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# Adsorption equilibria between liposome membrane formed of phosphatidylcholine and aqueous sodium chloride solution as a function of pH

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**Abstract**

The effect has been studied of the adsorption of ions ($H^+$, $Na^+$, $OH^-$, $Cl^-$) which are present in solution upon the electric charge of the liposome membrane formed of phosphatidylcholine (PC). The surface charge density of the membrane was determined as a function of pH and electrolyte concentration from electrophoretic mobility measurements. The measurements were carried out by the laser-Doppler microelectrophoresis method. A four-equilibria model has been proposed to describe the phenomena occurring on the membrane surface. The equilibria in which the adsorption of other ions on the liposome membrane surface was involved were assumed to exist beside the equilibria in which the $H^+$ and $OH^-$ ions were engaged. The idea was confirmed by mathematical calculations. Association constants of the liposome membrane surface with ions of solution ($K_{AH}$, $K_{ANa}$, $K_{BOH}$, $K_{BCl}$) were determined. The proposed model has been proved to be correct by comparing the resulting theoretic charge variation curves of the lecithin membrane with the experimental data.


*Keywords:* Surface charge density; Liposome membrane; Phosphatidylcholine; Adsorption equilibria

## 1. Introduction

Liposomes are spherical vesicles integrated by one or more phospholipid bilayers that encapsulate a part of aqueous media in which they are suspended. As the liposome membrane composition can be readily varied, the lipid vesicles are often used as the model system in the studies of biological membrane properties and structure [1,2].

Because of its ordered structure, a biological membrane can be considered from a physical point of view to be a distinct phase, different from neighbouring cytoplasm or intercellular liquid. Thus, the membrane surface can be considered to be an interface. Many physicochemical phenomena which are characteristic for an interface occur at the membrane–medium interface, e.g., asymmetric electric charge distribution [3]. This phenomenon is very well illustrated by the model of the phosphatidylcholine membrane. The total charge of a PC molecule is concentrated in the hydrophilic part of the phospholipid [4]. Two areas can be observed in this region: a negatively charged 1.8-nm-thick phosphate group and a positive choline group (2 nm). The specific orientation of these groups is made possible owing to this structure [5–7]. It is worth noting that the phosphatidylcholines are the most commonly used phospholipids for the preparation of liposomes. Over a wide range of pH (approximately 4–10) the phosphatidylcholine head group is zwitterionic with no net charge; however, liposomes prepared from pure phosphatidylcholines have non-zero zeta potentials over a wide range of ionic strength [8].

The change in the pH of the solution induces changes in electrical charge of the membrane due to the variations in the equilibria existing at the membrane surface. In our research, the surface charge density of the membrane was determined taking advantage of the electrophoretic method, which is useful for studying the properties of particles and molecules possessing the electric charge. Electrophoretic techniques had been applied to biological systems with different objectives for a long time.

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In 1960, the effects of the adsorption of ions on the colloid surfaces had been studied already using surfaces charge densities calculated from mobility measurements (Few et al.) [9]. Later, many studies on liposomes have applied the electrophoretic mobility to examine the electrokinetic properties of phospholipid vesicles. It provided many interesting results [10–12]. The recent literature presents examinations of the electrophoretic behavior of liposomes and effects of pH gradients on liposomal charge states realized by capillary electrophoresis [13].

The equilibria existing at the membrane surface occur between functional groups of the lipids and of other compounds of the cell membrane and outer medium components. The electric charge of the membrane is affected by these kind of equilibria and by their progress. The equilibria can be affected by many processes, e.g., adsorption, passive or active transport, leading to a membrane surface charge density variation.

It is known that the phosphatidylcholine molecule can exist in equilibrium with both the $H^+$ as well as with the $OH^-$ ions. However, a question may be asked whether only these ions are adsorbed at the phospholipid surface. It is the aim of this work to present the evidence that the acid–base equilibria are accompanied by the equilibria related to adsorption of other ions at the liposome membrane surface.

## 2. Experimental

### 2.1. Materials

Egg PC (99%) from Fluka was used in the experiment and it had the following fatty acid composition: 16:0 ~33%, 18:0 ~14%, 18:1 ~30%, 18:2 ~14%, 20:4 ~4%. Chloroform was chromatographic standard grade was from Aldrich. Water purified by Milli-Qll (18.2, Millipore, USA) was used to make all solutions.

