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HIGH-TEMPERATURE FLUORESCENCE OF LOW- AND HIGH CONCENTRATION AQUEOUS ATP SOLUTIONS[1]

UDC 539

The results of experimental studies of the luminescence of an aqueous solution of adenosine triphosphate (ATP) at room temperature are presented. High-temperature fluorescence of low- and high-concentrated solutions is experimentally detected. The shape of the fluorescence spectrum, the lifetime of excitations, and the temperature-based behavior of the emission intensity indicate the formation of rather stable dimer-like complexes in a high-concentration solution, which can form excimer states.

K e y w o r d s: high-temperature fluorescence, adenosine triphosphate, aqueous solution, excimer.

1. Introduction

Adenosine is a purine nucleoside that consists of adenine connected with ribose. Being a part of the intracellular medium, adenosine can play the role of a neuromodulator. In addition, it is a component of nucleic acids, some enzymes, *etc.* The derivates of adenosine (e.g., AMP, ADP,and ATP) are all important components of biochemical pathways in organic compounds.

ATP (Fig. 1) contains a π-system, so it absorbs in the UV-region. It is wel known that, at ambient temperature, the excitation of ATP aqueous solutions is relaxed through the conversion, and the luminescence is not observed [1]. However, at low temperatures, the fluorescence and/or phosphorescence with a high quantum yield do/does occur [2].

It is worth to note that, during 10–15 last years, a large number of papers reported to the investigations of the luminescence in various systems based on adenine or adenosine molecules at ambient temperature. As an example of these studies, we mention the luminescence in thin solid layers obtained by the adsorption of adenine on a highly oriented pyrolytic graphite surface (HOPG) [3] or the string-type system (poly-Adenine) [4]. The fluorescence of a highly concentrated aqueous solution of AMP at room temperature was recorded for the first time in [5]. The authors of work [6] studied the very weak fluorescence in a water solution of four bases: adenine, cytosine, guanine and, uracil. In the last 15 years, there were some publications in which the dimerization of adenine has

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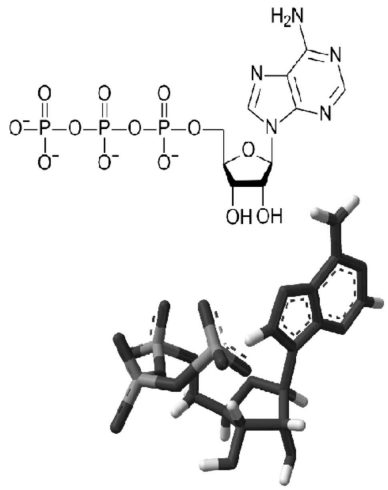

Fig. 1. Structural formula of ATP

been studied in detail from both an experimental and a theoretical standpoints. Different conditions for the appearance of dimers were considered both in solutions [7] and in solids.

It turned out that the process of self-formation of stable adenine dimers (or even chains or monolayers) is due to the formation of strong hydrogen adenine-adenine bonds. These bonds, in fact, are responsible for the formation of DNA pairs in living objects and, thus, can also aggregate AMP molecules in aqueous solutions into di- or trimers.

The above examples of systems are one- and two-dimensional formations in which adenine molecules are substantially restricted in motion. It is possible to make a preliminary assumption that the appearance of the adenosine fluorescence at room temperature may be due to a partial spatial fixation of the aromatic ring, which reduces the efficiency of the irradiation excitation channel and increases the quantum yield of fluorescence. Such fixation may have a different nature due to the presence of a rigid environment in the form of a substrate or frozen solvent or, for example, due to the formation of adenine-adenine dimers. In [7], a detailed theoretical study of the possible relative arrangement configurations of adenine molecules in dimers was carried out, and the authors concluded that the configuration with a parallel arrangement of aromatic rings is the most energy-efficient one.

Here, we will report the experimentally discovered high-temperature fluorescence of an ATP aqueous solution with a high quantum yield. It appears at very high, close to saturated, solution concentrations. Our results differ from those in [5], especially as we suggest another mechanism of detected fluorescence.

