Spectroscopic Methods

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Terahertz Absorption Spectroscopy of a Liquid Using a Polarity Probe: A Case Study of Trehalose/Water Mixtures**

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Water is important for the structure, stability, and function of biomolecules. It can simplify the energy landscape for molecular recognition or protein folding and often controls the native stability.[1,2] Proton transfer through local water networks requires correlated movement of H-bonds,[3] and conformational changes of proteins appear to be coupled to the dynamics of bulk and hydration water.[4] To understand such processes the vibrational absorption spectrum of the biomolecule–water interface must be observed. The underlying dynamics are widely distributed in time, from fast vibrational modes to slow diffusive reorientation. In low-viscosity liquids, like water, the diffusive regime comprises processes on the picosecond to nanosecond timescale (corresponding to wavenumbers $\nu < 1.5\ cm^{-1}$), which are captured by microwave dielectric spectroscopy. On the high-frequency side (in water above $1000\ cm^{-1}$) intramolecular vibrations are observed by infrared absorption spectroscopy. What is usually lacking is information on the intermolecular vibrational and librational dynamics, which are reflected in the intermediate segment of the spectrum, in the THz (up to $30\ cm^{-1}$) and far-infrared (FIR, 30–$250\ cm^{-1}$) regions. It is just this intermediate regime in which processes associated with H-bond dynamics are expected. Unfortunately, the generation and detection of light is difficult here. Furthermore, the response of the biomolecule–water interface is generally not so different from that of bulk water. There is thus a clear need to develop local spectroscopic schemes which avoid contributions from the bulk and confine absorption measurements to the interfacial region.

Using the polarity probe *N*-methyl-6-quinolone (MQ, inset Figure 3) we recently showed that the time-resolved Stokes shift (TRSS) of fluorescence reflects the infrared spectrum of the surrounding liquid.[5] The effective distance for the interaction ranges up to approximately 15 Å;[6] spatial resolution of this size may therefore be achieved by linking the probe to the supramolecular structure of interest. What is missing to date is how to extract the (unknown) dielectric properties from a measured TRSS curve. This key step is introduced herein and tested with aqueous trehalose solutions. The disaccharide trehalose[7–12] (inset Figure 1) is synthesized by some organisms in dry climates for protection against osmotic pressure and freezing;[7] it alters the H-bonding structure of water[8] and modifies the collective dynamics.[9,10] Trehalose is therefore an intriguing biomolecular model solute for demonstrating the practical use and spectroscopic potential of MQ.

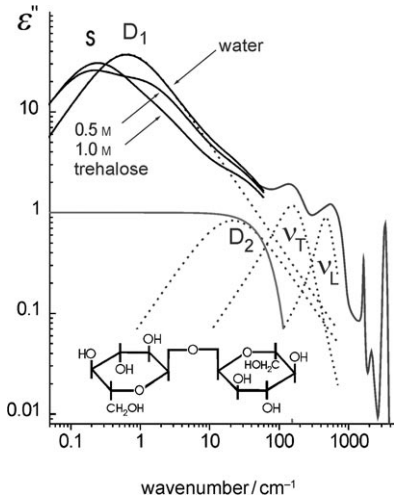

Figure 1. The dielectric loss of pure water (gray and corresponding black line) is modified by the addition of trehalose (inset). Intermolecular modes D_1, D_2, ν_T, and ν_L of pure water are shown separately as dashed lines. Mode S is assigned to rotational diffusion of the hydrated sugar. Black curves were determined from the time-resolved fluorescence Stokes shift of added methylquinolone. The observation window (gray) is determined by the effective time resolution.

To illustrate the kind of spectrum which must be recorded, Figure 1 shows the dielectric loss $\varepsilon''(\omega)$ of bulk water up to infrared frequencies.[13–16] This spectrum represents the background against which changes induced by the biomolecule have to be seen, therefore we describe it in a useful digression. $\varepsilon''(\omega)$ is essentially the oscillator strength distribution $\alpha(\omega)/\omega$, obtainable from the light attenuation coefficient $\alpha(\omega)$.[17,18] It determines[18] (apart from a constant) the dielectric dispersion $\varepsilon'(\omega)$ and thus the complex permittivity $\varepsilon(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega)$. The spectrum can be described by several bands.[16] The weak FIR band ν_T at $200\ cm^{-1}$ has substantial oscillator strength; it corresponds to translational vibration of the OH···O network.[16] Rotational diffusion is observed at lowest frequencies, in the microwave region around 20 GHz, and contributes

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a dominant Debye term D_1 together with a small Debye term D_2.[13,14] Librational water motion is observed in a band ν_L around 500 cm^{-1}.[14–16] All of these bands are broad (relative to the peak frequency) because intermolecular motion is easily perturbed, and thus the coherence strongly damped, compared to intramolecular oscillations, which are seen as narrow lines above 1000 cm^{-1}.

