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ELECTROMAGNETIC APPLICATIONS IN BIOLOGY AND MEDICINE

THz Monitoring of the Dehydration of Biological Tissues Affected by Hyperosmotic Agents

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Abstract—Different ways for monitoring the dehydration of tissues affected by hyperosmotic agents have been comparatively analyzed to increase the THz transparency of biological tissues. The data obtained with an original THz laser spectrometer, a Nicolet 6700 Fourier spectrometer, and a Callegari Soft Plus system for skin diagnostics are in good agreement. The corresponding responses of biological tissues (in the form of THz transmittance, reflectance, absorption coefficient, and hydration coefficient) to the effect of biologically compatible hyperosmotic agents have been studied.

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1. INTRODUCTION

THz range has been rather poorly studied, in particular, from the point of view of interaction of THz radiation with biological tissues and cells. To date, the most popular analytical technique in this frequency range is time-domain THz spectroscopy, which was used to demonstrate good prospects of THz radiation for applications in spectroscopy [1, 2] and in biology and medicine [3, 4].

The frequency dispersion of biological tissues and liquids in the THz range is known to be relatively weak; however, their THz absorption may be rather strong. Many biomolecules have spectral features in this range. In addition, inhomogeneities less than 10 μm in size, which lead to strong scattering in the visible and near-IR spectral ranges, do not contribute much to THz scattering. Using ultrashort pulses, one can investigate a wide frequency range in one measurement; implement a high temporal resolution; and obtain information about the phase and thus the refractive index.

Time-domain THz spectroscopy can be used to analyze liquids [5] and biological tissues due to its high sensitivity to the concentration and state of water [6]. This technique was applied to record absorption spectra of various liquids, including biological ones [7]. THz radiation is absorbed by liquids via the mechanism of dipole absorption, both the absorption inherent in the medium and that induced by the external-radiation field. Specifically for this reason the THz absorption in polar liquids is much higher than in nonpolar liquids [5, 8].

Fujioka et al. [9] investigated the IR spectra (at frequencies from 32 to 37 THz) of healthy stomach cells and cells with pathological changes and revealed differences in their absorption spectra. The absorption spectra of different tissues in the range from 0.5

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to 2 THz were reported in [10]. In addition, an attempt was made to develop a statistical optimization model of data processing with the purpose of classifying tissues in the absorption coefficient and refractive index [11, 12].

2. STATEMENT OF THE PROBLEM

The specificity of the properties of materials and tissues in the THz range suggests a possibility of their THz visualization with a contrast sufficiently high to distinguish pathological tissues [13, 14]. It has been shown that THz radiation is promising for medical diagnostics [15] and imaging pathological tissues, in particular, for establishing early stages of skin cancer [16, 17]. The portions of body affected by skin cancer are difficult to reveal by the naked eye or using optical cameras, because 85% cancer cells are concentrated in the epitheliums under the skin surface. THz waves, which easily penetrate the outermost layer of skin through the dehydrated dead cells of horny layer, make it possible to monitor the development of malignant processes in the very early stages. As compared with healthy tissues, cancer cells contain a larger amount of water [18], which intensively absorbs radiation in the THz and far-IR ranges [19].

The properties of aqueous solutions of proteins in the THz range are intensively studied [20, 21]. The interest in this problem is related to the ability of proteins to order the layers of water molecules that are in the immediate vicinity of protein molecule and reduce the mobility of water molecules and their ability to form hydrogen bonds. Thus, the presence of proteins changes the THz absorption spectra of water in comparison with those of bulk water [22–24]. In this context, the concepts of free and bound water have been introduced. The degree of hydration of tissues and the correlation between their optical parameters and water content in them are under study [25, 26]. Thus, the sensitivity of THz electromagnetic waves to the presence of water molecules in different states makes it possible to use them to diagnose cancer [27–29]. An elevated water content, along with other structural changes in tissue, results in a higher absorption coefficient and reduced reflection of tumor-containing tissues at THz frequencies [18]. However, despite the different ratios of the contents of two types of water in healthy and pathological tissues, visualization and diagnostics of tumors are hindered by specifically the presence of high water content in tissues because the absorption of radiation in the upper layers of tissue does not allow one to obtain information from the lower layers, where a tumor develops. This circumstance stimulates development of different methods for increasing the contrast of images formed by THz visualization.