2. Equipment and Materials

Chemically pure crystalline ATP (adenosine-5′-triphosphoric acid disodium salt, 99% purity, Bio-Chemika) was used as the test material. Aqueous solutions were prepared by adding a weighed amount of an ATP powder to bi-distillate to obtain the concentration $C = 2 \times 10^{-2}$ M. Serial two-beam spectrophotometers were used to record the absorption spectra: Specord 210 Plus (Analytik Jena) and SPECORD UV-VIS (Carl Zeiss). Recording was performed in standard quartz cuvettes 1×1 cm in size (transmission range 190–1100 nm) at room temperature. An SF-26 spectrometer was used to record the absorption spectrum in a 5-cm cell. Fluorescence spectra at room temperature were recorded on an FP-8200 JASCO fluorescence spectrometer; and standard quartz cells were used. The ATP molar extinction coefficient according to manufacturer's certificate is 15400 $\mathrm{l} \cdot \mathrm{mol}^{-1} \cdot \mathrm{cm}^{-1}$.

3. Experimental Results

The control over a water purity before the dissolution was carried out by recording the absorption spectrum (absorption registration region is 200–350 nm) and the luminescence with an excitation interval of 200–400 nm and the emission one of 210–700 nm.

The corresponding absorption spectrum of water is shown in Fig. 2, and the Excitation-Emission Map (EEM) is presented in Fig. 3. No sign of a specific signal other than that from the Raman scattering was detected. The control over the solution concentration was carried out by the standard method – measuring the optical density at the wavelength $\lambda = 260$ nm.

The UV absorption spectrum of ATP is well known and presented in Fig. 4. It was recorded for the samples with 2×10^{-2} M concentration in a cell 5 cm in length (curve *1*) with the use of an SF-26 spectrophotometer, 2×10^{-3} M (curve *2*), 1.1×10^{-3} M (curve *3*) recorded in a 1-cm-long cell with the use of a SPECORD UV-VIS spectrophotometer, and 4×10^{-4} M recorded in a 1-cm-long cell with the use of a SPECORD 210 Plus. The spectrum contains two absorption bands, with peaks at 260 and 218 nm

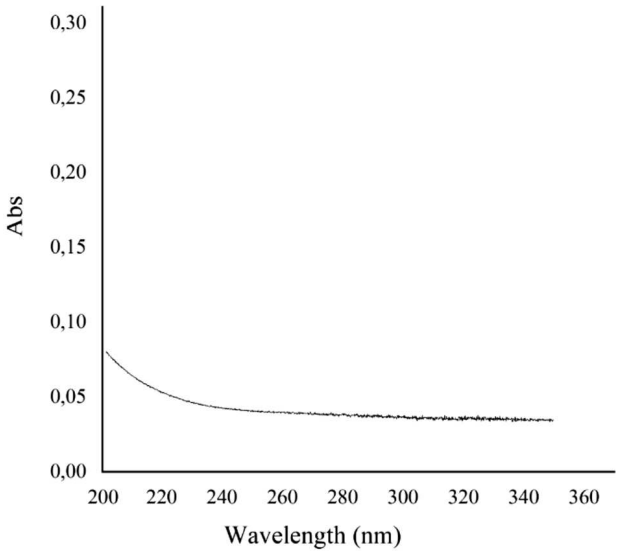

Fig. 2. Water absorption spectrum before the ATP solution preparation, SPECORD 210 Plus

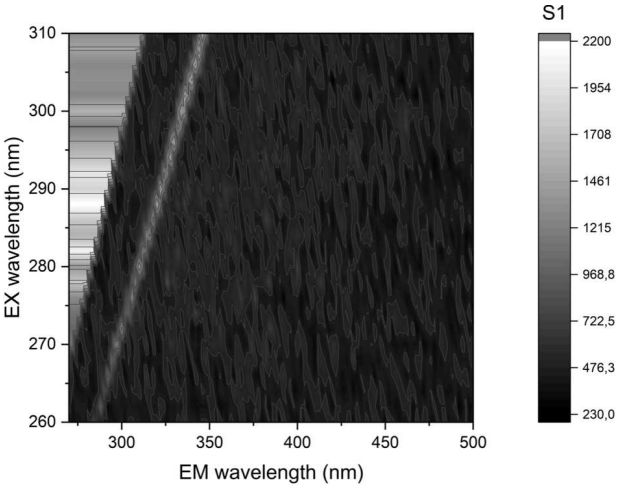

Fig. 3. Water EEM before the preparation of an ATP solution

(4.766 and 5.856 eV), which belong, respectively, to the first and second electronic transitions of the adenine π-electron system. The difference between the energies of these two levels is, thus, about 1.1 eV. The positions of the maxima do not depend on the concentration, and no new absorption bands appear, as the concentration of the solution increases.

