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INFLUENCE OF ZINC OXIDE NANOPARTICLES ON BILAYER LIPID MEMBRANE PROPERTIES

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The effect of zinc oxide nanoparticles (ZnO) on the stability and conductivity of the bilayer lipid membrane (BLM) in solution was studied. It has been shown that ZnO nanoparticles increase the stability of BLM in an electric field, and BLM becomes more stable with increasing their concentration. The increase in the stability of BLM in an electrical field is mainly due to the increase in the coefficient of linear tension of the pore edge, which is forming in BLM. It is also shown, that the presence of ZnO nanoparticles in the solution surrounding BLM leads to a decrease in the BLM conductivity.

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***Keywords*:** zinc oxide nanoparticles, stability and conductivity of BLM.

Introduction. It has now been established that the physical and chemical properties of nanoparticles and nanomaterials differ greatly depending on their sizes [1–3]. Often they acquire properties that the macroscopic phase does not possess. This circumstance led to the fact that they began to be actively implemented in almost all areas of scientific and practical human activity. On the other hand, there were well-founded fears of their damaging effects on biological systems [4, 5]. It is known that nanoparticles of metal oxides have a less damaging effect on a biological object than metallic nanoparticles [6]. Therefore, at present, both the properties of metal oxide nanoparticles and the possibility of their application in practice are being intensively studied. It should be noted that the practical application of nanoparticles, in particular nanoparticles of metal oxides, is often accompanied by their unfavorable attraction to the biosystem. There are good reasons to believe that the primary target of the action of nanoparticles on a biological object is the cell membrane [7]. Because of the fact that the cell membrane is very heterogeneous and has a complicated structure, it seems appropriate to investigate the effect of nanoparticles

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on a model membrane, a bilayer lipid membrane (BLM), which simulates the lipid bilayer of the biological membrane rather well [8, 9].

Previously, we investigated the effect of copper oxide nanoparticles on the BLM electrical parameters [10]. This paper is devoted to the study of the influence of zinc oxide nanoparticles on the most important characteristics of BLM – stability and conductivity. The results we obtained can be used to clarify the role of bilayer regions of biomembranes in the mechanism of action of zinc oxide nanoparticles on cells. It should be noted that zinc oxide nanoparticles are of great interest primarily because of their antibacterial and photocatalytic properties. ZnO nanoparticles affect the growth of *Escherichia coli* bacteria [11] and at high concentrations of ZnO (3–10 *mM*) all bacteria are killed. The photocatalytic activity causes the antiseptic properties of ZnO nanoparticles and, consequently, the possibility of their use in water treatment.

Materials and Methods. In all experiments BLM was prepared at room temperature (20–25°*C*) in accordance with the procedure described in [12]. The experiments were performed on BLM obtained from a mixture of 1, 2-dipalmitoyl-sn--glycero-3-[phospho-1-serine] (DPPS) and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) from the company "Avanti Polar Lipids" in a 1 : 1 ratio, dissolved in *n*-decane (4%). BLM was obtained in 0.1 *M* NaCl solution, pH 6. The diameter of the hole on which the BLM was formed was 1 *mm*. Because of the torus at the boundary of the hole, the flat area of the BLM was 0.65 mm^2. 1 *mg* of nanoparticles was dissolved in 1 *mL* acidic medium (0.1 *M* citric acid + 0.1 *M* sodium citrate, pH 3.8) and sonicated for 30 *min*. To measure the electrical parameters of the BLM, Keithley 427 current amplifier was used in accordance with the procedure described in [13]. Voltage in the range of $0.20 - 0.55$ *V* was applied to the BLM using chlorine-silver electrodes connected to an ADC (E14-140-M) and controlled by a computer. The geometrical parameters of the BLM were checked by measuring their electrical capacitance using cyclic current-voltage characteristics implemented in the Lab VIEW user program. These measurements made it possible to estimate the thickness of the BLM, which in all experiments was 45–55 Å. The effect of ZnO nanoparticles with an average size of 50 *nm* on the stability and conductivity of BLM was studied at ZnO concentrations of 5, 20 and 50 $\mu g/mL$. Nanoparticles were provided by the Institute of Medical Physics and Biophysics, University of Leipzig, Germany.

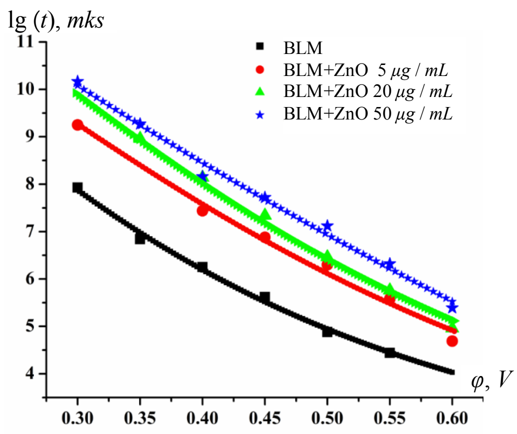
Results and Discussion. The stability of BLM was determined by the average lifetime of BLM at a given value of potential. The effect of ZnO nanoparticles on the average lifetime of a BLM in an electric field was investigated by the standard method described in [14–16]. The main parameter characterizing the degree of stability of BLM is their mean lifetime at a given voltage across the BLM. Investigation of the stability of BLM in an electric field was carried out according to the standard procedure using the dependence of the average lifetime $\bar{t}$ of BLM on the potential φ in the form $\bar{t}(\varphi)$:

$$\bar{t}(\varphi) = \frac{(k_B T)^{3/2}}{4\pi D c_0 S \gamma (\sigma + C\varphi^2/2)^{1/2}} \cdot \exp\left(\frac{\pi\gamma^2}{(\sigma + C\varphi^2/2)\, k_B T}\right), \quad (1)$$

where σ is the BLM surface tension; γ is the linear tension of the pore edge in BLM;

D is the diffusion coefficient of defects in the space of radii; φ is the potential difference across the membrane; k_B is the Boltzmann constant; T is the temperature; C is the reduced electrical capacitance, which is determined by the relation: $C = C_0(\varepsilon_w/\varepsilon_m - 1)$, where $C_0 = \varepsilon_0\varepsilon_m/h$ is the specific electrical capacitance of the BLM; $\varepsilon_w, \varepsilon_m$ and ε_0 are the dielectric constants of water, BLM and the vacuum respectively; c_0 is the pore concentration on the BLM; S is the area of the BLM. We rewrite Eq. (1) in a form convenient for its comparison with experimental data:

$$\lg\bar{t} = A - \frac{1}{2}\lg(1+M\varphi^2) + \frac{B}{1+M\varphi^2},$$
$$A = \lg\left(\frac{(k_BT)^{3/2}}{4\pi Dc_0S\gamma\sigma^{1/2}}\right),\ B = \frac{\pi\gamma^2\lg e}{\sigma k_BT},\ M = \frac{C}{2\sigma}. \tag{2}$$



The decrease of the average lifetime of BLM at increasing potential in the presence of ZnO nanoparticles at given concentrations. Points are experimental data (average of 5 measurements at each potential difference) and solid lines are theoretical curves from least-square fitting using Eq. (2).

The procedure for comparing the theoretical curve (2) with the experimental data was as follows. Initially, experimental points were obtained that depict the dependence of the average BLM lifetime on the potential in the absence of nanoparticles in solution, and then in the presence of zinc oxide nanoparticles. Then, using the least squares method, the curve was fitted according to Eq. (2) using experimental points. Figure presents the results of study of the ZnO nanoparticles concentration effect on the BLM stability in an electric field.

The parameters A, B and M can be determined by comparing the theoretical curves with experimental points in Figure. Then, using their values for Eq. (2), the values of the main parameters were determined, on which the BLM stability depends in the electric field: the BLM tension σ, the linear tension of the pore edge in the BLM γ and the parameter c_0SD.

The BLM stability parameter c_0SD, which is the product of the pore number c_0S on the BLM and the diffusion coefficient D of defects in the radius space, gives information on the number of pores on the BLM, and on the intensity of diffusion pore growth, on the other hand. The numerical values of the stability parameters of the BLM were found as follows. First, in the absence of nanoparticles in the surrounding BLM solution, the BLM tension was determined, which was found equal to $\sigma = 7.9 \cdot 10^{-3}$ N/m. Then, using this value, the value for the linear tension of the pore edge in the BLM in the absence of nanoparticles was determined $\gamma = 1.6 \cdot 10^{-11}$ N. These values are in good agreement with the literature data [14,17]. Then, in a similar way, we determined the values of the parameters σ, γ and c_0SD at various concentrations of ZnO nanoparticles in solution. The values of the parameters σ, γ and c_0SD thus determined are listed in Tab. 1.

Table 1

The parameters σ, γ and c_0SD, determined from a comparison of the theoretical curve (2) with the experimental data presented in Figure

ZnO, $\mu g/mL$	σ, N/m	$\gamma, \times 10^{-11}$ H	$c_0SD, \times 10^{-21}$ m^2/c
0	0.00791883	1.64692	0.53536
5	0.01518747	2.42858	6.91122
20	0.01322314	2.41266	7.45567
50	0.01706667	2.64834	5.97863

The experimental data in Figure show, that the mean lifetime of the BLM increases in the presence of ZnO nanoparticles with increasing concentration. Analysis and numerical calculations using Eq. (1) show that the average lifetime depends strongly on the parameter γ and with its increase the average lifetime of the BLM also increases. As can be seen from the experimentally determined values of the parameter γ, presented in the second column of Tab. 1, an increase in the parameter γ correlates with the growth of stability of the BLM in Figure.

The effect of zinc oxide nanoparticles on the conductivity of BLM was also investigated. It was shown that with an increase in the concentration of ZnO nanoparticles in an electrolyte solution, the conductivity of BLM decreases (see Tab. 2).

Table 2

Conductivity (g, $\times 10^{-9}$ Om^{-1}) of BLM in the presence of ZnO nanoparticles in solution

ZnO, $\mu g/mL$	0	5	20	50
Average Conductivity	0.27	0.26	0.25	0.23
Std. Error	0.01	0.01	0.02	0.02

Thus, an analysis of the experimental results showed, that the presence of zinc oxide nanoparticles in the surrounding BLM solution leads to an increase in the stability of the BLM in an electric field. Comparison of the experimental data with the results of theoretical calculations showed that the increase in stability of the BLM in an electric field is mainly due to an increase in the linear tension coefficient of the pore edge, which is formed in the BLM. Studies on the conductivity of BLM also showed that the presence of ZnO nanoparticles in the surrounding BLM solution leads to a decrease in the conductivity of the BLM.

Since the isoelectric point of ZnO is in the region of high pH (pH 9, 10), the ZnO nanoparticles were positively charged in the solutions we used [18, 19]. This circumstance suggests that the mechanism of its action on the stability and conductivity of BLM is similar to the mechanism of action of multiply positively charged ions, such as calcium [20, 21]. We can assume that, like calcium ions, positively charged ZnO nanoparticles are associated with negatively charged atomic groups on the BLM surface, resulting in a compaction of the BLM structure, which leads to an increase in stability and a decrease in the BLM conductivity.

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ՑԻՆԿԻ ՕՔՍԻԴԻ ՆԱՆՈՄԱՍՆԻԿՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԵՐԿՇԵՐՏ ԼԻՊԻԴԱՅԻՆ ԹԱՂԱՆԹՆԵՐԻ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԻ ՎՐԱ

Ուսումնասիրվել է ցինկի օքսիդի (ZnO) նանոմասնիկների ազդեցությունը երկշերտ լիպիդային թաղանթների (ԵԼԹ) վրա լուծույթում: Ցույց է տրվել, որ ZnO նանոմասնիկները մեծացնում են ԵԼԹ-ի կայունությունն էլեկտրական դաշտում և ԵԼԹ-ին առավել կայուն է դառնում նրանց կոնցենտրացիայի մեծացմանը զուգահեռ: ԵԼԹ-ի կայունության մեծացումն էլեկտրական դաշտում հիմնականում պայմանավորված է ԵԼԹ-ում ձևավորված ծակոտիների եզրագծերի գծային լարվածության գործակցի աճով: Ցույց է տրված նաև, որ (ZnO) նանոմասնիկների առկայությունը լուծույթում հանգեցնում է ԵԼԹ-ի հաղորդականության նվազմանը: