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SENSING ELEMENTS BASED ON PHOTOCHROMIC NANOCOMPOSITE FILM STRUCTURES OF THE BACTERIORHODOPSIN FOR BIOSENSORS OF ANESTHETICS

Potapchuk A.[2], Sichka M., Sakalosh I., Trikur I.[1], Shevchuk I.[1], Melnichuk D.[2], Rizak V.

[1]*Department of Solid State Electronics,*
[2]*Department of dentistry of postgraduate education Uzhhorod National University, Ukraine*

Summary. The investigation of influence of volatile general anesthetics and of local anesthetics on the parameters of nanocomposite film structures of bacteriorhodopsin photochromic biomolecules in porous polymeric and inorganic sol-gel matrices has been performed. It is shown that the change in spectral characteristics and photocycle parameters in film structures on the basis of bacteriorhodopsin under the influence of anesthetics enables to develop the technique of controlling the concentration of anesthetics on the basis of the given film structures and to create sensitive elements for optical and fiber-optic sensors for monitoring the concentration of anesthetic agents in vivo.

Key words: bacteriorhodopsin, purple membrane, anesthetics, film structures, nanocomposite structures, sensors.

Introduction. Using anesthetics in medicine and related fields leads to strict control of concentrations of in the body and their elimination speed. The base of anesthesia is the interaction between anesthetics molecules and biomembranes. The nature and characteristics of this interaction determine: the effectiveness and dynamics of anesthesia, the concentration of anesthetic required in certain circumstances, the absence of adverse effects both on individual organs and the body as a whole, the time of anesthesia and anesthetic metabolism. The results of many different studies do not provide complete answers about the features of this interaction. The non-specific nature of this interaction is shown by a wide range of structural built of anesthetics (from gas to organic solvents) and a strong correlation between the effectiveness of the anesthetic and its solubility in lipids [1]. However, the present results suggest that certain proteins change their activity at clinical concentrations of anesthetics that definitely requires a specific type of interaction [2]. Bacteriorhodopsin (BR) in the form of purple membrane (PM) – an attractive model to study the effects of anesthetics on the structure and properties of membranes. PM is a complex organic structure which is sensitive to various changes in the environment. Treatment PM by anesthetics leads to significant changes in characteristics of the membrane as a whole and individual BR molecules [3-6]. Characterization of the interaction of anesthetics with BR and establishment the changes of their physical properties and functioning can make a significant contribution to the study of anesthesia mechanism.

METHOD OF INVESTIGATION OF ANESTHETICS INFLUENCE ON STRUCTURE, PROPERTIES AND FUNCTION OF BACTERIORHODOPSIN

Considering the change of the spectral characteristics, the influence of anesthetics on the PM can be divided into three stages. The first stage – at low concentrations of anesthetics, there is a slight shift of the absorption maximum of 570 nm to 567 nm. The second stage – with increasing concentrations of anesthetics, there is a significant shift of the absorption maximum of 567 nm to 480 nm. The third stage – with a further increase in the concentration of some anesthetics leads to a shift in the absorption maximum in more short-wave region – up to 380 nm [3]. Important for the efficiency of interaction with anesthetic BR is the hydrate-level of PM [5] and pH [4,6]. All three forms have significant differences not only in the spectral characteristics. Process of interactions are accompanied by structural changes both in individual molecules and in the structure of PM that for certain anesthetic at high concentrations may be irreversible. The dynamic characteristics of photocycle are also changing [3].

To study the parameters of the BR films we have used a fiber-optic spectrophotometer *Ocean Optics USB4000* (spectral range 200–1100 nm, optical resolution of 0.25 nm.). As a light source was used a quartz halogen lamp *KTM 24-250*. To stabilize the lamp power was used rectifier *BCA-5A-K*. Transporting illumination to the sample and to the spectrophotometer was realized by using a fiber-optic 600-micron *Ocean Optics FIBER 600-UV*. To reduce the lightloss all connections of optical fibers were performed using standard connectors *Ocean Optics Premium-Grade SMA 905 Connector*. The light from the lamp was focused by system of lenses and introduced into an optical fiber with a special collimation lens (*Ocean Optics 74UV Colimating Lens*) was connected directly to SMA connector on the fiber end.

