ORIGINAL ARTICLES

ALUMINUM PHTHALOCYANINE NANOPARTICLES ACTIVATION FOR LOCAL FLUORESCENCE SPECTROSCOPY IN DENTISTRY

Zolotareva J.O.[1], **Farrakhova D.S.**[1, 2, 3], **Kupriyanova E.N.**[4], **Loschenov V.B.**[1, 2, 3]
[1]National Research Nuclear University MEPhI, Moscow, Russia
[2]Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow, Russia
[3]Dentospek LLC, Troitsk, Moscow, Russia
[4]SAHI «Dental Clinic No. 11 of the Moscow City Health Department», Moscow, Russia

Abstract

Early diagnosis of caries and tooth enamel microcracks is of great importance for preventing the destruction of healthy tooth enamel. In order to detect microcracks in the enamel and pathogenic microflora foci that can cause caries, nanoform of aluminum phthalocyanine (AlPc) can be used as a marker. In a colloidal solution, the nanoparticles do not fluoresce, unlike their molecular form. To convert the particle into its molecular form, it is necessary to have a solvent or specific environment (bacteria, macrophages, etc.). That is why the hydrophobic nanoparticles of aluminum phthalocyanine (nAlPc) can act as markers for detecting hidden pathogenic microflora during fluorescent diagnostics. Further reduction of the diagnosis time and increase the efficiency can be achieved by using biologically compatible surfactants as additional activators of nAlPc.

In order to carry out local fluorescence spectroscopy of enamel microcracks and pathogenic microflora foci on the enamel surface, a model compound containing surfactants, auxiliary components and nAlPc colloid at a concentration of 10 mg/l was prepared.

Studies on the interaction of the model compound with nAlPc and Protelan MST-35 with tooth enamel ex vivo have shown this surfactant to be a promising auxiliary activator of the nanoparticles, allowing conducting local fluorescence spectroscopy of the tooth enamel surface 3 min after application. In addition, statistical processing of the results showed the effectiveness of using the model compound for local fluorescence spectroscopy of the enamel surface in order to detect the enamel microcracks and the pathogenic microflora accumulation foci that can lead to the development of a cariogenic process.

Keywords: local fluorescence spectroscopy, nanoparticles, aluminum phthalocyanine, fluorescence, enamel microcracks, caries.

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Contacts: Zolotareva J.O., e-mail: JOKuznetsova@mail.ru

АКТИВАЦИЯ НАНОЧАСТИЦ ФТАЛОЦИАНИНА АЛЮМИНИЯ ДЛЯ ЛОКАЛЬНОЙ ФЛУОРЕСЦЕНТНОЙ СПЕКТРОСКОПИИ В СТОМАТОЛОГИИ

Ю.О. Золотарева[1], **Д.С. Фаррахова**[1, 2, 3], **Е.Н. Куприянова**[4], **В.Б. Лощенов**[1, 2, 3]
[1]Национальный исследовательский ядерный университет МИФИ, Москва, Россия
[2]Институт общей физики им. А.М. Прохорова Российской Академии Наук, Москва, Россия
[3]ООО «Дентоспек», Троицк, Москва, Россия
[4]АУЗ «Стоматологическая поликлиника №11 ДЗМ», Москва, Россия

Резюме

Ранняя диагностика кариеса и микротрещин эмали имеет большое значение для предотвращения разрушения здоровой эмали зубов. Для выявления микротрещин эмали и очагов скопления патогенной микрофлоры, которые могут стать причиной развития кариеса, в качестве маркера используется фталоцианин алюминия (AlPc) в виде наночастиц. В коллоидном растворе наночастицы не обладают собственной флуоресценцией, в отличие от молекулярной формы. Для перевода частицы в молекулярную форму необходимо присутствие растворителя или специфического окружения (бактерии, макрофаги и др.). Поэтому гидрофобные наночастицы фталоцианина алюминия (nAlPc) могут выступать в качестве маркера для обнаружения скрытых очагов скопления патогенной микрофлоры во время проведения флуоресцентной диагностики. Для сокращения времени диагностики и увеличения эффективности в качестве дополнительных активаторов nAlPc могут быть использованы биологически совместимые поверхностно-активные вещества (ПАВ).

Для проведения локальной флуоресцентной спектроскопии микротрещин эмали и очагов скопления патогенной микрофлоры на поверхности эмали была приготовлена модельная смесь, содержащая ПАВ, вспомогательные компоненты и коллоида nAlPc в концентрации 10 мг/л.
Исследования по взаимодействию модельной смеси с nAlPc и протеланом с эмалью зубов *ex vivo* показали перспективность использования этого ПАВ для дополнительной активации наночастиц, что позволяет проводить локальную флуоресцентную спектроскопию поверхности эмали зубов через 3 мин после нанесения. Также статистическая обработка результатов показала эффективность использования модельной смеси для локальной флуоресцентной спектроскопии поверхности эмали для выявления микротрещин эмали и очагов скопления патогенной микрофлоры, которая может привести к развитию кариесогенного процесса.

Ключевые слова: локальная флуоресцентная спектроскопия, наночастицы, фталоцианин алюминия, флуоресценция, микротрещины эмали, кариес.

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Контакты: Золотарева Ю.О., e-mail: JOKuznetsova@mail.ru

Introduction

According to the World Health Organization, dental caries affects approximately 60–90% of schoolchildren and almost 100% of adults worldwide [1]. Early diagnosis of the disease and its timely treatment will not only preserve dental health for many years, but also improve the quality of life.

Currently, the methods used for early diagnosis of caries and various types of tooth enamel damage include visual inspection and probing, X-ray and a number of optical methods. The most common optical methods include the use of Raman scattering, optical coherence tomography, light scattering spectroscopy and local fluorescence spectroscopy.

Raman scattering is used to diagnose periodontitis by saliva analysis [2, 3], as well as to detect caries on the basis of a change in the composition and structure of enamel [4-6]. The disadvantages of this method are its use mainly in *ex vivo* conditions, and in the presence of an already formed damage of tooth enamel. This method may not be used to diagnose enamel microcracks and hidden foci of accumulation of pathogenic microflora. Optical coherence tomography makes it possible to detect qualitative and quantitative morphological changes in hard dental tissues *in vivo*. Due to its good spatial resolution, the method is suitable for the early diagnosis of dental diseases, such as caries, as well as periodontal tissue diseases, including oral cancer. Three-dimensional imaging is another advantage that optical coherence tomography provides in dentistry applications. However, the application of this method is limited by the depth of penetration of optical radiation into biological tissue and the relatively high cost of the procedure [7]. Local fluorescence spectroscopy, which uses ultraviolet radiation for diagnostics, makes it possible to identify the extent and boundaries of enamel caries damage, but it does not solve the problem of diagnosis in the early stages of caries development, when the waste products of bacteria are present in small quantities [8].

Local fluorescence spectroscopy using laser radiation with a wavelength in the red region of the spectrum is a more advanced diagnostic tool. This method can be used to diagnose dental calculus [9, 10], caries [11–15] and inflammatory processes of periodontal tissues [16]. To conduct local fluorescence spectroscopy of enamel in the visible range of the spectrum, a sufficient amount of endogenous porphyrins is necessary, which are the product of the vital activity of bacteria accumulating in enamel areas damaged by caries, in microcracks or on the surface of dental calculus [8, 17]. But in the early stages of caries development, autofluorescence of pathogenic microflora is weakly expressed, which makes it difficult to conduct local fluorescence spectroscopy.

In 2007, it was proposed to use aluminum phthalocyanine nanoparticles (nAlPc) to detect pathogenic microflora localized in microdamage areas of tooth enamel [18]. Nanoparticles are used as a marker due to the fact that nAlPc are not fluorescent and photoactive in an aqueous medium [19], but when interacting with a specific biological environment, they begin to fluoresce and exhibit photodynamic activity. For fluorescence to occur, it is necessary that the phthalocyanine molecules separate from the particle surface or are in a partially bound state [20], which usually occurs in the presence of a solvent or a specific environment (bacteria, macrophages, etc.) [19-23]. The mechanism of the occurrence of fluorescence can presumably be described with a model involving the transition of molecules on the surface of a nanoparticle from the para position to the ortho position [19, 20, 22–24].

A number of authors [25, 26] indicate the possibility of fluorescence diagnostics of enamel microdamages 15 minutes after the application of colloid of nanoparticles to the enamel surface.

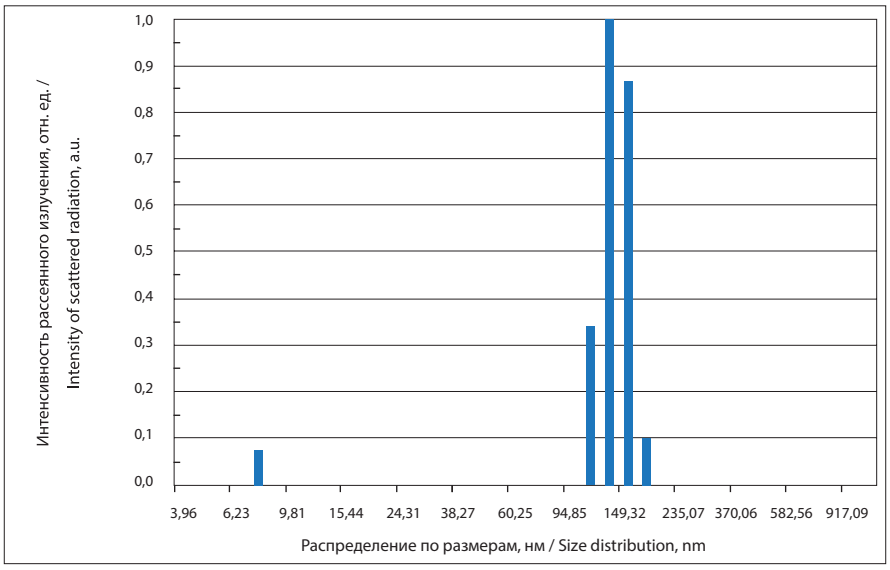


Рис. 1. Распределение nAlPc по гидродинамическому радиусу
Fig. 1. Distribution of nAlPc hydrodynamic radius

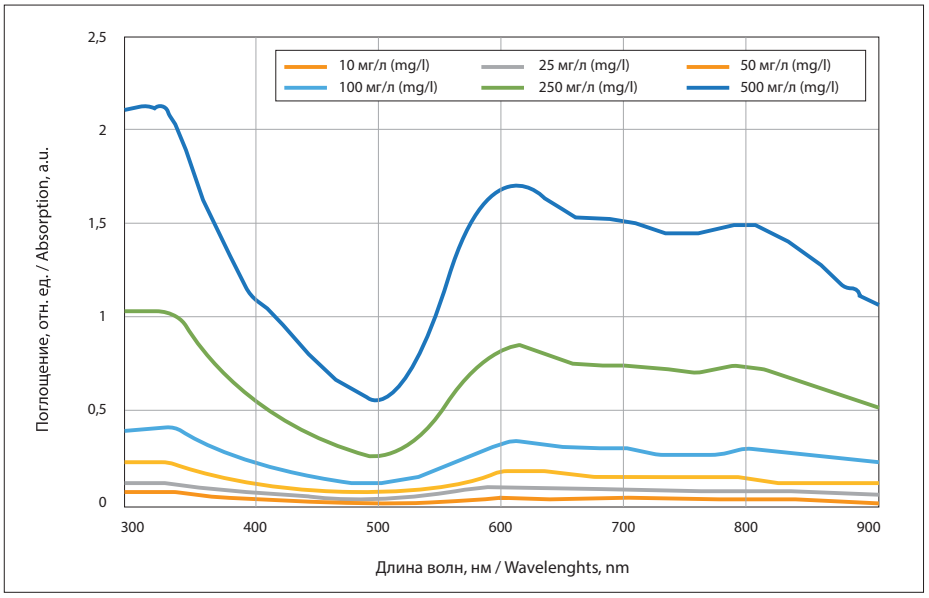


Рис. 2. Спектры поглощения коллоидных растворов nAlPc в концентрациях 10, 25, 50, 100, 250 и 500 мг/л
Fig. 2. Absorption spectra of nAlPc colloidal solutions at concentrations of 10, 25, 50, 100, 250 and 500 mg/l

