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THESIS APPROVAL

The abstract and thesis of Andreas Schilling for the Master of Science in Physics were presented July 11, 1994, and accepted by the thesis committee and the department.

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ABSTRACT

An abstract of the thesis of Andreas Schilling for the Master of Science in Physics presented July 11, 1994.

Title: Electrophoretic Studies of Ion Adsorption to Sarcoplasmic Reticulum and Phosphatidylcholine Membranes.

In this study, electrophoretic mobilities of native and two types of trypsin digested sarcoplasmic reticulum vesicles have been determined by microelectrophoresis using a Doppler Electrophoretic Light Scattering Analyzer to investigate the influence of hydrodynamic drag, caused by the Ca^{2+} , Mg^{2+} -ATPase protruding from the surface of native sarcoplasmic reticulum vesicles. After the prolonged digestion (protein:trypsin ratio of 20 for 3 hours at 25°C), the ATPase was cleaved and removed from the sarcoplasmic reticulum membrane as shown with SDS gel electrophoresis and an ATPase activity assay. Ionic strength and pH dependence of mobility showed a nearly pH independent increase in initial surface charge density after prolonged digestion. Adsorption isotherms for native, short (protein:trypsin ratio of 200 for 2 minutes at 25°C), and prolonged digested sarcoplasmic reticulum vesicles were recorded for TPhP^{+} (tetraphenylphosphonium), PCP^{-} (pentachlorophenol), and Ca^{2+} , and fitted to the Langmuir adsorption model. The most important result from the adsorption isotherms is that adsorption of the three ions did not increase significantly after prolonged digestion. From this it can be concluded that hydrodynamic drag does not have a measurable influence on electrophoretic mobility of sarcoplasmic reticulum vesicles and therefore cannot account for the big differences in mobility between sarcoplasmic reticulum ves-

icles and a comparable artificial membrane system (phosphatidylcholine/phosphatidylserine liposomes), which were observed in this lab earlier (Mense, M. 1993. Comparison of ion adsorption to phosphatidylcholine/phosphatidylserine and sarcoplasmic reticulum membranes. Master Thesis, Portland State University, library).

A thermodynamic analysis of adsorption was done for PCP^- adsorption to phosphatidylcholine liposomes, TPhP^+ adsorption to phosphatidylcholine liposomes, and TPhP^+ adsorption to sarcoplasmic reticulum vesicles, by recording adsorption isotherms at 10°C, 25°C, 40°C, and 55°C. The adsorption of PCP^- to phosphatidylcholine liposomes was clearly driven by enthalpy. In contrast, the adsorption of TPhP^+ to phosphatidylcholine liposomes and sarcoplasmic reticulum vesicles was characterized by a positive enthalpy and a still larger negative entropy term. The thermodynamic analysis of ion adsorption shows that the driving forces of adsorption are very similar for sarcoplasmic reticulum vesicles and the chosen artificial membrane system (phosphatidylcholine liposomes) in spite of the significant lower adsorption of biological membranes compared to artificial membrane systems.

**ELECTROPHORETIC STUDIES OF ION ADSORPTION TO SARCOPLASMIC
RETICULUM AND PHOSPHATIDYLCHOLINE MEMBRANES**

by

ANDREAS SCHILLING

A thesis submitted in partial fulfillment of the
requirements for the degree of

**MASTER OF SCIENCE
in
PHYSICS**

**Portland State University
1994**

To Susi

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During my first days at PSU, I decided pretty soon to do research in biophysics. That was a very fruitful decision, as I found out later, and the person who contributed by far most to this work and my research experience was my adviser Dr. Pavel Smejtek. It was not only his extensive knowledge in biophysics which I appreciated very much but also his way to guide through this year, and his gracious, sympathetic nature. With his always available help, it was possible to find my way in this completely new field, and to finish my studies in time.

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CHAPTER I

INTRODUCTION

A cell, one of the fundamental constituents of living organisms, consists of cytoplasm separated from its environment by a membrane. This compartmentalization is seen as an essential step in the evolution of life because it allows important chemicals to be concentrated and maintains the necessary conditions for the complicated biochemistry of life. These membranes, however, have more far-reaching functions than merely to establish boundaries to a system. They determine the nature of all communication between inside and outside of a cell. An example of intercellular communication is a phenomenon called "contact inhibition". Cells growing in a monolayer normally stop growing when they come into contact. The consequences of dysfunctional cell communication can be seen from cancer cells, which do not exhibit contact inhibition, and grow over each other to produce a tumor (Houslay and Stanley, 1982).

Biological membranes are also able to control the passage of materials moving in and out of the cell. This is largely achieved by membrane proteins. Sarcoplasmic reticulum membranes, for example, have the capability of regulating intracellular calcium concentration by pumping Ca^{2+} into storage against concentration gradients of several orders of magnitudes and by controlling the release from intracellular stores. This Ca^{2+} uptake and release mechanism is the molecular basis for the process of muscle contraction and relaxation (Suzuki and Ford, 1991, Trimm et al., 1988, among others). Cellu-

lar enzymes attached to the membrane are able to catalyze transmembrane reactions involving reactants on both sides of the membrane or molecular transport (Gennis, 1989).

Since biological membranes are necessarily complex objects, consisting of several types of lipids and a large number of proteins, they are difficult to study and understand. The fact that lipids provide the matrix for proteins in biomembranes led to the use of artificial membrane systems as models for biological membranes. The starting point for liposome research can probably be dated to 1961, when Rudin et al. were able to separate two aqueous compartments by a macroscopic membrane of a thickness of not more than two phospholipid molecules (Bangham, 1983). Bangham's "Liposome Letters" (1983) provide a refreshing impression about the development and diversification which the liposome membrane model experienced during the early years. An important result that validates the use of liposomes as models is that all major effects observed in artificial membranes have also been observed in biological membranes (Benz, 1985).

This study was motivated by a result from a Master's thesis that was obtained in this lab last year (Mense, 1993). From that work, it came out that SR vesicles, a representative for a biological membrane system, show significantly less ion adsorption than a comparable artificial membrane system (phosphatidylcholine/phosphatidylserine vesicles). Similar results were already been obtained by Conrad and Singer (1979 and 1981) who studied the binding of small amphipathic molecules to artificial (phosphatidylcholine) and biological membranes (erythrocytes, lymphoma cells, and sarcoplasmic reticulum vesicles). Their results, however, were questioned later because of the unreliability of one of their experimental methods. One part of this study was to investigate the influence of hydrodynamic drag, caused by the ATPase protruding from the surface of SR vesicles, on electrophoretic mobility of SR. If hydrodynamic drag had

a significant effect, then it could account for the big differences in electrophoretic mobility between liposomes and SR vesicles.

Another part of this work is the thermodynamic analysis of adsorption for phosphatidylcholine (PC) liposomes and SR vesicles. Measuring the temperature dependence of adsorption makes it possible to determine the contribution of enthalpic and entropic forces to the free energy of transfer, and therefore to get a deeper understanding of the driving forces of adsorption. The adsorption of lipophilic ions into model membranes or biological membranes is usually considered to be driven by entropy (Seelig and Ganz, 1991) due to the release of oriented water molecules from the hydrated ions (Tanford, 1980). This interpretation is based on measurements of water solubility of small organic molecules as a function of temperature. The free energy of transfer consisted of a large negative entropy term (at room temperature) and a small, slightly negative, enthalpy term. However, there is a possible conflict about the distribution of enthalpy and entropy to the free energy of transfer. Seelig and Ganz determined the enthalpy of transfer of several amphiphilic molecules (TNS, tetraphenylborate, amlodipine) from the aqueous phase to the lipid membrane with titration calorimetry. They found that the binding was clearly enthalpy driven and explained their results with van der Waals interactions between the nonpolar residues of the solute and the hydrophobic core of the lipid bilayer, or what they called the nonclassical hydrophobic effect.

CHAPTER II

MEMBRANE SYSTEMS

Two different membrane systems were used in this work for the adsorption studies: egg-phosphatidylcholine liposomes as an artificial model membrane system and sarcoplasmic reticulum as a representative of biological membranes. This chapter will provide a description and background information about both these membrane systems.

PHOSPHATIDYLCHOLINE LIPOSOMES

Lipids have a variety of biological roles: they serve as biological fuel, they are used for energy storage, and they are the main components of membranes. There are three major kinds of membrane lipids: phospholipids, glycolipids, and cholesterol. Phospholipids, the most abundant class, are derived either from the alcohol glycerol or sphingosine. The fatty acid chains in phospholipids contain typically between 14 and 24 carbon atoms and may be saturated or unsaturated (Stryer, 1981).

Phosphatidylcholine (PC), the lipid that was used in this study, is a neutral phosphoglyceride with a zwitterionic head group. It is found in most membranes of higher organisms and consists of a glycerol backbone, two fatty acid chains with varying length and a phosphorylated alcohol, choline. The structure formula of PC is given in Fig. 1. Since a large section of the lipid fraction of biological membranes consists of

PC (Yeagle, 1992), it is often used in adsorption studies (Smejtek et al., 1987, McLaughlin, 1978, Tatuljan, 1987, among others).

Figure 1. Molecular structure of phosphatidylcholine (from Stryer, 1981). R and R' stand for the fatty acid chains.

The formation of phospholipid bilayer vesicles in an aqueous solution can be explained through the combined effect of lipophilic and hydrophilic interactions which has its origin in the amphipathic structure of lipids. In the bilayer structure, the nonpolar fatty acid chains of the phospholipids are sequestered together away from contact with water, thereby maximizing lipophilic interactions. The polar head groups are at the same time in direct contact with water, therefore maximizing hydrophilic interactions (Singer and Nicolson, 1972). The preference for a bilayer structure is of critical biological importance. The lipid bilayer structures are approximately 40 - 60 Å thick, depending on the length of the hydrocarbon tail (Flewelling and Hubbell, 1986b). A lipid vesicle is presented in Fig. 2.

There are an amazing variety of research fields where liposomes are used. Virus infection of liposomes, liposomes in the treatment of cancer, and liposomes as models for drug binding studies are only a few examples from Bangham's book (1983), where other applications can also be found. A newer, very interesting, application is the use of liposomes as sensors for odorants (Nomura and Kurihara, 1987, Kashiwayanagi et al., 1990, Enomoto et al., 1991). This provides useful information for the development of new types of chemical sensors.

Figure 2. A lipid bilayer vesicle.

SARCOPLASMIC RETICULUM

Skeletal muscle is composed of a vast number of muscle fibers, aligned longitudinally in the direction of contraction. Each muscle fiber is composed of many con-

tractile units, called myofibrils, which are further divided into repeating units of contractile machinery, called sarcomeres. Muscle contraction is achieved through the simultaneous shortening of sarcomeres within a myofibril (Aidley, 1989).

Figure 3. Schematic diagram of the sarcoplasmic reticulum (Stryer, 1981).

The sarcoplasmic reticulum (SR) is an internal membrane system which regulates Ca^{2+} concentration in muscle. This regulation is achieved through the action of two membrane bound proteins, the Ca^{2+} , Mg^{2+} -ATPase and the Ca^{2+} release protein. The lipid bilayer profile structure as well as the protein distribution in SR is remarkably asymmetric. Approximately 50% of the mass of the total protein is localized externally

to the SR membrane lipid bilayer protruding from the outer lipid monolayer into the extravesicular medium as shown by Herbette et al. (1985).

The Ca^{2+} , Mg^{2+} -ATPase is a Ca^{2+} selective pump that lowers intracellular Ca^{2+} concentrations by actively sequestering free Ca^{2+} from the cell lumen via the hydrolysis of ATP into ADP and P_i . The Ca^{2+} release protein when opened, releases stored Ca^{2+} in response to an action potential emanating from the surface membrane of the muscle cell down the transverse tubules (Martonosi, 1984), raising intracellular Ca^{2+} concentration and initiating muscle contraction.

Figure 4. Transmission electron microscope picture of native SR. (A) SR vesicles negatively stained with 0.5% uranyl acetate. Outer projections are clearly seen extending from the edges of the vesicles. x 285,000 (from Scales and Inesi, 1976).

The release of Ca^{2+} from the SR into the cytoplasm is one of the key events in the process of muscle contraction and in spite of recent advances (Pessah et al., 1986, Inui et al., 1987) is not yet fully understood. SR membranes like cell membranes in general can be viewed as two-dimensional solutions of oriented proteins and lipids according to Singer and Nicolson's fluid mosaic model (Singer and Nicolson, 1972).

Figure 5. Transmission electron microscope picture of trypsin digested SR. (B) SR vesicles negatively stained with 0.5% uranyl acetate. After prolonged exposure to trypsin (SR protein/trypsin = 20 for 3 hours), the outer projections are absent. x 285,000. (from Scales and Inesi, 1976).

The protein components of SR are: Ca^{2+} , Mg^{2+} -ATPase, the major protein of SR, with a molecular weight of about 115 kDa (Gennis, 1989), which accounts for 70 to 80% of the vesicle's total protein content. The high-affinity calcium-binding protein

(55 kDa), calsequestrin (44 kDa), a glycoprotein (30 kDa), and proteolipid (12 kDa) (de Meis, 1981). Lipids account for about 50% of the total mass of SR vesicles (de Meis, 1981). About 90% of the lipid fraction is a mixture of phospholipids (MacLennan et al., 1971, among others). Phosphatidylcholine (PC) accounts for 50 to 70% of the phospholipids (de Meis, 1981).

