ANALYSIS OF PHOTOLUMINESCENCE DECAY KINETICS OF ALUMINUM PHTHALOCYANINE NANOPARTICLES INTERACTING WITH IMMUNE CELLS

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Abstract

This work is dedicated to the study of the photoluminescence kinetics of aluminum phthalocyanine nanoparticles in colloidal solutions at different pH and in the interaction with immune cells (macrophages). For measurements we used a registration system based on Hamamatsu streak camera (C10627-13 Hamamatsu Photonics) with picosecond temporal resolution (15 ps), conjugated with the fiber-optic spectrometer and picosecond laser pumping. The changes in fluorescence decay kinetics as additional lifetime components of fluorescence were found during the experiment. The number of components and duration of lifetimes changed while interacting with cells and depends on pH. At pH 2 the presence of two fluorescence lifetimes was recorded: the first one was 5 ns, which corresponded to the molecular form in solution, and 1,5 ns, which corresponded to bound state of phthalocyanine molecules. Due to the absence of other possible objects for bounding in the solution except of the nanoparticles we can suggest with a high degree of accuracy that the bounding occurs with the very these nanoparticles. Analysis of the fluorescence lifetimes of aluminum phthalocyanine nanoparticles in macrophages indicated the presence of two components: 9 ns and 4.5 ns. A model of surface molecules transitions from parallel to perpendicular position, regarding to the plane of the crystal nanoparticle was proposed.

Keywords: aluminum phthalocyanine, photoluminescence, nanoparticles, immune cells.

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Introduction

The use of crystalline nanoparticles of organic photosensitizers for photodiagnosis (PD) and photodynamic therapy (PDT) has a number of advantages compared to the molecular photosensitizers. One of such photosensitizers is the aluminum phthalocyanine nanoparticles (NP-AlPc). While in aqueous colloid, they do not fluoresce and are photodynamically inactive, but if they enter the biological environment they begin to show fluorescent properties. Intensive fluorescence and photodynamic activity happens predominantly in the pathologically changed tissues, while this effect is much weaker in healthy tissues. The work [1] studied the application of NP-AlPc to assess the risk of skin autografts rejection. It was noted that during the application of colloidal solution of NP-AlPc under the autografts the intensity of fluorescence in the case of inflammatory rejection increased. The work [2] assessed the applicability of NP-AlPc for PD and PTD of joint diseases. Distinct photobleaching, absenting during the use of molecular form of this photosensitizer, was observed in the studied area after laser action.

Materials and methods

In order to study the NP-AlPc photoluminescence kinetics, we developed a measurement system based on the streak-camera with picosecond temporal resolution (15 ps), Hamamatsu C10627-13, connected with the fiber-optic spectrometer. Semiconductor laser Hamamatsu with pulse duration of 67 ps and wavelength of 637 nm was used for excitation. The method called Time-correlated Single Photon Counting was used in the process of measurement [3, 4].

Fig. 2 represents the streak camera operating mechanism. Photoluminescence excited in the sample by a laser is gathered in an optic fiber and after that enters the monochromator. The beam, having passed through the monochromator, generates an image on the photocathode, which is converted into an electronic signal. Inside the streak scope, in parallel to the optical axis, the electrons are affected by the acceleration field, which makes them move from the photocathode to the phosphor screen. The variable field carrying out a time scan is applied to the electrons perpendicular to the direction of their

