectrum. This slowing down indicates that the

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solute molecules hinder the water molecules solvating TBA, TMAO and TMU in evolving to the bifurcated hydrogen-bond configuration. As this bifurcated hydrogen-bond state also forms the transition state for molecular reorientation [39] (successful switch), the slowing down of the spectral dynamics is expected to be strongly correlated with the slowing down of the molecular reorientation.

In Figure 4 it is seen that the concentration dependence of the slope offset A_0 perfectly correlates with that of the fraction B_0 of slowly reorienting of water molecules. To explore the correlation between the spectral diffusion and the reorientation in more detail, we plot in Figure 7 the fraction B_0 of slowly reorienting water molecules as a function of the slope offset A_0, for all measured solutions. The resulting points can be fitted well with a straight line, with a Pearson correlation coefficient of 0.97. This near-perfect correlation demonstrates that the slowing down of the spectral diffusion and the reorientation have a common origin. The solute molecules hinder the evolution to the bifurcated hydrogen-bonded structure, thereby preventing both rapid spectral diffusion (successful and unsuccessful switches) and rapid molecular reorientation (successful switches only). Probably, by filling the cavities in the hydrogen-bond network of water, hydrophobic solutes kinetically inhibit the collective reorganizations of the water liquid that would lead to the formation of bifurcated hydrogen-bonded configurations. A similar correlation between spectral dynamics and reorientation was recently observed for solutions of NaBr [44]. It was observed that the spectral diffusion and the reorientation show a very similar slowing down with salt concentration, which suggests that this slowing down finds a common origin in the influence of the ions on the hydrogen-bond dynamics of water, exactly as we find here for hydrophobic molecular groups.

In Figure 8 we plot the derivatives $\Delta A_0/\Delta w$ and $\Delta B_0/\Delta w$ for $w<0.1$ of the results presented in Figure 4 as a function of the number of methyl groups contained in the solute. It is seen that these derivatives fall on a line, meaning that the initial increases of A_0 and B_0 with w scale with the number of methyl groups contained in the solute. Again it should be noted that the increase of the effect on the water dynamics does not need to show a perfect scaling with the number of methyl groups, but for the solutes studied here the size of the

hydrophobic hydration shell appears to scale quite well with the number of methyl groups contained in the solute.

For all three solutions it is observed that at high concentrations A_0 and B_0 show a saturation effect due to the sharing of the hydration shells of neighboring solute molecules and aggregation of the solute molecules. For TBA and TMU the saturation effect is very strong, which can be explained from the aggregation. Due to aggregation, two different types of water molecules can be distinguished in highly concentrated TBA and TMU solutions: water molecules that are solvating the TBA/TMU molecules and water molecules that are embedded in water nanoclusters. Beyond a certain TBA/TMU concentration all additionally added TBA/TMU is completely embedded by the TBA/TMU molecules that are already present in the solution. As a result, beyond a certain TBA/TMU concentration the fraction of water showing bulk-like behavior within the nanoclusters and the fraction of water interacting with TBA/TMU (represented by A_0 and B_0) no longer change. The observation of clustering of TBA and TMU at high concentrations agrees with previous findings from neutron scattering studies and NMR [12,45–47]. For TMAO the saturation effects are much less strong, which indicates that the solutions containing TMAO remain quite homogeneous up to very high concentrations.

In recent classical molecular dynamics (MD) simulations (using SPC/E water) it was found that the effect of hydrophobic molecular groups on the reorientation and hydrogen-bond dynamics of water would be quite small [19,21]. A similar result was obtained in a quantum MD simulation of the effect of methanol on the reorientation dynamics of water [20]. We find that the effect of hydrophobic molecular groups on the reorientation of nearby O–H groups is much larger than is found in the MD simulations. In the MD simulations the reorientation of OH groups tangential to the hydrophobic methyl groups was calculated to slow down by a factor of 1.4-1.5 [19], whereas the experiments show a slowing down of these water molecules by a factor ~ 4 [18].

