https://doi.org/10.15407/ujpe69.12.913

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NON-THERMAL EFFECT OF MILLIMETER-WAVE RADIATION ON THE FLUORESCENCE OF THE RHODAMINE 6G AQUEOUS SOLUTIONS

With the help of fluorescence spectroscopy, the effect of millimeter-wave radiation on the aqueous solutions of the organic dye rhodamine 6G has been studied. By optimizing the dye concentration, the thermal effects are minimized, and the contribution of non-thermal mechanisms is identified. The results obtained indicate that millimeter-wave radiation induces structural changes in the aqueous medium, which, in turn, leads to changes in the fluorescent properties of the dye.

Keywords: millimeter waves, non-thermal effect, fluorescence, rhodamine 6G.

1. Introduction

Research on the effects of non-thermal interaction of millimeter-wave electromagnetic radiation (MMW) with biological objects has a long history spanning more than five decades. Despite the wide use of low-intensity MMW in biotechnology [1–3] and medicine [4–6], modern electromagnetic wave safety standards are mainly based on the assessment of a thermal influence [7, 8]. Exposure levels that do not induce a substantial temperature growth in biological tissues are considered safe. Thermal interaction mechanisms are well studied and can be described quantitatively using such a parameter as the Specific Absorption Rate (SAR).

Unlike thermal criteria, the non-thermal ones are focused on potential non-thermal effects on the human body, in particular, on the nervous, reproductive, and immune systems. The basis for non-thermal criteria comprises statistical data obtained from medicobiological studies, namely, epidemiological indicators of morbidity, demographic indicators (for example, the birth rate), and immunological parameters. A characteristic feature of non-thermal criteria is their high variability associated with the influence of numerous socioeconomic, climatic, and environmental factors. At the same time, the mechanisms of non-thermal effects of MMW at the cellular and molecular levels are not sufficiently studied today, thus, being a debatable topic of modern physics [4, 7, 9–11].

The lack of consensus with respect to the physical nature of the interaction between low-intensity MMW and biological systems is associated with the high nonlinearity and multiparametric origin of biological processes. The existing physical models cannot adequately describe the observed effects. This occurs due to the limitations of our understanding of the molecular mechanisms that underlie biological reactions. The lack of systematic studies of the interaction of MMW with non-biological systems, for which a more effective physical model can be developed in principle, complicates the extension of the obtained results to biological objects.

The unique physical and chemical properties of water, which are responsible for its central role in biological processes, make it a promising object for studying the non-thermal effects of electromagnetic radiation, especially in the millimeter-wave interval. Experimental data testify that the influence of microwaves on water leads to changes in its structural and dynamic characteristics that are not reduced to the thermal effect. In particular, reports were made about

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Citation: Yakunov A.V., Ilchuk D.T. Non-thermal effect of millimeter-wave radiation on the fluorescence of rhodamine 6G aqueous solution. *Ukr. J. Phys.* **69**, No. 12, 913 (2024). https://doi.org/10.15407/ujpe69.12.913.

the modification in the solubility of gases [12], the changes in thermophysical properties, adsorption capacity, electrical conductivity, and optical characteristics [13], the parameters of vibrational spectra [14], and the formation of reactive products such as hydrogen peroxide [15].

The results of dynamic molecular simulation make it possible to describe the mechanisms of interaction of millimeter waves with water at the molecular level. In those studies, it is assumed that electromagnetic radiation induces local energy fluctuations, which contribute to the development of hydrogen bonds, the transformation of the electromagnetic field energy into the kinetic energy of molecules and the energy of intermolecular interaction, and the violation of water structure near hydrated ions [16, 17]. At the same time, other authors do not reveal statistically significant changes [18, 19].