To study the influences of anesthetics on the parameters of the BR film were recorded the changes in spectrum's transmission and reflection of films. Changes in transmittance at wavelengths that undergo maximum change in absorption, namely 480 and 570 nm were recorded in real time, and regularly was recorded total spectrum of film transmission. To avoid accidental changes was held a parallel monitoring at 800 nm, where there are no changes made by the action of anesthetics on film.

To carry out the research in our laboratory has been developed and created sealed sensor camera Camera for studying the influence of volatile anesthetics was completely made of plexiglas as this material

rent, which facilitates installation and alignment s, and not susceptible to corrosion. Detachable l foramens for input and output of optical fibers :aled with technical silicone sealant and rubber . Valves for the inlet and outlet of gases and liq- re soldered in plexiglas. To create an atmosphere thetic – in cell was filled an aqueous solution of . anesthetic concentration. When concentration ıperature of aqueous solution are known we can :e equilibrium gas concentration in the air above ıtion. Reducing the concentration of anesthetic n a cell was achieved by draining the solution e cuvette and blowing air.

n the case of study the effect of aqueous anes- olutions on the parameters of the BR films as a camera was taken a gas cell, consisting of a cy- l glass body, which has two inlet valves for gas d, and the two flanges with threaded joints on the cheme of the camera is shown in Fig. 1. During ly film 1 was on the special object table 2 mount- ıe holder 3. Fiber 4 is fixed in a vertical position, licular to the table. Investigated film placed be- he fiber end and the object table. Study of reflec- pectra film was performed using a 600-micron ıtic Y-coupler. Common channel Y-coupler was irect contact with the film. Focused light from p was applied to one channel, and reflected from ı through another channel – was recorded by photometer.

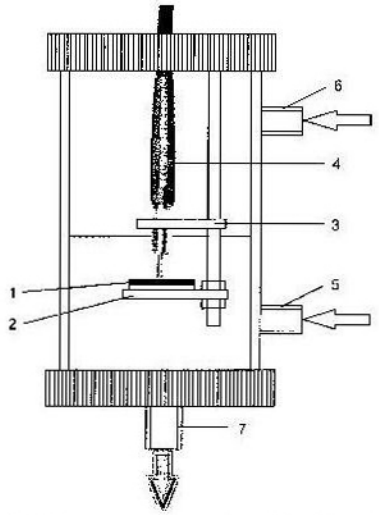

Fig. 1 - The camera construction for studying the e of an aqueous anesthetics solution on the pa- s of the BR films. 1 - the sample, 2 - object table, er, 4 - optical fiber, 5, 6, 7 – inlet and outlet valves ids.

Water is provided into the cell through valve 5 connected measuring beaker, that allows pre- ontrol amount of liquid. Aqueous anesthetics ıs were provided through valve 6 via plastic . Drain of liquids carried through the valve 7. ıce the concentration of anesthetic solution ed the washing of the cuvette with distilled For the film drying after experiment was used olow.

RESULTS AND DISCUSSION

Investigation the influence of chloroform on optical properties of BR films

To carry out all investigations was used BR, isolated from wild-type strain. BR was obtained in the form of fragments PM by standard methods [7] from Halobacterium salinarum with a ratio of protein/lipids in purple membranes 3:1 (molecular weight) and 1:10 (number of molecules). BR films in polymer matrices were prepared using standard techniques [8-10].

To study the influence of chloroform on the characteristics of BR was carried out a research of suspensions and film structures, made by using different matrices. To investigate the influence of chloroform to the suspension of BR was used an aqueous solution of PM concentration of 5 mg/ml. Change of concentration of analyte was carried out by introducing the calculated amount of chloroform in a cell or cuvette using microsyrenge. For suspension was carried out an investigation of spectrum transmission changes and its monitoring at fixed wavelengths.

In the study of film structures was used BR films in a polymer gelatin [7-10] and inorganic sol-gel matrices [11-15] obtained by coating. For the obtained films was carried fixation of changes the transmission and reflection spectra, and was monitored changes in transmittance and reflectance at fixed wavelengths in the process of change of chloroform concentration.

For BR films in gelatin matrix characterized by homogeneity and high optical quality. Methods of obtaining such film structures does not require expensive reagents, equipment, time and is well developed. All this makes the gelatin film of BR attractive for use in sensors for gases and volatile anesthetics [16, 17]. However, for use in solutions such films do not fit because of the aqueous solubility of gelatin. The film in water swells, changes its characteristics and is destroyed in the end.

On the Fig. 2 shows the changes in the spectrum transmission PM suspensions, with the adding of various amounts of chloroform to the solution. As seen in figure, introduction of chloroform solution leads to a decrease in absorption at 570 nm and the appearance of the absorption band at 480 nm. Moreover, the magnitude of changes depends on the concentration of chloroform.

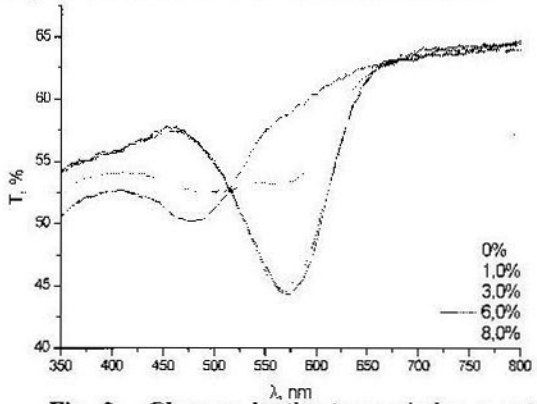

Fig. 2 - Changes in the transmission spectra of aqueous PM (C = 5 mg/ml) at different concentrations of chloroform.

It should be noted that the absorption spectrum returned to its original appearance decreasing the concentration of chloroform in the solution achieved by its evaporation.

To study the influence of chloroform on the films parameters based on BR we conducted a study to chloroform vapor spectra transmission of films in different matrices. Technologically easier is to obtain high-quality films on glass substrates. Therefore, initial experiments were carried out using of such films. The results of transmittance changes at different concentrations of chloroform is shown in Fig. 3.

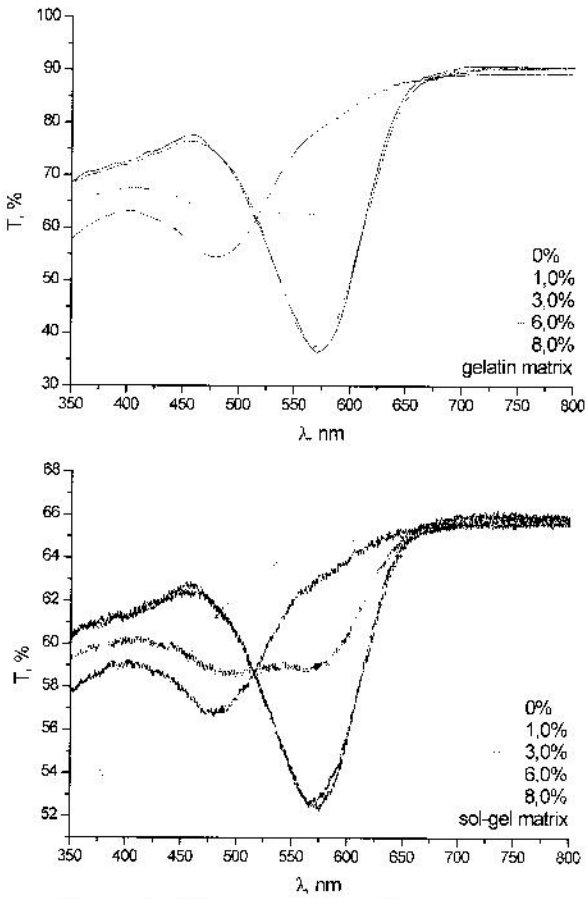

Figure. 3 - The spectra transmission of the BR film on a glass substrate in gelatin and sol-gel matrices at different concentrations of chloroform.

As it can be seen from the figure, for film spectral changes that occur under the influence of chloroform, similar to those observed in suspensions. Moreover for gelatin films, which are characterized by the best optical quality, transmissions changes have greater value with less noise.

