

Terahertz Spectroscopy of Mouse Blood Serum in the Dynamics of Experimental Glioblastoma

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Abstract. In this study terahertz (THz) absorption spectra of mouse blood serum in the dynamics of experimental U87 glioblastoma were investigated. Decrease of THz absorption with glioma growth was demonstrated. A two-component Debye model was used to analyze the experimental data. Analysis of the complex dielectric permittivity parameters of blood serum indicates an increase in the proportion of bound water in the samples in the dynamics of tumor growth. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: terahertz time domain spectroscopy; mouse blood serum; U87 glioblastoma; Debye model.

Paper #8990 received 3 Apr 2023; accepted for publication 21 Jun 2023; published online 15 Aug 2023. doi: [10.18287/JBPE23.09.030308](https://doi.org/10.18287/JBPE23.09.030308).

1 Introduction

Glioblastoma, a type of glioma, tumors developing in the glial tissue of brain, represents almost half of all malignant tumors of the central nervous system [1] and is among the most rapidly progressing cancers with the worst prognosis of survival [2]. One reason for this is late diagnosis and treatment [3]. Therefore, it is relevant and important for the early detection of glioblastoma and the development of non-invasive and minimally invasive methods to monitor the effectiveness of treatment. The study of oncological molecular markers, their causes and pathways of formation, is a major direction in the diagnosis and therapy of malignant tumors [4]. Conventional methods such as chromatography and mass spectrometry [5–7], as well as nuclear magnetic resonance [8, 9], due to complex sample preparation, long testing time and inability to provide timely intraoperative analysis cannot provide fully reliable information. Early diagnosis of glioblastoma can be achieved by analyzing body fluids [10, 11], including the

use of more technological and simple spectroscopic methods [12, 13].

Terahertz (THz) pulse spectroscopy, which has a number of features determining its application in creating new methods of medical diagnostics, has received intensive development [14–16]. The ability to directly measure the refractive index, absorption coefficient and dielectric function spectrum of the studied sample is a distinctive feature of this method [17, 18]. This makes it possible to obtain a detailed spectral characterization of the analyzed sample in a single measurement, giving the prospect of developing noninvasive rapid diagnostics on this basis [19]. Previously, THz spectroscopy methods have been used to study the features of THz images of mouse and rat brain tissue, freshly dissected [20, 21] and encased in paraffin blocks, in the dynamics of glioma development [22–28]. It was shown that the main contrast source in THz frequency range is water content, which increased with increasing tumor size and degree of malignancy [22, 27]. Other sources of contrast were increased cell density [22] and lipid content [26],

appearance of necrosis zones [28]. In these works, blood was not considered as an object of investigation. Meanwhile, blood composition significantly changes during the development of pathologies in organs and tissues of the body [29, 30], which can be used to create minimally invasive diagnostic methods [31, 32].

In this work we investigated THz absorption spectra of mouse blood serum in the dynamics of experimental U87 glioblastoma development. Currently, glioblastoma models with continuous cell lines such as U87 derived from primary human tumor cells transplanted intracranially into the mouse brain are most common [33, 34]. Using glioblastoma models in animals makes it possible to follow tumor development and blood composition changes in dynamics.

