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Article

Amphiphilic Planar Membranes in Ionic Equilibrium: a Study of pH Position-Dependent Values

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Os valores de pH na vizinhança de uma membrana ionizável são investigados pelo emprego da equação de Poisson-Boltzmann e de sua forma linearizada. Tais equações são resolvidas para uma simetria plana com a consideração de que a densidade de carga da superfície da membrana resulta de um processo de equilíbrio com a solução eletrolítica. Os resultados para o grau de ionização são apresentados em função da força iônica e da densidade superficial de carga da membrana. Nossos cálculos indicam que os valores de pH possuem uma apreciável variação dentro de uma distância de 2 nm a partir da membrana. É também apresentado que, uma membrana ionizável, na presença de um eletrólito com íons bivalentes, tem seu processo de ionização aumentado pela presença de tais íons. Em tal tipo de eletrólito, os valores de pH são mais estáveis do que em um eletrólito contendo apenas íons monovalentes.

The pH values near a planar dissociating membrane are studied under a mean field approximation using the Poisson-Boltzmann equation and its linear form. The equations are solved in planar symmetry with the consideration that the charge density on the dissociating membrane surface results from an equilibrium process with the neighboring electrolyte. Results for the membrane dissociation degree are presented as a function of the electrolyte ionic strength and membrane surface charge density. Our calculations indicate that pH values have an appreciable variation within 2 nm from the membrane. It is shown that the dissociation process is enhanced due to the presence of bivalent ions and that pH values acquire better stability than in an electrolyte containing univalent ions.

Keywords: pH control, ionic concentration, amphiphilic, Poisson-Boltzmann equation

Introduction

The pH value near charged dissociating surfaces plays important roles in the interactions between them,[1] in the adsorption of proteins to these surfaces,[2] and in a great number of biological process, such as permeation across a lipid bilayer membrane[3,4] and protein charge inversion.[5] It is also well known that H^+ gradients have an essential role in the energy production process in mitochondria.[6,7] A quantitative description of such effects requires the study of an inhomogeneous system comprising many interacting particles, such as ions in solution and charges due to the membranes' ionizable groups.[8] Such a description can be performed using concepts of Statistical Mechanics, as shown by Carnie and Torrie[9] for electrical double layers. Cevc[10] and Andelman[11] presented various perspectives and theoretical models related to the study of charged membranes. Considering a planar stiff membrane and employing a Poisson-Boltzmann-based model, Fleck *et al.*[12] related the membrane fluidity to the DNA adsorption on a negatively charged membrane. A single charged planar surface is a realistic and mathematically simple model that is applicable in describing a great number of biological systems.[13-19] More complex models comprising two parallel planar charged surfaces are reported in the literature.[20-26]

To analyze the physicochemical properties of a planar dissociating membrane, we develop a theoretical model

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based on the hypothesis of thermodynamic equilibrium in a mean field approximation.[27–29] To describe the ionic distribution near a charged membrane we utilize both the Poisson-Boltzmann equation and its linear form, denoted by LPB equation. Our model defines a role for bivalent ions in solution and allow us to determine the pH value from different distances from the membrane.

Mathematical Model

A planar dissociating membrane, immersed in a medium containing various ionic species, has an effective surface charge density resulting from the equilibrium between its constituent dissociating molecules and the solution. This density value can be inferred using the thermodynamic equilibrium condition, which requires the equality of the chemical potential value everywhere within the system. Symbolically, this condition is represented as:

$$\mu_i = \mu_{0i} + kT\ln\left(a_i\right) = \mu_{0i} + kT\ln\left(n_i\left(\vec{r}\right)\right) + kT\ln\left(\gamma\left(\vec{r}\right)\right) \quad (1)$$

In equation 1, for every ionic species *i*, the symbol μ stands for the chemical potential, μ_0 represents the standard chemical potential, *a* is the activity of the ionic species, γ is the local activity coefficient, $n_i\left(\vec{r}\right)$ is the local ionic concentration and *z* is the valence of the ion. The symbol *e* denotes the electron charge magnitude and *kT* is the Boltzmann constant times the absolute temperature.

The electrostatic interactions of an ion with others dispersed in solution may be evaluated by the mean electrostatic potential, $\psi\left(\vec{r}\right)$. The ionic concentrations are derived from equation 1 as described by Drigo Filho *et al.*[16] and Hill[30]:

$$n_i\left(\vec{r}\right) = n_{i0}\exp\left(-\frac{z_i e\psi\left(\vec{r}\right)}{kT}\right) \quad (2)$$

where n_0 is the number density unit in the International Unit System, the Avogadro's number of particles N_A *per* cubic meter.