As the simulations find the effect of the hydrophobic groups to be minor, it was also recently claimed that the reported effects of TBA, TMAO and TMU on water dynamics are

in fact due to the hydrophilic groups instead of the hydrophobic groups of these molecules [21]. As a result TMAO, which has a strongly polar N^+O^- group, was calculated to have the largest effect, even larger than TMU, while TBA, which has a hydroxyl group similar to the hydroxyl groups of water, was calculated to have a negligible effect on the spectral dynamics of water. However, these calculated results strongly differ from the experimental results of femtosecond mid-infrared, NMR, [9,10,12,13], and dielectric relaxation studies [15]. In all these studies it is found that the effect of the solute molecules on the water dynamics scales with the size of the hydrophobic part of the molecule. Probably the clearest example of this notion is the comparison of the hydrophylic urea and the amphiphilic tetramethylurea (TMU). Both NMR [12] and femtosecond mid-infrared [48] studies observe that the effect of urea on the reorientation dynamics of water is very small, while the effect of tetramethylurea is very large [12,17]. The present results show that TMU shows the largest effect and that the effects of TBA and TMAO are comparable, as illustrated in Fig. 8. All these results demonstrate that the hydrophylic group has in fact very little effect on the water dynamics, in contrast to the results of the molecular dynamics simulations of Ref. [21].

V. CONCLUSIONS

We studied the spectral and orientational dynamics of water in solutions of the amphiphilic solutes tertiary-butyl-alcohol (TBA), trimethyl-amine-oxide (TMAO), tetramethylurea (TMU) in isotopically diluted water, and we compare these dynamics with the dynamics of pure isotopically diluted water. The spectral dynamics are studied with femtosecond two-dimensional mid-infrared spectroscopy of the O–H stretch vibration of HDO in solutions of of TBA, TMAO, and TMU in HDO:D_2O. The reorientation dynamics are studied with polarization-resolved pump-probe spectroscopy of the O–D stretch vibration of HDO in solutions of TBA, TMAO, and TMU in HDO:D_2O. We observe that the addition of the amphiphilic solutes leads to a strong slowing down of both the spectral and the orientational dynamics.

The spectral and the orientational dynamics of the solutions can be described well with two components. One of these components possesses the same spectral/orientational dynamics as observed for pure isotopically diluted water, while the other component has much slower dynamics. At low concentrations ($w<0.1$), the amplitude of the slow component increases linearly with the solute concentration and the number of methyl groups contained in the solute. This result demonstrates that the slow water molecules hydrate the hydrophobic groups of the solute. The similarity of the results obtained at concentrations $w<0.1$ for TBA and TMAO demonstrates that the character of the hydrophylic group is relatively unimportant for the water dynamics. These findings agree with previous results of NMR [9,10,12,13] and dielectric relaxation studies [15], but differs from the results of recent molecular dynamics simulations [20,21].

For pure HDO:D_2O, we observe a fast and rapid spectral broadening following excitation in the blue wing of the absorption spectrum of the O–H stretch vibration, in agreement with previous experimental observations [37,38]. This process has been assigned to the rapid evolution to bifurcated, weakly hydrogen bonded water structures, and is proposed to play an essential role in the molecular reorientation of liquid water [39–42]. For solutions of TBA, TMAO, and TMU in HDO:D_2O we observe a much narrower and slower spectral broadening following excitation in the blue wing, indicating that in these solutions the evolution to the bifurcated hydrogen-bond state is strongly hindered for a significant fraction of the water molecules.

For all three studied solutes, the fraction of water showing slow spectral dynamics and the fraction of slowly reorienting water are highly correlated, showing the same saturation effect at high concentrations. This strong correlation demonstrates that the slow spectral dynamics and the slow molecular reorientation have a common origin. The effect of hydrophobes on the dynamics of water thus appears to be the hindrance of the formation of bifurcated hydrogen-bonded structures, as these structures are essential for both the spectral dynamics and the molecular reorientation.