2 Materials and Methods

2.1 Terahertz Time Domain Spectroscopy (THz-TDS)

Mouse serum samples were measured on a pulsed THz spectrometer, which is described in detail in previous articles [35–37]. A femtosecond laser (Spectra Physics Tsunami) with wavelength $\lambda = 800$ nm, pulse duration $\tau = 80$ fs, and modulation frequency $f = 80$ MHz was used as pumping. The THz generator was a multidipole photoconductive antenna (iPCA-21-05-1000-800-h, Batop, Germany), the detector was a $300 \mu\text{m}$ ZnTe crystal. Photoconductive antenna emission power was in the range of $70\div 75$ mW, bias supply voltage was 15 V. Spectral range for reliable measurements was from 0.6 to 1.8 THz, with small attenuation of the passed radiation. The range was determined by the signal-to-noise ratio, which reached 10^2 or 20 dB across the field in the absence of sample. The experimental samples were placed in a demountable liquid cuvette (Bruker, USA) with two identical polystyrene windows. The refractive index of windows was 1.5 at 0.6–1.8 THz. The thickness of the liquid layer given by PTFE spacer was $180 \pm 5 \mu\text{m}$, which provided a transmittance value in the range 0.05–0.50, depending on the sample type. The lowest transmittance coefficient corresponded to the transmittance of distilled water at high frequencies. Measurements were performed at temperature of $21 \pm 1^\circ\text{C}$ and 35% relative humidity. The cuvette was installed in THz beam tangle with a diameter of $300 \pm 15 \mu\text{m}$. The time shape of pulse that passed through the cuvette was recorded. To exclude the statistical error associated with changes in the laser power during the experiment, the transmission measurements of empty cuvette, cuvette with water and sample were carried out alternately. A time sampling of 25 ps from 1024 points with a signal accumulation time in each point of 300 ms was used. To increase the reliability of measurements, three independent measurements were averaged for each serum sample. The measurement error was calculated as the standard deviation from the group mean. The time shapes of group-averaged THz pulses while passing through air

(without a cuvette), an empty cuvette, and a cuvette filled with water and serum are shown in Fig. 1.

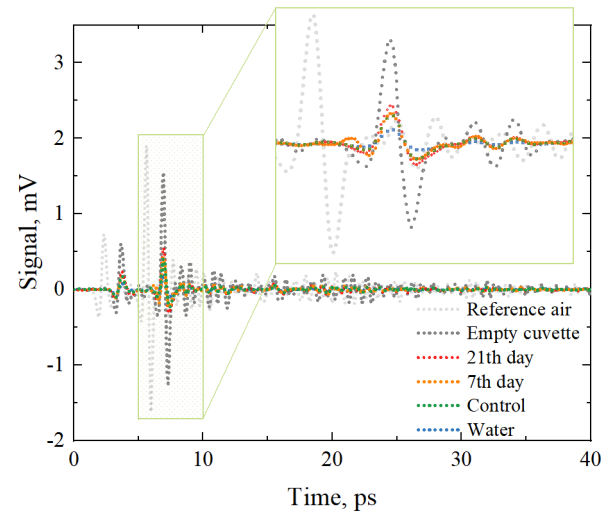


Fig. 1 Time shapes of group-averaged THz pulses: without cuvette (gray line), passed through empty cuvette (dark gray line), cuvette filled with water (blue line), serum of control mice (green line), after 7 (yellow line) and 21 days (red line) after injection of U87 glioblastoma cells.

Fourier transform of measured temporal shape of THz pulse gives complex spectrum $E(\omega)$ of radiation, i.e. amplitude $|E(\omega)|$ and phase $\arg(E(\omega))$ of spectrum, where E is electric component of electromagnetic wave field, $\omega = 2\pi f$, f is frequency. The transmittance spectrum of sample was calculated as follows:

$$T(\omega) = \frac{E(\omega)}{E_0(\omega)}, \quad (1)$$

where $E_0(\omega)$ is the reference spectrum of the THz pulse passing through an empty cuvette, $E(\omega)$ is the transmission of the cuvette with distilled water or blood serum.

The absorption coefficient and refractive index spectra were calculated using the methods described in detail in previous papers [14, 15, 31, 32, 35, 37, 38]; the sample absorption coefficient was calculated as follows:

$$\alpha(\omega) = \frac{-\ln|T(\omega)|}{d} + \frac{\ln(1-R_a^2)}{d}, \quad (2)$$

where d is sample thickness, $R_a = \frac{n_a - 1}{n_a + 1}$ is reflection coefficient for real part of refractive index averaged over all frequencies, $n_a = 1 + \Delta t \frac{c}{d}$ is averaged refractive index, Δt is pulse delay in passing through the sample. The refractive index was calculated as follows:

$$n(\omega) = \frac{-\arg(T(\omega)) \cdot c}{\omega \cdot d} + 1. \quad (3)$$

In this case, the complex permittivity has the form:

$$\varepsilon(\omega) = \left(n(\omega) - i\alpha(\omega) \cdot \frac{c}{\omega} + 1 \right)^2. \quad (4)$$

