Anomalous Dynamics in tert-Butyl Alcohol–Water and Trimethylamine N-Oxide–Water Binary Mixtures: A Femtosecond Transient Absorption Study

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Supporting Information

ABSTRACT: In this article, we have investigated the unusual dynamics of tert-butyl alcohol (TBA)—water and trimethylamine N-oxide (TMAO)—water binary mixtures using solvation dynamics as a tool. For this purpose, femtosecond transient absorption spectroscopy has been employed. Although these two molecules are isosteres to each other, a significant difference in water dynamics has been observed. The solvation times in TBA–water binary mixtures are found to be between 1.5 and 15.5 ps. On the contrary, we have observed very fast dynamics in TMAO–water binary mixtures (between 210 and 600 fs). Interestingly, unusual retardation in dynamics is observed at 0.10 mole fraction of TBA and TMAO in both the binary mixtures.

1. INTRODUCTION

In recent years, small amphiphilic molecules have gained a lot of attention from the researchers because of their fascinating behavior. For example, the molecule tert-butyl alcohol (TBA) does not favor the folded state of protein;1,2 whereas its isostere trimethylamine N-oxide (TMAO) stabilizes the native state.3–5 Another amphiphilic molecule dimethyl sulfoxide acts as a protein stabilizer in an aqueous binary mixture below the mole fraction of 0.15. After this concentration, an opposite effect has been observed.6 Therefore, the structural features of different amphiphiles and their extent of interactions with the solvent molecules are very much important to predict their behavior. Considering the two small organic molecules TBA and TMAO, both have the same hydrophobic composition (i.e., three methyl groups). On the contrary, TBA contains a tertiary butyl group, and TMAO has an N-oxide moiety in their hydrophilic counterparts (Scheme 1). In spite of this small difference, the aqueous solution behavior of the two molecules is quite different. Previous literature reports have suggested self-aggregation of TBA molecules in dilute aqueous solutions; however, TMAO does not exhibit a similar tendency.7,8 It is reported that TMAO forms an aggregate with water by strong hydrogen bonding.8 In a word, the solution behavior should depend on the type of aggregation (i.e., TBA–TBA or TMAO–water).

Scheme 1. Chemical Structures of the Materials Used in This Study

TBA–water binary mixtures have been extensively investigated by several groups using different techniques. Initial attempts, based on the “iceberg” model developed by Frank and Evans, clathrate-hydrate formation with water, and so forth,
have been taken to explain the nonideal behavior. However, recent neutron diffraction and small-angle neutron scattering experiments have pointed out the association of TBA molecules in TBA−water binary mixtures. Several molecular dynamics (MD) simulation studies have predicted the aggregation tendency of TBA molecules. It has been reported that the aggregation behavior of TBA depends on the applied force fields and the size of simulated systems. They have used four force fields to simulate TBA−water binary mixtures; two of them agree in favor of TBA aggregation. Using all the four force fields, they have observed demixing of TBA molecules above 0.1 mole fraction of TBA. However, TBA is soluble in water at any concentrations. Recently, Ben-Amotz and Rankin have observed the absence of hydrophobic contacts in alcohol−water binary mixtures. Shirota and Castner have reported the nonideal behavior of aqueous 1-propanol binary mixtures between 0.15 and 0.25 mole fraction of propanol by measuring the solvation dynamics of coumarin 153. Biswas and co-workers have found saturation in the activation energy for the conversion of the locally excited state (LES) to the charge-transfer state [of 4-(1-azetidinyl)-benzonitrile] at 0.10 mole fraction of TBA. In a recent study, we have shown the anomalous slowdown in solvation dynamics between 0.09−0.15 and 0.40−0.46 mole fractions of TBA in TBA−water binary mixtures. These two specific regions are completely correlated with the Bagchi and co-workers’ MD simulation work.

On the other hand, neutron-scattering experiments have suggested that 2−3 water molecules are strongly hydrogen-bonded to one TMAO. Mid-infrared femtosecond spectroscopy has been used to predict the immobilization of four water molecules by every methyl group of TMAO. The orientational dynamics of comparatively immobile (around the hydrophobic group of TMAO) hydroxyl groups of water are 4 times slower than the mobile one (i.e., bulk like). Interestingly, the reorientation time of mobile hydroxyl groups decreases with the increasing TMAO concentration. This is probably due to the TMAO-induced increased network defects of water. Similar slowdown is found in the spectral and orientational dynamics of D2O in aqueous TBA and TMAO solutions. Therefore, they have concluded that at low concentrations of the two solutes, the hydrophilic part does not play any role in the water dynamics. On the contrary, using the MD simulation study, Hynes and co-workers have not observed the slowing down of water motion. Ab initio MD (AIMD) and force field MD simulations have been employed to unravel the effects of TMAO on the reorientational dynamics of D2O. Interestingly, they have found that AIMD simulation is the right method to predict the slowdown of reorientational dynamics of D2O because of the formation of the strong O−D−O TMAO hydrogen bond. However, the force field model cannot predict the actual scenario because of the inability to account for a specific directionality of the hydrogen bond. In the TMAO−water mixture, water molecules do not get a chance to denature the protein because of their involvement in solvation of TMAO. The same group has also suggested the association of TMAO molecules in aqueous solution. Raman and infrared spectroscopy experiments have not found the aggregation propensity of TMAO molecules. Rather, the evidence for the TMAO−water complex has been provided by terahertz and Raman spectroscopy.