It is also known from the literature that, due to the photodynamic effect, water-soluble forms of aluminum phthalocyanine have an inhibitory effect on the growth of a number of bacteria that cause various types of damage of tooth enamel and periodontal tissues. In particular, photodynamic therapy with desulfurized aluminum phthalocyanine leads to the death *Streptococcus sanguis, Porphyomonas gingivalis, Escherichia coli, Streptococcus mutans, Candida albicans Actinobacillus actinotnycetetttcotnitans Streptococcus sobrinus, Lactobacillus casei* and *Actinomyces viscosus* [27–31], with chlorinated aluminum phthalocyanine, to the death of *Candida albicans* [32, 33], and with the use of a nanoemulsion, *Staphylococcus aureus* [34].

To reduce the waiting time for fluorescence enhancement, it was proposed to use a surfactant as an additional nAlPc activator, which would transfer a part of the surface molecules of nanoparticles to a more mobile

state. These molecules, without detaching themselves from the nanoparticle, can interact with the microenvironment and exhibit their fluorescence and photodynamic properties, which are close to their characteristics in molecular form.

The goal of this research is to study the interaction of laser radiation with nAlPc on the enamel surface for phototheranostics of initial caries. To achieve this goal, it is necessary to develop a method for controlling the concentration and size of particles in a colloid, and select the optimal surfactant in terms of health safety and fluorescence characteristics for additional activation of nAlPc.

To conduct local fluorescence spectroscopy of the enamel surface in order to detect enamel microcracks and foci of accumulation of pathogenic microflora, model mixtures were prepared containing different surfactants, nAlPc colloid and auxiliary components, and the spectro-fluorescence characteristics of *in vitro* mixtures and *ex vivo* tooth enamel were analyzed.

Materials and methods

Preparation of colloid of aluminum phthalocyanine nanoparticles

The colloidal solution of nAlPc was made with coarse-grained AlPc crystals produced by FSUE SSC NIOPIK (Russia) and distilled water. The crystals were dispersed with the use of Bandelin SONOPLUS HD2070 ultrasonic homogenizer with a KE76 tip (20 kHz, amplitude 165 μm) (Germany). The duration of dispersion was 30 minutes. The colloidal solution was then centrifuged in Centrifuge ELMI CM-6M unit for 10 minutes at a speed of 35,000 rpm. After centrifugation, large particles precipitated. Small particles in the upper layer were collected with the use of an automatic pipette dispenser. To control the particle size in the colloid, a static and dynamic light scattering spectrometer Photocor Complex (Russia) was used. A cuvette with a colloidal solution was irradiated with a low-intensity laser with an excitation wavelength of 635 nm to detect scattered light on the particles.

Fig. 1 shows the results of measuring the hydrodynamic radius of nAlPc in an aqueous medium at a concentration of 10 mg/l. nAlPc particles with a hydrodynamic radius of 140±36 nm and 9±2 nm scatter light in 97% and 3% of cases, respectively.

It is essential that an aqueous suspension of nanoparticles does not fluoresce. To study the interaction of nAlPc with enamel surface microflora, the colloid nAlPc at a concentration of 10 mg/L was used.

Monitoring the concentration of colloidal solution of aluminum phthalocyanine nanoparticles

To control the concentration of nAlPc in the colloid, a normalization curve was constructed based on experimental data. The absorption spectra (Fig. 2) of colloidal nAlPc solutions with known concentrations were measured with a Hitachi U-3400 spectrophotometer (Japan). The values of the optical density of colloids at an absorption wavelength of 538 nm which were plotted on a graph and were found to have a linear relationship were selected (Fig. 3). The concentration of the prepared nAlPc colloid was controlled with a normalization curve.

Preparation of experimental samples for studying the interaction of aluminum phthalocyanine nanoparticles with various surfactants

In order to study the possibility of using various surfactants as additional activators of nAlPc, experimental samples containing 0.5–2% surfactant and a colloidal solution of nAlPc at a concentration of 10 mg/L were prepared. The following were used as additional activators of nanoparticles: Tween 80, Propyleneglycol, Protelan MST-35, Plantacare 1200 UP, Lauryl Glucoside and sodium laurylethoxysulfate. Tween 80 is a hydrophilic non-ionic surfactant commonly used to increase bioavailability and targeted drug delivery in preclinical *in vivo* studies [35–37]. Propyleneglycol is used in the food industry as a food additive E1520. Plantacare, Protelan and sodium lauryl ethoxysulfate are approved as ingredients for toothpastes [38]. The control sample was an aqueous colloidal solution of nAlPc at a concentration of 10 mg/L without additives.

The creation of a model mixture with aluminum phthalocyanine nanoparticles and surfactants for local enamel fluorescence spectroscopy

To conduct *ex vivo* PD of tooth enamel, a model mixture was prepared containing nAlPc (10 mg / L), protelan (1%) and some additional components. The additional components were substances which are usually used in the production of toothpaste in accordance with GOST 7983–99 "Toothpastes. General specifications." Protelan is a surfactant that is produced from 100% of natural ingredients and does not harm living organisms or the environment where people live.

Before preparing the model compound, we optimized the structure and concentration of the components. A sample of the model mixture was studied immediately after preparation and after it had been held in a special thermostat (42°C, 1 month), which is equivalent to exposure at room temperature for 12 months.

To study the spectral properties of nAlPc, additional samples were used in the model mixture with Protelan: the basis of the model mixture without nAlPc and Protelan and the model mixture containing only nAlPc Additional samples of the model mixture were prepared to test the hypothesis stating that a surfactant can activate surface nanoparticle molecules like a solvent. The

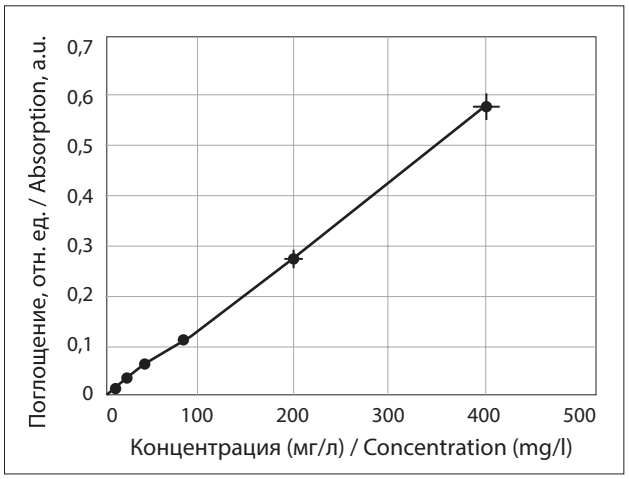


Рис. 3. График нормировочной кривой зависимости поглощения от концентрации коллоида nAlPc, применявшийся для контроля концентрации коллоидного раствора nAlPc
Fig. 3. The graph of the normalized curve used to control nAlPc colloid concentration: the dependence of the absorption on the concentration of the nAlPc colloid

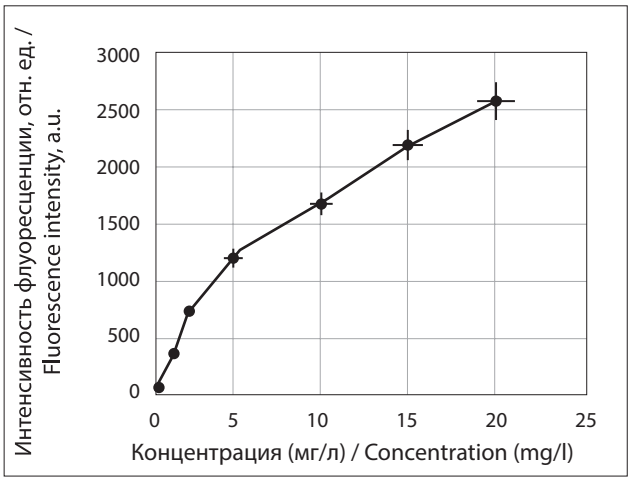


Рис. 4. Зависимость интенсивности флуоресценции nAlPc, перешедших в молекулярную форму, от концентрации при растворении в ДМСО
Fig. 4. The dependence of the fluorescence intensity of nAlPc, transformed into a molecular form, on the concentration at dissolution in DMSO

difference between the solvent of nanoparticles and the surfactant is that the solvent transfers surface molecules to a free state, while the surfactant makes them more mobile and capable of interacting with microflora without separating them from the surface of nanoparticles [38–40].

The control of the fraction of activated aluminum phthalocyanine nanoparticles in an aqueous solution and a model mixture

To quantify the fraction of activated nAlPc in the sample (converted to molecular form) depending on the concentration of surfactants and the interaction time, a calibration curve was produced. To construct, we used the experimental data obtained by the interaction of an organic solvent, dimethyl sulfoxide (DMSO) [41] with various concentrations of nAlPc (LESA-01-BIOSPEC spectrometer (Russia)). The findings are represented in Fig. 4. From the data obtained, it can be seen that for a concentration of nanoparticles of 10 mg/L at 100% solubility (complete transition to molecular form) in DMSO, the fluorescence intensity is 1700 relative units. Thus, knowing the concentration of nAlPc in the experimental sample and using the calibration curve, we can determine the fraction of activated nAlPc (converted to molecular form) as the ratio of the fluorescence intensity of nAlPc in the solution to the fluorescence intensity of nAlPc in DMSO.

To quantify the percentage of activated nanoparticles in the composition of the ready model mixture, a sample was prepared containing the model mixture and DMSO. The concentration of nanoparticles in the experimental sample was 2.5 mg/L. To achieve uniform composition, the sample was subjected to ultrasonic treatment. After that, the maxima of the fluorescence intensities of the model mixture and the experimental sample with DMSO were obtained where complete dissolution of nAlPc was observed, which signals the transition to the molecular form. The ratio between the fluorescence maxima was used to determine the fraction of activated nAlPc in the ready model mixture.

The biological samples for experimental research

To study the interaction of nAlPc with tooth enamel and pathogenic microflora contained on the surface and in microdamages of tooth enamel, human teeth were used (a total of 41 samples), which had been removed for various clinical indications. from the time of their removal to the time of the experiment, samples of extracted teeth were contained in an aqueous 0.9% sodium chloride solution for maximum preservation of microflora contained on the enamel surface.