### 2.2. Preparation of the phospholipid vesicles

Phospholipid vesicles were prepared according to the method proposed by Huang et al. [14]. 10 mg of lecithin was dissolved in 1–2 ml chloroform and the solvent was evaporated in a stream of argon (to prevent oxidizing) until 25–50 $\mu m^3$ lipid film remained in the beaker. Fifteen milliliters of an aqueous solution of NaCl or water Milli-Qll was then added, and the beaker was placed in a water bath (cooling ca. −7 °C was obtained). The head of a UD 20 ultrasound generator (Techpan, Poland) was immersed in the solution and the solution was subjected to ultrasound five times for 1.5 min each time. Liposomes of 10–20 nm diameters were obtained. The UD-20 apparatus is equipped with a vibratory unit ending with a 12 mm diameter, vibrating with a frequency ca. 22 kHz and amplitude to 16 μm. Ultrasound energy is produced in the piezoceramic transducer of the "sandwich" type. The maximal power output is 180 W.

### 2.3. Electrophoretic mobility measurement

The electrophoretic mobility of the phospholipid vesicle suspension was obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser-Doppler Velocimetry (LDV) with the Zetasizer Nano ZS (Malvern Instruments, UK). The measurements were carried out as function of hydrogen ion concentration and of electrolyte concentration. To obtain the pH dependence of electrophoretic mobility, the earlier prepared liposomes were suspended in sodium chloride solution or in water Milli-Qll and were titrated either with hydrochloric acid or with sodium hydroxide. To obtain the electrolyte concentration dependence of electrophoretic mobility, the liposomes were suspended in sodium chloride solution with an adequate concentration. The range of NaCl concentration was within limit range of $10^{-5}$ to 0.155 [M]. Measurements were executed with a pH ca. 7. Every result is a mean of six measurements at the given pH value and at the given concentration of sodium chloride value. Standard electrophoretic mobility deviation amounted to about 5% of the mean. All experiments were carried out three times.

### 2.4. Size measurement

The size of phospholipid vesicle suspension was determined at 25 °C by Dynamic Light Scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments, UK). This technique measures Brownian motion and relates this to the size of the particles. It does it by illuminating the particles with the laser and analysing the intensity fluctuations in the scattered light. The sample (lipid vesicles suspension solution) prepared in a syringe was injected through the folded capillary cell. The sample ports were closed by stoppers, then the cell was placed in a cell holder. A helium–neon laser was focused on the sample and scattered light was detected at 90 °C to the incidental beam. The scattering intensity signal for the detector was passed to a digital signal processing board called a correlator. The acquired information from the correlator was then passed to a computer, where the software analysed the derived size information data. The intensity size distribution of phosphatidylcholine liposomes obtained by Zetasizer Nano ZS is presented in Fig. 1.

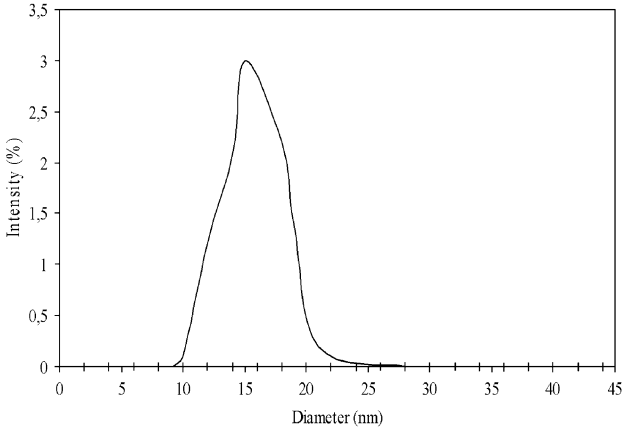


Fig. 1. The intensity size distribution of phosphatidylcholine liposomes. The *x* axis illustrates a distribution of size classes, while the *y* axis illustrates the relative intensity of the scattered light.

## 3. Theory

In an ionizing liquid, a colloidal particle is surrounded by an ionic atmosphere composed chiefly of oppositely charged single ions, and in considering the motion of the large particle it is necessary also to consider the effect of the atmosphere. In certain extreme cases (e.g., large non-conducting particles), the ionic atmosphere may be simply treated. The electrophoretic behavior of the particle is strongly influenced by the size of the electrical double layer. If the thickness of the diffuse double layer ($d$) is much smaller than the radius of curvature at any point on the surface ($a$) (i.e. $d \ll a$), it is possible to consider the particle with its double layer as a parallel plate condenser whose plates are at a distance apart given by the thickness of the diffuse double layer. Let the plates have a charge $q$ per unit area. When a steady state is reached in which the particle is moving at a constant speed through the liquid, there is equality between frictional and electrical forces. From the definitions of viscosity, velocity and mobility, we obtain [15]:

$$q = \frac{\eta\mu}{d} \tag{1}$$

Making use of the electrostatic expression:

$$q = \frac{\varepsilon\varepsilon_0\zeta}{4\pi d} \tag{2}$$

Further, introducing the mobility, we have Smoluchowski's equation [15]:

$$\mu = \frac{\varepsilon\varepsilon_0\zeta}{4\pi\eta} \tag{3}$$

where: $\eta$—viscosity of solution; $d$—thickness of diffuse double layer; $\mu$—electrophoretic mobility; ε—relatively permittivity of electrolyte; $\varepsilon_0$—permittivity of free space.

It follows from the above expression that the electrophoretic mobility of the non-conducting particle for which the ratio of particle radius to double layer thickness is large (at all points on the surface) is independent of its shape and size [16]. Thickness of diffuse double layer was calculated from the formula:

$$d = \sqrt{\frac{\varepsilon\varepsilon_0 \mathrm{RT}}{2F^2 I}} \tag{4}$$

where: $R$—gas constant; $T$—temperature; $F$—Faraday constant; $I$—ionic strength of electrolyte; $\zeta$—zeta potential.

The charge at the liposome membrane was calculated, from electrophoretic mobility measurements, according to Eq. (1).

Among other groups, the lecithin molecule contains the $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ group [17]. Therefore, it can exist in equilibrium not only with the $H^+$ and $OH^-$ ions but also with other ions existing in the solution, e.g., with the $Na^+$ and $Cl^-$ ions. The surface charge density of the lipid membrane results from the equilibria existing between the groups localized at the membrane surface and solution ions. Let us assume that the $H^+$, $OH^-$, $Na^+$, and $Cl^-$ ions are adsorbed at the phosphatidylcholine surface.The adsorption equilibria are described by the equations:

$$A^- + H^+ \leftrightarrow AH \tag{5}$$

$$B^+ + OH^- \leftrightarrow BOH \tag{6}$$

$$A^- + Na^+ \leftrightarrow ANa \tag{7}$$

$$B^+ + Cl^- \leftrightarrow BCl \tag{8}$$

where: $A^-$ is group $-PO^{(-)}$,$B^+$ is group $-N^{(+)}(CH_3)_3$.

Association constants are determined by surface concentrations of the membrane components and volume concentrations of the ions present in the solution according to the equations:

$$K_{AH} = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} \tag{9}$$

$$K_{BOH} = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \tag{10}$$

$$K_{ANa} = \frac{a_{ANa}}{a_{A^-} \cdot a_{Na^+}} \tag{11}$$

$$K_{BCl} = \frac{a_{BCl}}{a_{B^+} \cdot a_{Cl^-}} \tag{12}$$

The surface concentration of the lecithin is denoted by $C_L$

$$a_{A}{}^{-} + a_{AH} + a_{ANa} = C_L \tag{13}$$

$$a_{B}{}^{+} + a_{BOH} + a_{BCl} = C_L \tag{14}$$

where $a_{A}{}^{-}$, $a_{AH}$, $a_{ANa}$, $a_{B}{}^{+}$, $a_{BOH}$, $a_{BCl}$ — surface concentrations of membrane components [mol/m$^2$], $a_{H}{}^{+}$, $a_{OH}{}^{-}$, $a_{Na}{}^{+}$, $a_{Cl}{}^{-}$ — volume concentrations of ions in solution [mol/m$^3$].

It is not possible to take into consideration activity coefficients in the case of surface concentrations. Therefore, the symbol $a$ means the concentration, not the activity in this case. Whereas, in the case of ions presented in solution, we take into account activity coefficients in our calculations. So the symbol $a$ means the activity actually. We took the values of activity coefficients from the tables [18].

