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STUDY OF INTERACTION OF HOECHST 33258 WITH DNA AND HUMAN SERUM ALBUMIN UNDER THE INFLUENCE OF MILLIMETER RANGE ELECTROMAGNETIC WAVES

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In this work the effect of millimeter range electromagnetic waves (MM EMW) with the frequency 64.5 *GHz* on the complexes of Hoechst 33258 (H33258) with DNA and human serum albumin (HSA) has been studied by the methods of absorption and fluorescence spectroscopies. It was shown that the irradiation results in weakening of H33258 interaction with both macromolecules, which is connected with the fact that the frequency 64.5 *GHz*, being resonant for water, leads to the structurizing of water component around DNA and HSA, due to which the binding becomes weaker. This conclusion is based on the values of both binding constants and Stern–Volmer constants.

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***Keywords*:** millimeter range electromagnetic waves, macromolecule, ligand, absorption spectra, fluorescence spectra, binding constant, Stern–Volmer constant.

Introduction. Nowadays the studies of the effect of millimeter range electromagnetic waves (MM EMW) (1–10 *mm*, 30–300 *GHz*) on biological systems are of great interest, which is connected with the fact that the natural background of these waves is small and, apparently, living organisms have not adapted to this physical factor during the evolution [1]. Connected to the anthropogenic factor the intensity of MM EMW in the medium increase continuously and the peculiarity of the given physical factor is their action on biological systems, being on any level of organization [2–7]. From this point of view, along with the studies of MM EMW effect on organisms in general or on a cell separately, the experiments of this factor effect on biomacromolecules, particularly on the interaction with various high- and low-molecular weight compounds are of great interest [2–4, 8]. Among biomacromolecules DNA is important, which in a cell is in surrounding of various low-molecular weight compounds – ligands that interact and even change the structure and function of DNA. On the other hand, DNA also is exposed to the effect of MM EMW. From this point of view, it is interesting to study the effect of MM EMW on the structure and functions of DNA complexes with different ligands. The most important biomacromolecules are proteins that also are exposed to the effect of MM EMW. The effect of MM EMW on proteins is also an interesting topic to be studied,

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especially when they interact with ligands. In this work human serum albumin (HSA) serves as a protein example that mainly is a transport protein in blood plasma and interacts with various ligands, including drug preparations and contributes to the transport of these compounds throughout blood circulation. There exist many works, dedicated to MM EMW effect study on the structure and conformational changes of DNA and HSA [2, 3, 9]. It follows from these studies that the effect of MM EMW can lead to conformational changes of these macromolecules. On the other hand, as it was aforementioned, both DNA in a cell and HSA in blood always are in the surrounding of numerous small molecules that in any case interact with polymers and even form complexes with them [10–12]. From this point of view, the studies of MM EMW effect on the complexes of ligands with macromolecules are actual.

In the present work the effect of MM EMW on complex-formation of Hoechst 33258 (H33258) with DNA and HSA were studied. The choice of H33258 results from the fact that it is a ligand, specifically interacting with DNA (H33258 is known as AT-specific ligand, preferably binding in DNA minor groove) and it is worthwhile to examine how it interacts with HSA.

Materials and Methods. Calf thymus DNA (average GC-content – 42%), HSA, Hoechst 33258 ("Sigma", USA), physiological solution were used in the experiments. All preparations were used without additional purification. Concentrations of DNA and H33258 were determined spectrophotometrically, using the following values of the extinction coefficients: $\varepsilon_{260}=6600\ M^{-1}cm^{-1}$ for DNA and $\varepsilon_{343}=42000\ M^{-1}cm^{-1}$ for H33258. In experiments it was used HSA with 1% concentration.

DNA and HSA complexes with H33258 were studied by absorption and fluorescence spectroscopy methods. Absorption spectra of the complexes of DNA and HSA with H33258 were obtained using spectrophotometer Perkin Elmer Lambda 265 (USA) in the interval $300\leq\lambda\leq700$ *nm*. After the obtaining of absorption spectra of pure ligand, titration of the ligand solution was carried out by the solution of DNA and HSA at concentration ratios of ligand/DNA and ligand/HSA from 1/2 to 1/10.