3.1. Intrinsic fluorescence of an ATP aqueous solution at room temperature

Traditionally, a sample is excited at the absorption band maximum. Such an excitation of ATP at the

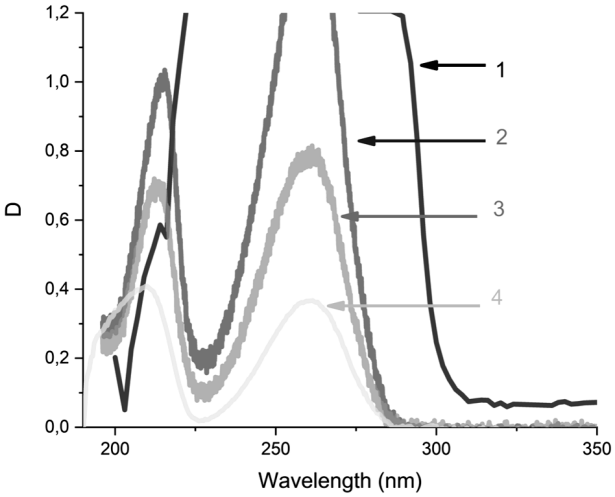

Fig. 4. ATP absorption spectrum $C = 2 \times 10^{-2}$ M, $L = 5$ cm, SF-26 (*1*); $C = 2 \times 10^{-3}$ M, $L = 1$ cm SPECORD UV-VIS (*2*); $C = 1.1 \times 10^{-3}$ M, $L = 1$ cm, SPECORD UV-VIS (*3*); $C = 4 \times 10^{-4}$ M, $L = 1$ cm, SPECORD 210 Plus (*4*)

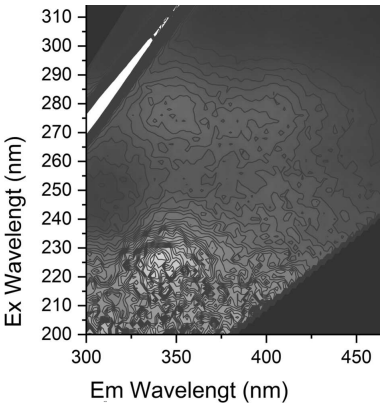

Fig. 5. EEM, ATP water solution, $C = 10^{-4}$ M, $T = 20$ °C

wavelength $\lambda = 260 \pm 5$ nm leads to a slightly noticeable emission [6]. However, we detected a luminescence, while exciting it at the edge of the absorption band – at 225 and 275 nm (see Fig. 5). The maximum of the detected fluorescence was at approximately 340 nm (2.94 μm^{-1}), it corresponds to $E = 3.65$ eV and is in good accordance with [6] (see Fig. 6). It is seen from Fig. 7 that the ratio of the intensities can be written as:

$$\frac{\text{max Intensity excited at 260 nm}}{\text{max Intensity excited at 225 nm}} \cong 1 \div 4.5.$$

The excitation function maxima correspond to energies of 5.6 eV and 4.5 eV, respectively, and the dif-

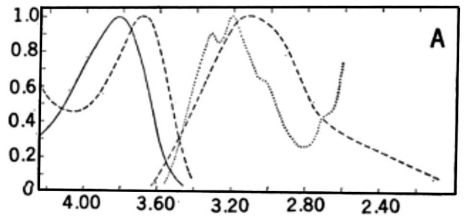

Fig. 6. Normalized adenosine fluorescence intensity (water solution, $T = 273$ K, $\lambda_{ex} = 261$ nm, dashed, right; EG solution $T = 78$ K, dotted, right), excitation function (water solution, $T = 273$ K, $\lambda_{em} = 320$ nm, dashed, left), absorption (solid, left), abscissa – wavenumber, μm^{-1} [6]

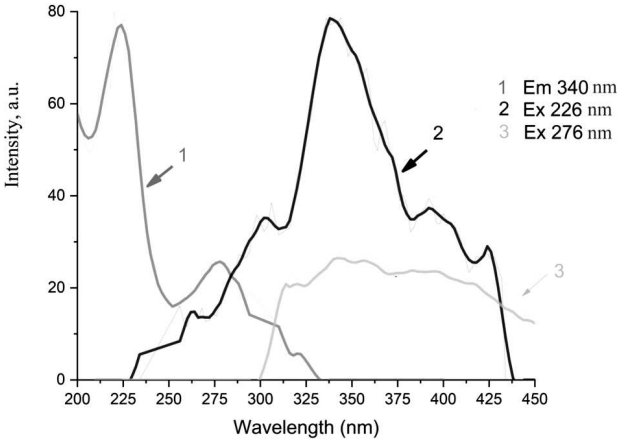

Fig. 7. Intensity of the fluorescence of ATP, $T = 20$ °C, $C = 2 \times 10^{-4}$ M

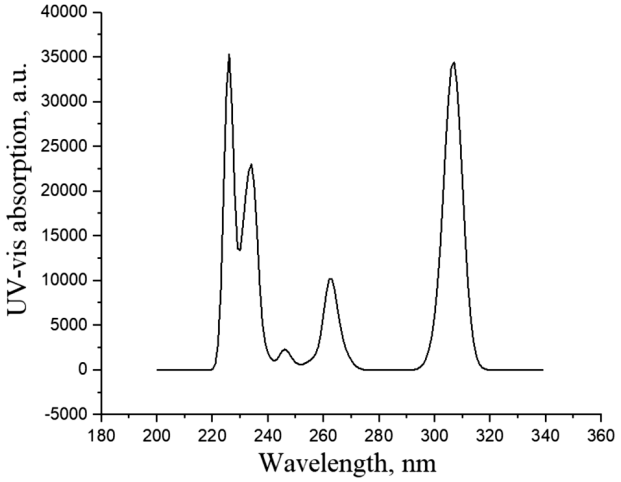

Fig. 8. Modeled absorption spectrum, ATP in water, Gaussian 03, DFT PBEPBE 6-311G+(d,p)

ference is 1.1 eV, which correlates with the data obtained from the absorption spectrum. However, we noticed a slight red shift of the excitation spectrum relative to the absorption one. Such red shift taken on the same instrument with identical bandwidths was found for every base [6]. Other examples of such behavior were reported for purines and pyrimidines under various conditions in [8–12].

Figure 8 and Table show the absorption spectrum of ATP obtained by a simulation using quantum-chemical calculations. It shows a quite good correspondence with experimental data, viz. two powerful bands at 225 and 263 nm. However, there was no experimentally detected longwave band at 307 nm.

The cross-sections of absorption and emission spectra give the $S_{00} - S_{01}$ transfer energy equal to about 4.3 eV.

The fluorescence lifetime was estimated to have two components: $\tau_1 = 4.4 \times 10^{-9}$ sec and $\tau_2 = 12.0 \times \times 10^{-9}$ sec (Fig. 9, curve *1*).

3.2. Fluorescence of a high-concentration aqueous ATP solution

The fluorescence of a highly concentrated aqueous solution of ATP was found to be significantly different

Results of quantum-mechanucal modelling (Gaussian 03 DFT)

Wavenumber, nm	Energy, eV	Oscillator force, f_{osc}
306.8	4.05190678	0.1256
301.34	4.125323555	0.0116
269.49	4.612879884	0.001
266.91	4.65746881	0.0059
262.61	4.733730627	0.0312
261.41	4.75546077	0.0065
260.83	4.766035349	4.00E-04
256.15	4.85311341	0.0038
251.76	4.937738322	0.0011
249.07	4.991066768	0.001
246.78	5.037381473	7.00E-04
245.84	5.056642532	0.0076
239.42	5.192235402	0.0057
234.62	5.298461342	0.0695
232.15	5.354835236	0.012
231.27	5.375210793	0.025
231.09	5.379397637	0.0119
230	5.404891304	0.002
226.97	5.477045425	0.0048
225.82	5.504937561	0.1269

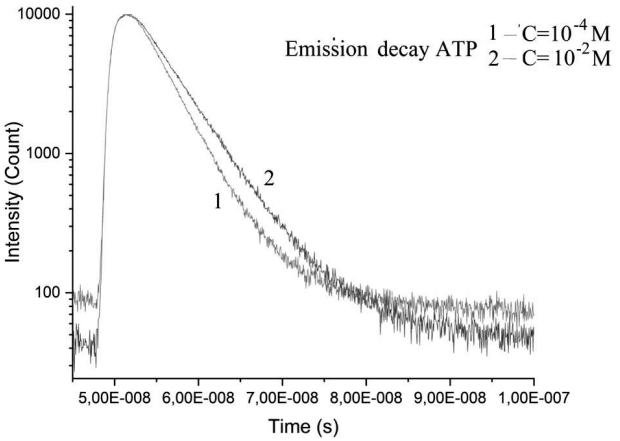

Fig. 9. $\tau_1 = 4.4 \times 10^{-9}$ sec, $\tau_2 = 12.0 \times 10^{-9}$ sec, $C = 2 \times 10^{-4}$ M; $\tau_3 = 4.2 \times 10^{-9}$ sec, $\tau_4 = 40.4 \times 10^{-9}$ sec, $C = 2 \times 10^{-2}$ M; $T = 20$ °C, $\lambda_{ex} = 280$ nm

