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Adsorption equilibria at interface separating electrolyte solution and phosphatidylcholine–stearylamine liposome membrane

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Abstract

The effect of the pH of an electrolyte solution on the electric surface charge of the liposome membrane was studied. The membrane of vesicles contained egg phosphatidylcholine (PC) with different proportions of stearylamine (ST). The surface charge density of the membrane was determined as a function of pH from electrophoretic mobility measurements. A six equilibria model describing the solution ions adsorption on the PC-ST liposome membrane surface was presented in this paper. The knowledge of the association constants of the $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups of PC with H^+, OH^-, Na^+, Cl^- ions: K_{A_1H}, K_{B_1OH}, K_{A_1Na}, K_{B_1Cl}, that had been presented earlier, allowed to determine the association constants of the $-N^{(+)}H_3$ group of ST with OH^- and Cl^- ions: K_{B_2OH}, K_{B_2Cl}. The proposed model has been proved to be correct by comparing the resulting theoretic charge variation curves of the PC-ST liposomal membrane with the experimental data.

Keywords: Phosphatidylcholine; Stearylamine; Surface charge density; Liposome membrane; Adsorption equilibria; Association constants

1. Introduction

In spite of being complicated, biological membranes are extremely interesting, research systems, contain many elements which influence their electric properties to a considerable extent. Carrying out studies of the complex structure is difficult, because of various kinds of interactions occurring between its components. For this reason, models of the membrane are used, e.g. liposomes which are simplified structures reflecting properties of natural membranes. Liposomes are spherical colloidal particles consisting of one or more concentric bilayers encapsulating part of the aqueous medium in which they float freely. Liposomes are made predominantly from amphiphiles, a special class of surfo-active molecules which are characterized by having a hydrophilic and a hydrophobic group on the same molecule [1]. The properties of liposomes and their subsequent applicability depend on the physical and physico-chemical characteristics of the liposomal membrane. Usually, a zwitterionic or non-ionic lipid is used as the basic lipid for the preparation of liposomes [2]. Phosphatidylcholines are the most widely used liposome-forming molecules because of their relevance to the behavior of these components in cell membranes. They are zwitterionic at physiological of pH because the quaternary ammonium group is neither basic nor acidic in these pH ranges [1]. The bilayer membranes mostly consist of either natural or synthetic phospholipids, but the application of other double-tail surfactants such as dialkyl quaternary ammonium compounds in pharmaceutical applications are also used. In addition, minor amounts of cholesterol, or single-tail surfactants, such as stearylamine (ST), may be added to affect specific characteristics such as the membrane permeability or electric charge density [3].

The electrical properties of liposomal surfaces can be conveniently investigated by microelectrophoresis in which the movement of the liposomes in an electric field is observed. In general, two parameters characterizing the liposomal surface can be calculated from the measured mobilities, firstly the electrical potential at the plane of shear and secondly the surface charge density [4]. An important property of a cell membrane is

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its surface charge density which controls several processes in biological membranes. Thus, it affects membrane-bound enzymes, insertion of newly synthesized proteins into membranes and host-pathogen interactions [5]. Since the surface charge is dependent only on the molecular composition of the cell membrane, the surface charge density is much closer to being an intrinsic property of a given cell than particle's surface potential, making it a more useful parameter for comparing the surface properties of two different cell types [6]. Since biological membranes are charged entities, aqueous solution next to these membranes contain counterions and electrolytes. The interactions between membranes strongly depend on the presence of ions and their specificity [7]. The charge of living cells is due to the dissociation of ionogenic, or charged, groups ($-PO_4^-$, $-NH_3^+$, $-COO^-$,) in the cell surface [8]. These are most often acidic-alkaline properties groups which make the adsorption of many substances and ions on surface of the membrane possible. The equilibria existing at the membrane surface occur between functional groups of the membranes and outer medium components. The equilibria can be affected by e.g. adsorption leading to a membrane surface charge density variation [9]. The change in the pH of the solution induces changes in surface charge of the membrane towards more positive values at lowering pH or towards more negative values at raising pH [10,11]. The surface charge of membranes also depends on ionic strength of the electrolyte and lipid composition of the membrane. The surface charge of liposomes can be modified by the incorporation of positively charged lipids, such as stearylamine, or negatively charged lipids, such as dicetylphosphate, phosphatidylglycerol or phosphatidylserine [2].