The surface charge density of a lecithin membrane is described by the equation:

$$q = \left(a_{B}{}^{+} - a_{A}{}^{-}\right)\mathrm{F} \tag{15}$$

elimination of $a_{AH}$, $a_{ANa}$, $a_{BOH}$, $a_{BCl}$ (from Eqs. (13) (14)) and of $a_{A}{}^{-}$, $a_{B}{}^{+}$, (from Eq. (15)) yields the following equation:

$$\frac{q}{F} = \frac{C_L}{1 + K_{BOH}a_{OH^-} + K_{BCl}a_{Cl^-}} - \frac{C_L}{1 + K_{AH}a_{H^+} + K_{ANa}a_{Na^+}} \tag{16}$$

The equation can be simplified to linear ones for high and low hydrogen ion concentrations. In the former case, the denominator of Eq. (16) was written in the order of decreasing power of hydrogen ion concentration. Thereafter, the numerator of

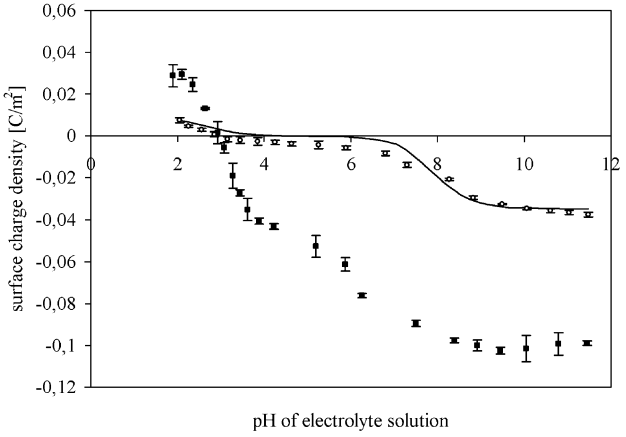


Fig. 2. The pH dependence of the surface charge density of liposomal membrane formed from phosphatidylcholine. The circle points denote experimental values obtained in 0.155 M NaCl solution, the solid line presents the theoretical data calculated from Eq. (16), obtained in the same solution and the square points denote experimental results obtained for liposomes suspended in water Milli-Qll.

each equation term was divided by a denominator and hence two initial terms only were left; the procedure yielded a straight line equation in the $a_{H^+}$ and $\frac{qa_{H^+}}{F}$ coordinates which was correct for high hydrogen ion concentrations.

$$\frac{qa_{H^+}}{F} = \frac{C_L}{1 + K_{BCl}a_{Cl^-}} a_{H^+} - \frac{C_L K_{BOH} K_W}{(1 + K_{BCl}a_{Cl^-})^2} \quad (17)$$

To obtain a linear equation in the low hydrogen ion concentration range the denominator of Eq. (16) was written in the order of increasing power of hydrogen ion concentration. Thereafter the procedure was as before yielding a straight line which in this case was correct in the low hydrogen ion concentration range.

$$\frac{q}{Fa_{H^+}} = -\frac{C_L}{(1 + K_{ANa}a_{Na^+})} \frac{1}{a_{H^+}} + \frac{C_L K_{AH}}{(1 + K_{ANa}a_{Na^+})^2} \quad (18)$$

The straight line equation coefficients can be determined using the linear regression method and they can be used to determine the association constants: $K_{BOH}$, $K_{BCl}$, $K_{AH}$, $K_{ANa}$. Knowledge of these parameters makes it possible to determine the liposome membrane surface charge value from Eq. (16) and to compare it with the experimental values.

The degree of coverage values of the phosphatidylcholine membrane surface, $\theta$, with the $H^+$, $OH^-$, $Na^+$, $Cl^-$ ions were determined from the relationships:

$$\theta_x = \frac{a_x}{C_L} \quad (19)$$

where $x = A^-$, AH, ANa, $B^+$, BOH, BCl.

The surface concentrations $a_{A^-}$, $a_{B^+}$ were determined from Eqs. (9)–(14); the results were:

$$a_{A^-} = \frac{C_L}{1 + K_{AH}a_H + K_{ANa}a_{Na}}$$

$$a_{B^+} = \frac{C_L}{1 + K_{BOH}a_{OH} + K_{BCl}a_{Cl}}$$

whereas $a_{AH}$, $a_{ANa}$, $a_{BOH}$, $a_{BCl}$ were obtained by transforming Eqs. (9)–(12).

