The interaction of double-charged metal ions with monolayers and bilayers of phospholipids

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Electrophoretic mobilities of hexadecane/water emulsions containing dimyristoylphosphatidylcholine (DMPC) or egg yolk lecithin (EYL) monolayers at the interface and those of liposomes prepared from the same lipids were measured as functions of the concentrations of Ca^{2+}, Mn^{2+}, Cu^{2+}, and Ni^{2+} cations in the aqueous phase. The surface potentials, surface charge densities (σ), and the Langmuir adsorption isotherms for various distances from the charged surface to the slip plane (d) were calculated on the basis of the Gouy-Chapman theory for 1 : 2 electrolytes and the values of ζ-potentials. The binding constants (K) and parameters d were determined under the assumption that the maximum σ values correspond to one ion per phospholipid molecule at the interface. In the case of DMPC, the ion binding constants (L mol^{-1}) at 25 °C are 230 and 87 for Ca^{2+}, 31.5 and 21 for Mn^{2+}, 11 and 6 for Cu^{2+}, and 7.5 and 5.3 for Ni^{2+} in liposomes and emulsions, respectively. The affinities of Cu^{2+} and Ni^{2+} ions for EYL monolayers and bilayers are lower than those for DMPC mono- and bilayers. The d parameters for all ions are smaller than the radii of the hydrated ions. In the case of Ca^{2+}, Cu^{2+}, and Ni^{2+}, the d values for mono- and bilayers are different. The differences in K values between monolayers and bilayers as well as those between DMPC and EYL mono- and bilayers can be attributed to the differences in the local environment and orientation of the interfacial phosphate groups in these systems.

Key words: phospholipids, liquid/liquid interfaces, bilayer membranes, double-charged ions, adsorption, electrophoresis, surface potential, electrokinetic potential.

Study of adsorption of double-charged cations on the surface of biological and model membranes is important from several viewpoints. Binding of ions to the phosphate groups of phospholipids (PL) can change the dynamic and ion-transport properties of mono- and bilayers.[1] Calcium ions are known to cause aggregation and coalescence of liposomes and cells.[2] The regularities of binding of paramagnetic metal ions (Ni^{2+}, Cu^{2+}, Mn^{2+}) to mono- and bilayers are also of considerable interest in connection with the use of paramagnetic relaxation of spin probes and labels, induced by these ions, to study the topography and the molecular dynamics of phospholipid layers.[3,4]

A number of studies have been devoted to the binding of double-charged ions, mostly Ca^{2+}, to bilayer lipid membranes.[5–11] However, interaction of these ions with monolayers at a liquid/liquid interface has scarcely been studied. These data are important because many chemical and biological processes occur at the interface between two liquid phases; these processes as well as the stability of disperse systems are largely determined by structural and dynamic parameters of monolayers.[12–15]

In the present work, we studied binding of double-charged cations to PL monolayers at the organic phase/water interface in emulsions and also, for comparison, to bilayers of the liposomes obtained from the same PL. Binding isotherms were obtained by the microelectrophoresis method, which we used previously to study adsorption of surface-active anions at the oil/water interfaces in macroemulsions.[16,17]

Experimental

Dimyristoylphosphatidylcholine (DMPC) (Sigma) and egg yolk lecithin (EYL) manufactured at the Kharkov Plant of Bacterial Preparations were used. Hexadecane ("pure" grade) was purified by distillation *in vacuo*. Calcium, manganese, copper, and nickel chlorides ("chemically pure" grade) were used as received.

Hexadecane/water emulsions containing PL monolayers at the interface were prepared using bidistilled water by ultrasonic dispersion on a UZDN-2 setup. A typical emulsion contained 50 mg of PL, 0.5 mL of hexadecane, and 9.5 mL of H_2O. An ice-cooled suspension was irradiated at a frequency of 22 kHz for 2 min; the operation was repeated three times at 2-min intervals. According to dynamic light-scattering measurements, the average size of the disperse particles was 0.5 μm. Previously,[18] it was shown by the spin probe method that dispersion under these conditions with the given component ratios mostly gives emulsions; the content of liposomes in the solution does not exceed 1.5% of the total PL concentration.