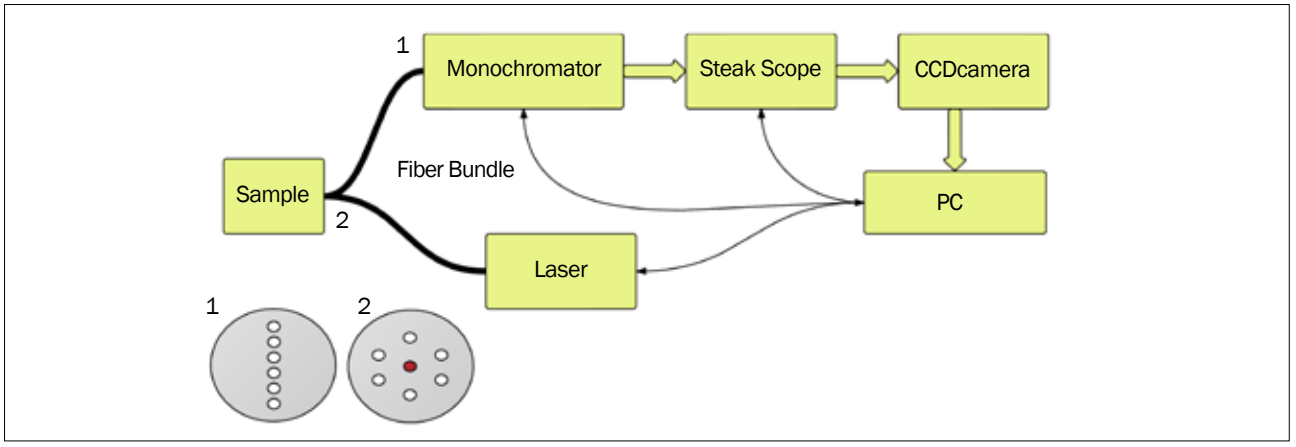

Fig. 1. Scheme of the experimental facility: 1 – outlet end of fiber bundle, 2 – distal end of fiber bundle

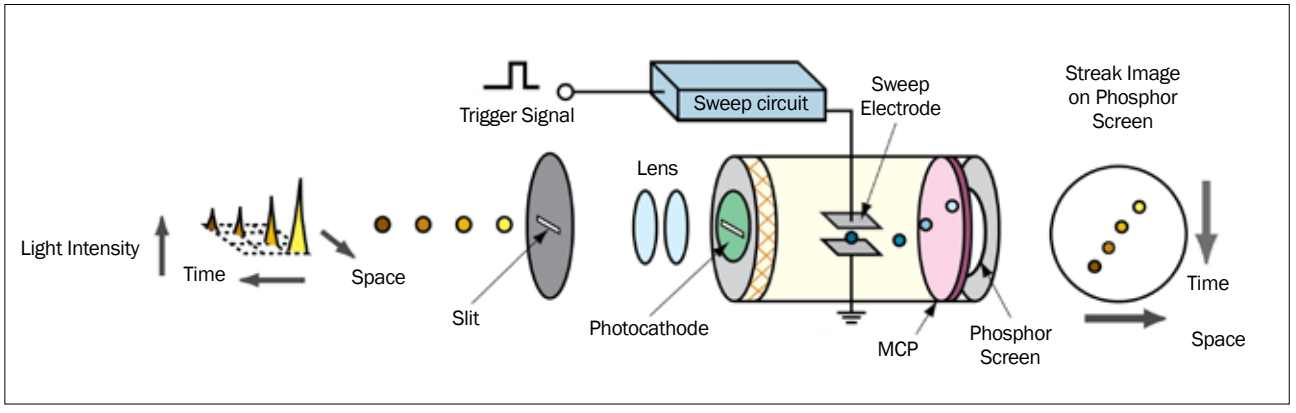

Fig. 2. Operating principle of the streak scope

motion. After the scanning, the electrons reach the MCP, after which the signal is captured by the digital camera and transmitted to the computer, where the obtained fluorescence quenching spectrum is mathematically processed to get information about the lifetime of fluorescence and the distribution of the number of photons registered by the streak-camera between the components of the lifetime.

At the preparatory stage of the work, the research was carried out on the study of the colloid fluorescence intensity dependence of aluminum phthalocyanine nanoparticles on pH and dependence of NP-AlPc colloid absorption on pH. The obtained dependencies of the absorption and fluorescence spectra of aluminum phthalocyanine nanoparticle colloid on pH are presented in Fig. 3 and 4 respectively. Absorption spectra were measured on Hitachi U-3400 spectrophotometer. Laser spectrometer LESA-01-BIOSPEC was used to measure the fluorescence spectra. Fluorescence was excited using He-Ne laser.