Fluorescence spectra of the complexes of DNA and HSA with H33258 were obtained by the spectrofluorometer Cary Eclipse (Australia). Excitation of the samples was carried out at the wavelength 343 *nm* and the spectra were registered in the interval $400\leq\lambda\leq600$ *nm*. After obtaining of fluorescence spectrum of pure ligand, titration of H33258 solution by the solutions of DNA and HSA was carried out at concentration ratio ligand/macromolecule from 1/2 to 1/10.

Irradiation was carried out in special glassy dishes. Width of irradiating layer of the solution was equal to ≈1 *mm*. For the irradiation the source of extremely high frequency signals G4-142 was used with working interval 53.57–78.33 *GHz*. Stability of signal frequency of the generator was equal to ±0.05% and frequency deviation of the output signal in the regime of continuous generation does not exceed 6 *MHz*. Irradiation of the samples was carried out at room temperature, power flux density in the sample place at the frequency 64.5 *GHz* was equal to $\approx50\ \mu W/cm^2$. For revelation of MM EMW effect on complex-formation of DNA and HSA with ligands the solutions of DNA and HSA were irradiated during 60 *min* by MM EMW 64.5 *GHz* frequency. Measurement error does not exceed 4%.

Results and Discussion. To determine the binding parameters of DNA and HSA with H33258 at the influence of MM EMW a spectrophotometric titration of H33258 solution by irradiated and non-irradiated solutions of macromolecules was carried out. Then from the obtained spectra (spectra are not presented) the adsorption isotherms were constructed by the Scatchard's method. The binding curves of H33258 with DNA and HSA are presented in Fig. 1. It is obvious from Fig. 1, a, that the binding curves of H33258 with DNA are non-linear, which indicates that the ligand interacts with DNA by more, than one mode.

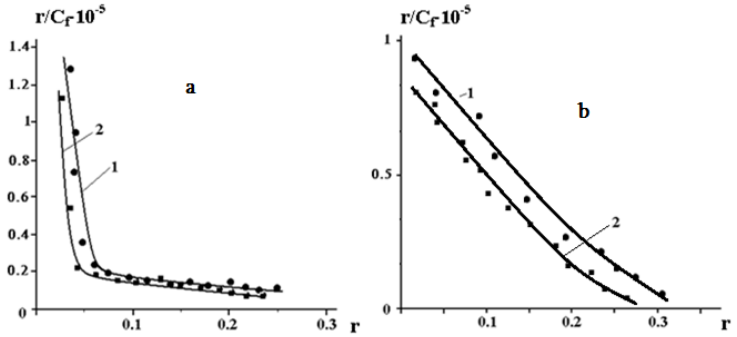

Fig. 1. Charactersitic binding curves of Hoechst 33258 with DNA (a) and HSA (b). Curves 1 correspond to non-irradiated samples; curves 2 correspond to irradiated samples by MM EMW with 64.5 *GHz* frequency.

The binding curves of H33258 with HSA are linear, which, in turn, means that the ligand interacts with HSA by one mode. From the binding curves the binding constants of the ligand to macromolecule were calculated that are presented in Tab. 1. Data, presented in Tab. 1, show that the effect of MM EMW results in decreasing of the binding constant of H33258 with both macromolecules, though, in the case of DNA both strong and weak binding modes weaken. In the case of DNA, a decrease of the binding parameters due to the irradiation was shown earlier in the work [13]. The binding constant of H33258 with DNA by the strong mode decreases by almost twice at the irradiation MM EMW by the frequency 64.5 *GHz*. It indicates that the effect of MM EMW leads to the binding weakening. Most apparently, the irradiation of MM EMW with the frequency 64.5 *GHz* leads to the structurizing of water molecules around macromolecule, due to which the binding between DNA and H33258 in the minor groove of DNA becomes weaker. At the weak binding the data, obtained from the binding curves, indicate that a weakening occurs in this case, which can be explained by the water structurization in the vicinity of DNA, screening the negatively charged phosphate groups from H33258 molecules.