Although a lot of individual studies have been performed on TBA−water and TMAO−water systems, comparative information from the experimental background is very few. Using heterodyne-detected vibrational sum frequency generation spectroscopy, the preferential orientation and expulsion/
accumulation of TMAO and TBA molecules in the air−water interface have been detected.45 They have found that TMAO is preferentially excluded from the air−water interface and remains at the water layer just below the interface. On the other hand, TBA is preferentially accumulated in the interface maintaining the “methyl-up” orientation. In a recent study, Nagata and co-workers have shown that TMAO is present near the air−water interface.46,47 A similar observation is also reported using MD simulations.48 In addition, TMAO induces a change in the angular distribution of the O−H group of water in the interface. Solvation dynamics is an important spectroscopic technique to measure the dynamic response of solvent molecules. Several groups have studied the solvation dynamics in binary mixtures and in neat solvents using different ultrafast measurements such as time-correlated single photon counting and fluorescence upconversion,49−57 femtosecond transient absorption spectroscopy,58−64 two-dimensional infrared,65 nuclear Overhauser enhancement spectroscopy, and so forth.65 In this article, we have investigated the solvation dynamics of TBA−water and TMAO−water binary mixtures up to 0.20 mole fraction of both solutes (TBA and TMAO) through femtosecond transient absorption measurements. For this purpose, coumarin 343 (C343) is used as a solvation probe. It has been observed that the timescales of water dynamics in TBA−water and TMAO−water systems are between 1.5−15 ps and 200−600 fs, respectively. Interestingly, we have found the retardation of solvation dynamics at 0.1 mole fraction of both solutes. To the best of our knowledge, this is the first report on the solvation dynamics of TBA−water and TMAO−water binary mixtures using femtosecond transient absorption spectroscopy.

2. RESULTS AND DISCUSSION

2.1. Steady-State Absorption and Emission Studies. The steady-state UV−visible absorption spectra of C343 in TBA−water and TMAO−water binary mixtures are shown in Figure 1a,b, respectively. In water, the absorption maximum of C343 is centered at 428 nm. With increasing TBA content up to $X_{TBA} = 0.10$, we have observed a red shift (up to 451 nm) after this concentration, a blue shift is found up to $X_{TBA} = 0.20$ (445 nm). A similar result is obtained in the case of TMAO−water binary mixtures. For this purpose, coumarin 343 (C343) is used as a solvaton probe. It has been observed that the timescales of water dynamics in TBA−water and TMAO−water systems are between 1.5−15 ps and 200−600 fs, respectively. Interestingly, we have found the retardation of solvation dynamics at 0.1 mole fraction of both solutes. To the best of our knowledge, this is the first report on the solvation dynamics of TBA−water and TMAO−water binary mixtures using femtosecond transient absorption spectroscopy.

Figure 2. Femtosecond TDAS of C343 in TBA−water [(a) $X_{TBA} = 0.06$, (b) $X_{TBA} = 0.10$, and (c) $X_{TBA} = 0.18$] and TMAO−water [(d) $X_{TMAO} = 0.06$, (e) $X_{TMAO} = 0.10$, and (f) $X_{TMAO} = 0.18$] binary mixtures [pumping wavelength: 415 nm].
up to $X_{TMAO} = 0.10$ (445 nm) and after that blue shift up to $X_{TMAO} = 0.20$ (438 nm). We have plotted the absorption maxima of C343 as a function of TBA and TMAO concentrations in Figure S1 (Supporting Information). On the other hand, the emission maximum of C343 in water is 490 nm. With increasing TBA and TMAO contents, we have observed a red shift in the emission maxima (Figure 1c,d). However, the extent of the red shift in TBA–water binary mixtures (490–501 nm) is greater than that in the TMAO–water system (490–494 nm).

Therefore, the UV–visible absorption results indicate that at $X_{TBA,TMAO} = 0.10$, the solution behavior is different from other concentrations (Figure S1). However, we have not found a similar behavior in the steady-state emission spectra. Sen and co-workers have found the composition-dependent steady-state absorption behavior of the DCM dye in chloroform–methanol binary mixtures. However, a similar behavior was absent in the steady-state emission spectra. Again, the composition-dependent behavior comes into picture in solvation dynamics measurements. Our observation is very much similar to their study. They have explained the composition-dependent absorption behavior in terms of synergistic solvation (probe molecules are solvated by the combined component of solvents in binary mixtures). However, the ground-state solvation structure becomes destroyed because of the generation of a large dipole moment upon excitation, and as a result, disappearance of excited-state synergism has been observed. This means that the synergism disappears slowly during the course of relaxation and vanishes completely at the relaxed state (RS) (steady-state emission). We can follow up the time-resolved relaxation process by studying the solvation dynamics. Therefore, we can verify whether the ground-state solution structure at $X_{TBA,TMAO} = 0.10$ affects the relaxation dynamics or not.

### 2.2. Solvation Dynamics Study in TBA–Water and TMAO–Water Binary Mixtures.

In this section, we would like to discuss the water dynamics in TBA–water and TMAO–water binary mixtures in detail. For this purpose, we have recorded femtosecond transient difference absorption spectra (TDAS) of C343 in TBA–water and TMAO–water binary mixtures with the increasing solute concentration up to $X_S = 0.20$ at a regular interval of $X_S = 0.02$ ($X_S$ represents the mole fraction of the solute). TDAS between 350 and 650 nm at $X_{TBA} = 0.06, 0.10$, and 0.18 and $X_{TMAO} = 0.06, 0.10$, and 0.18 are shown in Figure 2a–c,d–f, respectively. In each plot, TDAS at different delay times (time between pump and probe) are shown by using various colors. Cyan lines correspond to the TDAS at negative delay time, that is, the signal is not generated. As reported earlier, we have observed three bands in the transient signal of C343. Two excited-state absorption bands (positive signal) have been found between 350–400 nm and above 600 nm. Between this region, the bleach component from 400 to 600 nm consists of the ground-state bleach (GB) and induced fluorescence [stimulated emission (SE)]. The bump at around 460 nm is responsible for the GB. The band at the red side is for the SE. As it is reported that during the solvation process, a red shift is observed only in the SE band for coumarin derivatives, and it is very easy to monitor the SE signal of C343 for solvation calculations. To differentiate the phenomena between fluorescence and SEs, we have collected transient absorption spectra of C343 in the TBA–water system at 0.10 mole fraction of TBA [Figure S2, Supporting Information]. Below the zero delay condition (the probe pulse is followed by a pump pulse), we have observed fluorescence signals (inset of Figure S2) with a very small amplitude ($\sim 0.003$). On going from $\sim 0.5$ to 0.5 ps, an SE band is generated with an amplitude of $\sim 0.078$. The amplitude of the SE band is 26 times greater than that of fluorescence.