It can be seen from the fluorescence spectra presented in Fig. 4 that the fluorescence intensity of aluminum phthalocyanine nanoparticles in the colloid depends on pH. Thus, the most intensive fluorescence was registered at pH 11; fluorescence intensity at pH 2 was 3 times less than at pH 11 and is almost nonexistent at neutral pH 8.

Results and discussion

The spectra of fluorescence quenching kinetics of phthalocyanine nanoparticle colloid at different

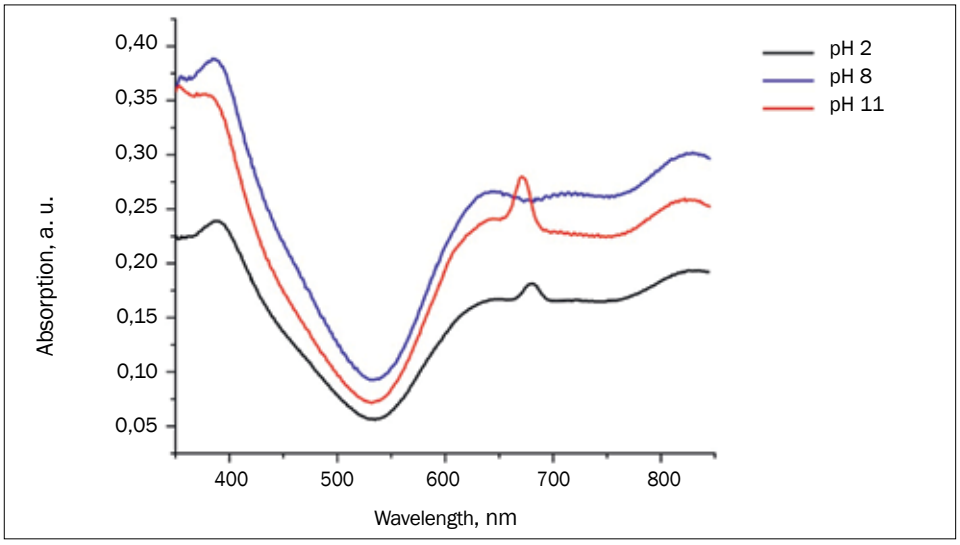

Fig. 3. Absorption spectra of NP-AlPc colloid depending on pH

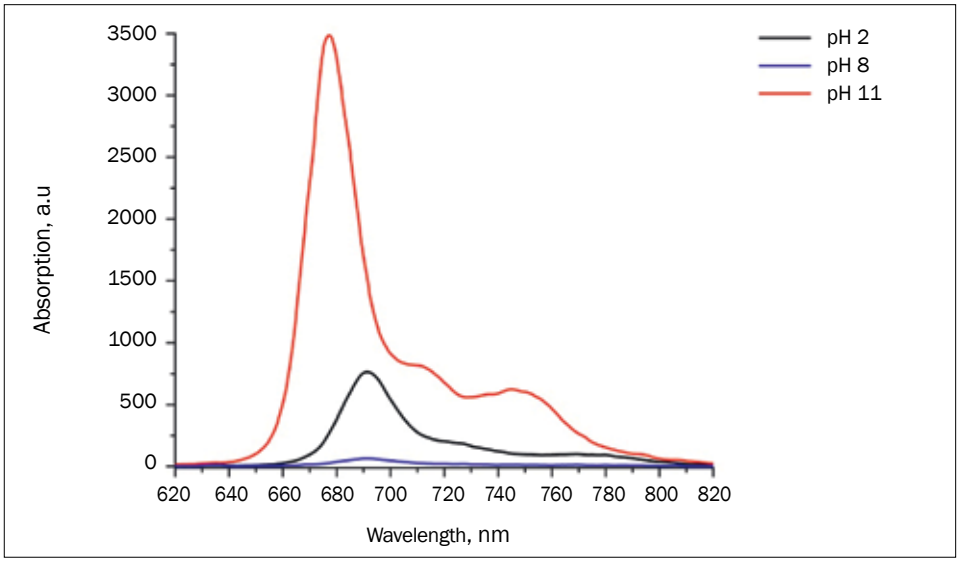

Fig. 4. Fluorescence spectra of NP-AlPc in colloid depending on pH

pH obtained using the streak-camera are provided in Fig. 5 and 6. The analysis of the spectrum for pH 2 determined visual resemblance with the fluorescence kinetics spectrum for molecular form of aluminum phthalocyanine. However, the mathematical processing of this spectrum showed presence of two fluorescence lifetimes: 5 ns (72% of the total number of photons) and 1.5 ns (28% of the total number of photons) with χ^2 = 1,092, whereas the duration of one lifetime of aqueous solution of the sulphureted aluminum phthalocyanine is 5 ns. Two lifetimes were obtained at pH 11: 3 ns (51% of the total number of photons) and 0.5 ns (49% of the total number of photons) with χ^2 = 1,009.

Value χ^2 is called a reduced distribution of chi-square and equal to:

$$\chi^2 = \frac{1}{V}\sum_i \frac{(M_i - C_i)^2}{M_i}$$

where M_i – value of experimentally researched quantity in the i point, Ci – mathematically approximated values of the researched quantity in the i point, V – number of degrees of freedom. More detailed description of the model used for assessment of the approximation quality is referred to in the work [5, 6].