The complicated character of the interaction between microwave electromagnetic radiation and water makes it necessary to perform additional experimental and theoretical research for a comprehensive analysis of the mechanisms underlying possible nonthermal biological effects. Our previous studies [20–24] carried out using fluorescence spectroscopy and the rhodamine 6G organic dye (R6G) in aqueous solutions revealed a high sensitivity of this technique to the electromagnetic radiation effects. In particular, we managed to distinguish the effects of microwave radiation with the frequency of household devices from purely thermal effects, which testifies to the availability of additional, non-thermal mechanisms of interaction between the radiation and the molecules in the environment. An analysis of the dynamics of changes in the spectral characteristics of R6G fluorescence during thermal cycling, as well as the disproportion between the radiation dose and the fluorescence intensity, confirm the hypothesis about the existence of non-thermal effects at microwave irradiation.

In this article, we present an experimental confirmation of the direct registration of non-thermal effects induced by MMW. The relevant data were obtained using fluorescence spectroscopy methods.

2. The Temperature Effect on the Fluorescence of Solutions

An increase in the temperature of solutions usually leads to an increase in the thermal motion of their molecules, which manifests itself in the growth of the frequency and energy of molecular collisions, as well as in the amplitude growth of intramolecular vibrations. Those factors make higher the probability of non-radiative processes of electronic excitation deactivation, such as internal conversion and intermolecular energy transfer. As a result, a reduction in the fluorescence quantum yield is observed.

At the same time, a non-standard temperature behavior of the fluorescence intensity was revealed, which manifests itself as the fluorescence intensity growth with the increasing temperature [25]. This phenomenon can be explained as a result of the molecular association processes. In particular, the formation of non-covalent complexes (dimers, trimers, and so forth) in the solution is characteristic of some organic molecules (such as rhodamine 6G). As a rule, the fluorescence quantum yield of associates is lower than that of isolated molecules. The total fluorescence intensity of the system is determined by the equilibrium between the monomeric and associated forms. As the temperature increases, the dissociation of the complexes is observed, which leads to an increase in the concentration of fluorescent monomers and, as a result, an increase in the fluorescence intensity.

The proposed research method is based on creating conditions under which temperature effects leading to the fluorescence quenching are compensated by oppositely directed processes associated with the temperature-induced dissociation of molecular aggregates. By optimizing the solution concentration, we were supposed to achieve the temperature independence of the fluorescence intensity in a certain frequency interval. In this case, any changes in the glow intensity caused by external factors can be interpreted as a result of the non-thermal effects.

3. Equipment and Materials

At the preliminary stage, we analyzed the temperature, t, dependences of the fluorescence spectral parameters of the R6G aqueous solutions with various concentrations. The spectra were registered using a high-rate spectrometer with video recording at various temperatures in the interval $t = (20 \div 30)$ °C. The dye concentration in the solution was varied from 10^{-3} to 10^{-2} g/l. Fluorescence was excited at a wavelength of 405 nm. A detailed description of specimen preparation, methods, and equipment, as well as the spectra processing algorithms, was given in work [24].

The resulting temperature dependencies of the relative fluorescence intensity change were approximated by the linear function

$$\frac{\Delta I}{I_0} = k(t - t_0),$$

where t_0 is the initial temperature, and k is the temperature coefficient. It was found that the temperature coefficient of the R6G solution depends on its concentration. At the concentration $C_0 \approx 0.004$ g/l, the value of k was close to zero (Fig. 1). When the concentration was varied from above to below C_0, k changed its sign from positive to negative. An aqueous solution of rhodamine C (RC) was used for the temperature calibration because its k-value weakly depends on the solution concentration, and this fact made it a popular marker in fluorescent thermometry.

At the main stage of measurements, a special waveguide unit was used (Fig. 2, *a*). A glass capillary 1 mm in diameter and containing the researched solution was placed in a rectangular waveguide with a cross-section of 5.20×2.60 mm^2, parallel to the wider walls. In order to excite fluorescence, radiation from a violet laser (405 nm, 60 mW) was directed onto the capillary through a hole in the wider wall. In order to heat the solution, infrared laser radiation (808 nm, 100 mW) was directed onto the capillary through a coaxial hole in the opposite wider wall. To enhance the temperature response, an area of the outer surface of the capillary was covered with a special absorbent, which provided the contact heating. The temperature regime was controlled by varying the intensity of the infrared laser beam.

