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Some problems of zeta potential determination in electrophoretic measurements on lipid membranes

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Electrophoretic mobilities were measured for cardiolipin, phosphatidylinositol and phosphatidylserine liposomes in solutions of potassium chloride. The zeta potential as a function of ionic strength deviates significantly from the predictions of the double-layer theory in the 10^{-3}–$5 \cdot 10^{-2}$ M range. This might be due to changes in pH in the course of the experiment and/or to the inapplicability of the Smoluchowski equation at low ionic strengths. Taking into account the relaxation effect results in theoretical curves, calculated for various particle sizes at low ionic strength, which are in better agreement with the experiment. However, for quantitative comparison with experimental data, further investigations are necessary.

Introduction

Ion interaction with lipid membranes results in potential changes at membrane/solution interfaces (see, for example, Ref. 1). As was pointed out by Mclaughlin [1] and later by Cherny and co-workers [2] and Abidor and co-workers [3], the Gouy-Chapman theory of the electrical double layer predicts, that for high surface charges and low ionic strengths in 1:1 electrolytes, the surface potential (ψ_s) plotted as a function of ionic strength represents a straight line with a slope about 60 mV per decade. There is some evidence showing that experimental values of surface potentials or those of surface potential changes ($\Delta\psi_s$) do not agree with these double-layer theory predictions. Abidor and co-workers [3] used the potentiodynamic method [2,4] for measurement of $\Delta\psi_s$ vs. log C * curves on bilayer lipid membranes (BLMs) from azolectin in monovalent electrolytes (chlorides and nitrates of alkali-metals, ammonium chloride and potassium perchlorate). Except for LiCl, in every case the slope was less then 60 mV per decade; in most cases $\Delta\psi_s$ plotted vs. log c showed almost no changes (plateau or small slope) between 0.001 and 0.01 M. Lakhdar-Ghazal and co-workers [5] determined the potential jump changes ($\Delta(\Delta V)$) on air/water interface for PG monolayers. In LiCl, NaCl and CsCl solutions below 10^{-4} M $\Delta(\Delta V)$ remained constant; for $C > 10^{-4}$ M $\Delta(\Delta V)$ increased with a slope 53 mV per decade. A slope less then 60 mV per decade was obtained also by Lau and co-workers [6] in ^{31}P-NMR studies of PG liposomes in NaCl.

We measured electrophoretic mobilities of liposomes from anionic lipids in 0.001–1 M KCl. Zeta potential values, calculated from the Smoluchowsky equation, were found to be less then those predicted by the Gouy-Chapman theory for 0.001–0.05 M; the zeta potential plotted vs. log C was found to have the same characteristic features (two regions with different slopes, both less then 60 mV per decade), as those described above for monolayers [5] and planar lipid membranes [3].

The question arises, whether these discrepancies may be interpreted as giving evidence against the validity of the Gouy-Chapman theory or alternatively, as showing some inaccuracy in experimental data? In the

Abbreviations: PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; CL, cardiolipin.

* This paper deals only with monovalent electrolytes; hence the ionic strength is considered to be identical to the salt concentration C.

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latter case we mean just the probability of unsuspected distortions resulting from the peculiarities of either a given model system or experimental procedure applied.

The purpose of the present work was to check the two possible sources of experimental error in zeta potential measurements: first, the pH changes accompanying the liposome preparation and the increase of salt concentration, and second, the inapplicability of the Smoluchowsky equation, commonly used to calculate the zeta potential from electrophoretic mobility values. The probability of pH changes in acidic lipid dispersions is usually supposed to be eliminated either by buffering the solution or by working with small lipid concentrations. As far as we can judge from the literature, however, this supposition is almost never justified by pH measurements, at least in those experiments where the ionic strength is changing. That is why we thought it useful to obtain some experimental evidence concerning both the pH changes in liposome suspensions and the related changes of the zeta potential.

