

Properties of aqueous solutions in THz frequency range

O Cherkasova¹, M Nazarov^{2,3} and A Shkurinov^{2,4}

¹Institute of Laser Physics of SB RAS, Novosibirsk 630090, Russia

²Crystallography and Photonics Federal Research Center, RAS,
Moscow 117342, Russia

³Kurchatov Institute National Research Center, pl. Akad. Kurchatova 1,
Moscow 123182, Russia

⁴Lomonosov Moscow State University, Moscow 119991, Russia

E-mail: o.p.cherkasova@gmail.com

Abstract. Terahertz time-domain spectroscopy has been used for measuring of bovine serum albumin and glucose solutions response. The transmission and the attenuated total internal reflection geometries have been combined for analyzing the dielectric properties of aqueous solutions spectra at 0.07-3.2 THz.

1. Introduction

It is known that the terahertz (THz) signal is sensitive to the glucose concentration in blood in the case of in vivo reflection studies of human or animal skin [1]. The origin of that sensitivity is the properties of water - the main component of tissues and fluids in a body. The state of the water itself, which is prevailing in biological samples, represents a characteristic that can be measured using broadband THz spectroscopy [2, 3]. Unfortunately, THz time-domain spectroscopy (TDS) usually captures not the centre of a low-frequency 'peak' of water absorption (in the region near 0.03 THz), but its high-frequency 'tail', starting from the frequency of 0.2 THz (figure 1) [2]. Nevertheless, THz-TDS has become an established method which complements the existing methods of diagnostics of biological tissues and solutions. However, there is still no consensus how to interpret the THz signal contrast in scanning the skin in vivo and how to predict the changes in the THz response of solutions.

In present work the THz-TDS has been used for measuring of bovine serum albumin (BSA) and glucose solutions in a wide range of concentrations and with additional low frequency part (0.07-0.2) of the THz spectra. To detect reliably small-scale changes in solutions we have performed measurements using both transmission and in attenuated total internal reflection (ATR) configurations. Combining the results of both configurations, the reliable range of the obtained complex dielectric function spectrum can be considerably broadened to the most important low frequency part. We also analyzed the change in the THz absorption during incubation of BSA with glucose. This is a model experiment for the study of the process of protein glycation. Protein glycation is accelerated under hyperglycemic conditions resulting in loss of the structure and biological functions of proteins.

2. Experimental setup and samples

The THz time-domain spectrometer [4, 5] and the calculation methods [6, 7] are described in detail in our previous works. The measurements of solution parameters were performed sequentially using two THz time-domain spectrometers: a 'low-frequency' spectrometer – in transmission configuration using



“thick” -500- μm cell, and a ‘high-frequency’ spectrometer – in attenuated total internal reflection configuration using a Dove prism and also in transmission configuration for high frequencies using “thin” cell (100 μm). In the low-frequency spectrometer [5] a multi-dipole emitting antenna at an average power of 10 μW (in the THz range) has been used and the detection was performed by conventional single-dipole antenna. This efficient emitter allows one to transmit through a 0.5- μm -thick water layer with frequencies up to 1 THz. For a stable measurement of the extremely low for THz-TDS frequencies (~ 0.07 THz), a long time sampling of 40 ps with respect to the THz pulse centre has been applied.