A G-141 millimeter-wave generator with an operating frequency range of 37.50–53.57 GHz was connected to one end of the waveguide section. The radiation frequency of 49.80 GHz was selected experimentally and corresponded to the resonance in this configuration of the waveguide line and the absorbing element [22] (Fig. 2, *b*). The output power of the generator was 20 mW. The absorbed wave power in the capillary was calculated as the difference between the incident power, on the one hand, and the sum of the reflected and transmitted powers, on the other hand. It was equal to 10 mW, which was enough to locally heat the aqueous solution by 3–4 °C [20]. Accountng for the size of the heated capillary region, the thermal relaxation time was estimated to be several seconds [20]. The fluorescence spectra were registered through the open end of the waveguide with an interval of 1 s.

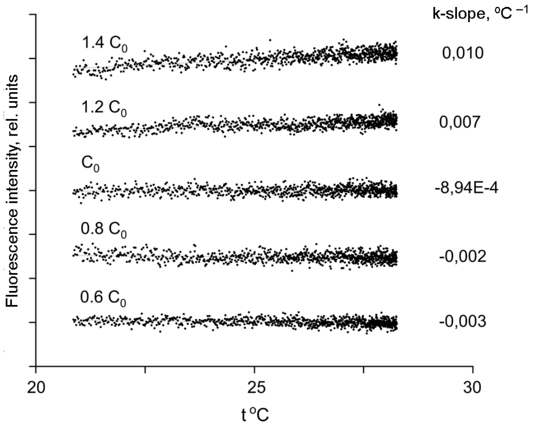
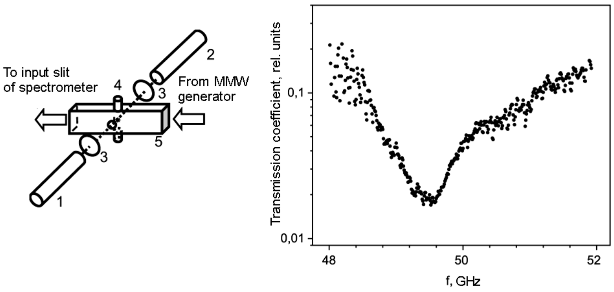


Fig. 1. Temperature dependences of fluorescence intensity of P6G solution at various concentrations close to the critical one



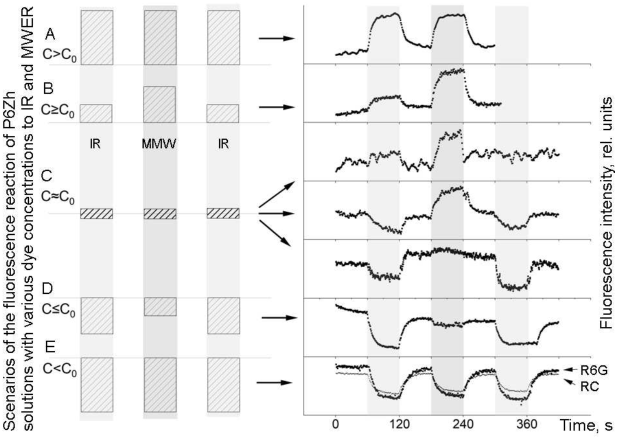
a *b*

Fig. 2. Schematic diagram of measurement unit (*a*): violet laser (*1*), infrared laser (*2*), focusing lenses (*3*), capillary with solution (*4*), waveguide section (*5*).Transmission characteristic of the waveguide section with the capillary (*b*)

The fluorescence of R6G solutions with concentrations close to the critical one was studied under the influence of millimeter waves and the contact heating. The experiments were carried out in three stages: using the sequential influence of the infrared heating, microwave irradiation, and infrared heating again. The duration of each stage was 60 s, and the same interval was held between the stages. For the sake of comparison, an aqueous RC solution with a concentration close to C_0 was subjected to a similar action.