The interactions between lipid membranes and surroundings are nowadays intensively developed. McLaughlin et al. [12] studied the adsorption of ClO_4^- and SCN^- ions on the phospholipid membranes; it was proved that the adsorption of these ions causes an increase of the negative surface potential. Grasdalen et al. [13], Gabrielska et al. [14], MacDonald and Seeling [15] studied the adsorption of multivalence ions, such as La^{3+}, Pr^{3+}, on the liposome surface. In our previous paper we presented the adsorption of Na^+ and Cl^- ions on the phosphatidylcholine (PC) surface [9]. The association constants (K_{A_1H}, K_{A_1Na}, K_{B_1OH}, K_{B_1Cl}) of the functional groups ($-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$) with the solution ions (H^+, Na^+, OH^-, Cl^-) for PC membrane were determined. The aim of this work is to examine and describe the phenomena occuring on the PC liposomal membrane surface modified by ST. The stearylamine molecules consist of two parts, a hydrophobic domain and a ionizable nitrogen atom positively charged at physiological pH [16]. We present changes of the electric charge caused by the solution ions adsorption on the PC-ST liposome surface. We also propose a six equilibria model describing the H^+, OH^-, Na^+, Cl^- ions adsorption on the PC-ST liposome membrane surface. The mathematical calculations enabled to determine the association constants of the $-N^{(+)}H_3$ group of ST with OH^- and Cl^- ions (K_{B_2OH}, K_{B_2Cl}).

Data presented in this work, obtained in result of mathematical derivation and confirmed experimentally, are of great importance for the interpretation of the phenomena occurring in lipid membranes. The knowledge of adsorption equilibria lets us understand the processes that take place on liposomal surface. The obtained results can be used in quantitative description of physical and chemical properties of biological membranes and, in our opinion, can help in a better understanding of biological membranes and in their biophysical studies.

2. Experimental

2.1. Materials

L-α-phosphatidylcholine from egg yolk was purchased from Fluka. Stearylamine, used as cationic charge-inducing agent, was supplied from Sigma. Chloroform was chromatographic standard grade (Aldrich). Water purified by Milli-Qll (18.2, Millipore, USA) was used to make all solutions and in all cleaning procedures.

2.2. Preparation of the phospholipid vesicles

Phospholipid vesicles were prepared by sonication. The lipids used were neutral PC and positively charged ST. Dry PC and ST were weighed, dissolved in chloroform (10 mg/1.4 ml), and mixed in molar ratios of PC-ST (10-1, 5-1, 3-1). Then the solvent was evaporated in a stream of argon to obtain 25–50 μm^3 of lipid film in a beaker. The film was hydrated with 15 ml isotonic saline solution (0.9% NaCl) and the beaker was placed in the water bath (at approx. 7 °C). 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.

2.3. Microelectrophoretic mobility measurements

The electrophoretic mobility of the phospholipid vesicle suspensions was determined 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 a function of hydrogen ion concentration. Every result is a mean of six measurements at the given pH value. Liposomes formed earlier were suspended in sodium chloride solution and were titrated either with hydrochloric acid or with sodium hydroxide to obtain the series of different pH solutions. Then the solutions were put into the measuring vessel one by one and the electrophoretic mobility was measured. All experiments were performed at least three times.

3. Theory

The measurement of electrophoretic mobilities is relatively straightforward, but their interpretation is more problematical. The parameter characterizing the liposomal surface is the surface charge density which can be calculated from the measured mobilities [4].

For liposomes, an accepted equation, that describes their electrophoretic behaviour, is the Smoluchowski's equation (Eq. (1)), which refers particles whose dimensions are greater than the double layer thickness. Liposomes are large relative to the double layer thickness even at relatively low ionic strength so that the equation is commonly used to calculate their electrophoretic mobility [4]:

$$\mu = \frac{\varepsilon\varepsilon_0\zeta}{4\pi\eta} \tag{1}$$

where: μ is the electrophoretic mobility, ε is the relative permittivity of electrolyte, ε_0 is the permittivity of free space, ζ is the zeta potential, η is the viscosity of the medium.