As for the practical use more interesting is the study of changes in spectra reflectance of films, we have carried out such studies using Y-coupler and camera (Fig. 1). To carry out the investigation used such interface *fiber end–film–substrate* as in this case, the best value was recorded the signal/noise ratio. The results of such investigations for films in various matrices are shown in Fig. 4. As can be seen from the results of qualitative changes reflectance spectra of films under the influence of chloroform practically indistinguishable from changes in the transmission spectrum. How it should be noted that the amplitude of the ch reflectance is much lower in comparison with ch es in bandwidth. However, even at this level of ch of spectral bands to feel the change of concentra chloroform at 1 %, which suggests the possib using such films as sensing element for sensors roform, as shown in Fig. 4 for sol-gel films inte reflected signal even greater than for gelatin fil lowing them to achieve a better signal/noise ratio is likely due to the fact that the film using a sol-ge trix have more defects in the structure which u ly leads to an increase in the total reflected sig enters into a common Y-coupler channel.

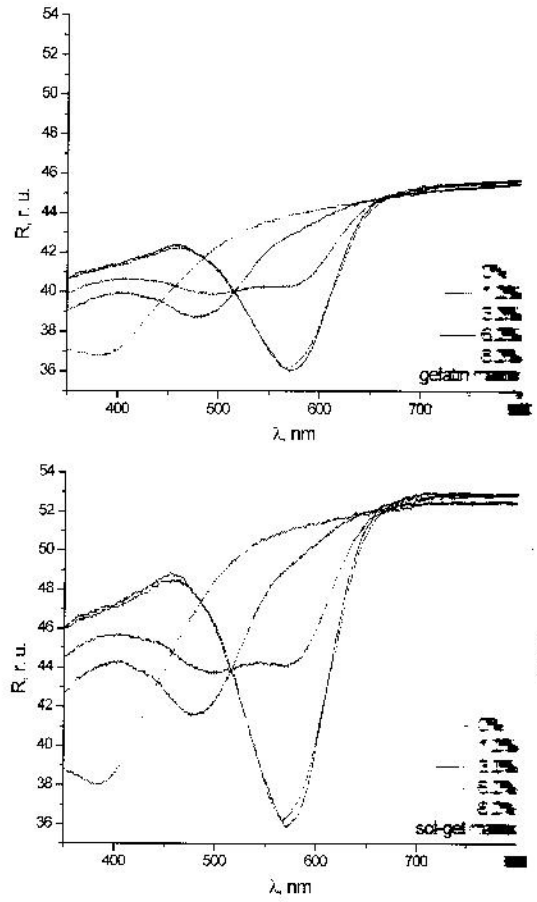

Figure. 4 - Reflection spectra of BR films substrate in gelatin and sol-gel matrices at differe centrations of chloroform.

As seen from the results, at low concen chloroform causes a slight shift of the main abso band of the BR film. With increasing concentra reduced absorption at 570 nm and the formation absorption band at 480 nm. With increasing conc tions above 7 % (by volume) is formed by the ab tion band at 380 nm. While the changes that occ concentrations below 7 % are reversible in nature. formation shape BR^{380} is no transition to the initial when removing anesthetic. In the reversibility mum change in transmission and reflection occ at 480 and 570 nm. Therefore, in order to track ch that occur in the film and estimate the concentra

esthetic sufficient to monitor the value of reflectance ransmittance at these two wavelengths.

The presence of chloroform leads to significant ctral changes in BR films. The change in signals and rsibility of changes determined by the concentra- of chloroform. The range of reversibility is in the e from 0 to 7.0 % (by volume). Minimal change concentration that can be unambiguously detected - 5 %. Average stabilizing signal by changing the con- ration is 57 s and 74 s for sol-gel and gelatin films. the time during which there is 90 % change in the e of the signal is 15 s for sol-gel and 22 s for gel- films. The response time does not change with an ease or decrease of concentration. In the reversibil- most noticeable changes occur at a wavelength of nm for both types of films. Mean changes in signal sity for the reduced reflectance in the case of sol- film is 29.3 % of the original signal level. For gela- films this value is 63.7 %.

Summarizing the results we can conclude that s of BR in gelatin and in sol-gel matrices can be as primary transducers for monitoring sensors of concentration of chloroform. Films in gelatin ma- es have a greater change in signal intensity, allow to the best sensitivity, however, are characterized by er response time. At the same time the film in sol matrices with a smaller amount of signal intensi- change with less response time. For use in aqueous ions will also be a significant advantage insoluble sol-gel films.