4. Results and Discussion

An analysis of the R6G solution reaction to the combined action of the contact heating and MMW at var-



a *b*

Fig. 3. Scenarios of the fluorescence reaction of R6G solutions with various dye concentrations to IR and MMW (*a*). Examples of corresponding fluorescence signals (*b*)

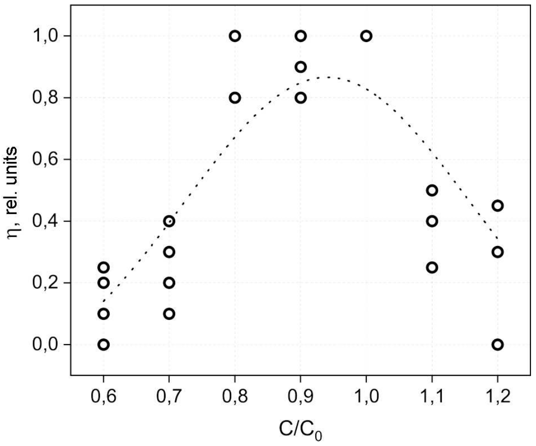


Fig. 4. Non-thermal effect manifestations at various dye concentrations

ious solution concentrations made it possible to identify five different behavioral models (Fig. 3, *a*). Experimental data (Fig. 3, *b*) demonstrate that, at concentrations that substantially differ from the critical one (scenarios A and E), the thermal mechanism of interaction dominates, and MMW does not make an additional contribution to the observed effects. The estimation of the heating level carried out using the temperature calibration gave a value of (3 ± 1) °C. Scenario E was found characteristic of all examined concentrations of the RC dye (Fig. 2, *b*, lower diagram).

In an immediate vicinity of the critical concentration (scenarios B and D), some differences in the system dynamics were revealed; namely, reactions of the same sign, but with different amplitudes were observed, which can be considered as a manifestation of non-thermal effects. In our previous study [22], the fluorescence reaction of the solution to the MMW action corresponded exactly to scenario B. The experimentally determined temperature equivalent turned out to be higher than the calculated one, which gave us grounds to suggest the availability of additional non-thermal processes.

Scenario C (with $C \approx C_0$) is characterized by an unusual behavior of the system, which manifests itself either in the absence of the reaction to either of the influences, but with the reaction to the other influence, or in the availability of the reactions with opposite signs. Such a contrast of reactions contradicts the expected thermal effects and testifies to the existence of complicated, potentially non-thermal mechanisms of the interaction between MMW and the solution. For the quantitative evaluation of the experimental data set, the dimensionless quantity

$$\eta = \left| \frac{\Delta I_{\mathrm{IR}} - \Delta I_{\mathrm{MW}}}{\Delta I_{\mathrm{IR}} + \Delta I_{\mathrm{MW}}} \right|$$

was introduced. This parameter characterizes the amplitude of the non-thermal component of system's response to a microwave disturbance, similarly to how the contrast coefficient determines the signal modulation amplitude. The dependence of the coefficient η on the solution concentration is plotted in Fig. 4. In scenario C, and partially in scenarios B and D, the signal turned out to be close to the level of noise fluctuations, which limited the accuracy of the quantitative evaluation of the parameter η. However, the general trend of the dependence is statistically significant and testifies to the availability of non-thermal effects.

Despite a substantial level of noise interference, a detailed analysis of the most representative experimental data made it possible to reveal additional aspects of the interaction of MMW with the researched specimen. In particular, the data presented in Fig. 5, *a* corresponding to one of the implementations of scenario C, testify to the presence of a two-stage fluorescence reaction to the MMW action. At the initial stage, a reduction in the fluorescence intensity, which is characteristic of thermal quenching, is observed. However, later, the non-thermal mechanism becomes dominant, which leads to an increase in the glow intensity.

A similar biphase fluorescence dynamics was registered in an experiment with a modified time profile of the exposition, whose results are depicted in Fig. 5, *b*. In this case, the duration of IR radiation pulses and subsequent pauses was shortened to 30 s, and the duration of MMW pulses and subsequent pauses was increased to 120 s. The obtained data demonstrate that the initial reaction of fluorescence to the MMW action has mainly a thermal character, which is evidenced by a reduction in the glow intensity during the first few seconds. However, a change in the reaction sign is observed afterwards, which leads to an intensity growth with some saturation signs. After the MMW was switched-off, the fluorescence intensity slowly decreased, and against the background of this fading, a faster process of temperature-induced quenching was observed. It should be noted that the characteristic time scale of fluorescence relaxation in response to the MMW exposition substantially exceeds the corresponding value for IR radiation and can be roughly evaluated to several tens of seconds.

