Generation of the Streaming Potential by Liposomes in Cylindrical Capillary. Experimental Data

Z. H. Zawada and B. Biliński

Department of Physical Pharmacy, Medical University of Silesia, Sosnowiec, Poland

Abstract. A model of creation a streaming potential U as a result of colloidal particle movement in flow in a capillary has been described previously (Zawada 1996) as well as the systems for measurement (Zawada 1990, 1991).

The filling of capillary with a solution of liposomes results in a labile adsorbance of liposomes on a capillary glass and changes the measured streaming potential. In order to minimalize these adverse effects, the capillary was covered with phospholipid layer of different composition. Some concentrations of stearylamine as a component of the phospholipid layer may fully compensate the surface charge of the glass capillary and can reduce the liposomes adsorption. The streaming potential of the liposomes solution depends on the ionic strength of the electrolyte and is smaller than the ζ potential for similar liposomes. This suggests that only a part of ions of the liposome ion atmosphere participate in creating of the streaming potential. These are the ions from the hydrodynamic slipping layer. The regression analysis of the relationships between streaming potential U and concentration of liposomes and next ionic strength of the electrolyte gave the value of the surface potential Ψ_0 and the thickness of the hydrodynamic slipping layer d, that is independent of the ionic strength.

Key words: Streaming potential — Surface potential — Liposome(s) — Hydrodynamic slipping layer

Introduction

The streaming potential is often measured for porous, both natural and synthetic materials, mainly membranes, agglomerates, soils, cartilages and bones. It characterises the electric properties of the inner surface of these materials.

Natural membranes bear net surface charge due to the presence of ionised groups of their lipids and proteins. The separation existing between these fixed

Correspondence to: Dr. Zygmunt H. Zawada, Department of Physical Pharmacy, Medical University of Silesia, Jagiellońska 4, 41–200 Sosnowiec, Poland.
E-mail: zzawada@slam.katowice.pl

charges and the free ions dissolved in the aqueous phase surrounding the membrane give rise to an interfacial electric field. It determines the surface potential Ψ_0. A large number of biological phenomena is influenced by the surface potential. In order to study the surface electrical parameters of liposomes, one should use liposomes which have some amphiphile probes incorporated into the bilayer. The electronic state of the probes depends on their polarity environment (Langner and Kubica 1999). The common feature of the methods using probes is the use of fluorescent (New 1990) or electron spin resonance (ESR) probes (Vistnes and Puskin 1981) which enable to estimate the changes of surface charge density σ or the surface potential Ψ_0. If the incorporation of suitable probes into the bilayer of liposomes is possible, these methods become useless.

Measurements of zeta potential (ζ) and electrophoretic mobility are also very important methods for studying the electrokinetic parameters of colloidal particles.

If both the diameter and concentration of liposomes are known, the measurement of the streaming potential U or streaming current I of liposomes solution make it possible to calculate the surface charge density σ and the surface potential Ψ_0 (Zawada 1996). Since the surface potential is a result of liposomes' movement along the hydrodynamic slipping layer, the detailed knowledge about structure of the diffuse layer of liposomes is necessary. The surface potential Ψ_0 of the liposome surface appointed by the measurements of the streaming potential has hydrodynamic nature, that means it is physically similar to ζ potential. It is always less than actual surface potential Ψ_0, particularly at high ionic strength.

The partial verification of the experimental data over the theoretical model (Zawada 1996) was the aim of this paper. The analysis of this phenomenon indicates complicated relationship among the streaming potential U, the surface potential Ψ_0 and diameter of the slipping layer d.

Materials and Methods

Phospholipids: Liposomes were prepared from egg phosphatidylcholine (PC) from the Serva (Heidelberg, Germany), cholesterol (Chol) from Mallinckrodt Inc. (St. Louis, MO), stearylamine (SA) from the Koch-Light Lab. (Haverhill, UK) and phospatidic acid (PA) of Sigma Chemical Co. (St. Louis, MO). PC was cleaned on a basic Al_20_3 column (Singleton et al. 1965). Addition of stearylamine or phosphatidic acid modified the phospholipid composition and surface charge of the membranes. KCl and phosphate buffer solutions saturated with AgCl were the electrolytes. All other chemicals and solvents were analytical grade obtained from commercial suppliers: Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland) and POCH (Gliwice, Poland).