The reduced ability of proteins of pathological tissues to bound water molecules suggests also a change in the ratio of the contents of free and bound water in tumor tissues in favor of free water. Thus, there is a new possibility of distinguishing healthy and pathological tissues proceeding not only from the total water content but also from the ratio of free- and bound-water contents. It was shown in [23] that removal of protein from a solution is accompanied by removal of bound water; a difference between healthy and pathological tissues caused by the difference in water contents (even after treating samples by formalin) was demonstrated in [30]. These data are indicative of strong bonding between a protein molecule and neighboring water molecules. The aforesaid suggests that a temporal removal of free water from tissue using dehydrating agents allows one to change the water content in tumors and thus increase the image contrast not only due to the reduced total amount of water in tissues but also as a result of change in the ratio of free- and bound-water contents. This approach is expected to help to classify tissues with respect to the contents of free and bound water in them and, in particular, diagnose cancer tumors.

Obviously, application in vivo of dehydration methods implies temporal and reversible character of changes in tissues caused by these methods. One of the most efficient dehydration methods is the use of hyperosmotic agents. This technique is based on the ability of agents to form a flow of free water out of tissue and their ability to penetrate a tissue and replace free water for some time. Promising agents of such kind are glycerol, polyethylene glycol, and glucose solution [31–36]. These materials are nontoxic and, as will be shown below, do not introduce significant errors into measurement results. In addition, they are characterized by rather high diffusion coefficients in tissues, which is a necessary condition for successful application in human tissue dehydration under clinical conditions.

In this paper, we report the results of studying the possibility of increasing the THz transparency of biological tissues under conditions of controlled dehydration caused by hyperosmotic agents.

3. EXPERIMENTAL

3.1. Study of Tissue Dehydration in the Range from 0.1 to 2.0 THz

Experiments were performed using time-domain THz radiation, obtained by converting ultrashort lasing pulses on a semiconductor surface. Under these conditions, radiation is generated due to the occur-

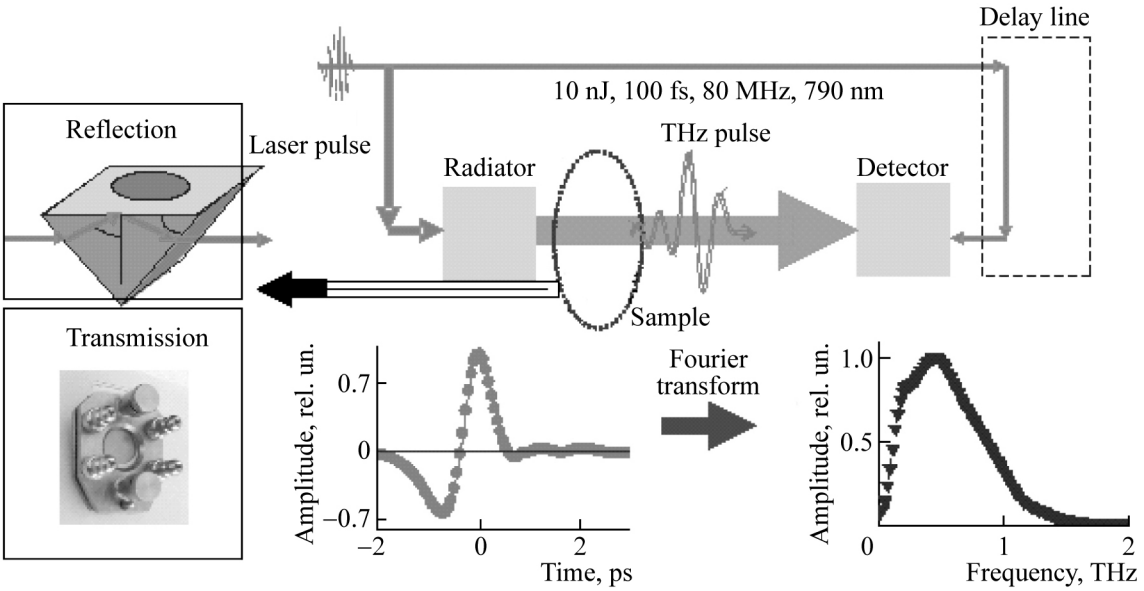

Fig. 1. Schematic of a time-domain THz spectrometer. A silicon prism and a metal mounting with polystyrene windows are used in the reflection and transmission geometries, respectively.

rence of time-dependent electron-hole photoconductivity of semiconductor (GaAs) irradiated by laser pulses and the peak of photocurrent induced by internal electric field. THz pulses were detected by an electro-optical ZnTe crystal. The experimental setup (THz spectrometer) is schematically shown in Fig. 1 [2].

Measurements were performed both in the transmission geometry (a sample was inserted in a metal mounting and clamped between two polystyrene plates) and in the reflection geometry (a sample was installed on a silicon prism). All data were normalized to the background signal recorded in the absence of sample (empty mounting or empty prism).

Hyperosmotic agents were polyethylene glycol (PEG) with a molecular weight of 600 (PEG-600), glycerol (99.9%), and propylene glycol (PG) (99.9%).

The objects of study were samples of bovine and pig muscular tissues.

3.2. Study of Tissue Dehydration in the Range from 16 to 30 THz

The transmission spectra of immersion dehydrating agents were measured on a Nicolet 6700 IR Fourier spectrometer (Thermo Electron Corporation, United States) (Fig. 2), which makes it possible to record reflection spectra in the mode of frustrated total internal reflection (FTIR) and transmission spectra in the mid-, far-, and near-IR ranges (from 4500 to 400 cm^{-1}). The instrument was aligned and the transmission spectra were calculated using the specialized Thermo Electron Omnic software package.

Table 1. Refractive indices of dehydrating agents

Dehydrating agent	Refractive index
30% solution of glucose in water	1.379
40% solution of glucose in water	1.391
PEG-600	1.464
Dehydrated glycerol	1.469
PG	1.428

We investigated the following dehydrating agents: 40% aqueous solution of glucose, PG (99.9%), PEG-600, and glycerol (99.9%). The refractive indices of the solutions used were measured on an IRF-454B2M Abbe refractometer (KOMZ, Russia) at a wavelength of 589 nm to be 1.391, 1.428, 1.464, and 1.469, respectively (Table 1).

Experimental studies were performed in vitro on samples of skin and transplanted hypodermic tumors (liver cancer) of white laboratory rats.

The experimental technique based on a Fourier spectrometer and FTIR and the results obtained were reported in [36].

3.3. In Vivo Study of Human Skin Parameters under Effect of Hyperosmotic Agents

The biophysical skin parameters were determined using a Callegari Soft Plus system for skin diagnostics (Soft Plus STANDART, Callegari, Italy) (Fig. 3).

Soft Plus STANDART makes it possible to estimate a number of functional skin parameters: hydration, temperature, fat content, pH (acid-alkaline balance), elasticity, melanin content, phototype, etc.

