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INFLUENCE OF ELECTROMAGNETIC RADIATION OF MILLIMETER RANGE ON THE OPTICAL PROPERTIES THE HEMOGLOBIN

The biological activity of millimeter electromagnetic radiation (MEMR) has long been known that is the basis for its use in medicine. But the primary biophysical mechanisms of this factor at the molecular level remain poorly understood and debated. Because of it the features of the influence of microwave radiation on the optical properties of water solutions of hemoglobin with the aim to characterize the certain structural state of hemoglobin were investigated in this research.

The object of the study was the optical properties of 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 (Shimadzu) 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 exposure of hemoglobin solutions was performed at wavelength of 7,1 mm with power that calculated to be approximately 6mW. This power of electromagnetic radiation corresponds to the conditional limit range between thermal and non-thermal action of this factor.

The dependence of the optical properties of hemoglobin in the spectral range of heme absorption on temperature was analyzed and effects of microwave radiation on the optical properties of hemoglobin solutions were considered in frame of hypochromic effects induced by temperature. It was revealed that the parameters of the absorption spectrum of an aqueous solution of hemoglobin depend on temperature. The optical density in the Sore band and the spectral bands of the oxygenated form of hemoglobin decreases upon temperature increase. The observed temperature hypochromic effect is associated with increased protein aggregation.

The effect of MEMR leads to a small but statistically significant hypochromism in the spectra of hemoglobin absorption that testifies on increase in protein aggregation under the action of this physical factor which cause the changes in the hydration of protein molecules. This effect depends on the protein concentration and is not detected at low concentrations.

Keywords: hemoglobin solutions, hypochromism, microwave electromagnetic radiation.

The biological activity of microwave electromagnetic radiation (MEMR) is well known [1]. This range of electromagnetic radiation is used in medicine as one of the physical factors for various therapeutic treatments [2]. However, the primary biophysical mechanisms of action of this electromagnetic factor remain unclear and actively debated.

It is believed that the main substance that interacts with MEMR in cells or model biological experiments is the water which is in different phase states [2–4]. The structural-dynamic state of water should in some way affect the hydration of biological molecules and their molecular dynamics that in turn should affect the structural-functional properties of biological macromolecules. Our previous studies found that MEMR-exposure of water solutions at a wavelength of 7,1 mm with an intensity of 6mW, which is into the limiting conditional range between thermal and non-thermal action of this factor, caused the significantly changes the optical properties of water in the nearinfrared range only in the presence of proteins [6]. A small but statistically significant hyperchromic effect indicated an additional MEMR-induced destruction of hydrogen bonds and an increase in the rotational-translational motion of water molecules in hemoglobin solutions.

It is known that protein macromolecular constructs have unique properties for the formation and coherent ordering of certain types of polariton states transmitted along the polypeptide chain and whose energy is used to perform chemical and molecular mechanical work, i. e., to perform its own specific functions of proteins in living organisms [7–9]. Such coherent states can arise due to the nonlinear interaction of external electromagnetic waves with own elementary excitations in macromolecular structures. Changing the structural and dynamic state of the aqueous environment of proteins can contribute to both the formation and destruction of such states in the proteins that in turn should affect their structural and functional properties.

Based on modern theoretical ideas and the results of previous studies the aim of this study was to elucidate the effects of microwave radiation on the optical properties of hemoglobin that characterize a certain structural and functional state of this protein. Hemoglobin is a key blood protein that transports oxygen to tissues, its structural and functional features are well known, so this protein is a convenient model for studying the primary mechanisms of action of various physical factors on the structural and functional properties of biomacromolecular structures.

Materials and methods. The object of this study was the optical properties of aqueous solutions of human hemoglobin. The study material was 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 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 higher than the partial pressure in arterial blood and 3 times higher than in venous [5]. Therefore, hemoglobin in aqueous solutions was in fully oxygenated form. Calculations of hemoglobin concentration in solutions are described in detail in [6].

The absorption spectra of distilled water and hemoglobin solutions were recorded on a BiospecMini spectrophotometer in the range of 190–1100 nm.