Investigation the influence of lidocaine on op- properties of BR films

By analogy with investigations carried out for oroform, a series of experiments were held to study influence of aqueous solutions of different concen- ions of lidocaine on the characteristics of BR. As the previous case, the investigations was carried out suspensions and films. For experiments was used a cial camera that allows to explore both the transmis- and reflection for the films deposited on glass sub- es as well as on the end of optical fibers. Accurate rol of the water and anesthetic allows to calculate concentration of lidocaine in each case. The camera ides a possibility to increase the concentration of sthetic liquid by introducing an additional amount concentrated solution and to reduce the total con- ration by washing chamber by certain amount of illate. By analogy with the previous experiments, was made a study of the influence of lidocaine on characteristics of the suspension. Bacteriorhodopsin centration was 5 mg/ml, the range of variation of centration of lidocaine – from 0 % to 2 %. The re- s are shown in Fig. 5.

The results are qualitatively similar to those ined for chloroform and consistent with the liter- e data. However, it should be noted that even at a centration of 0.5 % observed shift of the absorption is the same as at 1 % concentration of chloroform. o we used the maximum concentration that does not cause any irreversible changes. Since the suspension of BR demonstrates sensitivity to lidocaine, we carried out similar investigations for films in various matrices. In the case of films deposited on glass substrates, reflec- tion and transmission spectra are shown in Fig. 6 and Fig. 7.

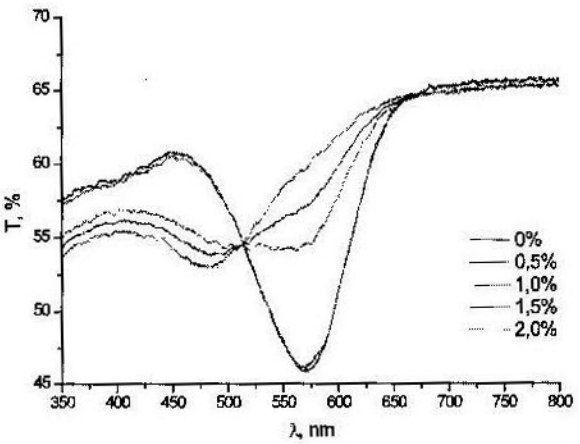

Figure. 5 - Transmission spectra of suspensions PM with adding different concentrations of lidocaine.

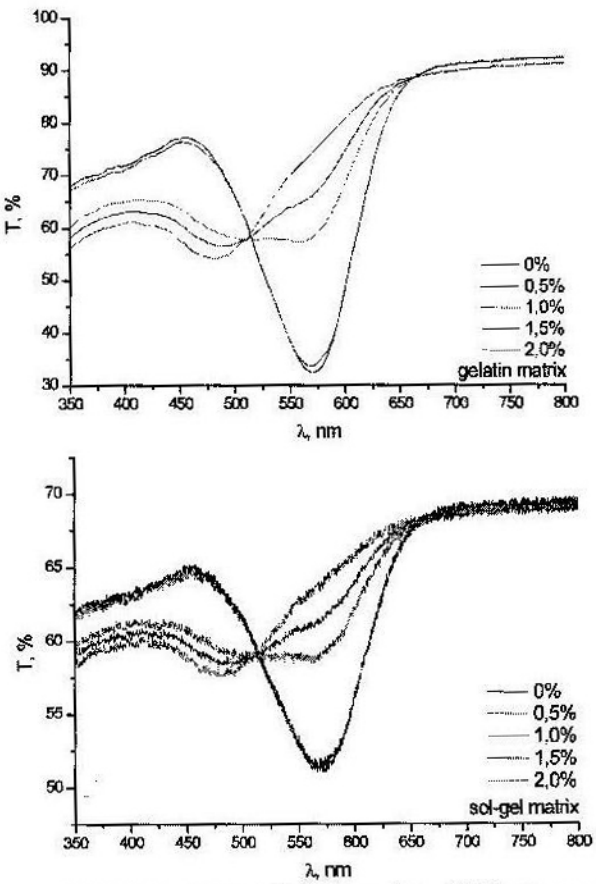

Figure. 6 - Transmission spectra of BR films on glass substrates and gelatin in the sol-gel matrices during immersion into aqueous solution of various concentrations of lidocaine.

For research on glass substrate films were placed in an aqueous solution of lidocaine. Introducing a certain amount of concentrated solution or distillate could change the overall concentration.