If we consider the electrical migration of such large particles it is possible to assume the particle with its double layer as a parallel condenser whose plates are at a distance apart given by the thickness of the 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 are equal between frictional and electrical forces. From the definitions of viscosity, velocity and mobility, we obtain [17]:

$$q = \frac{\eta\mu}{d} \tag{2}$$

where: q is the surface charge density, d is the thickness of diffuse double layer.

Thus, the measured mobilities of the PC-ST liposome membrane can be converted to surface charge density by means of Eq. (2).

The liposomal membrane surface observed from the aqueous solution side has uniformly distributed the functional groups of PC and ST, because it is built of molecules each having $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups and $-N^{(+)}H_3$ group. The surface charge density of the lipid membrane results from 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 PC-ST surface. Thus, the adsorption equilibria can be presented in the form:

$$A_1^- + H^+ \rightleftarrows A_1H \tag{3}$$

$$B_1^+ + OH^- \rightleftarrows B_1OH \tag{4}$$

$$B_2^+ + OH^- \rightleftarrows B_2OH \tag{5}$$

$$A_1^- + Na^+ \rightleftarrows A_1Na \tag{6}$$

$$B_1^+ + Cl^- \rightleftarrows B_1Cl \tag{7}$$

$$B_2^+ + Cl^- \rightleftarrows B_2Cl \tag{8}$$

where: A_1^- is group $-PO^{(-)}$, B_1^+ is group $-N^{(+)}(CH_3)_3$, of phosphatidylcholine and B_2^+ is group $-N^{(+)}H_3$ of stearylamine.

Association constants can be calculated from surface concentrations of the membrane components and volume concentrations of the ions present in the solution according to the equations:

$$K_{A_1H} = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \tag{9}$$

$$K_{B_1OH} = \frac{a_{B_1OH}}{a_{B_1^+} \cdot a_{OH^-}} \tag{10}$$

$$K_{B_2OH} = \frac{a_{B_2OH}}{a_{B_2^+} \cdot a_{OH^-}} \tag{11}$$

$$K_{A_1Na} = \frac{a_{A_1Na}}{a_{A_1^-} \cdot a_{Na^+}} \tag{12}$$

$$K_{B_1Cl} = \frac{a_{B_1Cl}}{a_{B_1^+} \cdot a_{Cl^-}} \tag{13}$$

$$K_{B_2Cl} = \frac{a_{B_2Cl}}{a_{B_2^+} \cdot a_{Cl^-}} \tag{14}$$

The concentrations balances are expressed as follows:

$$a_{A_1^-} + a_{A_1H} + a_{A_1Na} = c_L \tag{15}$$

$$a_{B_1^+} + a_{B_1OH} + a_{B_1Cl} = c_L \tag{16}$$

$$a_{B_2^+} + a_{B_2OH} + a_{B_2Cl} = c_{ST} \tag{17}$$

where: K_{A_1H}, K_{B_1OH}, K_{B_2OH}, K_{A_1Na}, K_{B_1Cl}, K_{B_2Cl} — association constants of the groups $-PO^-H^+$, $-N^+(CH_3)_3OH^-$, $-N^+H_3OH^-$, $-PO^-Na^+$, $-N^+(CH_3)_3Cl^-$, $-N^+H_3Cl^-$, respectively.

where: c_L, c_{ST} — surface concentrations of PC and ST, $a_{A_1^-}$, a_{A_1H}, a_{A_1Na}, $a_{B_1^+}$, a_{B_1OH}, $a_{B_2^+}$, a_{B_2OH}, a_{B_1Cl}, a_{B_2Cl} — surface concentrations of membrane components, a_{H^+}, a_{OH^-}, a_{Na^+}, a_{Cl^-} — volume concentrations of solution ions.

For membrane components, we assumed that their concentrations equal the activities, but for ions presented in solution we used the activity values in our calculations. We took the values of activity coefficients from the tables [18].