Liposomes: Liposomes were obtained by the modified reverse phase evaporation method (Szoka and Papahadjopoulos 1978). The modification consisted in substituting sonification by mechanical stirring of both the electrolyte (3 ml) and organic phase (9 ml CH_2Cl_2). The components of liposome membrane were previously diluted in a small quantity of $CHCl_3$ and next added to the CH_2Cl_2. By this method,

the whole of phospholipids diluted previously in organic phase was transformed to liposomes form.

In order to measure the streaming potential U at different ionic strengths, the liposomes were dialysed over water. From both the conductivity ω and volume data of dialysates, the ionic strength of samples was counted.

The volume of entrapped water V_w (as the volume of encapsulated aqueous phase over the mass of lipids) was established by adding carboxyfluorescein to the buffer (electrolyte) during preparation of liposomes and then 4-times dialysing over 100-fold volume of the electrolyte. Releasing it out of liposomes by addition of Triton X100 detergent, the encapsulation efficiency was determined from fluorescent data (New 1990). For some type of liposomes, methotrexate-4-amino-N^{10}-methylpteroyl-*L*-glutamic acid (MTX) was used as encapsulating indicator. After dialysis, MTX encapsulated within liposomes was measured photometrically ($\lambda_{max} = 305$ nm).

Heterogeneity and the liposomes radius r_0 were measured by laser light scattering method at a Zeta Sizer 5000 (Malvern) and the r_0 is an averaged radius of the liposomes from these data.

The streaming potential of liposomes prepared from PC only, PC/Chol (2/1 mol/mol) and PC/Chol/SA (5/2/1 mol/../mol) were measured in the KCl solution. The liposomes: PC/Chol (12/5), PC/Chol/SA (10/5/1) and PC/Chol/PA (10/5/1) with and without MTX were studied in the phosphate buffer.

System for the streaming potential measurement: The main part of the system is a thermostating (21 ℃) glass capillary (1) with diameter $\phi = 2R = 0.48$ mm and length $L = 200$ mm (Fig. 1). The capillary connects two containers of 80-ml

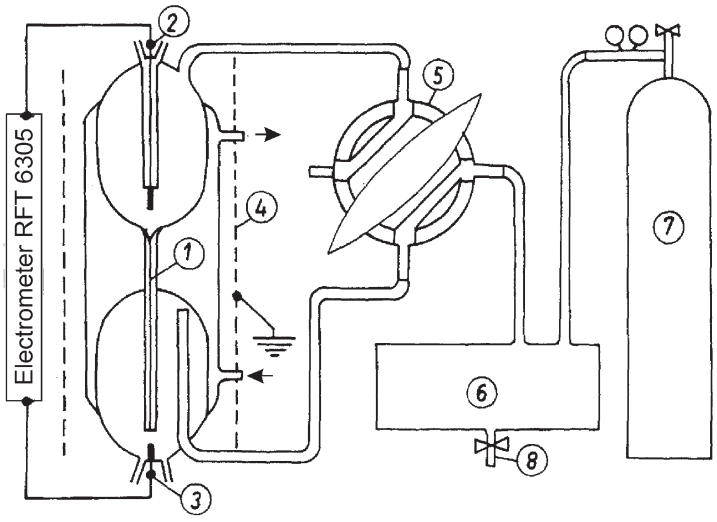

Figure 1. Experimental gauge and pressure-producing system for streaming potential measurements in a single capillary.

capacity each. At the end of the containers there are fitted exchangeable Ag/AgCl electrodes (2), (3). The capillary is electrostatically screened (4). The four-way valve (5) connects the capillary, the container (6) of 20-dm^3 capacity and high-pressure cylinder (7) with N_2. The capillary is filled with the studied solution through the hole up to the upper electrode.

Pressure P is measured by a digital manometer (not shown). Opening the valve (5) one can "push" the studied solution through the capillary in both directions. The valve (8) is used to control the pressure in the system and thus the velocity of the solution flowing through the capillary. The streaming potential U is measured for the upward and downward directions of the flow, next added and averaged to eliminate the influence of the gravitation. The streaming potential was measured with an analogue electrometer RFT 6305 Statron (GDR).

Covering the capillary with phospholipid membranes of different composition, the surface electrical charge of the capillary was modified. This was carried out in the following way: the electrodes were taken out and then 1–2 ml of chloroform solution of phospholipids was added to the upper container, and then the capillary was blown by nitrogen. The covering lipid layer was removed by successive washing the capillary with 95% v/v ethanol, acetone, chloroform and finally with anhydrous ethanol and dried by blow of nitrogen.

Theoretical background

According to the physical model, the streaming potential U is a result of velocity differences between the liposome and electrolyte surrounding it, both flowing through the capillary (Zawada 1996). Liposomes and other particles of colloidal size fix some hydration layer. The slipping layer of thickness d holds a part of the electrical double layer (EDL) of ions that are moving together with the liposome. If the Debye length $1/a$ for spherical surface is defined by the expression:

$$a^2 = \frac{e^2}{\varepsilon\varepsilon_0 kT} \sum c_i z_i^2 \tag{1}$$

and, if $a \cdot r_0 \gg 1$ and $r_0 \gg d$, then the surface potential Ψ_0 is defined as:

$$\Psi_0 \cong \frac{4\pi\sigma}{\varepsilon\varepsilon_0 a} \tag{2}$$

where: e, electron charge; c_i, concentration of ions; z_i, valency of ions; k, Boltzman constant; T, temperature; $\varepsilon_0 = 8.86 \cdot 10^{-12}$ [Farad/meter]; $\varepsilon = 87.74 - 0.4008[T - 273.15]$, dielectric permittivity of water (Gur and Ravina 1979).

The semi-surface potential Ψ_d (or σ_d) at the distance d from the liposome surface is equal:

$$\Psi_d = \Psi_0 \, \mathrm{e}^{-ad} \tag{3}$$

According to the model of (Zawada 1996) the measured streaming potential U is defined as:

$$U = \frac{8\pi \mathrm{Pr}_0^2 \sigma_d N_i}{3\eta\omega R^2} \tag{4}$$

where: η, viscosity of the electrolyte; R, the capillary radius; ω, conductivity of the liposome solution; P, hydrodynamic pressure; σ_d, semi-surface charge density at the distance d from the liposome surface; N_i, number of liposomes within the capillary ($N_\mathrm{i} \sim c_\mathrm{liposomes}$).

If mass m of phospholipids was transformed into N liposomes (single or multilamellar) and each enclose V_0 water phase, then the volume of encapsulation V_w may be defined as: $V_\mathrm{w} = \mathrm{N}V_0/m$. If N liposomes have an average radius r_0, then $N[(4/3)\pi r_0^3 - V_0]\rho = m$; where: ρ is bilayer density (1.014 g/ml, New 1990).

From these equations it is easy to determine the number of liposomes N forming from mass "m" of phospholipids:

$$N = 3m\frac{(1 + Vw\rho)}{4\pi r_0^3\rho} \tag{5}$$

If the capillary volume is $\pi R^2 L$ and volume of sample (after preparation) is V, then $N/N_\mathrm{i} = V/\pi R^2 L$. Connecting this equation with eqs. (2), (4) and (5) we obtain:

$$\Psi_{cd} = \frac{2\pi U r_0 \eta\rho\omega}{cPL\varepsilon\varepsilon_0(1 + V_w\rho)} \quad [\text{If } c \to 0 \text{ then } \Psi_{cd} \to \Psi_d] \tag{6}$$

where: $c = m/V$ (mass/volume) is the concentration of sample.

The surface potential of the solution with concentration of liposomes c we distinguished Ψ_{cd}, but Ψ_d is the surface potential of the solution with concentration of liposomes $c = 0$. The experimental relationships among U, ω and c are the most important factors, when the Ψ_0 or σ value has to be determined.

The thickness of the hydrodynamic slipping layer d should be a function of the ionic strength, temperature and changes of the viscosity in the closest neighbourhood of the liposome membrane.

Results

The aim of covering the capillary glass with phospholipid membrane was both to reduce the high streaming potential resulting from the electrolyte movement against the capillary glass and to decrease the surface fixation of the charged liposomes with the capillary glass. The relationship between the streaming potential U and hydrostatic pressure P for the capillary covered by phospholipid membranes of different composition: PC/Chol/SA from 2/1/1 to 2/1/0 [mol/../mol] was measured for KCl solution. This relationship U *versus* P (about 60 series, 15 points each) was linear and the regression coefficient was > 0.99 (for significance level $p = 0.01$). From these, the membrane of phospholipid composition PC/Chol/SA (2/1/1) [mol/../mol] only showed lack of any relationship between the streaming potential U and the pressure P. This membrane balanced the surface charge of the glass for KCl solution (Fig. 2). When phosphate buffer was used as an electrolyte, the streaming potential was measured in a capillary covered with PC/Chol/SA

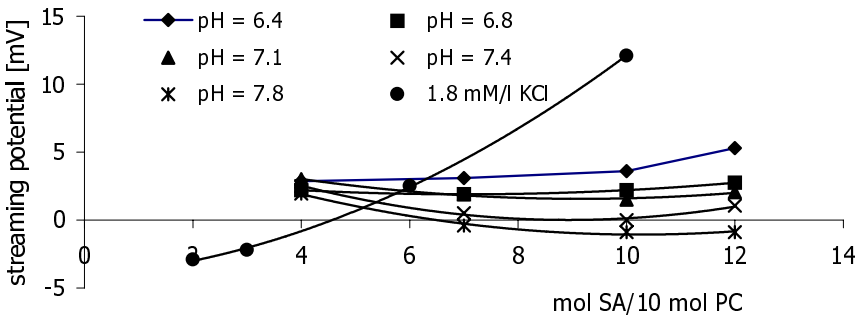

Figure 2. Streaming potential U for the capillary covered with membranes composed from PC/Chol (10/5 mol/mol) mixtured with SA. KCl solution or phosphate buffer were used as electrolytes.

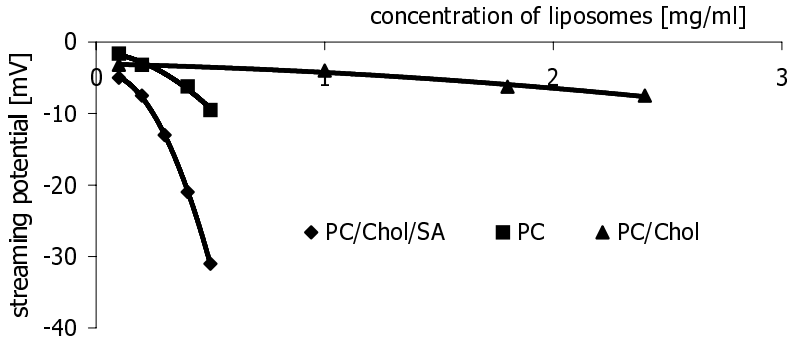

Figure 3. The streaming potential U for different concentrations of liposomes prepared from PC only, PC/Chol or PC/Chol/SA, all diluted in KCl. The inner surface of capillary was coated with PC/Chol/SA (2/1/1 mol/../mol) lipid layer.

membrane at different pH: 6.4, 6.8, 7.1, 7.4, 7.8. As can be seen on Fig. 2, the negative charge of the glass can be balanced by the PC/Chol/SA membrane in the presence of phosphate buffer of pH $\geq$ 7.4 only.

According to this model, the streaming potential U should be linearly related to the liposome concentration c (eq. 5). The relationships received by experimental measurements, however, were never linear, even for the lowest studied liposomes' concentrations. For low concentrations of liposomes (0.01–0.25 mg/ml), the best correlation was obtained for the relationship $U = f(c^2_{\text{liposomes}})$ (Fig. 3), for higher liposome concentrations this relationship was a saturated curve (not shown). Non-linear relationship is probably a result of very small ionic strength ($10^{-5} \div 10^{-4}$ mol/l) or/and high speed of liposomes in the capillary does not permit to reproduce a full ionic atmosphere.

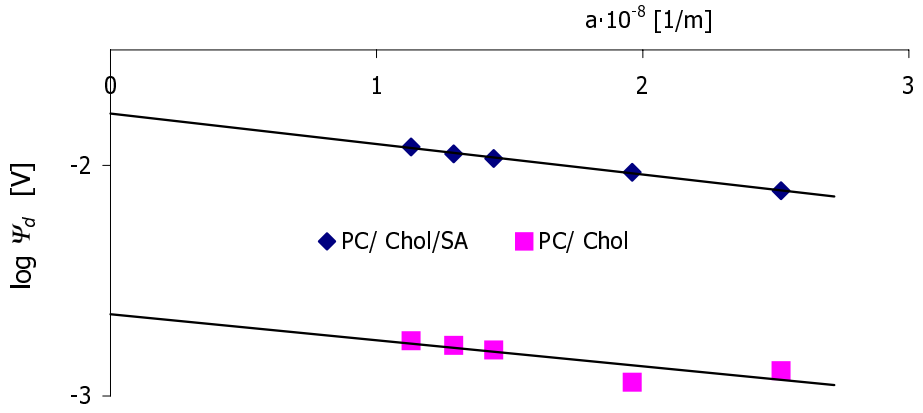

Figure 4. Regression analysis of the Ψ_{0d} potential for PC/Chol and PC/Chol/SA liposomes in 1.8 mmol/l KCl solution. The inner surface of capillary was coated with PC/Chol/SA (2/1/1 mol/../mol) lipid layer.

Table 1. Electrokinetic parameters of liposomes diluted in 1.8 mmol/l KCl solution

Type of liposomes [mol/mol]	c_{KCl} [mol·m^{-3}]	$a \cdot 10^{-8}$ [m^{-1}]	Ψ_d [mV]	Ψ_0 [mV]	$\sigma_d \cdot 10^{+3}$ [C·m^{-2}]	d [nm]	r_0 [nm]
PC/Chol/SA (10/5/2)	0.6	1.12	+11.6	+16.6	0.67	3.15 ± 0.7	825
	1.0	1.46	+10.5				
	1.8	1.96	+8.9				
	3.0	2.53	+7.5				
PC/Chol (2/1)	0.6	1.12	−1.74	−2.19	0.089	2.4 ± 0.7	980
	1.0	1.46	−1.58				
	1.8	1.96	−1.45				
	3.0	2.53	−1.29				
PC				≈ 0			

Fig. 4 shows the relationship $\log \Psi_d = f(a)$ of PC/Chol/SA and PC/Chol liposomes. These relationships are linear in the studied range of KCl concentrations and with the use of regression analysis it is possible to determine the Ψ_0 values (Table 1). The extrapolation of Ψ_d to $a = 0$ refers to a situation where the Debye length $1/a$ is infinitely large and the potential $\Psi_0 = \Psi_d$.

Before and after encapsulation of MTX and after removing non-encapsulated MTX by 5-fold dialysis, the streaming potential of PC/Chol, PC/Chol/SA and

Table 2. Streaming potential U of empty liposomes and of methotrexate encapsulated liposomes after 5-fold dialysis

Type of liposomes [mol/mol]	PC/Chol [12/5]	PC/Chol [12/5] (MTX)	PC/Chol/SA [10/5/1]	PC/Chol/SA [10/5/1] (MTX)	PC/Chol/PA [10/5/1]	PC/Chol/PA [10/5/1] (MTX)
U [mV]	+2.9	−3.4	+12.8	+4.8	−9.1	−3.7
V_w [m^3/kg]*	13.0 ± 3.2		9.2 ± 3.1		10.1 ± 2.9	
Encapsulation efficiency [%]*		17.2 ± 2.2		22.1 ± 3.7		26.8 ± 3.1
$r_0[\mu m]$	0.96		0.74		0.62	

* values are means of $3 \div 7$ experiments $\pm$ S.D.

PC/Chol/PA liposomes diluted within the phosphate buffer (pH = 7.4) was studied. If the liposomes contain MTX, the streaming potential U reaches only a few mV despite the dialysis was changed in relation to the liposomes non-coating MTX (Table 2). It suggests a strong surface binding of MTX with the phospholipid bilayer.