## 4. Results and discussion

The measurements of the electrophoretic mobility liposome membrane formed from phosphatidylcholine as a function of pH of electrolyte solution (0.155 M NaCl) were realized. The experimental control, in the absence of sodium chloride, was carried out to confirm our idea about adsorption of sodium and chlorine ions at the phospholipid surface. To obtain a complete set of measurements, the effect of the concentration of electrolyte solution was studied. The experimental values of electrophoretic mobility were converted to surface charge density using Eq. (1). The theoretical values of surface charge density were determined on the basis of Eq. (16). The association constants of the surface groups with the solution ions were determined from Eqs. (17) (18).

The pH dependence of the surface charge of the liposomal membrane is plotted in Fig. 2. The figure presents three curves; one of them is the experimental control curve, which was made in the absence of sodium chloride (presented as square points). The phosphatidylcholine liposomes used in the measurements were bathed in water. The other two curves were obtained in the presence of 0.155 M NaCl. The experimental values are presented as circle points and the theoretical values calculated from Eq. (16) are presented as a solid line. Compatibility of the two curves is good enough to confirm the correctness of both the equation and calculated parameters (association constants).

It can be observed that in basic solution in the presence of the sodium chloride, a decrease of negative charge occurs. $-N^{(+)}(CH_3)_3$ groups of phosphatidylcholine molecules are covered by $OH^-$ ions, whereas $-(PO)^{(-)}$ groups are uncovered. The fact indicates adsorption of $Na^+$ ions. A similar tendency can be observed in acid solution: in the presence of sodium chloride, a decrease of positive charge occurs. $-(PO)^{(-)}$

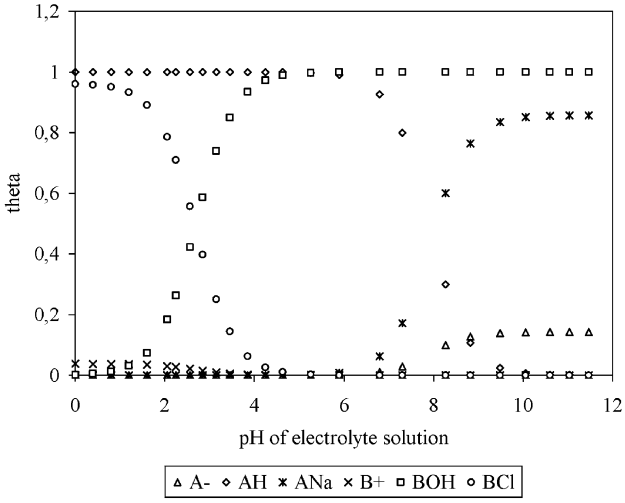


Fig. 3. The degree of coverage of the phosphatidylcholine membrane surface, $\theta$, with the $H^+$, $OH^-$, $Na^+$, $Cl^-$ ions, calculated for four equilibria model, from Eq. (19), as a function of pH of the 0.155 M NaCl solution.

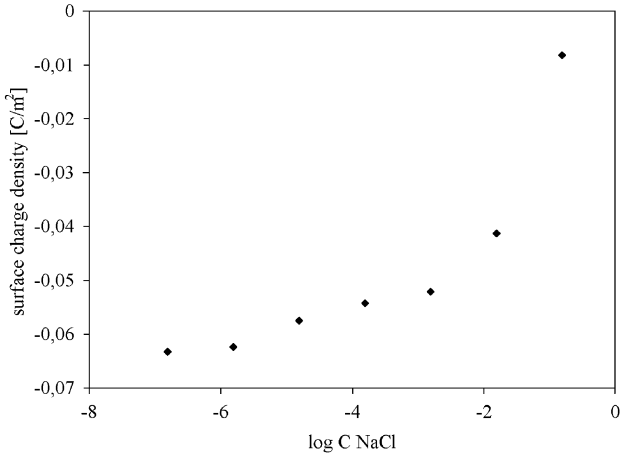


Fig. 4. The surface charge density of the liposomal membrane formed from phosphatidylcholine as a function of concentration of NaCl, pH 7.

groups are covered by $H^+$ ions, whereas $-N^{(+)}(CH_3)_3$ groups are uncovered. This fact indicates adsorption of $Cl^-$ ions.