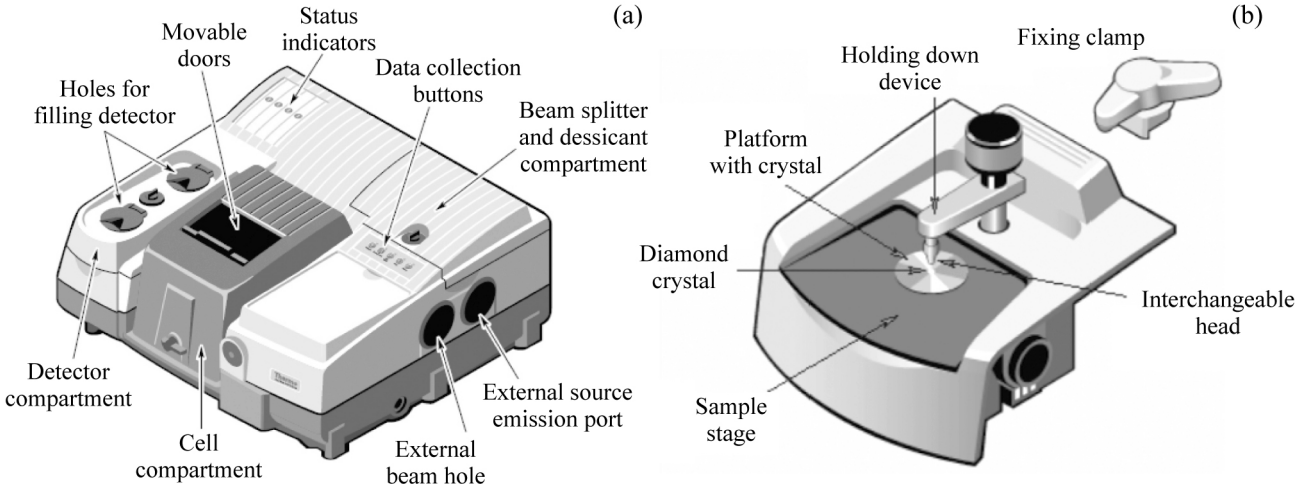

Fig. 2. Experimental setup: (a) Nicolet 6700 Fourier spectrometer and (b) Smart iTR FTIR attachment.

We used Soft Plus STANDART to measure the dependence of hydration (water content) in skin on the time of immersion agent action. The study was performed in vivo on a human skin. The experimental technique was described in detail in [35].

The dehydrating agents were chosen to be aqueous solutions of glucose with concentrations of 30 and 40%, PEG-600, glycerol (99.9%), and PG (99.9%); their refractive indices are listed in Table 1.

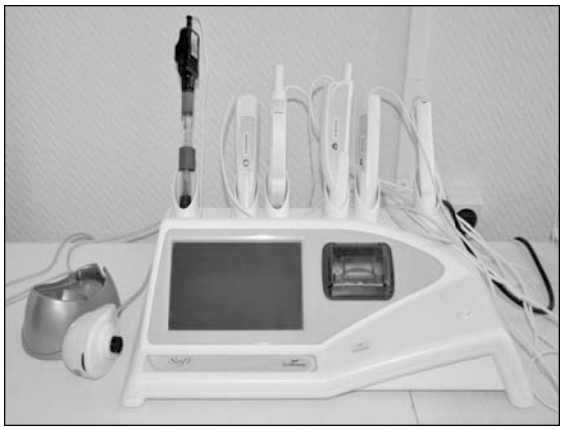

Fig. 3. Callegari Soft Plus system for skin diagnostics.

4. RESULTS AND DISCUSSION

Figures 4–6 show time dependences of the transmittance and reflectance of some biological tissues affected by hyperosmotic immersion agents. These data were obtained by time-domain THz spectroscopy (see Fig. 1) in the range from 0.1 to 2.0 THz. As was mentioned above, the background signal was recorded before each measurement, and all recorded spectra were normalized to this signal. Figure 4

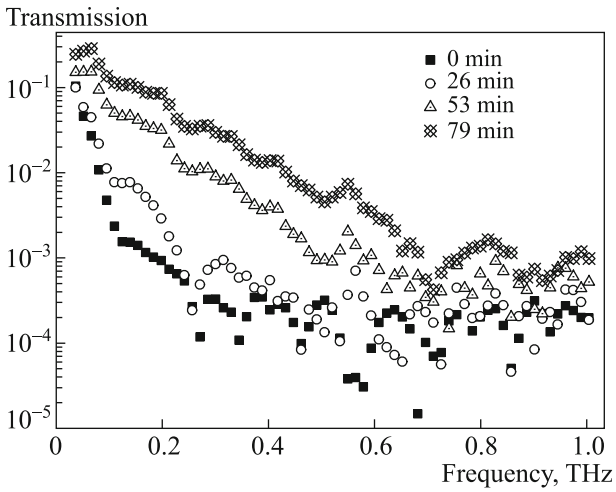

Fig. 4. Transmission spectra of pig muscular tissue affected by glycerol.

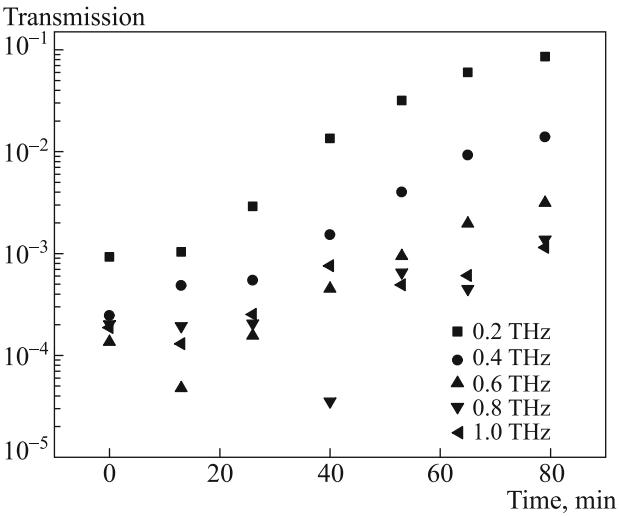

Fig. 5. Kinetics of change in the transmission of pig muscular tissue affected by glycerol at different frequencies.

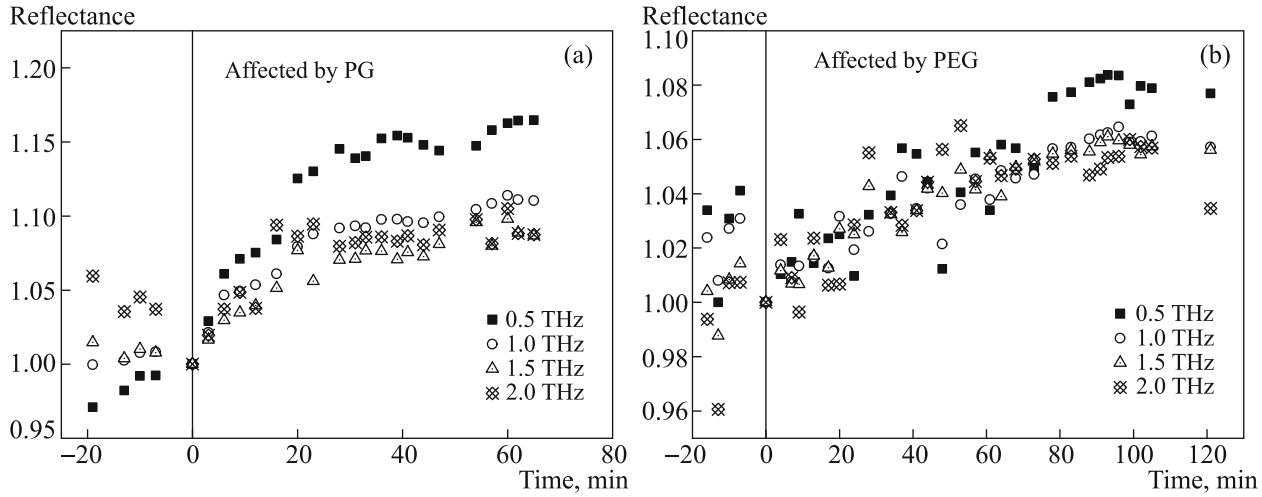

Fig. 6. Kinetics of relative reflected signal from a sample of muscular tissue affected by (a) PG and (b) PEG-600 at different frequencies. Zero instant corresponds to the time of agent deposition.

shows the transmission spectra of pig muscular tissue affected by glycerol. The frequency range in Fig. 4 is limited by 1 THz, because the signal-to-noise ratio is small at higher frequencies. The temporal dehydration, caused by hyperosmotic agent, determines the characteristic shape of the curve. It can be seen in Fig. 5 that the agent effect is most pronounced at low frequencies. In the course of time, as the agent and water flows were brought to equilibrium, dehydration was somewhat saturated; however, the saturation was not complete over 80 min. Table 2 contains quantitative data on the effect of glycerol on muscular tissue at individual frequencies: ratios of the transmittance by the end of the observation period to the initial value. The transmission of dehydrated tissue at low frequencies (from 0.1 to 0.2 THz) was found to decrease by almost two orders of magnitude. However, this effect can be related to the low detector sensitivity in the early stage of the experiment in this frequency range. Nevertheless, even disregarding the signals recorded during the first few minutes, the signal increased later by more than an order of magnitude.

