PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Physical and Mathematical Sciences 2023, **57**(1), p. 23–30

Physics

STABILITY AND CONDUCTIVITY OF BILAYER LIPID MEMBRANE IN PRESENCE Al_2O_3 NANOPARTICLES

Ts. M. JOMARDYAN [1*], G. V. ANANYAN [2**], V. B. ARAKELYAN [1***]

[1] *Chair of Molecular Physics, YSU, Armenia*
[2] *Interfaculty Research Laboratory of Structural Biophysics, YSU, Armenia*

The effect of aluminum oxide nanoparticles (Al_2O_3) on the stability and conductivity of BLM (the bilayer lipid membrane) in solution was studied. It has been shown that Al_2O_3 nanoparticles increase the stability of BLM in an electric field, and BLM becomes more stable with increasing their concentration. The experimental data are analyzed in terms of the well-known theory of BLM stability, which is based on the concept of spontaneous formation of microscopic pores on the BLM, the development of which leads to the loss of BLM stability. It is shown that aluminum oxide nanoparticles increase the value of the coefficient of linear tension of the pore edge, and with an increase in the concentration of nanoparticles, the linear tension also increases. It has been shown that the presence of nanoparticles in the solution leads to a decrease in BLM conductivity.

https://doi.org/10.46991/PYSU:A/2023.57.1.023

MSC2010: Primary: 81V55; Secondary: 82D80.

Keywords: aluminum oxide nanoparticles, stability and conductivity of BLM.

Introduction. In recent years, interest in nanoparticles in the field of fundamental and applied science, as well as industry and biomedicine gradually grows due to their unique properties. It has been established that the physical and chemical properties of nanoparticles significantly differ from their properties at macroscopic dimensions [1]. Sometimes nanoparticles acquire properties that are absent in the macroscopic phase. Therefore, nanoparticles began to be actively introduced into all areas of scientific and practical human activity. On the other hand, reasonable concerns have arisen about their damaging effect on biological systems [2]. As a rule, metal oxide nanoparticles have a less damaging effect on biological systems than metal

* E-mail: tsovinar.jomardyan@ysu.am
** E-mail: angay@ysu.am
*** E-mail: v.arakelyan@ysu.am

nanoparticles. The most widely used nanoparticles are metal oxides, especially copper, zinc, and aluminum oxides [3, 4]. The nanoparticles exhibit their biological activity at the stage of their interaction with cell membranes. Therefore, it can be considered that the cell membrane is the primary target for the action of nanoparticles on a biological object [5]. However, due to the fact that the cell membrane has a complex structure and is highly heterogeneous, it seems appropriate to study the effect of nanoparticles on a bilayer lipid model membrane (BLM), which almost unambiguously the lipid bilayer of a biological membrane [6]. This work is devoted to the study of the effect of nanoparticles of aluminum oxide (Al_2O_3) on the most important characteristics of BLM: stability and conductivity. For many years, aluminum oxide nanoparticles have been widely used in practice. Note that they are applied in the production of transparent and high-strength ceramics; as additives in the cosmetic industry; in the medical industry in the drug delivery system; as protection against microorganisms [7].

Materials and Methods. All experiments were carried out at room temperature (20–25°C) according to the procedure described in [8]. BLM was formed from a mixture of 1,2-dioleoyl-*sn*-glycero-3-[phospho-*l*-serine] (DPPS) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) from Avanti Polar Lipids in a ratio of 1:1, which were dissolved in *n*-decane (4%), in 0.1 *M* NaCl solution pH 6. BLM was formed on the hole with a diameter of 1 *mm*. Due to the torus at the whole boundary, the area of the flat part of the BLM was 0.65 mm^2. Nanoparticles weighing 1 *mg* dissolved in an acidic medium (0.1 *M* citric acid + 0.1 *M* sodium citrate, pH 3.8) and sonicated for 30 *min* (final concentration 1 mg/mL). The electrical parameters of the BLM were measured using Keithley 427 current amplifier. Voltage, in the range of 0.20–0.55 V, was applied to the BLM using silver-chlorine electrodes connected to the ADC (E14-140-M) and controlled by a computer. The geometric parameters of the BLM were checked by measuring their electrical capacitance using cyclic current-voltage characteristics implemented in the user program Lab VIEW. The capacitance of the BLM was measured by applying a symmetrical triangular voltage with a sweep rate of 0.07 V/s. These measurements made it possible to estimate the BLM thickness, which was 45–55 Å in all experiments. The effect of nanoparticles with an average size of 50 *nm* on the BLM stability and conductivity was tested at different concentrations of Al_2O_3 : 5, 20, and 50 $\mu g/mL$. Nanoparticles are represented by the Institute of Medical Physics and Biophysics of the University of Leipzig.