2.2 Samples

The model of orthotopic xenotransplantation of U87 human glioblastoma cells into immunodeficient SCID mice was used [39]. A 5 μ l cell suspension (500,000 U87 MG cells per animal) was injected in the subcortical brain structure through a hole in the animal's cranium. A 5 μ l culture medium was introduced to animals from the control group according to a similar scheme. Tumor size was measured in vivo on a horizontal tomograph with a magnetic field strength of 11.7 Tesla (Biospec 117/16, Bruker, USA) [40]. After that, animals of experimental and corresponding control groups were removed from the experiment by decapitation on days 7 and 21 after injection. Blood was collected in separate tubes, centrifuged and serum was frozen at -80°C until the day of analysis. There were 10 mice in each group. The study was carried out in accordance with the EU Directive 2010/63/EU and the ARRIVE 2.0 guidelines, and approved by the Inter-Institutional Commission on Biological Ethics at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Permission #78, 16 April 2021).

3 Results and Discussion

Tumor size increases significantly in the dynamics of U87 glioblastoma development. Thus, the tumor size is $2.6 \pm 0.4 \text{ mm}^3$ on day 7, and already $89.6 \pm 11.5 \text{ mm}^3$ on day 21 of the experiment. The THz response of blood serum also changes significantly, which is expressed in decrease of absorption coefficient at the last stage of the experiment (Fig. 2, a).

The high water content in blood serum determines the nature of its dielectric response in THz frequency range [15, 41]. Water can be in free or bound to the surrounding molecules. The reason for changes in the THz response of blood serum is the transition of water from free to bound state and back [15, 41]. The THz spectrum of water is characterized by the absence of well-defined spectral features in the frequency range of 0.1–3.0 THz. A similar type of spectra was obtained previously by us and some authors when studying of human blood [42], serum of rats with experimental liver cancer [31], diabetes [43, 44], as well as blood plasma from patients with thyroid disease [32]. The absorption spectra of control mice serum and on the 7th day of experiment are indistinguishable within the limits of error and have a smaller amplitude compared to the absorption spectrum of water. It is known that metabolic changes associated specifically with brain injury and the development of an inflammation reaction are observed in the first week after the introduction of culture medium or tumor cells into the brain [45]. Then blood composition changes associated with tumor development become

dominant [46]. The absorption coefficient of blood serum of mice on the 21st day of the experiment is significantly lower than the absorption coefficient of the other spectra in the entire range under study. At the same time, with increasing frequency, there is a greater degree of differentiation of absorption spectra for all samples. No significant differences in the refractive index spectra were observed (see Fig. 2, b).

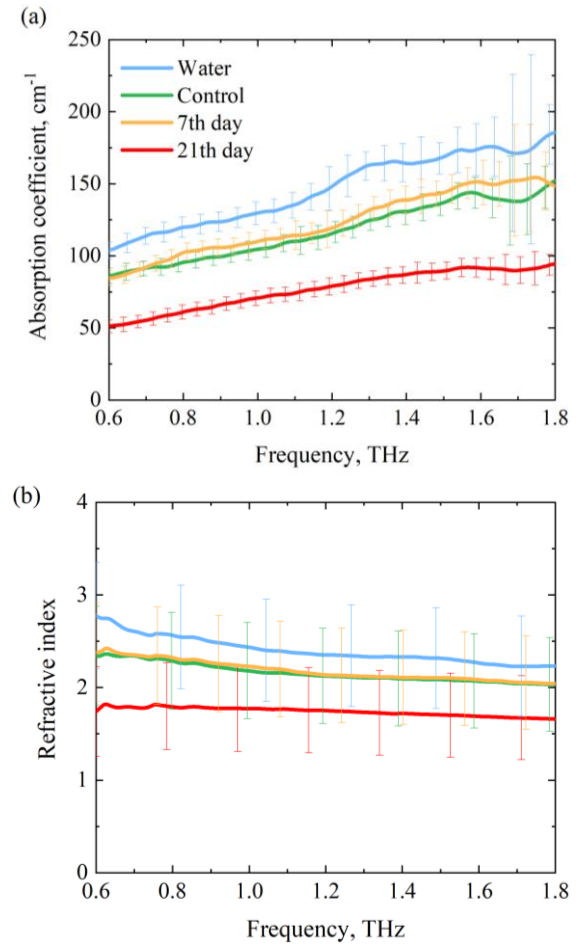


Fig. 2 Absorption coefficient (a) and refractive index (b) of water (blue line), blood serum of control mice (green line), and at 7 (yellow line) and 21 days (red line) after injection of U87 glioblastoma cells.