For equation 2 be valid, the number of ions must be small compared with the number of solvent molecules within the solution, or in other words, the ionic number density value must be much less than the solvent number density. If the ionic concentration is much less than the solvent concentration, then, as a first approximation, the solvent concentration in solution can be considered constant everywhere within the solution and equation 2 gives the relation for the ionic number density, which will be a position-dependent parameter. In equation 2 the exponential function depends on a continuous parameter specifying a point within the system. In planar symmetry, only a parameter to determine a plane parallel to the plane chosen as the origin is required, which for the studied system is the "plane" from which the ions are driven by the mean field.[26] For a biological membrane, some *conjectures* are made corresponding to the inner region, i.e., a dissociable group with its hydration layers is a site for counter ion binding. The counter ion is bounded by a hydrogen bond. A bivalent counter ion binding in the inner region enhances the proton dissociation because of the positive net charge of the complex bivalent ion, dissociable group, and hydration layers. In the following, the "plane" origin will sometimes be called the membrane surface. Agostinho-Neto *et al.*,[28] studying ion dissociation, concluded that the hydrated ion size, comprising the ion itself and hydration layers, depends on the interactions and specifically, on the ionic concentration. Near the membrane, under the conditions considered in this work, a "minimum area" of 0.5 nm^2 is available to each bounded hydrated ion. The overcrowding of the bounded ions in the membrane is improbable whenever the hydrated ion diameter is of the order of 0.7 nm, and the steric effects analyzed in previous studies[17,18] do not interfere in the conditions prevailing here.

Using equation 2, the relation between the Laplace operator of the electrostatic potential and the charge density can be written as:

$$\nabla^2\psi\left(\vec{r}\right) = -\frac{\rho\left(\vec{r}\right)}{\varepsilon} = -\frac{e}{\varepsilon}\sum z_i n_{i0}\exp\left(-\frac{z_i e\psi\left(\vec{r}\right)}{kT}\right) \quad (3)$$

Equation 3 is known as the Poisson-Boltzmann equation and ε represents the electrical permittivity of the medium. The analysis of the system is performed considering that, as an approximation, the medium dielectric constant value is not a position-dependent parameter. Defining the reduced potential as $\phi = e\psi\left(\vec{r}\right)/kT$ and measuring the distance from the membrane in terms of the length parameter $\lambda = \left(\varepsilon kT/2e^2 n_0\right)^{1/2}$, for a system with planar symmetry, equation 3 is reduced to:

$$\frac{d^2\varphi}{d\xi^2} = -\frac{1}{2}\sum_i z_i c_i \exp\left(-z_i\varphi\right) \quad (4)$$

where $\xi = x/\lambda$, *x* is the coordinate, and $c_i = n_{i0}/n_0$. Multiplying both sides of equation (4) by $\partial\phi/\partial\xi$ and integrating, a first integral is obtained:

$$\frac{1}{2}\left(\frac{d\varphi}{d\xi}\right)^2 - \frac{1}{2}\sum_i c_i \exp\left(-z_i\varphi\right) = -\frac{1}{2}\sum_i c_i \quad (5)$$

The right-hand side in equation 5 is equal to $-p/(2kTn_0N_A)$ with *p* representing the ionic contribution to the pressure value in the system. The pressure value is constant everywhere in the solution. This is a property related to the planar symmetry in equation 4 when referring to a system containing univalent and bivalent positive and negative ionic species, here denoted by PB4, which can be solved in terms of elementary functions.[14,15] Thereby, the reduced potential as a function of the distance ξ from a negatively charged surface is written as:

$$\varphi = \ln\left[b\frac{(1+a)\cosh\{c(\xi+d)\}+b}{b\cosh\{c(\xi+d)\}+(1+a)} - a \right] \quad (6)$$

Parameter d is an integration constant that depends on the normal component of the electric field at the surface, or equivalently, on the charge density value therein. The boundary condition $(d\varphi/d\xi) \to 0$, "rewritten" as $c(\xi + d) > 1$, is already incorporated in equation 6, and the reduced potential value goes to zero within the same limit. Parameter c is related to the ionic strength, expressed here in 10^{-3} mol L^{-1}. Using the neutrality condition in regions far from the membrane, $\sum_i z_i c_i = 0$, parameter c can be written as $c = (3c_{-2} + c_{-1} + c_{+2})^{½} = (3c_{+2} + c_{+1} + c_{-2})^{½}$. The meanings of the symbols a and b in equation 6 are: $a = 1 + c_{-1}/2c_{+2}$ and $b = (a^2 - c_{+2}/c_{-2})^{½}$.

The integration constant value d, in terms of the parameters above and the reduced potential value at the surface, is given by:

$$d = \frac{1}{c}\cosh^{-1}\left\{ \frac{(e^{\varphi(0)}+a)(1+a)-b^2}{b(1-e^{\varphi(0)})} \right\} \quad (7)$$

To determine the value of d, it is necessary to know the reduced potential value at the surface and the concentration values in the bulk.