Two alternative mechanisms can be proposed to explain the observed phenomenon. The first mechanism assumes a direct catalytic effect of MMW on the activation energy of dimerization reaction, and this scenario is consistent with the data of microwave chemistry [15]. However, taking the low intensity of the microwave field used in the experiment into account, such a mechanism seems less probable. The second mechanism consists the mediated effect of MMW on fluorescence through the aqueous medium. An analysis of previous studies testifies to the occurrence of considerable structural changes in water and aqueous solutions under the influence of microwave radiation. The mechanism of corresponding changes consists in the interaction of an ac electromagnetic field with the constant dipole moments of water molecules, which is accompanied by the reorientation of the dipoles and the modification of the hydrogen bond network. According to the percolation model of water structure [26], low-intensity microwave radiation induces the cumulative energy accumulation in the system, which gives rise to a gradual restructuring of the percolation cluster [27]. In this case, the characteristic relaxation time of the system demonstrates a substantial dependence on the specimen geometry. Namely, a considerable reduction of the relaxation time is observed, if changing from a bulk specimen (a standard cuvette) to capillaries, which testifies to the strengthening of the role of surface effects and dimensionality limitations. The results of dynamic molecular simulation confirm the possibility of the formation of long-term non-equilibrium states induced by collective fluctuations of the percolation cluster [17].

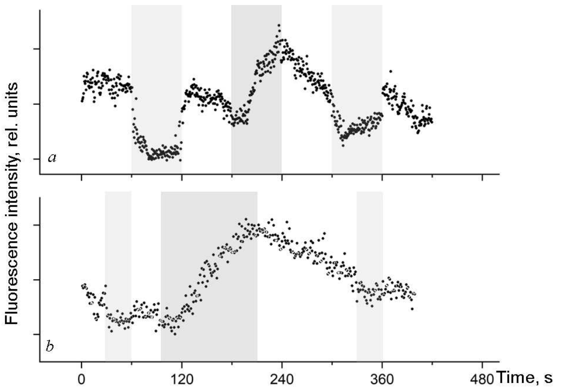


Fig. 5. Representative implementations of experiments with manifestations of non-thermal MMW effects

5. Conclusions

The existence of the non-thermal effect of millimeter-wave radiation on fluorescence in the rhodamine 6G aqueous solutions has been experimentally proven. When comparing the effects of the contact heating and millimeter-wave irradiation on the solutions with certain fluorophore concentrations, the opposite directions of fluorescence changes were revealed. A kinetic analysis of the fluorescence response to MMW showed that non-thermal effects develop more slowly than thermal ones, which testifies to the availability of complicated multistep processes induced by MMW in the aqueous environment. The obtained results open perspectives for the development of the physical models for the non-thermal interaction of MMW with biological systems and the identification of molecular targets for such an interaction.

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Received 08.08.24.
Translated from Ukrainian by O.I. Voitenko

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НЕТЕПЛОВИЙ ВПЛИВ МІЛІМЕТРОВОГО ВИПРОМІНЮВАННЯ НА ФЛУОРЕСЦЕНЦІЮ ВОДНОГО РОЗЧИНУ БАРВНИКА РОДАМІН 6G

За допомогою флуоресцентної спектроскопії досліджено вплив міліметрового випромінювання на водні розчини органічного барвника родамін 6G. Шляхом оптимізації концентрації барвника вдалося мінімізувати теплові ефекти і виділити внесок нетеплових механізмів. Отримані результати свідчать про те, що міліметрове випромінювання індукує структурні зміни у водному середовищі, що, своєю чергою, призводить до зміни флуоресцентних властивостей барвника.

Ключові слова: міліметрові хвилі, нетепловий ефект, флуоресценція, родамін 6G.