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The object of this study is the processes of interaction of fragments of purple membranes with certain chemicals, which affect the photochromic properties of bacteriorhodopsin films. This paper considers the possibility of modifying the properties of bacteriorhodopsin films by changing their chemical composition, which will make it possible to use such films to protect information. For this purpose, it has been determined which optical properties of bacteriorhodopsin are most important for its use in the field of information protection; films were made using various chemicals as impurities. It was shown that the introduction of impurities does not affect the absorption spectrum of bacteriorhodopsin, which indicates that it retains its properties. It has been established that impurity substances are evenly distributed in films. By studying the dynamics of photoinduced changes in films with different concentrations of chemical components, the effect of chemical modification on the photocycle of bacteriorhodopsin and the properties of film structures based on it was studied.

Based on the results of experimental studies, it was found that by changing the chemical composition of films, it is possible to change the time of storage of the information recorded on them in the range from several to hundreds of seconds continuously. The sensitometric sensitivity of film structures also depends on the chemical composition and can vary in the range from $3.9 \cdot 10^{-3}$ to $54 \cdot 10^{-3} \text{ m}^2/\text{J}$. At the same time, chemical modification is simpler from a technological point and cheaper from a financial point of view compared to other methods that make it possible to achieve similar results. The reported results suggest that chemically modified films based on bacteriorhodopsin have great potential for practical application in the field of information protection and access control

Keywords: bacteriorhodopsin, photocycle, film structures, photosensitivity, chemical modification, triethanolamine, information protection

DESIGN OF CHEMICALLY MODIFIED BACTERIORHODOPSIN FILMS FOR INFORMATION PROTECTION SYSTEMS

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1. Introduction

In materials science, the effectiveness of rethinking the properties and role of materials of biological origin in solving the problems of modern science and technology is becoming more and more clearly manifested. Bacteriorhodopsin (BR), one of the transmembrane protein complexes of halophilic microorganisms, can rightly be considered a pioneer and the most prominent representative of this trend. The possibilities of applied use of BR are impressive: optical recording with ultrahigh densities [1], switching of optical signals [2], photoelectric immunosensors [3], nonlinear optoelectronic elements [4], nanofluidics [5, 6], alternative energy [7–9], information protection [10], etc. The advantages of BR-based materials include high resolution and photosensitivity, almost unlimited aperture and reversibility, significant photochromic displacement and percentage of color change, long service life. All this allows it to compete with modern

materials used for optical information processing, renewable energy, and biosensorics. At the same time, since BR is produced by microorganisms in the process of their natural vital activity, the technology of its production is environmentally friendly, and the resources are practically inexhaustible.

In the natural environment, BR is embedded in the cell membrane of *Halobacterium salinarum* halobacterium. Areas of the membrane that contain only BR and lipids are called purple membranes (PM). Such areas of the membrane can be isolated while maintaining functional properties. Due to the crystalline structural organization and the fact that the bulk of the protein molecule is immersed in the lipid membrane, PM demonstrates high resistance to thermal and chemical denaturation. However, the uniqueness of the characteristics of PM-based materials is associated with the optical properties of BR. The absorption of the radiation quantum leads to the excitation of the molecule, after which, over a time of about 10 ms, it passes through a series of in-

intermediate states, called intermediates, and returns to its ground state. This cyclic reaction is called a photocycle. The energy of the absorbed quantum is spent on the transmembrane transfer of the proton. Intermediates are characterized by different lifetimes and highs of absorption. The scheme of the classical model of the photocycle, which contains the main intermediates and takes place under natural conditions, is shown in Fig. 1, *a*. The names of intermediate states are indicated in Latin letters and the indices indicate the corresponding maximums of absorption. Under certain conditions, the photocycle can take place in a different way from the natural path. For example, for long-term optical recording of information, the so-called branched photocycle is promising, in which, due to the additional optical excitation of the O_{640} intermediate, 9-*cis* isomerization of the retinal is achieved. In this case, the molecule can return to its ground state only with additional irradiation, and not thermally (Fig. 1, *b*). When BR molecules are irradiated with a powerful laser beam, direct two-photon absorption occurs, as a result of which the molecules pass into states that are not photochemically active and do not return to the original thermally. This results in a thermostable shift of the maximum absorption from the original magenta to the light blue color. In addition, as a result of this process, there is a constant local change in the polarization properties of BR. This effect can be used for long-term recording of information, with the possibility of encryption. A schematic image of such a process is shown in Fig. 1, *c*.