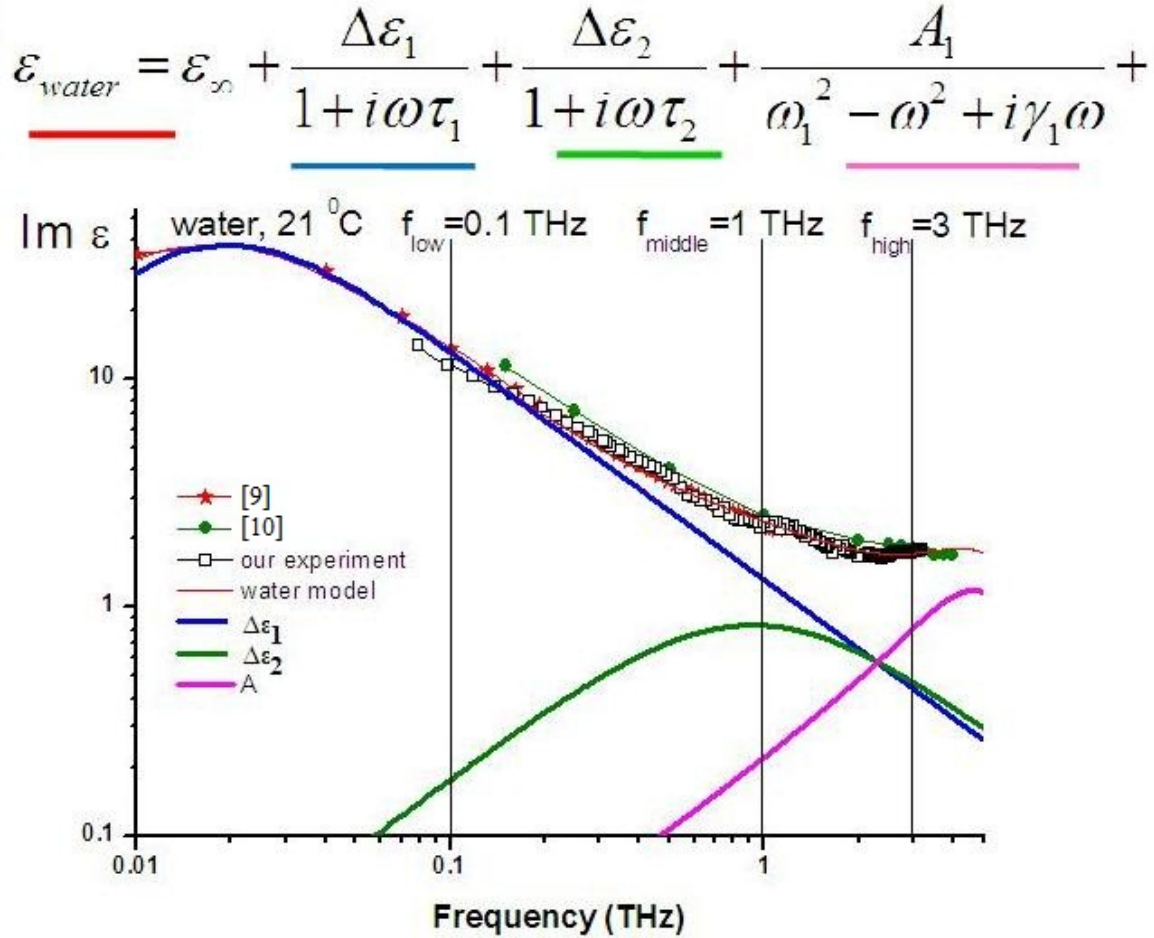


Figure 1. Spectra of the imaginary part of the dielectric permittivity $\text{Im}\{\varepsilon(f)\}$ of distilled water in the THz frequency range described by two-component Debye model.

In the high-frequency spectrometer [4], the LT-GaAs surface and a 1-mm-thick ZnTe crystal have been used as an emitter and a receiver, respectively [5]. The average-power THz radiation of 100 nW was sufficient to measure the reflection spectrum in the range of 0.3 – 3.2 THz. As the reference transmitted or reflected THz signal was used with the air or distilled water instead of the solution.

We used solutions of BSA and D-glucose (Sigma, USA) in a double-distilled water. Glucose solutions at a concentration of 270 – 840 mg mL^{-1} and BSA solutions at a concentration of 30 – 500 mg mL^{-1} were measured at the room temperature of 22°C. The solution volume for a single measurement in the ATR configuration constituted 800 μL (that provides a 1 mm thick layer on the prism surface), whilst in the transmission configuration, it was 400 μL (for 500 μm cell). To carry out

the glycation reaction, BSA was dissolved in phosphate buffer (50 mM, pH 7.4) at a concentration of 50 mg mL⁻¹ and incubated with glucose (0.5 M) for 96 hours at a temperature of $T = 47^\circ\text{C}$.

3. Approaches to the description of the dielectric function of solutions in the THz range

To fit dielectric function spectra $\varepsilon(f)$ we apply well-known two-component Debye model [7, 8] (figure 1). To clear out which process is responsible for THz sensitivity of biomolecule solution we divide available frequency range into three parts: “low” – slow relaxation dominates ($f \leq 0.1$ THz); “middle” – for fast relaxation ($0.1 < f < 3$) and “high” – for additional Lorentz term ($f > 3$ THz) (see figure 1). We choose appropriate experimental method for each spectral range (thick cell transmission (500 μm) for “low” frequencies, thin cell (100 μm) and ATR for “middle” frequencies, ATR for “high” frequencies). Actually all methods have considerable intersection in frequencies, so we combine the results obtained by different methods and this way obtain more precise data and a more broadband frequency range than ever published in THz literature for water solution spectroscopy.