The addition of trehalose should induce spectral changes throughout, including in the terahertz regime (1 THz = 33 cm^{-1}) of major interest here. In fact, as the concentration of trehalose is increased to 1.2 M, the attenuation at 80 cm^{-1} decreases by almost 20%.[11] Herein, we observe how the water bands in Figure 1 are modified and which new bands appear, using MQ dissolved in the bulk solution as a "molecular spectrometer". In practice, we assume a realistic expression for $\varepsilon(\omega)$ and analytically derive $R(t)$, a form for the relaxation function which is used to fit the time-resolved Stokes shift of fluorescence. This new kind of TRSS spectroscopy will be explained below.

The probe functions as a microscopic terahertz light source when its charge distribution is suddenly altered by femtosecond optical excitation (Figure 2a).[5,19,20] In the case

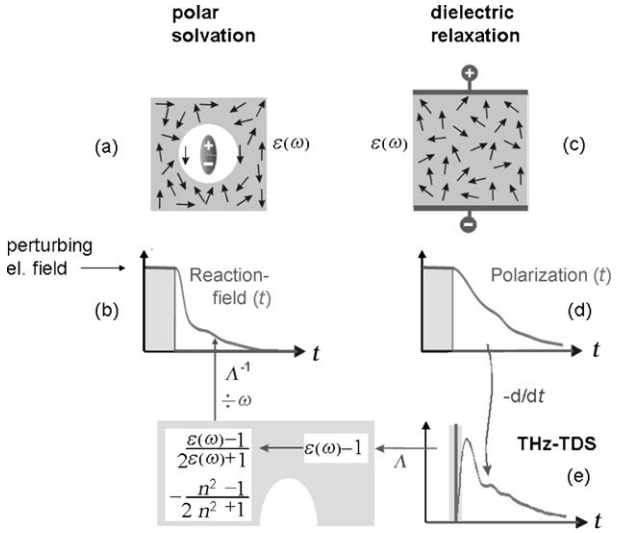

Figure 2. Bridge between spectroscopies: With a suitable molecular probe, polar solvation (a) may be described by continuum theory. A key quantity is the frequency-dependent permittivity $\varepsilon(\omega)$ of the medium. It is usually measured by dielectric relaxation (c) or terahertz time-domain spectroscopy (e). Herein, we measure the femtosecond solvation dynamics of methylquinolone (b) and find the corresponding value of $\varepsilon(\omega)$ quantitatively. (Λ is the Laplace transformation, and for simplicity $n_{cav} = 1$ has been assumed; see text.).

of MQ, $S_0 \rightarrow S_1$ excitation at 400 nm reduces the dipole moment μ from 10.8 to 5.8 D,[21] and the local electric field is switched down instantaneously. As the new field acts on nearby groups with partial charges, these reorient and collectively create the reaction field $R(t)$ (Figure 2b). The latter is reported by the polar probe molecule through an emission frequency which depends linearly on $R(t)$ (in this sense, the terms "reaction field" and "spectral relaxation function" are used synonymously). The probe is therefore not only light source but also detector. Compare this situation with classical dielectric relaxation measurements (Figure 2c), in which a capacitor containing the bulk liquid is suddenly discharged. The original polarization then relaxes with characteristic time behavior $P(t)$ (Figure 2d). By forming the time derivative the response function to a δ-shaped pulse is obtained (Figure 2e); the response can also be measured by terahertz time-domain spectroscopy (THz-TDS) more directly.[13,14] The dielectric susceptibility $\chi_P = \varepsilon(\omega) - 1$ is reached by Laplace transformation Λ of the response function.