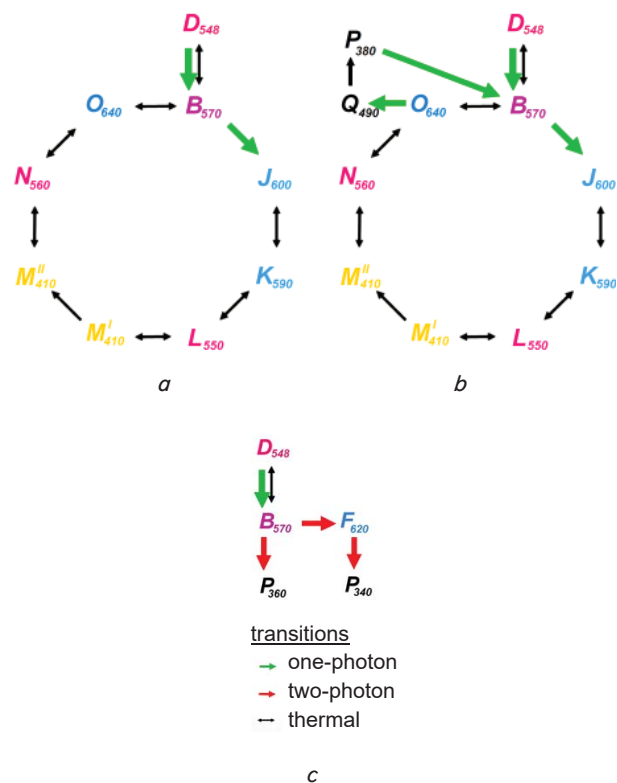


Fig. 1. Photoinduced changes in the bacteriorhodopsin molecule: *a* – classic photocycle; *b* – branched model with photoinduced 9-*cis* isomerization of retinal containing P- and Q-states; *c* – changes that occur during two-photon absorption [11, 12]

In addition to obtaining bacteriorhodopsin, halophilic microorganisms are interesting from a practical point of view for wastewater treatment [13, 14] and the production of biodegradable plastics and emulsifiers [15]. The most promising direction of using BR at present is the development of materials for optical processing and data storage [1]. At the same time, this material at the molecular level has a number of properties that can be effectively used in security, encryption, and information protection systems [10]. The technology of obtaining the material itself is environmentally friendly while the resources are practically inexhaustible. All these factors indicate the relevance of research on bacteriorhodopsin and materials based on it from both a fundamental and applied point of view.

2. Literature review and problem statement

Paper [10] systematized possible options for the use of bacteriorhodopsin for information security systems. It is shown that there are three main areas of use: optical recording of information with high densities and simultaneous encryption, the development of photochromic ink, and the possibility of molecular tracking of the material. However, the work does not note the problems that arise at the stage of practical use. The cause of the problematic issues are the features of the structure and functioning of the bacteriorhodopsin molecule, which in some cases must be adapted for specific tasks. Thus, in order to obtain a photochromic material on the basis of BR, which visually changes its color when illuminated by household light sources, it is necessary to increase the thermal lifetime of the M_{412} intermediate by several orders of magnitude. It should be noted that modification is not always needed. There are options for application use where the material almost perfectly meets all the requirements.

Thus, in [12], it is shown that the use of BR for recording and encrypting information, both due to changes in optical density and when using polarization recording, practically does not require any additional modification of the material. When illuminated by high-intensity light sources, photosensitivity is not a critical parameter, reversibility is not required in this case. For effective use, it is enough to obtain a high-quality uniform film of specified sizes.

Paper [16] investigated the possibility of tracking different batches of documents or goods through the use of dyes based on BR with a modified amino acid sequence. It is shown that the use of mass spectrometric analysis of ink makes it possible to clearly identify its belonging to a particular batch. The disadvantages of this method include the complexity of the amino acid sequence modification process and the need to use expensive mass spectrometric analysis to identify modified BR molecules. An option to overcome the corresponding difficulties may be the use of chemical impurities. In this case, the identifier, instead of BR molecules with a modified amino acid sequence, can be the substances that make up the film, and information about their exact mass ratios. At the same time, chemical modification can be used to increase the photosensitivity of the films. The introduction of impurities is much simpler from a technological point of view, this technique provides for the possibility of manufacturing films with different properties based on BR, obtained using optimized and well-developed classical meth-

ods, which will reduce total costs. In addition, more common and cheaper procedures such as raster electron microscopy can be used to detect certain specific impurities.

The possibility of manufacturing photochromic ink based on BR for protective marking and producing photochromic areas on access cards or passes is described in [16]. It is shown that for effective practical use in this direction it is necessary to be able to adjust the lifetime of the M_{412} intermediate and the photosensitivity of the material. The first will make it possible to visually observe the change in the color of the protective marking and save this change for a specified period of time, the second – to achieve visible changes even at household lighting levels. Photoinduced color change occurs due to the accumulation of the intermediate M_{412} . Under natural conditions, this intermediate has a lifetime of only a few milliseconds, and its population cannot be detected with the naked eye even with intense light. To obtain a photochromic material based on BR, which visually changes its color when illuminated by conventional light sources, it is necessary to increase the thermal lifetime of the M_{412} intermediate by several orders of magnitude. This can be achieved in different ways: by changing the humidity, pH, temperature, chemical composition of the medium around the PM or by replacing the chromophore with an analog. However, not all of the described methods are quite suitable for scientific research, can be effective for applied use.

The possibilities of using mutant variants of the BR molecule to change the parameters of the photocycle are described in [17]. It has been shown that the replacement of key amino acid residues can lead to an increase in the reprotonation time of the M-state and an increase in the photosensitivity of the material. However, although genetic modification procedures are well studied and worked out, they are still quite complex and involve obtaining fixed changes in the passage of the BR photocycle for each specific type of mutation. At the same time, in practice, to solve various problems, it is more convenient to be able to continuously change the parameters of the material in a certain range.

The use of retinal analogs to change the parameters of the photocycle, that is, photosensitivity and time characteristics of photochromic changes in materials based on BR, is described in [18]. The use of this technique makes it possible to change the position of the absorption bands of the ground state and intermediates, which may be of considerable interest for the manufacture of photochromic inks. In addition, the replacement of the chromophore can significantly affect the passage time of the photocycle. However, as in the previous case, the technology is quite complex, and the resulting changes are fixed for each retinal substitute.

