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INFLUENCE OF ELECTROMAGNETIC RADIATION OF MILLIMETER RANGE ON THE OPTICAL PROPERTIES OF WATER IN THE HEMOGLOBIN SOLUTIONS

The features of the influence of microwave radiation on the optical properties of pure water and water in solutions of hemoglobin in the near infrared range with the aim to characterize the certain structural and dynamic state of water are investigated. The object of the study was the optical properties of distilled water and aqueous solutions of human hemoglobin in the concentration range of 7 and 15 μm and in the temperature range 10–40°C. The optical spectra were recorded by using a BiospecMini spectrophotometer in the wavelength range of 190–1100 nm. In order to increase the accuracy of the analysis of spectral data the spectra were normalized relative to the baseline which was taken as a line that ran parallel to the horizontal axis due to the minimum value of the optical density in the absorption spectrum. The values of the absorption maxima and the optical density were used as the main parameters of the absorption spectra. The B-spline function was used to more accurately determine these parameters. Microwave exposures of distilled water and hemoglobin solutions was performed at a wavelength of 7.1 mm with a maximum 100% output power which was calculated to be approximately 24 mW. The dependence of the parameters of the water absorption spectrum in the range of the second overtone of the OH-valence oscillation of the water molecule on temperature is detailed. There were not found statistically significant changes in the distilled water absorption spectrum in the range of the second overtone OH-valence oscillation of the water molecule after one-hour exposure of the microwave EMR. In hemoglobin solutions in this spectral range there is a small but statistically significant increase in absorption. This may indicate on additional EM-induced activation of the libration motions of water molecules and an increase the probability of deformation oscillations that which are manifested in the near IR range in the band of the second overtone of water.

Keywords: water, hemoglobin solutions, microwave electromagnetic radiation, second overtone of OH-valence oscillation.

Introduction. In recent decades there is an active development of new telecommunications technologies of the new generation 5G and 6G which use microwave electromagnetic radiation (MEMR) in the millimeter range [1,2]. At the same time the autopilot technologies are rapidly evolving which also use MEMR for dynamic object observation [3]. Therefore, in the near future we expect a significant increase of the man-made electromagnetic background that potentially can provoke the harmful effects on humans and other living organisms. More than 180 scientists and doctors from 36 countries have appealed to the European Union to comply with Council of Europe Resolution 1815 "On the hidden dangers of electromagnetic fields and their impact on the environment" which indicates the public concern about the potentially harmful effects of microwave electromagnetic radiation on human health.

The biological activity of microwave electromagnetic radiation has long been known [4] and this fact is the basis for its use in biotechnology and medicine [5].

It is believed that the main substance that interacts with MEMR in cells or in model biological experiments is water which can exist in the different structure-dynamic states [5–7]. The structure-dynamic state of water must in some way affect the hydration of biological molecules and their molecular dynamics that in turn should affect the structural and functional properties of biological macromolecules. However, despite on certain array of theoretical ideas and experimental data on the biological effects of MEMR on living organisms, the molecular mechanisms of this physical factor remain in many cases unclear and debatable. Therefore, for present day the study of the influence of MEMR on the structural and functional properties of proteins remains an urgent task.

So, the aim of the study was to elucidate the features of the influence of microwave radiation on the optical properties of pure water and water in hemoglobin solutions in the near infrared range which characterize its specific structure-dynamic state.

Materials and methods. The object of the study was the optical properties of distilled water and aqueous solutions of human hemoglobin. The study material was distilled water and aqueous solutions of human hemoglobin at a concentration of 7 and 15 μM.

Hemoglobin solutions were obtained by osmotic shock of erythrocytes by adding distilled water to the erythrocyte mass obtained by centrifugation at 1500 rpm for 5 minutes. pH of solutions was 6.8. Given the fact that the content of hemoglobin in erythrocytes is 95–96 % of the total protein we believed that the optical properties of the obtained solutions are determined mainly by hemoglobin, especially in the visible and in near infrared range.

The partial pressure of oxygen in distilled water, which was used to prepare hemoglobin solutions, was at the level of 130–150 mm Hg, which is approximately 3 times higher than the partial pressure in arterial blood and 5 times higher than in venous. Therefore, hemoglobin in aqueous solutions was in fully oxygenated form and calculations of its concentration were carried out according to the law of Bouguer-Lambert-Ber using molar extinction of this protein at the maximum absorption of its oxygenated form 540–542 nm, which is is $\varepsilon_M = 14440\ M^{-1}cm^{-1}$ [8, 9]. The resulting aqueous hemoglobin solutions were diluted to a final hemoglobin concentration of 15 or 7 μM.

The absorption spectra of distilled water and hemoglobin solutions were recorded on a BiospecMini spectrophotometer in the range of 190–1100 nm. Measurements of the samples were performed against the air. This methodological approach allowed to register the optical properties of not only hemoglobin in the ultraviolet and visible range of electromagnetic waves, but also distilled water in the near infrared range of 800–1100 nm.

Analysis of absorption spectra was performed using Origin Pro software tools. In order to increase the accuracy of the analysis of spectral data the spectra were normalized relative to the baseline which was taken as the line that parallel to the horizontal axis on the minimum value of optical density in the absorption spectrum. The values of the absorption maxima and the optical density were used as the main parameters of the absorption spectra. The B-spline function was used to more accurately determine these parameters.

Microwave exposition of distilled water and hemoglobin solutions was performed directly in spectrophotometric quartz cells using a generator G4-141 at a wavelength of 7.1 mm (42.22 GHz) with a maximum 100% output power that according to the passport data of the device is not less than 10 mW. The working surface of the quartz cuvette was placed perpendicular to the axis of the waveguide at a distance of 2 mm (fig. 1).

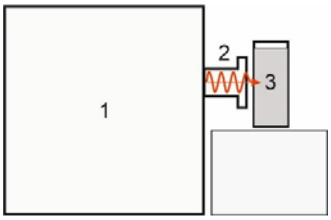

Fig. 1. Schematic diagram of irradiation of experimental samples
Notes: 1 – generator, 2 – waveguide,
3 – quartz cuvette with distilled water or hemoglobin solution

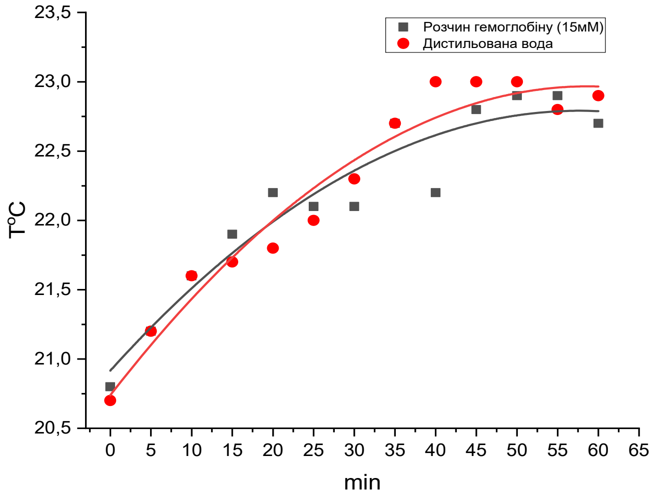

Fig. 2. An example of the temperature dynamics of samples of distilled water and aqueous solution of hemoglobin (15 μM) during exposition in MEMR of 7.1 mm at the maximum output power of the generator 100 %

Millimeter electromagnetic radiation entering the aqueous phase is actually completely absorbed that leads to heating of water or aqueous solution. The heat flux power and the amount of absorbed energy for the entire experiment were calculated using the formula of specific heat of water C = Q/(m•ΔT), where Q is the amount of heat, C is the specific heat of water in the temperature range 20–22 °C, which is equal to C = 4.182 kJ / (kg•K) [10].

Measurement of the temperature of water and aqueous protein solutions during exposure to MEMR showed that the presence of low concentrations of protein does not significantly affect the temperature dynamics of the samples and as a result the heat capacity (fig. 2). The temperature of water or aqueous protein solutions was increased by approximately 2.5°C during one-hour exposure in MEMR, after which the system reached equilibrium and the temperature did not change further. So, the optical spectra of the samples were exposed MEMR were measured in this equilibrium state and time range.

