Spectroscopy of solutions in the low frequency extended THz frequency range

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One of the most important biological components of the living systems is water. It is the main informative feature and simultaneously the main obstacle for tissue and bio-solution spectroscopy in THz range. Its intensive absorption in this frequency range, on the one hand, limits its penetration into the samples, but, on the other hand, allows one to consider it as the subject of a separate fruitful study [1-4]. Water molecules form hydrogen bonds (HB) with their neighbors to construct the water network. Currently, water can be divided on bulk water (it does not form bonds with biomolecules) and hydration or bounded water (it surrounds biomolecules and interacts with them). Thus, in the solution, a considerable part of water molecules are in the form of a hydration shell – Fig 1.
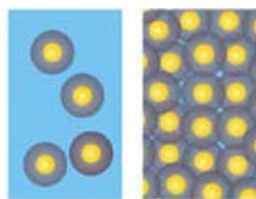

Fig. 1. Components of the solution: free water (blue background), bounded water (gray circles), solute molecules (yellow circles). Dilute solution (a) and saturated solution (b). After [4]

Bound or free water makes a valuable contribution to the THz response of biological objects. This allows us to use THz spectroscopy as a powerful tool to study different forms of water in a wide class of biological media, including the macromolecules in aqueous solutions. Actually, we do not measure the solute, its contribution is negligible. We measure how the solute modifies the solvent, the appearance and the structure of hydration shell around the solute molecules. To detect small changes in the solution smooth spectra, we should precisely describe solvent properties in a broad spectral range.

We describe a number of models of a water solution dielectric permittivity, which are applicable in the THz frequency range. We give a detailed description of biological solutions (protein and sugar solutions, blood components), analyze modern measuring techniques. All known processes (relaxation and dumped oscillation) in polar solutions below the tens of THz have very broadband responses, so it is essential to combine several experimental techniques, each for its own spectral range, to obtain spectra over many octaves. From the low frequency side, it is well established dielectric spectroscopy; from the high frequency side, it is FTIR and it is THz-time-domain spectroscopy (TDS) between them.

The main relaxation process (at 0.02 THz) reflects the cooperative reorientational dynamics of the dipole moment. It is assigned to the cooperative reorientation time of hydrogen-bonded bulk water molecules involving HB switching events. The oscillation processes indicates the overdamped modes, which correspond to several known vibration modes in the THz region: the bending mode between two water molecules forming the hydrogen-bond (at 1.5-2 THz); the intermolecular stretching of water is assigned to a hindered O...O translation (at 5.4 THz) *etc*.
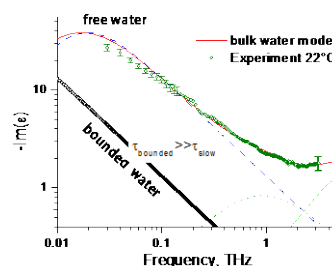

Fig. 2. Slow relaxation shift of bounded water spectra.

To fit dielectric function of water a well-known model with Debye-type relaxation and over-dumped oscillator components is usually applied [1-4].

$$\varepsilon(\omega, C) = \varepsilon_\infty + \frac{\Delta\varepsilon_1(C)}{1 + i\omega\tau_1} + \frac{\Delta\varepsilon_2}{1 + i\omega\tau_2} + \frac{A_1}{\omega_{01}^2 - \omega^2 + i\gamma_{01}\omega} + ... \tag{1}$$

where τ_1 and τ_2 are relaxation times for the first (main) relaxation process and the second ('fast') relaxation term; $\Delta\varepsilon_i$ are the contributions into permittivity from corresponding relaxation; A_1 is amplitude; w_{01} is frequency; γ_{01} is the linewidth of the oscillator term. Slow relaxation spectra of bounded water are shifted to much lower frequencies – Fig.2, thus, in GHz and THz range bounded water has smaller absorption and dispersion. That is the main reason of solution spectra changes. In general, we should use the effective medium approximation for the total spectrum; in a more simplified case, we should take into account the volume fractions of the three solution components with different spectral responses.