Even for pure water, the multi-parametric model of dielectric function is not exact and applicable in a very wide frequency range. In describing a real solution with a single varied parameter (for example, the concentration of dissolved substances), it does not make any sense to vary plenty of parameters in the expression for dielectric function of the solution, as it is now accepted in most of the publications.

Our approach to the description of the dielectric function of protein or sugar solutions in the THz range is the following:

1. To systematize the known parameters of water model and refine the range of reasonable values for each of them at room temperature.
2. In our view, the reference parameters of each relaxation process should be taken from a corresponding frequency range and appropriate measuring method.
3. The slow Debye γ -relaxation is the only significant process and other terms/processes introduce negligible contribution in the changes of dielectric permittivity of aqueous solutions in THz-TDS frequency range

Thus, for approximating the dielectric function spectrum, certain constraints should be set on each parameter, proceeding from the data taken from the frequency range appropriate to each of the processes. In our case, to analyse the data in the range of 0.07 – 3.2 THz at room temperature (20 – 25 $^\circ\text{C}$), we select the following parameter ranges, which do not contradict the most reliable published data: $\varepsilon_\infty = 2.1\text{--}2.7$, $\Delta\varepsilon_1 = 72\text{--}75$, $\tau_1 = 8.3\text{--}9.5$ ps, $\Delta\varepsilon_2 = 1.4\text{--}2.1$, $\tau_2 = 130\text{--}360$ fs, $\varepsilon_s = 77\text{--}80.2$. We obtain for a most simple model the relevant set of parameters which accurately enough describe both our experimental results and the data published by other authors [9, 10] for distilled water in the frequency range of 0.07 – 3.2 THz (figure 1).

4. Results

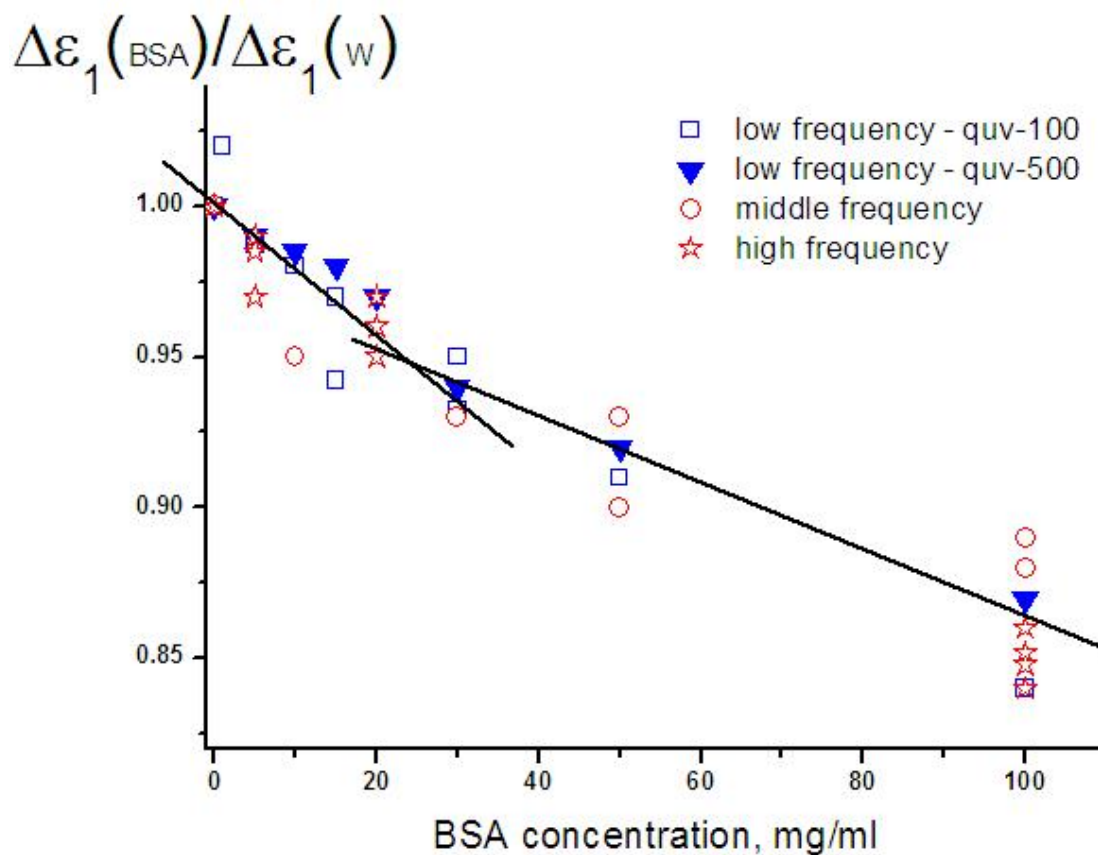
We analyzed the reasons for the THz transmission changes of studied solutions comparing experimental spectra to the model dielectric function of water. The insertion of glucose into water leads only to an increase of relaxation time τ_1 of the slow Debye process or as well to a decrease of relaxation amplitude of this solution (table 1). This simple approach describes observed spectral changes in a broad frequency range and for a number of concentrations from 10 mg/ml to saturated solution. The similar changes are observed for BSA spectra. Here ε_s – is the sum of amplitudes off all terms in the model, this value can be precisely measured by dielectric spectroscopy in low frequencies.