MEMR-exposure of hemoglobin solutions at the wavelength of 7,1 mm with power 6mW, was performed directly in spectrophotometric quartz cuvettes using a generator G4-141. The working surface of the quartz cell was placed perpendicular to the axis of the waveguide at a distance of 2 mm [6]. The total amount of energy absorbed by aqueous protein solution during 60-wave exposure in MEMR with a wavelength of 7,1 mm is equal to 21,16 J for

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this experimental protocol. This power of electromagnetic radiation corresponds to the conditional limit range between thermal and non-thermal action of this factor.

The temperature of the samples was measured in a quartz cuvette using a digital thermometer 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. According to previous studies [6], the optical properties of water in the near infrared range had a clear dependence on temperature, but one hour exposure of pure water in MEMR did not cause significant changes in the characteristics of the combination band of the second overtone OH-valence oscillation of the water molecule ($2\nu_1+\nu_2$, $\nu_1+2\nu_2$, $2\nu_1+2\nu_3$, $2\nu_2+2\nu_3$). However, a small but statistically significant hyperchromic effect in this spectral range was observed for water in hemoglobin solutions. It was concluded [5] that this is the result of additional MEMR-induced destruction of hydrogen bonds and an increase of the rotational translation of water molecules in hemoglobin solutions. Thus, the presence of protein in the solution was a key factor for changing the optical properties of water upon influence MEMR. But the question about changes of the properties of hemoglobin remained open.

In this study we find that the optical properties of hemoglobin solutions in the visible range of heme absorption also depend on the temperature of the solution (fig. 1 and 2). However, in contrast to the temperature dependence for water, the optical density in the range of hemoglobin absorption in the blue, yellow-green and yellow regions of the spectrum decreased with increasing temperature. The observed hypochromic temperature effects are small but statistically significant and should be considered if studies are performed at room temperatures ranging from ± 5 °C or more. At the other hand, the analysis of the absorption spectra showed no shifts of the position of the absorption maxima with increasing temperature are observed.

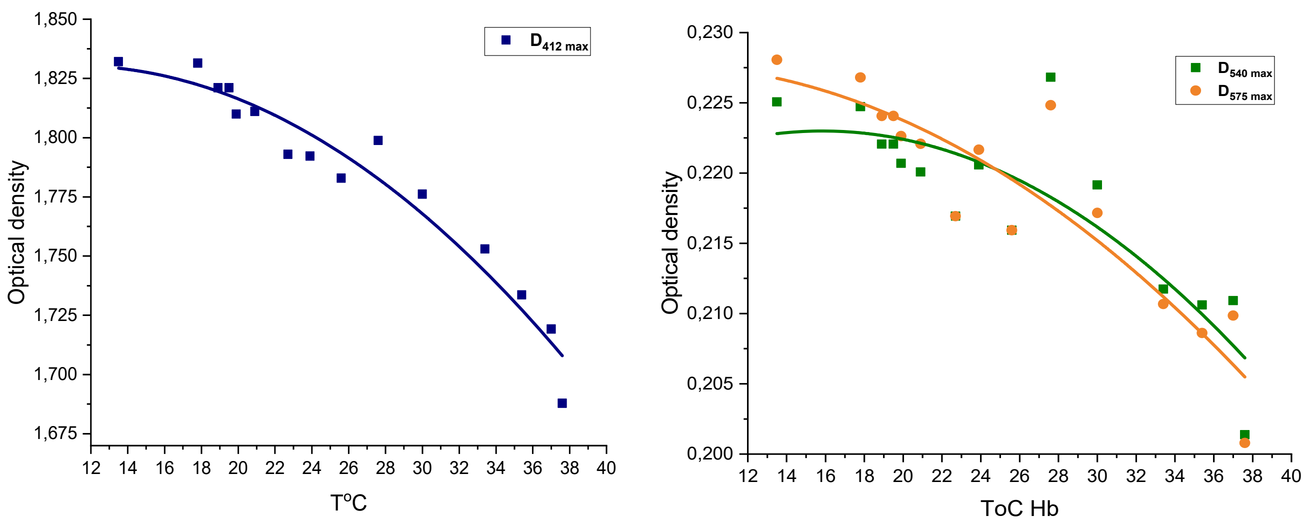

Fig. 1. Temperature dependence of the optical density in 15 μM hemoglobin solutions at the absorption maxima of the Sore band (412 nm – blue region) and the absorption maxima of the oxygenated form of hemoglobin (540 – [■] yellow-green and 575 – [●] yellow spectral region)