Our grounds for questioning the validity of the Smoluchowsky equation come from electrophoretic studies on inorganic disperse systems, dealing with similar discrepancies between the theoretical predictions and experimental results; the related papers are fully reviewed in Ref. 7. In classical colloid chemistry the problem was solved upon the development of theories more common, then the one suggested by Smoluchowsky; we mean here several theories based on the Gouy-Chapman-Stern model of the double layer, but taking into account also the influence of surface conductivity and double-layer relaxation on electrophoresis [7–12]. The thus obtained quite satisfactory description of numerous experimental data, otherwise inconsistent with the double-layer theory, led to the conclusion, that it was the incorrectness of the Smoluchowsky theory at low ionic strengths that accounted for the anomalies observed in inorganic disperse systems (see, for example, Refs. 13 and 14).

We are aware of only few papers [15–18] paying attention to the surface conductivity and relaxation effects and using the appropriate theoretical description in surface studies of lipid and biological membranes, including liposomes [16,18]. As far as we know, in the majority of cases the electrophoretic mobility data are generally interpreted within the limits of the Smoluchowsky theory; recent examples available are given in Refs. 19 and 20.

Suggesting the significance of surface conductivity or relaxation phenomena in membrane studies, we found it reasonable to supplement this paper with a section 'Theory' dealing briefly with the post-Smoluchowsky improvements in the theoretical descriptions of the electrophoresis; we give also our reasoning for choosing the Dukhin theory for comparison with our experimental results.

Materials and Methods

PI from yeast was purchased from Serva, CL from *Escherichia coli* from factory of bacterial preparations (Charkov, USSR), PS was produced in the laboratory of bioorganic chemistry of the Far Yeast University (Vladivostok, USSR) and also in the laboratory of biochemistry of the Novosibirsk State University (USSR). Lipids were checked for purity before use; all gave one spot by TLC control on silicagel (Desaga HP UVIS device, chloroform/methanol/water (65:25:4, v/v) has been used as solvent system, 0.1–0.2 mg of lipid has been loaded). Additional control for purity was provided by electrophoretic mobility measurements (see below). Potassium chloride (for spectral analysis) and Tris buffer (Calbiochem) were used without further purification. Solutions were prepared in twice distilled water ($\Omega = 2.5$ Mohm). The initial water solution used for liposome preparation contained 0.001 M KCl and 0.0005 M Tris-HCl, pH = 6.9 ± 0.1 ('buffer solution').

Multilamellar liposomes were prepared according to Bangham [21] by the evaporation of nonpolar solvent in a rotating flask and subsequent addition of the buffer solution. Lipid suspension (1 mg lipid/ml) was shaken vigorously for 5–10 min and used immediately for experimental purposes.

Electrophoretic mobility measurements were conducted with a laser IR-spectrometer Zetasizer-II (production of 'Malvern', UK), using the photon correlation spectroscopy technique [22]; a description of the standard procedure of mobility measurement may be found in Refs. 23 and 24. Liposome suspensions were analyzed in the measuring cell at 22°C. As a result, a Doppler shift frequencies spectrum was obtained, and the correspondent mobility values were also found; finally, the frequency/mobility spectrum was printed as graph, and the mean mobility value was used for calculation of the zeta potential by means of the Smoluchowsky equation, where dielectric constant and viscosity were taken to be 80 and 10^{-2} P, respectively. Thus obtained zeta potentials are designated further as ζ_{sm}; as will be shown below, ζ_{sm} may differ significantly from the true zeta potential at low ionic strengths. For any given C_{KCl}, ζ_{sm} was obtained as mean value from no less then three single measurements; the mean deviation was ±2–3 mV. Preliminary experiments showed, that even chromatographycally pure lipid preparations could give the additional peaks of small heights in the region of low mobilities on the mobility spectrum, indicative of some impurities in the liposome membranes. We have chosen therefore the samples showing only one peak in the region of high mobilities; among them, samples exhibiting the most negative values of ζ_{sm} in the buffer solution were selected for subsequent measurements. Addition of EDTA to the

latter samples led either to no changes of ζ_{sm}, or to a slight decrease in its negative value *, contrary to the expected increase induced by the complexation of multivalent cations; that is why we found it reasonable not to use EDTA in our measurements.

pH measurements in liposome suspensions were carried out with pH-meter OP-208 (Radelkis, Hungary), using the combined glass-calomel electrode; the device was calibrated with the standard buffer solutions (pH 6.88 and 7.38) at 22°C. The standard error was ± 0.03 pH units.