The most widely-exploited method of solution measurements using THz spectroscopy implies studying the THz-wave transmission through a thin (50–100 μm) cuvette. In such measurements, even a small

uncertainty of the analyte layer thickness might cause a noticeable error in the reconstructed THz dielectric response. Thus thick cuvette is preferable. It gives considerable advantages in low frequency part of the THz spectra, where water transmission is not so small. For the high THz frequencies, the attenuated total reflection (ATR) measurement approach is preferable, since it does not suffer from strong water absorption and implies measurements of a bulk sample; thus, eliminating an error of layer thickness measurements. Actually, these methods have considerable overlap of the spectral operation range; thus, we combine the results obtained by transmission and reflection in order to achieve a broader spectral operation range and more reliable data for water solution spectroscopy [1].

Once the water spectrum is precisely measured and described, one can proceed to water solution analysis. The spectra of aqueous protein or saccharides solutions with not extremely high concentration are almost completely determined by the spectral properties of the solvent water. Therefore, there are reasons for application of Eq. (1) to describe permittivity of such biological media as aqueous solutions. To detect small-scale changes in solutions we have normalized the transmittance (and reflectance) of the solution T_{BSA} to that of water – Fig 3.
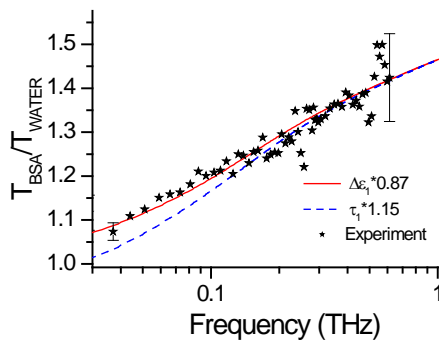
Fig 3. Transmission spectra of BSA 100 mg/ml water solution in a 500 μm cell, normalized to the case of water in the same cell, experimental data – open circles. Solid lines – model transmission with the same thickness and normalization. Red line with modified $\Delta\varepsilon_1 *0.87$ ($\Delta\varepsilon_1/\tau_1 = 0.87$) and blue line with modified $\tau_1 *1.15$ ($\Delta\varepsilon_1/\tau_1 = 0.87$).

To identify the reasons for the THz transmission changes of BSA solutions we have compared the experimental spectra to the relative transmission of modified model of water [1]. Such a simplified approach, implying variation of a single parameter $\Delta\varepsilon_1(C)$, describes well the experimental spectra of aqueous solutions (particularly, of sugars and proteins) in the frequency range of at least 0.03 to 3.2 THz, which is important for practical applications of THz-TDS systems. Such a difference is best fitted by a decrease in the amplitude of the slow relaxation amplitude $\Delta\varepsilon_1$. An alternative approach is to use $\tau_1(C)$ as the only varied parameter instead of $\Delta\varepsilon_1$. At frequencies above f=0.3 THz, it is mathematically almost equal to vary τ_1 or $\Delta\varepsilon_1$ because $\omega\tau_1 \gg 1$. In any case, in this THz band, 80 % of the spectral response is determined by the slow relaxation and its parameters, while contributions of the remaining processes in Eq. (1) are relatively small and weakly depend on the concentration.

Since there are practically no standards and calibrated instruments in the THz range, before studying a new system, it is necessary to achieve an agreement of the measured data with the well-known literature results on the THz dielectric response of some "reference" solute, for example, glucose.
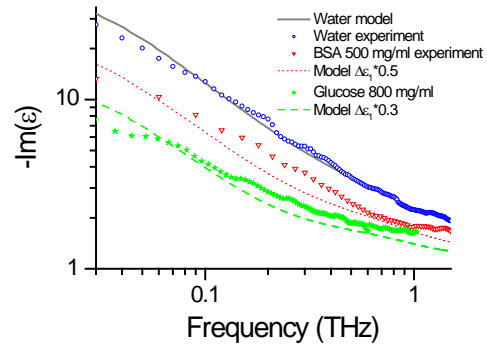
Fig 4. Dielectric function spectra of almost saturated solution of BSA (500 mg/ml) and of glucose (800 mg/ml) and of distilled water. Solid lines – model transmission with modified $\Delta\varepsilon_1 *0.5$ for BSA and $\Delta\varepsilon_1 *0.3$ for glucose. Symbols – experimental data.

Remarkable that different models lead to almost equal values of complex dielectric function in the frequency range where the models overlap.

Other important questions to be discussed in the talk are: Can we distinguish proteins from saccharides in water environment from the THz spectra shape? Are there narrow/resolvable spectral features of biomolecules in water environment below 5 THz? How can we extract the pure spectra of bounded water? What is useful in saturated solution spectra? When it is necessary to apply effective model for the solution?

Acknowledgements

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