During the experiment, an nAlPc colloid or model mixture was applied to the surface of the tooth enamel and washed off with water after 3 minutes.

An experimental unit for studying the interaction of a colloid of aluminum phthalocyanine nanoparticles with surfactants, a colloid of aluminum phthalocyanine nanoparticles and a model mixture of aluminum phthalocyanine nanoparticles and Protelan with tooth enamel surface microflora

An experimental unit was assembled for the research, consisting of a laser source for exciting fluorescence and a miniature universal spectrometer for recording and analyzing fluorescent signals.

A laser (632.8 nm) was used as a radiation source exciting fluorescence. The signal was detected with a LESA-01-BIOSPEC laser spectrometer (Russia) [42]. The measurement unit scheme is shown in Fig. 5.

Fluorescence measurement in samples containing nAlPc and surfactant was carried out at the following time intervals: 0–180 minutes, 1–6 days. For measurements, the cuvette with the sample was placed in a specially designed chamber (Fig. 4) with a sufficient level of protection against the impact of alien sources. The fiber optic probe (FOP) is in contact with the sample at an angle of 15° to prevent reflected light from the opposite wall of the Eppendorf device from entering the receiving fibers. The FOP consists of one light fiber and six receiving fibers. The distal end diameter is 1.8 mm.

During the studies on the interaction of the colloid nAlPc and the model mixture (with nAlPc and Protelan) with the surface microflora of tooth enamel, the FOP was placed in contact with the sample.

To visually assess the fluorescence image of the enamel surface before and after applying the model mixture to the tooth enamel, a video fluorescent system was used, which consists of a laser radiation source (635 nm), a light filter with a transmission range of 650–1500 nm, and a sensitive black-and-white camera.

Results processing

When studying the interaction of nAlPc with various surfactants, the processing of the results obtained in the form of spectra on LESA-01 Biospec unit was carried out in several stages. At the first stage, the spectra were averaged for each time point (with Uno Momento software supported in the MS Windows environment). The next step was normalization to the laser peak in order to be able to compare several spectra obtained at different time intervals and to take into account the influence of various factors (measurement geometry, laser power). Then, for each experimental sample, a chart was produced for the temporal dependence of the nAlPc fluorescence intensity upon interaction with various surfactants at various concentrations. The results were processed in a similar manner when studying the spectroscopic properties of a model mixture with nAlPc and Protelan.

The experimental results with biological samples included two groups. The first group consisted of a collagen solution of nAlPc and biological samples of human teeth, and the second group included a model mixture with nAlPc and Protelan and biological samples.

For each sample from both groups, a series of enamel autofluorescence spectra before and AlPc fluorescence spectra after applying a colloid of nanoparticles or a model mixture on tooth enamel were obtained. Each series of spectra was a set of data obtained for various areas on the surface of tooth enamel. The data obtained in the study of the interaction of the colloid nAlPc and the model mixture with tooth enamel *ex vivo* were normalized by the exposure time. Based on the series of

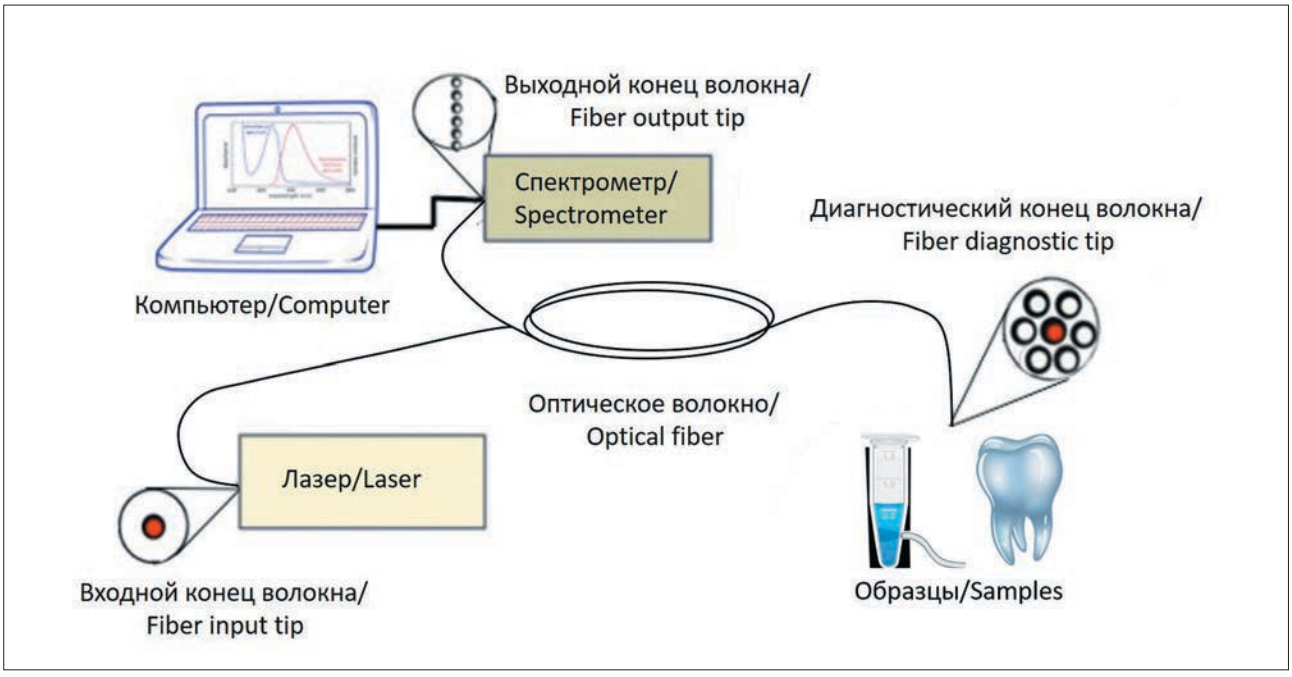


Рис. 5. Схематическое представление измерительной установки
Fig. 5. Experimental setup

spectra for each biological sample, the average value of the autofluorescence spectrum up to and AlPc fluorescence after applying a colloid of nanoparticles or a model mixture on tooth enamel was calculated.

Then, using the average spectrum value, for each sample, the enamel autofluorescence coefficient k_{af} was calculated as the ratio of the areas under the enamel autofluorescence spectrum to the area under the laser peak. Similar actions were performed to calculate the fluorescence coefficient k_{fl} of nAlPc after applying the colloid to the enamel, for a single sample.

Thus, for each experimental group of samples, an array of data was obtained with the enamel autofluorescence coefficients before and nAlPc fluorescence coefficients after applying a colloid of nanoparticles or a model mixture to the tooth enamel surface. To assess the difference in fluorescence from the enamel surface before and after application of experimental compositions with nAlPc, the fluorescence enhancement coefficient was calculated for each sample, which is the ratio of k_{fl} to k_{af}, and the area chosen for the calculation of k_{af} was the area under the autofluorescence spectrum which corresponded to the boundaries of the nAlPc fluorescence signal:

$$k_{DK} = \frac{k_{fl}}{k_{af}}$$

The fluorescence enhancement coefficients for each sample were used for statistical processing of experimental results, which was performed with the use of Statistics SPSS v23.0 computer program. The two groups of teeth (with the use of a colloidal solution of nAlPc or a model compound with nAlPc) were compared with Student's t-test.

Results and discussion

The results of the study of the interaction of aluminum phthalocyanine nanoparticles with various surfactants

It is known that surfactants affect the photophysical and photochemical properties of organic molecules. In the presence of surfactants, the acid-base properties of the molecules in the basic state and excited state change. The spectral and luminescent characteristics and the state of aggregation of the dyes change. A large number of chemical reactions in the presence of surfactants proceed differently from the way they occur in solutions [43].

Ethanol and surfactants act on the state of the medium in approximately the same way [40]. Since ethanol is a solvent of nAlPc nanoparticles [41], surfactants interacting with nanoparticles can activate them the way a solvent does [38, 39].

The study of the dynamics of interaction of aluminum phthalocyanine nanoparticles with propylene glycol

During the experiment, the dependence of the fluorescence index of nanoparticles upon interaction with propylene glycol (concentration 0.5–2%) over a time interval from 0 min to 7 days was investigated. It was found that propylene glycol does not interact with nanoparticles and does not cause nAlPc fluorescence.

The study of the dynamics of the interaction between aluminum phthalocyanine nanoparticles and Tween 80

The surface-active substance Tween 80 (polysorbate-80) is an emulsifier that is used in the food and cosmetic industries [39]. In [44], Tween 80 was used to prepare an emulsion to improve targeted delivery of chlorinated AlPc and to enhance the biodistribution of nanoparticles by coating the surface of AlPc particles [41]. Biologically compatible surfactants (Tweens) can be used to reduce the molecular aggregation that is observed for hydrophobic phthalocyanines, such as AlPc, in an aqueous medium. It is known that aggregation leads to deterioration of the effectiveness of photodynamic therapy and reduces the intensity of fluorescence [45].

Figure 6 shows a graph of the temporal dependence of nAlPc fluorescence intensity during interaction with Tween 80 at various concentrations. The graphs show that a strong increase in nAlPc fluorescence is observed at almost all time intervals when interacting with Tween 80. An increase in nAlPc fluorescence continues for up to 4 days. Then there is a decline followed by subsequent growth.

It was also noted that after 4 days, the nanoparticles in the experimental samples begin to precipitate. Shaking results in the formation of flakes, which do not dissolve. The maximum fraction of activated molecules located on the nAlPc surface varies in the range of 6–8% of their total calculated amount at Tween 80 concentrations of 0.5–2%.

The study of the dynamics of interaction between aluminum phthalocyanine nanoparticles and sodium lauryl ethoxysulfate

Sodium lauryl ethoxysulfate is an extremely hydrophilic surfactant [38]. At concentrations of 1% and 2% in the sample, it causes an increase in nAlPc fluorescence for up to 3-4 days, then the nanoparticles begin to precipitate, and the fluorescence decreases (Fig. 7).

The relatively low fluorescence of nAlPc is caused by the presence of 0.5% sodium lauryl ethoxysulfate in the sample. The fraction of activated molecules varies in the range of 0.5–3% when sodium lauryl ethoxysulfate has concentrations of 0.5–2%.