A bridge from dielectric relaxation to solvation of a dipolar probe is provided by Equation (1):[18]

$$\chi_{dip}(\omega) \propto \frac{\varepsilon(\omega)-1}{2\varepsilon(\omega)+n_{cav}^2} - \frac{n_\infty^2-1}{2n_\infty^2+n_{cav}^2} \quad (1)$$

Here $\chi_{dip}(\omega)$ is the susceptibility of the dipole reaction field $R(t)$ to changes of μ, n_∞ is the refractive index of the medium at optical frequencies, and n_{cav} represents the polarizability of the solute.[18,22] Equation (1) is based on simple continuum theory, which was empirically shown to be valid, quantitatively, for MQ.[5] For water up to 100 cm^{-1}, the complex permittivity $\varepsilon(\omega)$ can be described by two Debye terms (see Figure 1).[13,14] To allow for spectral changes upon addition of trehalose, we write generally for this range a triple-Debye ansatz plus a background correction $\varepsilon_\infty \cong n_\infty^2$ for electronic displacement polarizations in the optical regime [Eq. (2)]:

$$\varepsilon(\omega) = \frac{\varepsilon_0-\varepsilon_1}{1+i\omega\tau_1} + \frac{\varepsilon_1-\varepsilon_2}{1+i\omega\tau_2} + \frac{\varepsilon_2-\varepsilon_\infty}{1+i\omega\tau_3} + \varepsilon_\infty \quad (2)$$

Each mode is characterized by a Debye relaxation time τ_k and an amplitude $\Delta\varepsilon_k = \varepsilon_{k-1} - \varepsilon_k$.

After passage through Equation (1) and division by $s \equiv i\omega$ (equivalent to time integration, cf. Figure 2) the inverse Laplace transform Λ^{-1} is carried out analytically. The parameters which enter the calculation are τ_1, τ_2, τ_3, and $\varepsilon_0, \varepsilon_1, \varepsilon_2, \varepsilon_\infty$, and n_{cav}. They determine a triple-exponential form for the reaction field $R(t)$, as outlined in the Supporting Information. The femtosecond experiment consists of optical excitation of MQ at 400 nm and subsequent broadband fluorescence upconversion[23,24] with 85 fs full width at half maximum (FWHM) of the temporal apparatus function. Figure 3 shows the absorption spectrum and several time-gated fluorescence spectra ($\propto$ quantum distribution over wavenumbers). The average emission wavenumber $\langle\nu\rangle$ as a function of time constitutes the spectral relaxation function $R(t)$, which is completely determined by solvation.[5] Experimental curves are shown in Figure 4 for 0.0, 0.5, and 1.0 M trehalose solutions.

These key data are fitted by optimizing $\varepsilon(\omega)$, resulting in the black interpolation lines in Figure 4. The low-frequency behavior is obtained from separate microwave measurements, which are reported in the Supporting Information. For fitting, the input parameters $\varepsilon_1, \varepsilon_2, \varepsilon_\infty, \tau_1, \tau_2, \tau_3$ are allowed to vary, while ε_0 is fixed to the microwave value. (This link with microwave data will no longer be needed when $R(t)$ becomes more precise in the long-time region.) The cavity refractive

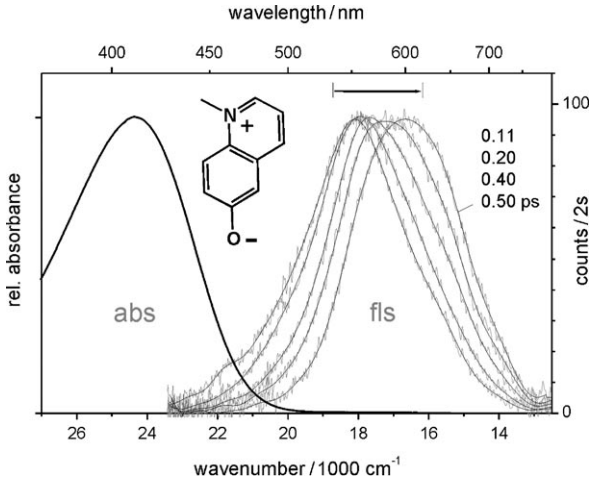

Figure 3. Absorption (abs) and fluorescence (fls) spectra of the polarity probe MQ (inset) in aqueous trehalose (1 M). After 40 fs excitation at 400 nm, the evolving emission was time-gated by broadband upconversion (85 fs FWHM resolution). The arrow indicates the observed range of the dynamic Stokes shift.