The procedure of creating BR films with a maximum lifetime in the state of M_{412} using photoinduced hydroxylaminolysis is described in [19]. The disadvantage of this method is the practical loss of photochromicity in such film structures. They are suitable for archiving information, but the lack of reversibility greatly limits the use in the field of information protection. In addition, the authors note that the photosensitivity of BR films with the addition of hydroxylamine decreases significantly over time after the film is made.

An option to overcome difficulties may be the use of chemical modification of BR films. This technique has several advantages while it can significantly affect their optical properties and the dynamics of photoinduced changes. First,

the introduction of impurities is much easier from a technological point of view. Second, different types of impurities can affect the specified properties of the films to varying degrees. Third, this modification procedure provides for the possibility of manufacturing films with different properties based on BR, obtained using optimized and well-developed classical methods, which will reduce the total costs.

Summing up the facts above, we can conclude that chemical modification has a number of significant advantages and can be effectively used to expand the scope of practical use of film structures based on BR. Therefore, the search for new substances that can affect the BR photocycle and the study of the properties of chemically modified film structures is of considerable scientific and applied interest.

3. The aim and objectives of the study

The aim of this study is to identify patterns of change in the lifetime of the M_{412} intermediate and the photosensitivity of BR films under the influence of chemical impurities. This will provide an opportunity to expand the possibilities of their use for information security systems.

To accomplish the aim, the following tasks have been set:

- to obtain chemically modified BR films and show that BR retains its spectral properties in such structures;
- to assess the uniform distribution of impurities in the obtained film structures;
- to study the dependence of the properties of films on their chemical composition by determining optical parameters and studying the dynamics of photoinduced changes.

4. The study materials and methods

The object of this study is the processes of interaction of BR molecules, in the form of fragments of purple membranes, with selected chemicals, which are reflected in the photochromic properties of film structures based on bacteriorhodopsin. The main hypothesis of the study assumes that by changing the chemical composition of the film, it is possible, in the right direction, to change the optical properties of the films, which will expand the range of their applied use.

Unlike the suspension of purple membranes where the number of water molecules necessary for conformational transformations of BR is present, in film structures dehydration limits the movement of individual groups of the BR molecule. The photocycle BR is somewhat simplified, the molecule under the action of the absorbed quantum of light passes from the ground state of BR_{570} , through the high-speed intermediates K, L in M_{412} , and, bypassing the intermediates N and O, returns to its original state. Transition $BR_{570} \rightarrow M_{412}$ is the most important since it is at this stage that the proton is separated from the Schiff base, which leads to significant changes in the optical properties of the film. Therefore, the optical properties of BR film structures were determined at 570 and 412 nm.

For research, we used BR obtained according to the standard methods described in [2]. Substances used to modify the properties of BR were selected according to a number of criteria. One of them is solubility in water since BR films are obtained from aqueous suspensions. The condition for the successful use of a substance as an impurity

or matrix material is its transparency in the region of 350–750 nm. The main function of BR is transmembrane proton transfer. Accordingly, substances that, due to the electrical capacity of their functional groups or atoms, can contribute to the process of deprotonation of the Schiff base and/or retain free protons, will effectively influence the course of the photocycle.

Guided by the requirements for the chemical modification of BR films, the following substances were used: triethanolamine (TEA), L-arginine hydrochloride (AGH), glutaraldehyde (GA), hexamethylenediamine hydrochloride (HMDAGH), ethyleneaminobromide hydrobromide (EABHB), and sodium dodecyl sulfate (SDS). The concentration of solutions used in the manufacture of films based on BR, was, for TEA, 0.4 M, and for all other substances – 0.01 M.

Film structures were obtained from PM suspensions with an optical density in the range of 30–35 (the concentration of PM was $(12.4\text{--}14.5\text{ kg/m}^3)$). Immediately before use, for the purpose of thorough mixing, the PM suspension was subjected to ultrasonic processing using a TESLA installation for 5 minutes. Gelatin was used as a matrix. Gelatin is easily mixed with an aqueous suspension of PM and with solutions of sensitizing substances, it forms a stable film-forming mixture that does not separate over time and, drying on a glass substrate, forms a homogeneous film. An aqueous solution of gelatin (6–10 %) was obtained by dissolving dry powder of the SIGMA brand in distilled water with a temperature of 333 K, with continuous stirring for 20 minutes. Gelatin pre-swelled in distilled water at room temperature for 30 minutes. Spectral analysis of gelatin and related substances used for chemical modification of the properties of BR films showed that they do not have absorption bands in the region of 350–750 nm.

Mixing of PM suspension, gelatin solution, and sensitizing agents in the appropriate ratio was carried out for 15–20 minutes on a magnetic agitator. The process was carried out in a desiccator in a double-walled dish, the design of which ensured the circulation of water thermostatic at 305 K. To remove air from the film-forming mixture, air was pumped out of the desiccator (until the moment of intensive appearance of foam on the surface of the mixture). The mixture prepared in this way in the amount of 0.2 milliliters was applied to a cleaned glass substrate with an area of about 5 cm^2 ($23\times 22\text{ mm}$) by irrigation and dried in a desiccator at room temperature.