Given the fact that the studied system of aqueous solution does not execute the work, the change in the internal energy of the experimental aqueous solution with a volume of 4 ml (10^{-4} kg) when its heated to 1oK under the action of microwave radiation is calculated as ΔQ = C•m•ΔT = 4182•0,004 * 1 = 16,728 J. Taking into account the time required to heat water or aqueous solution by 1°K, we have the heat flux power P = ΔQ/t, where t = 3000 s (correspond to the time of quasi-linear temperature output on the plateau (fig. 2)) і The power of electromagnetic radiation directly entering the aqueous phase is P = ΔQ / t = 16,728 / 3000 = 0,0056 J/s or about 6 mW. Traditionally in electromagnetic biology such power is considered as the boundary between thermal and non-thermal effects.

The total amount of energy absorbed by water or aqueous protein solution during 60-wave exposure in MEMR with a wavelength of 7.1 mm is equal to Q_{total} = ΔQ • t_{exp}= 0,0056*3600 = 21,16 J.

The temperature of the samples was measured in a quartz cuvette using digital thermometer with a probe with an accuracy of 0.1°C before recording the absorption spectra.

Statistical data processing was performed according to generally accepted algorithms of variation statistics. The average value and its standard deviation were calculated. To assess the reliability of the difference between the statistical samples was evaluated using the Mann-Whitney test

Results and discussion. Evaluation of the structural and dynamic state of distilled water and water in protein solutions was performed by analyzing the absorption spectra of water in the near IR range of 800–1100 nm. In this range the absorption band is registered with an absorption maximum of 975 nm at 25 °C that corresponds to the second overtone of the OH-valence oscillation of the water molecule. The name of this band is conditional because its spectral range consists of several combination of spectral lines 2v1 + v2; v1 + 2v2; 2v1 + 2v3; 2v2 + 2v3.

It is known that due to increasing water temperature there is a shift of this spectral band in the short-wavelength region with a small increase in the absorption intensity [11]. We found out in more detail the temperature dependence of the optical density on the maximum absorption of distilled water in the near infrared range in control samples of distilled water and hemoglobin solutions, as well as at one-hour exposure to microwave radiation (fig. 3–6).

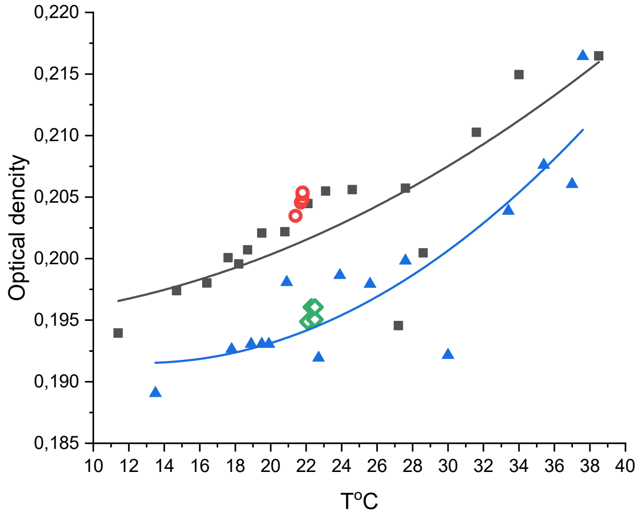

Fig. 3. Temperature dependence of the optical density for the absorption maximum in band of the second overtone of the OH-valence oscillation of the water molecule in distilled water (■) and in 15 μM solution of hemoglobin (▲) in control samples and under one-hour exposure to MEMR

(respectively – ○ і ◇)

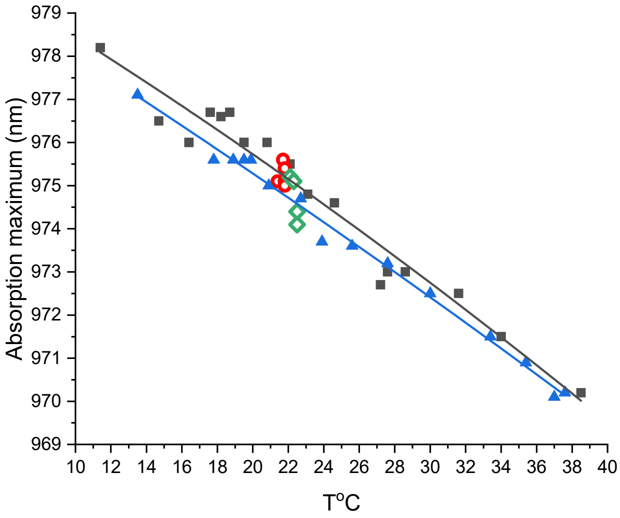

Fig. 4. Temperature dependence of the value of absorption maximum in band of the second overtone of the OH-valence oscillation of the water molecule in distilled water (■) and in 15 μM solution of hemoglobin (▲) in control samples and under one-hour exposure to MEMR