We perform fitting simultaneously for both Re and Im parts of $\varepsilon(f)$, which complicates an exact agreement in a wide frequency range, but increase the repeatability of obtained values. To describe the influence of solute concentration, we fit experimental complex spectra by a model only with the first Debye term's amplitude - $\Delta\varepsilon_1$ varied (see table 1). We suggest that, instead of complicating the models used for the analysis of experimental data, it is necessary to improve the accuracy and repeatability of experiments and to expand the spectral range of a measurement.

Table 1. The parameters of dielectric function for glucose and BSA solution.

C mg·mL ⁻¹	ϵ_s	ϵ_∞	$\Delta\epsilon_1$	τ_1 ps	$\Delta\epsilon_2$	τ_2 fs	ϵ 0.1 THz	ϵ 3.0 THz
0	78.5	2.5±0.1	75±6	9.5±0.8	1.5±0.05	230±30	7.8-i11.4	3.1-i1.7
270	60.0	2.49±0.1	53±2	9.5±0.3	1.6±0.05	230±20	6.9-i9.4	2.9-i1.5
810	34.3	2.5±0.02	29±2	9.5±0.3	1.7±0.03	230±20	6.4-i5.1	2.8-i1.3
100 (BSA)	64	2.5±0.02	65±2	9.5±0.3	1.6±0.03	230±20	7.3-i10.5	3.0-i1.5

As well we have found that increasing of BSA concentration in the solution results in a decrease of the amplitude $\Delta\epsilon_1$ (figure 2). We have not confirmed anomalous changes observed in papers [11, 12] at low concentrations and at low frequencies. The agreement with published data is only in following: the dependence of $\Delta\epsilon_1$ from concentration of a solute is nonlinear and has a bend at a particular concentration. We found this concentration for the case of BSA to be 30 ± 5 mg/ml.

**Figure 2.** The dependence of slow Debye process amplitude $\Delta\epsilon_1$ from BSA concentration in water.

Of practical interest is not a study of aqueous solutions of proteins or sugars separately, but a study of their mixtures. It is known that a high glucose level in human blood leads to glycation of proteins. The BSA ($50 \text{ mg}\cdot\text{mL}^{-1}$) was incubated in a phosphate buffer (50 mM, pH 7.4) in the presence of glucose (0.5 M) for 96 hours at $T = 47^\circ\text{C}$.

Figure 3 shows variations of transmission coefficient T_{int} during incubation of BSA with glucose. An averaged transmission coefficient of solution is normalized on an averaged transmission coefficient of pure water. The transmission coefficient of BSA incubated in buffer alone did not

change significantly. This indicates that BSA does not spontaneously modify under incubation conditions.