In the case of non-irradiated solution of HAS, the binding constant has a small value as compared to the strong binding to DNA. From the obtained data one can conclude that H33258 binding to HSA occurs by electrostatic mode. At MM EMW irradiation with the frequency 64.5 *GHz* a decrease of the binding constant takes place. This fact also can be explained by the structurization of water molecules around protein molecule induced by the irradiation with water resonant frequency – 64.5 *GHz* and water molecules screen the protein from H33258. It leads to a decreasing of H33258 binding constant with HSA.

Table 1

Values of the binding constants K of H33258 to non-irradiated and irradiated DNA and HSA by MM EMW with the frequency 64.5 GHz

		Non-irradiated sample, $K \cdot 10^{-5}$	Irriated sample by MM EMW with the frequency 64.5 *GHz*, $K \cdot 10^{-5}$
DNA–H33258	strong mode	93 ± 0.5	52 ± 0.2
	weak mode	1.2 ± 0.05	0.31 ± 0.05
HAS–H33258		0.26 ± 0.02	0.19 ± 0.02

The study of H33258 interaction with DNA and HSA by the fluorescence spectroscopy method has been carried out as well. The fluorescence spectra of H33258 and its complexes with HSA, being non-irradiated and irradiated by MM EMW with the frequency 64.5 *GHz* were presented in Fig. 2. Fluorescence spectra of H33258 and its complexes with DNA are not presented, since they are shape-similar. As it is seen from Fig. 2, a, the intensity of H33258 fluorescence spectra with HSA decreases. It indicates that at the binding to HSA, apparently, the surrounding of H33258 becomes more hydrophilic, due to which the fluorescence intensity decreases.

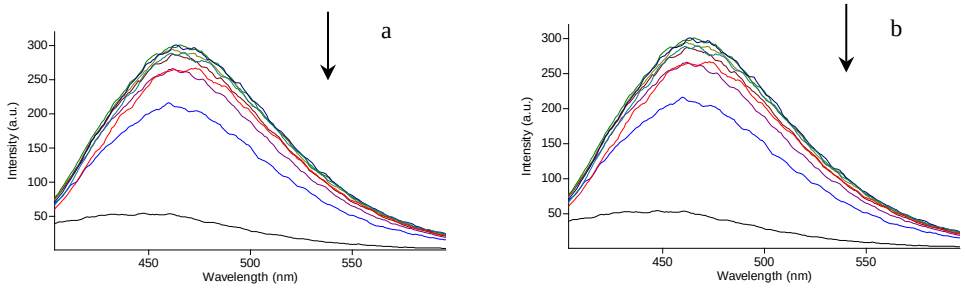

Fig. 2. Fluorescence spectra of H33258 and its complexes with HSA irradiated by MM EMW with the frequency 64.5 *GHz* (a) and non-irradiated (b).

Fluorescence intensity decreasing at the irradiation of the complex HAS–H33258 was shown earlier [14]. In the Tab. 2 the conditional values of maximal fluorescence intensities of H33258 at the addition of HSA were presented. It is obvious from table data, that the values of the intensities decrease. The values of fluorescence intensities of H33258 and its complexes with HSA at the irradiation MM EMW with the frequency 64.5 *GHz* were also presented in Tab. 2. The table data indicate, that along with increasing of HSA concentration a decrease of H33258 fluorescence intensity takes place. Though, as compared to the case of non-irradiated protein the values of fluorescence intensities decrease sufficiently less. It maintains the assumption that at the irradiation MM EMW with the frequency 64.5 *GHz*, water molecules are structurized around the protein and screen the latter from H33258 molecules that is why the binding of H33258 to HSA takes place more weakly, than for non-irradiated protein. Due to this fact the local surrounding of H33258 at the binding to HSA does not become more hydrophilic, than for non-irradiated protein, since the decrease of the fluorescence intensity is weaker.