Liposomes from EYL and DMPC were prepared in the absence of hexadecane under the same dispersion conditions and with the same PL concentrations as those used to prepare the emulsions.

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Electrophoretic measurements were carried out at 25 °C using the setup described previously.[16] Emulsions or liposomes with a PL concentration of 0.1 mg mL^{-1} were added to a solution of a salt with the required concentration. To determine the field intensity in the electrophoretic cell, the conductivity of the cell containing no suspension was measured by the bridge method with an accuracy of ±0.5%. The electrophoretic mobility (the ratio of the velocity of a particle to the field intensity) at each concentration was averaged over nine or ten measurements.

Results and Discussion

Theory. The binding of ions to the surface of particles in emulsions or liposomes can be studied using the electrophoresis technique by measuring the electrokinetic potential of a disperse particle (ζ) induced by the adsorbed ions. The ζ value is related to the electrophoretic mobility of the particle (μ), measured experimentally, by the Smolukhovsky—Helmholtz equation

$$\mu = \varepsilon\zeta/(4\pi\eta), \qquad (1)$$

where ε and η are the medium dielectric constant and viscosity, respectively.

The relationships between ζ and the surface potential (ϕ_0) and between ϕ_0 and the surface charge density (σ) can be derived from the Gouy-Chapman theory. It will be shown below that neglecting the binding of counterions, assumed in this theory, is consistent with the results obtained in this study and with published data. In the case of 1 : 2 electrolytes, an exact solution of the Poisson—Boltzmann equation for the diffuse double electric layer potential (ϕ) exists:[19,20]

$$th[\nu(\Psi)/4] = th[\nu(\Psi_0)/4]\exp(-\kappa d), \qquad (2)$$

where $$\nu(\Psi) = \ln\{[2\exp(\Psi) + 1]/3\}, \qquad (3)$$

$\Psi = e\phi/(kT)$, d is the distance from the surface on which bound charges are localized to the slip plane, and $1/\kappa$ is the length of the Debye shielding. For a 1 : 2 electrolyte,

$$\kappa = \{24\pi e^2 n/(\varepsilon kT)\}^{1/2}, \qquad (4)$$

where n is the number of cations in 1 cm^3 of the solution.

Equations (1)—(4) make it possible to calculate the surface potential ϕ_0 from the ζ-potential provided that the parameter d is known. The relationship between ϕ_0 and σ for 1 : 2 electrolytes has the following form:

$$\sigma = [\varepsilon kT\kappa/(4\pi e)]\cdot\{[2\exp(\Psi_0) + \exp(-2\Psi_0)]/3 - 1\}^{1/2}. \qquad (5)$$

In terms of the simple model assuming one type of binding sites, the binding constant (K) is determined from the relation

$$K = \vartheta/[(1 - \vartheta)c\exp(-2\Psi_0)], \qquad (6)$$

where c is the concentration of ions in solution, and ϑ is the fraction of the occupied binding sites on the surface; the Boltzmann factor $\exp(-2\Psi_0)$ takes into account the change in the ion concentration near the charged surface with respect to that in solution. The ϑ value can be expressed in terms of the surface charge density (σ) created by the adsorbed ions: $\vartheta = \sigma/\sigma_m$, where σ_m corresponds to binding by all the adsorption sites on the surface. Thus, the ϑ value depends on the ϕ_0 potential, which, in turn, is a function of the ϑ value and the ion concentration in solution, which determine the degree of its shielding. It is convenient to represent Eq. (6) as the dependence of s on the near-surface concentration of ions, $c_s = c\exp[-2e\phi_0/(kT)]$:

$$\sigma = \sigma_m Kc_s/(1 + Kc_s). \qquad (7)$$

When disperse particles are negatively charged (in the absence of metal ions) and when several (n) types of binding sites are present on the surface, Eq. (7) can be written in a more general form:

$$\sigma = \sum_{i=1}^{n}\sigma_{mi}\frac{K_i c_s}{1 + K_i c_s} - \sigma_0 . \qquad (8)$$

Results. The ϕ_0 and σ values at various ion concentrations in the solution were calculated using a specially composed program, which provided numerical solution of Eqs. (1)—(5) for specified d values. After that, the K and σ_m values were determined by the least-squares method using modeling of the $\sigma(c_s)$ dependences by Eq. (7) or (8).