Investigation of the interaction of aluminum phthalocyanine nanoparticles as a function of time of interaction with Protelan

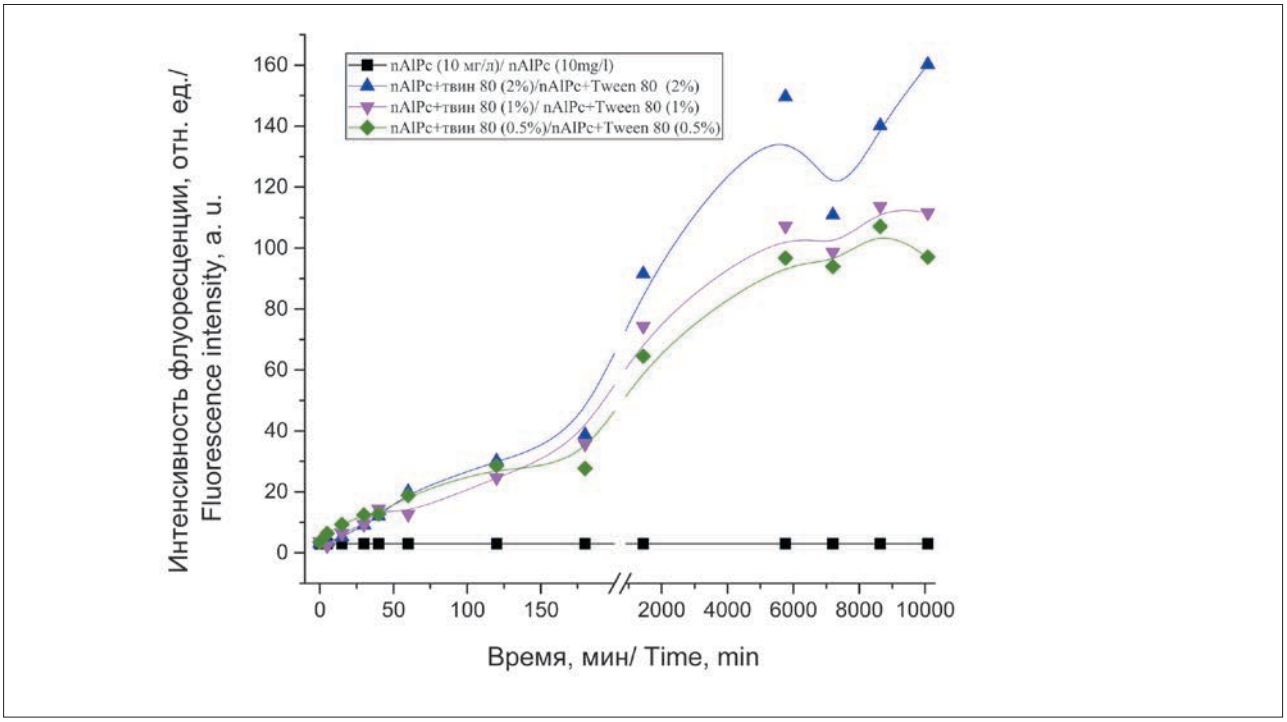


Рис. 6. Динамика интенсивности флуоресценции nAlPc при взаимодействии с твин 80 в концентрациях 0,5–2%
Fig. 6. The dependence of the fluorescence intensity on time in interaction with Tween 80 in concentrations of 0.5–2%

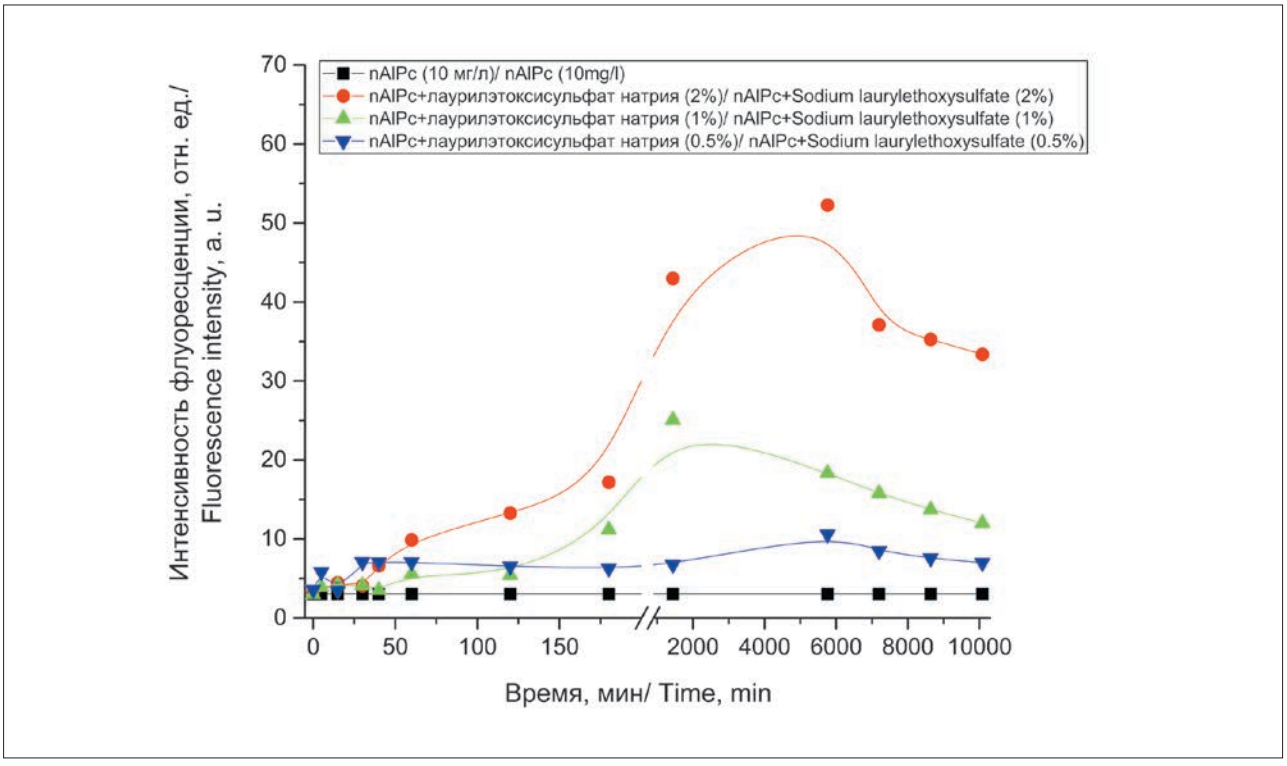


Рис. 7. Динамика интенсивности флуоресценции nAlPc при взаимодействии с лаурилэтоксисульфатом натрия в концентрациях 0,5–2%
Fig. 7. The dependence of the fluorescence intensity of nAlPc on time at interaction with sodium laurylethoxysulfate in concentrations of 0.5–2%

Protelan is obtained from 100% natural, renewable plant materials and does not contain sulfates, preservatives, EO groups and 1,4-dioxane. As a chemical substance, Protelan is characterized by high foaming ability and is used in the production of oral care products. This surfactant is also used in space, does not harm the body or the environment. It is easily biodegradable and approved by Cosmos standard [46]. In the interaction of nanoparticles with Protelan at concentrations of 0.5% and 1%, there is a strong increase in the maximum fluorescence intensity at 6 days after sample preparation, and at a concentration of 2% at 4 days (Fig. 8).

The decrease in fluorescence of nanoparticles is associated with their aggregation and precipitation. The fraction of activated nanoparticles varies in the range of 2–4.5% at surfactant concentrations of 0.5–2%.

The study of the dynamics of the interaction between aluminum phthalocyanine nanoparticles and Plantacare

Plantacare (Lauryl Glucoside) is synthesized from natural raw materials during the rectification of vegetable fats (coconut oil and glucose). In cosmetics, it acts as an emulsifier, dispersant, natural foaming agent, increases the viscosity; it is completely non-toxic and easily biodegradable. When interacting with nAlPc, it causes their fluorescence, the increase of which lasts up to 3–6 days, depending on the concentration. The decrease in fluorescence of nanoparticles is associated with their aggregation and subsequent precipitation. The fraction of activated nAlPc varies in the range of 0.6–2% at surfactant concentrations of 0.5–2% (Fig. 9).

Fluorescence spectra of aluminum phthalocyanine nanoparticles when interacting with various surfactants

During the experiment, it was revealed that there is a definite shift in the nAlPc fluorescence wavelength upon interaction with sodium lauryl sulfate compared to other surfactants by 15 nm, regardless of the concentration of substances and the time after the start of interaction. Figure 10 shows the fluorescence spectrum of nAlPc 40 min after the interaction with various types of surfactants (in 2% concentration).

It was hypothesized that the shift in the fluorescence wavelength may be due to the difference in the pH of the surfactant solution with nanoparticles or the absorbing characteristics of the surfactant.

For each surfactant, the pH values were: for Propyleneglycol, 5.5–8; for Tween, 80 - 5.5–7.5; for sodium lauryl ethoxysulfate, 7–9; for Protelane, 9–10; for Plantacare, 11.5–12.5. As can be seen from the data presented, there is no relationship between the pH of the samples and the shift in the wavelength of the nAlPc fluorescence upon interaction with sodium lauryl sulfate compared to other surfactants. It is also important to note that none of the experimental samples possessed a critical concentration of surfactant micellization, and the type of hydrophilic surfactant group does not affect the fluorescence of nanoparticles [39].

Plots of absorption spectra of various surfactants were obtained at a concentration of 1% by volume with the use of a Hitachi U-3400 spectrophotometer (Japan).

The measurement results showed that all surfactants do not absorb in the spectral range from 350 nm to 850 nm and do not strongly affect the nAlPc fluorescence spectra. Therefore, the reason for the change in the fluorescence wavelengths of the nanoparticles upon interaction with sodium lauryl ethoxysulfate relative to other spectra is not completely clear.

The spectroscopic properties of a model mixture with aluminum phthalocyanine nanoparticles and Protelan for local fluorescence enamel surface spectroscopy

The experiment for the study of the spectroscopic properties of nAlPc in the composition of the model mixture for the PD enamel state included the use of various samples: the basis of the model mixture without nAlPc and Protelan; model mix with nAlPc; model mix with nAlPc and Protelan. The main objective of the experiment was to test the hypothesis that surfactants interacting with nAlPc is capable of activating surface molecules like a solvent, the only difference being that a solvent transfers the molecules to the free state and a surfactant makes them more mobile and reactive without detaching them from the nanoparticle [38–40].

Samples of the model mixture were studied immediately after preparation and after exposure to a special thermostat at 42°C for 1 month, which is equivalent to being at room temperature for 12 months. The findings of the research are represented in Fig. 11. The peak at a wavelength of 632.8 nm corresponds to laser radiation scattered back from the sample surface, which was used to normalize the fluorescence spectra (670 nm) and to numerically estimate the concentration of fluorescent components.

As can be seen from the obtained experimental data, the spectroscopic properties of the model mixture immediately after preparation and after exposure which simulates 12-month storage, changed in the form of a two-fold fluorescence growth. Calculations show that fluorescent molecules are bound to nanoparticles. The pH of the model mixture was 6.27. The composition also did not contain any microflora: *Enterobacteriaceae, Pseudomonas aeruginosa, Staphylococcus aureus*, mold fungi and yeast. The model mixture is microbiologically pure and homogeneous, and its thickness is suitable for the use in a clinical setting.