To assess the optical characteristics of the films, an installation based on the SF-26 spectrophotometer was used. All studies were carried out in the ditch compartment of the spectrophotometer, which made it possible to ensure the accuracy corresponding to the parameters of the device. The introduction of external radiation into the ditch compartment was carried out using fiber optic. To irradiate the sample, in the study of the dynamics of photoinduced changes, a KGM lamp (power, 250 W, 24 V; a power density of 10.8 mW/cm^2) and spectral filters SEV-21 and OS-12 were used. Installation diagram is shown in Fig. 2.

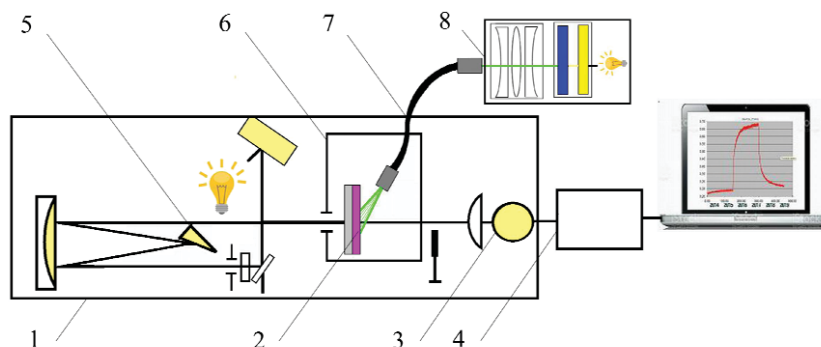


Fig. 2. Installation for studying the dynamics of photoinduced changes in bacteriorhodopsin films: 1 – spectrophotometer; 2 – sample; 3 – photodetector; 4 – analog-to-digital converter; 5 – diffraction lattice; 6 – ditch compartment; 7 – fiber optic harness; 8 – outer lighting unit

The analog signal from the photodetector of the spectrophotometer through an analog-to-digital converter was transmitted to the computer that registered and subsequently processed the results. All studies of film structures were carried out at room temperature and relative humidity of ~50 %. The films selected for research were kept for an hour in a darkened state.

The study of the transmission spectra of films was carried out according to the instructions of the spectrophotometer. The process of researching the dynamics of photoinduced changes involved three stages. At the first stage, the transmission of the darkened sample at the appropriate wavelength is monitored. At the second stage, to start the photocycle in the film, the external backlight is turned on and photoinduced transmission changes are recorded. At the last stage, the change in bandwidth is monitored after turning off the external light. All stages last 150 seconds. An example of the obtained curves, which reflect a photoinduced change in film transmission at 412 and 570 nm during the study is shown in Fig. 3.

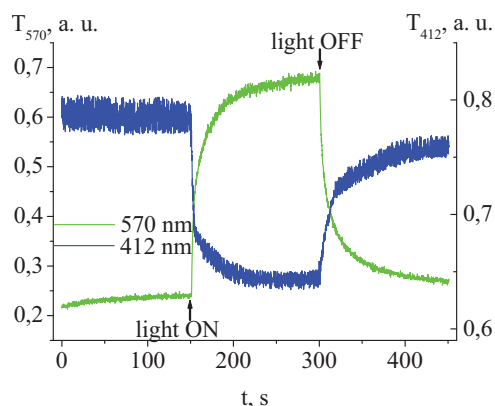


Fig. 3. Dynamics of photoinduced changes in the transmission of bacteriorhodopsin film mixed with triethanolamine at 570 and 412 nm

The measured values of the transmission coefficients determined the photoinduced changes in the optical density (ΔD_{570} , ΔD_{412}) of the film at the corresponding wavelength after 150 seconds of illumination according to the formula:

$$\Delta D = \log\left(\frac{T_0}{T_{150}}\right), \quad (1)$$

where T_0 is the transmission coefficient to lighting, T_{150} is the transmission coefficient 150 seconds after turning on the light.

We determined the monochromatic sensitometric sensitivity (S_{570} , S_{412}) according to the formula:

$$S = \frac{0.8}{P \cdot t}, \quad (2)$$

where P is the density of the irradiation power, t is the time required to change the optical density of the film by 0.1 under the action of lighting.

The coefficient of participation of molecules in the photocycle is estimated by the formula:

$$k = \frac{D_{\max}}{D_{\min}}, \quad (3)$$

where $D_{\max} = \log\frac{1}{T_{\min}}$, $D_{\min} = \log\frac{1}{T_{\max}}$ before and after 150 seconds of lighting.

The half-period of decay of the intermediate $M_{412} - \tau_{1/2}$ was defined as the time required to change the transmission coefficient by 50 % of the magnitude of the photoinduced changes after turning off the ambient lighting.

The optical parameters of BR films significantly depend on the concentration and ratio of impurities. Therefore, in each individual case, both for individual impurities and for their combinations, the composition of the film was optimized. The chemical composition of the film was considered optimal, in which clear highs were observed on the dependences of S , ΔD , and $\tau_{1/2}$ on the concentration of impurities.