The conditions for applicability of Eq. (1) were discussed previously.[16,21] An experimental criterion for its applicability is that the electrophoretic mobility should not depend on the particle size, which is the case for both liposomes and emulsions.

Figure 1 presents the dependences of the ζ-potential on the concentrations of Ca^{2+}, Mn^{2+}, and Ni^{2+} chlorides for both liposomes and emulsions prepared from

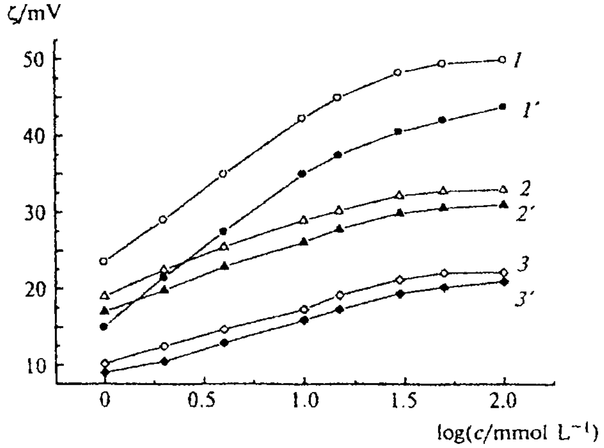

Fig. 1. Dependences of the ζ-potential on the concentrations of the Ca^{2+} (*1, 1'*), Mn^{2+} (*2, 2'*), and Ni^{2+} (*3, 3'*) ions in dispersions of DMPC liposomes (*1—3*) and hexadecane/DMPC/water emulsions (*1'—3'*) at 25 °C.

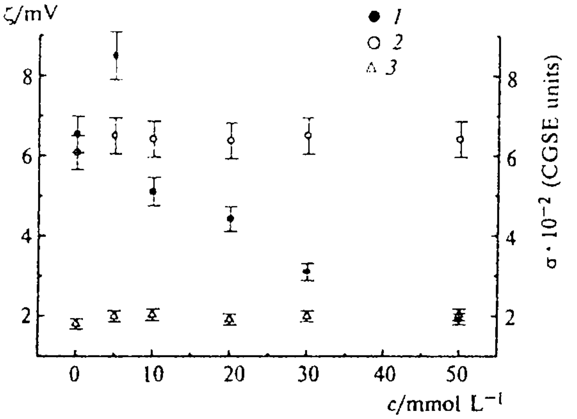

Fig. 2. Dependence of the ζ-potential (*1*) and the surface charge density of the Ni^{2+} ions (σ) (*2*, *3*) on the NaCl concentration in DMPC liposome dispersions in the presence of 5 (*1*, *2*) and 1 (*3*) mmol L^{-1} of $NiCl_2$.

DMPC. The plots for ζ(*c*) and $CuCl_2$ and for all of the salts in the case of EYL, which are not shown in Fig. 1, follow similar patterns.

The ζ values in the absence of divalent metal salts were measured in NaCl (1 mmol L^{-1}). For DMPC liposomes and emulsions, they varied from 0 to −3 mV; in the case of EYL, the maximum ζ values were −6 mV.

As noted above, Eqs. (1)—(5) are valid in the absence of binding of counterions, in this particular case, Cl^- anions. To verify this assumption, we measured the dependences of ζ-potentials on the NaCl concentration in the presence of $NiCl_2$ (1 and 5 mmol L^{-1}). These dependences were used to calculate the σ values for various *d* values. It can be seen from Fig. 2 that the σ value virtually does not depend on the NaCl concentration, indicating that no noticeable binding of Cl^- ions occurs. A similar conclusion confirming the absence of binding of Cl^- ions to lipid membranes has been made previously.[5]

The σ and ϕ_0 values can be determined from the experimental ζ(*c*) curves if the parameter *d* is known. In order to find all these parameters from the ζ(*c*) dependences for each type of ions in emulsion and liposome dispersions and for *d* values ranging from 0—0.2 nm, families of σ(*c*) and $\phi_0(c)$ curves and $\sigma(c_s)$ plots were calculated. Figure 3 shows the $\sigma(c_s)$ plots for the Mn^{2+} ions in DMPC liposomes and for the Ni^{2+} ions in hexadecane/DMPC/water emulsions as examples; the dashed lines correspond to the results of modeling of these dependences by Eq. (8) for $n = 1$ using the nonlinear least-squares method. The $\sigma(c_s)$ dependences for other ions in liposomes and emulsions follow similar patterns.

A typical feature of these binding isotherms is the pronounced dependence of their shape on the *d* value. Thus for the curves shown in Fig. 3, *a*, the parameter *K* decreases as *d* increases from 0 to 0.2 nm; when $d = 0.16$ nm, this parameters becomes negative, which is meaningless for a binding constant. Thus, for the Mn^{2+} ion adsorbed on DMPC liposomes, the *d* values should not be greater than 0.15 nm. The upper *d* values for other ions in liposomes and emulsions were estimated in a similar way: for Mn^{2+} in DMPC emulsions, the parameter *d* should not exceed 0.15 nm, for Cu^{2+}, the parameters should not be larger than 0.25 and 0.15 nm, respectively, those for Ni^{2+} should not exceed 0.18 and 0.13 nm, and those for Ca^{2+} should not exceed 0.1 and 0.09 nm.

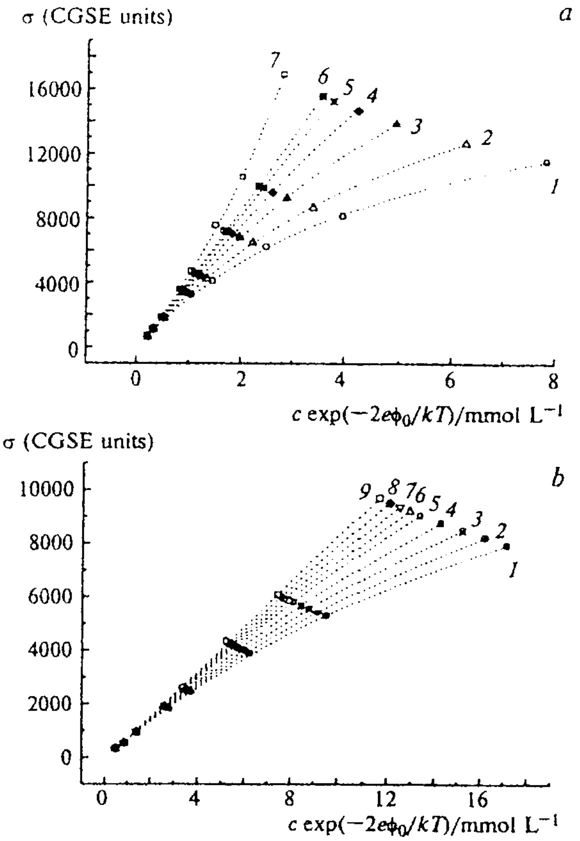

Fig. 3. Families of the plots for the surface charge density (σ) created by the adsorbed ions *vs* the concentrations of these ions near the surface $c_s = c\,\exp(-2e\phi_0/kT)$ (*c* is the concentration in the bulk of the aqueous phase, ϕ_0 is the surface potential) at various distances from the charged surface to the slip plane (*d*). The dots were obtained from the experimental plots for the ζ-potential *vs* ion concentration by calculating the ϕ_0 and σ values in terms of the Gouy-Chapman theory for 1 : 2 electrolytes; dashed lines correspond to the results of modeling in terms of Eq. (8) for $n = 1$. *a*. The Mn^{2+} ions in a DMPC liposome suspension at *d*/nm = 0 (*1*), 0.05 (*2*), 0.10 (*3*), 0.13 (*4*), 0.15 (*5*), 0.16 (*6*), 0.2 (*7*). *b*. The Ni^{2+} ions in a hexadecane/DMPC/water emulsion at *d*/nm = 0.05 (*1*), 0.07 (*2*), 0.09 (*3*), 0.11 (*4*), 0.13 (*5*), 0.14 (*6*), 0.15 (*7*), 0.16 (*8*), 0.17 (*9*).

The next obvious limitation imposed on the choice of *d* is that the σ_m values determined by modeling of the $\sigma(c_s)$ isotherms should not be greater than the value corresponding to binding of one ion to one PL mol-

Table 1. Parameters of binding of double-charged metal ions to phospholipid mono- and bilayers

Ion	Dispersion	d /nm	K /L mol^{-1}	$\sigma_m \cdot 10^{-4}$ /CGSE units
Ca^{2+}	DMPC liposomes	0.1	230±70	15.5±4.1
	DMPC emulsions	0.077	87±21	14.8±2.9
Mn^{2+}	DMPC liposomes	0.14	31.5±4.2	14.70±1.25
	DMPC emulsions	0.135	21.0±4.2	14.4±1.5
Cu^{2+}	DMPC liposomes	0.235	11±3	15.6±3.3
	DMPC emulsions	0.13	6.0±1.5	14.3±2.3
Ni^{2+}	DMPC liposomes	0.18	7.5±0.6	15.3±2.9
	DMPC emulsions	0.15	5.3±0.8	15.6±2.9
Cu^{2+}	EYL liposomes	0.195	3.75±1.5	15.5±2.5
	EYL emulsions	0.094	2.75±1.5	15.4±3.1
Ni^{2+}	EYL liposomes	0.07	2.0±0.4	15.6±2.9
	EYL emulsions	0.13	1.70±0.13	14.6±2.3

ecule. The area occupied by one PL molecule (A) in a fully hydrated lipid bilayer in the mesomorphic state[22] is 64 Å^2; hence, $\sigma_m = 2e/A \approx 15 \cdot 10^4$ CGSE units. Moreover, to quantitatively estimate the binding constants from binding isotherms, it is reasonable to assume that for all of the ions in lipid bilayers, the σ_m values are equal to the above-mentioned value to within the determination error for modeling parameters. Apparently, in monolayers, the A value is somewhat greater (correspondingly, σ_m is somewhat smaller) than that in bilayers. This assumption is based on the fact that rotational mobility of spin probes in monolayers is higher and the phase transition temperature is lower than those in bilayers.[18] However, the difference between the A values is apparently not larger than 10—20%, because it is known to be smaller than the difference between bilayers and cylindrical micelles.[22]

Table 1 contains binding constants and the parameters d, calculated from binding isotherms using the given σ_m values, for all the ions in DMPC-based liposome and emulsion dispersions and for the Cu^{2+} and Ni^{2+} ions in emulsions and liposomes from EYL.

Discussion. First of all, it is of interest to compare the resulting d values with the ion radii. According to Belov and Bokii,[23] the crystallographic radii of the Ca^{2+}, Mn^{2+}, Cu^{2+}, and Ni^{2+} ions are 0.104, 0.091, 0.080, and 0.074 nm, respectively. The radii of the aqua complexes of these ions, calculated for the octahedral coordination of water molecules using the tabulated values of the atom radii and bond lengths,[23] are 0.256, 0.243, 0.232, and 0.226 nm, respectively. Comparison of these values with the d values in Table 1 shows that for all of the ions, d is smaller than the radius of a hydrated ion. However, the sizes of nonhydrated Mn^{2+}, Cu^{2+}, and Ni^{2+} ions fall into the range of possible d values; in the case of the Ca^{2+} ion adsorbed on DMPC monolayers, the d value is somewhat smaller than the ion radius. Based on this comparison, it can be assumed that the adsorbed ions are partly dehydrated. The possibility that the symmetry of the ligand environment of an ion changes substantially due to its partial dehydration upon adsorption follows, among other reasons, from the fact that the ESR lines of the aqua complex of the Mn^{2+} ion are relatively narrow, whereas the ESR spectra of adsorbed Mn^{2+} ions are virtually not observed due to the large splitting in the zero field.[24]

It can be seen from Table 1 that the Ca^{2+} ions are characterized by the greatest constants of binding to both bilayers (liposomes) and monolayers (emulsions). This result, as well as the relative binding constants for Ca^{2+}, Mn^{2+}, Cu^{2+}, and Ni^{2+} ions, actually does not depend on the d magnitudes. The fact that the affinity of the Ca^{2+} ions for membranes is higher than that of Ni^{2+} ions is confirmed by independent data on spin-lattice relaxation of spin labels in lipid membranes: in the presence of Ca^{2+}, the relaxation induced by paramagnetic Ni^{2+} ions virtually disappears[25] as a result of the competing displacement of Ni^{2+} by Ca^{2+}.