Now, we explain the mechanism of solvation dynamics in brief. When C343 is excited using a pump pulse, this excitation causes redistribution of electronic charges within the molecule. As a result, there is a huge increase in the dipole moment on going from the ground state to the excited state. As soon as the dipole is created, the solvent molecules start to rearrange themselves around the newly formed dipole. We have successfully monitored this relaxation process by using white-light continuum (WLC) probe pulses and observed that two states are involved in the solvent relaxation process: that is, the LES (observed at very short delay time) and the RS (observed at long delay time). As the TDAS produce time-resolved spectra directly, we do not need to construct them like in other techniques. Additionally, nonemissive states can be detected using TDAS. These are the advantages of TDAS over the time-resolved fluorescence technique. By measuring the SE emission signal of C343, Cole has performed the solvation dynamics of C343 in aerosol-OT reverse micelles.

In our system, at $X_{TBA} = 0.06$ (Figure 2a), the SE band appears from 100 fs, and the maximum of this band is $\sim 470$ nm. With increasing delay time, we have observed a red shift in the SE signal, and the maximum reaches to $\sim 491$ nm (RS). The red shift continues up to the delay time of 43.2 ps. Note that the maximum of the SE band (491 nm) at 43.2 ps delay time exactly matches with the steady-state emission maxima [Figure 1c]. This is an indication of complete relaxation. On the other hand, at $X_{TBA} = 0.10$ and 0.18 (Figure 2b,c), the maxima of the SE band have been found $\sim 450$ nm (LES) at a delay time of 100 fs. Similar to $X_{TBA} = 0.06$, we have observed a red shift in the SE band up to 116 and 67.8 ps for $X_{TBA} = 0.10$ and $X_{TBA} = 0.18$, respectively. In all the three cases, the red shift with increasing delay time is an indication of solvation dynamics. The difference between $X_{TBA} = 0.06$ and $X_{TBA} = 0.10$ and 0.18 is that the fastest excited-state relaxation occurs in the former case (complete relaxation within 43.2 ps) than the other two cases (116 and 67.8 ps). The slowest relaxation is observed at $X_{TBA} = 0.10$. On the other hand, the red shift completes within 3–4.9 ps for all mole fractions of TMAO in TMAO–water binary mixtures (Figure 2d–f). Only in the case of $X_{TMAO} = 0.10$, the red shift continues up to the delay time of 4.9 ps. In other regions, a similar shift saturates within 3–3.5 ps. This indicates faster water dynamics in TMAO–water binary mixtures compared to the TBA–water system.

To get quantitative information about the solvation dynamics in these two binary mixtures, we have determined the maxima of the SE signal as a function of time. From these data, we have plotted the solvent correlation function $C(t)$, expressed by the following equation

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}$$

where $\nu(0)$, $\nu(t)$, and $\nu(\infty)$ are the peak frequencies of the SE band at time zero, an intermediate time, and infinity, respectively. The solvent correlation functions are fitted by using biexponential or triexponential functions depending on the nature of plots. A triexponential function $C(t)$ is expressed as
\[ C(t) = a_1 \exp^{-t/\tau_1} + a_2 \exp^{-t/\tau_2} + a_3 \exp^{-t/\tau_3} \]  

(2)

where \( \tau_1, \tau_2 \), and \( \tau_3 \) are the solvation times with amplitudes of \( a_1, a_2 \), and \( a_3 \), respectively. Solvent correlation functions at different mole fractions of TBA and TMAO in TBA–water and TMAO–water binary mixtures are shown in Figure 3a,b and c,d, respectively. From the individual time components of solvation dynamics and their relative contributions, we have calculated the average solvation time \( \langle \tau_s \rangle \) using the following equation.

\[ \langle \tau_s \rangle = a_1 \tau_1 + a_2 \tau_2 + a_3 \tau_3 \]  

(3)

Solvation parameters in TBA–water and TMAO–water binary mixtures are tabulated in Tables 1 and 2, respectively. It is reported that the major portion of solvation dynamics of C343 in neat water completes within 50 fs.67,68 Maroncelli and co-workers have observed the two time components (126 and 880 fs) of C343 in water.68 From Table 1, we have observed that with the increasing TBA concentration, one long-lifetime component is generated having a lifetime of 15–50 ps. The contribution of this component increases from 10 to 25% on going from \( X_{\text{TBA}} = 0.06 \)–0.10. After \( X_{\text{TBA}} = 0.10 \), the lifetime as well as the contribution of this component decreases up to \( X_{\text{TBA}} = 0.16 \). Again at \( X_{\text{TBA}} = 0.18 \) and 0.20, another long-lifetime component having a lifetime of 16–23 ps appeared. As the binary mixtures are complicated systems, instead of explaining individual components, we have discussed our results on the basis of the average solvation time. We have observed that the average solvation time in TBA–water binary mixtures is between 1.5 and 15.5 ps with a maximum at around \( X_{\text{TBA}} = 0.10 \) (\( \tau_s = 15.35 \) ps). On the other hand, in TMAO–water binary mixtures, the average solvation times are between 210 and 600 fs (Table 2). The lifetimes of fast components are between 90 and 120 fs which completely correlated with the

Table 1. Solvation Parameters of C343 in TBA–Water Binary Mixtures with Increasing TBA Content

| system \((X_{\text{TBA}})\) | \(\tau_1 (a_1) \) (ps) | \(\tau_2 (a_2) \) (ps) | \(\tau_3 (a_3) \) (ps) | \(\langle \tau_s \rangle \) (ps) |
|----------------|----------------|----------------|----------------|----------------|
| 0.02          | 0.10(0.51) | 3.20(0.49) | 1.62          | |
| 0.04          | 0.19(0.75) | 4.76(0.25) | 1.33          | |
| 0.06          | 4.67(0.90) | 15.26(0.10) | 5.73          | |
| 0.08          | 5.33(0.84) | 25.24(0.16) | 8.52          | |
| 0.10          | 0.05(0.46) | 9.90(0.29) | 49.84(0.25) | 15.35         |
| 0.12          | 5.82(0.79) | 12.17(0.21) | 7.15          | |
| 0.14          | 5.48(0.83) | 11.58(0.17) | 6.52          | |
| 0.16          | 5.06(0.80) | 12.05(0.20) | 6.48          | |
| 0.18          | 0.11(0.66) | 3.07(0.13) | 22.79(0.21) | 5.26          |
| 0.20          | 0.10(0.61) | 4.67(0.10) | 16.74(0.29) | 5.38          |

Error in experimental measurements ±5%.