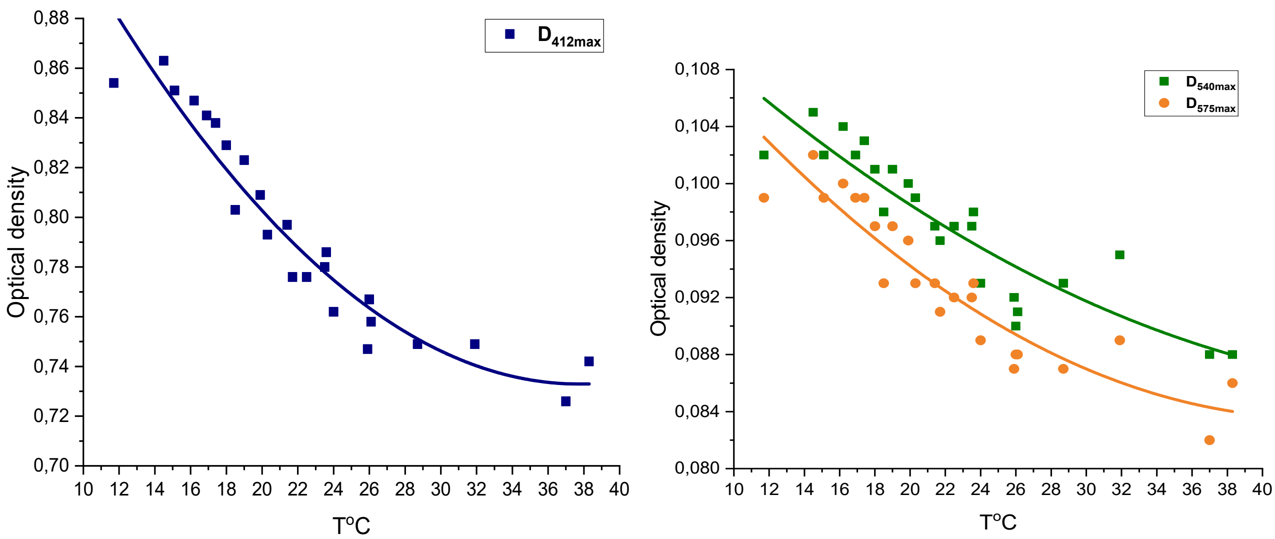

Fig. 2. Temperature dependence of the optical density in 7μM hemoglobin solutions at the absorption maxima of the Sore band (412 nm – blue region) and the absorption maxima of the oxygenated form of hemoglobin (540 – [■] yellow-green and 575 – [●] yellow spectral region)

The question arises as to the causes of the detected temperature hypochromism. It is known [10, 11] that hypochromism occurs due to two main reasons. The first cause is related to the dipole-dipole interaction of chromophores and the polarization interaction of dipole transition moments in an ordered system of biomacromolecules. According to this exciton mechanism a wide absorption band is formed due to the collinear and parallel interaction of chromophores. Hypochromism occurs due to the fact that the parallel interaction of dipole moments of the transition of chromophores begins to prevail. But in this case, from the two separate bands that forming the integrated spectral band, the low-frequency band should dominate, and the intensity of the high-frequency band should decrease. According to this mechanism, we should expect a shift of the maximum of the integral absorption band to the red region. In our case such noticeable shifts were not observed.