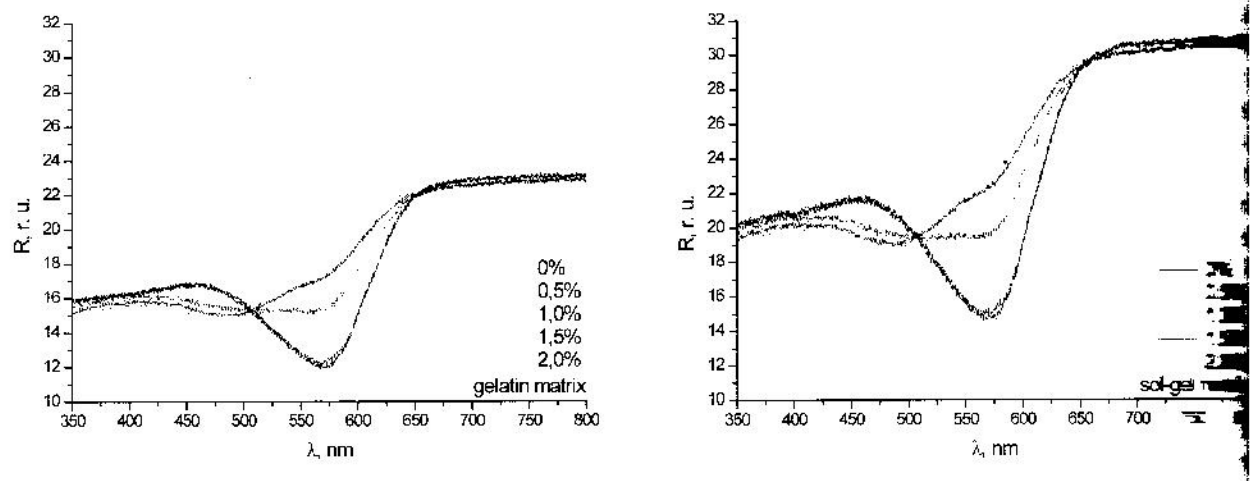

Figure. 7 - Reflection spectra of BR films on glass substrates and gelatin in the sol-gel matrices during im into aqueous solution of various concentrations of lidocaine.

Study of the characteristics of gelatin films BR matrix in aqueous lidocaine confirmed that these films can be used effectively for control of a vapor-gas mixture. After placing the film in an aqueous solution, depending on the concentration of lidocaine were corresponding changes range of the transmission. However, within about 15 minutes of the film was destroyed by water. Fig. 8 shows photos of the film before and after the film reflection spectrum investigation in aqueous solution. As you can see from the photos, homogeneous film collapsed at the site of contact with the fiber. Swelling gelatinous matrix during exposure to water led to a redistribution of the material film and after drainage of the liquid film forming material is concentrated around the fiber end, while at the same end BR almost disappeared.

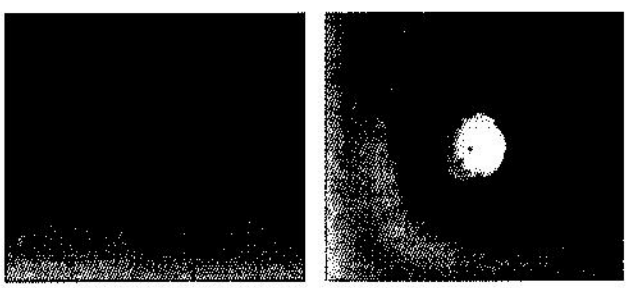

Figure. 8 - Film BR in gelatin matrix before and after exposure to an aqueous solution of lidocaine.

These results are confirmed by monitoring data, that are shown in Fig. 9. As can be seen from the results of the initial spectrum observed changes similar to those that occur in suspension under the action of lidocaine. To obtain spectra at different concentrations and to prevent the destruction of the film, we placed the film in a solution of given concentration, measurements were carried out and, after stabilization of indices, washed the sample by distillate and dried the film. Then studies were carried out using different concentrations.