The surface concentrations of PC and ST in the membrane built of their mixture at different molar ratios are determined from the equations:

$$n = \frac{c_L}{c_{ST}} \tag{18a}$$

$$c_L A_L + c_{ST} A_{ST} = 1 \tag{18b}$$

where:

A_L surface area occupied by a PC molecule (66 Å^2/molecule [19])

A_{ST} surface area occupied by a ST molecule (19 Å^2/molecule [20])
n molar ratio of PC-ST

The surface charge density of PC-ST membrane is described by the equation:

$$q = (a_{B_1^+} + a_{B_2^+} - a_{A_1^-})F \tag{19}$$

where:
F=96487 C/mol — Faraday constant
Elimination of a_{A_1H}, a_{A_1Na}, a_{B_1OH}, a_{B_2OH}, a_{B_1Cl}, a_{B_2Cl} (from Eqs. (15)–(17)) and of $a_{B_1^+}$, $a_{B_2^+}$, $a_{A_1^-}$ (from Eq. (19)) yields the following equation:

$$\frac{q}{F} = \frac{c_{ST}}{1 + K_{B_2OH}a_{OH^-} + K_{B_2Cl}a_{Cl^-}} + \frac{c_L}{1 + K_{B_1OH}a_{OH^-} + K_{B_1Cl}a_{Cl^-}} - \frac{c_L}{1 + K_{A_1H}a_{H^+} + K_{A_1Na}a_{Na^+}} \tag{20}$$

It is difficult to carry out the regression of Eq. (20) to determine the association constants. In order obtaining these values, the equation was simplified to linear ones for high and low hydrogen ion concentrations. In the former case, the denominators of Eq. (20) were written in the order of decreasing power of hydrogen ion concentration.

$$\frac{q}{F} = \frac{c_{ST}\cdot a_{H^+}}{a_{H^+}(1 + K_{B_2Cl}a_{Cl^-}) + K_{B_2OH}K_w} + \frac{c_L\cdot a_{H^+}}{a_{H^+}(1 + K_{B_1Cl}a_{Cl^-}) + K_{B_1OH}K_w} - \frac{c_L}{K_{A_1H}a_{H^+} + (1 + K_{A_1Na}a_{Na^+})} \tag{21}$$

Thereafter, the numerator of each equation term was divided by a denominator and thence two initial terms only were left; the procedure yielded a straight line equation (y=ax+b) in the a_H^+ and $\frac{qa_{H^+}}{F}$ coordinates which was correct for high hydrogen ion concentrations ($a_{H+}\to\infty$).

$$\frac{qa_{H^+}}{F} = \left(\frac{c_{ST}}{1 + K_{B_2Cl}a_{Cl^-}} + \frac{c_L}{1 + K_{B_1Cl}a_{Cl^-}}\right)a_H^+ - \left(\frac{c_L K_{B_1OH}K_w}{(1 + K_{B_1Cl}a_{Cl^-})^2} + \frac{c_{ST}K_{B_2OH}K_w}{(1 + K_{B_2Cl}a_{Cl^-})^2} + \frac{c_L}{K_{A_1H}}\right) \tag{22}$$

Where: $\frac{c_{ST}}{1+K_{B_2Cl}a_{Cl^-}} + \frac{c_L}{1+K_{B_1Cl}a_{Cl^-}}$ is the slope line (coefficient a of a straight line equation) and $-\left(\frac{c_L K_{B_1OH}K_w}{(1+K_{B_1Cl}a_{Cl^-})^2} + \frac{c_{ST}K_{B_2OH}K_w}{(1+K_{B_2Cl}a_{Cl^-})^2} + \frac{c_L}{K_{A_1H}}\right)$ is its intersection point with the ordinate axis (coefficient b of a straight line equation).