Based on comparison of experimentally obtained dielectric permittivity spectra with the model dielectric function of water $\varepsilon(\omega)$, which can be described by the two-component Debye model and the Lorentz term [15, 31, 37, 47–50], we analyzed the observed differences in THz spectra:

$$\varepsilon(\omega) = \varepsilon_{\infty} + \frac{\Delta\varepsilon_1}{1+i2\pi f\tau_1} + \frac{\Delta\varepsilon_2}{1+i2\pi f\tau_2} + \frac{A_1}{\omega_{01}^2 - (2\pi f)^2 + i2\pi f\gamma_{01}}, \quad (5)$$

where τ_1 and τ_2 are relaxation time of first (“slow”) and second (“fast”) Debye terms, $\Delta\varepsilon_i$ is contributions to dielectric permittivity of first and second Debye terms, A_1 is amplitude, ω_{01} is resonance frequency, γ_{01} is half-

width from the Lorentz term and ε_∞ is dielectric permittivity of water at high frequencies.

The following model parameters for water were obtained: $\Delta\varepsilon_1 = 74 \pm 9$, $\tau_1 = 9.47 \pm 0.5$ ps, $\Delta\varepsilon_2 = 1.67 \pm 0.03$, $\tau_2 = 0.24 \pm 0.01$ ps, $A_1 = 31$ THz², $\omega_{01} = 5.3$ THz, $\gamma_0 = 7$ THz, $\varepsilon_\infty = 2.3 \pm 0.2$. For frequency range of 0.05–2.5 THz at a temperature of 20–22 °C the given set of values is optimal. Comparison of the experimental imaginary $\text{Im}(\varepsilon)$ and real $\text{Re}(\varepsilon)$ parts of dielectric permittivity with model spectra for water are shown by blue line and dotted line in Fig. 3, respectively.

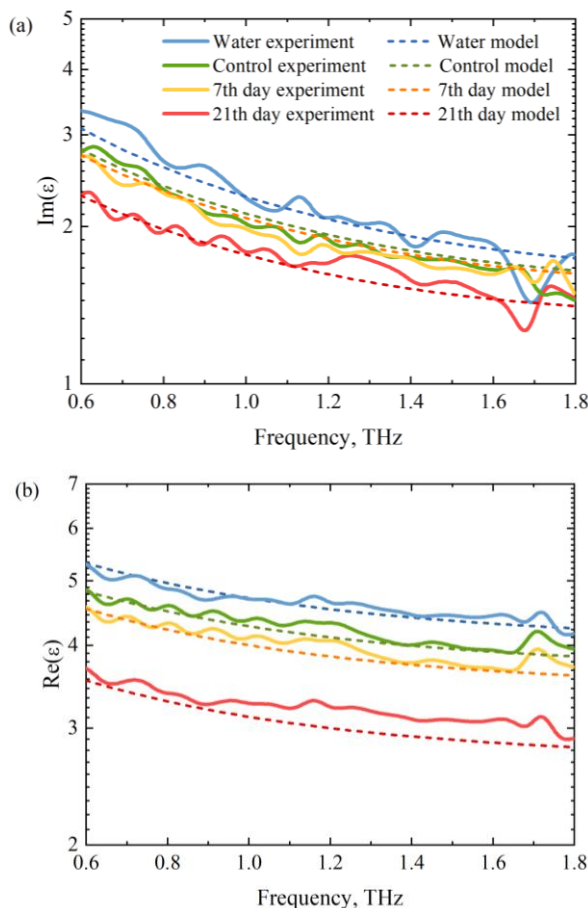


Fig. 3 Imaginary $\text{Im}(\varepsilon)$ (a) and real $\text{Re}(\varepsilon)$ (b) parts of dielectric permittivity for experimental data presented in Fig. 1 and their model curves: for water (blue line), blood serum of control mice (green line), and after 7 (yellow line) and 21 days (red line) after injection of U87 glioblastoma cells.