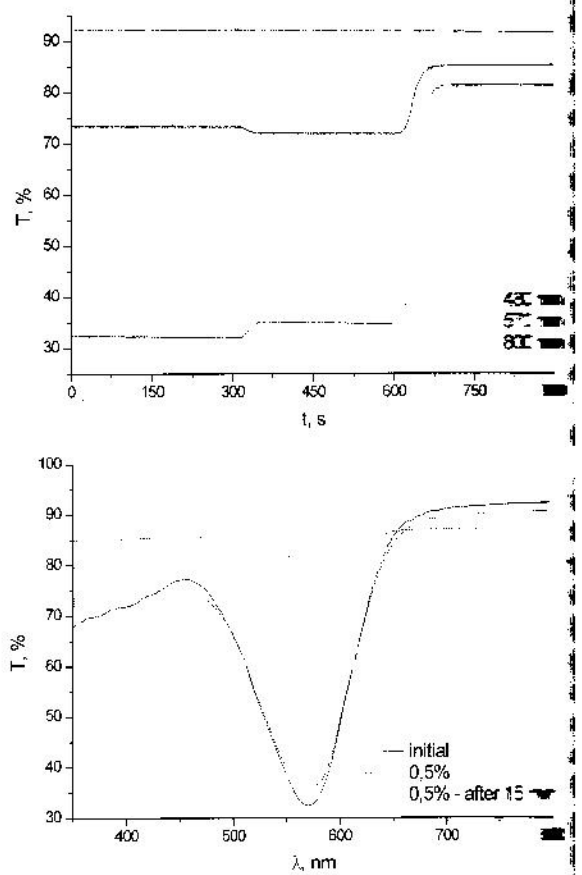

Figure. 9 - Dynamics of change of reflectance fixed length and change the transmission spectra of films on a glass substrate in a gelatin matrix during sure in aqueous solution with a concentration of lido 0.5 % (by volume) for an extended period of time.

From these results we can conclude that of BR in gelatin matrix are sensitive to changes in concentration of lidocaine, but because of its aq solubility gelatin is not suitable for the role of the trix in the films that will be used for chemical senso aqueous solutions. Therefore, further studies were formed using films in sol-gel matrices.

For films that have used the sol-gel matri lation of homogeneity and structure of the films was

ved even when exposure to an aqueous solution
1 24 hours, regardless of the concentration of the
on. For sol-gel films is typical greater scattering
insequently lower values of transmittance changes
; changes of the concentration of anesthetic com-
to gelatin films. At the same time, the intensity
reflected signal amplitude and its change for the
l films is higher. So in the case of reflective type
such films will demonstrate better sensitivity.
The presence of lidocaine in the film environ-
eads to significant spectral changes. The change
ismittance and reflectance is determined by the
ntration of lidocaine. In the concentration range
6 – 2.0 % (by volume) all changes are fully re-
le. Minimal change in concentration that can be
iguously detected – 0.25 %. Average stabilizing
by changing the concentration is 54 s and 71 s
l-gel and gelatin films respectively. And time
which there is 90 % change in the value of the
is 14 s for sol-gel and 19 s for gelatin films.
nse time does not change when the concentra-
changed. In the reversibility most noticeable
:s occur at a wavelength of 570 nm for both
of films. Average changes in signal intensity for
luced reflectance in the case of sol-gel film is
6 of the original signal level. For gelatin films this value is 17.7 %.

Summarizing the results of studies we can conclude that films of BR in gelatin matrices can not be used for measurements in aqueous solutions, though are demonstrating sensitivity to the presence and concentration of lidocaine. BR Films in sol-gel matrices can be used as primary transducers for sensors monitoring the concentration of lidocaine in the solution. In the range of 0 – 2.0 % sensitive elements based on such film structures will have low response times with good sensitivity.

Conclusions

Changes of spectral characteristics and photocycle parameters in film structures of bacteriorhodopsin under the influence of anesthetics enable the development of anesthetics concentration controlling technique on their basis. Is shown that the film structure based on bacteriorhodopsin is sensitive to changes in the concentration of chloroform and lidocaine and can be effectively used as sensing elements for optical and fiber-optic sensors. The range of the reversibility of these sensors will be 0 % – 7.0 % for chloroform and 0 % – 2.0 % for lidocaine. Reversibility, high sensitivity and high response speed of bacteriorhodopsin at the expence of matrices microporosity allows to create a minimally invasive biosensors of anesthetics, that work *in vivo* in real time.

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