To obtain a linear equation in the low hydrogen ion concentration range the denominator of Eq. (20) was written in the order of increasing power of hydrogen ion concentration.

$$\frac{q}{F} = \frac{c_{ST}\cdot a_{H^+}}{K_{B_2OH}K_w + a_{H^+}(1 + K_{B_2Cl}a_{Cl^-})} + \frac{c_L\cdot a_{H^+}}{K_{B_1OH}K_w + a_{H^+}(1 + K_{B_1Cl}a_{Cl^-})} - \frac{c_L}{(1 + K_{A_1Na}a_{Na^+}) + K_{A_1H}a_{H^+}} \tag{23}$$

Thereafter the procedure was as before yielding a straight line equation which in this case was correct in the low hydrogen ion concentration range ($a_{H+}\to 0$).

$$\frac{q}{Fa_{H^+}} = -\left(\frac{c_L}{(1 + K_{A_1Na}a_{Na^+})}\right)\frac{1}{a_{H^+}} + \left(\frac{c_L}{K_{B_1OH}K_w} + \frac{c_{ST}}{K_{B_2OH}K_w} + \frac{c_L K_{A_1H}}{(1 + K_{A_1Na}a_{Na^+})^2}\right) \tag{24}$$

Where: $-\left(\frac{c_L}{(1+K_{A_1Na}a_{Na^+})}\right)\frac{1}{a_{H^+}}$ is the slope line (coefficient a of a straight line equation) and $\left(\frac{c_L}{K_{B_1OH}K_w} + \frac{c_{ST}}{K_{B_2OH}K_w} + \frac{c_L K_{A_1H}}{(1+K_{A_1Na}a_{Na^+})^2}\right)$ is its intersection point with the ordinate axis (coefficient b of a straight line equation).

The straight line equation coefficients can be determined using the linear regression method and they can be used to calculate sought-after parameters. The knowledge of the association constants of the surface group of PC with the H^+, OH^-, Na^+ and Cl^- ions (K_{A_1H}, K_{B_1OH}, K_{A_1Na}, K_{B_1Cl}) [9] can be used to determine the association constants of the surface group of ST with the OH^- and Cl^- ions: K_{B_2OH}, K_{B_2Cl}. On the basis of calculated parameters it is possible to determine the theoretical liposome membrane surface charge value from Eq. (20) and to compare it with the experimental data.

The degree of coverage values of the liposomal surface occupied by ST with the OH^-, Cl^- ions were determined from the relationship:

$$\theta_x = \frac{a_x}{c_{ST}}, \tag{25}$$

where: x=B_2^+, B_2OH, B_2Cl

The degree of coverage values of the liposomal surface occupied by PC with the H^+, OH^-, Na^+, Cl^- ions were determined earlier [9].

The surface concentration, $a_{B_2^+}$ was obtained by transforming Eqs. (11), (14), (17); the result was:

$$a_{B_2^+} = \frac{c_{ST}}{1 + K_{B_2OH}a_{OH^-} + K_{B_2Cl}a_{Cl^-}}$$

whereas a_{B_2OH}, a_{B_2Cl} were determined from Eqs. (11) and (14).

4. Results and discussion

The measurements of the electrophoretic mobility of PC-ST system in different molar ratios were performed as a function of

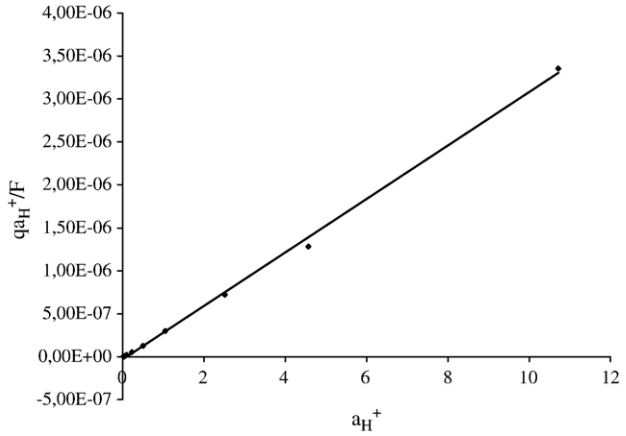

Fig. 1. Method of determination of a and b coefficients of a straight line equation from Eq. (22) for PC-ST system (5-1) — the experimental values are marked by points and the theoretical ones by line.