Figures 6(a) and 6(b) show time dependences of the reflectance (along with the normalization to the background, the data are also normalized to the signal recorded directly before depositing an agent). Prior to the agent deposition (negative time), the sample was placed on the prism without being affected. The signal instability before the agent deposition is most likely to be caused by natural sample drying. Application of both PG and PEG-600 led to pronounced saturation of signal, which corresponded to the end of dehydration. The saturation time for PG is likely to range from 40 to 60 min, whereas for PEG-600 saturation occurred somewhat later: over a time from 80 to 100 min. This can be explained by different sample thicknesses (1.05 and 1.25 mm in the experiments with PG and PEG-600, respectively), because the agents were deposited on samples from above, and reflected signals were recorded from the opposite side. It should be noted that, when a sample was affected by PG, the signal amplitude increased by 6–16% (depending on frequency) by the end of the experiment, whereas in the case of PEG-600 the corresponding increment was 3 to 8%.

Figure 7 shows time dependences of normalized transmittance, recorded on a Nicolet 6700 Fourier spectrometer (see Fig. 2). Despite the rather strong spread of data, which is caused by both the individual features of tissue samples and the measurement technique based on FTIR prism (which implies a close contact of thin tissue sample), one can observe an increase in transmission with the agent application time and with somewhat more rapid and significant increase in transmission for the transplanted-tumor tissue. These data were normalized to the signal corresponding to the instant before agent deposition.

Table 2. Transmission kinetics of pig muscular tissue affected by glycerol

Frequency, THz	Increase in transmission (ratio of signal amplitudes in the end and beginning of experiment)
0.2	92.2
0.4	56.7
0.6	23.2
0.8	6.8
1.0	6.1

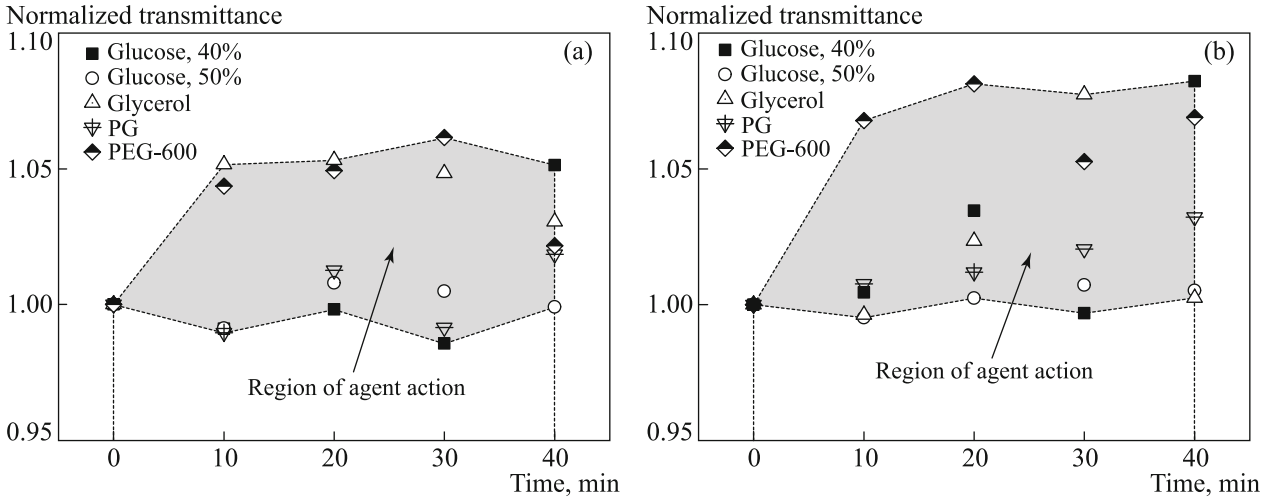

Fig. 7. Time dependences of the normalized transmittance of (a) healthy skin and (b) tissue tumor affected by different agents, measured at a frequency of 20 THz.