The second cause of hypochromism is related to the so-called "sieve" effect, i. e., the uneven distribution of chromophores due to formation of the molecular aggregates that in turn leads to a violation of the Bouguer-Lambert-Behr law. A classic manifestation of this effect is hypochromism of hemoglobin optical specters in erythrocyte suspensions [12]. Given the fact that in this study no significant shifts in the position of absorption maxima occurred, the detected changes in the absorption spectra of hemoglobin can be explained by the effect of "sieve" which occurs due to aggregation of hemoglobin molecules. The driving causeof the protein aggregation in this case is likely the destruction of hydrogen bonds in the aqueous phase and the increase of the rotational-translation motion of water molecules with increasing temperature thatresultsto decrease of the hydrate shell of protein macromolecules and increase their ability to aggregation.

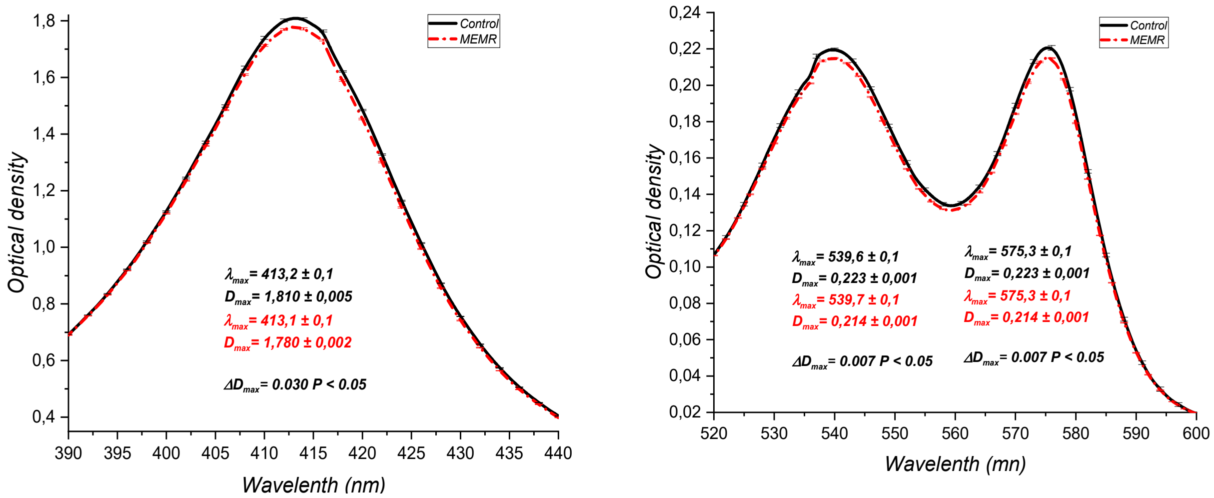

Fig. 3. Absorption spectra in the blue, as well as in the yellow-green and yellow region of the spectrum of 15 μM solutions of hemoglobin in control samples (solid black line) and upon 1-hour exposure to MEMR (7,1 mm, 6 mW, 60 min exposure, dash dot red line), (*T* = 22,5°C)

Note: here and below – the spectral lines shown in the figures are obtained by averaging the absorption spectra for 10 repeated studies (*n* = 10).