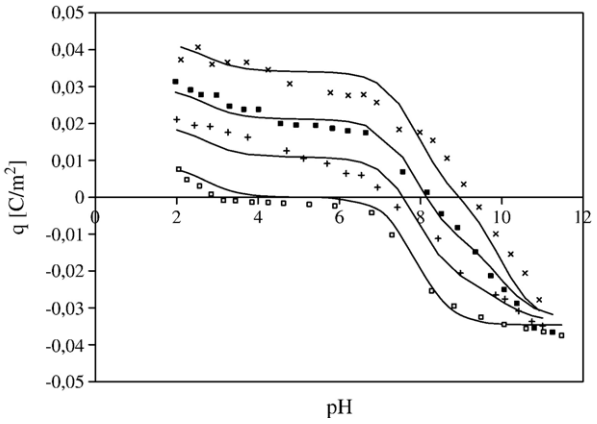

Fig. 3. The pH dependence of the surface charge density of PC-ST membrane and of pure PC membrane. The points denote experimental values; □ — PC, + — PC-ST 10-1, ■ — PC-ST 5-1, × — PC-ST 3-1, the solid lines present the theoretical data for each molar ratio of PC-ST calculated from Eq. (20) and the theoretical data for pure PC membrane calculated in our work [9].

pH of electrolyte solution (0.155 M NaCl). The experimental values of surface charge density were calculated from measured electrophoretic mobility values using Eq. (2). The theoretical values of the surface charge density were determined on the basis experimental data from Eq. (20). The association constants of the surface groups of the phosphatidylcholine with the H^+, Na^+, OH^-, Cl^- ions (K_{A_1H}, K_{A_1Na}, K_{B_1OH}, K_{B_1Cl}) were determined in an earlier paper [9]. Whereas the association constants of the surface groups of the stearylamine with the OH^- and Cl^- ions (K_{B_2OH}, K_{B_2Cl}) were determined with the use of Eqs. (22) and (24). Eq. (22) was used to calculate coefficients of a straight line equation ($y=ax+b$) in the a_H^+ and $\frac{qa_{H^+}}{F}$ coordinates. Fig. 1 presents a method of determination of coefficients a and b. The plot presents experimental results obtained for PC-ST system at molar ratio 5–1. Analogous Eq. (24) was used to calculate a and b coefficients of a straight line equation in the $\frac{1}{a_{H^+}}$ and $\frac{q}{Fa_{H^+}}$ coordinates. A typical determination of those values in the case of PC-ST system (5–1) is shown in the Fig. 2.

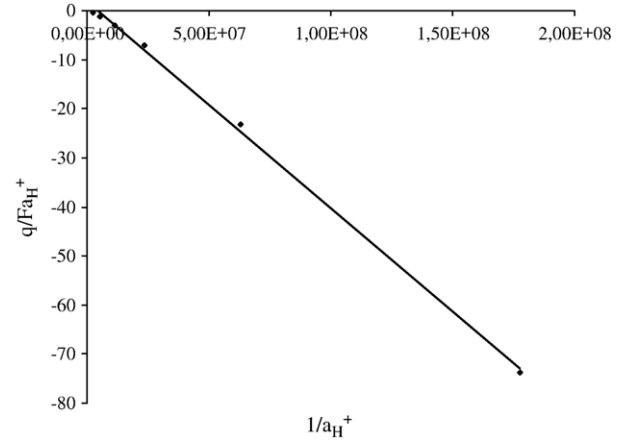

Fig. 2. Method of determination of a and b coefficients of a straight line equation from Eq. (24) for PC-ST system (5-1) — the experimental values are marked by points and the theoretical ones by line.

The knowledge of the calculated coefficients allowed us to determine the K_{B_2OH}, K_{B_2Cl} association constants.

The pH dependence of the surface charge of the PC-ST system (at molar ratios 10-1, 5-1, 3-1) is plotted in Fig. 3. The experimental values are marked by points and the theoretical ones, obtained from Eq. (20), by solid lines. The experimental and theoretical curves for pure PC liposomal membrane are also presented in the Fig. 3. It isn't possible to present curves for pure ST, because this component doesn't create the membrane [21].