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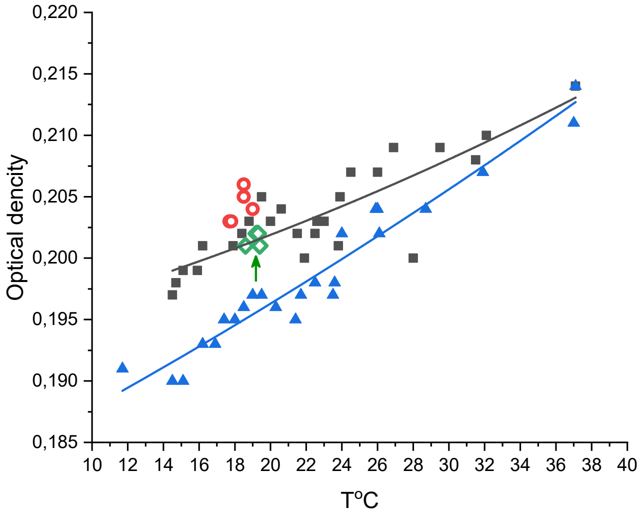

Fig. 5. Temperature dependence of the optical density for the absorption maximum in band of the second overtone of the OH-valence oscillation of the water molecule in distilled water (■) and in 7 μM solution of hemoglobin (▲) in control samples and under one-hour exposure to MEMR

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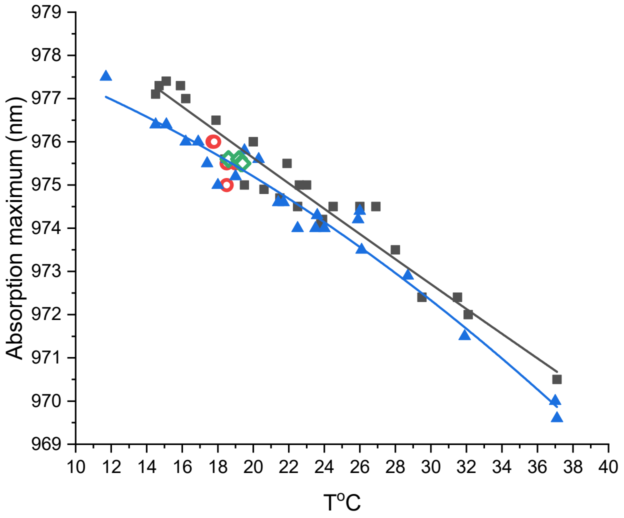

Fig. 6. Temperature dependence of the value of absorption maximum in band of the second overtone of the OH-valence oscillation of the water molecule in distilled water (■) and in 7 μM solution of hemoglobin (▲) in control samples and under one-hour exposure to MEMR

(respectively – ○ і ◇)

***Table* 1. Influence of millimeter electromagnetic radiation on the parameters of the absorption band in the range of the second overtone of the OH-valence oscillation of the water molecule**

	Optica density *Dmax* (*M* ± *SD*)		**Absorption maximum λmax (*M* ± *SD*)**	
	Control samples	MEMR influence	Control samples	MEMR influence
Water	0,204 ± 0,002	0,205 ± 0,001	975,4 ± 0,6	975,2 ± 0,2
15 μMgemoglobin solution	0,194 ± 0,00**	0,195 ± 0,001**	974,9 ± 0,2	974,6 ± 0,5
Water	0,202 ± 0,001	0,204 ± 0,001	975,9 ± 0,5	975,6 ± 0,4
7 μMgemoglobin solution	0,196 ± 0,0001**	0,201 ± 0,0005 *,**	975,5 ± 0,3	975,6 ± 0,1

Notes: * – statistically significant difference relative to control samples P < 0,05, ** – statistically significant difference relative to samples with distilled water *P* < 0,05.