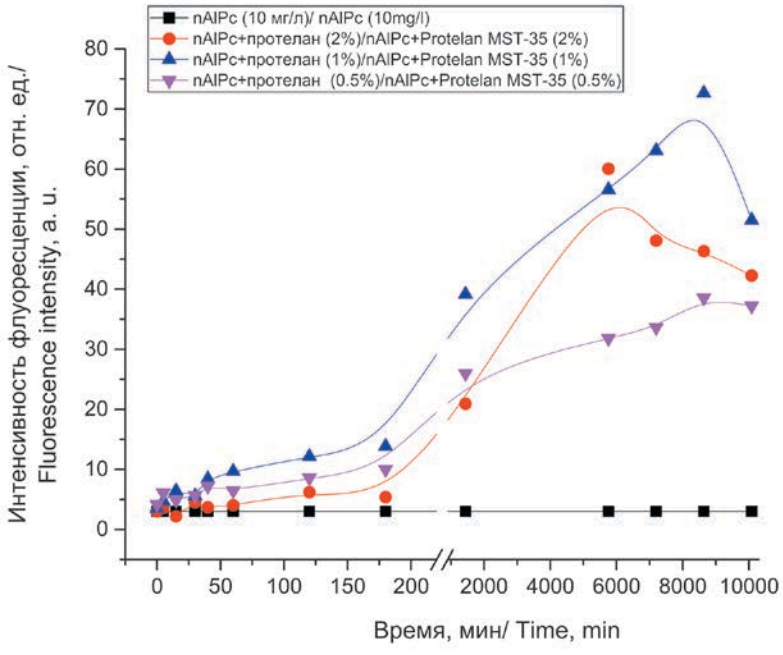


Рис. 8. Динамика интенсивности флуоресценции nAlPc при взаимодействии с протеланом в концентрациях 0,5–2%.
Fig. 8. The dependence of the fluorescence intensity of nAlPc on time at interaction with Protelan MST-35 in concentrations of 0.5–2%

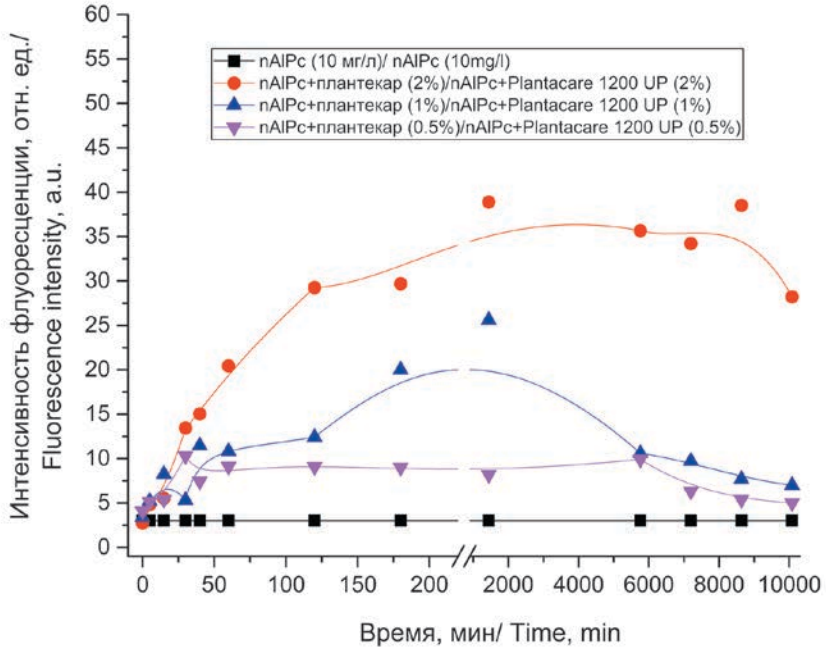


Рис. 9. Динамика интенсивности флуоресценции nAlPc при взаимодействии с плантекаром в концентрациях 0,5–2%.
Fig. 9. The dependence of the fluorescence intensity of nAlPc on time at interaction with Plantacare 1200 UP in concentrations of 0.5–2%

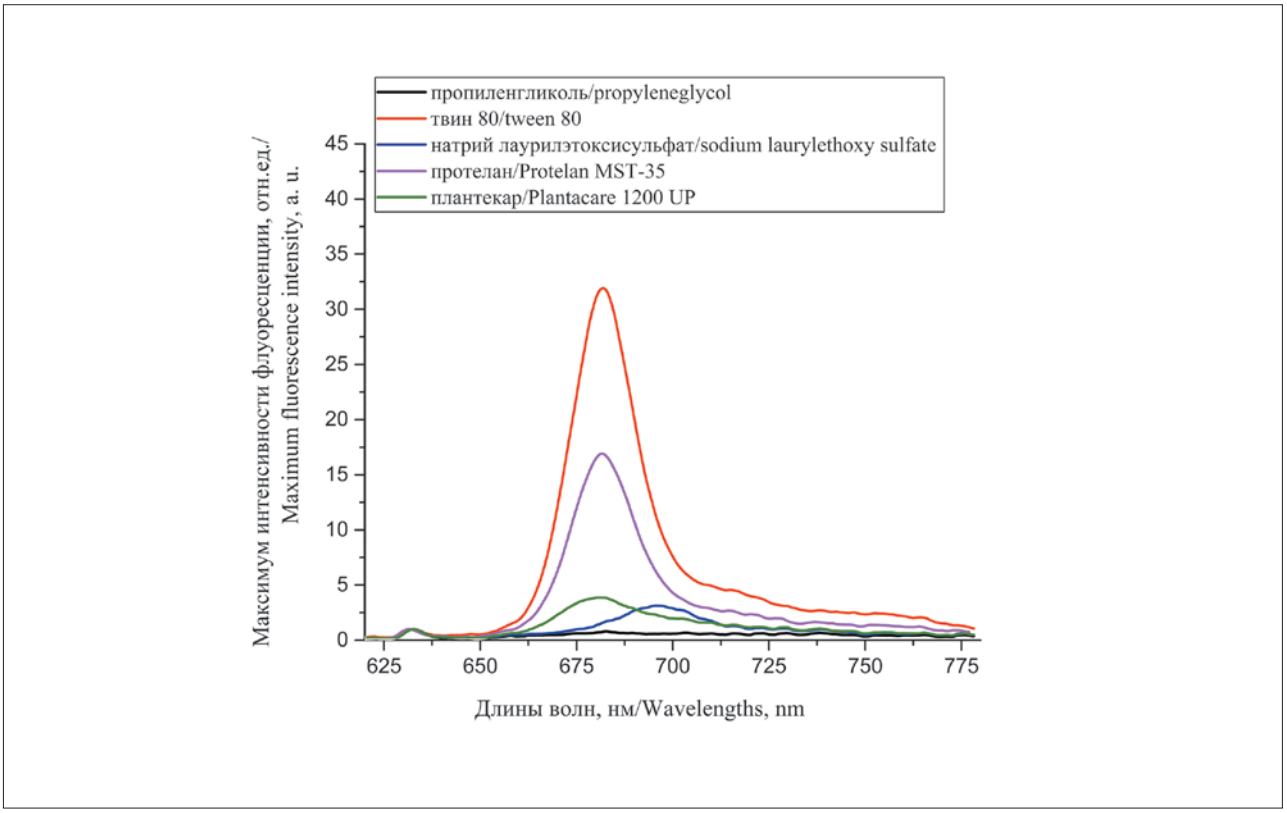


Рис. 10. Спектры флуоресценции nAlPc при взаимодействии с различными ПАВ через 120 мин после приготовления образцов
Fig. 10. Fluorescence spectra of nAlPc interacting with various surfactants 120 min after sample preparation

The preliminary shelf life of the model mixture is at least 12 months.

To quantify the percentage of activated molecules in the composition of the finished model mixture with nAlPc and Protelan, a sample was prepared containing the model mixture and DMSO, which is a solvent of nanoparticles. The concentration of nanoparticles in the experimental sample was 2.5 mg/L. The results of measuring the fluorescence of nanoparticles in the sample, as well as in the model mixture, are presented in Fig. 12.

The fluorescence intensity of all molecules upon dissolution of nAlPc in DMSO is about 100 relative units, at a concentration of nAlPc in the sample of 2.5 mg/L. At the stated concentration in the model mixture of 10 mg/l, the fluorescence intensity of the phthalocyanine molecules has to be 400 relative units. As can be seen from fig. 10 and 11, the fluorescence intensity in the composition of the model mixture is approximately 4–8 relative units. Thus, it can be concluded that about 1–5% of molecules in the activated model are in the activated state. Such a small number of molecules in a free state will allow not only to increase the efficiency and reduce the time of PD, but also to distinguish between the fluorescence of nanoparticles in the presence and in the absence of pathogenic microflora in the area under examination.

The results of a study of the interaction of colloid nanoparticles of aluminum phthalocyanine with enamel ex vivo

The results of studies to identify the nature of the interaction of the colloidal solution of nAlPc with enamel *ex vivo* tooth samples showed that after 3 min there is a low fluorescence due to autofluorescence of microflora and slight activation of surface nanoparticle molecules. A marked increase in the fluorescence of nAlPc occurs 1 h after the application of the colloidal solution. This suggests that it takes some time for the activation of surface nanoparticle molecules by pathogenic microflora located on the surface of the tooth enamel.

To conduct fluorescence diagnostics in a clinical setting, one hour required for fluorescence enhancement is too long an interval for the procedure. This problem is solved by introducing an additional nAlPc activator into

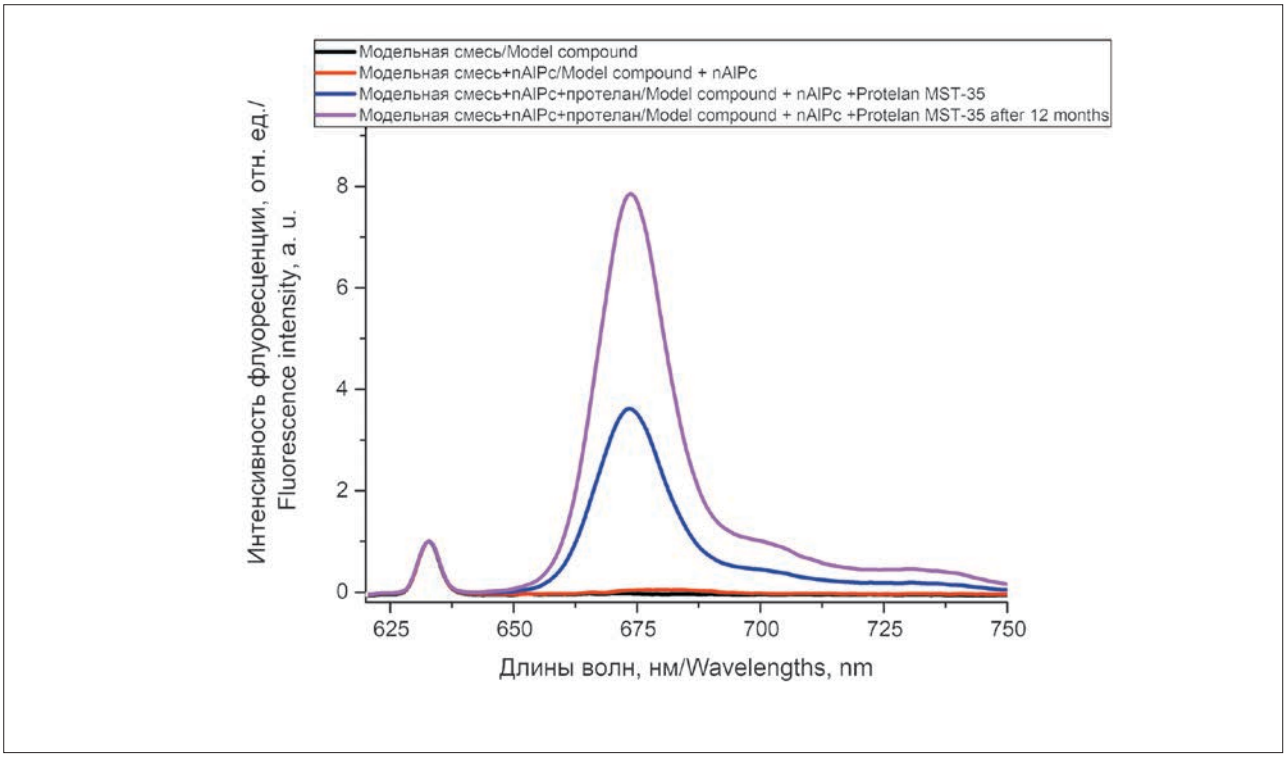


Рис. 11. Спектры флуоресценции модельной смеси, модельной смеси с nAlPc и модельной смеси с nAlPc и протеланом, полученные сразу после приготовления и через 12 мес
Fig. 11. Fluorescence spectra of the model compound, the model compound with nAlPc and the model compound with nAlPc and Protelan MST-35 measured immediately after preparation and 12 months later