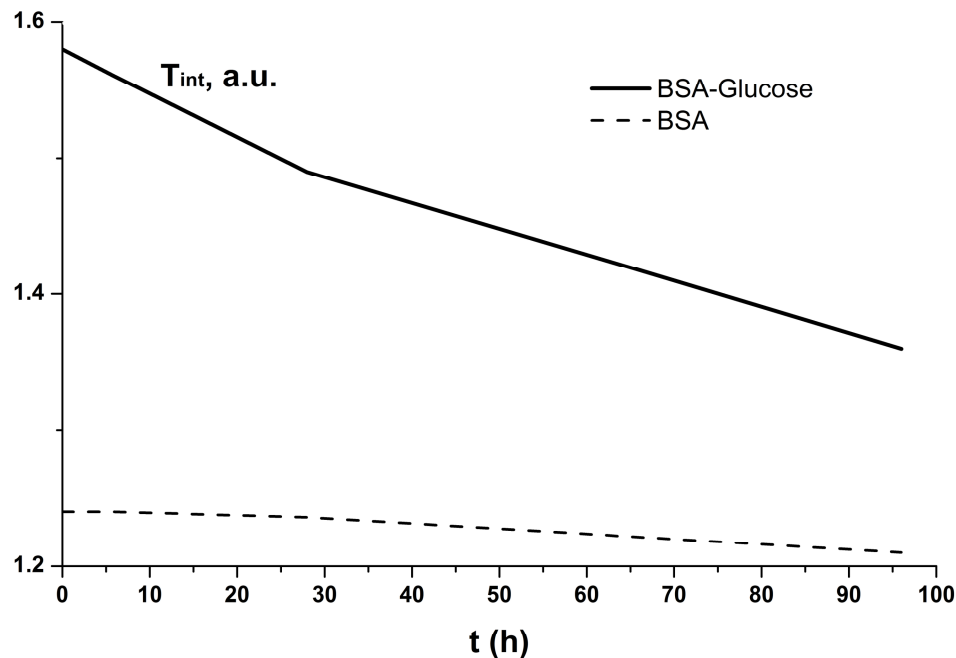


Figure 3. Dependence of an averaged transmission coefficient T_{int} at 0.1-1.0 THz from time t (h) after the start of incubation of BSA with glucose.

It was found that value of THz absorption coefficient of glycated albumin solution varies considerably during incubation. At the early stage of incubation, the amplitude of the first Debye term $\Delta\epsilon_1$ was 88% relative to the value for pure water. After 96 hours of incubation BSA with sugar the amplitude of the first Debye term $\Delta\epsilon_1$ was 92% relative to that for pure water [13]. During incubation sugar molecules form covalent bonds with protein molecule. The incubation mixture contains a significantly smaller part of sugar molecules bound with water molecules. In other words, the amount of free water molecules is increased at the final stage of incubation. As a result, the imaginary part of the dielectric constant is increased and this in turn leads to a reduction in the transmission of incubation mixture at 96 hour of incubation.

5. Conclusion

Dielectric properties of BSA and glucose solutions were systematically measured at 0.07-3.2 THz frequency range. It was found that the most significant is the reduction $\Delta\epsilon_1$ (or increase τ_1) of the slow Debye relaxation process with increasing concentrations of solute. Glycation of BSA results in a change the parameters of slow Debye relaxation. During incubation of BSA with sugars the incubation mixture contains a significantly smaller part of sugar molecules bound with water molecules and the amount of free water molecules is increased at the final stage of incubation.

Acknowledgments

This work has been supported by RFBR (grant № 14-02-00846, № 14-02-00979 and № 16-52-00222).

References

- [1] Cherkasova O, Nazarov M and Shkurinov A 2016 *Opt. Quant. Electron.* **48** 217
- [2] Nazarov M, Cherkasova O and Shkurinov A 2016 *Quantum Electron.* **46** 488–95

- [3] Cherkasova O, Nazarov M, Angeluts A and Shkurinov A 2016 *Optics and Spectroscopy* **120** 50
- [4] Nazarov M, Shkurinov A, Kuleshov E and Tuchin V 2008 *Quantum Electron.* **38** 647–54
- [5] Nazarov M, Shkurinov A, Angeluts A and Sapozhnikov D 2009 *Radiophysics and Quantum Electronics* **52** 595
- [6] Angeluts A, Balakin A, Evdokimov M, Esaulkov M, Nazarov M, Ozheredov I, Sapozhnikov D, Solyankin P, Cherkasova O and Shkurinov A 2014 *Quantum Electron.* **44** 614
- [7] Yada H, Nagai M and Tanaka K 2008 *Chem. Phys. Lett.* **464** 166
- [8] Fitzgerald A, Pickwell-MacPherson E and Wallace V 2014 *PLOS ONE* **9** e99291
- [9] Vinh N, Allen S and Plaxco K 2011 *Am. Chem. Soc.* **133** 8942
- [10] Shiraga K, Suzuki T, Kondo N, De Baerdemaeker J and Ogawa Y 2015 *Carbohydr. Res.* **406** 46
- [11] Bye J, Meliga S, Ferachou D, Cinque G, Zeitler J and Falconer R 2014 *J. Phys. Chem. A* **118** 883–8
- [12] Sushko O, Dubrovka R and Donnan R 2015 *The Journal of Chemical Physic* **142** 055101-1–9
- [13] Cherkasova O, Nazarov M and Shkurinov A 2016 *Proc. SPIE* **9917** Saratov Fall Meeting 2015: Third International Symposium on Optics and Biophotonics and Seventh Finnish-Russian Photonics and Laser Symposium (PALS) 991706 doi:10.1117/12.2229741