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Sensor coating based on lipid Langmuir monolayer with the glucose oxidase enzyme molecules

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Abstract: **The sensor coating was created based on a Langmuir monolayer of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) with immobilized molecules of the enzyme glucose oxidase and its sensor properties were studied. The process of incorporation of glucose oxidase enzyme molecules into a Langmuir monolayer of phospholipid DPPE molecules was studied using the compression isotherm method. Adsorption of glucose oxidase molecules has an expanding effect on the Langmuir monolayer of DPPE and leads to an increase in the area per molecule from 32.5 Å^2 to 49 Å^2 and a decrease in the compression modulus of the monolayer from 133 mN/m to 83 mN/m. Also, the adsorption of glucose oxidase leads to an increase in the desorption coefficient of the monolayer into the k_d subphase from $0.5 \cdot 10^{-3}$ to $1.2 \cdot 10^{-3}$. The morphology of films with immobilized enzyme molecules at different adsorption times was characterized by atomic force microscopy. Increasing the enzyme adsorption time leads to a decrease in the surface roughness of the formed film from 3.6 nm at 5 minutes to 2.4 nm at 60 minutes. The formed DPPE monolayers with immobilized enzyme molecules were transferred to graphite electrodes. Their sensory properties were studied using cyclic voltammetry and impedance measurements. The resulting coatings were sensitive to glucose in solutions with concentrations from 0 to 1 mg/ml. The presence of a linear concentration dependence of the maximum and minimum currents and resistances in the specified concentration range makes the created films promising for use as touch coatings.**

Keywords: **amperometric glucose sensor, enzymatic sensors, Langmuir-Blodgett films, biosensors**

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Contents

1. INTRODUCTION.

Polysaccharides, and in particular glucose, are actively used in the food industry, so the creation of glucose sensors with increased sensitivity and selectivity is a fairly urgent task for monitoring its content in technological processes, in final products, as well as in production waste. A promising solution to this problem is the development of a touch coating for a biosensor based on molecules of the enzyme glucose oxidase (GO). The main task in this case is to fix the enzyme molecule in the sensor layer and maintain its activity over a long period of time. One of the approaches that allows, within one technological operation, to form a film with enzyme molecules included in it and ensure its protection from environmental influences is the Langmuir-Blodgett technology [1-4]. Its use makes it possible to form a bilayer membrane-like film with included enzyme molecules. In such a film, the enzyme molecules will be protected from external influences by a thin layer of lipid molecules. Therefore, the urgent task is to study the process of incorporation of enzyme molecules into Langmuir monolayers of various surfactants [5-8]. A similar approach can also be used to dope monolayers and Langmuir-Blodgett films with metal ions dissolved in the subphase [9]. Authors [10] The effect of the charge of the head group of surfactant molecules on the process of incorporation of enzyme molecules into a lipid monolayer was studied. At the same time, the authors did not take into account the influence of the acidity of the subphase on the charge of the enzyme molecule. The influence of the length of the hydrophobic part of the lipid molecule on the enzyme adsorption process was studied in [11]. The work also studied the time of adsorption of glucose oxidase molecules on the surface of water before the onset of compression to phase transitions in the monolayer and its surface properties. Studying the effect of adsorption time on the properties of Langmuir monolayers is an urgent task, since in order to produce reproducible samples of touch coatings it is necessary to precisely control the number of enzyme molecules adsorbed by the film. The stability of dipalmitoylphosphate acid monolayers was studied in [12]. The authors paid more attention to the surface properties of monolayers during enzyme adsorption and did not focus on changes in the morphology of the films. It is worth noting the works that studied the process of adsorption of glucose oxidase by mixed monolayers of phospholipid molecules and nanoparticles [13]. The work also studied the effect of the thickness of films with an immobilized enzyme on the sensitivity of sensors based on them. It has been shown that at film thicknesses up to 10-11 nm, the concentration dependence of the sensor parameters remains linear over large concentration ranges. At the same time, there is not enough information about the stability of monolayers on the surface of the aqueous subphase. This aspect becomes especially relevant when trying to mass produce touch coatings using the Langmuir-Blodgett technology.

Therefore, in this work, the sensory properties of a coating based on a lipid Langmuir monolayer with molecules of the enzyme glucose oxidase formed were studied. For this purpose, the stability of monolayers of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine during the adsorption of glucose oxidase molecules was studied using compression isotherms and the morphology of films obtained on their basis was studied.

2. EXPERIMENTAL PART

2.1. Formation of Langmuir monolayers

To prepare a solution of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE, Sigma Aldrich, 99%), DPPE powder was dissolved in chloroform (Sigma Aldrich, 99%), resulting in a solution with a concentration of 10^{-3} M.