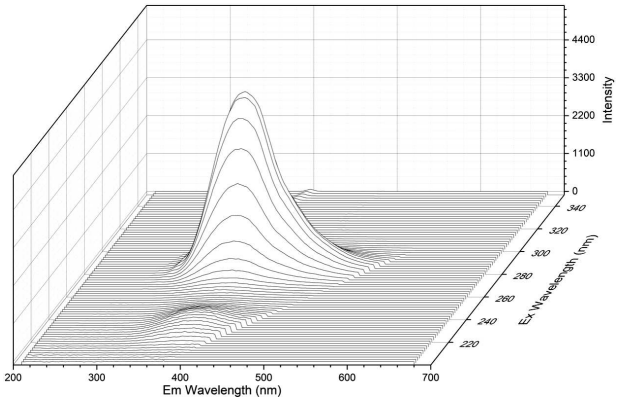

Fig. 10. EEM, $T = 293$ K, $C = 2 \times 10^{-2}$ M

from the fluorescence discussed above (see Fig. 10, the concentration $C = 2 \times 10^{-2}$ M). Figure 11 shows the excitation and emission spectra of the fluorescence. The fluorescence spectrum has a high-intensity band with a half-width of about 100 nm. It is unstructured and has a Gaussian-like shape with a maximum at approximately 390 nm (25640 cm^{-1}), which corresponds to $E = 3.18$ eV. The intersection of the long-wave edge of the absorption spectrum of the highly concentrated solution and the short-wave edge of its fluorescence spectrum gives the excited state energy of about 4 eV (corresponding to 3.2 μm^{-1}). The fluorescence lifetime for a highly concentrated solution also has two components – $\tau_1 = 4.2 \times 10^{-9}$ sec, $\tau_2 = 40.4 \times 10^{-9}$ sec (Fig. 9, curve *2*). The fluorescence intensity also reveals a temperature de-

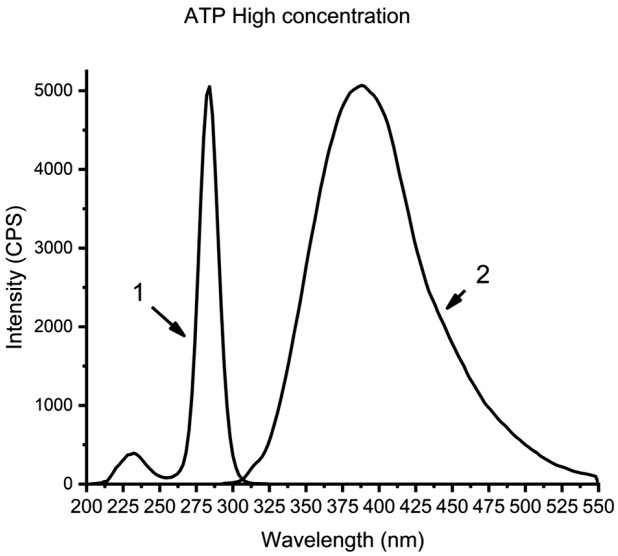

Fig. 11. Fluorescence of an ATP water solution, (*1*) – $\lambda_{em} = 390$ nm, $\lambda_{ex} = 280$ nm, $T = 293$ K, $C = 2 \times 10^{-2}$ M

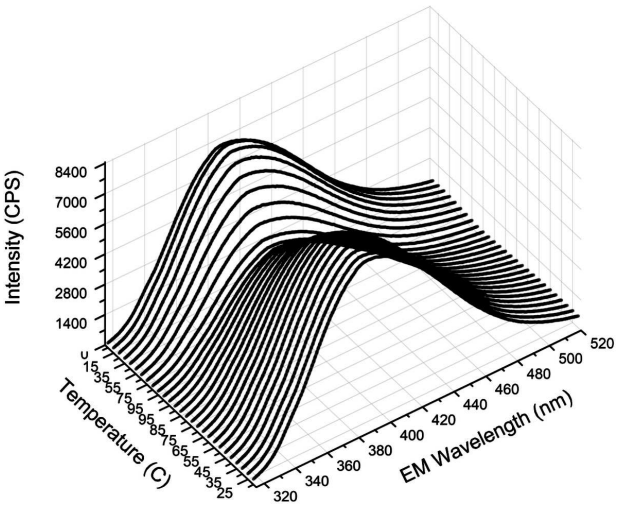

Fig. 12. Fluorescence spectrum of ATP, when heated and cooled in the temperature interval $T = 20$–100 °C, $C = 2 \times \times 10^{-2}$ M, $\lambda_{ex} = 280$ nm

pendence. Figures 12–15 show the fluorescence spectra recorded, when the cuvette is heated and cooled back. The shape of the emission band and the position of its maximum are independent of the temperature. Figure 15 shows the dependence of the fluorescence intensity on the temperature (the intensity value was taken near the emission maximum). Heating the solution from room temperature (20 °C) to 95 °C results in a significant (almost to a half) flu-