Table 2

Characteristic values of maximal fluorescence intensities of H33258 and its complexes with HSA at the irradiation and without it

Volume of HSA, added to H33258 solution, μL	Fluorescence intensity (a.u.) of H33258 spectra	Fluorescence intensity (a.u.) of H33258 spectra at the irradiation of the protein by MM EMW, 64.5 *GHz*
0	305.454	305.454
20	295.158	298.964
40	275.065	286.948
60	244.777	266.292
80	193.401	216.455
100	183.648	211.455

Analogous scenery is observed for H33258 binding to DNA. In this case, the fluorescence spectra of H33258 complexes with DNA decrease, as compared to the fluorescence spectrum of pure ligand, which indicates the fluorescence quenching of H33258 by DNA.

Values of maximal intensities of the fluorescence spectra of H33258 complexes with DNA are presented in Tab. 3. As it is shown from Tab. 3, at non-irradiated samples a decrease of fluorescence intensities is pronounced, while at the irradiation with 64.5 *GHz* a decrease of the fluorescence intensities is observed, but in less degree. This indicates that despite the fact of H33258 fluorescence quenching due to DNA at the presence of the irradiation the quenching is expressed less, than at the absence of irradiation.

Table 3

Characteristic values of fluorescence maximal intensities of H33258 and its complexes with DNA at the irradiation and without irradiation

DNA volume, added to H33258 solution, μL	Fluorescence intensity (a.u.) of H33258 spectra	Fluorescence intensity (a.u.) of H33258 spectra at MM EMW irradiation of DNA, 64.5 *GHz*
0	305.454	305.454
20	205.321	280.453
40	193.569	249.236
60	185.432	234.562
80	173.468	219.263
100	162.465	201.528

It is connected to the fact that MM EMW irradiation leads to weakening of H33258 binding with DNA, due to which the fluorescence spectrum decreases less, than for non-irradiated samples. From the fluorescence spectra of H33258 and its complexes with DNA the Stern–Volmer curves were constructed (Fig. 3) and the quenching constants for non-irradiated and irradiated complexes (curve 1 and 2, respectively) were calculated.

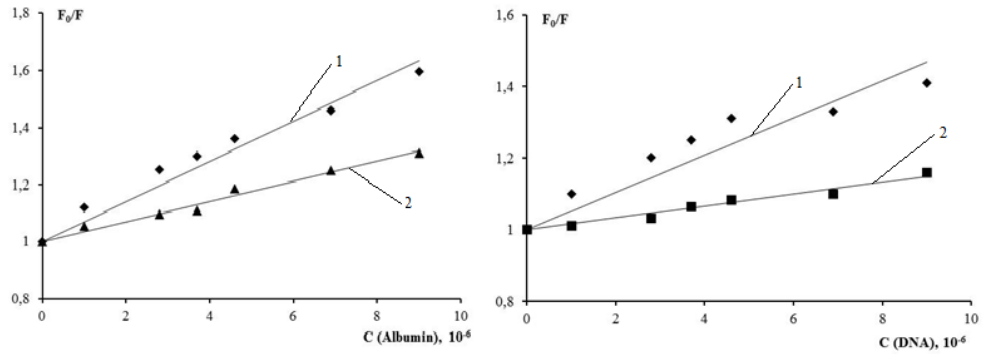

Fig. 3. Characteristic curves of Stern–Volmer for fluorescence quenching of H33258 at its interaction with HSA (above) and DNA (beneath). Curves 1 correspond to non-irradiated samples, curves 2 correspond to irradiated samples.

In the Tab. 4 the values of quenching coefficients of H33258 fluorescence are presented. It is obvious from table data, that the values of K_{SV} decrease at MM EMW irradiation for the complexes H33258–HSA by almost 50%, but for the complexes H33258–DNA – by 30%. This fact also indicates that MM EMW irradiation with the frequency 64.5 *GHz* results in weakening of H33258 binding to HSA and DNA that is why the quenching coefficient decreases. One can assume that a hydrophilization of H33258 surrounding takes place, while binding to macromolecule.