Table 2. Solvation Parameters of C343 in TMAO–Water Binary Mixtures with Increasing TMAO Content

| system \((X_{\text{TMAO}})\) | \(\tau_1 (a_1) \) (ps) | \(\tau_2 (a_2) \) (ps) | \(\langle \tau_s \rangle \) (ps) |
|----------------|----------------|----------------|----------------|
| 0.02          | 0.10(0.83) | 0.84(0.17) | 0.23          | |
| 0.04          | 0.09(0.84) | 0.87(0.16) | 0.21          | |
| 0.06          | 0.09(0.81) | 0.85(0.19) | 0.23          | |
| 0.08          | 0.10(0.75) | 0.95(0.25) | 0.31          | |
| 0.10          | 0.12(0.70) | 1.48(0.30) | 0.53          | |
| 0.12          | 0.11(0.69) | 1.68(0.31) | 0.60          | |
| 0.14          | 0.10(0.83) | 0.88(0.17) | 0.23          | |
| 0.16          | 0.11(0.85) | 0.82(0.15) | 0.22          | |
| 0.18          | 0.11(0.84) | 0.83(0.16) | 0.22          | |
| 0.20          | 0.12(0.83) | 0.81(0.17) | 0.24          | |

Error in experimental measurements ±5%.
previous study. Interestingly, the long component lifetime increases from 0.95 to 1.48 ps from $X_{\text{TMAO}} = 0.08−0.10$. At $X_{\text{TMAO}} = 0.12$, and it reaches to 1.68 ps (nearly 2 times of the long component of neat water). It is to be noted that the water–water hydrogen bond lifetime is increased 3.8 times in the presence of 1 M TMAO. The contribution of this (long) component also increases from 19 to 31% from $X_{\text{TMAO}} = 0.06$ to 0.12. We have obtained the highest solvation time between $X_{\text{TMAO}} = 0.10$ and 0.12. Although the average solvation times are significantly different for both the systems, we have observed retardation of water dynamics at around $X_{\text{TMAO}} = 0.10$.

Recently, we have reported the retardation of solvation dynamics between $X_{\text{TBA}} = 0.09−0.15$ and $X_{\text{TBA}} = 0.40−0.46$ in TBA–water binary mixtures using femtosecond fluorescence upconversion measurements of coumarin 480 (C480). In this study, we have also observed the slowdown in solvation dynamics at around $X_{\text{TBA}} = 0.10$ using femtosecond transient absorption measurements of C343 (Figure 4a). Additionally, at around $X_{\text{TBA}} = 0.10$, the average solvation time is almost similar for two cases (18.84 and 15.35 ps). This observation indicates that the solvation dynamics of the TBA–water system is independent of the probe and measurement techniques. To see whether there is any effect of C343 on the dynamics of the TMAO–water system, we have employed a different dye, C480. We have measured the transient absorption spectra of C480 in the TMAO–water binary mixture (at $X_{\text{TMAO}} = 0.10$) [Figure S3, Supporting Information]. Similar to the case of C343, a red shift in the SE band is observed with increasing probe delay. The red shift saturates at 4.75 ps. In C343, similar saturation occurs at 4.9 ps. Therefore, the dynamics of the TMAO–water system is probe-independent.

Generally, solvation dynamics are related to physical observables (viscosity, thermodynamics, etc.). Thermodynamic properties exhibit deviation from the ideal behavior at 0.04 mole fraction of TBA. The viscosity of the binary mixture reaches a maximum at around $X_{\text{TBA}} = 0.40$. However, there is no report of a similar deviation at $X_{\text{TBA}} = 0.10$. Therefore, the retardation of the solvation time at $X_{\text{TBA}} = 0.10$ is not related to the physically observable. The possible explanation for this slowdown at $X_{\text{TBA}} = 0.10$ is the structural transition induced by TBA aggregation from the waterlike network to the alcohol-like zigzag structure. Biswas and co-workers have shown the structural transition from the waterlike tetrahedral network to the zigzag alcohol-like structure at around $X_{\text{TBA}} = 0.10$ in TBA–water binary mixtures. It has been reported that with the increasing TBA concentration, aggregation tendency increases and reaches its maximum in the range of $X_{\text{TBA}} = 0.14−0.17$. The aggregation of TBA molecules at around $X_{\text{TBA}} = 0.10$ is further supported by other groups. Hence, our result is completely in line with the other reports. Therefore, the long-lifetime component of 15−50 ps is probably responsible for large TBA clusters. On the other hand, similar slowdown in solvation dynamics is also found at $X_{\text{TMAO}} = 0.10−0.12$ in TMAO–water binary mixtures (Figure 4b). Several groups have reported the formation of strong hydrogen bonds between the O–H group of water and the $O_{\text{TMAO}}$ atom. Therefore, this strong hydrogen bond reduces the number of free water molecules that are available to hydrate the biomolecules. Thus, TMAO can act as a stabilizer of protein. Indra and Biswas have shown that long hydrogen-bond fluctuation lifetime is because of the strong TMAO–water interaction. Interestingly, TMAO stabilizes the compact state of a polymer up to 1 M concentration; after this concentration, it exerts an opposite action.

This observation indicates that TMAO is unable to form a strong hydrogen bond with the water molecules after a certain concentration and thus destabilizing the compact state of the polymer. Using broadband dielectric spectroscopy, one slower relaxation mode is found at a 4 M concentration of TMAO ($X_{\text{TMAO}} = 0.10$). Sudden increase in the lifetime as well as in the contribution of slow solvation component between $X_{\text{TMAO}} = 0.10$ and 0.12 indicates that the TMAO–water complexes become most stable, and this reduces the availability of free water molecules to slowdown the relaxation process. After this concentration, the TMAO–water complexes may not remain intact. As a result, the dynamics become faster again. Therefore, we feel that our probable explanation of observed composition dependence of the TMAO–water system is in complete correlation with the previous literature report.