Electrophoretic mobility and pH were measured in two series of experiments. In the first series, measurements were conducted in the conventional way: starting with the sample in buffer solution, both parameters were determined after each addition of the concentrated KCl. In the second series, after liposome formation in buffer solution pH was adjusted to 7.00 by 0.01 M KOH; for this sample the electrophoretic mobility was measured. Afterwards, ionic strength was changed by the addition of concentrated KCl, again pH was adjusted to 7.00 and the electrophoretic mobility measured; this typical procedure was repeated in every point, that is, after each addition of concentrated KCl. The same procedure was realized in the absence of buffer; the electrophoretic mobilities were found to be practically the same as those defined in the buffered suspensions. Thus, in the second series we obtained the dependence of electrophoretic mobility (hence, of ζ_{sm}) on ionic strength at constant pH = 7.00. As will be seen below (Fig. 1), this minute transformation of the experimental procedure (compared to the first series) leads to quite appreciable changes of ζ_{sm}. Each series was repeated no less then three times for a given lipid species.

Theory

In the well-known classical Smoluchowsky theory [25] the relationship between the electrophoretic mobility ν and the electrokinetic potential, ζ, is given by

$$\nu = \frac{\varepsilon \varepsilon_0}{\eta} \zeta \qquad (1)$$

where ε and η are, respectively, the relative dielectric permittivity and dynamic viscosity of the disperse medium, and ε_0 is the permittivity of free space.

In the subsequent decades significant progress was achieved in theoretical description of electrokinetic phenomena. The early studies in this field were fully reviewed by Overbeek [8] and Booth [9]; more recent developments were extensively discussed by Overbeek and Wiersema [23], Dukhin and Deryaguin [7], Overbeek and Bijsterbosch [24] and Hunter [14]. The main results were:

(1) Correction for the relaxation effect; the analytical expressions were obtained by Overbeek [8] and Booth [9] and the computer solutions were suggested by Wiersema et al. [11] and more recently by O'Brien and Hunter [12].

(2) Correction for the surface conductivity; the influence of surface conductivity on the electrophoretic mobility was taken into account by Henry [10] and Booth [9]; their expressions are seldom used, however, because the 'normal' surface conductivity effects (see below) are involved in the treatment of the relaxation effect.

(3) The 'polarized double layer' model developed by S.S. Dukhin and his colleagues for thin double layers ($\varkappa \cdot a \gg 1$) [7,28–30]. The most detailed description of this approach is given in Ref. 7. The 'polarization' means here the distortion of the double layer from its equilibrium state caused by the motion of a charged particle. Polarization of the double layer is regarded as being responsible for the relaxation effect [28]. At the same time, the S.S. Dukhin approach gives an elaborate description of the ion flows in the vicinity of a particle surface, thus taking into account the surface conductivity effects on electrophoresis. In this treatment the double-layer model is used, where the shear plane is not necessarily placed at the Stern layer boundary, but may shift away from the surface into the diffuse layer. Thus, the assumption $\zeta = \psi_d$, accepted in the treatment of the relaxation effect [12], is not used in the S.S. Dukhin approach. Hence, the latter takes into account both 'normal' surface conductivity (i.e., that existing outside the plane of shear), and 'abnormal' surface conductivity (i.e., that existing in the hydrodynamically immobile layer, in the diffuse layer inside the plane of shear).

At this point, it seems possible to form a more clear-cut notion about the relationship between the ground terms used in this field. It is obvious, that surface conductivity and double-layer relaxation (identical to the 'polarization') represent the two closely interconnected phenomena accompanying the charged particles' movement in the electric field. In the electrophoresis process of a spherical particle, the two phenomena can not be really separated (see, for example, Ref. 7); in this case, considering the relaxation effect one takes into account also the surface conductivity effect and vise versa. This complex of phenomena may be treated theoretically on the basis of more or less complicated model of the electrical double layer: (1) that requiring the plane of shear to coincide with the Stern layer boundary (Overbeek-Booth-Wiersema

* The EDTA-induced decrease of ζ_{sm} in our case is presumably connected with the experimentally measured decrease of pH in lipid suspension after the addition of EDTA.

[8,9,11] and O'Brien and White [12]) or (2) that allowing the plane of shear to be placed at any distance from the surface in the diffuse layer (Dukhin, S.S., and his colleagues [7,30]). In the first case, one considers both double-layer deformation and ion fluxes only outside the plane of shear; in the second case, both processes are considered outside as well as inside the plane of shear. In the latter case we obtain the more complete description of either surface conductivity or relaxation effects; such a description was suggested by S.S. Dukhin in his 'polarized double layer' approach.

According to Midmore and Hunter [31], the last approach proved to be useful for finding the ζ-potential of polystyrene latexes; the comparison of different theoretical approaches led the authors to the conclusion, that the condition $\zeta = \psi_d$ assumed by O'Brien and White is not obeyed by the latex/electrolyte system for electrolyte concentration less then approx. 0.01 M. It was shown, that in this case, equations suggested either by Dukhin and Semenikhin [30] or by Henry [10] allow the more reliable calculations of zeta potential from electrophoretic mobility data, than those used by O'Brien and White [12].

In this paper we use the S.S. Dukhin theory for clearing out the effect of surface conductivity and double-layer polarization in our experimental conditions. This choice is justified by a small values of $1/\varkappa \cdot a$ (in PS suspension used for theoretical analysis, $a > 0.5$ μm and $1/\varkappa > 10^{-2}$ μm); besides, we can hardly use the procedures of both Wiersema and O'Brien, and White: the former has a upper limit in zeta potential values ($\zeta < 150$ mV) which is sure to be succeeded by highly charged lipid membranes at low ionic strengths (see Fig. 1, curve 3); the latter requires that ζ should be equal to ψ_d, a condition which is not satisfied in our case, because for lipid membranes the plane of shear is placed usually at some distance from the Stern layer (see, for example, Ref. 16).

There are several expressions in the S.S. Dukhin theory, that give the relationship between ν, ζ and ψ_d [7,14,30]. For our purposes, we found it reasonable to use the Dukhin-Deryaguin equation [7] in its form, adapted for the case of strongly charged particles; the adaptation [32] resulted in the simple expression:

$$\nu = \frac{\varepsilon\varepsilon_0}{\eta}\zeta\left[\frac{(1+\mathrm{Rel})\cdot\dfrac{z|\tilde{\zeta}|}{4} - \mathrm{Rel}\ln\mathrm{ch}\dfrac{z\tilde{\zeta}}{4}}{\dfrac{z|\tilde{\zeta}|}{4}(1+2\,\mathrm{Rel})}\right] \tag{2}$$

where z is ion valency, $\tilde{\zeta} = e\zeta/kT$, $\mathrm{Rel} = e^{|\tilde{\psi}_d|/2}/\varkappa a = \varkappa^\sigma/K_e a$, where $\tilde{\psi}_d = e\psi_d/kT$, $\varkappa$ is reciprocal Debye length, a is particle radius, $\varkappa^\sigma$ is specific surface conductivity, K_e is conductivity of water solution. Stern potential ψ_d was taken equal to the surface potential ψ_s *. Note, that in the approach of S.S. Dukhin Rel value reflects the influence both of surface conductivity and double-layer relaxation on the electrophoretic mobility of strongly charged particles (e.g. Ref. 7).

ζ was calculated from the well-known equations of Gouy-Chapman-Stern theory [1]:

$$\psi(x) = \frac{2kT}{e}\ln\frac{1+\alpha\exp(\varkappa x)}{1-\alpha\exp(-\varkappa x)} \tag{3}$$

where

$$\alpha = \frac{\exp(ze\psi_s/2kT)-1}{\exp(ze\psi_s/2kT)+1} \tag{3a}$$

and ψ_s is determined from

$$\frac{A\sigma}{C^{1/2}} = \mathrm{sh}\frac{e\psi_s}{2kT} \tag{4}$$

If the plane of shear is placed at distance L from a surface, then $\zeta = \psi(L)$. σ is membrane charge density, C is electrolyte concentration, $A = 1/(8\varepsilon\varepsilon_0 kT)^{1/2}$. Specific adsorption of monovalent cation was taken into account by

$$\sigma = \frac{\sigma^{\max}}{1 + KC_0\exp(-e\psi_s/kT)} \tag{5}$$

where $\sigma^{\max}$ is the maximal charge density (corresponding to the density of molecules in lipid monolayer at membrane/solution interface); C_0 is bulk ion concentration, K is the cation binding constant.

The effect of surface conductivity and double layer relaxation may be detected by the comparison of experimental mobilities with those calculated from Eqn. 2. In our case, however, we preferred for convenience to compare instead the experimental zeta potentials already available (i.e. ζ_{sm}) with the correspondent theoretical values, calculated from Eqn. 2. Indeed, multiplying both parts of Eqn. 2 by $\eta/\varepsilon\varepsilon_0$, we obtain in the right-hand part the product of ζ-potential and the term in brackets; this product is formally identical to ζ and is designated further as ζ^t_{sm}. Hence, ζ^t_{sm} represents the theoretical prediction for the behaviour of ζ_{sm} within the framework of the Gouy-Chapman-Stern model, corrected for the surface conductivity and relaxation effects.

* Stern potential is used for marking the surface layer of adsorbed ions. For lipid bilayers, ion adsorption boundary is not clearly defined, because of the possibility of ion penetration into the polar headgroup region. Thus, in lipid membrane studies one generally assumes $\psi_d = \psi_s$.

Results and Discussion

Fig. 1 represents the experimental values of ζ_{sm} for CL(A), PI(B) and PS(C) in potassium chloride solution. Curves 1 were obtained in the usual way, by the successive additions of the concentrated salt solution to the lipid suspension in buffer solution. The data demonstrate the marked deviations from the Gouy-Chapman theory prediction (curves 3), obtained for the reasonable values of $\sigma^{max}(1e/60\ \text{Å}^2)$ and $L(2\ \text{Å})$ (see, for example Ref. 1). One may assume, that this discrepancy results from the ζ_{sm}-change, brought about by the pH-changes in liposome suspensions. To test this version, we measured the pH-changes during the liposome formation (ΔpH_f) as well as the pH dependence on ionic strength. ΔpH_f in buffer solution turned out to

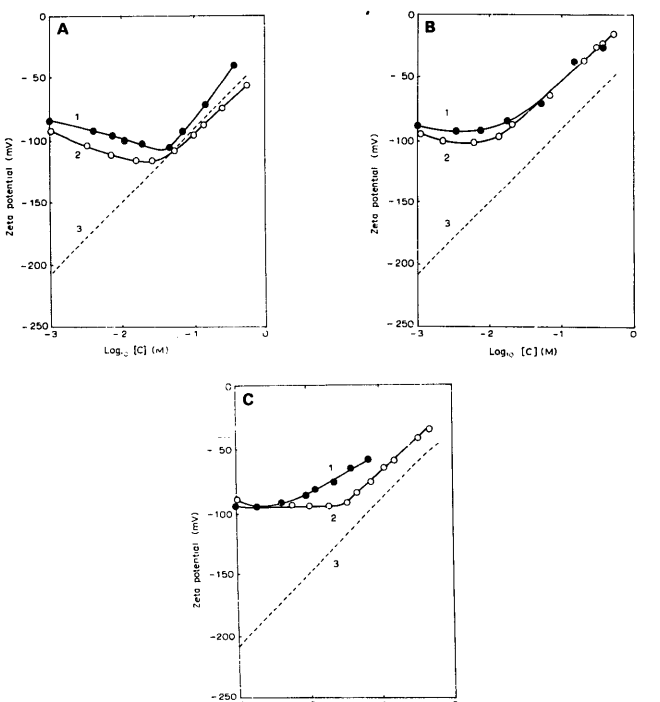

Fig. 1. Zeta potential plotted as a function of KCl concentration. (A) CL; (B) PI; (C) PS. Measurements were started in buffer solution (0.001 M KCl + 0.0005 M Tris-HCl, pH 6.9). Circles: ζ_{sm}, measured at pH, changing in the course of experiment (1) and pH 7.00 (2); the experimental error lies within each circle. Line 3, the zeta potential, calculated from the Gouy-Chapman theory for $\sigma^{max} = 1e/60\ \text{Å}^2$, $K = 0$, $L = 2\ \text{Å}$ (see Theory).