Table 4

Values of Stern-Volmer constants for H33258 at the binding to HSA and DNA

	Values of quenching constants, $K_{SV}\cdot10^{-4}$, *L/mol*	
	H33258–HSA	H33258–DNA
Non-irradiated sample	7.04 ± 0.08	5.2 ± 0.06
Irradiated sample by MM EMW with the frequency 64.5 *GHz*	3.4 ± 0.04	1.6 ± 0.05

Conclusion. Thus, from the data, obtained in the work, one can conclude that MM EMW irradiation with the frequency 64.5 *GHz* results in weakening of the interaction between H33258 and macromolecules. It occurs being resulted from the structurizing of water molecules around macromolecule, which in turn is induced by MM EMW irradiation with the frequency 64.5 *GHz* – water resonant frequency. Consequently, the ligand binding to HSA and DNA weakens. It is indicated by the data, obtained by absorption spectroscopy method. On the other hand, this fact is revealed by fluorescence analysis as well. If in non-irradiated samples the fluorescence intensity of H33258 decreases, indicating that a binding to HSA and DNA occurs and the macromolecule quenches the intensity, in MM EMW irradiated samples with the frequency 64.5 *GHz* the fluorescence intensity decreases, but remains higher as compared to non-irradiated samples. It indicates that the macromolecule is not able to quench the fluorescence intensity well, because the structurized water molecules screen the molecule HSA and DNA from H33258, as a

result of which the binding takes place weakly. This fact is also maintained by the values of Stern–Volmer constants.

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REFERENCES

1. Ongel K., Gumral N., Ozguner F. The Potential Effect of Electromagnetic Field: A Review. *Cell Membrane and Free Radical Research* **1** (2009), 85–89.
2. Kalantaryan V.P., Babayan Y.S., et al. Influence of Low Intensity Coherent Electromagnetic Millimeter Radiation (EMR) on Aqua Solution of DNA. *Progress in Electromagnetics Research Letters* **13** (2010), 1–9. https://doi.org/10.2528/PIERL09110605
3. Zhao R., Zhang S., et al. Studying Gene Expression Profile of Rat Neuron Exposed to 1800 *MHz* Radio-Frequency Electromagnetic Fields with cDNA Microassay. *Toxicology* **235** (2007), 167–175. https://doi.org/10.1016/j.tox.2007.03.015
4. Zhang M., Li X., et al. Effects of Low Frequency Electromagnetic Field on Proliferation of Human Epidermal Stem Cells: An *in vitro* Study. *BioElectroMagnetics* **34** (2013), 74–80. https://doi.org/10.1002/bem.21747
5. Romanenko S., Siegel P.H., et al. Effects of Millimeter Wave Irradiation and Equivalent Thermal Heating on the Activity of Individual Neurons in the Leech Ganglion. *J. Neurophysiology* **112** (2014), 2423–2431. https://doi.org/10.1152/jn.00357.2014
6. Trosic I., Busljeta I., Modlic B. Investigation of the Genotoxic Effect of Microwave Irradiation in Rat Bone Marrow Cells: *in vivo* Exposure. *Mutagenesis* **19** (2004), 361–364. https://doi.org/10.1093/mutage/geh042
7. Abdolmaleki A., Sanginbadi F., et al. The Effect of Electromagnetic Waves Exposure on Blood Parameters. *Int. J. of Hemat. Oncol. and Stem Cell Res*. **6** (2012), 13–16.
8. Shahinyan M.A., Antonyan A.P., et al. Study of the Influence of Millimeter Range Electromagnetic Waves on Methylene Blue Complexes with Human Serum Albumin. *J. of Electromagnetic Waves and Applications* **33** (2019), 2317–2327. https://doi.org/10.1080/09205071.2019.1677508
9. Vardevanyan P.O., Antonyan A.P., et al. Influence of Millimeter Electromagnetic Waves on Fluorescence of Water-saline Solutions of Human Serum Albumin. *J. Applied Spectroscopy* **83** (2016), 486–489. https://doi.org/10.1007/s10812-016-0316-z
10. Willis B., Arya P. Recognition of B-DNA by Neomycin–Hoechst 33258 Conjugates. *Biochemistry* **45** (2006), 10217–10232. https://doi.org/10.1021/bi0609265
11. Vardevanyan P.O., Parsadanyan M.A., et al. Spectroscopic Study of Interaction of Various GC-content DNA with Hoechst 33258 Depending on Na^+ Concentration. *J. Biomol. Struct. and Dyn*. **39** (2020), 1519–1523. https://doi.org/10.1080/07391102.2020.1730244
12. Vardevanyan P.O., Antonyan A.P., et al. Study of Interaction of Methylene Blue with DNA and Albumin. *J. Biomol. Struct. and Dyn*. (2021) https://doi.org/10.1080/07391102.2021.1902397