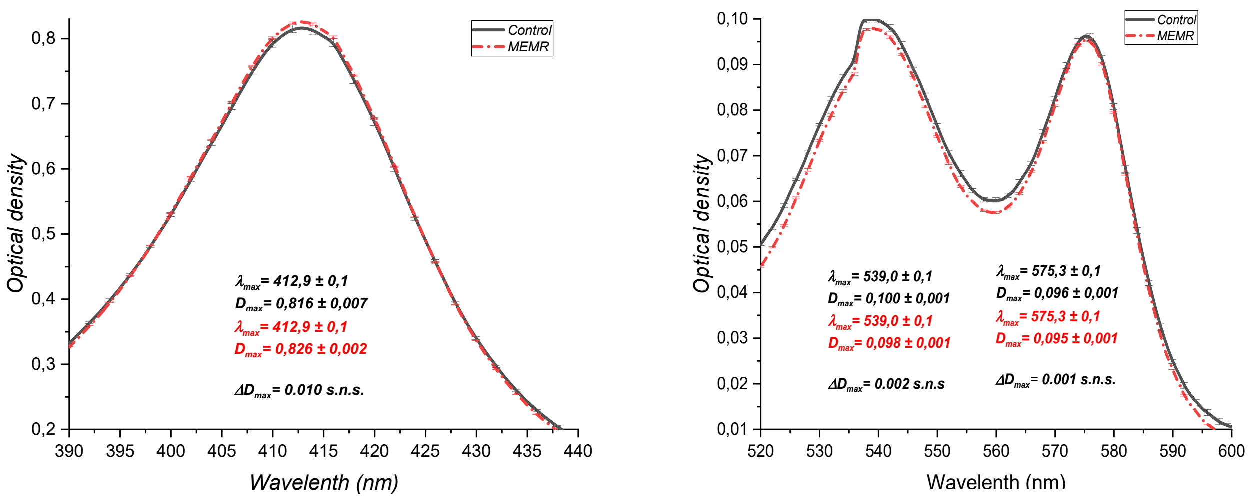

Fig. 4. Absorption spectra in the blue, as well as in the yellow-green and yellow region of the spectrum of 7μM solutions of hemoglobin in control samples (solid black line) and upon 1-hour exposure to MEMR (7,1 mm, 6 mW, 60 min exposure, dash dot red line), (*T* = 19,5°C). s. n. s – statistically insignificant difference)

The influence of MEMR on hemoglobin solutions causes the statistically significant decrease of the optical density at the absorption maxima in the optical spectra under conditions of equal temperatures in the experimental and control samples (fig. 3). This hypochromic effect is also not accompanied by changes of the position of absorption maxima. Thus, we can conclude that the effect of MEMR on the optical properties of hemoglobin also occurs by the temperature mechanism. This conclusion is confirmed by previously obtained data [6] showed the additional destruction of hydrogen bonds and growth of rotational translation motion of water molecules under conditions of equal temperatures in the experimental samples compared to control.

Thus, MEMR with threshold thermal intensity is a physical factor that causes aggregation of hemoglobin molecules due to the impact on the structural-dynamic properties of water. It is important to note that at low protein concentrations such effects are not observed (Fig. 4) also as for water phase too [6]. Such a lack of statistically significant effects of MEMR on the properties of water in low-concentration hemoglobin solutions [6] indicates that the biological effects of electromagnetic fields are manifested in highly concentrated aqueous-colloidal systems and probably such effects should be much stronger in the case of intact cells and biological fluids.

Conclusion. The parameters of the absorption spectrum of an aqueous solution of hemoglobin depend on temperature. As the temperature increases, the optical density in the Sore band and the bands of the oxygenated form of hemoglobin decreases. The observed hypochromic temperature effect is associated with increased protein aggregation.

The effect of MEMR leads to a small but statistically significant hypochromism in the spectra of hemoglobin absorption that indicates the increase of protein aggregation unon the action of this physical factor which cause the changes of hydration of protein molecules. This effect depends on the protein concentration and is not detected at low concentrations.

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ВПЛИВ ЕЛЕКТРОМАГНІТНОГО ВИПРОМІНЮВАННЯ МІЛІМЕТРОВОГО ДІАПАЗОНУ НА ОПТИЧНІ ВЛАСТИВОСТІ ГЕМОГЛОБІНУ

Біологічна активність мікрохвильового електромагнітного випромінювання (МЕМВ) відома давно, що є основою його застосування в медицині. Однак біофізичні механізми цього фактора на молекулярному рівні залишаються недостатньо вивченими, щодо них ідуть активні дискусії. З огляду на це було досліджено особливості впливу МЕМВ на оптичні властивості водних розчинів гемоглобіну з метою характеристики певного структурного стану цього білка.

Об'єктом дослідження були оптичні властивості водних розчинів гемоглобіну людини в діапазоні концентрацій 7 і 15 мкМ та в діапазоні температур 10–40 ° С.

Оптичні спектри реєстрували за допомогою спектрофотометра Biospec Mini в діапазоні довжин хвиль 190–1100 нм. З метою підвищення точності аналізу спектральних даних спектри були нормалізовані щодо базової лінії, що проходила паралельно горизонтальній осі через мінімальне значення оптичної щільності у спектрі поглинання. Як основні параметри спектрів поглинання були використані значення максимумів поглинання та оптичної щільності. Для точнішого визначення зазначених параметрів застосовано функцію В-сплайну.