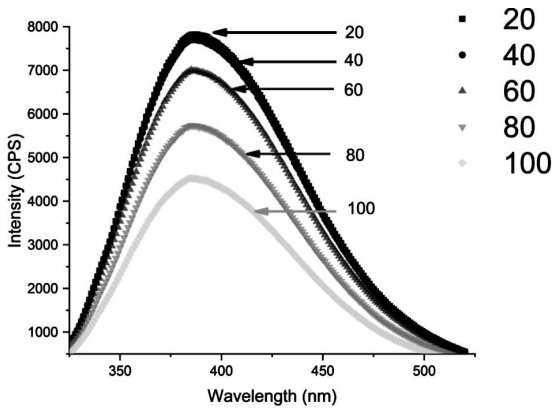

Fig. 13. Fluorescence spectrum of ATP under the heating in the temperature interval T = 20–100 °C, $C = 2 \times 10^{-2}$ M, $\lambda_{ex} = 280$ nm

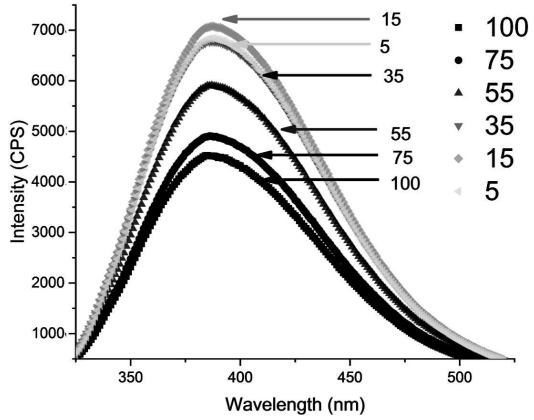

Fig. 14. Fluorescence spectrum of ATP under the cooling in the temperature interval T = 100–5 °C, $C = 2 \times 10^{-2}$ M, $\lambda_{ex} = 280$ nm

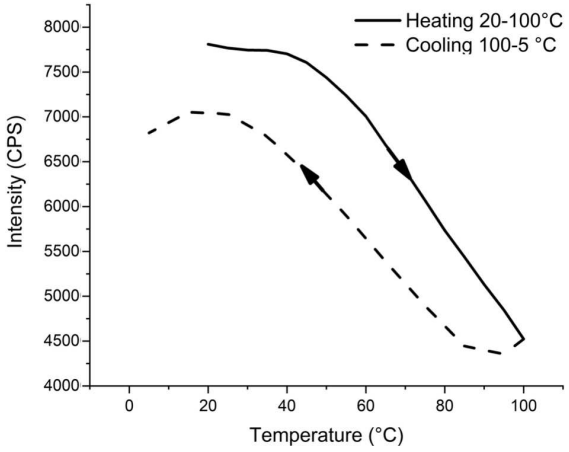

Fig. 15. ATP fluorescence intensity, when heated and cooled in the temperature interval T = 20–100 °C, $C = 2 \times 10^{-2}$ M, $\lambda_{ex} = 280$ nm

orescence intensity decrease. At the subsequent cooling back, the fluorescence is restored to almost the starting level, with a temperature hysteresis taking place. In addition, the fluorescence intensity showed a dependence on the dilution rate of the stock high concentration solution, decreasing with a decrease in the sample concentration of AMP and reaching the noise level at a dilution of 4000 times relative to the starting concentration.

4. Analysis of Experimental Results

We have experimentally obtained the following:

• It has been confirmed that it is possible to excite the ATP fluorescence at room temperature.

• The absorption spectrum does not show the dependence on the solution concentration in the concentration region of $2 \times 10^{-2} \div 1.1 \times 10^{-4}$ M, and no additional absorption bands were detected.

• The absorption spectrum coincides with the fluorescence excitation spectrum. However, a slight red shift of the fluorescence excitation function relative to the absorption spectrum was detected.

• The fluorescence emission spectrum of a low-concentration solution has a maximum at 340 nm (29400 cm^{-1}). For the high-concentration one, it is at 390 nm (25640 cm^{-1}). The difference is about 3500 cm^{-1} (0.5 eV).