Interestingly, on going from $X_{\text{TBA}} = 0.02$ to 0.04, we have observed a decrease in the average solvation time (Table 1). The contribution of the fast component increases from 51% to 75%. It is reported that aggregation of TBA excludes nearby water molecules to the bulk. As a result, the polarity increases and the solvation time decreases. Several thermodynamic properties of aqueous TBA exhibit deviation from the ideal behavior at $X_{\text{TBA}} = 0.04$. It should be mentioned here that in our previous study, we have identified this region by measuring the solvation dynamics using the “spectral reconstruction method”. However, the “single wavelength measurement method” could not detect it. Therefore, to extract the solvation information, the TDAS method is more accurate and time-saving than the time-resolved fluorescence measurements.

To further corroborate our results, we have fitted the experimental decay curves obtained from TDAS measurements.
We have chosen a particular probe wavelength (550 nm) and fitted the data for TBA–water and TMAO–water systems at $X_{TBA,TMAO} = 0.10$. Experimental decay curves have been fitted using an exponentially decaying function, $\sum A_i \exp(-t/\tau_i)$, convoluted with an instrument response function ($\tau_i$ and $A_i$ are the decay times of individual components and their corresponding contributions, respectively). Fitted decay curves are shown in Figure 5a,b. From fitting, three time constants obtained at $X_{TBA} = 0.10$ are 0.60 ps (0.25), 5.7 ps (0.35), and 41.4 ps (0.40). On the other hand, the values for $X_{TMAO} = 0.10$ are 0.15 ps (0.82), 2.25 ps (0.17), and 15.6 ps (0.01). It has been reported that the three time components of C343 in ethanol are 100−300 fs, 3 ps, and 28 ps. These are attributed to the solvation time scales. In our case, the timescales of C343 are completely correlated with the previous literature report. There are two methods available in the literature to calculate the solvation time (spectral reconstruction and single wavelength method). In the “spectral reconstruction” method, fluorescence transients of a probe are measured at different emission wavelengths in a particular medium using the fluorescence upconversion technique. Time-resolved emission spectra (TRES) can be obtained by proper fitting of fluorescence transients. One can calculate the solvent correlation function $C(t)$ (this provides solvation timescales) with the help of TRES. In the “single wavelength” method, the timescales of a single wavelength transient can successfully represent the solvation dynamics of a system. In our previous studies, we have shown that the results of “spectral reconstruction” and “single wavelength” measurement methods agree with one another. In this study, we have tried to correlate the solvation times of both the systems calculated through the “spectral reconstruction” method (by measuring the red shift in the SE band) with the time constants of a 550 nm transient. Discussion regarding the choice of the single wavelength is given in our earlier publication. Interestingly, we have found a nice agreement between the timescales of the two methods. In addition, the results of “single wavelength” fitting provides a similar dynamical trend with the information of the “spectral reconstruction” method (the dynamics of the TBA–water system is much more retarded compared to the TMAO–water system at $X_{TBA} = 0.10$). We have shown in our earlier study that although the timescales of the two methods are different, they can provide a similar dynamical trend. Therefore, we feel that in this study, the result of the “spectral reconstruction” method is completely corroborated by the information from “single wavelength” fitting.

The aggregation of TBA molecules creates a tremendous heterogeneity within the solution. As a result, we have observed a massive slowdown (greater than 25 fold) in the solvation dynamics compared to its isosteric response. Irrespective of the timescales of solvation dynamics, we have observed deviations from the ideal behavior in both the binary mixtures. According to the ideal behavior, any solution property should change linearly between two neat solvents. However; we have observed deviation at 0.10 mole fraction of both solutes. These deviations exactly match with the results of absorption measurements (see Figure S1). Therefore, the ground-state solution structure clearly affects the relaxation dynamics and completely disappears at RES. The difference in dynamics between these two systems is due to different hydrophilic groups of two isosteric molecules. Sen and co-workers have reported synergistic solvation (combined component of solvent participates in solvation dynamics) in hydrogen bond-donating and hydrogen bond-accepting solvent pairs (binary mixture). If one of the components of the binary mixtures has no hydrogen bond-donating or hydrogen bond-accepting groups, the mechanism of solvation is preferential (probe molecule is solvated by a particular solvent). As both of our systems (TBA–water and TMAO–water) contain hydrogen bond-donating and hydrogen bond-accepting solvent pairs, we feel that the mechanism of solvation is synergistic in our case. It is very much important to provide a connection between our two studies (present study and ref 30). Several MD simulation studies have reported the self-aggregation of alcohol molecules in alcohol–water binary mixtures. However, Patey and co-workers have pointed out the force field-dependent aggregation behavior of alcohol molecules. This study motivates us to perform a thorough investigation of the TBA–water system experimentally. In a comparative study, we have shown that the aggregation tendency of TBA is more prominent compared to ethanol (as the dynamics of the TBA–water system is slower than that of the ethanol–water system).

Previously, we have studied the dynamics of two self-aggregated systems. TBA and TMAO show very different behaviors in terms of their aggregation in water and toward the vapor–water interface. In view of their (TBA vs TMAO) very different behavior to the stability of proteins, it is very interesting to investigate the water dynamics. We have tried to probe the solvation dynamics of TBA–water and TMAO–water binary mixtures using the fluorescence upconversion technique [Figure S4, Supporting Information]. For this purpose, we have collected fluorescence upconversion traces of C343 for $X_{TBA} = 0.10$ and $X_{TMAO} = 0.10$ (maximum retarded regions) at two different emission wavelengths (530 and 510 nm). In the TBA–water system, a significant rise component (signature of solvation dynamics) is observed at the red-end.
side of the emission spectrum (Figure S4a,b). However, the dynamics of the TMAO–water system is very fast, and rise components are not at all clear (or very small contribution, Figure S4c,d). It is very difficult to construct the solvent correlation function \([C(t)]\) by measuring the wavelength-dependent fluorescence of C343 in the TMAO–water system using fluorescence upconversion [the cross-correlation measured between the second harmonic and the fundamental had a full-width at half-maximum of 300 fs]. On the other hand, transient absorption spectroscopy (pulse width 50 fs) directly measures the time-dependent emission spectra of C343 in the TMAO–water system, and we have successfully resolved the difference in dynamics at \(X_{\text{TMAO}} = 0.10\) (time-dependent red shift in the SE spectrum saturates at 4.9 ps) with the other regions (the same shift saturates between 3 and 3.5 ps). To compare this result (TMAO–water system) with that of the TBA–water system, we have re-investigated the system (TBA–water) with transient absorption spectroscopy. Interestingly, the dynamics of the TBA–water system measured through two different ultrafast techniques agree with one another and indicate the slowdown in the solvation response at \(X_{\text{TBA}} = 0.10\) within a window of \(X_{\text{TBA}} = 0.0–0.20\). In view of this good agreement between the two methods, we feel that transient absorption spectroscopy provides interesting dynamical information about the TMAO–water system (which is not possible using fluorescence upconversion measurements) and also makes it possible to compare the TBA–water and TMAO–water binary mixtures.

Solvation dynamics is an important tool to probe the dynamics of binary mixtures. In contrast to the literature debate, the similar dynamical results of the TBA–water system between our two studies and a nice dynamical comparison between TBA–water and TMAO–water systems (this is not possible using fluorescence upconversion techniques) are the novelties of our work.

3. CONCLUSIONS

In conclusion, unusual dynamics of TBA–water and TMAO–water binary mixtures have been investigated using solvation measurements. We have observed that the solvation response in TMAO–water binary mixtures is much faster than that in the TBA–water system. Interestingly, retardation in solvation dynamics has been found at 0.10 mole fraction of both the solutes. TBA aggregation-induced structural transition and formation of TMAO–water complexes are the probable reasons for this retarded solvation dynamics at 0.10 mole fraction of TBA and TMAO.

4. EXPERIMENTAL SECTION

4.1. Materials. Laser-grade C343 and C480 are bought from Exciton. Spectroscopic grade TBA and TMAO are purchased from Spectrochem Pvt. Ltd. and Sigma-Aldrich, respectively. Triple-distilled Milli-Q water is used to prepare TBA–water and TMAO–water solutions. We have maintained pH 7 for all solutions. Scheme 1 represents the chemical structures of all the materials used for this experiment.

4.2. Instruments and Methods. Steady-state UV–vis and fluorescence measurements are performed using a Shimadzu (model UV 2450) UV–vis spectrophotometer and a Hitachi (model no. F-7000) spectrophuorimeter (wavelength resolution is 0.5 nm), respectively. Ultrafast transient absorption measurements are carried out with a commercially available transient absorption spectrometer from Newport Corp. For this experiment, a Ti:sapphire (Coherent Inc., Libra) regenerative amplifer laser system is used as the main laser source. The output of the regenerative amplifier is centered at 808 nm, at a repetition rate of 1 kHz with an average pulse energy of 3 mJ and a pulse width of 50 fs. The whole beam is split into two parts by a 70/30 (R/T) beam splitter. The major portion of the beam is used to generate a pump beam at 415 nm from an optical parametric amplifier (TOPAS-Prime, Light Conversion). A small portion of the fundamental beam is allowed to pass through a computer-controlled motorized delay stage (Newport Corp, ILS-LM) and is used to generate a WLC probe beam with a spectral range of 350–850 nm by focusing it onto a 3 mm CaF\(_2\) crystal. Pump and probe beams are focused on the sample with spot sizes of 210 and 80 \(\mu\)m, respectively. Maximum time delay that can be achieved between the pump and the probe is 3 ns. After passing through the sample, the WLC probe is collected by a fiber-coupled spectrometer with an Si photodiode array (Oriel Instrument, MS-260i). Samples are kept in a cuvette of path length 1 mm for measurement, and they are continuously stirred with a magnetic stirring system. A block diagram of the instrument is provided in Scheme S1 (Supporting Information).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01595.

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Notes
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REFERENCES

(1) von Hippel, P. H.; Wong, K.-Y. On the Conformational Stability of Globular Proteins: The Effects of Various Electrolytes and Nonelectrolytes on the Thermal Ribonuclease Transition. J. Biol. Chem. 1965, 240, 3909–3923.
(2) Cordone, L.; Izzo, V.; Sgroi, G.; Fornili, S. L. Effect of Some Monoalcoholic Alcohols on the Functional Stability of Bovine Liver Beta-Galactosidase. Biopolymers 1979, 18, 1965–1974.

(3) Yancey, P.; Clark, M.; Hand, S.; Bowls, R.; Somero, G. Living with Water Stress: Evolution of Osmolyte Systems. Science 1982, 217, 1214–1222.

(4) Zou, Q.; Bennon, B. J.; Daggett, V.; Murphy, K. P. The Molecular Mechanism of Stabilization of Proteins by TMAO and Its Ability to Counteract the Effects of Urea. J. Am. Chem. Soc. 2002, 124, 1192–1202.

(5) Bennon, B. J.; DeMarco, M. L.; Daggett, V. Preventing Misfolding of the Prion Protein by Trimethylamine N-Oxide. Biochemistry 2004, 43, 12955–12963.

(6) Banerjee, S.; Roy, S.; Bagchi, B. Enhanced Pair Hydrobicity in the Water—Dimethylsulfoxide (DMSO) Binary Mixture at Low DMSO Concentrations. J. Phys. Chem. B 2010, 114, 12875–12882.

(7) Paul, S.; Patey, G. N. Why tert-Butyl Alcohol Associates in Aqueous Solution but Trimethylamine-N-oxide Does Not. J. Phys. Chem. B 2006, 110, 10514–10518.

(8) Freda, M.; Onori, G.; Santucci, A. Infrared Study of the Hydrophobic Hydration and Hydrophobic Interactions in Aqueous Solutions of tert-Butyl Alcohol and Trimethylamine-N-oxide. J. Phys. Chem. B 2001, 105, 12714–12718.

(9) Frank, H. S.; Evans, M. W. Free Volume and Entropy in Condensed Systems III. Entropy in Binary Liquid Mixtures; Partial Molal Entropy in Dilute Solutions; Structure and Thermodynamics in Aquoelectrolytes. J. Chem. Phys. 1945, 13, 507–532.

(10) Murthy, S. N. Detailed Study of Ice Clathrate Relaxation: Evidence for the Existence of Clathrate Structures in Some Water–Alcohol Mixtures. J. Phys. Chem. A 1999, 103, 7927–7937.