As can be seen from figures 3 and 5, the optical density at the maximum of the second overtone of the OH-valence oscillation of water molecules increases with increasing temperature. At the same time, there is a very strong negative correlation between the value of maximum absorption and temperature. So, the increasing of temperature causes the shift of the absorption maximum to the region of higher energies. These results confirm and significantly detail the data obtained in [11]. Fluctuations can be observed in the temperature range of 20–34 °C. Such fluctuations in the temperature dependence of heat capacity, electrical conductivity and other parameters are revealed of water by different researchers [11–15].

The high positive correlation between temperature and optical density at the maximum of the second OH-overtone can be explained on the basis of generally accepted ideas about the influence of temperature on water properties. As the temperature increases the libration-translational movement of water molecules increases that leads to the weakening of hydrogen bonds and their rupture. But the results of studies of the IR spectra of water in other ranges indicate a more complex nature of the temperature dependence of the IR spectra of water [16]. In particular, as the temperature increases the optical density at the fundamental frequency of the OH-bond (3400–3500 cm-1) decreases and the frequencies value shifts to the region of higher energies, that is to the region of shorter waves. At the same time, the intensity of the bands responsible for deformation (1640–1670 cm-1) and libration (600–800 cm-1) oscillations increases with increasing temperature. In the temperature range of 10–40°C, the position of the maximum absorption is changed weakly. This behavior of the absorption spectra in the IR range is due to the fact that the energy of hydrogen bonds, which form a complex dynamic network, is characterized by a certain distribution of energy. As the temperature rises the hydrogen bonds with low energy are broken first that leads to decrease in the total number of OH-bonds and, accordingly, to decrease of light absorption. The remaining OH-bonds are characterized by higher energy that is reflected in the absorption spectra to the form of a shift of the fundamental band of the OH-oscillation to the region of shorter waves with higher energy. As a result of the rupture and deformation of hydrogen bonds upon elevated temperatures the number of molecules with deformation and libration modes increases that leads to an increase of light absorption on these spectral lines. The position of these lines in the spectrum shifts to the region of higher energies much weaker compared to the fundamental mode.

Thus, based on the data [16] and taking into account the fact that the band of the second OH-vibration overtone is a combination of several 2v1+v2; v1+2v2; 2v1+2v3; 2v2+2v3, the temperature dependences shown in fig. 4–6, can be explained as follows. The reason for the shift of the absorption maximum to the region of higher energies is mainly the shift of the fundamental mode of OH-oscillations, and the increase of optical density is the result of superposition of two opposite effects, that is decrease of the fundamental mode of OH-bond and increase of deformation mode. The contribution of the latter is dominant that ultimately determines the positive correlation between temperature and optical density.

One-hour of exposure in MEMR led to heating of the experimental samples to about 2.5°C after which the temperature stabilized (fig. 2). Nevertheless, in this equilibrium state the values of optical density at the maximum absorption of the second OH-bond overtone for distilled water were within revealed temperature dependence (fig. 3 and 5; table 1). Thus, with increasing temperature the distilled water changes its optical properties regardless of the method of heating, that is by infrared or millimeter radiation heating.

It is necessary to note that the values of optical density in hemoglobin solutions are statistically significantly lower compared to samples of pure distilled water (fig. 3, 5).

There may be several explanations for this fact. The first is, the proportion of water in the protein solution is slightly lower compared to pure water samples. The decrease of the mass fraction of water should lead to a decrease in the optical density on the fundamental line of OH-bond and on its overtones. However, the mass fraction of protein in aqueous solution is very small, and when it is reduced twice the difference in light absorption between distilled water and protein solutions decreases very weakly (fig. 5). Therefore, the such explanation is not complete.

The second explanation may be related to the fact that in the protein solution part of the water molecules is involved to the formation of the hydrate shell of proteins where water molecules are structured by certain way and, accordingly, their mobility is limited. If we take into account the results [11, 16] the consequence of such structural ordering of water in the hydrated shells of proteins should be decrease of absorption in the IR region. The reducing of the protein concentration leads to decrease of this difference between the absorption of light by pure water and protein solutions (fig. 3 and 5, table 1). Probably in the experiment we have combination of both reasons.