• The difference of energies between $S_{00} - S_{01}$ electronic transitions in low- and high-concentration solutions is 0.3 eV.

• The fluorescence excitation lifetime for the low-concentration ($C = 2 \times 10^{-4}$ M) solution has two closely related components $\tau_1 = 4.4 \times 10^{-9}$ sec and $\tau_2 = 12.0 \times 10^{-9}$ sec. The fluorescence excitation lifetime for the high-concentrated solution also has two components – $\tau_3 = 4.2 \times 10^{-9}$ sec and $\tau_4 = 40.4 \times 10^{-9}$ sec ($C = 2 \times 10^{-2}$ M), which differ by an order of magnitude.

• In addition, the fluorescence spectrum intensity for the high-concentration solution shows the temperature dependence. When heated, the fluorescence intensity decreases, while maintaining a constant position of the spectral maximum. At the cooling back, the fluorescence intensity increases. We detected a temperature hysteresis.

For low-concentration solutions, our results correspond to [6]. The authors detected an excitation function red shift corresponding to the absorption spectrum. The maximum of the excitation function was

established at 275 nm. But, despite this, they excited the fluorescence at 260 nm, following tradition. So, they recorded a very low signal and had to, in their own words, "collect it by scanning the specimen several times and utilizing the techniques of digital signal accumulation and right-angle fluorescence detection from finite absorbing solution". The nature of this red shift is a disputable question for the moment. Processes that may explain this type of a shift include the existence of two or more tautomeric structures for the first absorption band, some of which are fluorescent with different lifetimes, and some are not; the variable efficiency of radiationless deactivation processes as a function of the excitation energy, and the emission from an n-π^* or π-π^* state hidden in the red edge of the absorption band. The set of short lifetimes τ_1, τ_2, and τ_3 may correspond to different tautomers [6]. At the same time, we have considered the presence of specific 10-times-longer lifetime component ($\tau_4 = 40.4 \times 10^{-9}$ sec) in the high-concentration specimen intensity decay. Apparently, this red shift is the reason for why the high-temperature fluorescence of an ATP aqueous solution was considered insignificant for many years and was not experimentally recorded.

The room-temperature fluorescence of a high-concentration solution differs from that of a low-concentration one; it has another shape of spectrum and the maximum position shifts at 3500 cm^{-1}. Figure 16 shows a photo of the luminescent solution in a spectrophotometric cuvette; it has a bright violet-blue color, while the fluorescence of the low-concentration sample is completely in the ultraviolet region.

In our opinion, all these facts can be explained by the presence of certain dimer-like complexes in the high-concentration specimen, which are able to form an excimer state. This statement corresponds to authors' conclusion in [5]. According to calculations [7], two adenine π-electron systems in a dimer are most likely to have a sandwich orientation with an extremely short distance of about 2 Å, and the monomers are possibly linked by hydrogen bonds responsible for the DNA formation or even by weak van der Waals forces. Obviously, for the formation of dimeric ATP complexes, a close relative arrangement of molecules is required, that appears at a high concentration of the solution. Such objects, once formed, remain in the solution even upon a further concentration decrease, as observed experimentally. If the concentration of such complexes is low, they no longer manifest themselves in the absorption spectra.

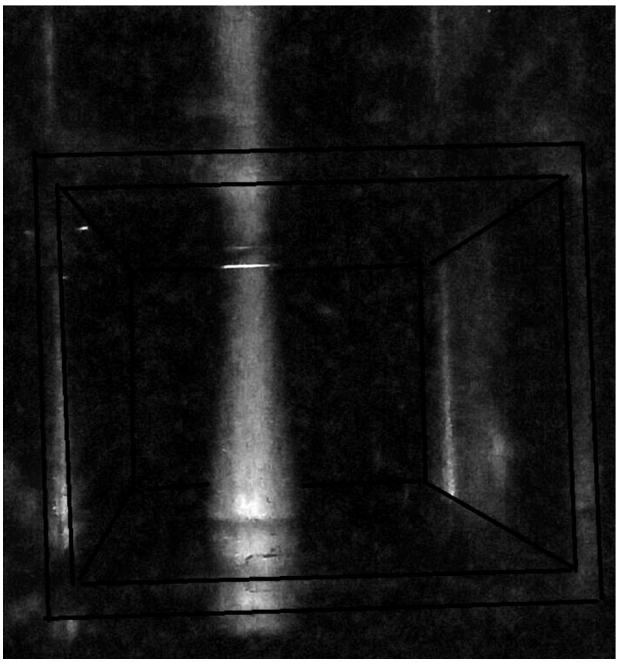

Fig. 16. Photo of the cuvette taken through the open top of the cuvette. Fluorescence of an aqueous solution of ATP, the radiation source is in the photo from below, $t = 20$ °C, $C = 2 \times 10^{-2}$ M, $\lambda_{\text{ex}} = 280$ nm