CHAPTER III

ION INTERACTIONS WITH MEMBRANES

This chapter provides theoretical background on how to describe ion adsorption to membranes using the Guoy-Chapman-Stern theory. Afterwards, a model for the total membrane potential is introduced that accounts for the vast difference in adsorption between anions and cations. The last part, microelectrophoresis, presents background about the method of how adsorption of ions to membranes is measured.

ADSORPTION THEORY

In order to treat ion adsorption to artificial and biological membranes, a theory describing the potential in an electrolyte adjacent to a charged surface has to be combined with an adsorption model. In this study the Guoy-Chapman theory of the diffuse electrical double layer was combined with the Langmuir adsorption isotherm. This method of treating adsorption was introduced by Stern (1924) and is known as the Guoy-Chapman-Stern theory.

Guoy-Chapman Theory

The theory of the electrical double layer had its starting point in 1879, when the formation of an electrical double layer was first mentioned by Helmholtz (1879). A major advance was made by Guoy (1910) and independently by Chapman (1913). They used the Poisson equation to describe the electrostatic potential near a plane surface

and the Boltzmann relation to take into account the statistical tendency of the counter-ions to diffuse away from a region of high concentration. The model for the electrolyte used in the Guoy-Chapman theory is quite simple. All ion-ion correlations in the double layer are ignored except through the effect of the mean potential (McLaughlin, 1989). The Poisson equation in SI units, describing the electrostatic potential $V(x)$ at a distance x from a planar surface, is given by

$$\frac{d^2V(x)}{dx^2} = -\frac{\rho(x)}{\epsilon_r \epsilon_0} \quad (1)$$

where $\rho(x)$ is the volume charge density, ϵ_r the dielectric constant of the electrolyte and ϵ_0 the permittivity of free space. The one dimensional Poisson equation restricts the application of the theory to surfaces with radii of curvature much larger than the Debye length. The volume charge density, ρ , is, by definition, related to the ion density $n_i(x)$ through

$$\rho(x) = e \sum_i z_i n_i(x) \quad (2)$$

with e the proton charge and z_i the valence of the i th ion, including sign. Assuming a dielectric constant in the aqueous phase that is uniform up to the surface of the membrane, the electrochemical potential of an ion must be constant. This condition manifests itself in the Boltzmann relation.

$$n(x) = n(\infty) \exp\left(-\frac{qV(x)}{k_B T}\right) \quad (3)$$

k_B is Boltzmann's constant and T the absolute temperature. The combination of equations (1)-(3) results in the nonlinear Poisson-Boltzmann relation that, together with the appropriate boundary conditions

$$\lim_{x \rightarrow \infty} V(x) = \lim_{x \rightarrow \infty} \frac{dV(x)}{dx} = 0 \quad (4)$$

can be solved analytically for the one-dimensional case (McLaughlin, 1977). The expression for the potential $V(x)$ is rather complicated but can be linearized for $zeV_0 < 2k_B T$ and reduces to

$$V(x) = V(0)\exp(-\kappa x) \quad (5)$$

with $1/\kappa$ the characteristic Debye length, given by

$$\kappa = \sqrt{\frac{2e^2}{\epsilon_r \epsilon_0 k_B T} \sum_i z_i^2 C_{i0}} \quad (6)$$

where C_{i0} is the bulk concentration of the i th ion. Typical values for the Debye length $1/\kappa$ in adsorption studies are in the range of 1 nm (0.1M monovalent salt solution) to 10 nm (0.001M monovalent salt solution) (McLaughlin, 1989).

Electroneutrality of the electrolyte implies that the surface charge density, σ , can be obtained by integrating over the volume charge density of the electrolyte, ρ .

$$\sigma = -\int_0^\infty \rho(x) dx \quad (7)$$

This integration can be carried out using equations (1) and (2), yielding the desired relation between surface charge density and surface potential, V_0 , which is known as Grahame's equation (Smejtek, 1990).

$$\sigma = \text{sgn}(V_0) \sqrt{2k_B T \epsilon_r \epsilon_0 \sum_i C_{i0} \left[\exp\left(-\frac{z_i e V_0}{k_B T}\right) - 1 \right]} \quad (8)$$

In addition to the assumptions already mentioned above, surface charges are smeared uniformly over the membrane solution interface and image charge effects are considered to be negligible in the Guoy-Chapman theory.

A newer, very sophisticated theory has been developed by Kjellander and Marcelja (1986a and 1986b) that takes into account discreteness and finite size of charges as well as image charge effects and correlation effects in the electrolyte. The predictions of this theory agree remarkably well with the Guoy-Chapman theory. This can be partly explained by statistical mechanics calculations (Carnie and Torrie, 1984, Joensson et al., 1980).

Discreteness of charge is most important at low charge densities (if the distance between charges is large compared to $1/\kappa$), high ionic strengths, and if polyvalent lipids are used (McLaughlin, 1989). As Monte Carlo simulations and real experiments on phospholipid bilayers have shown, the finite size of ions can be ignored if they are smaller than the Debye length (McLaughlin, 1989). A wide variety of experimental work that shows the applicability of the Guoy-Chapman theory to describe the potential adjacent to a phospholipid bilayer membrane is given in McLaughlin's review articles from 1977 and 1989. The applicability of the Guoy-Chapman theory to describe the potential in an electrolyte adjacent to SR vesicles is shown in the work of Arrio et al. (1984).

A biologically important consequence from the Boltzmann relation (3) is that close to a negatively charged membrane the concentration of positive counterions is strongly increased and therefore the pH drops according to the following equation.

$$(pH)_{Membrane} = (pH)_{Bulk} + \frac{V_0}{0.059} \quad (9)$$

V_0 is the surface potential in mV. This may effect many enzymatic reactions near or at biological membranes.

The Langmuir Adsorption Model

In the Langmuir adsorption model for an ion X , the surface density of adsorbed ions, $(X)_{\text{Membrane}}$, is obtained as a function of the bulk ion concentration, $[X]_0$, using two adsorption parameters. The association constant, K , accounts for the strength of the ion-membrane interactions, and P_s , the membrane surface area of one adsorption site, takes into account that a certain region is associated with each adsorbed ion, and is therefore excluded from further adsorption. The Langmuir adsorption isotherm is given through the following equation where $[X]_{\text{Interface}}$ is the interface volume density of adsorbing species

$$(X)_{\text{Membrane}} = K[X]_{\text{Interface}} \left(\frac{1}{P_s} - (X)_{\text{Membrane}} \right) \quad (10)$$

The term in the brackets on the right hand side represents the number of unoccupied sites per unit area (Smejtek and Wang, 1990). The concentration of adsorbing species at the interface, where the adsorption takes place, is related to the bulk concentration, the experimentally controlled quantity, with a Boltzmann factor.

$$\frac{[X]_{\text{Interface}}}{[X]_0} = \exp \left(- \frac{qV_{\text{Membrane}}}{k_B T} \right) \quad (11)$$

The ion charge of adsorbing species is q , V_{Membrane} is the surface potential, T the absolute temperature, and k_B denotes Boltzmann's constant.

In the case of low surface coverage with adsorbing species ($1/P_s \gg (X)_{\text{Membrane}}$), the Langmuir isotherm reduces to equation 12 where β is the linear partition coefficient.

$$\frac{(X)_{\text{Membrane}}}{[X]_0} = \frac{K}{P_s} = \beta \quad (12)$$

In order to relate parameters directly obtained from the experiment, like β , with thermodynamic parameters of ion adsorption, a relation between the bulk partition coefficient, γ , and Gibb's free energy change, ΔG , has to be applied.

$$\gamma \equiv \frac{[X]_{\text{Membrane}}}{[X]_0} = \frac{\beta}{t} = \exp\left(-\frac{\Delta G}{RT}\right) \quad (13)$$

$[X]_{\text{Membrane}}$ is the membrane volume density of adsorbing species, R the gas constant, and t the width of the membrane adsorption layer. The Gibb's free energy change is given through equation 14.

$$\Delta G = \Delta H - T\Delta S \quad (14)$$

where ΔH and ΔS stand for the change in enthalpy and entropy.

In this study a value of $t = 0.4$ nm was assumed with reference to the work of Flewelling and Hubbell (1986a), and Smejtek and Wang (1990). It follows from equation (13) that a plot of $\ln(\beta/t)$ versus $1/T$ will result in a linear dependence with slope $-\Delta H/R$ and a y-axis intersection at $\Delta S/R$. It is therefore possible to determine separately the enthalpy and entropy term of Gibb's free energy change from the temperature dependence of adsorption.

The applicability of the Guoy-Chapman-Stern theory to describe ion adsorption to membranes has been shown by several studies (McLaughlin and Harary, 1976, McLaughlin et al., 1978, Puskin and Coene, 1980, Lau et al., 1981, among others).

THE TOTAL MEMBRANE POTENTIAL MODEL

One class of molecules plays the major role in the study of lipid bilayers: hydrophobic ions. These charged molecules are characterized by their common feature to readily permeate across and strongly bind to lipid bilayers. Examples of hydrophobic ions are dipicrylamine (DPA⁻), tetraphenylborate (TPhB⁻), tetraphenylphosphonium (TPhP⁺), and pentachlorophenol (PCP⁻). Due to their importance, these ions and cations are frequently used in current research (Smejtek et al., 1989, Barstad et al., 1993, Smejtek and Wang, 1990, Flewelling and Hubbell, 1986a, among others). TPhP⁺ and PCP⁻ were also used in this study.

Flewelling and Hubbell developed a theoretical model for the total membrane potential which is consistent with the complete set of thermodynamic parameters for the two hydrophobic structural analogues TPhP⁺ and TPhB⁻ (Flewelling and Hubbell, 1986a and 1986b). Flewelling and Hubbell's major contribution was the introduction of a specific model for the membrane dipole potential, ≈ 240 mV, inside positive, treating lipids as a two-dimensional array of point dipoles located near each membrane water interface. This was done to take into account the fact that anions bind orders of magnitude more strongly to and translocate orders of magnitude more rapidly across lipid bilayers than structurally analogous cations.

The total energy profile can be written as the sum of four terms:

$$W_{\text{total}}(z) = W_{\text{Born}}(z) + W_{\text{image}}(z) + W_{\text{dipole}}(z) + W_{\text{neutral}}(z) \quad (15)$$

W_{neutral} is essentially the free energy of transfer apart from electrical contributions. It stands for all neutral terms as hydrophobic, van der Waals and steric factors.

W_{Born} is the Born expression for the energy of transfer of an ion with charge, q , and radius, r , moved from a region of dielectric constant ϵ_2 to a region with dielectric constant ϵ_1 . This relationship in SI units is given by the following equation where ϵ_0 is the permittivity of vacuum.

$$W_{\text{Born}} = \frac{1}{4\pi\epsilon_0} \frac{q^2}{2r} \left(\frac{1}{\epsilon_1} - \frac{1}{\epsilon_2} \right) \quad (16)$$

W_{image} accounts for the interactions of the charged molecule with the interfaces. This expression is important because of the relative thinness of membranes (approximately 40 Å for a phosphatidylcholine bilayer). Neumcke and Lauser (1969) presented a complicated expression that combined Born and image energy. It is the general theoretical expression for the Born and image energies for a charge within a dielectric slab sandwiched between two bulk dielectrics. Under normal conditions of ion adsorption to membranes (radius of ion $r \leq 4$ Å, thickness of membrane $d \approx 40$ Å, $r \leq x \leq d/2$) this reduces to a more convenient expression:

$$W_{\text{B+I}}(x) = \frac{1}{4\pi\epsilon_0} \frac{q^2}{2\epsilon_1 r} \left[1 - \frac{r}{2x} - 1.2 \left(\frac{r}{d} \right) \left(\frac{x}{d} \right)^2 \right] \quad (17)$$

The distance x is measured from one interface and ϵ_1 is the dielectric constant of the membrane. For lipid bilayers the full expression and the approximate solution rarely differ more than 4%.

There are three possible sources for the membrane dipole potential W_{dipole} : oriented water molecules at the surface, the lipid headgroups, and the lipid carbonyls. The contribution of the quite large dipole moment of water molecules to the dipole

potential is fairly complicated and depends significantly on the perturbing effects of adsorbing species. The charged or zwitterionic phospholipid headgroups may contribute to the membrane dipole potential but because the negatively charged phosphate groups are most likely closer to the membrane than the rest of the headgroup, the dipole potential would be negative inside rather than positive. The relatively large dipole moment of the ester groups, that link the two fatty acid chains to the glycerol backbone, could produce a substantial dipole potential that is positive inside as observed.

The dielectric constant represents a macroscopic concept and therefore has to be applied very carefully at the molecular scale of a lipid bilayer. The effective dielectric constant of the hydrocarbon portion of a lipid bilayer has a value of approximately 2, the headgroup and ester group regions have values between 20 and 50 (Flewelling and Hubbell, 1986b). For their theoretical model, Flewelling and Hubbell (1986b) assumed the following dielectric function.

$$\epsilon(z) = \epsilon_h + \frac{\epsilon_{aq} - \epsilon_h}{1 + 10^{y(z)}} \quad (18)$$

with
$$y(z) = 4 \frac{l_{diel} - z}{2r} \quad (19)$$

Bulk membrane and water dielectric constants are ϵ_h and ϵ_{aq} , respectively. z is the position from the center of the bilayer and r the radius of the ion. The total membrane potential, including the various contributions, for both cations and anions, is graphically presented in Fig. 6.