Results and Discussion. Currently, the most widely used method for assessing BLM stability is based on measuring the average lifetime of BLM under certain conditions. The stability of the BLM in an electric field is defined as the average lifetime of the BLM at a given voltage. In [9–11], the BLM stability in an electric field was experimentally studied and its theoretical description (the theory of BLM electrical breakdown) was developed in detail. These studies made it possible to reveal the main parameters on which the stability of the BLM in an electric field depends and to determine them by comparing the experimental data with the theoretical curve of the dependence of the average BLM lifetime $\bar{t}(\varphi)$ on the potential φ. This method was used to study the effect of Al_2O_3 nanoparticles in the surrounding BLM solution on the average BLM lifetime in an electric field. It was shown in [9], that the dependence of the average BLM lifetime on the potential has the form:

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

where σ is the lateral tension of the BLM; γ is linear tension of the pore edge in BLM; D is diffusion coefficient of pores in the space of radii; φ is potential difference across the membrane; k_B is Boltzmann's constant; T is temperature; C is the reduced electric capacitance, which is determined by the relation $C = C_0(\varepsilon_w/\varepsilon_m - 1)$, where $C_0 = \varepsilon_0\varepsilon_m/h$ is specific electric capacitance of BLM; ε_w; ε_m and ε_0 are the dielectric constants of water, BLM and the vacuum respectively; c_0 is concentration of pores on BLM; S is BLM area. For the convenience of comparing theoretical formula (1) with experimental data, we rewrote (1) in the form [10]:

$$\lg\bar{t} = A - \frac{1}{2}\lg\left(1 + M\varphi^2\right) + \frac{B}{1 + M\varphi^2}, \tag{2}$$

$$A = \lg\left(\frac{(k_BT)^{3/2}}{4\pi D c_0 S\gamma\sigma^{1/2}}\right), \quad B = \frac{\pi\gamma^2\lg e}{\sigma k_BT}, \quad M = \frac{C}{2\sigma}. \tag{3}$$

The experiment and procedure for comparing the experimental data with the theoretical curve (2) were thoroughly described in [11]. Experimental points, which depicted the dependence of the average BLM lifetime on the potential, were obtained at first in the absence of nanoparticles in the solution, and then in the presence of aluminum oxide nanoparticles in different concentrations. Then, the curve was fitted according to Eq. (2), using experimental points by the least squares method. Then determined the values of the parameters on which depend the BLM stability. The Figure presents the results of the study of the concentration effect of Al_2O_3 nanoparticles on the BLM stability in an electric field.

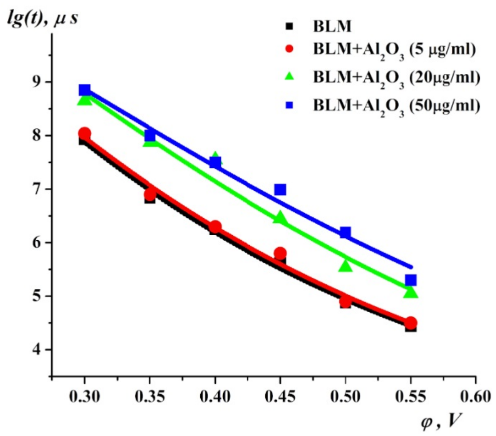

Fig. 1. Dependence of the average BLM lifetime on the potential in the presence of Al_2O_3 nanoparticles at concentrations of 5 $\mu g/mL$, 20 $\mu g/mL$, and 50 $\mu g/mL$: dots are experimental data (average of 5 measurements at each potential), solid lines are theoretical curves drawn according to Eq. (2) by the least squares method.

As shown in Fig. 1, with an increase in the concentration of Al_2O_3 nanoparticles, the average BLM lifetime increases. An analysis of Eq. (1) shows that the average BLM lifetime strongly depends on the parameter γ, and with an increase in γ, the average BLM lifetime also increases. The parameters A, B, and M were determined from a comparison of the theoretical curves with the experimental points presented in Figure. Then, using Eq. (3), were found the values of the main parameters, which characterized the stability of the BLM in an electric field: the BLM tension (σ), the coefficient of linear tension of the pore edge (γ) and the parameter c_0SD. The values of the parameters σ, γ, and c_0SD are given in Tab. 1.

Table 1

Parameters σ, γ and c_0SD determined from a comparison of the theoretical curve with the experimental data presented in Figure

Al_2O_3, $\mu g/mL$	σ, N/m	$\gamma \cdot 10^{-11}$, N	$c_0SD \cdot 10^{-22}$, m^2/s
0	0.0079	1.6	5.3536
5	0.0093	1.7894	6.2821
20	0.0159	2.4390	6.7068
50	0.0199	2.6603	5.4986

The lateral tension of the BLM is equal to $\sigma = 7.9 \cdot 10^{-3}$ N/m in absence of Al_2O_3 nanoparticles in the surrounding BLM solution (Tab. 1). Further, this tension value makes it possible to determine the value of the linear tension of the pore edge in the BLM $\gamma = 1.6 \cdot 10^{-11} N$. These values are in good agreement with the literature data [12, 13]. Parameter c_0SD, which is considered the product of numbers c_0S of BLM diffusion coefficient D of defects in the space of radii, on the one hand, gives information about the number of pores on the BLM and, on the other hand, gives information about the intensity of diffusion growth of the pore. From Tab. 1 it follows that the parameter c_0SD has a numerical value equal to $5.3 \cdot 10^{-22} m^2/s$. Note that certain values of the parameters σ, γ, and c_0SD depend on the phospholipid composition of BLM, the presence or absence of charges on the BLM surface, and on the type of solvent during BLM formation. However, the numerical values of σ, γ, and c_0SD for different BLMs turn out to be in approximately the same order. Specific values of the parameters σ, γ, and c_0SD, given in the first row of Tab. 1, in order of magnitude, fit into the range of their changes in accordance with literature data [12–14].

The values of the parameters were determined in a similar way σ, γ, and c_0SD at different concentrations of Al_2O_3 nanoparticles in solution: 5, 20, and 50 g/mL (Tab. 1). As follows from Tab. 1, the increase in the stability of the BLM correlates with the increase in the main parameter (γ), on which the stability of the BLM depends most strongly. The increase in σ and γ is also expected, since sometimes a rude estimate of γ assumes that γ is directly proportional to σ [15]. From Tab. 1

follows that with an increase in the concentration of nanoparticles the parameter c_0SD also increases, this fact should lead to a decrease in the average lifetime of the BLM, as seen from Eq. (1). However, since γ increases with an increase in the concentration of nanoparticles, and the average BLM lifetime grows exponentially with an increase in γ, then under the combined action of γ and c_0SD on $\bar{t}(\varphi)$ the influence of γ is stronger. So, with an increase in the concentration of nanoparticles, an increase in the stability of the BLM is observed (see Figure).

The influence of nanoparticles of aluminum oxide Al_2O_3 on the specific conductivity (g) of BLM was also studied. This conduction is commonly referred to as background conduction, in contrast to conduction induced by carriers or channel formers. It has been shown that with an increase in the concentration of Al_2O_3 nanoparticles in the electrolyte solution, a slight decrease in the BLM specific conductivity is observed (Tab. 2).

Table 2

The BLM specific conductivity (g) in the presence of Al_2O_3 *nanoparticles in solution (0.1 M NaCl)*

	g, 10^{-9} Om^{-9} cm^{-2}			
BLM + Al_2O_3	0	$5\mu g/mL$	$20\mu g/mL$	$50\mu g/mL$
Average g	0.27	0.24	0.24	0.23
Std. Error	0.01	0.01	0.01	0.01

As seen from Tab. 2, the decrease in BLM conductivity with an increase in the concentration of nanoparticles in solution correlates with an increase in the BLM stability (see Fig. 1). This correlation can be explained using the results of [15]. As it was shown in [16], the background conductivity of the BLM could be explained as a result of the spontaneous formation of hydrophilic pores on the BLM. Since the loss of BLM stability in an electric field can also be due to the spontaneous formation of hydrophilic pores and their development to critical sizes [9–11], in our case, an increase in the BLM stability should be accompanied by a decrease in the BLM conductivity, which is observed experimentally.

A possible mechanism of the influence of aluminum oxide on the stability and conductivity of BLM may be a consequence of the electrostatic interaction of aluminum oxide with the surface of BLM. It is known that aluminum oxide has an isoelectric point in the range of pH within $7.5-8.0$ [17,18]. Since all experiments were carried out in the 0.1 NaCl solutions with pH 6, the Al_2O_3 nanoparticles are positively charged. We can assume that the mechanism of action of Al_2O_3 nanoparticles on the stability and conductivity of BLM is similar to the mechanism of action of positively charged multiply charged ions [19, 20]. Positively charged Al_2O_3 nanoparticles are adsorbed on the negatively charged atomic groups of the BLM surface, which leads to the compactization of the BLM structure, which contributes to an increase in the stability and a decrease in the conductivity of the BLM.