To obtain a direct evidence for skin dehydration under exposure to agents, we performed clinical studies on eight volunteers using a Callegari Soft Plus system (see Fig. 3). Figure 8 shows the time dependences of relative skin hydration, averaged over all examinees. Before averaging, the data were normalized for each individuum to take into account the individual features of skin. The hydration values obtained 3 min after the agent deposition were used for normalization.

It can clearly be seen that the degree of human skin hydration sharply decreases 13–17 min after applying the agent (due to the action of hyperosmotic agent) and then sharply increases 20 min after (rehydration with higher moistening of the upper part of skin occurs). Specifically this effect is the manifestation of agents, the moistening effect for most of which is based on the formation of a water flow from the water-saturated deep layers of skin. Concerning the practical application of THz radiation in diagnostics, one must take into account that the skin transparency window exists for 13 to 17 min.

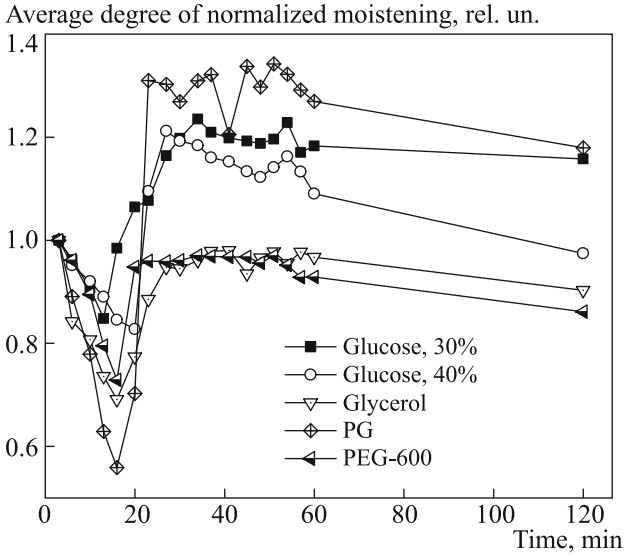

Fig. 8. Time dependences of the average normalized hydration (moistening) for the skin of volunteers ($N = 8$) affected by hyperosmotic agents.

When analyzing data obtained in different ways, one must take into account that the profiles of time characteristics completely describe the dehydration process. For in vitro studies, this process can be separated into two stages:

(i) a sharp increase in the THz signal after depositing agent, which can be explained by biological tissue dehydration;

(ii) saturation of signal when equilibrium between water and agent flows is reached.

At the same time, in vivo studies are characterized by the third stage, in which the upper skin layers are rehydrated due to the physiological skin response.

5. CONCLUSIONS

An analysis of the data obtained in this study suggests the following:

(i) a common feature of all results is the similar behavior of characteristics in time: almost all curves have pronounced extrema related to the onset of maximum tissue dehydration;

(ii) the time-domain THz laser spectroscopy data demonstrate that the characteristics change almost immediately (during the first minute) after the agent deposition and then this change significantly increases;

(iii) the time dependences of the transmission of healthy and pathological tissues recorded in the range from 16 to 30 THz are more difficult to interpret; nevertheless, the signal on the whole increases with time;

(iv) dehydration in vivo occurs generally more rapidly than in vitro (the characteristic times are 13 to 17 and 20 to 80 min, respectively).

The comparison of the results obtained by different methods showed qualitative similarity of the time dependences of THz signals, with some quantitative discrepancy. The dehydration caused by application of hyperosmotic agents has a temporal character, with a pronounced instant of saturation and subsequent rehydration under in vivo conditions.

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