5. Results of investigating the properties of chemically modified films of bacteriorhodopsin

5.1. Results of investigating the spectral characteristics of bacteriorhodopsin films of different chemical composition

The conditions for passing the BR photocycle in suspensions and in film structures are somewhat different. This is due to a number of factors such as a decrease in the hydration of certain amino acid residues, an alteration in the mobility of some parts of the amino acid chain due to the influence of the matrix, etc. Certain chemicals that do not result in the loss of the photocycle in suspension can lead to irreversible loss of photochromicity in the films obtained from such suspensions. On the transmission spectra of such films, the BR absorption band in the region of 570 nm disappears, which indicates a retinal separation and loss of photochrome.

In order to make sure that after the films have dried, bacteriorhodopsin has not lost its properties, a study of the spectral characteristics of the obtained film structures with an optimal content of various impurities was carried out. The results of the research are shown in Fig. 4.

As can be seen from the results of the study of spectral characteristics (Fig. 4), the absorption spectra of chemically modified films practically do not differ from the absorption spectrum of the film without impurities. The absence of

changes in the spectral characteristics of films with impurities is one of the proofs that BR in such structures retains its properties.

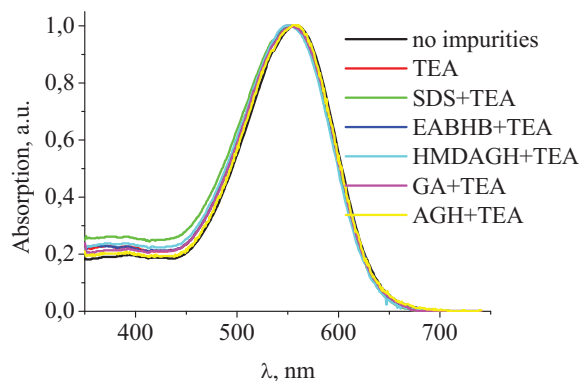


Fig. 4. Spectra of absorption of bacteriorhodopsin films in gelatin matrices without impurities and with optimal concentrations of various chemicals

5.2. Results of investigating the uniform distribution of impurities in the obtained film structures

The introduction of photosensitizing impurities into the composition of films based on BR poses an additional task of determining the homogeneity of the distribution of these substances in the film. With the help of existing analytical procedures, selective determining of the distribution of impurities is problematic since the chemical elements that make up them are identical to those that make up the BR and the matrix. However, there are a number of impurities that contain atoms unique to this composition – these are substances that contain bromine and sulfur atoms. The study of films with impurities of dodecyltrimethylaminobromide (DTMAB) and phenylthiosemicarbazide (FTSC) using raster electron microscopy makes it possible to determine the distribution of bromine and sulfur over the film area. Analyzing the uniform distribution of these elements in the film, we can conclude that the distribution of impurities in the film is homogeneous, and there is influence of the methods used to obtain films on this process. The results of investigating the distribution of chemical elements over the area of the BR film in the gelatin matrix with the impurities DTMAB and FTSC, which is obtained by irrigation, are shown in Fig. 5.

From the results obtained (Fig. 5) it can be seen that in the selected area of the film there are atoms of carbon, oxygen, bromine, sulfur, and platinum (Fig. 5, a). The presence of platinum in the chemical composition is associated with a conductive film with a thickness of ~50 nm applied to the surface of the samples, for charge runoff during studies using a raster electron microscope.

The distribution of the elements included in the selected substances according to the film area is shown in Fig. 5, b–d. Different chemical elements on the same part of the film surface are reflected in different colors. Probably, the uneven distribution of the studied elements is due to the structural features of the morphology of the film surface.

A similar pattern was observed for films obtained by molding and centrifugation (spin coating). From the data obtained, we can conclude that the methods used to prepare the film-forming mixture and apply chemically modified BR films ensure uniform distribution of chemicals.