All experiments on the formation and study of the surface properties of Langmuir DPPE monolayers were carried out on a KSV Nima LB Trough KN2001 installation (Finland) with

a working area of 243 cm^2. The formation of Langmuir monolayers with immobilized molecules of the enzyme glucose oxidase was carried out according to the following procedure. A solution of DPPE in chloroform was applied to the surface of the aqueous subphase in an aliquot of 50 µl. Deionized water with a resistivity of 18 MOhm×cm and a glucose solution with a concentration of 0.015 mg/ml were used as a subphase. After 30 minutes, the monolayers were compressed by movable barriers at a constant rate of monolayer area loss equal to 0.7 cm^2/min. During the compression of the monolayer by movable barriers, the $\pi(A)$ isotherm was automatically recorded – the dependence of the change in surface pressure (π) on the area occupied by one molecule in the monolayer (A). Compression isotherms of DPPE monolayers formed in the absence and presence of dissolved GO molecules in the subphase are shown in **Fig. 1**.

By analyzing the compression isotherms, such monolayer parameters as the specific area per molecule in the non-tilting condensed phase (A_0) and the compression modulus (k) were obtained. The value of A_0 coincides with the abscissa of the intersection point of the tangent drawn to the condensed phase of the graph of the monolayer compression isotherm. To calculate the compression modulus we used the formula [14]:

$$k = -A_0 \frac{d\pi}{dA}, \quad (1)$$

where A_0 is the specific area per molecule in the non-obliquely condensed phase, k is the compression modulus of the monolayer, A is the area occupied by the monolayer. The values of k, A_0 are given in **Table** 1.

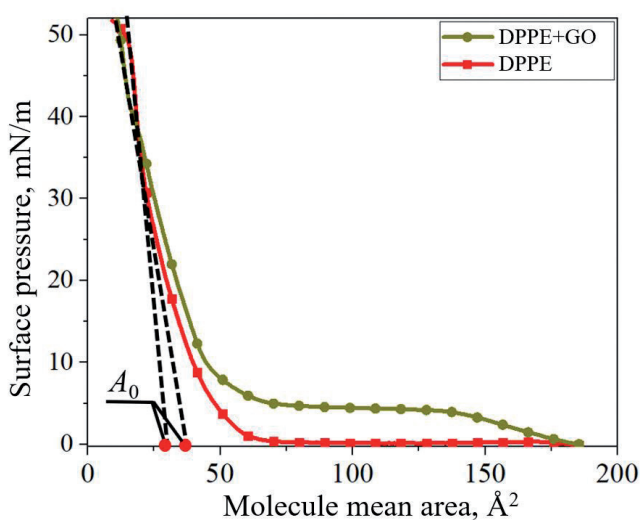

Fig. 1. *Compression isotherms of a DPPE monolayer formed on the subphase in the presence and absence of dissolved glucose oxidase enzyme molecules. Points A_0 – areas occupied by one molecule in a non-tilting-condensed phase.*

Table 1

Compression modulus (k) and area occupied by one molecule in the non-tilt-condensed phase of a DPPE monolayer (A_0), formed in the presence (DPPE+GO) and in the absence (DPPE) of enzyme molecules dissolved in the subphase, π_e – equilibrium pressure

Monolayer parameter	DPPE	DPPE+GO
k, (mN/m)	133	87
A_0, (Å^2)	32.5	49
π_e, (mN)	37.5	42

The transfer of monolayers to solid substrates was carried out using the Langmuir-Blodgett method at an equilibrium value of surface pressure (πe). Atomically smooth highly oriented pyrographite was used as a substrate.

2.2 Study of film surface morphology

The study of surface morphology was carried out using the atomic force microscopy method on an NT-MDT Ntegra installation in a hybrid mode with a single line scanning speed of 0.79 Hz. An NT-MDT NSG10 series cantilever with a tip radius not exceeding 10 nm was used. Image processing and calculation of the average roughness and film thickness were performed in the open source software Gwyddion 2.63.

2.3. Studying the sensory properties of Langmuir-Blodgett films with an immobilized enzyme using electrophysical methods

To measure the electrophysical parameters of the created films, a P-45X potentiostat was used. The sensory properties of the formed coatings were studied using the following method.

The formed Langmuir monolayers with the immobilized GO enzyme were compressed to a pressure of πe. Next, the monolayers were transferred by the Langmuir-Blodgett method onto graphite electrodes with a diameter of 2 mm at a speed of electrodes passing through the water surface of 1 mm/min. Thus, sensor coatings were formed, consisting of 2 Langmuir monolayers with an immobilized glucose oxidase enzyme. The electrodes were placed at a distance of 5 mm from each other in a reservoir with 2 ml of glucose solution of various concentrations. To eliminate the influence of electromagnetic interference, the measuring cell was placed in a steel box with a metal lid. Voltammograms of the measured solutions were obtained in the potential range from –1 V to +1 V with a voltage sweep rate of 0.2 mV/s. Impedance measurements were carried out in the frequency range from 50 KHz to 4 KHz with a bias voltage of 0.8 V.

3. RESULTS AND DISCUSSION

3.1. Study of the adsorption process of glucose oxidase molecules

An analysis of the compression isotherms presented in Fig. 1 shows that the adsorption of enzyme molecules leads to a change in the type of compression isotherm. The moment of phase transition between the liquid phase and the inclined-condensed phase is extended. Section II-III of the compression isotherm appears in cases where the contribution to the growth of surface pressure from the interaction of the head groups of surfactant molecules increases. Adsorption of GO molecules led to a decrease in the monolayer compressive modulus k from 133 mN/m to 87 mN/m and an increase in A_0 from 32.5 Å^2 to 49 Å^2. Thus, we can conclude that the adsorption of GO molecules by a DPPE monolayer has an expanding effect on the monolayer [15]. It is worth noting that after the collapse pressure was reached, a kink was observed in the compression isotherm, after which the value of the surface pressure remained constant. The presence of such a section is explained by the beginning of the formation of a multilayer membrane-like structure [16].

The study of the adsorption of GO molecules by DPPE film occurred in two stages. At the first stage, the value of the equilibrium pressure at which the monolayer was subsequently transferred to solid substrates, πe, was established. To do this, the monolayer was compressed to a surface pressure value corresponding to the middle of the non-obliquely condensed phase, after which the compression process stopped. A further change in the surface pressure is associated with the occurrence of relaxation processes in the monolayer. To analyze them, changes in surface pressure (π) versus time (t) were recorded automatically in automatic mode. The graph of the dependence $\pi(t)$ is shown in **Fig. 2.** The coordinate of the intersection point of the projection of the linear part of the graph $\pi(t)$ and the ordinate axis (π) corresponds to the value of the equilibrium pressure π_e, at which the rates of adsorption and desorption of the monolayer substance into the subphase are equalized [17]. The value of the tangent of the angle of inclination of the linear part of the graph $\pi(t)$ corresponds to the rate of decrease in surface pressure (k_d) due to the structural

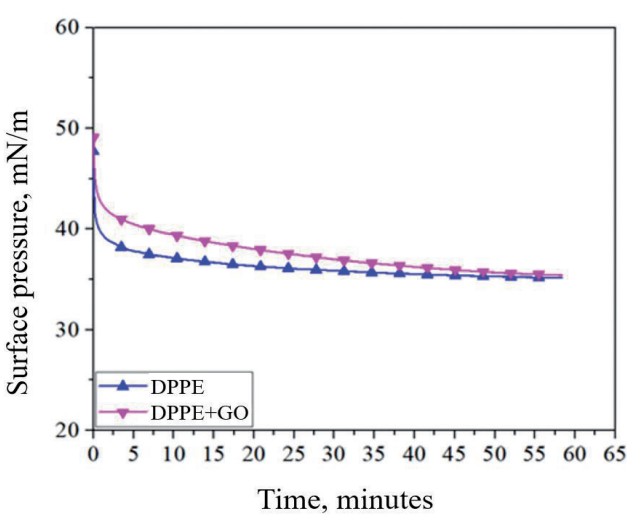

Fig. 2. *Graphs of the decrease in surface pressure of DPPE monolayers (a), obtained in the subphase in the presence and absence of dissolved enzyme molecules, at a constant monolayer area.*

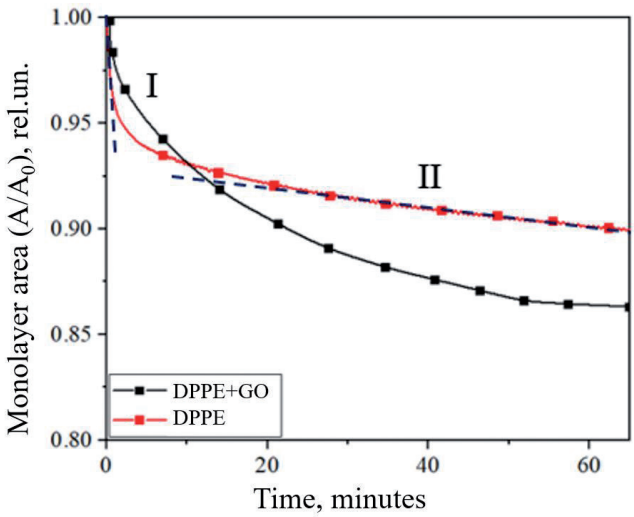

Fig. 3. *Graphs of the decrease in the area of DPPE monolayers, obtained in the subphase in the presence and absence of dissolved enzyme molecules, at a constant monolayer area.*

reorganization of molecules in the monolayer, as well as due to the possible loss of part of the substance of the monolayer under water [18,19]. It can be seen from Fig. 2 that the adsorption of GO molecules led to an increase in k_d and π_e from $0.5 \cdot 10^{-3}$ and 37.5 mN/m to $1.2 \cdot 10^{-3}$ and 42 mN/m, respectively.