It can be observed that in a basic solution with an increasing content of ST in the membrane, a decrease of negative charge occurs and the isoelectric point of the membrane is shifted to a strongly basic solution. $-N^{(+)}(CH_3)_3$ groups of PC molecules and $-N^{(+)}H_3$ groups of ST molecules are covered by OH^- ions. The K_{B_1OH} association constant determined in our previous paper [9] is equal to 5.35×10^9 [m^3/mol] whereas K_{B_2OH} association constant was determined from Eq. (24) and is equal to 23.9 [m^3/mol]. We also proved that the Na^+ ions from the electrolyte solution are adsorbed on free $-PO^{(-)}$ groups of PC ($K_{A_1Na}=0.051$ [m^3/mol]) [9]. However, in acid solution with an increasing content of ST in the PC membrane, an increase of positive charge occurs. $-PO^{(-)}$ groups of PC molecules are covered by H^+ ions, whereas the Cl^- ions from electrolyte solution are adsorbed on $-N^{(+)}(CH_3)_3$ groups of PC and $-N^{(+)}H_3$ groups of ST. The K_{A_1H} and K_{B_1Cl} association constants were determined earlier [9] and are equal to 5.58×10^5 and 0.218 [m^3/mol]. The K_{B_2Cl} association constant was determined from Eq. (22) and is equal to 0.0099 [m^3/mol]. The association constant of the $-N^{(+)}H_3$ group of ST with the Cl^- ions (K_{B_2Cl}) was calculated from Eq. (22) whereas the association constant of the $-N^{(+)}H_3$ group of ST with the OH^- ions (K_{B_2OH}) was calculated from Eq. (24). It is also possible to calculate the K_{B_2OH} constant on the basis of Eq. (22). However it turns out that the value of the intersection point with the ordinate axis (b coefficient in a straight line equation) in the Eq. (22) is burdened with such a large standard error that it

isn't possible to make reliable use of it and, equally, this applies to the K_{B_2OH} value. Casals et al. [22] examined whether pH influences the electrophoretic mobility of PC-ST vesicles. Similar results were obtained; an increase of pH causes a decrease of zeta potential and with an increase in content of ST in the membrane, an increase of zeta potential occurs (and the surface charge density the same).

In order to get a complete picture of the encountered difficulties, we also performed a model calculation of the surface charge density vs. pH for a membrane formed from pure ST (Fig. 4). These are only theoretical considerations because, as we have mentioned above, this component doesn't create a membrane. Fig. 4 presents two curves; one of them was made according to the assumption that only the OH^- ions are adsorbed on the ST membrane surface (presented as solid line). The other curve was obtained according to the assumption that except for the adsorption of OH^- ions, Cl^- ions also are adsorbed (presented as broken line). It can be observed that in pH < 10, in the case when we take the adsorption of Cl^- ions into consideration, a decrease of positive charge occurs compared to the case when we assume the adsorption of OH^- ions only. It can be determined from the comparison of the association constants of the surface groups of ST with the OH^- and Cl^- ions, that the OH^- ion is more strongly adsorbed than the Cl^- ion. However, in pH < 10 the adsorption of the Cl^- ions is observed (Fig. 5).

Coverage of the ST membrane surface by ions vs. pH of electrolyte solution is presented in Fig. 5. The degree of coverage of the PC membrane surface was presented earlier [9]. Beside coverage with OH^- ions, coverage with Cl^- ions was considered to check if coverage with these ions is as high as to affect the PC-ST system membrane surface charge.

As can be seen in Fig. 5, the adsorption of the Cl^- ions begins when the amount of the OH^- ions begins to decrease (at pH < 10). Coverage of the membrane surface by the Cl^- ions remains unchanged in the pH range below 10 and the degree of coverage value is ca. 0.54. Therefore the Cl^- ions cover the ST surface only to some extent, whereas there are some free $-N^{(+)}H_3$ groups at the ST membrane surface. The