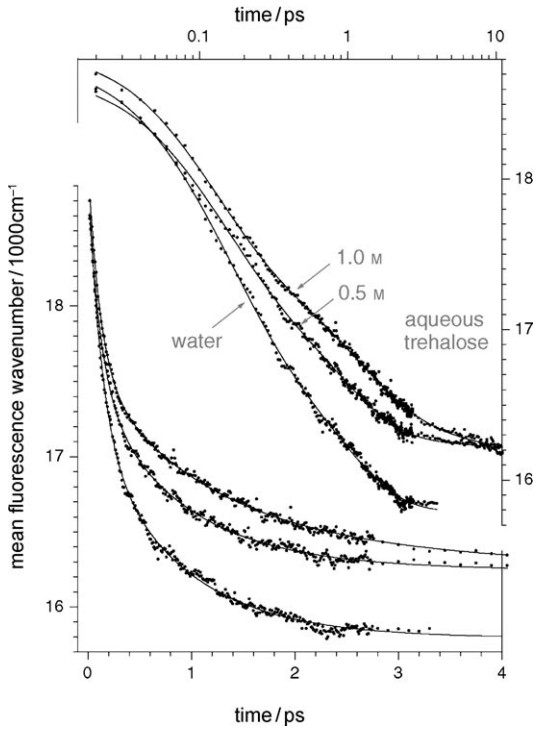

Figure 4. The mean fluorescence wavenumber (dots) relaxes on several timescales after femtosecond optical excitation. Fits by continuum theory (lines) yield $\varepsilon(\omega)$ curves for each solution (black lines in Figure 1).

index n_{cav} is governed by the effective polarizability of the probe and the cavity volume;[18,5] at this exploratory stage it must be treated specially as follows. For pure water the known $\varepsilon(\omega)$ is used to find $n_{cav} = 2.3$ from the time-resolved Stokes shift of MQ. The THz absorption spectrum of pure water is also calculated for reference.[21] Regarding the sugar solutions, note that the dielectric permittivity curve which is extracted from the femtosecond measurements depends on the value that was assumed for n_{cav}, and so does the attenuation coefficient $\alpha(\omega)$, which is derived.[17,18] That dependence can be used to obtain n_{cav} from absorption measurements at a fixed frequency. Havenith and co-workers[11] found that 0.5 M (1.0 M) trehalose reduces the absorption coefficient of water around 80 cm^{-1} by 7.2 (16.4) %. To reproduce this observation with the present data, we need to change n_{cav} slightly to 2.26 (2.0), which implies that the cavity volume increases with sugar concentration (see below).

The resulting $\varepsilon''(\omega)$ curves are shown in Figure 1 as black lines. They connect smoothly with the microwave results and obey the known attenuation at 80 cm^{-1}. When the trehalose concentration is raised to 0.5 M and then to 1.0 M, characteristic changes are observed:

1) A new relaxational mode S appears at 7.1 GHz. It is assigned to rotational relaxation of the hydrated trehalose solute by analogy to results from dielectric relaxation of maltotriose[25] and glucose[26,27] solutions, which were confirmed by depolarized Rayleigh scattering.[28]
2) The dynamics of the solution differ from those of pure water. The rotational water mode D_1 loses amplitude and is blue-shifted (becomes faster) as the trehalose concentration reaches 0.5 M, and it is strongly reduced when the concentration is raised further to 1.0 M. The water mode D_2 at higher frequency (ca. 0.14 THz or 5 cm^{-1}) seems less affected. Our observation agrees with dynamical studies,[9,10,29,30] which showed that a sugar solute alters the tetrahedral configuration of water molecules.
3) Between D_2 and ν_L no further distinct processes are observed. No new information is obtained for the single point at 2.5 THz, since here the FIR attenuation data from Havenith and co-workers[11] were used. Non-ideal quadratic behavior with increasing trehalose concentration was attributed to overlap of dynamical hydration shells. The dissolved polarity probe MQ should reside increasingly in such overlap regions, which explains why its cavity volume changes similarly with sugar concentration.

In summary: from the spectral relaxation $R(t)$ of *N*-methyl-6-quinolone fluorescence on 0.100–100 ps timescales, the frequency-dependent permittivity $\varepsilon(\omega)$ of the surrounding medium was extracted up to about 100 cm^{-1}. The key consists of an appropriate analytical connection $\varepsilon(\omega) \rightarrow R(t)$, which is needed for data fitting. Measurements with bulk trehalose/water solutions served to establish and test the method. Its unique feature is locality, that is, the possibility to measure $\varepsilon(\omega)$ around a supramolecular structure with a covalently connected or embedded probe, and across a broad spectral range.

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