(11) Dixit, S.; Crain, J.; Poon, W. C. K.; Finney, J. L.; Soper, A. K. Molecular Segregation Observed in a Concentrated Alcohol–Water Solution. Nature 2002, 416, 829–832.

(12) Soper, A. K.; Finney, J. L. Hydration of Methanol in Aqueous Solution. Phys. Rev. Lett. 1993, 71, 4346–4349.

(13) Finney, J. L.; Bowron, D. T.; Daniel, R. M.; Timmins, P. A.; Roberts, M. A. Molecular and Mesoscale Structures in Hydrophobically Driven Aqueous Solutions. Biophys. Chem. 2003, 105, 391–409.

(14) Dougan, L.; Bates, S. P.; Hargreaves, R.; Fox, J. P.; Crain, J.; Finney, J. L.; Réat, V.; Soper, A. K. Methanol-Water Solutions: A Biperculating Liquid Mixture. J. Chem. Phys. 2004, 121, 6456–6462.

(15) Bowron, D. T.; Finney, J. L.; Soper, A. K. Structural Investigation of Solute-Solute Interactions in Aqueous Solutions of Tertiary Butanol. J. Phys. Chem. B 1998, 102, 3551–3563.

(16) Bowron, D. T.; Soper, A. K.; Finney, J. L. Temperature Dependence of the Structure of a 0.06 Mole Fraction Tertiary Butanol-Water Solution. J. Chem. Phys. 2001, 114, 6203–6219.

(17) D’Arrigo, G.; Teixeira, J. Small-Angle Neutron Scattering Study of D2O–Alcohol Solutions. J. Chem. Soc., Faraday Trans. 1990, 1503–1509.

(18) Tanaka, H.; Nakanishi, K. Structure of Aqueous Solutions of Amphiphiles: t-Butyl Alcohol and Urea Solutions. Fluid Phase Equilib. 1993, 83, 77–84.

(19) Kusak, P. G.; Lyubartsev, A. P.; Bergman, D. L.; Laaksonen, A. Computer Simulation Study of tert-Butyl Alcohol. 2. Structure in Aqueous Solution. J. Phys. Chem. B 2000, 104, 9533–9539.

(20) Lee, M. E.; van der Vegt, N. F. A. A New Force Field for Organic Solutes. J. Chem. Phys. 2009, 131, 074509.

(21) Shulgin, I.; Ruckenstein, E. Kirkwood-Buff Integrals in Aqueous Solutions. J. Phys. Chem. Lett. 2015, 6, 1696–1701.

(22) Rack, M.; van der Post, S. T.; Bakker, H. J. Contacts between Alcohols in Water Are Random Rather Than Hydrophobic. J. Phys. Chem. Lett. 2015, 6, 688–692.

(23) Shirotai, H.; Castner, E. W. Solvation in Highly Nonideal Solutions: A Study of Aqueous 1-Propanol Using the Coulmarin 153 Probe. J. Chem. Phys. 2000, 112, 2367–2376.

(24) Pradhan, T.; Ghoshal, P.; Biswas, R. Excited State Intra-molecular Charge Transfer Reaction in Binary Mixtures of Water and Tertiary Butanol (TBA): Alcohol Mole Fraction Dependence. J. Phys. Chem. A 2008, 112, 915–924.

(25) Banik, D.; Roy, A.; Kanda, N.; Sarkar, N. Picosecond Solvation and Rotational Dynamics: An Attempt to Reinvestigate the Mystery of Alcohol–Water Binary Mixtures. J. Phys. Chem. B 2015, 119, 9905–9919.

(26) Meersman, F.; Bowron, D.; Soper, A. K.; Koch, M. H. J. Counterraction of Urea by Trimethylamine-N-oxide is Due to Direct Interaction. Biophys. J. 2009, 97, 2559–2566.

(27) Rezus, Y. L. A.; Bakker, H. J. Observation of Immobilized Water Molecules Around Hydrophobic Groups. Phys. Rev. Lett. 2007, 99, 148301.

(28) Rezus, Y. L. A.; Bakker, H. J. Destabilization of the Hydrogen-Bond Structure of Water by the Osmolyte Trimethylamine-N-Oxide. J. Phys. Chem. B 2009, 113, 4038–4044.

(29) Bakulin, A. A.; Pshenichnikov, M. S.; Bakker, H. J.; Petersen, C. Water Molecules Slow Down the Hydrogen-Bond Dynamics of Water. J. Phys. Chem. A 2011, 115, 1821–1829.

(30) Laghe, D.; Stirmann, G.; Hynes, J. T. Why Water Reorientation Slows without Iceberg Formation Around Hydrophobic Solutes. J. Phys. Chem. B 2009, 113, 2428–2433.

(31) Usui, K.; Hunger, J.; Sulpiz, M.; Ohito, T.; Bonn, M.; Nagata, Y. Ab Initio Liquid Water Dynamics in Aqueous TMAO Solution. J. Phys. Chem. B 2015, 119, 10597–10606.

(32) Sarma, R.; Paul, S. Trimethylamine-N-oxide’s Effect on Polyol Hydration at High Pressure: A Molecular Dynamics Simulation Study. J. Phys. Chem. B 2013, 117, 9056–9066.

(33) Hunger, J.; Tielrooij, K.-J.; Buchner, R.; Bonn, M.; Bakker, H. J. Complex Formation in Aqueous Trimethylamine-N-oxide (TMAO) Solutions. J. Phys. Chem. B 2012, 116, 4783–4795.

(34) Mazur, K.; Heisler, I. A.; Meech, S. R. THz Spectra and Dynamics of Aqueous Solutions Studied by the Ultrafast Optical Kerr Effect. J. Phys. Chem. B 2011, 115, 2563–2573.

(35) Kuroda, Y.; Kimura, M. Vibrational Spectra of Trimethylamine Oxide Dihydrate. Spectrochim. Acta 1966, 22, 47–56.

(36) Freda, M.; Onori, G.; Santucci, A. Infrared and Dielectric Spectroscopy Study of the Water Perturbation Induced by Two Small Organic Solutes. J. Mol. Struct. 2001, 565–566, 153–157.