Earlier we demonstrated for the THz range that any aqueous solution of biomolecules, including blood serum, can be described with sufficient accuracy mainly by changes in the amplitude $\Delta\varepsilon_1$ and the relaxation time τ_1 of the first Debye term [31, 37, 43, 47–49]. This approach is valid also for a model spectrum of serum dielectric permittivity in the dynamics of glioma development. By changing $\Delta\varepsilon_1$ and τ_1 , all other terms of the model (5) have fixed values corresponding to water parameters in the chosen frequency range. Results of comparison of experimental and model dielectric

permittivity spectra are shown in Fig. 3. The parameters of Debye model $\Delta\varepsilon_1$ and τ_1 for all investigated samples are given in Table 1.

Table 1 Parameters $\Delta\varepsilon_1$ and τ_1 of studied samples.

Sample/Parameter	$\Delta\varepsilon_1$	τ_1 (ps)
Distilled water	74.9 ± 4.1	9.28 ± 0.51
Blood serum of control mice	74.3 ± 4.9	9.56 ± 0.31
Blood serum of mice after 7 days	68.2 ± 5.1	10.42 ± 0.32
Blood serum of mice after 21 days	57.9 ± 5.4	11.80 ± 0.29

It should be noted that for frequencies above 0.1 THz we cannot separate the contribution of the decrease $\Delta\varepsilon_1$ or increase τ_1 to the model spectrum. Only the change in the ratio $\Delta\varepsilon_1/\tau_1$ can be clearly discerned [47]. We have previously shown a 1.2-fold decrease in the ratio compared to water for blood plasma of rat with experimental diabetes, which is associated with a high level of glucose in the blood plasma [43]. It was also shown that as both glucose concentration and protein concentration increase in solutions, the proportion of water bound to biomolecules increases, which has a lower value of $\Delta\varepsilon_1$ and a high value of relaxation time τ_1 as compared to water molecules not bound by hydrogen bonds [47, 49].

Table 1 shows that in the dynamics of U87 glioblastoma there is a decrease in $\Delta\varepsilon_1$ and an increase in τ_1 , which leads to a decrease in the ratio $\Delta\varepsilon_1/\tau_1$. As noted above, on day 21 of the experiment, there is a more than 34-fold increase in tumor size compared to 7 days after the introduction of glioblastoma U87 cells. This leads to a significant change in blood serum composition [46]. The decrease of $\Delta\varepsilon_1$ contribution to dielectric permittivity of samples in the dynamics of tumor growth is related to the decrease of free water fraction with simultaneous increase of bound water fraction. The increase of relaxation time τ_1 in the dynamics of tumor growth indicates a “slowing down” of the process of water molecules reorientation in the hydrogen bonding network. Thus, a change in blood serum composition in the dynamics of glioma development leads to a change in complex dielectric permittivity parameters.

4 Conclusion

The THz absorption spectra of mouse blood serum in frequency range 0.6–1.8 THz in the dynamics of experimental U87 glioblastoma were studied. It is shown that the absorption coefficients of healthy mouse serum and on the 7th day of experiment do not differ. The absorption coefficient of mouse serum on 21st day of the experiment is significantly lower compared to water and serum of other experimental groups. This difference in THz response is explained by changes in blood composition at different stages of U87 glioblastoma

development. Analysis of complex dielectric permittivity parameters of blood serum in the dynamics of glioblastoma development in mice indicates an increase in proportion of bound water with tumor growth.

Acknowledgements

This work was performed partly within the State Assignment of IA&E SB RAS, ILP SB RAS and FSRC “Crystallography and Photonics” RAS. This work has been supported by the Interdisciplinary Scientific and Educational School of Moscow University “Photonic and

Quantum Technologies. Digital Medicine” for part of the results analysis. The research was carried out partly with the support of the Tomsk State University Development Program (Priority-2030). The work of D. V. and Yu. K. was supported by the Ministry of Science and Higher Education of the Russian Federation (budget funds for the V. E. Zuev Institute of Atmospheric Optics of the Siberian Branch of the Russian Academy of Sciences).

Disclosures

The authors declare no conflict of interest.

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