In our model, the membrane is composed, in part, by amphiphilic dissociating molecules such as cardiolipin or phosphatidylserine. In nature, the amount of dissociating molecules contained in the membrane depends on the membrane kind. For example, cardiolipn might represent 20% of the mitochondrial membrane total composition.[31,32] Here, the fraction of dissociated molecules in the membrane may be inferred under the following considerations: (*i*) the local charge rearrangement of the dissociating groups depends on the local microscopic electric field; and (*ii*) it is expected that the probability of dissociation does not depend on the state of movement of the molecule.

Under a mean field description, where the electric potential plays the role of the potential of mean force for the hydrated ions, the dissociation constant value is not influenced by the mean electric potential value; thus, by equation 2, the electric potential dependence is canceled. The same is true for any dissociating molecule whose products are ions; in particular, the water ionic product does not depend on the mean field value. This particular property of the mean field approach makes it a suitable methodology in the description of hydrogen ionic concentration, because the solvent (water) has dissociative properties that cannot be neglected.

The proton concentration near the membrane is given by $n_{0p}\exp(-\phi(\xi = 0))$, where n_{0p} is the proton concentration far from the membrane, which is a concentration related to the bulk pH value. In terms of the dissociation constant and the reduced potential value at the membrane, the dissociation degree α can be written as:

$$\alpha = \frac{1}{1+\frac{n_{0p}e^{-\varphi(\xi=0)}}{K}} \quad (8)$$

The surface charge density value σ comes in the form:

$$\sigma = -\frac{\alpha\, e}{A} \quad (9)$$

where A represents the area *per* dissociating molecule. The boundary condition is:

$$\left.\frac{d\varphi}{d\xi}\right|_{\xi=0} = -\frac{\sigma\, e\,\lambda}{\varepsilon\, k\, T} = \frac{\alpha\, e^2\,\lambda}{\varepsilon\, k\, T\, A} \quad (10)$$

The value of the reduced potential derivative at $\xi = 0$, as a function of the reduced potential value at the surface, can be obtained from the combination of equations 8-10. For a negatively charged membrane, the reduced potential is a monotonically increasing function of parameter ξ, written as:

$$\left.\frac{d\varphi}{d\xi}\right|_{\xi=0} = (e^{-\varphi(0)}-1)\,[c_{+2}+(c_{-1}+2c_{+2})\; e^{\varphi(0)}+c_{-2}\; e^{2\varphi(0)}]^{1/2} \quad (11)$$

Equations 10 and 11 lead to the relation:

$$\frac{\alpha\, e^2\,\lambda}{\varepsilon\, kTA} = (e^{-\varphi(0)}-1)\,[c_{+2}+(c_{-1}+2c_{+2})\; e^{\varphi(0)}+c_{-2}\; e^{2\varphi(0)}]^{1/2} \quad (12)$$

which when combined with equation 10 results in a transcendental equation for the reduced potential value at the membrane surface, given by:

$$e^{\varphi(0)} = \left\{ \frac{A\varepsilon kT}{e^2\,\lambda}\left(e^{\varphi(0)}+\frac{n_{0p}}{K}\right)(1-e^{\varphi(0)})\left[c_{+2}+(c_{+1}+2c_{+2})e^{\varphi(0)}+c_{-2}\; e^{2\varphi(0)}\right]^{1/2} \right\}^{1/2} \quad (13)$$

The parameter n_{0p} is the hydronium concentration in the bulk and it is added to the total monovalent positive ionic concentration c_{+1}. Determining the reduced potential function, it allows the calculation of ionic species concentration values everywhere within the solution. The pH values as a function of distance ξ from the membrane, is given by the equation:

$$pH(\xi) = pH + \log_{10}(e) \times \varphi(\xi) \tag{14}$$

The reduced potential function for univalent ions electrolyte, obtained from equation 6, is written as:

$$\varphi(\xi) = \ln\left\{ \tanh^2\left[\left(\frac{c_1}{4}\right)^{1/2} (\xi + d) \right] \right\} \tag{15}$$

where c_1 is the concentration of the univalent ions, and equation 7 provides:

$$d = \frac{1}{c_1^{1/2}} \cosh^{-1}\left(\frac{1 + e^{\varphi(0)}}{1 - e^{\varphi(0)}} \right) \tag{16}$$

We analyze this system solving two coupled transcendental equations. One of them, equation 17, results from the boundary condition, equation 10, and the derivative of the reduced potential function from equation 15, calculated with the value $\xi = 0$.

$$\sinh[cd] = -\frac{1}{\alpha}\left(\frac{2c\,\varepsilon\,kT A}{e^2\,\lambda} \right) \tag{17}$$

The other transcendental equation is:

$$\frac{1}{\alpha} = \left[\frac{K(T)}{n_{0p}} + \coth^2\left(\frac{cd}{2} \right) \right] \frac{n_{0p}}{K(T)} \tag{18}$$

obtained from equation 8 using the ionic strength parameter $c^2 = c_1$.

Linear form of the Poisson-Boltzmann equation

An alternative theoretical description for the systems under consideration is the linear form of the Poisson-Boltzmann equation. The corresponding equation is usually derived with the assumption that the reduced potential value is much less than one. Within this approximation, the reduced potential is given by:

$$\varphi(\xi) = \frac{\sigma e \lambda}{c \varepsilon k T} \exp(-c\xi) \tag{19}$$

The exponential function behavior for the reduced potential, as shown in equation 19, can be obtained from equations 6 or 15 by writing the hyperbolic functions in terms of exponentials and giving to the argument of the *ln* function the form of a sum of 1 plus the exponential terms, which allows the approximation $\ln(1 + u) \approx u$. The linear form approach leads to:

$$\left.\frac{d\varphi}{d\xi}\right|_{\xi=0} = -c\varphi(0) \tag{20}$$

where the reduced potential value on the surface is given by:

$$\varphi(0) = -\frac{\alpha}{2c\lambda A N_A} \tag{21}$$

Carrying the value above into equation 8, the equation for the dissociation degree α in the linear approximation becomes a transcendental equation in relation to α:

$$\alpha = \frac{1}{1 + \dfrac{n_{0p}}{K(T)} \exp\left(\dfrac{\alpha}{2c\,\lambda\,N_A\,A} \right)} \tag{22}$$

Results and Discussion

The main parameters referring to electrolyte composition, lipid area *per* dissociation group and membrane composition used in our calculations for dissociation degree and pH values are obtained from the literature and should, in principle, describe those concentrations found in cells. As reported by Gennis[32] and Cevc,[33] some biomembranes phospholipids head groups have areas from 0.5 to 5 nm^2; in our model these two values are utilized to represent the area *per* dissociating group when the groups are gathered together (0.5 nm^2) or somewhat uniformly spread in the membrane (5 nm^2). Ionic concentration values, “far” from the membrane are specified by the ionic strength parameter. For a system containing four ionic species, the ionic strength range is computed assuming the ionic concentration values to be in the same proportion as the specific concentration: 126 × 10^{-3} mol L^{-1} of univalent positive ions, 15.5 × 10^{-3} mol L^{-1} of bivalent positive ions, 17 × 10^{-3} mol L^{-1} of negative univalent ion, and 70 × 10^{-3} mol L^{-1} of bivalent negative ions.[34] The simple ionic intracellular concentration values are 5-15 × 10^{-3} mol L^{-1} of Na$^+$, 140 × 10^{-3} mol L^{-1} of K$^+$, 0.5 × 10^{-3} mol L^{-1} of Mg^{+2}, 10^{-7} mol L^{-1} of Ca^{+2}, 7 × 10^{-8} mol L^{-1} of H$^+$, and an anion concentration value between 5 and 15 × 10^{-3} mol L^{-1} of Cl$^-$. The total concentration of Mg^{+2} is about 20 × 10^{-3} mol L^{-1} and a value of between 1 and 2 × 10^{-3} mol L^{-1} of Ca^{+2}, mostly bound to proteins and other substances.[35] Other intracellular anions, apart from Cl$^-$, are HCO_3^- and PO_4^{-3}. The univalent and bivalent ion concentration values used in the present study have quantities of cations and anions in a proportion to produce an electrically neutral system. The dissociation of the membrane is analyzed adopting a *pK* value of 4.72 for a phosphatidylserine-like[33] molecule and a *pK* value of 2.5 for a cardiolipin-like molecule.[36]

The temperature value of the system is taken as 301.15 K, (37 ºC). The solvent (water) is considered a medium with a dielectric constant value of 74.151 in the entire volume of the solution and the water dissociating properties are described by the ionic product K_w.

Figures 1 to 5 show the dissociation degree α as function of the electrolyte ionic strength, with bulk pH equals to 7.2. The results displayed in Figures 1 and 4 are obtained using a dissociation constant value equal to 3.1623 (High α); Figures 2 and 5 are instead obtained using a dissociation constant value equal to 0.019 (Low α). In all of these five figures, "scattered" corresponds to an area value of 5 nm^2 *per* dissociating group and "compact" represents an area of 0.5 nm^2 *per* dissociating group. Figures 1 and 2 show the results for Poisson-Boltzmann equation for an electrolyte containing univalent and bivalent ions. Figure 3 displays

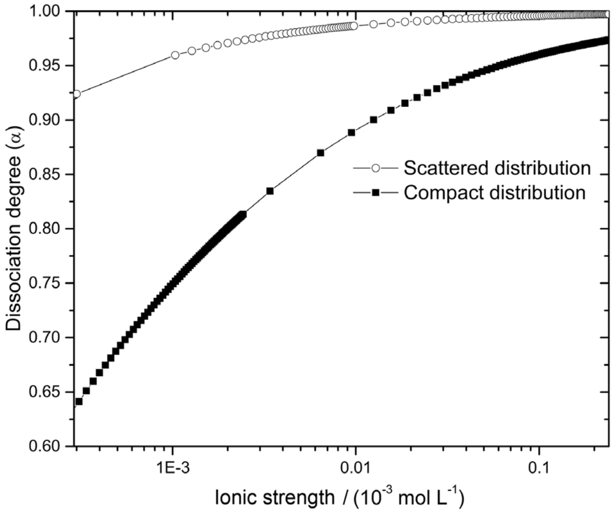

Figure 1. Dissociation degree as a function of the ionic strength for a membrane composed by dissociating groups with α = 3.1623. Scattered corresponds to an area value of 5 nm^2 *per* dissociating group and compact represents an area of 0.5 nm^2 *per* dissociating group. Results obtained by Poisson-Boltzmann in an univalent and bivalent ions containing electrolyte.