Analysis of the effect of microwave radiation on the optical properties of water showed that the value of the optical density of distilled water after its exposure in MEMR fully coincide with the temperature effects for control water samples and deviations from temperature dependence are not observed (fig. 3 and 5). But it is impossible to say unequivocally for hemoglobin solutions. If statistically significant deviations from the temperature dependence of the optical density on the second overtone of the OH-bond was not observed in more concentrated solutions of protein (15 μM) (fig. 3), there was small but statistically significant increase of light absorption in more dilute solutions of hemoglobin (7 μM) (fig. 5). According to the detected temperature dependence of the optical properties for the hemoglobin solution at this concentration the action of the MEMR led to additional "heating" of the sample by 5–6°C that is a very large value. At the same time the effect of MEMR on hemoglobin solutions in no way affects the value of the absorption maximum in the band of the second overtone of water (fig. 4, 6). Thus, the follow certain contradiction arises: the temperature valuesmeasured in protein solutions by a usual contact thermometer and by the optical method can differ significantly. What are the reasons for this discrepancy?

It is known that substances when dissolved in water change its structural and dynamic state that is reflected in changes of the thermodynamic parameters of water and aqueous solutions [17–19]. Dissolved substances depending on their chemical nature affect the structural and dynamic properties of water in different ways. Some substances limit the translation motion of water molecules and thus contribute to the formation of hydrogen bonds between them, while others, on the contrary, contribute to the destruction of hydrogen bonds. Thus, solute substances affect such key properties of aqueous solutions as their viscosity, heat capacity, solubility for other substances, etc. If external physical factors, in particular MEMR, affect the libration-translational motion of water molecules, this in turn also affects the thermodynamic state of the entire water or water-colloidal system. To explain such effects in the early 80s of the 20th century Ueberreiter K. [17], comparing structured and unstructured liquids, introduced the concept of structural temperature which for water, according to the author, is in the range of 140–150oK. This is the imaginary temperature that corresponds to the transition of the liquid into a fully structured solid state. Thus, according to the idea of Ueberreiter K., the structural temperature shows the degree of structure of the liquid. If the structural temperature of the fluid increases it means its further structuring, and if it decreases, it corresponds to the destructuring of the fluid.

Using this approach, as well as the results of our study and other data [11, 16], the revealed effects can be explained as follows. The MEMR energy is weak enough to break hydrogen bonds but is sufficient to excite the rotational motions of water molecules and, as a result, increases the probability of deformation oscillations that appear in the near IR range in the second overtone band as an increase in optical density. Due to the fact that the deformation oscillations show much weaker dependence on temperature [16], the absorption maximum at the second overtone is practically unchanged.

Thus, the results of our study prove that MEMR in relation to the aqueous phase should be considered as a destructive factor that reduces the structural temperature of solutions due to activation of the rotational-translational motion of water molecules, which in turn should reduce the viscosity of aqueous solutions, heat capacity and other thermodynamic parameters. The results obtained by other researchers confirm existence of such effects [20]. In addition, according to the results of our study such effects can be highly nonlinear and show a complex dependence on the concentration of solute substances, in particular proteins.

Conclusions. The parameters of the water absorption spectrum in the range of the second overtone of the OH-valence oscillations of the water molecule depend on the temperature. As the temperature increases, the optical density increases almost linearly and the absorption maximum shifts to the blue region that indicates the destruction of hydrogen bonds and the growth of the rotational-translational motion of water molecules.

One-hour of exposure of the distilled water in the MEMR does not cause significant changes in the absorption spectrum of water in the range of the second overtone OH-valence oscillations of the water molecule, but in the dilute hemoglobin solutions in this spectral range there is a small but statistically significant increase of light absorption. Additional MEMR-induced activation of libration motion of water molecules increases the probability of deformation oscillations which are manifested in the near IR range on the band of the second overtone of the valence oscillations of water molecules.