It also can be observed that the pH at the isoelectric point appears to be the same both for the presence and absence of sodium chloride. It testifies a nearly identical adsorption of both $Na^+$ and $Cl^-$ ions [19] or a noticeable affinity for one ion [20].

Association constants of the surface groups with the solution ions were determined from Eqs. (17) and (18) by the linear regression method using the Excel 97 program. The $K_{BOH}$, $K_{BCl}$, $K_{AH}$, and $K_{ANa}$ determined in this way are equal to $5.35 \times 10^9 \pm 1.56 \times 10^8$, $0.218 \pm 0.0105$, $5.58 \times 10^5 \pm 2.0275 \times 10^4$, $0.051 \pm 0.0016$ [$m^3$/mol], respectively.

It can be determined from comparison of the association constants that the $H^+$ ion is more strongly adsorbed than the $Na^+$ ion and the $OH^-$ ion also more strongly adsorbed than the $Cl^-$ ion. However, in acid solution, in the absence of the $OH^-$ ions, the adsorption of the $Cl^-$ ions is observed. And in basic solution, in the absence of the $H^+$ ions, the adsorption of $Na^+$ is observed (Fig. 2).

The degree of coverage of the phosphatidylcholine membrane surface by ions as function of pH of 0.155 M NaCl is presented in Fig. 3. Beside the coverage with the $H^+$ and $OH^-$ ions, the coverage with other ions ($Na^+$ and $Cl^-$) was considered to check if the coverage with these ions is as high as to affect the phosphatidylcholine membrane surface charge.

As can be seen in Fig. 3 the $Na^+$ ions adsorption starts when the amount of the $H^+$ ions becomes low (at pH > 6). In basic solution, the degree of coverage of the membrane by the $Na^+$ ions is over 0.8, i.e. in this pH range the membrane is covered by the $Na^+$ ions. A similar tendency can be observed for the $Cl^-$ ions: the adsorption of the $Cl^-$ ions begins when the amount of the $OH^-$ ions begins to decrease (at pH < 4). In a strongly acid solution the degree of coverage of the membrane by the $Cl^-$ ions is almost 1, i.e., the surface is covered by the $Cl^-$ ions. Thus, the adsorption of the $Na^+$ and $Cl^-$ ions must be taken into account as the electric charge is affected by this phenomenon.

The experiments were also conducted as a function of electrolyte concentration. Variation in surface charge of the phosphatidylcholine membrane as a function of concentration of sodium chloride is presented in Fig. 4. The sodium and chlorine ions only slightly reduce the negative value of surface charge density at low concentrations of NaCl, however, at higher concentrations, the reduction is meaningful. The measurements were done at neutral pH value, therefore the concentrations of the $H^+$ and $OH^-$ ions are very low. It is obvious that for low concentrations of electrolyte solution, we should not expect meaningful changes in the surface charge density values. However, for high concentrations of sodium chloride in pH of ca. 7, a competition in the adsorption between the $H^+$ and $Na^+$ ions appears (Fig. 3). The increase of the $Na^+$ ions concentration causes the decrease of the negative charge, and the same observation proves the adsorption of the $Na^+$ ions.

Matsumura et al. [21] studied the electrostatic interaction between liposomes. They measured electrophoretic mobilities as a function of various concentrations of metal chloride (NaCl, $CaCl_2$). Similar results for sodium chloride were observed.

The two-equilibria model taking into account the adsorption of the $H^+$ and $OH^-$ ions only has been observed in the literature [17,22] dealing with the cell membrane charge. As has been demonstrated, it resulted in neglect of charge variations caused by adsorption of the $Na^+$ and $Cl^-$ ions. The assumption that only two equilibria (5 and 6) exist unavoidably results in falsified numerical values of these equilibrium constants. Taking into account the equilibria in which the $Na^+$ and $Cl^-$ ions are involved makes it possible to obtain more reliable association constants. The association constants $K_{BOH}$ and $K_{AH}$ calculated in terms of the two-equilibrium model would differ from those obtained in terms of the four-equilibria model. The former would be burdened by the error due to neglect of the $Na^+$ and $Cl^-$ ions adsorption on the membrane surface. Thus, the idea of assuming existence of liposome membrane surface equilibria in which other ions are involved in addition to the acid–base equilibria has been proved to be correct.

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