VI. ACKNOWLEDGEMENT

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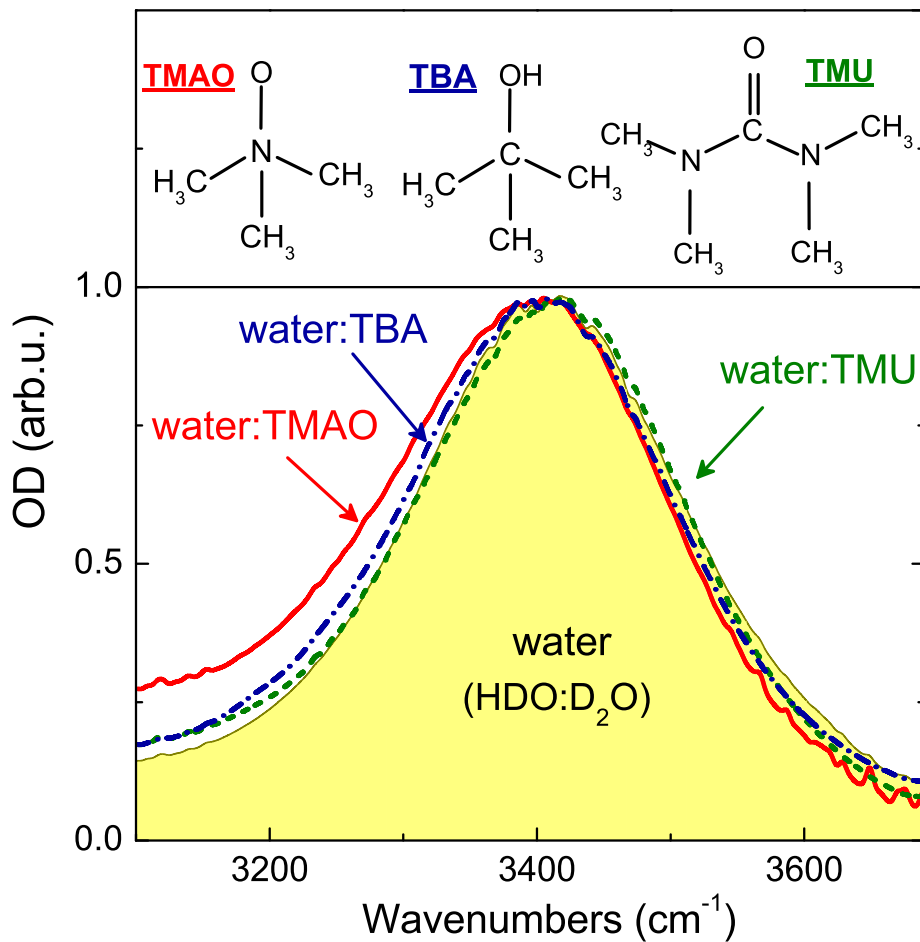[48] Rezus, Y. L. A.; Bakker, H. J. *Proc. Nat. Acad. Sci. USA* **2006**, *103*, 18417.