To determine the mechanism of loss of the monolayer substance into the subphase, a method was used based on the analysis of desorption curves in the coordinates of the relative loss of area versus time. For this purpose, curves of the decrease in monolayer area were recorded at constant surface pressure near the point of phase transition of the monolayer two-dimensional liquid - condensed film. **Fig. 3** shows graphs of changes in the relative monolayer area over time for DPPE monolayers formed on the subphase in the presence and absence of dissolved GO molecules. In the presented desorption curves, two sections can be distinguished, approximated by a linear dependence. This is due to the presence of several mechanisms of desorption of the monolayer substance into the near-surface layer. In the literature, two types of desorption are distinguished. The first is desorption, controlled by the departure of the monolayer substance into a near-surface layer of finite thickness. And the second is the diffusion transition of monolayer molecules from a static near-surface layer deep into the subphase. In the desorption graph presented in Fig.3, areas with the first and second desorption mechanisms are marked with numbers (I) and (II), respectively. To describe the desorption processes (I) and (II), expressions (1) and (2) were used [20,21]:

$$ln\frac{A}{A_0} = -k_s t, \tag{2}$$

$$ln\frac{A}{A_0} = -k_v t, \tag{3}$$

where A/A_0 is the relative change in the monolayer area over time t, k_v and k_s are the desorption and diffusion coefficients, respectively.

The numerical values of the coefficients k_s and k_v correspond to the angle of inclination of the linear sections of the area loss curves presented in **Fig. 4**. The values of ks and kv are given in **Table 2**.

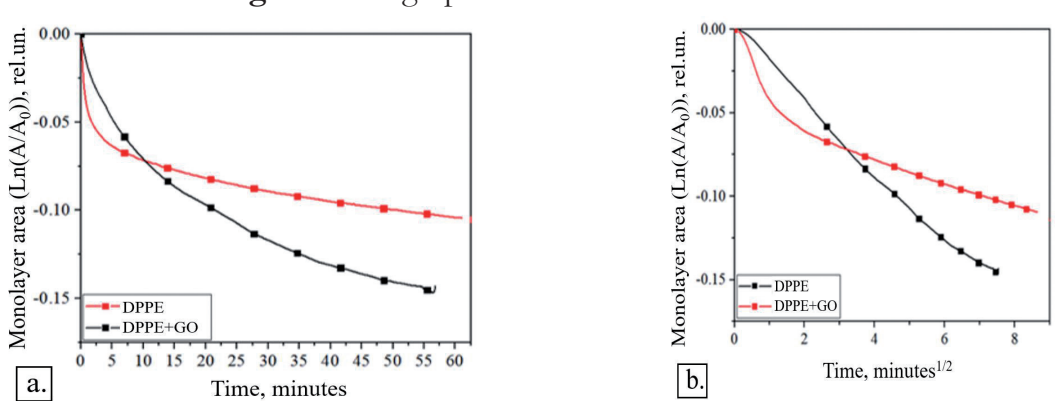

Fig. 4. *Curves of the decrease in the area of DPPE monolayers (a, b), formed in the presence and absence of enzyme molecules dissolved in the subphase.*

Table 2

Desorption coefficients of DPPE monolayers formed on the subphase in the presence and absence of dissolved GO molecules

	DPPE	DPPE+GO
T, min	3	12
k_s	$5 \cdot 10^{-4}$	$6 \cdot 10^{-4}$
k_v	$3.3 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$
k_d	$0.5 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$

Table 3

Average roughness (R_a), thickness (L) and surface area (S_q) of monolayer DPPE films transferred at different enzyme adsorption times.

GO adsorption time	5	30	60
R_a, nm	3.6	3.3	2.4
S_q, mkm^2	0.3	0.3	0.3
L, nm	10.7	10.4	8.7

From Table 2 it can be seen that the adsorption of GO molecules by a DPPE monolayer leads to a change in desorption coefficients, controlled by the transition of the monolayer substance to the surface layer and the transition of the substance from the surface layer deep into the subphase. The coefficient k_s increases from $5 \cdot 10^{-4}$ to $6 \cdot 10^{-4}$, and k_v decreases from $3.3 \cdot 10^{-2}$ to $1.1 \cdot 10^{-2}$ in the presence of enzyme molecules in the subphase. The presence of GO molecules in the subphase leads to a decrease in the rate of desorption, which is controlled by the transition of the monolayer substance to the near-surface layer, and an increase in the rate of transition of molecules of the substance from the near-surface layer deep into the subphase.

3.2. Effect of enzyme adsorption time on the surface morphology of the formed films

Fig. 5 shows images of the surface morphology of monolayer DPPE films with adsorbed aggregates to GO molecules transferred at different adsorption times. **Table 3** shows the values of the roughness of the formed films, the average thickness and the average surface area of the films.

Adsorption of GO by monolayers leads to a change in the morphology of films obtained on their basis. When GO is adsorbed by a DPPE monolayer for 5 minutes, regions with a height of 12 to 24 nm are formed on the film surface. The GO molecule has dimensions of 6×5.2×7 nm [22], while the DPPE monolayer has a thickness of about 3 nm. Thus, we can conclude that these areas are aggregates of GO molecules adsorbed by a monolayer of DPPE molecules. When the adsorption time increases to 30 minutes, the area occupied by GO islands increases. In this case, the thickness of individual sections increases to 25-30 nm. The average film roughness decreases from 3.6 nm to 3.3 nm, which can be explained by an increase in the area occupied by GO aggregates. A further increase in the adsorption time to 60 minutes leads to a decrease in surface roughness and average film thickness. This behavior can be associated

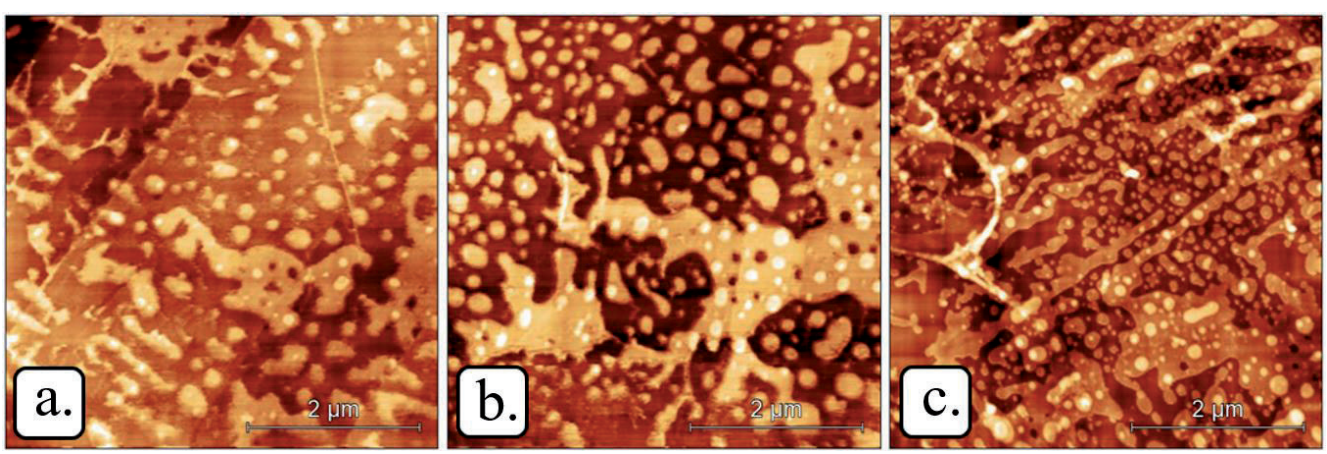

Fig. 5. *Images of the surface morphology of monolayer DPPE films formed on a subphase containing GO at GO adsorption times of 5 minutes (a), 30 minutes (b) and 60 minutes (c).*

with a decrease in the rate of transition of the monolayer substance into the diffusion layer. As a result, the area covered by aggregates of GO molecules increases. At the same time, the height of individual aggregates decreases to 22-25 nm.

An increase in the adsorption time of the GO enzyme on the film leads to a change in its morphology. The most developed morphology is observed in the film transferred 5 minutes after holding the monolayer at constant pressure (the total adsorption time, including the time of compression of the monolayer by movable barriers, is 125 minutes). Further adsorption leads to a decrease in the average surface roughness and average film thickness. Therefore, a monolayer with an adsorption time of 30 minutes was used to form the sensor coating.

3.3. Studying the sensory properties of the formed coatings using voltammetric and impedancemetric methods

The formed sensor coatings were studied by cyclic voltammetry. Cyclic voltammograms and graphs of changes in maximum currents between the electrodes at different concentrations of glucose in the solution are shown in **Fig. 6**. For a biofilm with the immobilized GO enzyme, a linear dependence of the current on the concentration of glucose in the solution is observed. The increase in current with increasing concentration to 0.9 mg/ml is 0.25 μA for forward bias and 0.35 μA for reverse bias. In the absence of biofilm on the electrodes, concentration changes in current are random. The large operating currents are explained by the absence of a film of lipid molecules, which is a dielectric, on the electrodes.