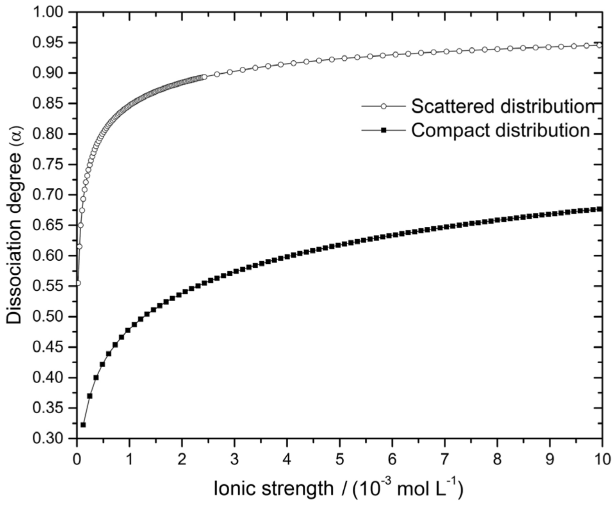

Figure 2. Dissociation degree as a function of the ionic strength for a membrane composed by dissociating groups with α = 0.019. Scattered corresponds to an area value of 5 nm^2 *per* dissociating group and compact represents an area of 0.5 nm^2 *per* dissociating group. Results obtained by Poisson-Boltzmann in an univalent and bivalent ions containing electrolyte.

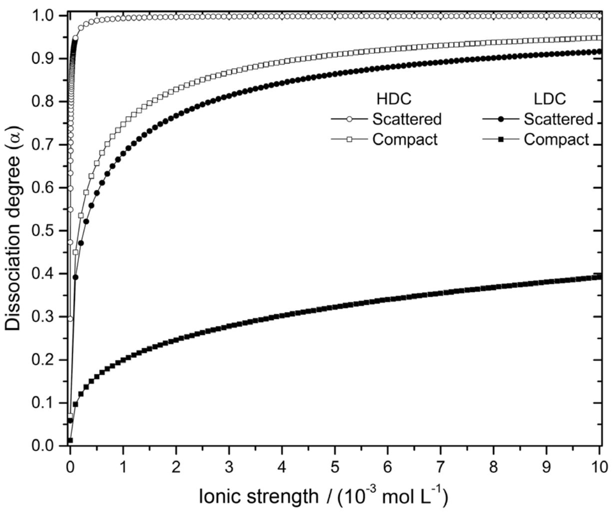

Figure 3. Dissociation degree as a function of the ionic strength for a membrane composed by dissociating groups: HDC denotes groups with α = 3.1623 (empty points) and LDC denotes points with α = 0.019 (full points). Results obtained by Poisson-Boltzmann in an univalent ions containing electrolyte.

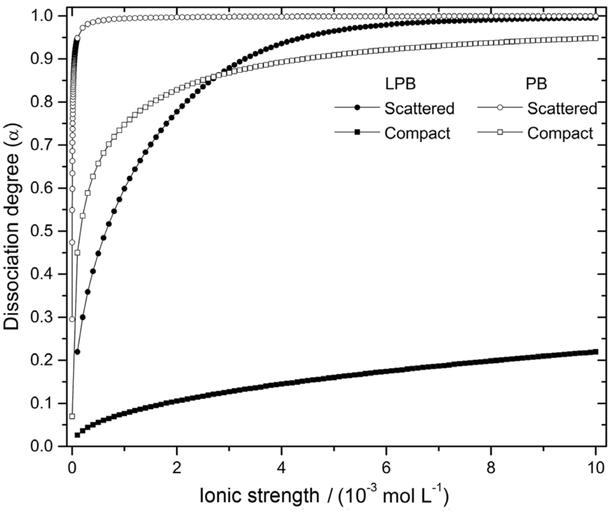

Figure 4. Dissociation degree as a function of the ionic strength for a membrane composed by dissociating groups with α = 3.1623. Scattered corresponds to an area value of 5 nm^2 *per* dissociating group and compact represents an area of 0.5 nm^2 *per* dissociating group. PB denotes results obtained by the Poisson-Boltzmann equation (empty points) and LPB denotes results obtained by its linear form (full points). The were calculations done in univalent and bivalent ions containing electrolyte.

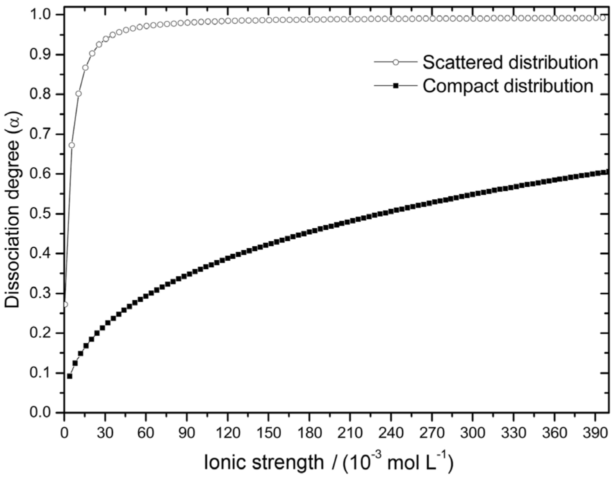

Figure 5. Dissociation degree as a function of the ionic strength for a membrane composed by dissociating groups with α = 0.019. Scattered corresponds to an area value of 5 nm^2 *per* dissociating group and compact represents an area of 0.5 nm^2 *per* dissociating group. Results obtained by the linear form of Poisson-Boltzmann equation in an univalent ions containing electrolyte.

the results for the PB equation analyzing membranes composed by dissociating groups with high and low valued dissociation constant in a univalent ions electrolyte.

To compare the results obtained by the solution of PB equation and by its linear form (LPB), Figures 4 and 5 show the dissociation degree as a function of the ionic strength for scattered and compact distributions of dissociating groups. Comparing the two approaches, the PB equation and LPB for a system in equal conditions, LPB gives a lower value of dissociation degree than the results obtained with the "full" PB description. Figure 5 shows the results obtained by LPB and demonstrate the effect of area *per* dissociating group in the dissociation degree: the dispersed (scattered) dissociating groups always exhibit a higher value of α than the gathered groups (compact). For low values of ionic strength, the value of dissociation degree shows great variation and an appreciable dependence on the other parameters considered. The membrane with a high valued dissociation constant and dispersed/scattered dissociating groups, shows an almost complete dissociation degree in an ionic strength of less than 1 × 10^{-3} mol L^{-1}. Comparing the results shown in Figures 1 and 2 with the remaining figures, it can be concluded that the presence of the bivalent ionic species enhances the dissociating process.

Figure 4 also shows that both approaches, PB and LPB, give equivalent results for a membrane with dispersed dissociating groups in an electrolyte with an ionic strength value greater than 10 × 10^{-3} mol L^{-1}. Another fact that can be observed is that a higher valued dissociation constant leads to a higher value of the dissociation degree. Figures 6 and 7 display pH values as a function of the distance from the membrane in a 242.5 × 10^{-3} mol L^{-1} ionic strength electrolyte. This calculation was performed using the PB equation and the composition of the electrolyte considered for describing a system with four ionic species is presented in Table 1. An ionic concentration of 242.5 × 10^{-3} mol L^{-1} is

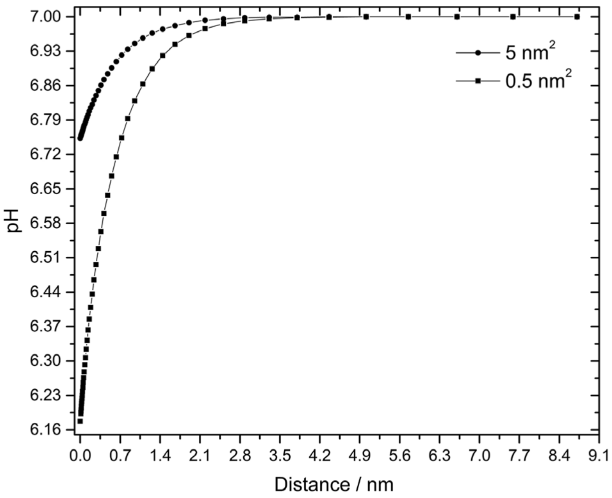

Figure 6. pH values as function of the distance from a membrane in a high valued ionic strength electrolyte for two distinct area *per* dissociating group: 0.5 nm^2 and 5 nm^2.

Table 1. Ionic composition of a system with four ionic species[34,35]

Ion kind	Ionic concentration / (10^{-3} mol L^{-1})
Univalent positive	126
Bivalent positive	15.5
Univalent negative	17
Bivalent negative	70
Univalent ions as the unique species	242.5

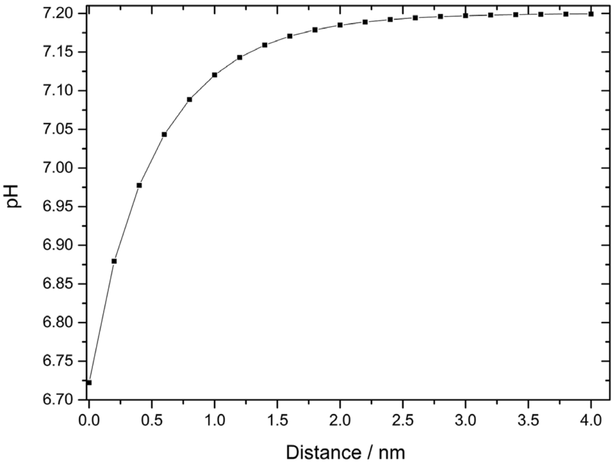

Figure 7. pH values as function of the distance from a membrane in a high valued "ionic strength" electrolyte. The dissociating groups are in a scattered distribution on the membrane (5 nm^2 *per* dissociating group).

used when the electrolyte is treated as containing univalent ions as the unique species.