FIG. 1. Structural formulae of tertiary-butyl-alcohol (TBA), trimethyl-amine-oxide (TMAO), tetramethylurea (TMU) and linear absorption spectra of the O–H stretch vibration of HDO for solutions of TBA, TMA, and TMU in 4% HDO:H_2O and for pure 4% HDO:H_2O. The concentration of the solutions is 10 water molecules per solute molecule (w=0.1).
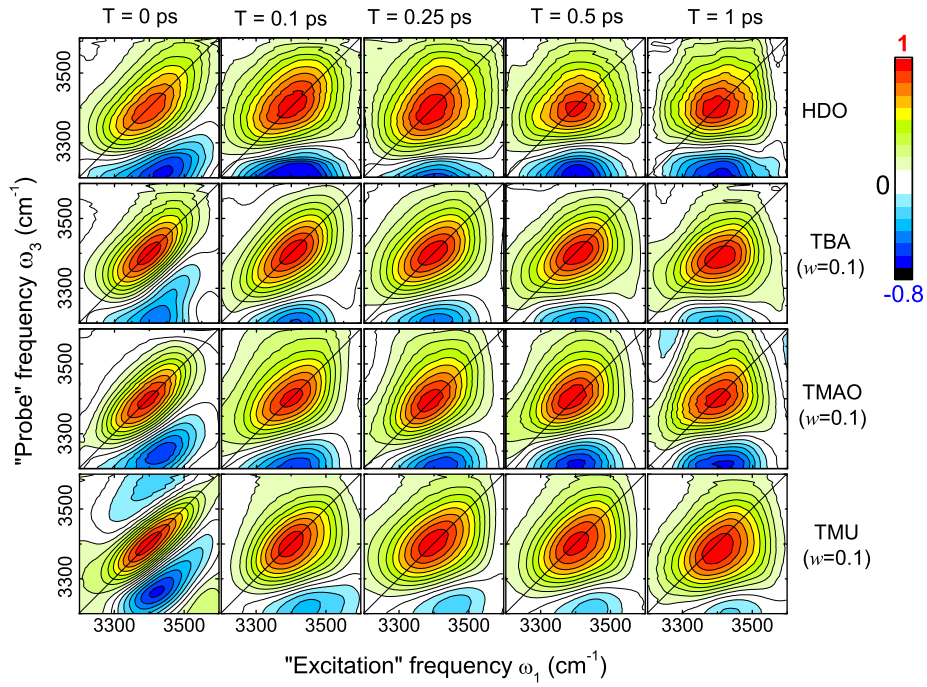
FIG. 2. Two-dimensional correlation spectra of pure HDO:D_2O and of solutions of TBA, TMAO and TMU in HDO:D_2O with w=0.1, measured at different waiting times. Positive signals (red color) correspond to bleaching of and stimulated emission from the fundamental transition while the excited state absorption is represented by negative values (blue color). Each 2D spectrum is normalized onto its maximum. The equilateral contours are drawn in 10% steps of the maximum amplitude.