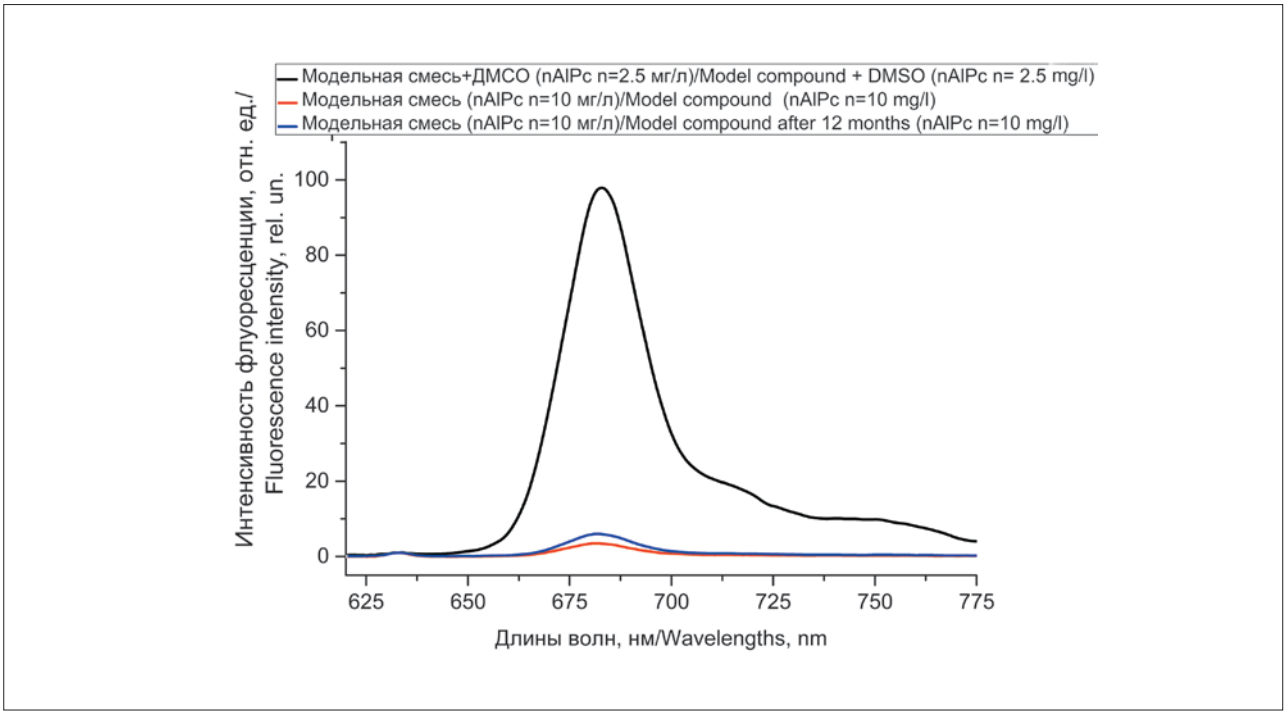


Рис. 12. Спектры флуоресценции модельной смеси с nAlPc (2,5 мг/л) и ДМСО и модельной смеси с nAlPc (10 мг/л) сразу после приготовления и спустя 12 мес
Fig. 12. Fluorescence spectra of the model compound (nAlPc 2.5 mg/l) with DMSO, and model compound (nAlPc 10 mg/l) immediately after preparation and 12 months later

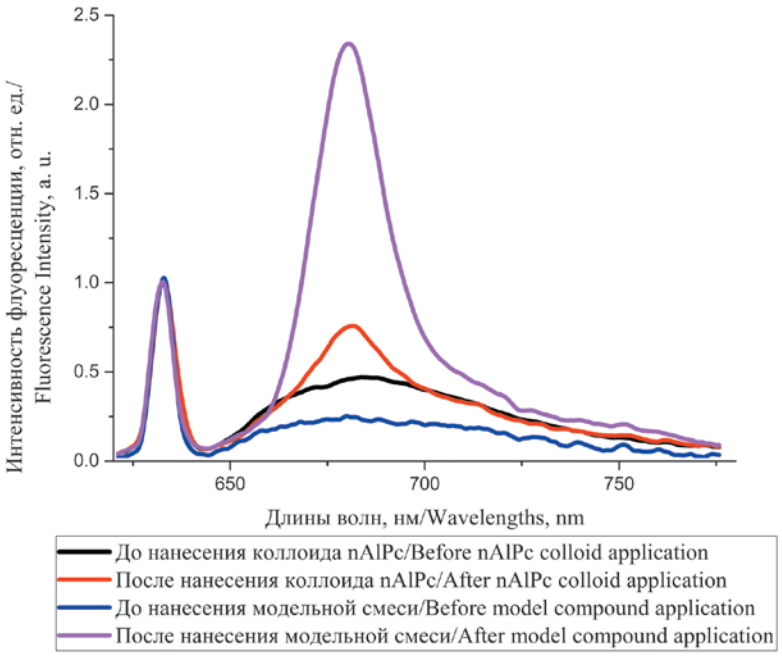
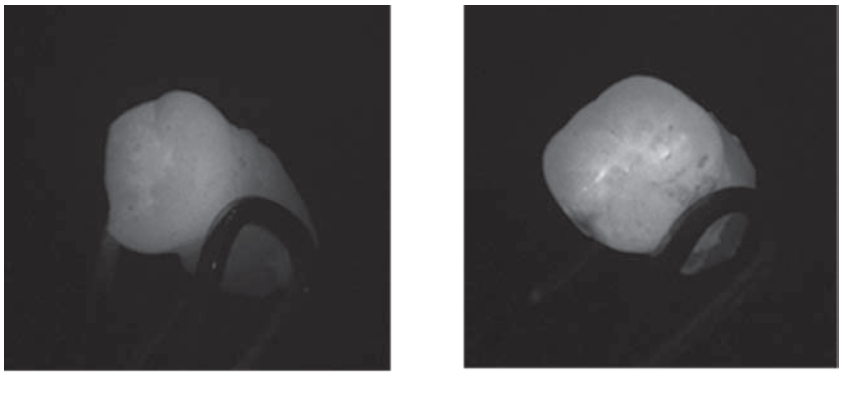


Рис. 13. Спектры флуоресценции nAlPc до и после нанесения модельной смеси с ПАВ и коллоида nAlPc на поверхность эмали зубов (экспозиция 3 мин)
Fig. 13. nAlPc fluorescence spectra before and after the application of the model compound with surfactant and nAlPc colloid on the tooth enamel surface (3 min exposition)



a b

Рис. 14. Видео-флуоресцентные изображения поверхности эмали зубов:
а – до нанесения модельной смеси;
б – через 3 мин после нанесения модельной смеси
Fig. 14. Video-fluorescent images of the tooth enamel surface:
a – before the model compound application;
b – 3 min after the model compound application

the colloid, which will reduce the time of the diagnostic process.

For this experimental group, fluorescence amplification factors were calculated for each sample (3 minutes after colloid application), which were used in the further statistical calculations.

The results of a study of the interaction of the model mixture and the surface microflora of tooth enamel

The study examined the interaction of the model mixture (with nAlPc and Protelan) with the surface microflora of tooth enamel. For each sample, the fluorescence enhancement coefficient was calculated as described in the Materials and Methods section. Figure 13 shows an example of enamel autofluorescence spectra before application and fluorescence of nAlPc after application of the model mixture.

Figure 14 shows video fluorescence images of the surface of the tooth enamel before applying the model mixture (*a*) and after 3 minutes (*b*).

Statistical processing of two groups of measurements on human teeth *ex vivo* (with a colloidal solution of nAlPc and a model mixture with nAlPc) using Student's t-test of reliability showed that the calculation results are statistically significant ($p < 0.05$), and the use of Protelan for additional activation of nAlPc is justified.

Conclusions

Experimental studies have shown reliable effectiveness of use of nAlPc in the composition of the model mixture for local fluorescence spectroscopy of the enamel surface to identify microcracks of enamel and potential foci of pathogenic microflora accumulation.

Based on the analysis of the results of studying the interaction of the nAlPc colloid with various surfactants, we can conclude that in the initial time period, it is the smallest particles and molecules in the colloid that interact with the surfactant, and after the lapse of time, molecules related to larger particles contribute to the fluorescence intensity.

Spectroscopic studies of the model mixture with nAlPc and Protelan showed the effectiveness of the use of this surfactant for additional activation of nanoparticles, which makes it possible to perform PD on the enamel surface of teeth 3 minutes after application. The addition of Protelane to the model mixture for conducting PD of dental enamel activates no more than 1–5% of nAlPc molecules.

In the future, the combined use of nAlPc with surfactants will increase not only the sensitivity and efficiency of PD of hard tooth tissues, but also the effectiveness of PDT of periodontal tissues.

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REFERENCES

1. Thomas S.S., Mohanty S., Jayanthi J.L., Varughese J.M., Balan A., Subhash N. Clinical trial for detection of dental caries using laser-induced fluorescence ratio reference standard, *J. Biomed. Opt.*, 2013, Vol. 15, No. 2, pp. 1–8.
2. Gonchukov S.A., Sukhinina A.A., Bakhmutov D.N., Biryukova T.B., Tsvetkov M.C., Bagratashvily V.C. Periodontitis diagnostics using resonance Raman spectroscopy on saliva, *Laser Phys. Lett.*, 2013, Vol. 10, No. 7, 075610.
3. Gonchukov S., Sukhinina A., Bakhmutov D., Minaeva S. Raman spectroscopy of saliva as a perspective method for periodontitis diagnostics, *Laser Phys. Lett.*, 2012, Vol. 9, No. 1, pp. 73–77.
4. Ramakrishnaiah R., Rehman G., Basavarajappa S., Alkhuraif A., Durgesh B.H., Khan A.S., Rehman I. Applications of Raman Spectroscopy in Dentistry: Analysis of Tooth Structure, *Appl. Spectrosc. Rev.*, 2014, Vol. 50, No. 4, pp. 332–350.
5. Tsuda H., Arends J. Raman Spectroscopy in Dental Research: A Short Review of Recent Studies, *Adv. Dent. Res.*, 1997,Vol. 11, No. 4, pp. 539–547.
6. Buchwald T., Okulus Z., Szybowicz M. Raman spectroscopy as a tool of early dental caries detection–new insights, *J. Raman Spectrosc.*, 2017, Vol. 48, No. 8, pp. 1094–1102.
7. Hsieh Y.-S., Ho Y.C., Lee S.Y., Chuang C.C., Tsai J.C., Lin K.F., Sun C.W. Dental Optical Coherence Tomography, *Sensors (Basel)*, 2013, Vol. 13, No. 7, pp. 8928–8949.
8. Chen Q.G., Zhu H.H., Xu Y., Lin B., Chen H. Quantitative method to assess caries via fluorescence imaging from the perspective of autofluorescence spectral analysis, *Laser Phys.*, 2015, Vol. 25, No. 8, pp. 1–9.