13. Antonyan A.P., Shahinyan M.A., et al. Effect of MM EMW on Hoechst 33258 Binding to DNA. *Biolog. J. of Armenia* **64** : 1 (2012), 62–68 (in Russian).
14. Vardevanyan P.O., Shahinyan M.A., et al. Spectroscopic Study of Protein Complexes with Low-molecular Compounds. *J. Cont. Physics* (*Arm. Acad. of Sci*.) **56** (2021), 60–64. https://doi.org/10.3103/s1068337221010126

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ИССЛЕДОВАНИЕ ВЗАИМОДЕЙСТВИЯ HOECHST 33258 С ДНК И СЫВОРОТОЧНЫМ АЛЬБУМИНОМ ЧЕЛОВЕКА ПОД ВОЗДЕЙСТВИЕМ МИЛЛИМЕТРОВЫХ ЭЛЕКТРОМАГНИТНЫХ ВОЛН

В работе исследовано влияние миллиметровых электромагнитных волн (ММ ЭМВ) с частотой 64,5 *ГГц* на комплексы Hoechst 33258 (H33258) с ДНК и сывороточным альбумином человека (САЧ) методами абсорбционной и флуоресцентной спектроскопии. Показано, что облучение приводит к ослаблению взаимодействия H33258 с обеими макромолекулами, что связано с тем, что частота 64,5 *ГГц*, будучи резонансной для воды, структурирует водную составляющую вокруг ДНК и САЧ, из-за чего связывание становится слабее. Данное заключение основывается на значениях как констант связывания, так и констант Штерна–Фольмера.

Պ. Հ. ՎԱՐԴԵՎԱՆՅԱՆ, Մ. Ա. ՇԱՀԻՆՅԱՆ, Ա. Վ. ՎԱՐԴԱՆՅԱՆ, Ս. Վ. ԳՐԻԳՈՐՅԱՆ

HOECHST 33258-Ի ՓՈԽԱԶԴԵՑՈՒԹՅԱՆ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆԸ ԴՆԹ-Ի ԵՎ ՄԱՐԴՈՒ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՀԵՏ ՄԻԼԻՄԵՏՐԱՅԻՆ ՏԻՐՈՒՅԹԻ ԷԼԵԿՏՐԱՄԱԳՆԻՍԱԿԱՆ ԱԼԻՔՆԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ՆԵՐՔՈ

Աշխատանքում հետազոտվել է 64,5 *ԳՀց* հաճախությամբ միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների (ՄՄ ԷՄԱ) ազդեցությունը ԴՆԹ-ի և մարդու շիճուկային ալբումինի (ՄՇԱ) հետ Hoechst 33258-ի (H33258) կոմպլեքսների վրա կլանման և ֆլուորեսցենտային սպեկտրադիտման մեթոդներով: Ցույց է տրվել, որ ճառագայթահարումը հանգեցնում է H33258-ի փոխազդեցության թուլացմանը երկու մակրոմոլեկուլների հետ, ինչը կապված է այն բանի հետ, որ 64,5 *ԳՀց* հաճախությունը, լինելով ռեզոնանսային ջրի համար, կառուցվածքավորում է ԴՆԹ-ի և ՄՇԱ-ի շուրջը ջրային բաղադրիչը, ինչի հետևանքով կապումը դառնում է ավելի թույլ: Տվյալ եզրակացությունը հիմնվում է կապման հաստատունների և Շտերն–Վոլմերի հաստատունի արժեքների վրա: