

Generalized Gouy-Chapman Potential of Charged Phospholipid Membranes with Divalent Cations

Barbara Abraham-Shrauner*, St. Louis, Mo.

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Summary

An exact result for the electrostatic potential of a phospholipid membrane modeled by a fixed charge sheet and diffuse double layer of the Gouy-Chapman theory is given. The dependence of the potential with distance is expressed in simple form for positive divalent ions (alkaline earth cations) added to monovalent cations and anions in the bathing solution. The result has applications in the study of the effect of divalent cations on nerve or muscle excitation and on the formation of blood clots.

Introduction

Electrostatic interactions near biological membrane surfaces are important for many biological phenomena. Two biological examples where electrostatic interactions are important are nerve excitation and blood clotting reactions. These processes are influenced by the surface charge and the concentration of mobile ions in the solutions bathing the nerve or phospholipid surfaces.

In the studies of nerve excitation the increase in the divalent ion concentration in the bathing solution of a nerve or a muscle was shown to produce a shift in the conductance voltage curves along the positive voltage axis (Frankenhauser and Hodgkin, 1957). Attempts to explain this shift have been made (Takata et al., 1966; McLaughlin et al., 1970; Gilbert and Ehrenstein, 1970; McLaughlin et al., 1971; and Träuble and Eibl, 1974) but agreement on the explanation has not yet been reached. The relative importance of the binding of divalent ions to the membrane surface or the screening by the divalent ions in the diffuse double layer in causing the voltage shift has not been decided but electrostatic interactions are assumed to be crucial.

Several early blood clotting studies (Bangham, 1961; Papahajopoulos and Hanahan, 1964 and Barton et al., 1970) suggested that varying the surface charge of the phospholipids altered the clotting times and that Ca^{++} was essential

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in certain reactions. More recently the conversion of prothrombin to thrombin has been studied in detail (Gitel et al, 1973) and the role of the negatively charged phospholipid vesicles elucidated. The presence of phospholipid vesicles greatly accelerates the conversion by binding prothrombin, activated factor X and divalent ions (usually Ca^{++}) to the phospholipid surface. The concentration of Ca^{++} is of interest because the binding of prothrombin to the phospholipid varies with Ca^{++} concentration.

In both the above phenomena the ionic strength does not correctly predict the relative screening effect of the electrostatic force by monovalent and divalent counterions near a surface with a large, fixed surface charge. Hence, one must find the ionic concentration by using the nonlinear Poisson-Boltzmann equation or by generalizing the Gouy-Chapman (Gouy, 1910; Chapman, 1913) theory of the diffuse double layer for a 1-1 electrolyte.

The Gouy-Chapman model has been solved exactly for a 1-1 electrolyte facing a plane sheet of fixed surface charge. If the biological membrane surfaces have a small curvature, the membrane may be assumed planar. Corrections to the potential in the diffuse double layer for a sphere of small curvature have been given (Abraham-Shrauner, 1973 a, 1973 b). In addition an exact result for the potential of two parallel, plane fixed charge sheets separated by electrolyte with divalent and monovalent cations has been given (Ninham and Parsegian, 1971). The result involves Jacobian elliptic functions which are complicated to use.

Since we have not found any derivations of the potential and the counterion concentrations of the Gouy-Chapman model with divalent and monovalent cations, we present that result here. In addition some numerical results for the potential and Ca^{++} concentration near a phospholipid vesicle such as is involved in the conversion of prothrombin to thrombin are given.

The dimensionless potential Φ in electrolyte facing a fixed sheet of charge for a 2-1-1 electrolyte is

$$\Phi = \ln \left\{ (1 + 3K) \tanh^2 \left[\frac{\sqrt{1 + 3K} (X + F)}{2} \right] - K \right\} - \ln(1 + 2K) \quad (1)$$

where K is the density ratio of $Z = +2$ to $Z = +1$ ions, F is an integration constant and X is the dimensionless distance which together with Φ is defined below.

This result for the potential in eq. (1) is evident once it is derived. Nevertheless, the result is extremely useful because it is analytically simple, it is quite similar in form to the original Gouy-Chapman result for symmetrical electrolytes, it applies to a realistic case which includes calcium ions and no tedious integrations of differential equations are required. Previous approaches have included approximations on the equation for $\frac{d\Phi}{dx}$ and numerical integration of the differential equation. The analytic result is preferable for the potential if the function appears in further calculations. The analytic result for the charge sheet potential is a good first approximation near curved surfaces of radius large compared to a

Debye length and appears in the next order corrections. The analytic result is also preferable when the free energy is calculated since the potential appears in a complicated form in the free energy expression. The electrostatic free energy is useful in the analysis of calcium binding to protein molecules and phospholipids, for example.

Derivation of the Potential

We start from the nonlinear Poisson-Boltzmann equation. Our system consists of a fixed charge sheet at $x=0$ to simulate the net negative charge of the phospholipid membrane. To the right, $x>0$, is an electrolyte solution containing monovalent cations and anions and divalent cations which are alkaline earth cations. To the left of the charge sheet, $x<0$, is a uniform dielectric to simulate the hydrocarbons of the membrane interior (see Fig. 1).

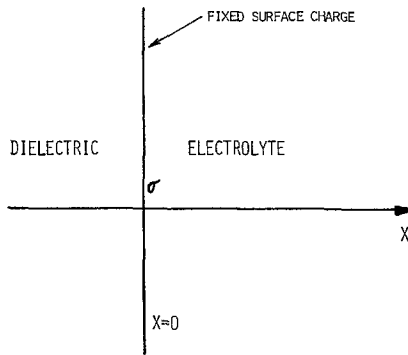


Fig. 1. Model system for phospholipid membrane facing electrolyte

We start from the Poisson-Boltzmann equation in dimensionless variables

$$\frac{d^2 \Phi}{dX^2} = -\frac{1}{2} \sum_{j=1}^N Z_j \eta_j e^{-Z_j \Phi}, \quad (2)$$

where Z_j is the charge number, the dimensionless potential Φ , the dimensionless ion density η_j and the dimensionless distance X are

$$\Phi = \frac{q \phi}{k T}, \quad \eta_j = \frac{n_j}{n}, \quad X = \frac{x}{\lambda_D} \quad (3)$$

with the Debye length λ_D given by $\lambda_D^2 = \frac{k T \epsilon}{2 q^2 n}$. Here q is the unit positive charge, ϕ is the electrostatic potential, n_j is the number density of the j -th species, n is the number density of the monovalent cation and ϵ is the permittivity. M. K. S. units are used. The boundary conditions are the usual ones that charge neutrality holds in the bulk fluid, $\sum_{j=1}^N Z_j \eta_j = 0$ as $X \rightarrow \infty$, or that the potential and electric

field (proportional to $\frac{d\Phi}{dX}$) be zero in the bulk electrolyte. The electric field vanishes for $X < 0$ and at the charge sheet $\left. \frac{d\Phi}{dX} \right|_{X=0} = -2\gamma$, $\gamma = \frac{q\sigma\lambda_D}{2\epsilon kT}$ where σ is the fixed surface charge density of the sheet.

Next eq. (2) can be integrated by the standard trick of multiplying by $\frac{d\Phi}{dX} dX$ to find the equation for $\frac{d\Phi}{dX}$. Equating $\frac{d\Phi}{dX}$ to -2γ at the charge sheet gives the Graham equation which is written later on for the 2-1-1 electrolyte. The second integration results in X in terms of an integral over Φ .

$$X + D = \int \frac{d\Phi}{\left[\sum_{j=1}^N \eta_j (e^{-Z_j\Phi} - 1) \right]^{\frac{1}{2}}} \quad (4)$$

where D is an integration constant.

To integrate the above integral in eq. (4) we can factor the square root term. This had been shown previously for 1-1 electrolyses, 2-1 electrolytes (Abraham-Shrauner, 1973 b) and we first showed it for 2-1-1 electrolytes by trial and error. However, a general proof exists for all Z_j for this *geometry* that considerably simplifies this integration. Hence simpler representation of the potential can be found for the $Z = \pm 1$, ± 2 case or even with one value of $Z_j = 3$ than might have been expected.

One can show that the term in the square root can be written as

$$\sum_{j=1}^N \eta_j (y^{Z_j} - 1) = (y-1)^2 f(y) \quad (5)$$

where $y = e^{-\Phi}$ and $f(y)$ is a polynomial (some powers of y may be negative). To show eq. (5) holds one first notes that $(y-1)$ must be a factor of each term in the sum over j in eq. (5) from elementary algebra. Next assume eq. (5) is correct and differentiate. The resultant terms on the right hand side of the equation contain at least one factor of $y-1$ or the right hand side vanishes at $y=1$ if $(y-1)^2$ is a factor. However, at $y=1$, $\Phi=0$, $\sum_{j=1}^N \eta_j Z_j = 0$ by charge neutrality or the left hand side of the differentiated equation must vanish. Hence $(y-1)^2$ is a factor.

For a 1-1 electrolyte the entire term factors in the square root and the two forms of the potential are found.

$$\begin{aligned} \Phi &= -4 \tanh^{-1} (e^{-X} \tanh M_{eff}) \\ &= 2 \ln \tanh \left(\frac{X}{2} + \tanh^{-1} e^{-2M_{eff}} \right). \end{aligned} \quad (6)$$

for $M_{eff} = \frac{1}{2} \sinh^{-1}(-\gamma) = \frac{-\Phi(0)}{4}$. The first form is the usual Gouy-Chapman potential but the second form which is equivalent is included for comparison with the 2-1-1 electrolyte result.

We integrate eq. (4) for a 2-1-1 electrolyte by letting $Z_1 = -1$, $Z_2 = 1$, $Z_3 = 2$, $\eta_2 = 1$, $\eta_3 = K$, and $\eta_1 = 1 + 2K$ where the last follows by charge neutrality. The integral was done by bringing the $(e^{-\Phi} - 1)^2$ factor outside the square root, letting $W = 1 - e^{\Phi}$ and expanding the integral in partial fractions.

Our result for the potential is then eq. (1) above. The potential for the 2-1-1 electrolyte given by eq. (1) is very similar to the second form of eq. (6) for the 1-1 electrolyte and in the limit of no divalent ions ($K \rightarrow 0$) the two are the same. The integration constant F may be determined from

$$F = \frac{2}{\sqrt{1+3K}} \tanh^{-1} Z \quad (7)$$

where Z is a solution of the cubic equation

$$\sqrt{1+3K} Z^3 - 2\gamma Z^2 + \sqrt{1+3K} Z + \frac{2\gamma K}{1+3K} = 0$$

or

$$Z = \left[\frac{K + (1+2K)e^{\Phi(0)}}{1+3K} \right]^{\frac{1}{2}}$$

If we substitute Z into the equation containing 2γ we find Graham's equation

$$2\gamma = -(e^{-\Phi(0)} - 1) [K + (1+2K)e^{\Phi(0)}]^{\frac{1}{2}} \quad (8)$$

We can check the large increase in divalent cation concentration near a highly charged surface for model calculations for the calcium ion concentration $[Ca^{++}(X)]$

$$[Ca^{++}(X)] = [Ca^{++}(\infty)] e^{-2\Phi(X)}$$

near a phospholipid vesicle surface. The following parameters were used, $T = 23^\circ \text{C}$, $q = 4.80 \times 10^{-10}$ esu, $k = 1.38 \times 10^{-16}$ ergs/ $^\circ \text{K}$, $\epsilon_R = 79.1$, Avogadro's number $A = 6.022 \times 10^{23}$ particles/mole, area per charge group $A = 99 \text{ \AA}^2$, bulk concentration of +1 cations (mostly Na^+) = .128 M. The Debye length was found to be $8.51 \text{ \AA} \left(\lambda_D^2 = \frac{\epsilon_R k T}{8 \pi n q^2} \text{ in cgs units} \right)$, and $2\gamma = -7.70$. For the bulk calcium concentration $[Ca^{++}(\infty)] = 2 \times 10^{-3} \text{ M}$, $K = 1.5625 \times 10^{-2}$ and for $[Ca^{++}(\infty)] = 4 \times 10^{-5} \text{ M}$, $K = 3.125 \times 10^{-4}$.

The tables for the extremes of bulk calcium concentration given above give the potential in thermal potentials (1 thermal potential = 25.5 mV here) and the molar strength of calcium $[Ca^{++}(X)]$ as a function of distance from the fixed charge sheet in Debye lengths. The Gouy-Chapman potential $\Phi_G(X)$ for zero calcium concentration in a 1-1 electrolyte is given for purposes of comparison.

The parameters chosen above were used since they apply to experiments in the conversion of prothrombin to thrombin in the presence of phospholipid vesicles, Ca^{++} and activated factor X [Jackson, private communication]. The binding of Ca^{++} to the phospholipids was not considered. The effect of binding calcium to the vesicle surface is to reduce the fixed charge density or increase the area per charged molecule. The present relations for the potential and ion concentrations can always be applied for the correct surface charge density. Hence the results in the tables are correct only if the actual areas per charged molecules are the ones assumed.

Table 1. *Potential and Calcium Concentrations as a Function of Distance from Charge Sheet for 2-1-1 Electrolyte and Gouy-Chapman Potential. $[Ca^{++}(\infty)] = 4 \times 10^{-5} M$*

X	$\Phi(X)$	$\Phi_G(X)$	$[Ca^{++}(X)]$
0	-4.04	-5.48	$1.28 \times 10^{-1} M$
.1	-3.41	-4.34	3.65×10^{-2}
.2	-2.94	-3.62	1.43×10^{-2}
.3	-2.57	-3.11	6.80×10^{-3}
.4	-2.26	-2.70	3.70×10^{-3}
.5	-2.01	-2.38	2.21×10^{-3}
1.0	-1.15	-1.34	4.03×10^{-4}
1.5	-6.88×10^{-1}	-7.94×10^{-1}	1.58×10^{-4}
2.5	-2.51×10^{-1}	-2.89×10^{-1}	6.61×10^{-5}
4.0	-5.59×10^{-2}	-6.44×10^{-2}	4.47×10^{-5}
5.0	-2.05×10^{-2}	-2.37×10^{-2}	4.17×10^{-5}

Table 2. *Potential and Calcium Concentration as a Function of Distance from Charge Sheet for 2-1-1 Electrolyte and Gouy-Chapman Potential. $[Ca^{++}(\infty)] = 2 \times 10^{-3} M$*

X	$\Phi(X)$	$\Phi_G(X)$	$[Ca^{++}(X)]$
0	-3.55	-5.48	2.29 M
.1	-2.95	-4.34	7.25×10^{-1}
.2	-2.53	-3.62	3.17×10^{-1}
.3	-2.21	-3.11	1.66×10^{-1}
.4	-1.94	-2.70	9.75×10^{-2}
.5	-1.72	-2.38	6.25×10^{-2}
1.0	-9.81×10^{-1}	-1.34	1.42×10^{-2}
1.5	-5.78×10^{-1}	-7.94×10^{-1}	6.36×10^{-3}
2.5	-2.06×10^{-1}	-2.89×10^{-1}	3.02×10^{-3}
4.0	-4.43×10^{-2}	-6.44×10^{-2}	2.19×10^{-3}
5.0	-1.59×10^{-2}	-2.37×10^{-2}	2.06×10^{-3}

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Dr. Barbara Abraham-Shrauner
Department of Electrical Engineering
Washington University
St. Louis, MO 63130, U.S.A.