be small for CL and PS (about 0.5 pH units), but quite appreciable for PI (about 1.5 pH units), probably indicative of significant dissociation of the latter lipid in 10^{-3} M KCl. For the three lipids studied addition of salt causes first a slight decrease (in the range 0.001–0.05 M KCl) and then some increase of pH value (data not shown). For each concentration step, the pH change was small (not exceeding 0.15 pH units), but quite noticeable; it could hardly have been the result of the improper choice of the solution constituents: trying various buffers, different salt/buffer ratios in 10^{-3} M KCl or smaller lipid concentrations (0.5 mg/ml $< C_{lip}$ $<$ 1 mg/ml) *, we failed to obtain constant pH 7.00 in the 'blank' experiments, i.e., changing ionic strength without the mobility measurements.

To find out, whether the observed pH-changes are able to modify the true behaviour of ζ_{sm} at pH = 7.00 we measured ζ_{sm} vs. log c curves, correcting the pH at each point in the whole range of ionic strengths; constant pH 7.00 was maintained by the addition of the appropriate amounts of KOH (or HCl) either in the presence or in the absence of buffer. The results are shown in Fig. 1 (curves 2). Comparison with similar data uncorrected for the constant pH (curves 1) reveals the increase of negative ζ_{sm} for all three lipids studied. The effect turned out to be different for various lipid species: CL(A) demonstrates the changes of ζ_{sm} in the whole range of ionic strengths; for PI(B) the change is less pronounced and lies at low C_{KCl}; in the case of PS(C) ζ_{sm} remains practically unchanged below 0.01 M KCl, then increases rapidly with obvious shift of the whole curve at higher ionic strengths. The PS behaviour may look contradictory to the fact, that ζ_{sm} for this lipid was reported to remain constant at 5 $<$ pH $<$ 7 in 0.05–0.2 M solutions of monovalent salts (see, for example, Ref. 33). The problem may be solved, however, by the allowance for the H-bonding between the PS polar headgroups (see, for example, Ref. 34), that affects the pK values of ionizable groups. In our case, increase of ionic strength leads presumably to the decrease of pK_{COO^-} and hence, to the increase of σ^{max}, this process being dependent on the conditions used for changing of salt concentration. Thus, at higher ionic strengths, curves 1 and 2 in Fig. 1C differ not only in the pH value, but also in membrane charge density; this difference may be responsible for the discrepancy of the two curves in the 10^{-2}–10^{-1} M range. This assumption may be proved, in particular, by the direct measurements of pK_{COO^-} for PS membranes at different ionic strengths between 10^{-3} and 10^{-2} M; that and some other related evidence both for PS and for the two other lipids will be reported in our future publications.

Summarizing the results of Fig. 1 we may conclude, that (1) elimination of minor pH changes, arising in the course of experiment, results in quite noticeable changes of zeta potential vs. salt concentration plots, these changes being probably more complicated in nature, than those that may be expected from the corresponding ζ_{sm}(pH)-curves and (2) maintenance of a constant pH of 7.00 makes it possible to specify the ζ_{sm} values, although the pH has almost no influence on the characteristic 'two-slope' behaviour of the zeta potential.

Searching for another way of explanation, we turned to the problem of surface conductivity and double-layer relaxation in electrophoresis. As follows from the section Theory, in our case the effect of both factors on the electrophoretic mobility may be detected by the comparison of experimental zeta potentials already available (i.e., ζ_{sm}) with the corresponding theoretical values (ζ^t_{sm}) calculated from Eqn. 2. Dealing with the polydisperse suspensions studied in this work, we calculated ζ^t_{sm} for a certain set of particle sizes, determined for a given lipid species by means of electron microscopy technique.