Figure 6. The total membrane potential model (Smejtek, 1994).

MICROELECTROPHORESIS

In this study adsorption of ions to artificial and biological membrane systems was investigated using microelectrophoresis. Other possible methods to study this kind of adsorption are electron paramagnetic resonance spectroscopy (EPR) (Flewelling and Hubbell, 1986, Langner et al., 1990 among others), nuclear magnetic resonance spectroscopy (NMR) (Altenbach and Seelig, 1985, Scherer and Seelig, 1989 among others), fluorescence measurements (Enomoto et al., 1991, Kashiwayanagi et al., 1990 among others), and conductance measurements (McLaughlin et al., 1975 among others).

Microelectrophoresis has an advantage over these methods from the point of view that there are no probes or currents through the membrane necessary to monitor changes in surface potential. The measurement is therefore more direct.

When a charged particle suspended in an electrolyte is subjected to an electric field, the particle accelerates and, since it experiences viscous drag that is increasing with increasing velocity, it reaches a terminal velocity. This equilibrium between the electric and the friction force is reached within microseconds. The electrophoretic mobility μ of a charged particle is defined by the following equation.

$$\mu = \frac{v}{E} \quad (20)$$

v : terminal velocity, typically $\frac{\mu m}{s}$

E : applied electric field, typically $\frac{V}{cm}$

The electrophoretic mobility depends on pH, ionic strength, viscosity, temperature and dielectric constant of the suspending electrolyte. These parameters are either specified at measurement or taken into account in the adsorption model.

The zeta potential, ζ , of a particle is defined as the electrostatic potential at the hydrodynamic shear plane. The shear plane is an imaginary sphere around a particle inside which the electrolyte moves with the particle as it moves through the solution. For a Newtonian fluid and for particles which are large compared to the Debye-Hueckel screening distance $1/\kappa$, which is the exponential decay distance of the electrostatic potential and can be regarded as the double layer thickness, the zeta potential is proportional to the electrophoretic mobility (Cafiso et al., 1989).

$$\zeta = \frac{\eta}{\epsilon \epsilon_0} \mu \quad (21)$$

"Helmholtz-Smoluchowski equation" (in SI units)

η and ϵ are the viscosity and the dielectric constant of the electrolyte, respectively.

Using the values for ϵ and η for water from the handbook of chemistry and physics, the following relations between mobilities and zeta potentials are obtained:

$$10^{\circ}\text{C} : 1 \frac{\mu\text{m cm}}{\text{s V}} \Leftrightarrow 17.58 \text{ mV} \quad (22\text{a})$$

$$25^{\circ}\text{C} : 1 \frac{\mu\text{m cm}}{\text{s V}} \Leftrightarrow 12.83 \text{ mV} \quad (22\text{b})$$

$$40^{\circ}\text{C} : 1 \frac{\mu\text{m cm}}{\text{s V}} \Leftrightarrow 10.08 \text{ mV} \quad (22\text{c})$$

$$55^{\circ}\text{C} : 1 \frac{\mu\text{m cm}}{\text{s V}} \Leftrightarrow 8.334 \text{ mV} \quad (22\text{d})$$

The connection between the zeta potential obtained from the electrophoretic measurements and the surface potential, V_{surface} , that is to be determined in order to apply the Guoy-Chapman theory, is made by the following equation where s is the shear plane distance.

$$\zeta = V_{\text{surface}} \exp(-\kappa s) \quad (23)$$

This is an approximate solution from the Debye-Hueckel theory (Brown, 1974) which holds for the case that the diffuse double layer is much thicker than the shear plane distance ($s \ll 1/\kappa$).

Typical values for s and $1/\kappa$ in this study were $s \approx 0.2 \text{ nm}$ and $1/\kappa \approx 1\text{-}10 \text{ nm}$, so that the above condition was always fulfilled. The location of the shear plane as a function of ionic strength was determined in this lab earlier (Smejtek et al., 1987).

The electrophoretic mobility method of obtaining surface potentials from electrophoretic mobilities cannot be applied to any membrane system because in order to have a defined shear plane distance the membrane surface has to be sufficiently smooth.

A rough surface would cause additional drag that is not taken into account in this model.

One aim of this study was to determine whether proteins which protrude from the surface of SR vesicles cause enough hydrodynamic drag to account for the significant differences in electrophoretic mobilities between SR vesicles and phosphatidylcholine/phosphatidylserine liposomes that were obtained in this lab earlier (Mense, 1993). In that work the differences in electrophoretic mobility were interpreted in terms of the Helmholtz-Smoluchowski equation (21) as a difference in surface charge density which means that the biological membranes (SR) adsorb much less than the used artificial membranes (PC/PS liposomes).

An example of the non-applicability of this model is reported by Woodle et al. (1992). According to them is it not possible to combine the Guoy-Chapman theory with the Helmholtz-Smoluchowski equation in order to describe the observations made with red blood cells (Levine et al., 1983). The difference may be attributed to contributions either of hydrodynamic drag due to the presence of bulky sugars or to differences in the location of the charge relative to the surface.

CHAPTER IV

MATERIALS AND METHODS

CHEMICALS

Sodium chloride (NaCl), potassium chloride (KCl), sodium pentobarbitol, leupeptin, dithiothreitol (DTT), sodium dodecyl sulfate (SDS), trypsin (from bovine pancreas, type III, 10,200 U/mg protein), trypsin inhibitor (soybean, type 1-S, 1 mg will inhibit 1.5 mg trypsin of 10,000 U/mg activity), tris(hydroxymethyl)aminoethane base, ammonium persulfate, glycine, bromphenol blue, Coomassie Brilliant Blue R, adenosine triphosphate (ATP), ethyleneglycol-bis(β -aminoethylether)tetracetic acid (EGTA), lactate dehydrogenase (LDH, suspension in 2.1 M $(\text{NH}_4)_2\text{SO}_4$ at pH = 6.0), magnesium dichloride, NADH, phosphoenolpyruvate (PEP), pyruvate kinase (PK), sodium azide (NaN_3), and the molecular weight standard mix (SDS-7B) were from Sigma Chemical Company, St. Louis, MO. Pentachlorophenol (PCP), tetraphenylphosphonium (TPhP), and calcium chloride dihydrate were from Aldrich Chemical Company, Milwaukee, WI. The male New Zealand white rabbit was purchased at the Western Oregon Rabbit Company. Egg-phosphatidylcholine (PC) in a concentration of 20 mg/ml, dissolved in chloroform, was 99% pure (Avanti Polar Lipids, Birmingham, AL). Potassium phosphate dibasic trihydrate, boric acid, and glacial acetic acid came from Mallinckrodt Chemical Works, St. Louis, MO. N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid (HEPES) was from Research Organics Inc., Cleveland, OH, methanol

from VWR Scientific (Cleveland, OH), acrylamide (>99.9%) and N,N'-methylene-bis-acrylamide(bis) from BioRad. The pH standards were bought from Scientific Instruments Corning Glass Works (Medfield, MA), potassium hydroxide from Matheson, Coleman and Bell Manufacturing Chemists (Norwood, OH), and potassium citrate monohydrate from EM Science (Cherry Hill, NJ). Chloroform came from American Burdick and Jackson (Muskegon, MI), tetramethylethylenediamine (TEMED) from Eastman Kodak Company (Rochester, NJ), and ultra pure sucrose from ICN Biomedicals Inc. (Irvine, CA). All chemicals were of reagent grade or higher purity and were used without further purification. Solutions were prepared with highly purified deionized water.

SAMPLE PREPARATION

Phosphatidylcholine Liposomes

Multilayered vesicles were prepared within 24 hours of use by a method similar to that of Bangham (1974). Egg-PC from the stock solution was diluted with chloroform in a 50 ml round bottom flask. The chloroform was evaporated using a rotary flash evaporator (Buchler Instruments, Fort Lee, NJ). The lipid solution was heated carefully using a warm water bath (35°C) and the collector flask for the evaporated chloroform was cooled with ice in order to get as complete an evaporation of the chloroform as possible. This resulted in a thin lipid film in the flask. A mechanical pump was used to pump for at least one more hour at a pressure of about 0.1 torr. A buffer solution (0.002 phosphate, 0.002 citrate, 0.0005 borate at pH = 8.5) was added so that a final lipid concentration of 0.20 mg/ml was obtained. The flask was shaken

until the aqueous buffer no longer beaded up on the inner surface of the round bottom flask, indicating that all the lipid was taken up in the aqueous solution.

The vesicle size obtained with this method is about 1-3 μm (Blochel, 1992). The electrophoretic measurements were done with a final lipid concentration of 20 $\mu\text{g/ml}$. The liposomes showed a zeta potential of approximately -10 mV (at 25°C) in the absence of any adsorbing species. This was not in agreement with data from Bangham et al. (1974) who found that egg-PC vesicles have no initial surface charge density over a pH range from 3 - 9.5. This problem already occurred earlier in this lab (Blochel, 1992). At that time the possibilities of adsorption of buffer anions and distortion of the flow profile due to a pH dependent surface charge on the channel wall were excluded. One possible explanation for this phenomena could be that an ageing process caused an initial surface charge. In this study the effect was taken into account by a corresponding initial surface charge for the PC liposomes.

Sarcoplasmic Reticulum Vesicles

New Zealand white rabbit skeletal muscle SR was isolated from back and white leg muscle tissue by a multiple centrifugation procedure, following the method of MacLennan (1970). A 2 to 3 kg male rabbit was anesthetized with sodium pentobarbital then bled. The back and hind leg muscle were then removed, and after fat and connective tissue were trimmed off, homogenized, and centrifuged 5x at accelerations between 1,600 g and 78,000 g. These steps resulted in the removal of soluble proteins, mitochondria, actin and myosin. All SR containing solutions were kept under 5°C. The resultant SR was then re-suspended in buffer solution (100 mM KCl, 20 mM HEPES at pH = 7.0) and stored frozen at -50°C in a freezer. The vesicles formed by resealing of the fragmented SR are right-side out. The outer surface of the vesicle membrane corresponds to the surface of the SR that faces the cytoplasm (de Meis, 1981).

The protein concentration of the stock solution was determined following the method of Kalckar (1947) by recording absorbance measurements with a Beckman DU-7 HS UV/VIS spectrophotometer (Beckman Instruments, Fullerton, CA) at 230, 260 and 280 nm. From these measurements, the final protein concentration was calculated to be 6.1 mg/ml.

The final solutions for the electrophoretic measurements were prepared in either 5 mM HEPES or B⁻³ (a buffer solution containing 2 mM phosphate, 2 mM citrate, 0.5 mM borate) with a protein concentration of either 20 or 40 µg/ml. Occasionally, the final solution contained particles visible to the naked eye (coming from the SR stock solution). When this occurred, the sample was centrifuged for 5 minutes with an Adams Sero-Fuge (Clay Adam, Parsippany, NJ) at approximately 2,000 g and the supernatant was taken for the measurement.

ELECTROPHORETIC MEASUREMENTS

All electrophoretic measurements were done with a Doppler Electrophoretic Light Scattering Analyzer (DELSA 440, Langley Ford Instruments Division of Coulter Electronics, Inc., Hialeah, FL). Electrophoretic Light Scattering (ELS) is a measurement method that combines two technologies: Electrophoresis and Laser Doppler Velocimetry (LDV), which is a method for measuring the speed of particles by analyzing the Doppler shifts of scattered light.

The DELSA 440 measures the electrophoretic mobility distribution, zeta potential and average hydrodynamic radius of particles in liquid suspension by analyzing the scattered light from a 5 mW He/Ne laser ($\lambda = 632.8$ nm). The measurements are limited by sedimentation and the particle size which may lie between 0.01 and 30 µm.

The size of liposomes and SR vesicles used in this study was in the range of about 1 μm . The particles are analyzed by making independent laser Doppler measurements at four different angles simultaneously with 256-channel resolution each.

The Electrophoretic Cell

The sample is analyzed in the sample chamber which is located in the optical bench. Total sample volume is about 1 ml. A rectangular channel with a height of approximately 1 mm runs through the 5 mm thickness of the glass insert which is provided with an anti-reflective coating, connecting the hemispherical cavities in each electrode. The sample is injected through a fill tube using a syringe, filling both hemispheres and the channel. The filling process has to be executed very carefully in order to make sure that there are no air bubbles in the channel because they would destroy the flow profile in the channel and the stationary layer would become unknown.

During electrophoresis the silver surfaces of the hemispheres act as electrodes. Light scattering data is collected from a small scattering volume in the channel where the scattering volume is defined by the intersection of the main beam with the reference beam for that angle. It is approximately 0.01-0.03 μl . The data acquisition and analysis is performed using software on an IBM Personal System/2 computer, Model 50.