It is interesting to compare the binding parameters for mono- and bilayers. The fact that the affinity for monolayers is lower than for bilayers for all four of these ions may be due to different orientations or different local environments of the PL phosphate groups in mono- and bilayers. In this connection, note that the reciprocals of K (dissociation constants) are two or three orders of magnitude greater than the equilibrium concentrations of these ions in saturated solutions of the corresponding inorganic phosphates, which had been estimated using the data on the solubility products.[23] This difference is apparently due to the influence of the local environment at the interface of lipid layers on the binding constants of ions. The different orientations of polar groups in mono- and bilayers may also account for the slight differences between the d values for the Ca^{2+}, Cu^{2+}, and Ni^{2+} ions (see Table 1).

It can also be seen from Table 1 that the constants of binding of the Cu^{2+} and Ni^{2+} ions to DMPC bilayers and monolayers are greater than those for the EYL layers. This result is consistent with the data[5] indicating that the constant of binding of the Ca^{2+} ions increases several-fold on going from EYL to saturated PL. DMPC is a PL with saturated alkyl chains, whose lipid layers undergo a gel—liquid crystal phase transition at 23 °C. EYL contains the same polar group, phosphatidylcholine, as DMPC; however, EYL is heterogeneous in its acyl residues, which are mostly formed from unsaturated hydrocarbon chains of various lengths consisting of equilibrium mixtures of *cis*- and *trans*-isomers; as a consequence, the lipid bilayer is less ordered than DMPC.[22] The dependence of the binding constant on the structure of hydrocarbon chains is apparently due to the fact that they have an influence on the packing and orientation of the PL polar groups, which results in different local environments of the binding sites.

Potentiometric and conductometric studies of the binding of cations to liposomes and emulsions are difficult because they require the use of high concentrations of disperse particles, and this results in their aggregation. The methods of equilibrium dialysis and radioactive labels do not allow one to distinguish between the adsorbed ions and the ions occurring in the diffuse double electric layer. ^{31}P NMR spectroscopy provides valuable information on both the fraction of bound phosphate groups and on the lifetime and the structure of the complex; however, it is applicable only to paramagnetic ions with very short spin-lattice relaxation times.[5]

The electrophoresis method used in the present study is free from the above limitations. However, it can be used only in those cases where the Smolukhovskii—Helmholtz equation (1) and the Gouy-Chapman theory are applicable to the liposome and emulsion dispersions; in addition, the distances from the adsorbed ions to the slip plane need to be known. It can be seen from the results presented here that the dependences of the ζ-potential on the metal ion concentration are described adequately in terms of the simple adsorption model in which the ion concentration near the interface, which depends on the surface potential, is used as the concentration of free ions; the relationship between the surface potential and the density of the adsorbed charges is determined using the Gouy-Chapman theory.

The adsorption of double-charged metal ions by PL multilayers was studied[6] by an indirect method based on the measurement of the electrostatic repulsion between bilayers. It was concluded that binding of Ca^{2+} ions in this system cannot be described by the binding constant and the surface potential. However, the use of a fluorescence probe (1-aniline-8-naphthalenesulfonate) gave[7—9] dissociation constants for the Ca^{2+} ions and lipid membranes of the order of 10^{-3} mol L^{-1}, which is in satisfactory agreement with the results of the present study.

The published data on the Mn^{2+} binding by bilayer membranes are also contradictory. The dissociation constant was found[10] to be 0.11 mol L^{-1}; determination by a different method[11] gave a value of 0.02 mol L^{-1}, which is consistent with our results.

Thus, in the present study, the constants of binding of the Ca^{2+}, Mn^{2+}, Cu^{2+}, and Ni^{2+} ions to PL monolayers at the liquid/liquid interface were determined for the first time; the difference between the affinities of monolayers and bilayer membranes for these ions and the influence of the structure of the PL hydrocarbon chains on the affinity were established;

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