Discussion

Description of the streaming potential and other electrokinetic phenomena using the non-equilibrium thermodynamics concerns mainly the porous or multicapillary system which has a complicated capillary geometry (Katschalski and Curran 1965; Rizvi and Zaidl 1984). It is impossible for such systems to describe the movement of the EDL ions against a stable solid phase according to the Nevier-Stokes equation. In the second extreme case the movement of the EDL ions against a flat wall of a rectangular capillary was initialized and there the hydrodynamic description according to Nevier-Stokes was accurate. The three-dimensional structure of the EDL in such systems was described according to the Guy-Chapman-Stern or later theories. According to these theories, the distribution of EDL ions is a complicated function of distance from the inner surface, viscosity and permitivity of solution (Gurr 1979; Gurr and Ravina 1979; Bowen 1985). The strictly defined phospholipid interface of liposomes with uniformly or point wise distributed electrical charge is a common feature of the above described system.

Liposomes are the simplest model of biological membranes. For biological systems such as tissues, cells or biological membranes the interface is broadened due to transition from clean electrolyte to the hydrophobic core of biological membranes

through a layer of strongly hydrated proteins or surface lipopolysaccharides and a polar layer headgroup of phospholipids.

From the most part of measurements one can obtain only the changes of the surface potential of liposomes. This is a result of the used methods of measurement. A lot of measurements of the $\Delta\Psi$ were carried out using fluorescent probes (Mikes and Kovar 1981), dyes (Nakagaki et al. 1981) or ESR markers; putting them into hydrophobic phase of the liposome and in the water phase. The hydrophobity of the membrane, position of the marker within the bilayer (Langner et al. 1990; Langner and Kubica 1999), pH (Lukac 1983), ionic strength of the electrolyte and the three dimensional EDL structure have a significant influence on the markers state. In these measurements the phospholipid membrane of PC or PC/Chol was the reference material of zero surface charge only under neutral pH conditions. For other pH values there is no standard (Grit and Crommelin 1992).

There is no defined notion of the synonymous value of the surface potential Ψ, for instance at infinitely large or zero ionic strength of electrolyte. All of the data of the surface potential Ψ or the surface charge density σ were obtained for different electrolytes with varying ion concentration.

The Ψ_0 (+16.6 mV) for PC/Chol/SA liposomes defined here as a potential of zero ionic strength is not in agreement with other experimental data ($+4.6 \div 5.6$ mV, Nałęcz et al. 1980) for high ionic strength. The surface potential counted from the streaming potential data is different from the surface potential obtained with the use of the other techniques and the former is more comparable with the zeta-potential from electrophoretic data (Hattenbach et al. 1985). Measurements of electrophoretic mobility of liposomes in solutions of high ionic strength 0.1–0.01 mol/l are usually carried out. For these values of ionic strength the streaming potential is small, often below 1 mV. Finally, random electrostatic disturbances have then critical influence on the accuracy of the streaming potential measurements.

The changes of Ψ_d *versus* a result in large thickness of the hydrodynamic slipping layer: $d = 2.4$ nm for PC/Chol liposomes and 3.15 nm for PC/Chol/SA liposomes (Table 1). The electrophoretic measurements evaluate the thickness as 0.2 nm (Hattenbach et al. 1985). The viscosity, sedimentation and diffusion measurements give values from 1.5 to 14 nm, and the values obtained increase with temperature (Watts et al. 1978).

The EDL ions at the largest distance from the liposome surface are important in generating the streaming potential U. Thus the real structure of the EDL far from the interface becomes crucial. It gives a possibility to correlate the experimental data with the models of ion atmosphere both in the neighbourhood of and far away from the interface. The most often used models of EDL: Gouy-Chapman, Debye-Hückel, Gouy-Chapman-Stern and discreteness-of-charge (Nelson and McQuarrie 1975) apply approximation based on assuming linear relationship between the EDL ions density and the electric field from these ions. It does not agree with the primary Boltzman distribution of co-ions and counter-ions (Libuś and Libuś 1987). This approximation has a relatively large influence on thermal movements of ions in conditions of electrostatic equilibrium within the ion atmosphere for low

ionic strength. All these factors are probably the reason of differences between the surface potential obtained by the above described measurements and by other means. The kinetics of diffusion that reproduced EDL structure was not taken into account during fast flow of the liposomes through the capillary. It probably underrates the registered streaming potential U.

The changes of the streaming potential of liposomes encapsulating MTX were estimated as a result of the surface adsorption MTX, impossible to be removed by manifold dialysis. The presence of MTX at the outer surface of liposome may be important at evaluation of physiology for liposomal MTX, when MTX is present on the outer liposome surface.

References

Bowen B. D. (1985): Streaming potential in the hydrodynamic entrance region cylindrical and rectangular capillaries. J. Colloid Interface Sci. **106**, 367—376

Gur Y. (1979): Electrokinetic and rheological parameters of water in a single capillary. J. Colloid Interface Sci. **72**, 222—232

Gur Y., Ravina I. (1979): Rheological electrokinetic phenomena in a single capillary. J. Colloid Interface Sci. **72**, 442—457

Grit M., Crommelin D. A. (1992): The effect of surface charge on the hydrolysis kinetics of partially hydrogenated egg phosphatidylcholine and egg phosphatidylglycerol in aqueous liposome dispersions. Biochim. Biophys. Acta **1167**, 49—55

Hattenbach A., Gundel J., Haroske D., Muller E. (1985): The interaction of a charged amphiphile with lipid model membranes. A test of the Gouy-Chapman-Stern theory. Studia Biophys. **105**, 39—47

Katschalski A., Curran P. F. (1965): Non-equilibrium thermodynamic in biophysics. Harvard University Press, Cambridge, Mass.

Langner M., Cafiso D., Marcelija S., McLaughlin S. (1990): Electrostatics of phosphoinoside bilayer mambranes. Biophys. J. **57**, 335—349

Langner M., Kubica K. (1999): The electrostatics of lipid surfaces. Chem. Phys. Lipids **101**, 3—35

Libuś W., Libuś Z. (1987): Elektrochemistry. Polish Scientific Publishers PWN, Warszawa

Lukac S. (1983): Surface potential at surfactant and phospholipid vesicles as determined by amphiphilic pH indicators. J. Phys. Chem. **87**, 5045—5050

Mikes V., Kovar I. (1981): Interaction of liposomes with homologus series of fluorescent berberine derivatives. Biochim. Biophys. Acta **640**, 341—351

Nakagaki M., Kotoh I., Honda T. (1981): Surface charge of lipid membrane estimated from the portitioning of methylene blue into liposomes. Biochemistry **20**, 2208—2212

Nałęcz M. J., Zborowski K., Famulski S., Wojtczak L. (1980): Effect of phospholipid composition on the surface potential of liposomes and the activity of enzymes incorporated into liposomes. Eur. J. Biochem. **112**, 75—80

Nelson A. P., McQuarrie D. A. (1975): The effect of discrete charges on the electrical properties of a membrane. J. Theor. Biol. **55**, 13—27

New R. R. (1990): Physical methods of study. In: Liposomes: A Practical Approach. pp. 183–219, Oxford University Press, Oxford

Rizvi S. A., Zaidl S. B. (1984): Nonequilibrium thermodynamic studies of electrokinetic effects across an artifical lipid-coated membrane. J. Colloid Interface Sci. **97**, 536—540

Singleton W. S., Gray M. S., Brown M. L., White J. L. (1965): Chromatografically homogeneous lecithin from egg phospholipids. J. Am. Oil Chem. Soc. **42**, 53—59

Szoka F., Papahadjopoulos D. (1978): Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Biochemistry **75**, 4194—4198

Vistnes A. I., Puskin J. S. (1981): A spin label method for measuring internal volumes in liposomes or cells, applied to Ca-dependent fusion of negatively charged vesicles. Biochim. Biophys. Acta **644**, 244—250

Watts A., Marsh D., Knowles P. F. (1978): Characterisation of dimyristoylphosphatidylcholine vesicles and their dimensional changes through the phase transition: molecular control of membrane morphology. Biochemistry **17**, 1792—1801

Zawada Z. (1990): Measuring apparatus for electrokinetic characteristics of liposomes. PAK **5**, 91—92 (in Polish)

Zawada Z. (1991): Investigation of electrical characteristics of liposome surface using flow potential measurements. PAK **1**, 8—11 (in Polish)

Zawada Z. (1996): Generation of streaming potential by liposomes in cylindrical capillary. A physical model. Gen. Physiol. Biophys. **15**, 165—173

Final version accepted: October 31, 2002