Fig. 2 represents the theoretical curves (ζ^t_{sm}) and experimental values (ζ_{sm}) for PS. According to the electron microscopy data, 90% of the particles in 0.005 M KCl have the diameters between 0.5 and 2 μm; among them, about 60% of the particles are about 1 μm. We calculated therefore ζ^t_{sm} for the corresponding radii: 0.25 μm, 0.5 μm and 1 μm. ζ_{sm} was obtained at pH 7.00 (Fig. 1C, curve 2). For comparison, we present in Fig. 2 also 'true' zeta potentials (ζ) obtained from the Gouy-Chapman-Stern theory for the same σ^{max}, K and L as those used for calculation of ζ^t_{sm}.

Fig. 2A shows the ζ^t_{sm}-variation with ionic strength for $\sigma^{max} = 1e/60$ Å^2, $K = 0$ and $L = 2$ Å. For the three sizes chosen, ζ^t_{sm} plotted as a function of log C exhibits a flat minimum, dividing the whole curve into two parts with different slopes to the concentration axis. Minimum is localized at different ionic strengths for the different particle sizes, shifting to the lower ionic strengths with increasing radius a. In the region where $\sigma > 0.1$ M, ζ^t_{sm} changes linearly with ionic strength and is practically equal to ζ for all particle sizes. For $C < 0.1$ M, ζ^t_{sm} becomes less then ζ, and the less is the particle, the more is the difference.

In the region, where ζ^t_{sm} is independent on the particle size ($C > 0.1$ M, Fig. 2A), we can reach the quantitative agreement between ζ^t_{sm} and ζ_{sm}, varying the three parameters: σ^{max}, L and K. We considered σ^{max} to be constant and equal to $1e/60$ Å^2, and searched for either L or K necessary to superpose the theoretical curve with experimental points between 0.1 and 1 M.

* There are concentration limits inherent to Zetasizer II: the reliable mobility data could be obtained for lipid concentration no less than 0.5 mg/ml.

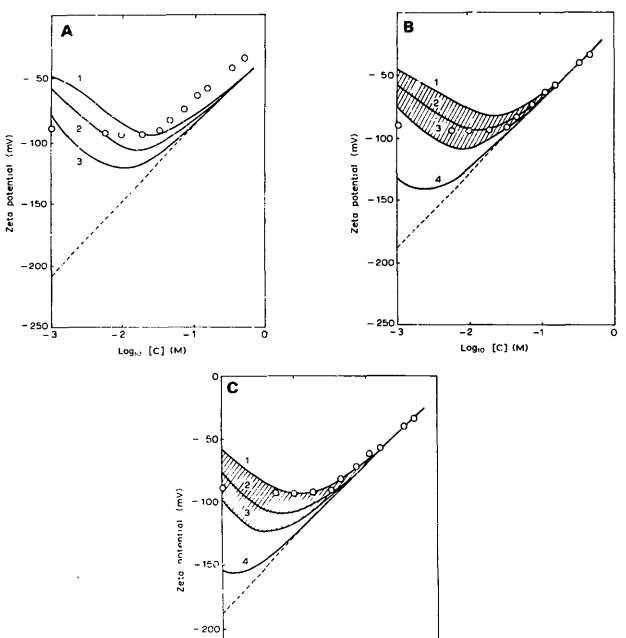

Fig. 2. Comparison of ζ_{sm} (○) with ζ^{L}_{sm}, calculated for PS from the S.S. Dukhin theory (Eqn. 2). Theoretical curves were obtained for $\sigma^{max} = 1e/60$ Å^2 and the following sets of other parameters: A: $L = 2$ Å, $K = 0$; $a = 0.25$ μm (1), 0.5 μm (2) and 1 μm (3); B: $L = 3.5$ Å, $K = 0$, $a = 0.25$ μm (1), 0.5 μm (2), 1 μm (3) and 5 μm (4); C: $L = 2$ Å, $K = 0.25$ M^{-1}; $a = 0.25$ μm (1), 0.5 μm (2), 1 μm (3) and 5 μm (4). The hatched region contains the set of values ζ^{L}_{sm}, calculated from Eqn. 2 for the particles, present in PS suspension studied. This region should obviously include also the experimental values ζ_{sm}, providing that the electrophoretic mobility suffers from the relaxation effect. Dashed line, the 'true' zeta potential (ζ), calculated from the Gouy–Chapman-Stern theory for $\sigma^{max} = 1e/60$ Å^2; K and L, respectively, 0 and 2 Å (A), 0 and 3.5 Å (B), 0.25 M^{-1} and 2 Å (C).