(37) Di Michele, A.; Freda, M.; Onori, G.; Santucci, A. Hydrogen Bonding of Water in Aqueous Solutions of Trimethylamine-N-oxide and tert-Butyl Alcohol: A Near-Infrared Spectroscopy Study. J. Phys. Chem. A 2004, 108, 6145–6150.

(38) Munro, K. L.; Rogers, D. H.; Hammer, N. I. Raman Spectroscopic Signatures of Noncovalent Interactions Between Trimethylamine N-oxide (TMAO) and Water. J. Phys. Chem. B 2011, 115, 7699–7707.

(39) Knake, L.; Schwaab, K.; Kartaschew, K.; Havenith, M. Solvation Dynamics of Trimethylamine N-Oxide in Aqueous Solution Probed by Terahertz Spectroscopy. J. Phys. Chem. B 2015, 119, 13842–13851.
(45) Ahmed, M.; Namboodiri, V.; Mathi, P.; Singh, A. K.; Mondal, J. A. How Osmylate and Denaturant Affect Water at the Air–Water Interface and in Bulk: A Heterodyne-Detected Vibrational Sum Frequency Generation (HD-VSFG) and Hydration Shell Spectroscopic Study. J. Phys. Chem. C 2016, 120, 10252−10260.

(46) Otto, T.; Backus, E. H. G.; Mizukami, W.; Hunger, J.; Bonn, M.; Nagata, Y. Unveiling the Amphiphilic Nature of TMAO by Vibrational Sum Frequency Generation Spectroscopy. J. Phys. Chem. C 2016, 120, 17435−17443.

(47) Otto, T.; Hunger, J.; Backus, E. H. G.; Mizukami, W.; Bonn, M.; Nagata, Y. Trimethylamine-N-Oxide: Its Hydration Structure, Surface Activity, and Biological Function, Viewed by Vibrational Spectroscopy and Molecular Dynamics Simulations. Phys. Chem. Chem. Phys. 2017, 19, 6909−6920.

(48) Fiore, A.; Venkateshwaran, V.; Garde, S. Trimethylamine N-Oxide (TMAO) and tert-Butyl Alcohol (TBA) at Hydrophobic Interfaces: Insights from Molecular Dynamics Simulations. Langmuir 2013, 29, 8017−8024.

(49) Zhang, X.; Liang, M.; Hunger, J.; Buchner, R.; Maroncelli, M. Dielectric Relaxation and Solvation Dynamics in a Prototypical Ionic Liquid + Dipolar Protonic Liquid Mixture: 1-Butyl-3-Methylimidazolium Tetrafluoroborate + Water. J. Phys. Chem. B 2013, 117, 15356−15368.

(50) Mukherjee, S.; Sahu, K.; Roy, D.; Mondal, S. K.; Bhattacharyya, K. Solvation Dynamics of 4-Aminophthalimide in Dioxane−Water Mixture. Chem. Phys. Lett. 2004, 384, 128−133.

(51) Molotsky, T.; Huppert, D. Solvation Statics and Dynamics of Coumarin 153 in Dioxane−Water Solvent Mixtures. J. Phys. Chem. A 2003, 107, 8449−8457.

(52) Paul, A.; Samanta, A. Effect of Nonpolar Solvents on the Solute Rotation and Solvation Dynamics in an Iodimetric Ionic Liquid. J. Phys. Chem. B 2008, 112, 947−953.

(53) Jarzba, W.; Walker, G. C.; Johnson, A. E.; Barbara, P. F. Nonexponential Solvation Dynamics of Simple Liquids and Mixtures. Chem. Phys. 1991, 152, 57−68.

(54) Gardeczi, J. A.; Maroncelli, M. Solvation and Rotational Dynamics in Acetonitrile/Propylene Carbonate Mixtures: A Binary System for Use in Dynamical Solvent Effect Studies. Chem. Phys. Lett. 1999, 301, 571−578.

(55) Chichos, F.; Willert, A.; Rempel, U.; von Borczyskowski, C. Solvation Dynamics in Mixtures of Polar and Nonpolar Solvents. J. Phys. Chem. A 1997, 101, 8179−8185.

(56) Sahu, K.; Mondal, S. K.; Roy, D.; Karmakar, R.; Bhattacharyya, K. Slow Solvation Dynamics of 4-AP and DCM in Binary Mixtures. J. Photochem. Photobiol., A 2005, 172, 180−184.

(57) Banik, D.; Kundu, N.; Kuchlyan, J.; Roy, A.; Banerjee, C.; Ghosh, S.; Sarkar, N. Picosecond Solvation Dynamics—a Potential Viewer of DMSO−Water Binary Mixtures. J. Chem. Phys. 2015, 142, 054505.

(58) Kwak, K.; Park, S.; Fayer, M. D. Dynamics around Solute and Solute Complexes in Mixed Solvents. Proc. Natl. Acad. Sci. U.S.A. 2007, 104, 14221−14226.

(59) Levinger, N. E.; Davis, P. H.; Fayer, M. D. Vibrational Relaxation of the Free Terminal Hydroxyl Stretch in Methanol Oligomers: Indirect Pathway to Hydrogen Bond Breaking. J. Chem. Phys. 2001, 115, 9352−9360.

(60) Piletic, I. R.; Gaffney, K. J.; Fayer, M. D. Structural Dynamics of Hydrogen Bonded Methanol Oligomers: Vibrational Transient Hole burning studies of Spectral Diffusion. J. Chem. Phys. 2003, 119, 423−434.

(61) Gaffney, K. J.; Davis, P. H.; Piletic, I. R.; Levinger, N. E.; Fayer, M. D. Hydrogen Bond Dissociation and Reformation in Methanol Oligomers Following Hydroxyl Stretch Relaxation. J. Phys. Chem. A 2002, 106, 12012−12023.

(62) Gupta, S.; Rafiq, S.; Sen, P. Dynamics of Solvent Response in Methanol−Chloroform Binary Solvent Mixture: A Case of Synergistic Solute. J. Phys. Chem. B 2015, 119, 3135−3141.

(63) Ghosh, R.; Mondal, J. A.; Palit, D. K. Ultrafast Dynamics of the Excited States of Curcumin in Solution. J. Phys. Chem. B 2010, 114, 12129−12143.