5. Conclusions

We can state that the high-temperature fluorescence of an ATP water solution is a proven experimental fact. At a low concentration of the solution, the optical centers are ATP monomers, and the quantum yield of fluorescence is quite low, with a maximum at 340 nm. The energy of the excited state is estimated at 4.3 eV; the decay time is in a region of 4–15 ns. At a high concentration of the solution, the optical centers are excimers formed in dimer-like complexes. The quantum yield of excimer fluorescence is supposedly higher, but this suggestion will be considered additionally. The maximum excimer fluorescence occurs at 390 nm. The non-structural shape of the emission spectrum, the characteristic lifetime of the excitation (40 ns), the characteristic temperature behavior of the emission spectrum (a decrease in the fluorescence intensity under the heating; hysteresis), and

the specific red-shift of the emission maximum (about 3500 cm^{-1}) are the main signs of the excimer formation in a high-concentrated ATP water solution. The difference between the first electronic excited levels of the monomer and excimer states is, according to our estimates, about 0.3 eV.

1. J.R. Lakowicz. *Principles of Fluorescence Spectrosscopy* (Springer, 2006).
2. V. Yashchuk *et al.* Optical response of the polynucleotides-proteins interaction. *Mol. Cryst. Liq. Cryst.* **535**, 93 (2011).
3. I. Bald *et al.* Two-dimensional network stability of nucleobases and amino acids on graphite under ambient conditions: adenine, L-serine and L-tyrosine. *Phys. Chem. Chem. Phys.* **12**, 3616 (2010).
4. C. Su, C.T. Middleton, B. Kohler. Base-stacking disorder and excited-state dynamics in single-stranded adenine homo-oligonucleotides. *J. Phys. Chem. B* **116** (34), 10266 (2012).
5. A.I. Kononov, M.N. Bukina. Luminescence excitation spectra reveal low lying excited states in stacked adenine bases. *J. Biomol. Struct. Dyn.* **20** (3), 465 (2002).
6. M. Daniels, W. Hauswirth. Fluorescence of the purine and pyrimidine bases of the nucleic acids in neutral aqueous solution at 300 K. *Science, New Series* **171**, 3972 (1971), 675 (2016).
7. F. Plasser, H. Lischka. Electronic excitation and structural relaxation of the adenine dinucleotide in gas phase and solution. *Photochem. Photobiol. Sci.* **12** (8), 1440 (2013).
8. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, Jr. Vreven, T. Kudin, K.N. Burant *et al. Gaussian 03.* (Gaussian, Inc., Pittsburgh, PA, 2003).
9. H. C. Börresen. Effects of cooling on the fluorescence of biological guanine derivatives. *Acta Chem. Scand.* **19**, 2100 (1965).
10. S. Udenfriend, P. Zaltzman. Fluorescence characteristics of purines, pyrimidines, and their derivatives: Measurement of guanine in nucleic acid hydrolyzates. *Anal. Biochem.* **3**, 49 (1962).
11. J.E. Gill. The fluorescence excitation spectrum of thymine: Evidence for wavelength dependence of the quantum yield. *J. Mol. Spectrosc.* **27**, 539 (1968).
12. K. Berens, K.L. Wierzchowski. Luminescence of tautomeric forms of thymine monoanions. *Photochem. Photobiol.* **9**, 433 (1969).

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ВИСОКОТЕМПЕРАТУРНА ФЛЮОРЕСЦЕНЦІЯ ВИСОКОКОНЦЕНТРОВАНОГО ВОДНОГО РОЗЧИНУ АТФ

В статті наведено результати експериментального дослідження люмінесценції водного розчину аденозинтрифосфату (АТФ) за кімнатної температури. Виявлена високотемпературна люмінесценція (флюоресценція) висококонцентрованого розчину АТФ. Вона має функцію збудження в УФ-області, відмінну від спектра поглинання АТФ. Вивчено температурну поведінку цієї люмінесценції, зроблено висновок про те, що центрами люмінесценції, на наш погляд, є АТФ-ексимери, які утворюються при високій концентрації АТФ.

Ключові слова: високотемпературна флюоресценція, аденозинтрифосфат, водний розчин, ексимер.