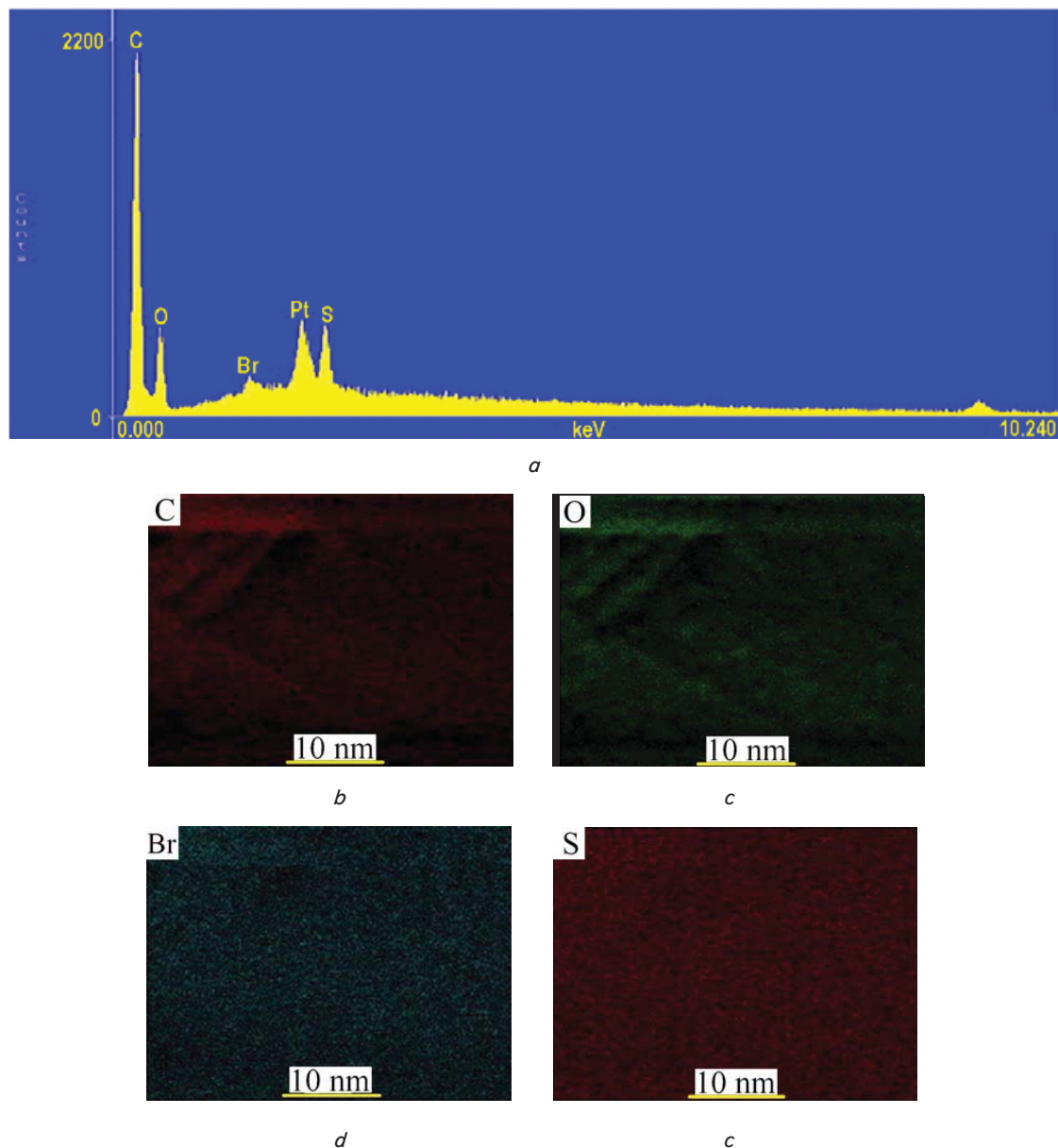


Fig. 5. Analysis of the chemical composition and distribution of elements in bacteriorhodopsin films with impurities of dodecyltrimethylaminobromide and thiourea using raster electron microscopy: *a* – chemical elements that make up the film; *b* – distribution of carbon atoms over the area of the film; *c* – distribution of oxygen atoms over the area of the film; *d* – distribution of bromine atoms over the area of the film; *e* – distribution of sulfur atoms over the area of the film

5.3. Results of investigating the dependence of the dynamics of photoinduced changes in films on their chemical composition

To assess the effect of chemical modification on the photochromic properties of the films, a study was conducted on the effect of the concentration of selected chemicals or their combinations on the sensitivity of the film to external radiation. Based on studies of changes in optical absorption at 570 and 412 nm, the maximum values of the optical parameters of BR films, which can be achieved using combinations of selected chemicals, were established. The maximum values of the studied parameters obtained for different impurities and their combinations are given in Table 1.

Table 1

Optical parameters of bacteriorhodopsin films in a gelatin matrix with an optimal content of various sensitizing substances

Chemical substances	ΔD_{570} , a.u.	ΔD_{412} , a.u.	K_{412} , %	$S_{570} \cdot 10^{-3} \text{ m}^2/\text{J}$	$S_{412} \cdot 10^{-3} \text{ m}^2/\text{J}$	$\tau_{1/2}$, s
No impurities	0.315	0.121	10.7	3.9	1.4	3
AGH	0.290	0.163	16.3	19.3	1.0	5
TEA	0.995	0.624	45.5	36.3	14.8	157
AGH+TEA	0.879	0.554	48.3	45.5	16.6	160
GA+TEA	0.963	0.582	50.0	28.4	12.8	135
HMDAGH+ +TEA	1.047	0.610	47.6	54.0	17.3	156
EABHB+ +TEA	0.976	0.473	39.9	48.0	24.0	158
SDS+TEA	0.740	0.440	37.0	43.2	12.7	190

From the results given in Table 1, it can be seen that under the action of lighting there are changes in transmission at characteristic wavelengths. This indicates that a photocycle is stored in all films, that is, BR retains its photochromic properties. The chemicals used for the modification do not provoke the separation of retinal or any other negative processes that would interfere with the practical use of the obtained films for solving applied problems. At the same time, significant differences are observed for different chemical impurities, both in the sensitivity of the films to lighting and in the lifetime of intermediate intermediates and the number of molecules involved in the passage of the photocycle. In the absence of changes in the spectral characteristics and identity of all other research parameters, these changes can be explained only by the influence of chemicals on the passage of the photocycle.

6. Discussion of results of the chemical modification of bacteriorhodopsin films and their use to protect information

As can be seen from Fig. 4, the position of the main absorption maximum at 570 nm remains virtually unchanged. The presence of a band of absorption of the ground state of BR and the absence of the influence of chemical impurities on the transmission spectrum of darkened films is the first evidence that the BR in such films retains its photochromic properties. For some films, a slight increase in absorption in the region of 412 nm can be noted. As further studies have shown (Table 1), the increase in absorption in the region of 412 nm correlates with an increase in the photosensitivity and lifetime of the intermediate M_{412} . That is, under the action of the corresponding chemicals, the properties of the films change so much that even the insignificant intensity of the probing beam of the spectrophotometer is sufficient to create photoinduced changes visible on the absorption spectra.