The electrophoretic cell was disassembled and cleaned before each series of experiments. The cell constant is used by the DELSA 440 to calibrate the electric field and therefore has to be determined after each disassembly. This is done by using a conductivity calibrating solution (YSI 3161, Yellow Springs Instruments Co., Inc., Yellow Springs, OH).

Figure 7. Electrophoretic cell (from DELSA manual).

The conductivity of the calibrating solution was specified to be $1000 \pm 5 \mu\text{S/cm}$ at 25°C by the manufacturer. The conductivity at 10°C , 40°C and 55°C was measured in a separate experiment using a conductivity meter (YSI Model 34, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) and a temperature bath (temperature was constant within $\pm 0.1^\circ\text{C}$). The obtained values (error smaller than 1%) are:

$$10^\circ\text{C} : \text{conductivity} = 721 \mu\text{S/cm} \quad (24a)$$

$$40^\circ\text{C} : \text{conductivity} = 1300 \mu\text{S/cm} \quad (24b)$$

$$55^\circ\text{C} : \text{conductivity} = 1612 \mu\text{S/cm} \quad (24c)$$

The Optical Bench

In the system, the main beam and the four reference beams are generated from the same laser using a diffraction grating which acts as a five way beam splitter. The main beam carries about 80% of the intensity whereas the remaining 20% is split into the four reference beams. Before entering the sample, the beams pass through collimating and focusing optics and also through a swivel polarizer that is used to manually adjust the intensity.

The main beam additionally passes through a frequency shifter that allows detection of the direction of particle motion, thus, the sign of mobility. Each beam can be blocked separately by a beam blocker. A converging lens makes all five rays intersect within the channel of the sample chamber which can be viewed with a 24x microscope.

After the sample area, four photo diode detectors sense the scattered light at four angles to the main beam. The angles are 7.5° , 15° , 22.5° and 30° with respect to the main beam. At the three larger angles, the appropriate reference beam and the light scattered from the scattering volume for that angle pass directly to the photo diode. At the lowest scattering angle, the mixed light passes first through an aperture and then a lens that focuses the light onto a detector.

Detection of the Doppler Shift

To describe the scattering process, the scattering vector is introduced. The scattering vector, $\vec{k} = \vec{k}_1 - \vec{k}_0$, where $\vec{k}_0$ and $\vec{k}_1$ are the wave vectors before and after the scattering, is in the plane defined by the incident light beam and the detector at an angle bisecting the angle between the laser beam and the line from the scattering volume to the detector.

Figure 8. Optical bench of the DELSA (from DELSA manual).

The magnitude of $\vec{k}$ is given by :

$$k = |\vec{k}| = \frac{4\pi}{\lambda} n \sin(\theta/2) \quad (25)$$

n stands for the refractive index of the solution, θ is the scattering angle (angle between incident beam and the line connecting the scattering volume and the detector), and λ is the laser wavelength in vacuum.

Figure 9. The scattering vector (from DELSA manual).

Due to the movement of the particles, the scattered beam experiences a frequency shift of

$$\Delta\nu = \frac{2nv}{\lambda} \cos(\alpha) \sin(\theta/2) \quad (26)$$

where v is the velocity of the particle with respect to the detector and α the angle between scattering vector and particle velocity.

The interference between the scattered and the reference beam results in a sinusoidal intensity fluctuation at the photo diode detector. The frequency of the fluctuation is proportional to the Doppler shift $\Delta\nu$ and, therefore, to the speed of the particles.

Figure 10. A typical frequency spectrum. The spectrum was recorded with native SR during measurements for the ionic strength dependence.

The detector converts the intensity fluctuation into a time varying current which, aside from a dc contribution of no importance, is given by

$$I(t) = \sum_i a_i \sin(2\pi\Delta\nu_i t) \quad (27)$$

where the sum extends over the different scattering species. The a_i are constants which

Figure 11. A typical zeta potential spectrum. The spectrum was recorded with native SR during measurements for the TPhP⁺ adsorption isotherm.

take into account the concentration and scattering intensity of the different scattering species. The detector current is converted into a time varying autocorrelation function which is then Fourier transformed into a power spectrum

$$S(\nu) = \sum_i a_i [\delta(\nu - \Delta\nu_i) + \delta(\nu + \Delta\nu_i)] \quad (28)$$

using a Fast Fourier Transform (FFT) algorithm where each pair of spikes corresponds to one kind of particles.

As the power spectrum indicates one could not determine the sign of mobility without having the main beam frequency shifted. The frequency shift is one half of the selected frequency range (0-1000 Hz). Knowing the position of the spikes and the magnitude of the applied electric field, E , one can calculate the electrophoretic mobilities according to

$$\mu_i = \frac{2\pi\Delta\nu_i}{EK \cos(\alpha)} \quad (29)$$

These mobilities can be converted into zeta potentials using the Helmholtz-Smoluchowski equation. The software allows us to view the frequency, the mobility and the zeta potential spectrum.

Diffusion Broadening

The power spectrum above is of course an idealized case, because small particles have a significant diffusive motion (Brownian motion) superimposed on the motion caused by the applied electric field. This causes the so-called diffusion broadening. It also means that the δ -peaks in the power spectrum have to be replaced by Lorentzian curves centered at $\pm \Delta\nu_i$:

$$S(\nu) = \sum_i a_i \left[\frac{(\gamma_i / 2\pi)^2}{[(\nu - \Delta\nu_i)^2 - (\gamma_i / 2\pi)^2]} + \frac{(\gamma_i / 2\pi)^2}{[(\nu + \Delta\nu_i)^2 - (\gamma_i / 2\pi)^2]} \right] \quad (30)$$

$$\gamma_i \equiv D_i k^2 \quad (31)$$

γ_i and D_i are the spectral broadening and the diffusion coefficient of the i th particle, respectively. Diffusion broadening is directly proportional to the diffusion coefficient and proportional to the square of the scattering vector. Since for spherical particles the diffusion coefficient is inversely proportional to the radius, diffusion broadening can be used to measure the particle size, knowing temperature and viscosity of the suspension. The DELSA 440 measures particle size by recording angular dependence of diffusion broadening in the frequency spectrum at zero applied electric field.

Electroosmosis

In order to obtain true electrophoretic mobilities with this microelectrophoresis method, one has to take into account an electrokinetic effect called electroosmosis. Electroosmosis occurs because the channel walls of the sample chamber are, in general, at a different electrostatic potential than the bulk solution in the chamber. This difference causes a non uniform distribution of the different ions in the channel which means that the potential of the electrolyte in the channel becomes dependent on the distance from the channel walls.

When an external electric field is applied parallel to the channel, the electrolyte in the channel moves with a speed and direction dependent on its electrostatic potential, which in turn depends on its position within the channel. Because the volume of the sample chamber is fixed and the electrolyte is assumed to be incompressible, there is zero net flow across any cross section of the channel. For the rectangular cross section channel of the DELSA 440, a calculation gives a parabolic flow profile .

The zero net flow condition implies the existence of a so-called stationary layer, a position at which the liquid velocity is zero. For the parabolic flow profile in the DELSA channel, the stationary layers have been calculated to be at 16% (lower) and 84% (upper) of the cell height. Only measurements of mobility made at the stationary layer are the true electrophoretic mobilities. In order to reduce the effect of sedimentation in the channel all measurements in this study were done at the upper stationary layer because it is less affected by sedimentation.

Figure 12. Electroosmotic flow profile (from DELSA manual).

Measurements

The laser was allowed to warm up for at least 1 hour before measurements were done. The electrophoretic cell was rinsed with 100-200 ml deionized water using an aspirator and flushed with air for 5-10 minutes until completely dry. Afterwards it was filled with sample and inserted into the sample chamber compartment. For the

measurements at 40°C and 55°C the sample chamber and the sample solution were warmed up before the filling process was carried out. This was done in order to prevent bubble formation within the sample chamber due to decreased solubility of gases at higher temperatures.

With an adjustment wheel the beam intersection was focused into the middle of the channel, using the viewing microscope. The heterodyne ratio, the strength of the reference beam divided by the strength of the scattered beam on the detector, was adjusted to a recommended value between 2 and 30 for each angle with the beam intensity adjustment wheel. Beam intersection and heterodyne ratio were checked once or twice a day.

Run Parameters. The following run parameters had to be specified before each measurement run.

Temperature. The temperature range of the DELSA 440 is 10°C to 60°C with increments of 0.1°C. The temperature dependent studies were done at 10°C, 25°C, 40°C and 55°C, all the other experiments were carried out at 25°C. After an equilibrium was reached the temperature was kept constant within $\pm 0.4^\circ\text{C}$ at 10°C, 40°C, 55°C and within $\pm 0.2^\circ\text{C}$ at 25°C.

Viscosity, refractive index and dielectric constant of the electrolyte. These parameters were set to values for water (from the handbook of chemistry and physics) because the effect of salt in the solution can be neglected.

Cell constant. The cell constant was set to the value that was obtained with the conductivity calibrating solution.

Frequency range. The frequency range of the DELSA 440 that can be chosen reaches from 50-1000 Hz. Due to the fact that the DELSA uses a 256 channel analyzer the

TABLE I
VISCOSITY, REFRACTIVE INDEX AND DIELECTRIC CONSTANT
OF WATER AT DIFFERENT TEMPERATURES

Temperature [°C]	Viscosity [poise]	Refractive index	Dielectric constant
10	0.01307	1.3334	83.95
25	0.00890	1.3329	78.36
40	0.00653	1.3309	73.15
55	0.00504	1.3285	68.30

resolution of the measurement is given by frequency range divided by 256. Three points had to be taken into account when the frequency range was chosen. First, the Doppler shift of the particles has to lie within the chosen range. Second, the duration of measurement and achievable frequency resolution are related inversely to each other. And third, the signals gets noisier when a smaller frequency range is selected. Most of the measurements were done with a frequency range of 500 Hz, a few with 250 Hz.

Field on/off time, run time. In the DELSA 440 a measurement is done by applying an electric field to the sample for a selectable time (on time), letting the sample recover for a selectable time (off time), applying the electric field with polarity reversed for a second on time, allowing the sample to recover again, then repeating the whole process. The total period during which measurements are made is called run time. The reason for reversing polarity of the field is to avoid polarization effects of the electrodes and the relaxation period is to allow Joule heating to dissipate.

Field on time must equal or exceed the frequency range dependent minimum duration. During our experiments, field on time was always 2.0 s which was in agreement with the chosen frequency range, field off time was 0.5 s. Run time was set to either 30 s or 60s.

Shift frequency. The shift frequency is one-half of the selected frequency range. The frequency shifter was always on.

Current/voltage mode. Measurements with the DELSA 440 can be made in two different modes so that either the current or the voltage across the electrodes is kept constant. The range is 0 to 30 mA in the current mode. The Joule heating caused by the electrical current passing through the electrolyte can have two major effects. In the first case, convection can occur in the channel making it impossible to measure mobilities. Very rarely was this a problem. The second effect of a temperature rise due to Joule heating is the conductivity change (about 2% per degree). In most cases, except for very low conductivities, the current mode is preferred, because the conductivity change, which is mainly due to a change in viscosity, is compensated for by a change in mobility which depends inversely on viscosity. Therefore, the electrophoretic mobility of the particles, to first order, remains constant even though the temperature in the channel is changing. During all experiments for this work the DELSA 440 was operated in the current mode. Experience showed that good results were obtained if the current (in mA) was set to 1-2 times the square root of conductivity (in mS).

Determination of the Stationary Layer. During the time that was needed for the sample to reach the preset temperature, usually up to ten minutes, the laser beam was placed at the theoretical stationary layer. The theoretical value for the upper stationary layer is at 84% of the cell height. The cell height was obtained by measuring the vertical distance between two detector minima for the 15° detector with a digital gauge (Mitutoyo Digimatic Indicator IDC 543, Mitutoyo Corp., Tokyo, Japan). These very sharp minima correspond to the top and bottom of the channel because the laser light is strongly scattered at the edges of the channel and therefore minima in the detector

current are produced. Due to residual sedimentation, the cell height varied within 25 μm during all experiments.

The accuracy of the digital gauge was 1 μm , and the reproducibility of the cell height was within 1 to 2 μm . A difference of 10 μm in cell positioning results in a difference of about 1-1.5 mV in zeta potential as shown earlier in this lab (Blochel, 1992, for egg-PC liposomes at 25°C).

After positioning the laser beam, two or three measurement runs were executed. The current level was increased by about 20% for two successive runs. Since for the stationary layer the obtained mobilities should be independent of the chosen current level, data were only taken if the different values fell into the normal scattering range of approximately 0.08 mobility units. If the values differed too much, the cell height was measured again and the laser beam position corrected.

Data Analysis. All data were checked to see if the peaks in the frequency spectrum showed the required proportionality with increasing angle (approximation for small angles) according to equation (26). In some cases peaks at zero Hz were observed. They were identified as peaks due to particles adsorbed onto the surface of the cell, because the peaks did not show diffusion broadening. These peaks were disregarded in the determination of electrophoretic mobility or zeta potential. In either the mobility or the zeta potential spectrum a symmetrical area around the peaks was selected and the peak analysis was performed using the software. The mean values and standard deviations of all four angles were averaged. Consequently, each data point of an isotherm is the result of two or three runs at different currents, each run at four different angles.

TRYPSIN DIGESTION

The enzyme trypsin is known to cleave the major protein of SR, the Ca^{2+} , Mg^{2+} -ATPase (Scales and Inesi, 1976). Tryptic proteolysis of SR is mostly used to gain insight into the mechanism of active Ca^{2+} transport by analyzing the structure function relationship between tryptic fragments of the Ca^{2+} , Mg^{2+} -ATPase (Stewart et al., 1976, Scott and Shamoo, 1982). The purpose of the trypsin digestion in this study was to cleave and to remove the outer projections of the Ca^{2+} , Mg^{2+} -ATPase protruding from the membrane surface. Two different types of digestion were carried out: a mild digestion and a prolonged digestion. Both were done at room temperature.

The mild trypsin digestion was done as described in Trimm's dissertation (Trimm, 1988). The prolonged digestion was carried out following the method given in Scales and Inesi (1976). The SR vesicles from the stock solution were suspended in 20mM HEPES, containing 100 mM NaCl, at pH = 7.4 in both cases. Then trypsin was added so that a final SR:trypsin ratio of 200 for the mild and a ratio of 20 for the prolonged digestion was obtained. The incubations were stopped by addition of a 40-fold excess of trypsin inhibitor after 2 minutes for the mild digestion and by addition of a 2-fold excess of trypsin inhibitor after 3 hours for the prolonged digestion. The samples were then centrifuged at 145,000 g for 1.5 hours and the pellet re-suspended in either 20 mM HEPES, containing 100 mM NaCl, at pH = 7.4 for the DELSA measurements or in a 0.125 M Tris (Tris(hydroxymethyl)aminoethane base) buffer solution, containing 15% sucrose and 2.1% sodium dodecyl sulfate (SDS), at pH = 6.8 for the SDS gel electrophoresis. Buffer solution for the re-suspension was added so that the SR concentration in the final solution was the same as it was in the SR stock solution.

SDS GEL ELECTROPHORESIS

Discontinuous SDS gel electrophoresis, according to the method of Laemmli (1970), was done with native and trypsin digested SR to prove the cleavage of the Ca^{2+} , Mg^{2+} -ATPase by trypsin. The SDS-polyacrylamide gels contained 5% polyacrylamide in the stacking gel ($\text{pH} = 6.8$) and 10% polyacrylamide in the running gel ($\text{pH} = 8.8$). The samples were incubated with 0.5 mg/ml bromphenol as tracking dye in the buffer solution described under trypsin digestion and then applied to the gels. The gels were run at a current between 10 and 30 mA for 12-24 hours. The staining solution that was used contained 50% methanol (v/v), 10% acetic acid (v/v), and 0.1% Comassie Brilliant Blue (w/v). The solution used for destaining contained 50% methanol (v/v) and 10% acetic acid (v/v). The following prestained molecular weight standards were used for reference: α_2 macro globulin (190 kDa, 180 kDa), β -galactosidase (125 kDa, 116 kDa), fructose-6-phosphate kinase (88 kDa, 84 kDa), pyruvate kinase (65 kDa, 58 kDa), fumarase (56 kDa, 48.5 kDa), lactate dehydrogenase (38 kDa, 36.5 kDa), and triosephosphate isomerase (33.5 kDa, 26.6 kDa). The first number in the parentheses stands for the apparent molecular weight during SDS gel electrophoresis, while the second number is the original molecular weight.

ATPASE ACTIVITY ASSAY

The purpose of this assay is to monitor the Ca^{2+} , Mg^{2+} -ATPase activity of SR. The assay is run at 37°C in a timed reaction following the description in Chu et al. (1988). In this assay Ca^{2+} is pumped into the vesicles by the Ca^{2+} , Mg^{2+} -ATPase consuming energy from the hydrolysis of ATP (adenosine triphosphate).

In a second step, ADP (adenosine diphosphate) is phosphorylated to ATP using energy from the reaction of phosphoenolpyruvate (PEP) to pyruvate where pyruvate kinase (PK) acts as an enzyme. In the last step, pyruvate reacts to lactate using lactate dehydrogenase (LDH) as an enzyme, where the necessary energy is provided through the oxidation of NADH.

The Ca^{2+} , Mg^{2+} -ATPase activity is therefore coupled to a decrease in NADH which was measured with a Beckman DU-7 HS UV/VIS spectrophotometer (Beckman Instruments, Fullerton, CA) by recording the absorbance at 366 nm for four minutes. The reactants for 1 ml reaction mixture (pH = 7) were: 500 μl 50 mM HEPES, 100 μl 1M KCl, 10-20 μl 50 mM ethylene glycol bis (β -aminoethyl ether)-N,N,N',-N'-tetraacetic acid (EGTA), 20 μl 150 mM NADH, 10 μl 100 mM PEP, 5 μl LDH (25 units) and PK (37 units), 10 μl 500 mM NaN_3 , 10 μl 1 M MgCl_2 , 20 μl 300 mM ATP, 10 μl 100 mM CaCl_2 , and 10-20 μg SR sample. The reaction is started by adding the sample (Mg^{2+} stimulated ATPase activity). After two minutes, Ca^{2+} stimulated ATPase activity was initiated by addition of CaCl_2 .

FITTING METHODS

The experimentally obtained mobility data were fitted using a FORTRAN 77 program which has been developed particularly for these electrophoretic mobility studies. The program plotted experimental data and, after specifying initial surface charge density, association constant, and adsorption site area, it also plotted the theoretical isotherm from the Guoy-Chapman-Stern theory as described in chapter III. The

theoretical isotherm was obtained numerically. It also calculated chi-square, given through equation (34), which is a quantity that describes the goodness of a fit (Bevington, 1969). The fits were optimized by varying association constant and adsorption site area. For this purpose experimental and theoretical data were compared, chi-square calculated, and the adsorption parameters changed until a best fit, minimizing chi-square, was obtained.

$$\chi^2 = \frac{1}{N-1} \sum_{i=1}^N \frac{(\mu_{i,ex} - \mu_{i,theo})^2}{\sigma_i^2} \quad (34)$$

The initial surface charge density was obtained by a best fit from the ionic strength dependence where the isotherm is fixed through the screening effect alone without adsorption. For the adsorption isotherms, association constant and adsorption site area were varied until a best fit was obtained.

For the PCP⁻ adsorption to egg-PC liposomes, a more sophisticated FORTRAN 77 program was available, that was developed in this lab earlier for adsorption studies. It minimized chi-square using the Marquardt-Algorithm (Bevington, 1969) by varying adsorption site area and association constant.

CHAPTER V

RESULTS AND DISCUSSION

TRYPSIN DIGESTION

The successful digestion of the ATPase protein of SR is shown with SDS gel electrophoresis for both kinds of trypsin digestion. The gel is presented in Fig. 13. Lane 1 is native SR, treated exactly the same way as the mild digested SR in lane 2, only without trypsin.

Figure 13. SDS gel electrophoresis with native, short, and prolonged digested SR. Lane 1 is native SR (control to lane 2), lane 2 is short digested SR, lane 3 are the 7 molecular weight standards, lane 4 is native SR (control to lane 5), and lane 5 is prolonged digested SR. A stands for the Ca^{2+} , Mg^{2+} -ATPase (100 kDa) and C for calsequestrin (54 kDa).

In lane 3 are the molecular weight standards and lane 4 is again the control to the prolonged digested SR in lane 5. The molecular weights of the protein standards, plotted semi-logarithmic vs. migration distance, were used to obtain the calibration curve (Fig. 14). The curve showed good agreement with a reference calibration curve from Sigma Chemical Company (St.Louis, MO) where the standards were bought.

Figure 14. Calibration curve for SDS gel electrophoresis. The following molecular weight standards were used: α_2 macro globulin (190 kDa, 180 kDa), β -galactosidase (125 kDa, 116 kDa), fructose-6-phosphate kinase (88 kDa, 84 kDa), pyruvate kinase (65 kDa, 58 kDa), fumarase (56 kDa, 48.5 kDa), lactate dehydrogenase (38 kDa, 36.5 kDa), and triosephosphate isomerase (33.5 kDa, 26.6 kDa). The calibration curve is a locally weighted least square fit.

The control lanes with native SR show two major bands: the ATPase at 100 kDa (marked as A in Fig. 13) and calsequestrin with 54 kDa (marked as C in Fig. 13).

Reference values for the molecular weight of the ATPase are 100 kDa (Trimm, 1988), 115 kDa (Fleischer and Tonomura, 1985), and 100-120 kDa (de Meis, 1981). Reported values for calsequestrin are 60 kDa (Trimm, 1988) and 44 kDa (de Meis, 1981). Calsequestrin is not cleaved by a mild digestion, due to its location within the SR membrane, and still appears in the gel in lane 2. It is, however, digested after the prolonged digestion as can be seen in lane 5. Lanes 2 and 5 show that the ATPase is cleaved after the mild as well as after the prolonged digestion. In the case of the mild digestion, the ATPase yields two complementary fragments (Chu et al., 1988) while during the prolonged digestion the fragments are further digested.

ATPASE ACTIVITY ASSAY

A known phenomenon (Chu et al., 1988) is that the ATPase of SR still shows activity after a mild trypsin digestion (SR protein:trypsin ratio (w/w) between 2000 and 250 for 2 minutes) although the successful cleavage can be shown by gel electrophoresis. This is explained by the way that the cleaved part of the ATPase still sticks to the membrane after digestion and thus activity can be maintained.

This assay was run to get supporting evidence that the cleaved ATPase of SR no longer sticks to the membrane after prolonged trypsin digestion. The assay was run with native SR and SR after prolonged digestion with the results of the absorbance measurements presented in Figs. 15 and 16.

The first step in both assays was to record the NADH baseline (1) which was constant. After adding sample, native SR showed a clear Mg^{2+} -stimulated ATPase activity (2). The following addition of CaCl_2 resulted in an approximate 15% increase of Mg^{2+} , Ca^{2+} -stimulated ATPase activity (3 and 4).

λ	ABS	SOURCE	MODE
366.0	0.3090	Visible	Time

Figure 15. ATPase activity for native SR. The figure shows absorbance measurements at 366 nm vs. time (in minutes). 1 is the NADH baseline, 2 is absorbance after adding sample (Mg^{2+} stimulated ATPase activity), 3 and 4 is absorbance after adding Ca^{2+} (Ca^{2+} , Mg^{2+} stimulated ATPase activity).

λ	ABS	SOURCE	MODE
366.0	0.3694	Visible	Time

Figure 16. ATPase activity for prolonged digested SR. The figure shows absorbance measurements at 366 nm vs. time (in minutes). 1 is the NADH baseline, 2 is absorbance after adding sample (Mg^{2+} stimulated ATPase activity), 3 is absorbance after adding Ca^{2+} (Ca^{2+} , Mg^{2+} stimulated ATPase activity).

For the prolonged digested SR, the Mg^{2+} -stimulated activity dropped to approximately 30% (2) and only a slight further increase through the Ca^{2+} -stimulation after adding $CaCl_2$ (3).

The result of these absorbance measurements is that the major part of the ATPase protein of SR is cleaved and removed from the membrane after prolonged trypsin digestion even though a small fraction of the ATPase still sticks to the membrane, as indicated by the remaining activity.

IONIC STRENGTH DEPENDENCE OF NATIVE AND DIGESTED SR MOBILITY

Ionic strength dependence of mobility was measured for native and both types of digested SR vesicles in 5 mM HEPES at pH = 7.3. Ionic strength in the solution was varied by varying the KCl concentration from 15 mM to 200 and 300 mM KCl, respectively. The experimental and theoretical isotherms are presented in Figs. 17 and 18 with corresponding data in Tables 8-11.

All four mobility isotherms could be fitted satisfactorily with an association constant of $K_a = 0$ for K^+ , indicating that potassium cations adsorb to neither native nor digested SR vesicles within the investigated concentration range and the change in mobility is solely due to screening according to the Guoy-Chapman theory. The increasing ionic strength of the solution has in principle two effects, which are both taken into account in the adsorption model. First, the shear plane distance decreases with increasing ionic strength. Second, the Debye length decreases strongly with increasing ionic strength due to the screening effect of the counterions in the solution. The first effect alone would cause slightly greater absolute values of mobility with increasing ionic strength, whereas the second effect would cause the opposite.

Figure 17. Ionic strength dependence of native SR mobility.

Figure 18. Ionic strength dependence of digested SR mobility.

Since the first effect is very small, the shape of the isotherm is dominated by the screening effect of the counterions.

The initial surface charge densities of the SR vesicles were determined by the best fit. The two experiments with native SR yielded an initial surface charge density of $-7.1 \times 10^{-3} \text{ C/m}^2$ (one electron charge per 22 nm^2). SR vesicles after a short digestion showed $-8.5 \times 10^{-3} \text{ C/m}^2$ (one electron charge per 19 nm^2), and after the prolonged digestion, a value of $-19 \times 10^{-3} \text{ C/m}^2$ (one electron charge per 8 nm^2) was obtained. Values for native SR vesicles are reported by Arrio et al. (1984) who determined the surface charge density of SR vesicles in 10 mM HEPES at $\text{pH} = 7.2$ and 21°C to be $-5.4 \times 10^{-3} \text{ C/m}^2$ (ionic strength: 4.7 mM). Liu and Oba (1990) found $-9.2 \times 10^{-3} \text{ C/m}^2$. Earlier in this lab (Mense, 1993), a value of $-6.1 \times 10^{-3} \text{ C/m}^2$ was obtained for a different SR preparation.

The results of the ionic strength dependence clearly indicate that the short digestion has only a small effect on the SR vesicles. The change in surface charge density is only slightly larger than the experimental error. The prolonged digestion, however, has a considerable impact and causes a surface charge density change of altogether twice the value observed for the short digested SR vesicles.

pH DEPENDENCE OF NATIVE AND DIGESTED SR MOBILITY

The pH dependence of electrophoretic mobility was determined for both native and trypsin digested SR vesicles (prolonged digestion) at an ionic strength of 10 mM KCl and is presented in Fig. 19. The experiment was done in a pH range between 3 and 10 due to the buffering capacity of the phosphate/citrate/borate buffer. The mobilities of native and digested SR show a qualitatively very similar behavior. They are both

positive for very low pH due to the protonation of membrane proteins and decrease with increasing pH as expected. At high pH the mobilities become pH independent and level off due to complete deprotonation.

Figure 19. Mobility of native and trypsin digested SR vesicles vs. pH.

Isoelectric points for native and digested SR were determined graphically to be at $\text{pH} = 4.3 \pm 0.2$ and $\text{pH} = 3.7 \pm 0.2$, respectively. The value for native SR is in agreement with the isoelectric point for ATPase, the major protein of SR, which is reported to be 4.5 in detergent (Tenu, J.P., and Dupaix, A., unpublished results, as reported in Arrio et al., 1984).

The mobilities of the native SR vesicles level off at a pH of about 5.5-6 whereas the leveling off for the digested SR is at a higher pH of approximately 6.5-7 with a slight tendency to drop further at pH = 9. The most important result obtained from the pH dependence is that the digested vesicles show a nearly pH independent shift to more negative mobilities of about 1 mobility unit, indicating an additional pH independent negative surface charge caused by the trypsin digestion in comparison to native SR vesicles. The curves shown in the graph are not fitted to a model, but locally weighted least-squares to obtain smooth curves.

TPhP⁺ ADSORPTION TO SR

TPhP⁺ was chosen as a typical monovalent lipophilic cation to monitor the possible changes in electrophoretic mobility of SR vesicles after trypsin digestion. Isotherms for TPhP⁺ adsorption to native, short digested, and long digested SR vesicles were measured in phosphate/citrate/borate buffer at pH = 7.3 in the concentration range of 0.5 to 100 mM TPhP⁺. The isotherms are presented in Figs. 20, 21, and 22 while the corresponding data is given in Tables 13 and 14.

The isotherms could all be fitted to the adsorption model. The parameters for adsorption, association constant and adsorption site area, were determined for each isotherm and are summarized in Table 2. The adsorption characteristics stay approximately the same for native and short digested SR as well as for long digested SR. The adsorption site area is approximately the same for all three isotherms and the association constants for native and short digested SR are also identical. The slightly increased association constant for long digested SR is partly compensated by the increased adsorption site area. The concentration point where the mobility of the

vesicles reverses is 57 mM TPhP⁺ for native SR, 70 mM for short digested SR, and 70 mM for long digested SR. This also indicates that the interaction of TPhP⁺ with the membrane stays relatively unchanged after trypsin digestion.

TABLE II
PARAMETERS FOR TPhP⁺ ADSORPTION TO SR

	Native SR	Short digested SR	Long digested SR
Association constant [l/mol]	12	12	40
Adsorption site area [nm ²]	3.5	3	4

Figure 20. Mobility isotherm for TPhP⁺ adsorption to native SR vesicles.

Figure 21. Mobility isotherm for TPHP⁺ adsorption to short digested SR vesicles.

Figure 22. Mobility isotherm for TPHP⁺ adsorption to long digested SR vesicles.

PCP⁻ ADSORPTION TO SR

In this study PCP⁻ was chosen as an environmentally significant monovalent lipophilic anion for the adsorption to SR vesicles. Adsorption isotherms were measured for native, short digested, and long digested SR vesicles in phosphate/citrate/borate buffer at pH = 9.2 in a concentration range of 3 μ M to 3 mM. The experimental and theoretical isotherms are shown in Figs. 23, 24, and 25 with corresponding data in Tables 15 and 16.

A pH of 9.2 was chosen to guarantee the complete ionization of PCP in the solution ($pK_a = 4.8$). The association constants and adsorption site areas obtained from the fit to the model are given in Table 3.

TABLE III
PARAMETERS FOR PCP⁻ ADSORPTION TO SR

	Native SR	Short digested SR	Long digested SR
Association constant [l/mol]	2000	2000	2000
Adsorption site area [nm ²]	18	15	15

The adsorption of PCP⁻ to SR was altogether weak for a lipophilic anion due to the negative initial surface charge density of SR. The measurements, especially for digested SR were somewhat difficult, resulting in larger standard deviations for the data points. The reason for this is not known.

Figure 23. Mobility isotherm for PCP⁻ adsorption to native SR.

Figure 24. Mobility isotherm for PCP⁻ adsorption to short digested SR.

Figure 25. Mobility isotherm for PCP⁻ adsorption to long digested SR. The theoretical curve for the PCP⁻ adsorption to SR cannot be seen as a best fit, but as an illustration that the shown theoretical curve is consistent with the measured data points.

All three isotherms could be fitted with nearly the same set of adsorption parameters, meaning that for a negatively charged monovalent ion the adsorption characteristics stay unchanged within the error of measurement after short as well as after prolonged trypsin digestion.

Ca²⁺ ADSORPTION TO SR

Ca²⁺ as an adsorbing ion was primarily chosen because it is a divalent ion. Additionally, Ca²⁺ is especially important in connection with SR, first because of the not yet fully understood calcium release and uptake mechanism of SR, and second because recent studies in this lab (Mense, 1993) suggested that native SR has two different adsorption sites for Ca²⁺.

Figure 26. Mobility isotherm for Ca²⁺ adsorption to native SR.

Figure 27. Mobility isotherm for Ca²⁺ adsorption to short digested SR.

Figure 28. Mobility isotherm for Ca^{2+} adsorption to long digested SR.

Ca^{2+} adsorption isotherms were measured for native, short digested, and long digested SR vesicles in 5 mM HEPES at pH = 7.3. Ca^{2+} concentration was varied in a concentration range of 0.05 mM to 200 mM by dissolving appropriate amounts of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in buffer. The experimental mobilities and the theoretical isotherms are graphically presented in Figs. 26, 27, and 28.

TABLE IV

PARAMETERS FOR Ca^{2+} ADSORPTION TO SR

	Native SR	Short digested SR	Long digested SR
Association constant [l/mol]	8	8	8
Adsorption site area [nm ²]	10	10	10

Measurements with Ca^{2+} concentrations above 200 mM were not possible because due to the high conductivity of the solution, the maximum possible current did not give a large enough frequency shift to get reliable data. All three mobility isotherms could be fitted to the adsorption model, parameters in Table 4, implying that Ca^{2+} adsorption to SR vesicles can be described with the standard Langmuir adsorption model, assuming one kind of adsorption sites.

The results in Table 4 show that for an adsorbing divalent cation the electrophoretic mobility of native, short and prolonged digested SR vesicles can be described with the same set of adsorption parameters. The concentration points where the vesicles show a zero mobility, indicating that adsorbed Ca^{2+} compensates for the initial surface charge, were determined to be 17.5 mM for native SR, 19 mM for short digested SR, and 19.5 mM for long digested SR.

THERMODYNAMIC ANALYSIS OF ADSORPTION

PCP Adsorption to PC Vesicles

PCP⁻ was chosen as a typical lipophilic anion for the temperature dependent studies. The electrophoretic measurements were done in phosphate/citrate/borate buffer (pH = 8.4) at 10°C, 25°C, 40°C, and 55°C. Two experiments were done to check the reproducibility of the linear partition coefficient which agreed in both experiments within the experimental error.

The error bars for the linear partition coefficient are no real standard deviations but reasonable estimates obtained from the fit. The thickness of the membrane adsorption layer was assumed to be $t = 4 \text{ \AA}$ which is the commonly accepted value (Flewelling and Hubbell, 1986a and 1986b, Smejtek and Wang, 1990). The mobility isotherms are

presented in Figs. 29 and 30 with experimental and theoretical data in Tables 19, 20a, and 20b. The linear partition coefficient, β , was calculated from the adsorption parameters (obtained from a best fit to the model), as explained in chapter 3, and then $\ln(\beta/t)$ plotted vs. $1/T$ as shown in Fig. 31. A linear regression for both experiments yielded $y(x) = 3.03x + 1.11$ from where the enthalpy and entropy of adsorption were determined to $\Delta H = -25 \pm 3$ kJ/mol and $\Delta S = 9 \pm 12$ J/(mol K).

The error for the entropy term, ΔS , was obtained from the plot program (Axum, version 3.0 (1993), TriMetrix, Inc.) that did the linear regression. The value seems to be relatively small and the real uncertainty of ΔS is maybe larger. The errors for ΔS for the TPhP^+ adsorption to PC and SR were determined in the same way and are also maybe larger.

Figure 29. Mobility isotherms for PCP^- adsorption to PC vesicles at 10°C and 25°C (exp.1).

Figure 30. Mobility isotherms for PCP⁻ adsorption to PC vesicles at 10°C, 25°C, 40°C, and 55°C (exp.2).

TABLE V

PARAMETERS FOR PCP⁻ ADSORPTION TO PC VESICLES

	Experiment 2				Experiment 1	
	10°C	25°C	40°C	55°C	10°C	25°C
Assoc. constant [l/mol]	120000	83000	43000	20000	130000	88000
Adsorption site area [nm ²]	4.2	4.2	4.2	4.2	4.2	4.2
Linear partition coeff. [10 ⁻⁵ m]	4.6	3.3	1.7	0.76	5.3	3.5

Figure 31. Linear regression for the temperature dependence of the linear partition coefficient β for PCP^- adsorption to PC.

The results show that PCP^- adsorption to PC liposomes is driven by enthalpy change rather than by entropy change. The value of $-T\Delta S$ for room temperature amounts to less than -3 kJ/mol compared to -25 kJ/mol for the enthalpy. This finding is new, there are no data available in the literature. However, thermodynamic parameters for PCP^- can be compared with those for other lipophilic ions published in the literature. Seelig and Ganz (1991), who determined ΔH and ΔS for the binding of negatively charged 2-(p-toluidinyl)naphtalene-6-sulfonate (TNS^-) and tetraphenylborate (TPB^-) to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) from calorimetry studies, pointed out that it will be necessary to revise the present concept of hydrophobic binding since the experimental results are inconsistent with the current models. They

found for TNS⁻ $\Delta H = -38$ kJ/mol and $-T\Delta S = -7$ kJ/mol (25°C), and for TPB⁻ $\Delta H = -39$ kJ/mol and $-T\Delta S = -2$ kJ/mol (25°C). They explained the dominating enthalpy term, compared to the small entropy term, with van der Waals interactions between the hydrophobic part of the lipid bilayer and the nonpolar part of the adsorbing ion.

TPhP Adsorption to PC Liposomes

In order to determine the thermodynamic parameters for the adsorption of a positively charged ion to PC liposomes, TPhP⁺ was chosen. The measurements were done at pH = 7.3 in phosphate/citrate/borate buffer at 10°C, 25°C, and 40°C. The mobilities are shown in Fig. 32, with corresponding data in Tables 21 and 22. For TPhP⁺ adsorption to PC, the adsorption parameters (Table 6) were again determined from the fit to the model, β computed from association constant and adsorption site area, and then $\ln(\beta/t)$ plotted vs. $1/T$. From the linear regression $y(x) = -3.20x + 17.20$ we obtained $\Delta H = 27 \pm 5$ kJ/mol and $\Delta S = 145 \pm 17$ J/(mol K) and thus at 25°C $-T\Delta S = -43$ kJ/mol. The adsorption of TPhP⁺ to PC is clearly entropy driven, in contrast to PCP⁻ adsorption.

TABLE VI
PARAMETERS FOR TPhP⁺ ADSORPTION TO PC VESICLES

	Temperature		
	10°C	25°C	40°C
Association constant [l/mol]	600	950	1030
Adsorption site area [nm ²]	6.0	4.6	3.5
Linear partition coefficient [10 ⁻⁷ m]	1.7	3.4	4.9

Figure 32. Mobility isotherms for TPhP⁺ adsorption to PC liposomes at 10°C, 25°C, and 40°C.

Figure 33. Linear regression for the temperature dependence of the linear partition coefficient β for TPhP⁺ adsorption to PC.

Flewelling and Hubbell (1986a) determined change in enthalpy and entropy for TPhP⁺ adsorption to egg-PC vesicles with two different experimental methods: equilibrium dialysis and spin label techniques. They found $\Delta H = 14.6 \pm 1.3$ kJ/mol (equilibrium dialysis) and $\Delta H = 16.3 \pm 3.4$ kJ/mol (spin label). For TPhP⁺ the entropy term was found to be $\Delta S = 88 \pm 4$ J/(mol K) from equilibrium dialysis and $\Delta S = 113 \pm 46$ J/(mol K) from the spin label methods. The origin for the positive enthalpy term for the positively charged ion TPhP⁺ is unknown.