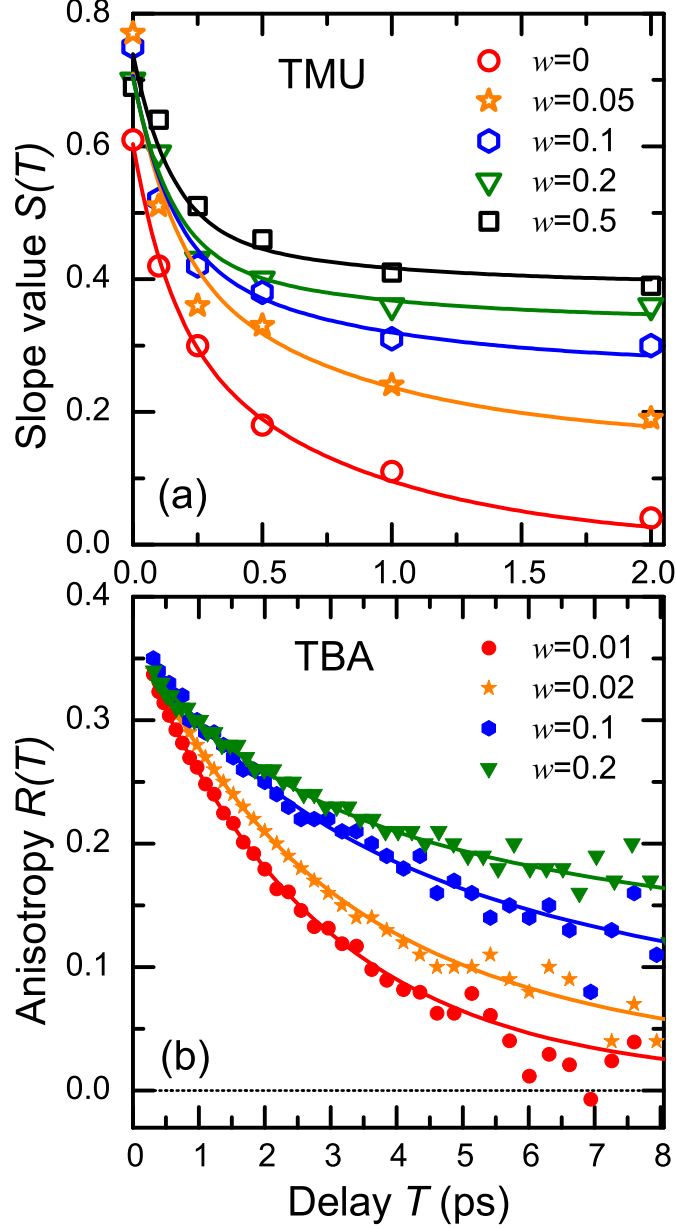
FIG. 3. Slope value $S(T)$ (Fig. a) and anisotropy $R(T)$ (Fig. b) as a function of the delay T for different concentrations. The slope values are calculated as an average over the range of +/- 50 cm^{-1} from the absorption maximum. The curves represent bi-exponential fits to the data using equations (3) and (4).
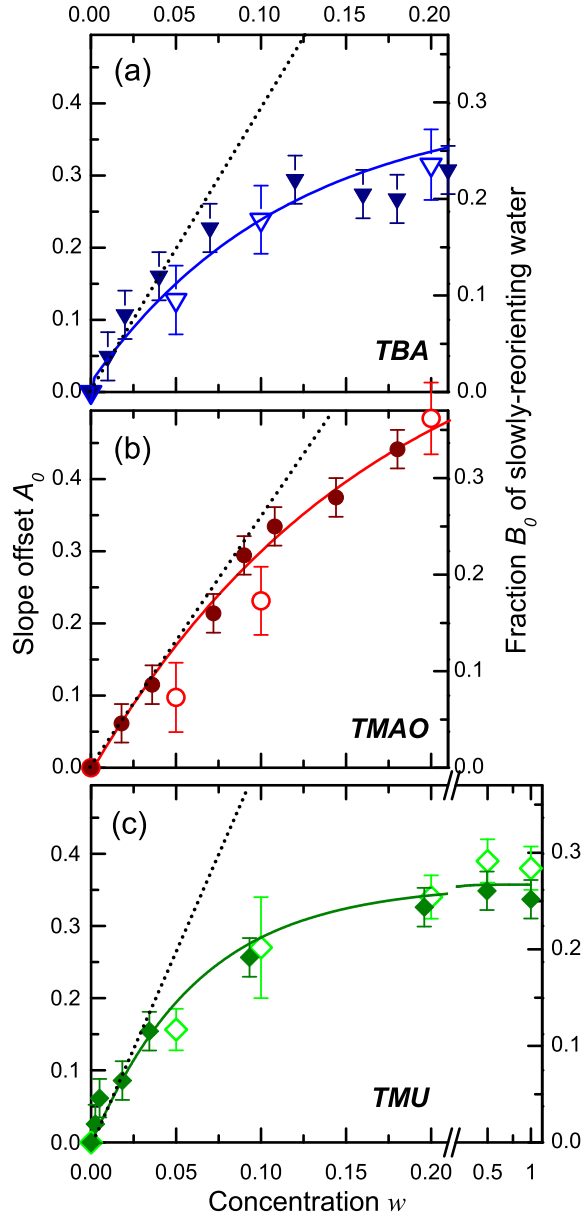
FIG. 4. Slope offset A_0 (left axis, open symbols) representing water showing slow spectral diffusion, and the fraction B_0 of slowly reorienting water (right axis, filled symbols) as a function of solute concentration w for solutions of TBA (a), TMAO (b) and TMU (c). The values of A_0 and B_0 are obtained from fits