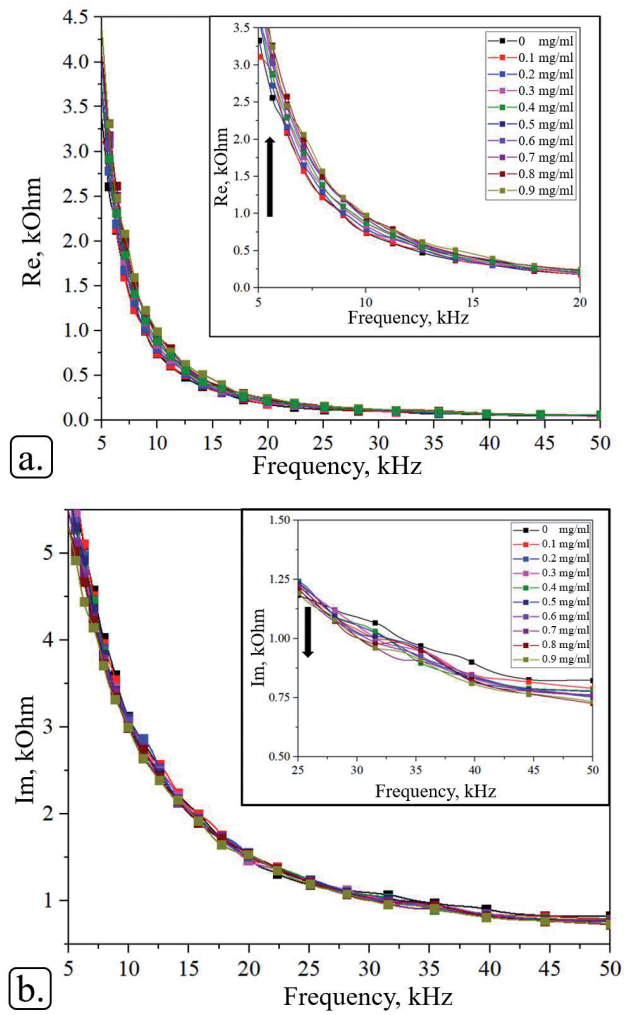

Fig. 7. *Frequency dependences of the real (a) and imaginary (b) parts of the total impedance of the system at different concentrations of glucose molecules in solution.*

Fig. 7 shows the frequency dependences of the real (a) and imaginary (b) parts of the total

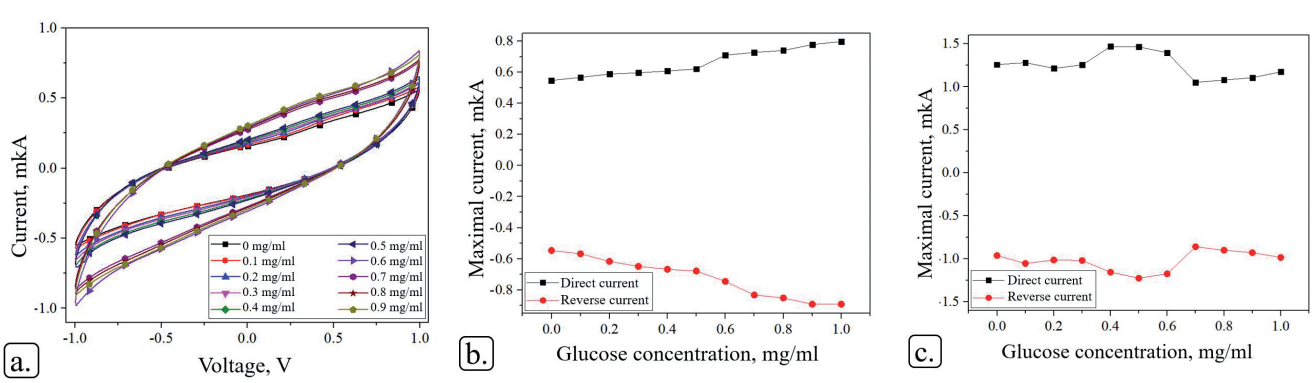

Fig. 6. *Cyclic voltammograms (a) and changes in the maximum values of forward and reverse currents in the presence (b) and in the absence of a sensor film (c) at different concentrations of glucose in the solution.*