From Figure 6, it can be concluded that appreciable variations from the bulk pH value to those near the membrane, occur in a region within 2.0 nm of the membrane. Figure 7 shows the results considering a membrane with dispersed dissociating groups. The pH values for a high ionic strength electrolyte are independent of the other parameter values, mainly the value of the membrane dissociation constant and all the model equations give practically the same results. The major difference for the calculated pH values is less than 0.3% at the membrane surface. The dissociation degree values are 0.999 for the groups with the higher valued dissociation constant and 0.990 for the lower valued ones. It can be verified using the equations presented in the model section that the reduced potential values at the membrane (i.e., in $\xi = 0$) are around –1.1.

It is concluded that in a system with a high ionic strength and with the dissociating molecules scattered in the membrane, the pH value are stable.

Considering the situation of gathered dissociating groups in the membrane, the calculated values in a PB4 electrolyte are shown in Figure 8. For high and low valued dissociating constants the results are practically the same, and the difference between calculated pH values and bulk pH is ca. 1.7 units, which means that in the membrane the hydronium ions concentration is forty times greater than in the bulk.

Figure 9 shows the results for PB and LPB equations describing an electrolyte composed by univalent ions bathing a membrane with dissociating groups gathered together. In this figure, it is noticeable that while PB produces points that are almost coincident for two dissociation constant values, LPB gives appreciably different pH values, mainly near the membrane.

Using gathered dissociating groups for High α and Low α, we obtain values for the dissociation degrees in three electrolytes: *i*) for PB4 electrolyte, 0.999 and 0.894; *ii*) for the same electrolyte and using LPB, 0.997 and 0.790; *iii*) for an electrolyte containing univalent positive and negative ions, 0.828 and 0.508. The reduced potential values near the membrane, resulting from the PB equation, are in the interval –3.6 to –4.8. These values are outside the range in which LPB is a good approximation, and this fact indicates that LPB is not appropriate to describe the dissociation process of compact structures of dissociating molecules.

To verify the consistency of the bivalent ion concentration values obtained using the PB equation, some results are shown in the Table 2. The groups dissociating constant α was equal to 3.1623.

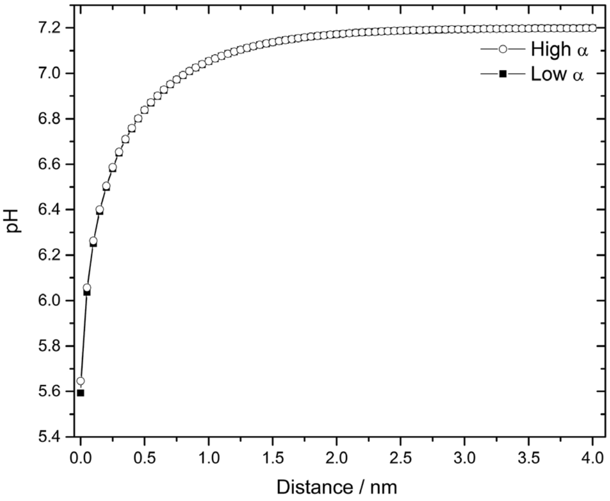

Figure 8. pH values as function of the distance from a membrane in a high valued "ionic strength" electrolyte. The dissociating groups are in a compact distribution on the membrane (0.5 nm^2 *per* dissociating groups). High α = 3.1623 and Low α = 0.019.

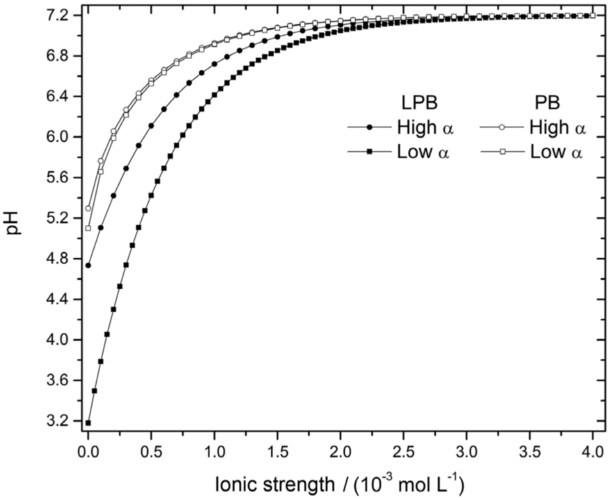

Figure 9. pH values as function of the ionic strength. PB denotes the Poisson-Boltzmann equation and LPB denotes its linear form. High α = 3.1623 and Low α = 0.019.

At a distance of 1 nm from the membrane, for the area *per* dissociating group and ionic strength equal to 5 nm^2 and 2.425 × 10^{-3} mol L^{-1}, we obtain a reduced potential of –0.18294 and 22 × 10^{-3} mol L^{-1} for the concentration. The pH value at the abovementioned distance is 7.12, which is slightly different to the bulk pH value of 7.2.

The value of 29 mol L^{-1} is completely outside the range in which the PB equation may be used to describe the system. High concentration values are possible for elements that may be considered completely dissociated in the bulk, near the membrane, because hydrated ions are not rigid spheres. Under such conditions, the hydrated ion loses part of its hydrating molecules. The radius of a hydrated ion in a concentration of 29 mol L^{-1} is about 0.2 nm, meaning

Table 2. Area *per* dissociating groups, ionic strength, reduced potential, distance from the membrane and bivalent ions concentration (abbreviated as B.I. Concentration)

Area *per* dissociating groups / nm^2	Ionic strength / (10^{-3} mol L^{-1})	Reduced potential	Distance from the membrane / nm	B.I Concentration / (10^{-3} mol L^{-1})
0.5	245.5	−3.70	0.0	25.0
0.5	245.5	−1.02	0.4	119
0.5	24.55	−10.04	1.35	11.6
0.5	2.455	−6.07	0.0	29.0
0.5	2.455	−1.08	4.0	1.30
5	242.5	−1.10	0.0	140
5	2.425	−3.70	0.0	253
5	2.425	−2.185	1.0	12

that the number of solvent molecules of the hydration layer, attributed to the ion, reaches lower values as the bivalent ions approach the membrane. There is a transition region between the physical membrane and the plane from where the ions are driven by the mean field potential. The hypothesis used to deduce the mean field is not applicable in this transition region, due to processes involving the dissociation of the complex formed by the ions (bivalent or univalent in the bulk), and the water molecules in the hydration layer. In this way, bivalent cations may form complexes with valences +2, +1 or 0 by the unbinding of protons from the hydration molecules. One also realizes that near the membrane, the concentration of the bivalent and univalent hydrated ions will have lower values.

The criterion used in the development of the model requires that the local and total ionic concentration must be lower than 550×10^{-3} mol L^{-1}. To obtain the results presented in this work, the hypothesis adopted is that the ions are considered completely dissociated and as having a stable structure of hydration layers. The hypothesis is not fulfilled by ions in regions where the concentration values are high. The hydrated ion radius value corresponding to the concentration value of 140×10^{-3} mol L^{-1} is about 1.1 nm. In these conditions, the complex dissociation is not expected to occur.

These results show that the amount of ions that may be considered bivalent ions in the bulk, and that may bind in the membrane, depends mainly on the density of the dissociable groups in the membrane surface and on the relative concentration of bivalent ions. This effect may be related to cellular processes involving, locally, contraction and expansion. The binding of bivalent ions occurs in a contracted state of the structure, and in an expansion produced by an additional pressure on the surface resulting from this binding, the unbinding of part of the ions follows.

Furthermore, for a membrane with dissociable groups and showing elastic properties, fewer bivalent counter ions will bind if the membrane is in its expanded state. The dissociation of the ionic complex is important for bivalent and univalent ions in high concentration values near the membrane; however, the dissociation property of the solvent (water) is manifested only when the ionic concentration values are very low, i.e., ca. 10^{-7} mol L^{-1}.

As a final note in the discussion, the value of 1.7 in the difference between the pH value near the membrane and in the bulk may be considered an upper limit for planar membranes; lower pH values require non-planar geometries.

Conclusion

The analysis of the inhomogeneous hydronium ion distribution near charged membranes using a mean field approximation and the Poisson-Boltzmann equation, or it's linear form, leads to equivalent results when the electrolyte ionic strength is greater than 200×10^{-3} mol L^{-1}, and the charge density value in the membrane has a small value as one electronic charge over an area of 5 nm^2. Under these circumstances, the reduced potential value at the membrane is about −1.1, which implies that the mean value of the electrostatic interaction energy is around −1.1 kT. The descriptions of the system using the PB and LPB equations do not agree when high values for the surface charge density are considered, for instance, a charge density value corresponding to one electronic charge in an area 0.5 nm^2. In this case, the reduced potential value is within the range of −5 to −3 and the LPB equation is not a good approximation.

Two cases of electrolyte composition were studied: *i*) an electrolyte with positive and negative bivalent ion and univalent ions; *ii*) an electrolyte with positive and negative univalent ions. The presence of bivalent ions leads to an enhancement of the membrane dissociation process and to a higher stability in the hydronium ion concentration values around the membrane.

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