Research of fluorescence quenching kinetics of NP-AlPc located inside cells and participating in the implantation process was of special interest in this work. The THP-1 monocyte culture widely used for imitation of macrophages was used for this study. Monocytes transform into macrophages if there is an inflammation. Phytohemagglutinin-P (PHA) and Concanavalin A (Con A) were used in the culture for induction of THP-1 proliferation. PHA and Con A were added to monocytes in a concentration of 10 mcg/ml. After 2 days, NP-AlPc colloid was added to the monocytes activated in this way. A day after the addition of NP-AlPc, the cells were washed, pelleted, and the resulting cellular mass was examined using time-resolved spectroscopy.

For the reference sample of monocytes without activation with lectins the fluorescence decay kinetics analysis (Fig. 7) showed the presence of two lifetimes: 6 ns (93% of the total number of registered photons) and 1.5 ns (7% of the total number of the registered photons) with χ^2 = 1,024.

Kinetics of NP-AlPc fluorescence decay in macrophages activated by PHA is presented in Fig. 8. Analysis of fluorescence kinetics of this sample showed presence of two lifetimes.

Similar results were obtained for NP-AlPc in macrophages activated by Con A. Total results of the research of NP-AlPc luminescence kinetics in macrophages are presented in Table.

The results of the research show that interaction of NP-AlPc with monocytes/macrophages leads to the change of their fluorescence lifetime. Similar observations were reported in work [7] where it was proposed to assess the efficiency of cancer treatment by fluorescence lifetime. This work used monoexponential approximation for fluorescence decay kinetics. The results showed considerable difference in the fluorescence lifetime in tumor and in surrounding tissues before treatment and 7 days after treatment when the size of the tumor became comparable with its size before treatment. Strong correlation between