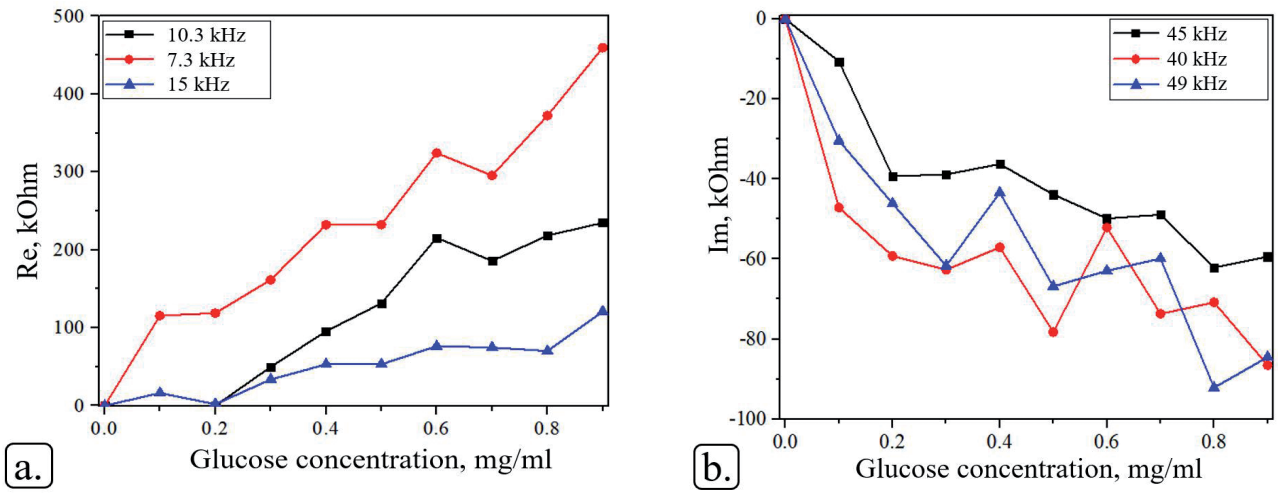

Fig. 8. *Concentration dependences of the real (a) and imaginary (b) parts of the total impedance of the system for the corresponding frequencies.*

impedance of the measured cell at different concentrations of glucose molecules in the solution. In the frequency range from 5 to 20 kHz and from 30 to 50 kHz, frequencies can be distinguished for which the concentration dependence of the real and imaginary parts of the impedance is monotonic.

Thus, the concentration dependence of the real part of the impedance at frequencies of 7.3 kHz, 10 kHz and 15 kHz has a sublinear form. The concentration dependence of the imaginary impedance particles at frequencies of 40 kHz, 45 kHz and 49 kHz can be approximated by an exponential function. The corresponding concentration dependences are shown in **Fig. 8**.

4. CONCLUSION

In this work, the influence of the adsorption time of glucose oxidase molecules on the surface properties of Langmuir DPPE monolayers and their morphology was studied. Based on the formed monolayers, sensor bilayer coatings were created. Differences in the adsorption time of the enzyme lead to differences in the morphology of the films. The process of incorporation of glucose oxidase enzyme molecules into a Langmuir monolayer of DPPE lipid molecules was studied. The adsorption of GO molecules has an expanding effect on the Langmuir DPPE monolayer. Adsorption of GO molecules leads to a structural restructuring of the monolayer, accompanied by an increase in the specific area of the monolayer, a decrease in the rate of decrease in surface pressure and an increase in pressure πe. When GO is adsorbed by a DPPE monolayer, the filling rate of the near-surface diffusion layer decreases, but at the same time the rate of transition of molecules from the diffusion layer into the bulk of the subphase increases. The increase in the specific area per molecule during the adsorption of an enzyme by a monolayer is associated with the floating of the enzyme and the formation of an adsorption Gibbs monolayer at the water-air interface, which is subsequently compressed by the emerging monolayer of lipid molecules. As a result, when the monolayer is compressed, the position of all formed phases shifts to a region of large areas. An increase in the adsorption time of GO molecules leads to a decrease in the average roughness of the film and its thickness.

Voltammetry and impedance measurements of the films showed the sensitivity of the sensor coating to glucose molecules in aqueous solutions. The presence of a linear concentration dependence of maximum and minimum currents and resistances in the glucose concentration range from 0 to 1 mg/ml makes such sensor coatings promising for use as sensor coatings.

Thus, the possibility of creating an enzymatic glucose biosensor based on a LB film from DPPE phospholipid molecules with immobilized glucose oxidase enzyme molecules has been demonstrated. The main advantage of such biosensors is their high selectivity and sensitivity with respect to the molecules being detected.