to the data using equations (3) and (4). The dotted lines represent linear fits to the low-concentration data. The anisotropy data for the TMAO and TMU solutions are obtained from previous work [16,17].
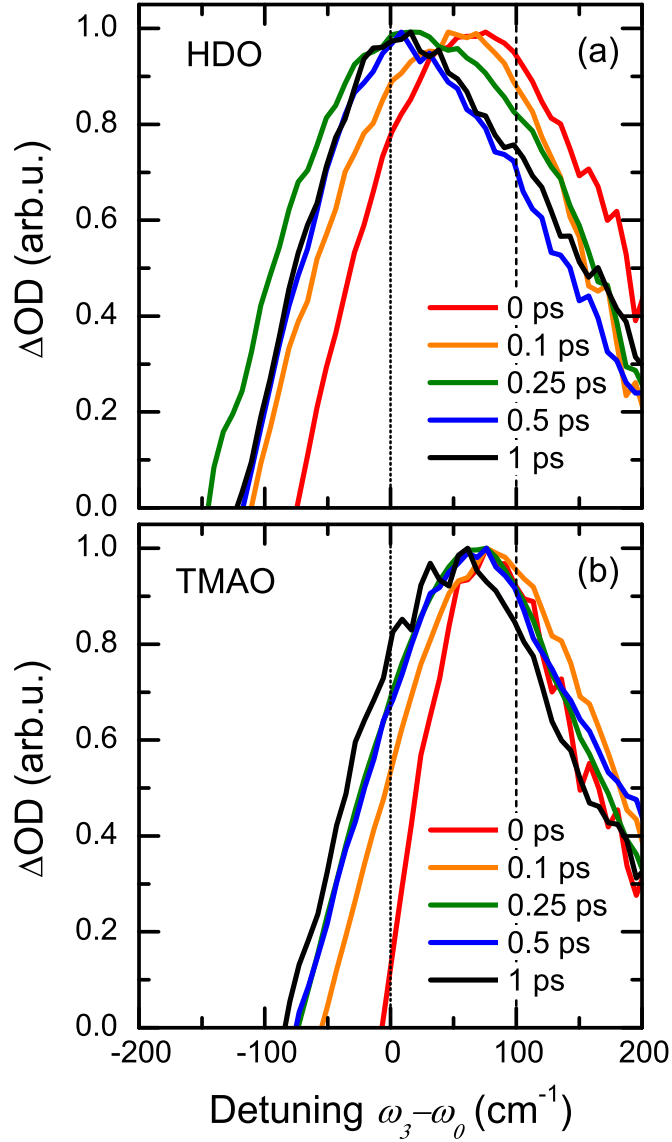
FIG. 5. Cross-sections through the 2D spectra (Fig. 2) for pure HDO:D_2O (a), and a solution of TMAO in HDO:D_2O with $w = 0.2$ (b) measured for $\omega_1 - \omega_0 = 100$ cm^{-1}. Only the positive part (i.e. due to the ground-state bleach and excited-state stimulated emission) of the cross-sections is presented. The dashed and dotted lines show frequencies of the on-diagonal excitation and equilibration, respectively
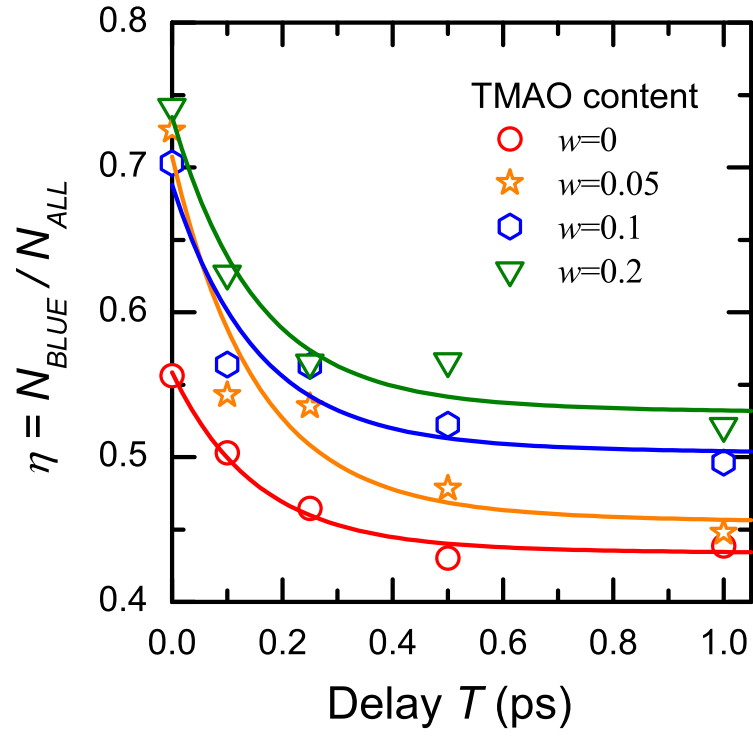
FIG. 6. Fraction η of high-energy oscillators after excitation at $\omega_1 - \omega_0 = 100$ cm^{-1} as a function of waiting time T for four different solutions of TMAO dissolved in HDO:D$_2$O. The lines are guides to the eye.
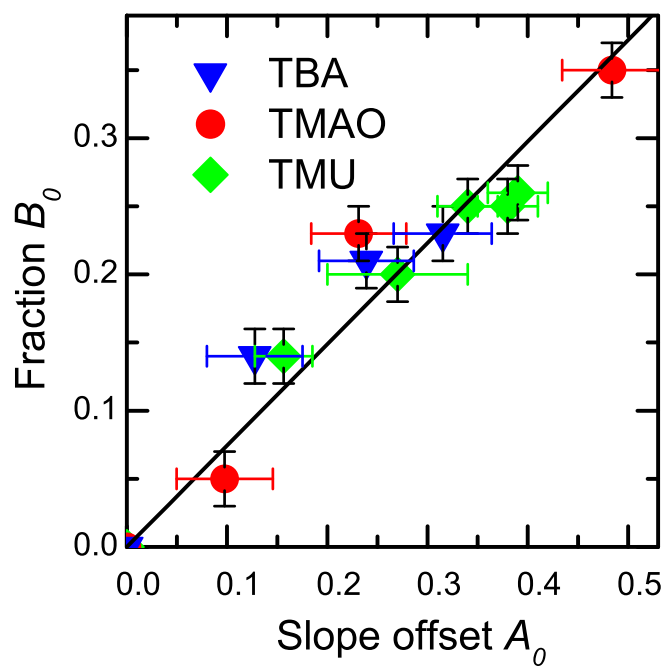
FIG. 7. The fraction B_0 of slowly reorienting water molecules as a function of the slope offset A_0 representing the water molecules showing slow spectral diffusion, for all measured solutes and concentrations.
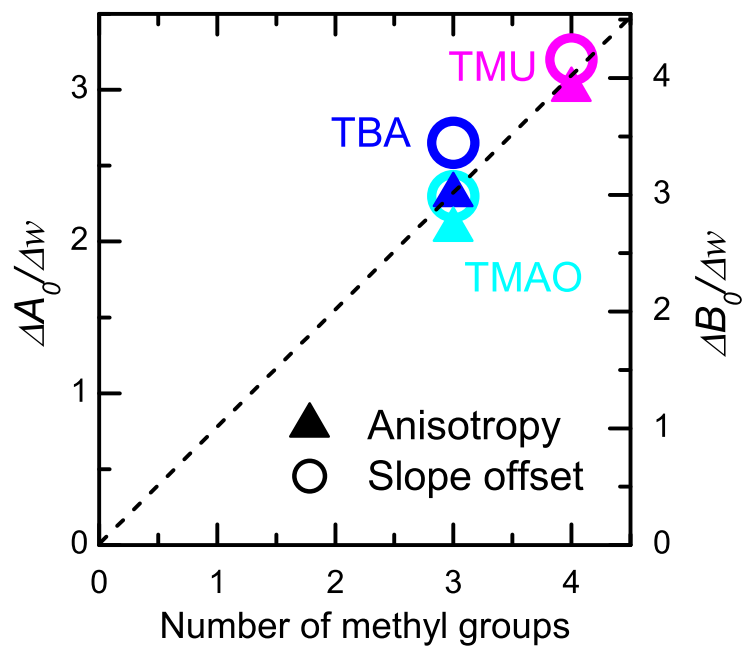
FIG. 8. Slopes of the dotted lines of Figure 4 (= $\Delta A_0/\Delta w$) as a function of the number of methyl groups contained in the solute (open circles). For comparison also the derivative of the fraction B_0 of slowly reorienting water molecules with respect to the concentration (= $\Delta B_0/\Delta w$) is presented (solid triangles).