ЛИТЕРАТУРА

1. Thomas S.S., Mohanty S., Jayanthi J.L., et al. Clinical trial for detection of dental caries using laser-induced fluorescence ratio reference standard // J. Biomed. Opt. – 2013. – Vol. 15, No. 2. – P. 1–8.
2. Gonchukov S.A., Sukhinina A.A., Bakhmutov D.N., et al. Periodontitis diagnostics using resonance Raman spectroscopy on saliva // Laser Phys. Lett. – 2013. – Vol. 10, No. 7. – 075610.
3. Gonchukov S., Sukhinina A., Bakhmutov D., Minaeva S. Raman spectroscopy of saliva as a perspective method for periodontitis diagnostics // Laser Phys. Lett. – 2012. – Vol. 9, No. 1. – P. 73–77.
4. Ramakrishnaiah R., Rehman G., Basavarajappa S., et al. Applications of Raman Spectroscopy in Dentistry: Analysis of Tooth Structure // Appl. Spectrosc. Rev. – 2014. – Vol. 50, No. 4. – P. 332–350.
5. Tsuda H., Arends J. Raman Spectroscopy in Dental Research: A Short Review of Recent Studies // Adv. Dent. Res. – 1997. – Vol. 11, No. 4. – P. 539–547.
6. Buchwald T., Okulus Z., Szybowicz M. Raman spectroscopy as a tool of early dental caries detection–new insights // J. Raman Spectrosc. – 2017. – Vol. 48, No. 8. – P. 1094–1102.
7. Hsieh Y.-S., Ho Y.C., Lee S.Y., et al. Dental Optical Coherence Tomography // Sensors (Basel). – 2013. – Vol. 13, No. 7. – P. 8928–8949.
8. Chen Q.G., Zhu H.H., Xu Y., et al. Quantitative method to assess caries via fluorescence imaging from the perspective of autofluorescence spectral analysis // Laser Phys. – 2015. – Vol. 25, No. 8. – P. 1–9.

9. Buchalla W., Lennon A.M., Attin T. Fluorescence spectroscopy of dental calculus, *J. Periodontal Res*., 2004, Vol. 39, No. 5, pp. 327–332.
10. Bakhmutov D., Gonchukov S., Sukhinina A. Fluorescence spectroscopy of dental calculus, *Laser Phys. Lett*., 2010, Vol. 7, No. 5, pp. 384–387.
11. Bakhmutov D.N., Gonchukov S.A., Kharchenko O., Nikiforova O., Vdovin Yu. Early Dental Caries Detection by Fluorescence Spectroscopy, *Laser Phys. Lett*., 2004, Vol. 1, No. 11, pp. 565–569.
12. Bakhmutov D.N., Gonchukov S.A., Kharchenko O. Early caries naked-eyed examination, *Laser Phys. Lett*., 2008, Vol. 5, No. 5, pp. 375–378.
13. Borisova E.G., Uzunov T.T., Avramov L.A. Early Differentiation between Caries and Tooth Demineralization Using Laser-Induced Autofluorescence Spectroscopy, *Lasers Surg. Med*., 2004, Vol. 34, No. 3, pp. 249–253.
14. Drakaki E., Makropoulou M., Khabbaz M., Serafetinideset A.A. Reflectance, scattering and laser induced fluorescence for the detection of dental caries, *Proc. SPIE*, 2003, 5141_348.
15. Drakaki E.A., Makropoulou M.I., Khabbaz M., Serafetinides A.A. Laser Induced Fluorescence in diagnosis of dental caries, *Proc. SPIE*, 2003, Vol. 5149, pp. 45–52.
16. Sinyaeva M.L., Mamedov A.A., Vasilchenko S.Y., Volkova A.I., Loschenov V.B. Fluorescence Diagnostics in Dentistry, *Laser Phys*., 2004,Vol. 14, No. 8, pp. 1132–1140.
17. Schoenly J.E., Seka W., Featherstone J.D., Rechmann P. Near-UV laser treatment of extrinsic dental enamel stains, *Lasers Surg. Med*., 2012, Vol. 44, No. 4, pp. 339–345.
18. Sinyaeva M.L., Vasil'chenko S.Yu., Volkova A.I., Korovin S.B., Mamedov Ad.A., Kuz'min S.G., Luk'yanets E.A., Loshchenov V.B., Konov V.I. Use of aluminum phthalocyanine nanoparticles for tooth enamel microdamage detection, Rossiyskie nanotekhnologii, 2007, Vol. 2, No. 11–12. pp. 58–63. (in Russian)
19. Steiner R., Breymayer J., Rueck A., Loschenov V.B., Ryabova A.V. Crystalline organic nanoparticles for diagnosis and PDT, *Proc. SPIE*, 2015, Vol. 9308, pp. 1–7.
20. Breymayer J., Rück A., Ryabova A.V., Loschenov V.B., Steiner R.W..Fluorescence investigation of the detachment of aluminum phthalocyanine molecules from aluminum phthalocyanine nanoparticles in monocytes/macrophages and skin cells and their localization in monocytes/macrophages, *Photodiagnosis Photodyn. Ther*., 2014,Vol. 11, No. 3, pp. 380–390.
21. Vasilchenko S.Y. Volkova A.I., Ryabova A.V., Loschenov V.B., Konov V.I., Mamedov A.A., Kuzmin S.G., Lukyanets E.A. Application of aluminum phthalocyanine nanoparticles for fluorescent diagnostics in dentistry and skin autotransplantology, *J. Biophotonics*, 2010, Vol. 3, No. 5–6, pp. 336–346.
22. Kuznetsova J.O., Makarov V.I. Application of nanophotosensitizers (aluminum phthalocyanine nanoparticles) for early diagnosis and prevention of inflammatory diseases, *J. Phys. Conf. Ser*., 2016, Vol. 737, No. 1, pp. 1–3.
23. Kuznetsova J.O., Farrakhova D.S., Yassin M.G. Aluminum phthalocyanine nanoparticles as a contrast agent for the detection of tooth enamel microcracks, *Photon Lasers Med*., 2016, Vol. 5, No. 4, pp. 267–322.
24. Bystrov F.G., Makarov V.I., Pominova D.V., Ryabova A.V., Loshchenov V.B. Analysis of photoluminescence decay kinetics of aluminum phthalocyanine nanoparticles interacting with immune cells, *Biomedical Photonics*, 2016, Vol. 5, No. 1, pp. 3–8. (in Russian)
25. Sinyaeva M.L., Panchenko V.Y., Sabotinov N.V., Mamedov A.A., Lervkin V.V., Kharnas S.S., Volkova A.I., Loschenov V.B., Berezin A.N., Kiselev G.L. Optimization of parodontium tissue irradiation method for fluorescent diagnostic (FD) and photodynamic therapy (PDT), *Proc. SPIE*, 2004, Vol. 5449, pp. 462–465.

9. Buchalla W., Lennon A.M., Attin T. Fluorescence spectroscopy of dental calculus // J. Periodontal Res. – 2004. – Vol. 39, No. 5. – P. 327–332.
10. Bakhmutov D., Gonchukov S., Sukhinina A. Fluorescence spectroscopy of dental calculus // Laser Phys. Lett. – 2010. – Vol. 7, No. 5. – P. 384–387.
11. Bakhmutov D.N., Gonchukov S.A., Kharchenko O., et al. Early Dental Caries Detection by Fluorescence Spectroscopy // Laser Phys. Lett. – 2004. – Vol. 1, No. 11. – P. 565–569.
12. Bakhmutov D.N., Gonchukov S.A., Kharchenko O. Early caries naked-eyed examination // Laser Phys. Lett. – 2008. – Vol. 5, No. 5. – P. 375–378.
13. Borisova E.G., Uzunov T.T., Avramov L.A. Early Differentiation between Caries and Tooth Demineralization Using Laser-Induced Autofluorescence Spectroscopy // Lasers Surg. Med. – 2004. – Vol. 34, No. 3. – P. 249–253.
14. Drakaki E., Makropoulou M., Khabbaz M., Serafetinideset A.A. Reflectance, scattering and laser induced fluorescence for the detection of dental caries // Proc. SPIE. – 2003. – 5141_348.
15. Drakaki E.A., Makropoulou M.I., Khabbaz M., Serafetinides A.A. Laser Induced Fluorescence in diagnosis of dental caries // Proc. SPIE. – 2003. – Vol. 5149. – P. 45–52.
16. Sinyaeva M.L., Mamedov A.A., Vasilchenko S.Y., et al. Fluorescence Diagnostics in Dentistry // Laser Phys. – 2004. – Vol. 14, No. 8. – P. 1132–1140.
17. Schoenly J.E., Seka W., Featherstone J.D., Rechmann P. Near-UV laser treatment of extrinsic dental enamel stains // Lasers Surg. Med. – 2012. – Vol. 44, No. 4. – P. 339–345.
18. Синяева М.Л., Васильченко С.Ю., Волкова А.И. и др. Использование наночастиц фталоцианина алюминия для детектирования микроповреждений эмали зубов // Российские нанотехнологии. – 2007. – Т. 2, № 11–12. – С. 58–63.
19. Steiner R., Breymayer J., Rueck A., et al. Crystalline organic nanoparticles for diagnosis and PDT // Proc. SPIE. – 2015. – Vol. 9308. – P. 1–7.
20. Breymayer J., Rück A., Ryabova A.V., et al. Fluorescence investigation of the detachment of aluminum phthalocyanine molecules from aluminum phthalocyanine nanoparticles in monocytes/macrophages and skin cells and their localization in monocytes/macrophages // Photodiagnosis Photodyn. Ther. – 2014. – Vol. 11, No. 3. – P. 380–390.
21. Vasilchenko S.Y. Volkova A.I., Ryabova A.V., et al. Application of aluminum phthalocyanine nanoparticles for fluorescent diagnostics in dentistry and skin autotransplantology // J. Biophotonics. – 2010. – Vol. 3, No. 5–6. – P. 336–346.
22. Kuznetsova J.O., Makarov V.I. Application of nanophotosensitizers (aluminum phthalocyanine nanoparticles) for early diagnosis and prevention of inflammatory diseases // J. Phys. Conf. Ser. – 2016. – Vol. 737, No. 1. – P. 1–3.
23. Kuznetsova J.O., Farrakhova D.S., Yassin M.G. Aluminum phthalocyanine nanoparticles as a contrast agent for the detection of tooth enamel microcracks // Photon Lasers Med. – 2016. – Vol. 5, No. 4. – P. 267–322.
24. Быстров Ф.Г., Макаров В.И., Поминова Д.В. и др. Исследование кинетики затухания фотолюминесценции молекулярных нанокристаллов фталоцианина алюминия при взаимодействии с иммунокомпетентными клетками // Biomed. Photonics. – 2016. – Т. 5, № 1. – P. 3–8.
25. Sinyaeva M.L., Panchenko V.Y., Sabotinov N.V., et al. Optimization of parodontium tissue irradiation method for fluorescent diagnostic (FD) and photodynamic therapy (PDT) // Proc. SPIE. – 2004. – Vol. 5449. – P. 462–465.
26. Васильченко С.Ю., Волкова А.И., Коровин С.Б. и др. Исследование флюоресцентных свойств наночастиц фталоцианина алюминия в микроповреждениях эмали зуба // Рос. биотер. журн. – 2006. – Т. 5, № 2. – С. 77–80.