Conclusion. Thus, the analysis of the obtained results shows that the presence of aluminum oxide nanoparticles in the surrounding BLM solution increases the stability of BLM in an electric field, and BLM becomes more stable while increasing their concentration. It is shown that aluminum oxide nanoparticles increase the value of the coefficient of linear tension of the edge of the pore, and with an increase in the concentration of nanoparticles, the linear tension also increases. On the other hand, it has been shown that the presence of nanoparticles in the solution leads to a decrease in BLM conductivity.

Positively charged Al_2O_3 nanoparticles are adsorbed on the negatively charged atomic groups of the BLM surface, which leads to the compactization of the BLM structure, which contributes to an increase in the stability and decreases the conductivity of the BLM.

The work of the first author was supported by the Science Committee of RA, in the frames of the research project No. 21T-1F244.

Received 10.01.2023
Reviewed 01.02.2023
Accepted 10.02.2023

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Ծ. Մ. ՋՈՄԱՐԴՅԱՆ, Գ. Վ. ԱՆԱՆՅԱՆ, Վ. Բ. ԱՌԱՔԵԼՅԱՆ

ԵՐԿՇԵՐՏ ԼԻՊԻԴԱՅԻՆ ԹԱՂԱՆԹԻ ԿԱՅՈՒՆՈՒԹՅՈՒՆԸ ԵՎ ՀԱՂՈՐԴՈՒՆԱԿՈՒԹՅՈՒՆԸ Al_2O_3 ՆԱՆՈՄԱՍՆԻԿՆԵՐԻ ԱՌԿԱՅՈՒԹՅԱՄԲ

Ուսումնասիրվել է ալյումինի օքսիդի նանոմասնիկների (Al_2O_3) ազդեցությունը ԵԼԹ-ի (երկշերտ լիպիդային թաղանթ) կայունության և հաղորդունակության վրա լուծույթում: Ցույց է տրվել, որ Al_2O_3 նանոմասնիկները մեծացնում են ԵԼԹ-ի կայունությունը էլեկտրական դաշտում, որն առավել արտահայտիչ է դառնում նանոմասնիկների կոնցենտրացիայի մեծացման դեպքում: Փորձարարական փվյալները վերլուծվում են ԵԼԹ-ի կայունության հայտնի տեսության տեսանկյունից, համաձայն որի կայունության կորուստը պայմանավորված է ԵԼԹ-ի վրա միկրոսկոպիկ ծակոտիների ինքնաբուխ ձևավորմամբ: Ցույց է տրված, որ ալյումինի օքսիդի նանոմասնիկների առկայությամբ մեծանում է ծակոտիների եզրերի գծային լարվածության գործակցի արժեքը, իսկ նանոմասնիկների կոնցենտրացիայի ավելացման դեպքում մեծանում է նաև գծային լարվածությունը: Ցույց է տրվել նաև, որ լուծույթում նանոմասնիկների առկայությունը հանգեցնում է ԵԼԹ հաղորդունակության նվազմանը:

Ц. М. ДЖОМАРДЯН, Г. В. АНАНЯН, В. Б. АРАКЕЛЯН

УСТОЙЧИВОСТЬ И ПРОВОДИМОСТЬ ДВУХСЛОЙНОЙ ЛИПИДНОЙ МЕМБРАНЫ В ПРИСУТСТВИИ НАНОЧАСТИЦ ОКСИДА АЛЮМИНИЯ

Показано, что наличие наночастиц оксида алюминия в окружающем двухслойную липидную мембрану (ДЛМ) растворе приводит к повышению устойчивости ДЛМ в электрическом поле. Показано также, что увеличение концентрации наночастиц увеличивает устойчивость ДЛМ. Экспериментальные данные анализируются в рамках хорошо известной теории устойчивости ДЛМ, в основе которой лежат представления о спонтанном образовании на ДЛМ микроскопических пор, развитие которых и приводит к потере устойчивости ДЛМ. Из сопоставления экспериментальных кривых зависимости устойчивости ДЛМ от подаваемого на ДЛМ напряжения с теоретическими кривыми определены значения коэффициента линейного натяжения кромки поры. Показано, что наночастицы оксида алюминия повышают значение коэффициента линейного натяжения кромки поры и с увеличением концентрации наночастиц растет также линейное натяжение. Показано, что наличие наночастиц в растворе приводит к уменьшению проводимости ДЛМ.