TPhP Adsorption to SR Vesicles

TPhP⁺ adsorption to SR vesicles was measured in phosphate/citrate/borate buffer (pH = 7.3) at 10°C, 25°C, 40°C, and 55°C. The isotherms are presented in Figs. 34 and 35. The experimental data and the theoretical isotherms are given in Tables 23 and 24. The fit to the model yielded the adsorption parameters (Table 7), from which the linear partition coefficient, β , was computed. A linear regression was done for the plot of $\ln(\beta/t)$ vs. $1/T$ with the result $y(x) = -2.33x + 10.34$. The thermodynamic parameters for TPhP⁺ adsorption to SR vesicles are therefore $\Delta H = 19 \pm 5$ kJ/mol and $\Delta S = 86 \pm 15$ J/(mol K) ($-T\Delta S = -26$ kJ/mol for 25°C) suggesting that the adsorption is dominated by entropy change.

The comparison of TPhP⁺ adsorption parameters for PC liposomes with those for SR shows some similarities. For both PC and SR, the enthalpy terms are comparable and positive, while the entropy terms, $-T\Delta S$, even larger and negative, dominate the energetics of adsorption.

Figure 34. Mobility isotherms for TPhP⁺ adsorption to SR vesicles at 10°C and 25°C.

Figure 35. Mobility isotherms for TPhP⁺ adsorption to SR vesicles at 40°C and 55°C.

TABLE VII
PARAMETERS FOR TPhP⁺ ADSORPTION TO SR VESICLES

	Temperature			
	10°C	25°C	40°C	55°C
Association const. [l/mol]	5.5	7.0	9.0	41
Adsorption site area [nm ²]	2.5	2.4	2.5	5.6
Linear part. coeff. [10 ⁻⁹ m]	3.7	4.8	6.0	12

Figure 36. Linear regression for the temperature dependence of the linear partition coefficient β for TPhP⁺ adsorption to SR.

CONCLUSIONS

In this study, electrophoretic mobilities of native and two types of digested SR vesicles were measured to investigate the possible influence of hydrodynamic drag, caused by the ATPase protruding from the surface of SR vesicles. After the prolonged digestion, the ATPase was cleaved and removed from the membrane as shown with the ATPase activity assay and SDS gel electrophoresis. Ionic strength and pH dependence of mobility showed a nearly pH independent increase in initial surface charge density after prolonged digestion. Adsorption isotherms for all three kinds of SR vesicles, native, mildly and strongly modified by trypsin digestion, were measured for the monovalent cation TPhP^+ , the monovalent anion PCP^- , and the divalent cation Ca^{2+} . The most important result from the adsorption studies is that adsorption of the three ions did not increase significantly after prolonged trypsin digestion. From this it can be concluded that hydrodynamic drag does not have a measurable influence on the electrophoretic mobility of SR vesicles and therefore cannot account for the big differences in mobility between SR vesicles and a comparable artificial membrane system (PC/PS liposomes).

In order to gain insight into the driving forces of adsorption to artificial and biological membrane systems, a thermodynamic analysis was done for PCP^- and TPhP^+ adsorption to PC vesicles, and for TPhP^+ adsorption to SR vesicles. The adsorption of the negatively charged monovalent ion PCP^- to PC liposomes was clearly driven by enthalpy and only a small contribution to the free energy of transfer came from the entropy. In contrast, the adsorption of TPhP^+ to PC liposomes was driven by entropy. The free energy of transfer consisted of a positive enthalpy term and a still larger, negative entropy term. The adsorption of TPhP^+ to SR vesicles showed the same features as the TPhP^+ adsorption to PC vesicles. In contrast to PCP^- , its free energy of

transfer was determined by the positive enthalpy and by a dominating entropy term. The thermodynamic analysis of ion adsorption shows that the driving forces of TPhP^+ adsorption for the artificial phospholipid and the biological membrane SR are very similar despite the significant lower ion adsorption to biological membranes compared to that for artificial membranes.

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APPENDIX

TABLES

TABLE VIII

EXPERIMENTAL MOBILITY DATA FOR IONIC STRENGTH
DEPENDENCE OF NATIVE SR

Experiment 1		Experiment 2	
Ionic strength [mM KCl]	Mobility [($\mu\text{m/s}$)/(V/cm)]	Ionic strength [mM KCl]	Mobility [($\mu\text{m/s}$)/(V/cm)]
5.33	- 2.16 (0.09)	8.33	- 1.74 (0.09)
10.33	- 2.01 (0.09)	15.33	- 1.37 (0.08)
30.33	- 1.29 (0.09)	30.33	- 1.09 (0.07)
50.33	- 0.98 (0.08)	50.33	- 0.88 (0.08)
100.33	- 0.73 (0.09)	80.33	- 0.72 (0.07)
200.33	- 0.62 (0.11)	100.33	- 0.60 (0.07)
---	---	200.33	- 0.51 (0.18)

TABLE IX

EXPERIMENTAL MOBILITY DATA FOR IONIC STRENGTH
DEPENDENCE OF DIGESTED SR

Short digestion		Long digestion	
Ionic strength [mM KCL]	Mobility [($\mu\text{m/s}$)/(V/cm)]	Ionic strength [mM KCl]	Mobility [($\mu\text{m/s}$)/(V/cm)]
15	- 1.62 (0.15)	15.33	- 3.15 (0.11)
30	- 1.25 (0.13)	30.33	- 2.79 (0.14)
50	- 1.09 (0.10)	50.33	- 2.31 (0.12)
80	- 0.97 (0.07)	80.33	- 2.15 (0.12)
100	- 0.86 (0.07)	100.33	- 1.87 (0.10)
200	- 0.70 (0.07)	200.33	- 1.30 (0.08)
---	---	300.33	- 1.09 (0.08)

TABLE X
THEORETICAL MOBILITY DATA FOR IONIC STRENGTH
DEPENDENCE OF NATIVE SR

Fit for experiment 1		Fit for experiment 2	
Ionic strength [mM KCl]	Mobility [($\mu\text{m/s}$)/(V/cm)]	Ionic strength [mM KCl]	Mobility [($\mu\text{m/s}$)/(V/cm)]
1.00	- 3.42	1.00	- 3.12
1.58	- 3.20	1.58	- 2.92
2.51	- 2.92	2.51	- 2.66
3.98	- 2.60	3.98	- 2.36
6.31	- 2.25	6.31	- 2.04
10.0	- 1.90	10.0	- 1.72
15.8	- 1.57	15.8	- 1.41
25.1	- 1.28	25.1	- 1.15
39.8	- 1.02	39.8	- 0.92
63.1	- 0.82	63.1	- 0.73
100	- 0.65	100	- 0.58
158	- 0.52	158	- 0.47
251	- 0.42	251	- 0.38
398	- 0.35	398	- 0.31
631	- 0.30	631	- 0.26
1000	- 0.26	1000	- 0.23

TABLE XI
THEORETICAL MOBILITY DATA FOR IONIC STRENGTH
DEPENDENCE OF DIGESTED SR

Short digestion		Long digestion	
Ionic strength [mM KCL]	Mobility [($\mu\text{m/s}$)/(V/cm)]	Ionic strength [mM KCL]	Mobility [($\mu\text{m/s}$)/(V/cm)]
6.31	- 2.50	6.31	- 4.66
10.0	- 2.12	10.0	- 4.09
15.8	- 1.76	15.8	- 3.51
25.1	- 1.43	25.1	- 2.95
39.8	- 1.15	39.8	- 2.43
63.1	- 0.92	63.1	- 1.98
100	- 0.73	100	- 1.61
158	- 0.59	158	- 1.30
251	- 0.48	251	- 1.06
398	- 0.39	398	- 0.88
631	- 0.33	631	- 0.75
1000	- 0.29	1000	- 0.65
1580	- 0.23	1580	- 0.52

TABLE XII

pH DEPENDENCE OF MOBILITY FOR NATIVE AND DIGESTED SR

Native SR vesicles		Trypsin digested SR vesicles	
pH	mobility [($\mu\text{m/s}$)/(V/cm)]	pH	mobility [($\mu\text{m/s}$)/(V/cm)]
3.01	2.08 (0.05)	3.06	1.05 (0.09)
3.75	1.19 (0.06)	3.62	0.14 (0.16)
4.07	0.61 (0.06)	4.32	- 1.30 (0.13)
4.57	- 0.78 (0.08)	4.81	- 1.61 (0.14)
5.17	- 1.52 (0.09)	5.53	- 2.22 (0.14)
5.57	- 1.54 (0.09)	6.30	- 2.46 (0.20)
6.12	- 1.61 (0.12)	7.10	- 2.65 (0.11)
6.84	- 1.62 (0.08)	7.96	- 2.60 (0.19)
7.54	- 1.78 (0.09)	8.40	- 2.67 (0.16)
8.48	- 1.65 (0.12)	9.11	- 2.85 (0.15)
9.28	- 1.69 (0.11)	9.94	- 2.88 (0.14)
10.10	- 1.76 (0.09)	---	---

TABLE XIII

EXPERIMENTAL MOBILITY DATA FOR TPhP⁺ ADSORPTION TO SR

TPhP ⁺ concentration [mM]	Mobility of native SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of short digested SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of long digested SR [($\mu\text{m/s}$)/(V/cm)]
0.3	- 1.83 (0.07)	---	- 2.94 (0.18)
0.5	---	---	- 2.73 (0.12)
1	- 1.52 (0.06)	- 2.32 (0.11)	- 2.48 (0.14)
3	- 1.54 (0.06)	- 2.16 (0.13)	- 2.00 (0.13)
5	- 1.25 (0.06)	- 1.81 (0.11)	- 1.65 (0.11)
8	- 1.14 (0.07)	- 1.54 (0.09)	---
10	- 0.77 (0.07)	- 1.21 (0.09)	- 1.15 (0.10)
15	- 0.55 (0.08)	- 0.93 (0.05)	---
30	- 0.11 (0.06)	- 0.43 (0.04)	- 0.45 (0.07)
50	- 0.13 (0.06)	- 0.08 (0.05)	- 0.21 (0.05)
83	0.26 (0.07)	0.15 (0.06)	---
100	---	---	0.03 (0.05)

TABLE XIV
THEORETICAL MOBILITY DATA FOR TPhP⁺ ADSORPTION TO SR

TPHP ⁺ concentration [mM]	Mobility of native SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of short digested SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of long digested SR [($\mu\text{m/s}$)/(V/cm)]
0.100	- 1.84	- 2.70	- 3.16
0.159	- 1.83	- 2.68	- 3.11
0.251	- 1.81	- 2.64	- 3.03
0.398	- 1.78	- 2.60	- 2.93
0.631	- 1.74	- 2.53	- 2.79
1.00	- 1.68	- 2.43	- 2.61
1.58	- 1.58	- 2.30	- 2.39
2.51	- 1.46	- 2.12	- 2.13
3.98	- 1.30	-1.90	- 1.84
6.31	- 1.10	-1.64	- 1.53
10.0	- 0.86	- 1.34	- 1.20
15.8	- 0.60	- 1.01	- 0.88
25.1	- 0.33	- 0.68	- 0.57
39.8	- 0.07	- 0.35	- 0.29
63.1	0.18	- 0.05	- 0.05
100	0.40	0.22	0.13
158	0.57	0.43	0.24
251	0.70	0.60	0.31
398	0.78	0.70	0.33
631	0.82	0.76	0.33
1000	0.80	0.76	0.30

TABLE XV
THEORETICAL MOBILITY DATA FOR PCP⁻ ADSORPTION TO SR

PCP ⁻ concentration [μM]	Mobility of native SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of short digested SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of long digested SR [($\mu\text{m/s}$)/(V/cm)]
1.0	- 2.05	- 2.05	- 2.81
1.6	- 2.05	- 2.05	- 2.81
2.5	- 2.05	- 2.05	- 2.81
4.0	- 2.05	- 2.05	- 2.81
6.3	- 2.05	- 2.05	- 2.81
10.0	- 2.05	- 2.05	- 2.81
15.8	- 2.06	- 2.06	- 2.82
25.1	- 2.06	- 2.06	- 2.82
39.8	- 2.07	- 2.07	- 2.82
63.1	- 2.08	- 2.09	- 2.83
100	- 2.10	- 2.11	- 2.84
159	- 2.12	- 2.14	- 2.86
251	- 2.16	- 2.18	- 2.89
398	- 2.21	- 2.24	- 2.92
631	- 2.27	- 2.31	- 2.97
1000	- 2.34	- 2.39	- 3.03
1585	- 2.42	- 2.49	- 3.10
2510	- 2.49	- 2.57	- 3.16
3980	- 2.55	- 2.65	- 3.22
6310	- 2.59	- 2.69	- 3.26
10000	- 2.58	- 2.70	- 3.25