REFERENCES

1. Ariga K, Nakanishi T, Michinobu T. Immobilization of Biomaterials to Nano-Assembled Films (Self-Assembled Monolayers, Langmuir-Blodgett Films and Layer-by-Layer Assemblies) and Their Related Functions. *Journal of Nanoscience and Nanotechnology*, 2006, 6:2278-2301.
2. Caseli L. Enzymes immobilized in Langmuir-Blodgett films: Why determining the surface properties in Langmuir monolayer is important?. *An Acad Bras Cienc*, 2018, 90:631-644.
3. Rodrigues RT, Nordi CFS, Siqueira Junior JS, Caseli L. Effect of interfering agents for urease immobilized in Langmuir-Blodgett films of controlled molecular architecture. *Thin Solid Films,* 2020, 704:138043.
4. Gorbachev IA, Smirnov AV. Biosensor based on Langmuir-Blodgett film with alcohol oxidase enzyme. *RENSIT: Radioelectronics. Nanosystems. Information Technologies*, 2023, 15(3):307-316. DOI: 10.17725/rensit.2023.15.307.
5. Zaitsev SY. Functional Langmuir films with glucose oxidase. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 1993, 75:211-216.
6. Wang K-H, Syu M-J, Chang C-H, Lee Y-L. Immobilization of glucose oxidase by Langmuir–Blodgett technique for fabrication of glucose biosensors: Headgroup effects of template monolayers. *Sensors and Actuators B*, 164:29-36.
7. Furtado FAS, Caseli L. Enzyme activity preservation for galactose oxidase immobilized in stearic acid Langmuir-Blodgett films. *Thin Solid Films,* 2020, 709:138253.
8. Saha S, Chowdhury J. Sustained and improved enzymatic activity of trypsin immobilized in the Langmuir Blodgett film of DPPC: A rapid enzyme sensor for the detection of Azocasein. *Materials Chemistry and Physics,* 2020, 243:122647.
9. Gorbachev IA, Smirnov AV, Kuznetsova IE, Kolesov VV. Temperature influence on the formation of Langmuir monolayers with Ni arachidic acid and Ni arachidate clusters. *RENSIT: Radioelectronics. Nanosystems. Information Technologies*, 2023, 15(3):295-306. DOI: 10.17725/rensit.2023.15.295.
10. Li J, Rosilio V, Boissonnade M-M, Baszkin A. Adsorption of glucose oxidase into lipid monolayers: effect of a lipid headgroup charge. *Colloids and Surfaces B: Biointerfaces*, 2003, 29:13/20.
11. Zhang J, Rosilio V, Goldmann M, Boissonnade M-M, Baszkin A. Adsorption of Glucose Oxidase into Lipid Monolayers. Effect of Lipid Chain Lengths on the Stability and Structure of Mixed Enzyme/Phospholipid Films. *Langmuir,* 2000, 16:1226-1232.
12. Lee Y-L, Lin J-Y, Lee S. Adsorption Behavior of Glucose Oxidase on a Dipalmitoylphosphatic Acid Monolayer and the Characteristics of the Mixed Monolayer at Air/Liquid Interfaces. *Langmuir*, 2007, 23:2042-2051.
13. Ohnuki H, Saiki T, Kusakari A, Endo H, Ichihara M, Izumi M. Incorporation of Glucose Oxidase into Langmuir-Blodgett Films Based on Prussian Blue Applied to Amperometric Glucose Biosensor. *Langmuir,* 2007, 23:4675-4681.
14. Vu TT, Kharitonova NV, Maiorova LA, Gromova OA, Torshin IY, Koifman OI, Compression Speed as a Parameter Changing the Dimensionality of Corrole Nanostructures in Layers at the

Air-Water Interface. *Macroheterocycles,* 2018, 11(3):286-292.

15. Yanklovich AI. Regular multimolecular structures of surfactants - Langmuir-Blodgett films. *Advances in colloidal chemistry.* Leningrad, Khimiya Publ., 1991, 262-291.
16. Gopal A, Lee KYC. Headgroup Percolation and Collapse of Condensed Langmuir Monolayers. *J. Phys. Chem. B,* 2006, 110:22079-22087.
17. Sanchez CC, Nino MRR, Patino JMR. Relaxation phenomena in monoglyceride films at the air–water interface. *Colloids and Surfaces B: Biointerfaces,* 1999, 12:175-192.
18. Rabinovitch W, Robertson RF, Mason AG. Relaxation of surface pressure and collapse of unimolecular films of stearic acid. *Can. J. Chem.*, 1960, 38:1881-1890.
19. Hac-Wydro K, Wydro P, Dynarowicz-Latka P. Metal ions binding to tri-n-octylphosphine oxide (TOPO) monolayer spread at the air/water interface. *Thin Solid Films,* 2008, 516:8839-8843.
20. Gorbachev IA, Shtykov SN, Brezesinski G, Glukhovskoy EG. Studying of Quantum Dots Langmuir Monolayers Stability at the Different Subphase Temperature. *BioNanoSci.,* 2017, 7:686-691.
21. Ter-Minassian-Saraga L. Recent work on spread monolayers, adsorption and desorption. *Journal of Colloid Science,* 1956, 11:398-418.
22. Hecht HJ, Kalisz HM, Hendle J, Schmid RD, Schomburg D. Crystal Structure of Glucose Oxidase from Aspergillus niger Refined at 2·3 Å Reslution. *Journal of molecular biology,* 1993, 229(1):153-172.