Експозицію розчинів гемоглобіну в МЕМВ проводили на довжині хвилі 7,1 мм з потужністю 6 мВт. Ця потужність МЕМВ відповідає умовному граничному діапазону між тепловою та нетермічною дією цього фактора.

У статті проаналізовано залежність оптичних властивостей гемоглобіну у спектральному діапазоні поглинання гемму від температури та розглянуто вплив мікрохвильового випромінювання на оптичні властивості розчинів гемоглобіну в контексті гіпохромних ефектів, індукованих температурою. Виявлено, що параметри спектра поглинання водного розчину гемоглобіну залежать від температури. Оптична щільність у смузі Соре та спектральних смуг ахоксигенованої форми гемоглобіну зменшується за підвищення температури. Спостережуваний температурний гіпохромний ефект пов'язаний зі збільшенням агрегації білка.

Вплив МЕМВ призводить до невеликого, але статистично значущого гіпохромізму у спектрах поглинання гемоглобіну, що свідчить про збільшення агрегації білка під дією цього фізичного фактора, який спричиняє зміни в гідратації білкових молекул. Зазначений ефект залежить від концентрації білка і не виявляється за низьких концентрацій.

Ключові слова: розчини гемоглобіну, гіпохромізм, мікрохвильове електромагнітне випромінювання.

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ВЛИЯНИЕ ЭЛЕКТРОМАГНИТНОГО ИЗЛУЧЕНИЯ МИЛЛИМЕТРОВОГО ДИАПАЗОНА НА ОПТИЧЕСКИЕ СВОЙСТВА ГЕМОГЛОБИНА

Биологическая активность микроволнового электромагнитного излучения (МЭМИ) известна давно, что является основой его применения в медицине. Но биофизические механизмы этого фактора на молекулярном уровне остаются недостаточно изученными и активно дискутируются. Поэтому были исследованы особенности влияния МЭМИ на оптические свойства водных растворов гемоглобина с целью характеристики определенного структурного состояния этого белка.

Объектом исследования стали оптические свойства водных растворов гемоглобина человека в диапазоне концентраций 7 и 15 мкм и в диапазоне температур 10–40 ° С.

Оптические спектры регистрировали с помощью спектрофотометра BiospecMini в диапазоне длины волн 190–1100 нм. С целью повышения точности анализа спектральных данных спектры были нормализованы относительно базовой линии, проходившей параллельно горизонтальной оси через минимальное значение оптической плотности в спектре поглощения. В качестве основных параметров спектров поглощения были использованы значения максимумов поглощения и оптической плотности. Для более точного определения этих параметров использовали функцию В-сплайна.

Экспозицию растворов гемоглобина в МЭМИ проводили на длине волны 7,1 мм с мощностью 6 мВт. Эта мощность МЭМИ соответствует условному предельному диапазону между тепловым и нетепловым действием этого фактора.

В статье проанализировано зависимость оптических свойств гемоглобина в спектральном диапазоне поглощения гемма от температуры и рассмотрено влияние МЭМИ на оптические свойства растворов гемоглобина в контексте гипохромных эффектов, индуцированных температурой. Выявлено, что параметры спектра поглощения водного раствора гемоглобина зависят от температуры. Оптическая плотность в полосе Соре и спектральных полосах оксигенированной формы гемоглобина уменьшается при повышении температуры. Наблюдаемый температурный гипохромный эффект связан с увеличением агрегации белка.

Влияние МЭМИ приводит к небольшому, но статистически значимому гипохромизму в спектрах поглощения гемоглобина, что свидетельствует об увеличении агрегации белка под действием этого физического фактора, вызывающего изменения в гидратации белковых молекул. Этот эффект зависит от концентрации белка и не проявляется при низких концентрациях.

Ключевые слова: растворы гемоглобина, гипохромизм, микроволновое электромагнитное излучение.