Fig. 2B shows the results for the selection of L, with $K = 0$. In the range 0.1–1 M the best agreement was achieved for $L = 3.5$ Å. With this L value, at low ionic strengths we have also rather good agreement between changes of ζ^{L}_{sm} and ζ_{sm}; in the range 0.002–0.03 M experimental values of ζ_{sm} lie within the limits of the hatched region, restricted by the curves $\zeta^{L}_{sm}(\log C)$, calculated for the particles sizes, found for the given lipid suspension. It may be seen also, that for high ionic strengths ζ_{sm} coincides with 'true' zeta potentials (ζ), calculated from Eqn. 3; the deviations begin at 0.02–0.03 M.

Fig. 2C represents the results for the chosen K with $L = 2$ Å. The coincidence of ζ_{sm} with 'true' zeta potential was obtained in the range 0.04–1 M; K was found to be 0.25 M^{-1}, in agreement with $K_K = 0.215$ M^{-1} obtained for PS in Ref. 16. Deviation of ζ'_{sm} from ζ is observed between 0.001 and 0.03 M; for these ionic strengths all the experimental points are found to be inserted between the ζ'_{sm}(log C)-curves, calculated for the given particle sizes (hatched region).

Summarizing the results shown in Fig. 2 we may say here, that ζ'_{sm} calculated according to the Dukhin equation behaves similarly to the experimental values of ζ_{sm} at the ionic strengths studied. The similarity expresses itself, first, in the change of slope in one and the same range of ionic strengths and second, in the observation, that experimental values of ζ_{sm} lie within the limits of ζ'_{sm} values, calculated for the particle sizes, found for the given lipid suspension.

Thus, we have the right to conclude, that the anomalous behaviour of zeta potentials in our case may really be brought about by the relaxation effect. If this is true, the Smoluchowsky equation is not valid because ζ_{sm} is not equal to the 'true' ζ, but represents rather a certain parameter proportional to the electrophoretic mobility. The coincidence of ζ_{sm} and ζ takes place where the relaxation effect is not significant; this happens, when the combination of surface charge, ionic strength and particle radius satisfies to the condition Rel $\ll 1$ [7]; our calculations show, that difference between ζ and ζ_{sm} is less then experimental error for Rel < 0.02.

In electrophoretic measurements on polydisperse suspensions, quantitative comparison of ζ'_{sm} and ζ_{sm} is not justified, since ζ'_{sm} is calculated for a given radius of a particle, while ζ_{sm} is determined for a certain set of particles with different radii, it being known that size distribution for charged amphiphiles may vary significantly with ionic strength. For this reason, we affirm here just the possibility of the relaxation effect on the electrophoretic mobility; the final conclusion may be achieved only in the course of measurements on monodisperse suspension (or alternatively, using the microelectrophoresis technique), providing that particle size remains constant in the wide range of ionic strengths.

As for the reasons of disagreement of experimental data for ζ_{sm} with double-layer theory predictions, the question remains unsolved for the time being; one may think, however, that, using the electrophoresis technique, for finding a well-founded answer the estimation of error, brought in by the Smoluchowsky equation, is extremely desirable. Such an estimation proves to be useful not only for the relatively small particles present in our experimental case, but also for the large particles, which may be encountered in other lipid dispersions. Fig. 2 (B and C) shows the change of ζ'_{sm} for the particles having the diameter 10 μm (curves 4); it may be seen, that the difference between ζ'_{sm} and ζ is small in the range 0.01–1 M, but becomes quite noticeable below 0.01 M. Hence, even for the large particles a significant difference may be expected between ζ'_{sm} and true zeta potential for large surface charges and low ionic strengths.

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