For practical use, an equally important parameter will be the uniform distribution of impurities in the film. After all, it is this factor that will determine the reproducibility of parameters over the entire area of the sample. As can be seen from the results shown in Fig. 5, the method used to prepare the film-forming mixture and apply the film makes it possible to obtain a uniform distribution of the film material (gelatin, PM, and impurities) over the surface of the substrate.

The absence of a negative effect of impurities on the functioning of the BR molecule (Fig. 4) can be explained by careful analysis, selection, and coordination of the parameters of the matrix material and impurities with the properties of BR. Detailed preparation of the film-forming mixture, compliance with temperature and time regimes when applying and drying films explain the uniform distribution of material and impurities in the film (Fig. 5).

Although impurities have practically no effect on the transmission spectra of darkened films (Fig. 4), the dynamics of photoinduced changes can largely depend on the chemical composition. As can be seen from the data given in Table 1, the half-period of life of the M_{412} intermediate, that is, the time of preservation of information recorded on the BR film, can be from a few seconds to several minutes. The required time is easy to change to the desired value by changing the concentration or type of impurity. The most noticeable contribution to the modification of the parameters of the photocycle, in particular the increase in the lifetime of M_{412} , is made by triethanolamine, which indicates a

high sensitivity of BR to this substance. The action of TEA is manifested in the shift in equilibrium between the intermediates of the BR photocycle, there is an accumulation of a large number of molecules in the state of M_{412} due to a decrease in the concentration of intermediates K , L and a delay in the transition to the ground state of BR_{570} . An increase in the concentration of TEA does not significantly affect the process of formation of M_{412} at the initial stage of the photocycle. Its main action is manifested in the microsecond time interval and affects the relaxation process of the M_{412} intermediate, which leads to an increase in the amplitude of the change in absorption and slowing of relaxation to hundreds of seconds.

It was found that with an increase in the amount of TEA in the film to 15.42 % (weight), the amplitude of optical absorption increases, the relaxation time of the M_{412} intermediate increases. Maximum changes were observed at a concentration of about 15 weight percent. Further increase in the concentration of TEA in the BR film leads to a decrease in the photoinduced change in optical absorption and an increase in the relaxation rate of the intermediate M_{412} . The optimal parameters are possessed by films in which the concentration of PM is 28 % (by weight), TEA – 15.4 % (by weight), gelatins – 56.6 % (by weight). For such a chemical composition, the half-period of decomposition of the intermediate M_{412} increases by more than 50 times, the coefficient of participation of molecules in the photocycle and the sensitometric sensitivity of the films increase.

Other substances, among those studied, have a significantly smaller impact on the parameters of the photocycle. For example, the introduction of arginine hydrochloride into the film led to much smaller changes in the properties of the film. As can be seen from Table 1, at an optimal concentration of AGH, the semi-periodic decay of the intermediate M_{412} increases by less than 2 times. Other parameters have also changed, but insignificantly.

At the same time, the use of a combination of TEA and AGH made it possible to “sum up” their influence on the film parameters. When optimizing the composition of film structures based on BR, it was found that using a combination of two sensitizing substances in the BR film, their maximum concentration can be 14 % (weight). At the same time, the optimal concentration of TEA in the BR film is no more than 12.8 % (by weight).

Glutaraldehyde (GA) interacts with amino acid residues and charged lipid bilayer groups [20]. Studies of BR films, which together with TEA included glutaraldehyde, showed that the use of this substance also makes it possible to influence the optical parameters of the films. The following optical parameters are characteristic of the optimal composition of films with such impurities: S_{570} and S_{412} – $28.4 \cdot 10^{-3}$ and $12.8 \cdot 10^{-3} \text{ m}^2/\text{J}$, respectively, $\tau_{1/2}$ – 135 s with a coefficient of participation of BR in the photocycle of 50 %. The optimum concentration of GA was 0.53 % (by weight), at a concentration of TEA of 12.8 % (by weight) and PM – 28 % (by weight). In addition to changing optical properties, the gelatin film structures of BR with TEA and the optimal GA content demonstrate increased resistance to dissolving them in water. This property may be important in the technological aspects of obtaining and applying materials based on BR.

Salts of amines EABHB and HMDAGH when used in combination with TEA contribute to an increase in photosensitivity, which means a decrease in the energy barriers to the transition of $BR_{570} \leftrightarrow M_{412}$ in the BR molecule. There is reason to believe that an increase in the sensitivity of BR

films is associated with the emergence of interactions between the active centers of sensitizing substances and the hydrophilic region of the PM surface. Such interactions can stimulate conformational changes in the retinal and the separation of the proton from the Schiff base during the formation of the M_{412} intermediate. In addition, the result of the interaction of nitrogen-containing impurities with the polar heads of the liquid bilayer PM may be the redistribution of the electron density of the surface amino acid residues of opsin. The lifetime of the M_{412} intermediate, compared to AGH, does not change much. The half-period of decay remains about 160 seconds while the sensitometric sensitivity of the films increases.