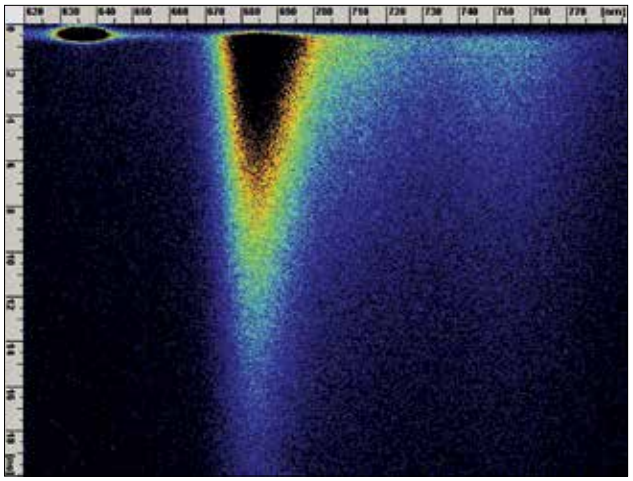

Fig. 5. Spectrum of fluorescence kinetics of NP-AlPc in colloid at pH 2

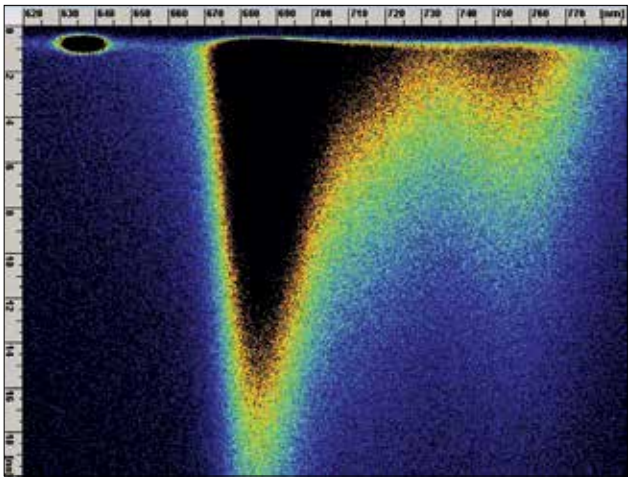

Fig. 6. Spectrum of fluorescence kinetics of NP-AlPc in colloid at pH 11

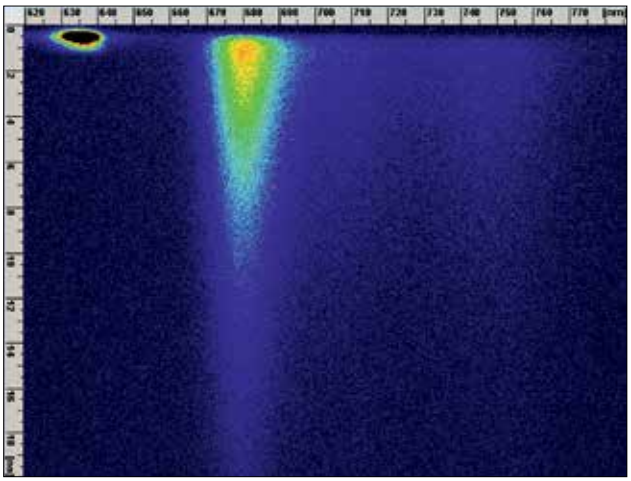

Fig. 7. Spectrum of fluorescence kinetics obtained from the cellular mass of THP-1 monocytes incubated with NP-AlPc

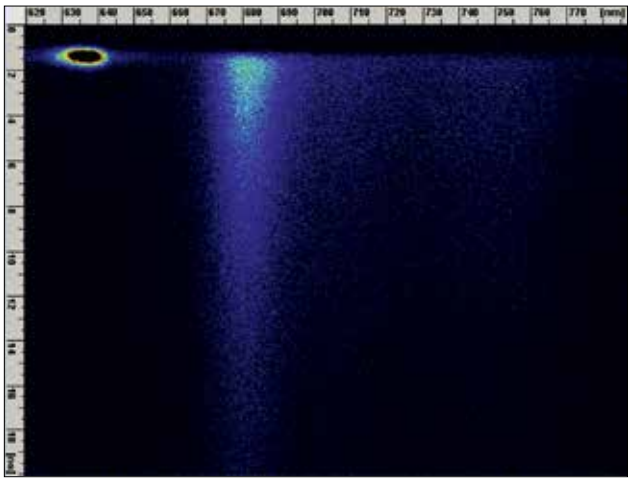

Fig. 8. Spectrum of fluorescence kinetics obtained from the cellular mass of THP-1 monocytes, activated by PHA and incubated with NP- AlPc