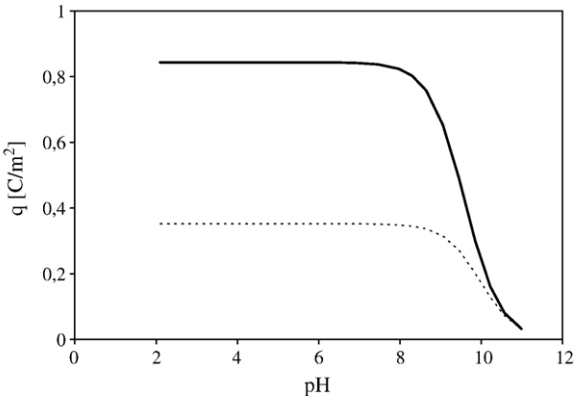

Fig. 4. The theoretical curves of the surface charge density vs. pH for a membrane formed from pure ST. The solid line denotes values obtained after considering only adsorption of OH^- ions at the surface, the broken line denotes values obtained after considering adsorption of Cl^- as well.

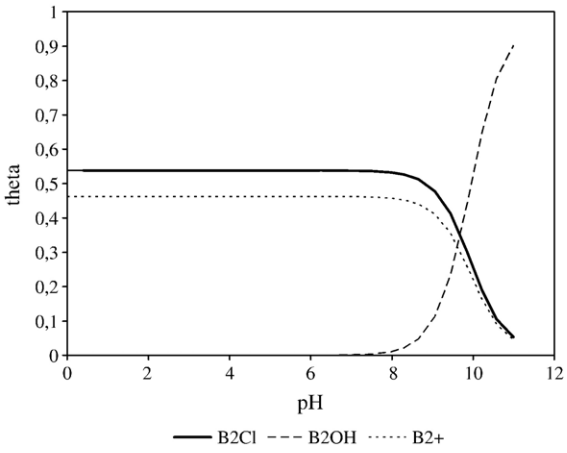

Fig. 5. The degree of coverage of the ST membrane surface, θ, with the OH^- and Cl^- ions, calculated from Eq. (25), as a function of pH of electrolyte.

amount of free $-N^{(+)}H_3$ groups also remains unchanged in a pH range below 10, so almost half of the ST surface in the PC-ST system is not covered. In the case of the membrane formed from PC, the amount of free $-N^+(CH_3)_3$ groups was not significant and the degree of coverage values of those groups amounted to ca. 0.02 (max) within the whole pH range. This is reflected in the association constants values of the PC-ST membrane groups with OH^- and Cl^- ions which are equal to: $K_{B_1Cl}=0.218$, $K_{B_1OH}=5.35\times10^9$, $K_{B_2Cl}=0.0099$, $K_{B_2OH}=23.9$ [m^3/mol]. Differences in the association constant values of amine groups of the PC and ST molecules in the PC-ST membrane can be explained as distinctions in number of carbon atoms bounded with nitrogen atom of amine group. Stearylamine is a primary amine, whereas the choline group of phosphatidylcholine molecule contains a tertiary amine (trimethyloamine). The literature values of the dissociation constants of primary amines are higher than that of tertiary amines [23] and so for the association constants values we have an inverse situation. It is necessary to emphasize that these are values obtained as the volumetric dimension; however our reflections refer to the surface dimension. Nonetheless it is possible, on the basis of these data, to explain differences in the amount of free amine groups of the PC and ST molecules at the PC-ST liposomal membrane surface. We suppose that low values of K_{B_2Cl} and K_{B_2OH} obtained in our work can explain a large quantity of free $-N^{(+)}H_3$ groups of ST molecules, whereas high values of K_{B_1Cl} and K_{B_1OH} explain a low quantity of free $-N^{(+)}(CH_3)_3$ groups of PC molecules.

5. Conclusions

The influence of pH on surface charge density of the PC-ST liposomal membrane was described on the basis of mathematical equations. The association constants values of the functional groups of the membrane components with the electrolyte ions confirmed the adsorption, except H^+ and OH^-, also Na^+ and Cl^- ions at PC-ST membrane surface. The K_{B_1Cl}, K_{B_2Cl} and K_{A_1Na}constants are low compared to K_{B_1OH}, K_{B_2OH}, K_{A_1H} nonetheless the adsorption of Na^+ and Cl^- ions

should be take into account by considering phenomena occurring on the membrane surface. The proposed six equilibria model has been proved to be correct by good agreement of the experimental and theoretical charge variation curves of the PC-ST liposomal membrane.

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