The maximum lifetime values of the intermediate M_{412} were obtained using sodium sulphur-containing dodecyl sulfate (DDSN) in combination with TEA. At optimal concentration of impurities, the decomposition semi-period was 190 seconds, which is more than 60 times higher than the value obtained for films without impurities. An interesting fact is that the maximum lifetime is observed at the lowest coefficient of participation of molecules in the photocycle among the studied combinations of impurities, which may indicate the absence of the influence of impurities on the energy barriers of transitions between intermediates. In the case of using DDSN, the presence of partially negatively charged centers (S and O atoms), the action of which is shielded by a long hydrocarbon radical, is responsible for changes in optical parameters.

If we summarize the results given in Table 1 and literary data [18], it is possible to formulate a number of features that are characteristic of substances that have been used to effectively slow down the decomposition of M_{412} . First, almost all substances are characterized by the presence of nitrogen in amine or guanidine groups or the inherent strong ability to complexate with positively charged ions. For nitrogen, which in all these substances acts as trivalent, only 3 p-electrons of the external orbitals, with two s-electrons being used to temporarily bind free hydrogen ions. Second, if we express the concentrations of substances in the form of the ratio of the molar concentrations of impurities and BR, it turns out that in all cases hundreds of impurity molecules per BR molecule are needed for a tangible effect. If we assume the presence of a direct interaction of the impurity with the BR molecule (a certain amino acid residue or their group), one, maximum of several, impurity molecules will be enough to change the properties of the material.

That is, different substances, with different masses, chemical formulas, overall dimensions of molecules have a similar effect on the course of the photocycle. Moreover, these molecules must be in large numbers, and their presence significantly affects the sensitivity of the photocycle to the number of free H^+ ions in the medium, which is determined by the pH level. All these facts can be interpreted as evidence of the absence of direct interaction of impurity molecules with BR molecules. At the same time, the effect of exposure can be realized through active correction, for example, through temporary complexation, the amount of free hydrogen ions in the medium around the BR molecule. Reducing the number of free hydrogen ions directly affects the reprotonation time of the Schiff base and the possibility of the molecule moving to the ground state, thereby increasing the lifetime of M_{412} . That is why in order to "catch" all the free protons, a large number of impurity molecules per BR molecule are needed.

As can be seen from the results given in Table 1, due to the modification of the chemical composition of the film, it was possible to increase the conservation time of photoin-

duced changes by more than 60 times, up to 190 seconds. Such time is quite enough, for example, to implement feedback, when the terminal can record a specific message for the user on the photochromic area of the identification card. After 190 seconds, the message will disappear and cannot be authorized to read even if the card is lost while this time is enough for the cardholder to read it. The increase in the number of molecules in the M -state, due to an increase in its lifetime, leads to a change in the color of photochromic inks even at household lighting levels. This also contributes to an increase in the photosensitivity of films under the action of impurities. As can be seen from Table 1, the sensitometric sensitivity of the films was increased by more than an order of magnitude.

The limitation of this study is a relatively small amount of substances used. Despite the fact that it was possible to significantly influence the passage of the photocycle and the photosensitivity of the films, the obtained data are not enough to clearly formulate the microscopic mechanism of interaction of impurities with BR. The lack of an interaction mechanism limits the possibility of further theoretical substantiation of the results obtained within the framework of the generally accepted theory of the photocycle.

Further development of this area of research is possible due to an increase in the number of chemicals used that can modify the optical properties of bacteriorhodopsin films while maintaining their photochromic properties. Identification and detailed study of the effects of new chemical impurities will make it possible to understand how the mechanism of their influence is implemented and describe it at the microscopic level.

Although the general mechanism of interaction of BR molecules and impurities is not yet fully understood, we can confidently say that using appropriate combinations of chemical impurities, it is quite realistic to obtain films with predetermined parameters. It is the film structures with high photosensitivity and increased thermal lifetime of the M_{412} intermediate that are necessary to create protective markings and other applications in the field of information protection. It should be noted that the magnitude of the change in the corresponding parameters depends on the concentration of impurities and can be optimized for a specific task. Different parts of the document can be protected by elements of different parts chemical composition. The absolute lifetime of the M_{412} intermediate, subject to the possibility of its continuous change, can be predetermined, easily determined by the terminal and serve as an additional element of protection against counterfeiting.

By selecting the appropriate chemical components correctly, it is possible to increase the thermal lifetime of the M_{412} intermediate to a given value in a wide time range and control the photosensitivity of the film. With much simpler technology and the presence of a large selection of sensitizing substances, the effectiveness of the directional change in the optical properties of the films using chemical modification may be greater than that obtained by more complex methods of site-specific mutagenesis or retinal replacements.

7. Conclusions

1. A series of films with different combinations and concentrations of sensitizing substances were made, as well as their complex studies were carried out. It is shown that BR in these films retains its spectral and photochromic properties.
2. The results of studies of films by raster electron microscopy allow us to assert that the method used to prepare

the film-forming mixture and apply the films ensures uniform distribution of bacteriorhodopsin and impurities.

3. Investigating the dynamics of photoinduced changes in films of different compositions has shown that the use of impurities makes it possible to obtain film structures based on bacteriorhodopsin with a given level of photosensitivity and the time of preservation of the information recorded on them. Varying the compositional and quantitative composition of the films, it is possible to change the time of storage of information in the range from several to hundreds of seconds

and change the sensitometric sensitivity of S_{570} from $3.9 \cdot 10^{-3}$ to $54 \cdot 10^{-3} \text{ m}^2/\text{J}$ continuously.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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