Table.
Lifetime of NP-AlPc luminescence in activated and non-activated macrophages

Sample	Lifetime , τ ns	Photon distribution in τ	Number of photons during time t	Fitting criterion χ^2
THP-1 monocytes incubated with NP- AlPc	τ_1 = 6 ns τ_2 = 1,5 ns	93% photons 7% photons	6450 photons	χ^2 = 1,024
THP-1 cells activated by Con A, incubated with NP- AlPc	τ_1 = 9 ns τ_2 = 4,5 ns	32% photons 68% photons	7335 photons	χ^2 = 1,013
THP-1 cells activated with PHA, incubated with NP-AlPc	τ_1 = 9,3 ns τ_2 = 4,1 ns	31% photons 69% photons	5391 photons	χ^2 = 1,037

the fluorescence lifetime and binding of fluorophore molecules with receptors was determined.

Thus, AlPc nanoparticle fluorescence lifetime strongly depends on a certain type of their interaction with macrophages, and proceeding from the obtained results, we can make an assumption that NP-AlPc have at least two possible states:

- NP-AlPc are passive, the superficial molecules of AlPc are in para-position relative to the nanoparticle surface (lying on the surface). NP-AlPc does not fluoresce in this state. But as a result of the interaction, the surrounding bioorganic molecules can tear off individual AlPhc molecules. In this case, the interaction between a phthalocyanine molecule and a biomolecule can be characterized by weak coordinate or Van der Waals bonds and hence the fluorescence lifetime will not differ from the lifetime of water-soluble, molecular form of aluminum phthalocyanine.
- NP-AlPc are active, the superficial AlPc molecules are in the ortho-position (floating perpendicular to the NP-AlPc surface). With one bond they hold on to the nanoparticle's surface, with the other – connect with the bioorganic molecules with covalent bond. In this case, the fluorescence decay time is 1–2 ns. If we deliver energy sufficient for activation of the photodynamic reaction to this pair, then the third component in the fluorescence quenching spectrum appears, the duration of which will be less than 1 ns. This effect is noticeable because the NP in this way can deactivate the macrophages.

Conclusion

This work studies the kinetics of fluorescence decay of aluminum phthalocyanine nanoparticles incubated with macrophages. In the course of the work, NP-AlPc colloids with various pH were prepared and spectra of fluorescence and absorption of prepared colloids were measured to assess the bond type.

Using the developed system, the fluorescence lifetime values were obtained for each sample. For pH 2, the calculation showed the presence of two lifetimes: 5 ns (72% of the total number of registered photons) and 1.5 ns (28% of the total number of the registered photons), however, the component with the lifetime of 5 ns coincides with the fluorescence lifetime of the molecular (dissolved) form of aluminum phthalocyanine. For pH 11, the calculation showed the components at 3 ns (51% of the total number of the registered photons) and 0.5 ns (49% of the total number of the registered photons). For the samples with macrophages activated by Con A, we obtained two lifetimes: 9 ns (32% of the total number of the registered photons) and 4.5 ns (68% of the total number of the registered photons). For the samples with macrophages activated by PHA, we obtained two lifetimes: 9.3 ns (31% of the total number of the registered photons) and 4.1 ns (69% of the total number of the registered photons). Whereas for the reference sample of monocytes without activation, the fluorescence decay kinetics analysis showed presence of two lifetimes: 6 ns (93% of the total number of the registered photons) and 1.5 ns (7% of the total number of the registered photons).

Based on the fact that the nanoparticle fluorescence of aluminum phthalocyanine is susceptible to photobleaching, which is not characteristic for the molecular form of AlPc, we can make an assumption that fluorescence appears in the pathologically changed tissues not only due to regular dissolution, but due to the transition of superficial molecules to other states each of which can be described by the separate nature of interaction with the environment. This is confirmed by appearance of several fluorescence lifetime components, each one being specific for a certain state of the molecules on the surface of the nanoparticle and their interaction with the environment.

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