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ВПЛИВ ЕЛЕКТРОМАГНІТНОГО ВИПРОМІНЮВАННЯ МІЛІМЕТРОВОГО ДІАПАЗОНУ НА ОПТИЧНІ ВЛАСТИВОСТІ ВОДИ В РОЗЧИНАХ ГЕМОГЛОБІНУ

Досліджено особливості впливу мікрохвильового випромінювання на оптичні властивості чистої води і води в розчинах гемоглобіну у ближньому інфрачервоному діапазоні, що характеризують її певний структурно-динамічний стан. Об'єктом дослідження були оптичні властивості дистильованої води і водних розчинів гемоглобіну людини в концентрації 7 і 15 мкМ у температурному діапазоні 10–40 °С. Реєстрацію спектрів здійснювали на спектрофотометрі BiospecMini у діапазоні 190–1100 нм. З метою підвищення точності аналізу спектральних даних проводили нормування спектрів щодо базової лінії, за яку брали лінію, що проходила паралельно горизонтальної осі через мінімальне значення оптичної густини у спектрі поглинання. Значення максимумів поглинання й оптичну густину використовували як головні параметри спектрів поглинання. Для точнішого визначення зазначених параметрів застосовували функцію B-spline. Мікрохвильове опромінення дистильованої води і розчинів гемоглобіну здійснювали на довжині хвилі 7,1 мм з максимальною 100-відсотковою вихідною потужністю, яка згідно з розрахунком становила приблизно 24 мВт. Деталізовано залежність параметрів спектра поглинання води в діапазоні другого обертону ОН-валентного коливання молекули води від температури. З'ясовано, що одногодинна експозиція дистильованої води в мікрохвильовому ЕМВ не викликає достовірних змін у спектрі поглинання води в діапазоні другого обертону ОН-валентного коливання молекули води, але в розбавлених розчинах гемоглобіну в цьому спектральному діапазоні спостерігаємо мале, але статистично достовірне зростання поглинання ІЧ-світла. Це може свідчити про додаткову ЕМ-індуковану активацію лібраційних рухів молекул води і зростання ймовірності деформаційних коливань, що виявляються у ближньому ІЧ-діапазоні на смузі другого обертону води.

Ключові слова: вода, розчини гемоглобіну, мікрохвильове електромагнітне випромінювання, другий обертон ОН-валентного коливання.

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ВЛИЯНИЕ ЭЛЕКТРОМАГНИТНОГО ИЗЛУЧЕНИЯ МИЛЛИМЕТРОВОГО ДИАПАЗОНА НА ОПТИЧЕСКИЕ СВОЙСТВА ВОДЫ В РАСТВОРАХ ГЕМОГЛОБИНА

Исследовано влияние микроволнового излучения на оптические свойства дистиллированной воды и воды в растворах гемоглобина в ближнем инфракрасном диапазоне, который характеризует ее определенное структурно-динамическое состояние. Объектом исследования были оптические свойства дистиллированной воды и водных растворов гемоглобина человека в концентрации 7 и 15 мкМ в температурном диапазоне 10–40 °С. Регистрацию спектров осуществляли на спектрофотометре BiospecMini в диапазоне 190–1100 нм. С целью повышения точности анализа спектральных данных проводили нормирования спектров относительно базовой линии, проходящей параллельно горизонтальной оси через минимальное значение оптической плотности в спектре поглощения. Значение максимумов поглощения и оптическую плотность использовали в качестве главных параметров спектров поглощения. Для более точного определения указанных параметров использовали функцию B-spline. Микроволновое облучение дистиллированной воды и растворов гемоглобина осуществляли на длине волны 7,1 мм с максимальной 100-процентной выходной мощностью, составляющей примерно 24 мВт. Детализировано зависимость параметров спектра поглощения воды в диапазоне второго обертона ОН-валентного колебания молекулы воды от температуры. Выяснено, что одночасовая экспозиция дистиллированной воды в микроволновом ЭМИ не вызывает достоверных изменений в спектре поглощения воды в диапазоне второго обертона ОН-валентного колебания молекулы воды, но в разбавленных растворах гемоглобина в этом спектральном диапазоне наблюдается малый, но статистически достоверный рост поглощения ИК-света. Это может свидетельствовать о дополнительной ЭМ-индуцированной активации либрационных колебаний молекул воды и росте вероятности деформационных колебаний, проявляющихся в ближнем ИК-диапазоне на полосе второго обертона воды.

Ключевые слова: вода, растворы гемоглобина, микроволновое электромагнитное излучение, второй обертон ОН-валентного колебания.