TABLE XVI

EXPERIMENTAL MOBILITY DATA FOR PCP⁻ ADSORPTION TO SR

PCP ⁻ concentration [μM]	Mobility of native SR [[$\mu\text{m/s}$]/(V/cm)]	Mobility of short digested SR [[$\mu\text{m/s}$]/(V/cm)]	Mobility of long digested SR [[$\mu\text{m/s}$]/(V/cm)]
3	- 1.96 (0.07)	- 1.96 (0.15)	---
6	- 2.02 (0.08)	- 2.07 (0.12)	- 2.75 (0.15)
10	- 1.89 (0.05)	- 1.89 (0.11)	- 2.92 (0.15)
30	- 1.98 (0.07)	- 2.22 (0.12)	- 2.98 (0.10)
60	- 2.09 (0.06)	- 2.28 (0.07)	- 2.87 (0.17)
100	- 2.14 (0.06)	- 2.20 (0.12)	- 2.71 (0.12)
300	- 2.24 (0.08)	- 2.41 (0.08)	- 2.71 (0.30)
600	- 2.35 (0.07)	- 2.37 (0.11)	- - -
1000	- 2.38 (0.08)	- 2.44 (0.14)	- 2.92 (0.29)
3000	- 2.38 (0.06)	- 2.62 (0.18)	---

TABLE XVII

EXPERIMENTAL MOBILITY DATA FOR Ca²⁺ ADSORPTION TO SR

Ca ²⁺ concentration [mM]	Mobility of native SR [[$\mu\text{m/s}$]/(V/cm)]	Mobility of short digested SR [[$\mu\text{m/s}$]/(V/cm)]	Mobility of long digested SR [[$\mu\text{m/s}$]/(V/cm)]
0.05	- 2.02 (0.06)	---	---
0.1	- 1.83 (0.07)	- 2.15 (0.15)	- 2.15 (0.15)
0.2	- 1.65 (0.07)	- 1.76 (0.06)	- 1.91 (0.13)
0.5	- 1.32 (0.05)	- 1.36 (0.09)	- 1.50 (0.10)
1	- 1.05 (0.05)	- 1.32 (0.03)	- 1.21 (0.08)
2	- 0.84 (0.06)	- 0.88 (0.06)	- 0.90 (0.07)
5	- 0.56 (0.04)	- 0.47 (0.04)	- 0.71 (0.07)
10	- 0.11 (0.05)	- 0.17 (0.03)	- 0.28 (0.11)
20	0.13 (0.08)	- 0.05 (0.04)	- 0.11 (0.09)
50	0.22 (0.20)	0.15 (0.07)	0.11 (0.08)
100	0.24 (0.05)	0.23 (0.09)	0.22 (0.09)
200	---	---	0.37 (0.13)

TABLE XVIII

THEORETICAL MOBILITY DATA FOR Ca^{2+} ADSORPTION TO SR

Ca^{2+} concentration [mM]	Mobility of native SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of short digested SR [($\mu\text{m/s}$)/(V/cm)]	Mobility of long digested SR [($\mu\text{m/s}$)/(V/cm)]
0.0100	- 2.14	- 2.38	- 2.54
0.0158	- 2.12	- 2.36	- 2.51
0.0251	- 2.09	- 2.33	- 2.47
0.0398	- 2.05	- 2.27	- 2.42
0.0631	- 1.99	- 2.20	- 2.34
0.100	- 1.90	- 2.10	- 2.23
0.159	- 1.79	- 1.97	- 2.09
0.251	- 1.64	- 1.82	- 1.92
0.398	- 1.47	- 1.63	- 1.73
0.631	- 1.28	- 1.42	- 1.51
1.00	- 1.08	- 1.20	- 1.28
1.58	- 0.87	- 0.97	- 1.04
2.51	- 0.66	- 0.75	- 0.81
3.98	- 0.47	- 0.55	- 0.60
6.31	- 0.30	- 0.36	- 0.40
10.0	- 0.14	- 0.19	- 0.23
15.8	- 0.01	- 0.05	- 0.07
25.1	0.11	0.08	0.06
39.8	0.21	0.19	0.17
63.1	0.30	0.28	0.27
100	0.38	0.36	0.35
158	0.45	0.44	0.43
251	0.52	0.51	0.50
398	0.55	0.54	0.53
631	0.52	0.51	0.50
1000	0.47	0.46	0.45

TABLE XIX

EXPERIMENTAL AND THEORETICAL MOBILITY DATA FOR PCP-
ADSORPTION TO PC IN EXPERIMENT 1

EXPERIMENT 1				
PCP- concentration [10 ⁻⁶ M]	Mobility at 10°C (exp.) [(μm/s)/(V/cm)]	Mobility at 10°C (fit) [(μm/s)/(V/cm)]	Mobility at 25°C (exp.) [(μm/s)/(V/cm)]	Mobility at 25°C (fit) [(μm/s)/(V/cm)]
1	- 0.91 (0.02)	- 0.97	- 1.32 (0.03)	- 1.15
2	- 1.07 (0.02)	- 1.21	- 1.42 (0.03)	- 1.42
5	- 1.61 (0.02)	- 1.64	- 1.90 (0.03)	- 1.94
10	- 2.03 (0.02)	- 2.02	- 2.42 (0.03)	- 2.43
20	- 2.59 (0.03)	- 2.43	- 2.97 (0.03)	- 2.98
50	- 3.04 (0.03)	- 2.97	- 3.79 (0.03)	- 3.72
100	- 3.34 (0.03)	- 3.37	- 4.36 (0.04)	- 4.28
200	- 3.66 (0.03)	- 3.74	- 4.90 (0.04)	- 4.80
500	- 4.30 (0.04)	- 4.12	- 5.42 (0.05)	- 5.39

TABLE XXa

EXPERIMENTAL AND THEORETICAL MOBILITY DATA FOR PCP-
ADSORPTION TO PC IN EXPERIMENT 2

EXPERIMENT 2				
PCP- concentration [10 ⁻⁶ M]	Mobility at 10°C (exp.) [(μm/s)/(V/cm)]	Mobility at 10°C (fit) [(μm/s)/(V/cm)]	Mobility at 25°C (exp.) [(μm/s)/(V/cm)]	Mobility at 25°C (fit) [(μm/s)/(V/cm)]
2	- 1.14 (0.04)	- 1.06	- 1.26 (0.05)	- 1.37
10	- 1.92 (0.05)	- 1.88	- 2.35 (0.04)	- 2.38
50	- 2.85 (0.07)	- 2.84	- 3.66 (0.07)	- 3.66
200	- 3.55 (0.08)	- 3.62	- 4.78 (0.09)	- 4.75
900	- 4.13 (0.08)	- 4.20	- 5.51 (0.11)	- 5.61

TABLE XXb

EXPERIMENTAL AND THEORETICAL MOBILITY DATA FOR PCP-
ADSORPTION TO PC IN EXPERIMENT 2

EXPERIMENT 2				
PCP ⁻ concentration [10 ⁻⁶ M]	Mobility at 40°C (exp.) [(μm/s)/(V/cm)]	Mobility at 40°C (fit) [(μm/s)/(V/cm)]	Mobility at 55°C (exp.) [(μm/s)/(V/cm)]	Mobility at 55°C (fit) [(μm/s)/(V/cm)]
2	- 0.74 (0.10)	- 0.99	- 0.42 (0.11)	- 0.72
10	- 2.23 (0.09)	- 2.12	- 1.67 (0.14)	- 1.68
50	- 3.80 (0.09)	- 3.76	- 3.73 (0.12)	- 3.43
200	- 5.40 (0.10)	- 5.23	- 5.64 (0.19)	- 5.22
900	- 6.66 (0.15)	- 6.51	- 7.41 (0.20)	- 6.96

TABLE XXI

EXPERIMENTAL MOBILITY DATA FOR
TPhP⁺ ADSORPTION TO PC

TPhP ⁺ concentration [10 ⁻⁴ M]	Mobility at 10°C [(μm/s)/(V/cm)]	Mobility at 25°C [(μm/s)/(V/cm)]	Mobility at 40°C [(μm/s)/(V/cm)]
5	0.22 (0.03)	0.37 (0.04)	0.65 (0.06)
10	0.32 (0.04)	0.58 (0.06)	0.97 (0.06)
20	0.52 (0.04)	0.98 (0.05)	1.72 (0.04)
50	0.82 (0.04)	1.29 (0.05)	2.16 (0.08)
200	0.93 (0.06)	1.67 (0.07)	2.66 (0.08)
450	0.90 (0.07)	1.49 (0.10)	2.44 (0.11)

TABLE XXII
THEORETICAL MOBILITY DATA FOR
TPhP⁺ ADSORPTION TO PC

TPhP ⁺ concentration [10 ⁻⁴ M]	Mobility at 10°C [(μm/s)/(V/cm)]	Mobility at 25°C [(μm/s)/(V/cm)]	Mobility at 40°C [(μm/s)/(V/cm)]
1.00	- 0.07	- 0.24	- 0.33
1.58	- 0.02	- 0.10	- 0.08
2.51	0.05	0.07	0.20
3.98	0.14	0.27	0.51
6.31	0.24	0.47	0.84
10.0	0.35	0.68	1.15
15.8	0.47	0.89	1.46
25.1	0.59	1.08	1.74
39.8	0.69	1.24	1.99
63.1	0.79	1.38	2.19
100	0.86	1.48	2.35
158	0.92	1.56	2.46
251	0.95	1.59	2.53
398	0.96	1.60	2.55
631	0.94	1.58	2.53
1000	0.90	1.52	2.47

TABLE XXIII
THEORETICAL MOBILITY DATA FOR TPhP⁺ ADSORPTION TO SR

TPhP ⁺ concentration [10 ⁻⁴ M]	Mobility at 10°C [(μm/s)/(V/cm)]	Mobility at 25°C [(μm/s)/(V/cm)]	Mobility at 40°C [(μm/s)/(V/cm)]	Mobility at 55°C [(μm/s)/(V/cm)]
0.100	- 1.30	- 2.05	- 2.72	- 3.57
0.158	- 1.30	- 2.05	- 2.72	- 3.57
0.251	- 1.30	- 2.04	- 2.71	- 3.56
0.398	- 1.30	- 2.04	- 2.71	- 3.55
0.631	- 1.29	- 2.04	- 2.70	- 3.53
1.00	- 1.29	- 2.03	- 2.69	- 3.50
1.59	- 1.28	- 2.02	- 2.67	- 3.45
2.51	- 1.28	- 2.00	- 2.64	- 3.38
3.98	- 1.26	- 1.97	- 2.60	- 3.28
6.31	- 1.24	- 1.93	- 2.53	- 3.14
10.0	- 1.21	- 1.87	- 2.43	- 2.95
15.8	- 1.16	- 1.78	- 2.29	- 2.70
25.1	- 1.09	- 1.65	- 2.11	- 2.39
39.8	- 1.00	- 1.49	- 1.86	- 2.03
63.1	- 0.88	- 1.28	- 1.57	- 1.63
100	- 0.73	- 1.03	- 1.22	- 1.20
158	- 0.56	- 0.76	- 0.83	- 0.77
251	- 0.37	- 0.46	- 0.43	- 0.37
398	- 0.18	- 0.16	- 0.03	- 0.01
631	0.01	0.13	0.35	0.28
1000	0.19	0.40	0.69	0.49
1580	0.35	0.63	0.99	0.61
2510	0.48	0.82	1.23	0.66
3980	0.59	0.98	1.41	0.65
6310	0.68	1.10	1.54	0.62
10000	0.73	1.14	1.56	0.57

TABLE XIV
EXPERIMENTAL MOBILITY DATA FOR TPhP⁺ ADSORPTION TO SR

TPhP ⁺ concentration [10 ⁻⁴ M]	Mobility at 10°C [(μm/s)/(V/cm)]	Mobility at 25°C [(μm/s)/(V/cm)]	Mobility at 40°C [(μm/s)/(V/cm)]	Mobility at 55°C [(μm/s)/(V/cm)]
0.3	---	---	---	- 3.37 (0.18)
0.5	---	---	---	- 3.46 (0.16)
1	---	---	---	- 3.39 (0.17)
3	- 1.22 (0.04)	- 1.93 (0.07)	- 2.50 (0.10)	- 3.17 (0.16)
5	- 1.10 (0.05)	- 1.87 (0.07)	- 2.61 (0.09)	- 3.14 (0.16)
10	- 1.10 (0.04)	- 1.73 (0.06)	- 2.55 (0.08)	- 3.09 (0.14)
21	---	---	- 2.21 (0.09)	---
30	- 1.18 (0.06)	- 1.81 (0.08)	---	- 2.55 (0.13)
40	---	---	- 1.87 (0.07)	---
50	- 1.10 (0.06)	- 1.51 (0.12)	---	- 2.04 (0.13)
70	---	---	- 1.60 (0.07)	- 1.40 (0.10)
100	- 0.94 (0.07)	- 1.28 (0.09)	---	- 0.86 (0.10)
125	- 0.64 (0.07)	- 1.03 (0.08)	- 0.97 (0.10)	---
200	- 0.45 (0.05)	- 0.58 (0.09)	- 0.48 (0.08)	---
300	- 0.25 (0.07)	- 0.37 (0.08)	---	0.11 (0.10)
400	- 0.14 (0.05)	- 0.17 (0.07)	- 0.07 (0.10)	---
500	0.02 (0.04)	0.07 (0.06)	0.06 (0.09)	0.42 (0.10)
1000	---	---	---	0.56 (0.10)
1670	---	---	---	0.47 (0.12)