26. Vasil'chenko S.Yu., Volkova A.I., Korovin S.B., Loshchenov V.B., Sinyaeva M.L., Mamedov Ad.A., Luk'yanets E.A., Kuz'min S.G. Investigation of aluminum phthalocyanine nanoparticles fluorescent properties in tooth enamel microdamage, *Ros. bioter. zhurn.*, 2006, Vol. 5, No. 2, pp. 77–80. (in Russian)
27. Dobson J., Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser, *Arch. Oral Biol.*, 1992, Vol. 37, No. 11, pp. 883–887.
28. Lacey J.A., Phillips D. The photobleaching of disulfonated aluminium phthalocyanine in microbial systems, *Photochem. Photobiol. Sci.*, 2002, Vol. 1, No. 2, pp. 120–125.
29. Lacey J.A., Phillips D. Fluorescence lifetime measurements of disulfonated aluminium phthalocyanine in the presence of microbial cells, *Photochem. Photobiol. Sci.*, 2002, Vol. 1, No. 6, pp. 378–383.
30. Wilson M., Dobson J., Sarkar S. Sensitization of periodontopathogenic bacteria to killing by light from a low-power laser, *Oral Microbiol. Immunology*, 1993, No. 8, pp. 182–187.
31. Carrera E.T., Dias H.B., Corbi S.C.T., Marcantonio R.A.C., Bernardi A.C.A., Bagnato V.S., Hamblin M.R., Rastelli A.N.S. The application of antimicrobial photodynamic therapy (aPDT) in dentistry: a critical review, *Laser Phys.*, 2016, Vol. 26, No. 12. doi: 10.1088/1054–660X/26/12/123001
32. Yin R., Hamblin M.R. Antimicrobial Photosensitizers: Drug Discovery Under the Spotlight, *Curr. Med. Chem.*, 2015, Vol. 22, No. 18, pp. 2159–2185.
33. Carmello J.C., Alves F., Ribeiro A., Basso F.G., de Souza Costa C.A., Tedesco A.C., Primo F.L., Mima E.G., Pavarina A.C. In vivo photodynamic inactivation of Candida albicans using chloro-aluminum phthalocyanine, *Oral Dis.*, 2016, Vol. 22, No. 5, pp. 415–422.
34. Ribeiro A.P., Andrade M.C., Bagnato V.S., Vergani C.E., Primo F.L., Tedesco A.C., Pavarina A.C. Antimicrobial photodynamic therapy against pathogenic bacterial suspensions and biofilms using chloro-aluminum phthalocyanine encapsulated in nanoemulsions, *Lasers Med. Sci.*, 2015, Vol. 30, No. 2, pp. 549–559.
35. Ragelle H., Crauste-Manciet S., Seguin J., Brossard D., Scherman D., Arnaud P., Chabot G.G. Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice, *Int. J. Pharm.*, 2012, Vol. 427, No. 2, pp. 452–459.
36. Zhang H., Yao M., Morrison R.A., Chong S. Commonly used surfactant, Tween 80, improves absorption of P-glycoprotein substrate, digoxin, in rats, *Arch Pharm Res*, 2015, Vol. 26, No. 9, pp. 768–772.
37. Natarajan J., Baskaran M., Humtsoe L.C., Vadivelan R., Justin A. Enhanced brain targeting efficacy of Olanzapine through solid lipid nanoparticles, *Artif. Cells. Nanomedicine. Biotechnol.*, 2016, Vol. 45, No. 2, pp. 364–371.
38. Salaguer J.-L. *Surfactants Types and Uses*. Venezuela, Laboratorio FIRP, 2002. 49 p.
39. Kholmberg K., Yensson B., Kronberg B., Lindman B. *Poverkhnostno-aktivnye veshchestva i polimery v vodnykh rastvorakh* [Surfactants and polymers in aqueous solutions]. Moscow, BINOM, Laboratoriya znaniy Publ., 2013. 513 p.
40. Savvin S.B. *Poverkhnostno-aktivnye veshchestva* [Surfactants]. Moscow, Nauka Publ., 1991. 251 p.
41. Asem H., El-Fattah A.A., Nafee N., Zhao Y., Khalil L., Muhammed M., Hassan M., Kandil S. Development and biodistribution of a theranostic aluminum phthalocyanine nanophotosensitizer, *Photodiagnosis Photodyn. Ther.*, 2016, Vol. 13, pp. 48–57.
42. Loschenov V., Konov V., Prokhorov A. Photodynamic therapy and fluorescence diagnostics, *Laser Phys.*, 2000, Vol. 10, No. 6, pp. 1188–1207.
43. Ivanov V.L., Lyashkevich S.Yu. Influence of surfactants on the chain reaction of halogen photosubstitution by a sulfonyl hydroxide group in halogenhydroxynaphthalenes, *Khimiya*
27. Dobson J., Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser // Arch. Oral Biol. – 1992. – Vol. 37, No. 11. – P. 883–887.
28. Lacey J.A., Phillips D. The photobleaching of disulfonated aluminium phthalocyanine in microbial systems // Photochem. Photobiol. Sci. – 2002. – Vol. 1, No. 2. – P. 120–125.
29. Lacey J.A., Phillips D. Fluorescence lifetime measurements of disulfonated aluminium phthalocyanine in the presence of microbial cells // Photochem. Photobiol. Sci. 2002. – Vol. 1, No. 6. – P. 378–383.
30. Wilson M., Dobson J., Sarkar S. Sensitization of periodontopathogenic bacteria to killing by light from a low-power laser // Oral Microbiol. Immunology. – 1993. – No. 8. – P. 182–187.
31. Carrera E.T., Dias H.B., Corbi S.C.T., et al. The application of antimicrobial photodynamic therapy (aPDT) in dentistry: a critical review // Laser Phys. – 2016. – Vol. 26, No. 12. doi: 10.1088/1054–660X/26/12/123001
32. Yin R., Hamblin M.R. Antimicrobial Photosensitizers: Drug Discovery Under the Spotlight // Curr. Med. Chem. – 2015. – Vol. 22, No. 18. – P. 2159–2185.
33. Carmello J.C., Alves F., Ribeiro A., et al. In vivo photodynamic inactivation of Candida albicans using chloro-aluminum phthalocyanine // Oral Dis. – 2016. – Vol. 22, No. 5. – P. 415–422.
34. Ribeiro A.P., Andrade M.C., Bagnato V.S., et al. Antimicrobial photodynamic therapy against pathogenic bacterial suspensions and biofilms using chloro-aluminum phthalocyanine encapsulated in nanoemulsions // Lasers Med. Sci. – 2015. – Vol. 30, No. 2. – P. 549–559.
35. Ragelle H., Crauste-Manciet S., Seguin J., et al. Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice // Int. J. Pharm. – 2012. – Vol. 427, No. 2. – P. 452–459.
36. Zhang H., Yao M., Morrison R.A., Chong S. Commonly used surfactant, Tween 80, improves absorption of P-glycoprotein substrate, digoxin, in rats // Arch Pharm Res. – 2015. – Vol. 26, No. 9. – P. 768–772.
37. Natarajan J., Baskaran M., Humtsoe L.C., et al. Enhanced brain targeting efficacy of Olanzapine through solid lipid nanoparticles // Artif. Cells. Nanomedicine. Biotechnol. – 2016. – Vol. 45, No. 2. – P. 364–371.
38. Salaguer J.-L. Surfactants Types and Uses. – Venezuela: Laboratorio FIRP, 2002. – 49 p.
39. Холмберг К., Йёнссон Б., Кронберг Б., Линдман Б. Поверхностно-активные вещества и полимеры в водных растворах. – Москва: БИНОМ. Лаборатория знаний, 2013. – 513 с.
40. Саввин С.Б. Поверхностно-активные вещества. – М.: Наука, 1991. – 251 с.
41. Asem H., El-Fattah A.A., Nafee N., et al. Development and biodistribution of a theranostic aluminum phthalocyanine nanophotosensitizer // Photodiagnosis Photodyn. Ther. – 2016. – Vol. 13. – P. 48–57.
42. Loschenov V., Konov V., Prokhorov A. Photodynamic therapy and fluorescence diagnostics // Laser Phys. – 2000. – Vol. 10, No. 6. – P. 1188–1207.
43. Иванов В.Л., Ляшкевич С.Ю. Влияние поверхностно-активных веществ на цепную реакцию фотозамещения галогена сульфогруппой в галогенгидроксинафталинах // Химия высоких энергий. – 2013. – Т. 47, No. 4. – С. 293–297.
44. Silva E.P.O., Franchi L.P., Tedescoa A.C. Chloro-aluminium phthalocyanine loaded in ultradeformable liposome for photobiology studies on human glioblastoma // RSC Adv. – 2016. – Vol. 83. – P. 1–10.
45. Muehlmann L.A., Ma B.C., Longo J.P., et al. Aluminum-phthalocyanine chloride associated to poly(methyl vinyl ether-

vysokikh energiy, 2013, Vol. 47, No. 4, pp. 293–297. (in Russian)
44. Silva E.P.O., Franchi L.P., Tedescoa A.C. Chloro-aluminium phthalocyanine loaded in ultradeformable liposome for photobiology studies on human glioblastoma, *RSC Adv.*, 2016, Vol. 83, pp. 1–10.
45. Muehlmann L.A., Ma B.C., Longo J.P., Almeida Santos Mde F., Azevedo R.B. Aluminum-phthalocyanine chloride associated to poly(methyl vinyl ether-co-maleic anhydride) nanoparticles as a new third-generation photosensitizer for anticancer photodynamic therapy, *Int. J. Nanomedicine*, 2014, Vol. 9, pp. 1199–1213.
46. Cath T.Y., Adams D., Childress A.E. Membrane contactor processes for wastewater reclamation in space: II. Combined direct osmosis, osmotic distillation, and membrane distillation for treatment of metabolic wastewater, *J. Memb. Sci.*, 2005, Vol. 257, No. 1–2, pp. 111–119.

co-maleic anhydride) nanoparticles as a new third-generation photosensitizer for anticancer photodynamic therapy // Int. J. Nanomedicine. – 2014. – Vol. 9. – P. 1199–1213.
46. Cath T.Y., Adams D., Childress A.E. Membrane contactor processes for wastewater reclamation in space: II. Combined direct osmosis, osmotic distillation, and membrane distillation for treatment of metabolic wastewater // J. Memb. Sci. – 2005. – Vol. 257, No. 1–2. – P. 111–119.