Biophysical Chemistry 24 (1986) 33–39
Elsevier

BPC 01051

CALCULATION OF IONIC FLOCCULATION CONCENTRATIONS FOR BIOLOGICAL MEMBRANES BEARING IONIZABLE GROUPS

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Received 1st October 1985
Revised manuscript received 20th January 1986
Accepted 18th February 1986

Key words: Flocculation; Charge-regulated membrane; DLVO theory

The flocculation concentrations in the DLVO theory of colloid stability have previously been calculated under conditions of constant plate potential or constant plate charge. These boundary conditions are not appropriate in the case of biological membranes bearing ionizable surface groups. In this case the surfaces undergo charge regulation as they approach each other and both the surface charge and potential vary. In this paper a numerical method is used to calculate the ionic flocculation concentrations for the two membranes undergoing surface charge regulation. It is shown that the flocculation concentration lies between that for constant charge and that for constant potential. Flocculation concentrations are calculated as a function of the surface density of ionizable groups and are shown to be dependent on both the pK_a of the surface groups and the bulk pH of the solution.

1. Introduction

There are many examples of biological structures whose equilibrium can be explained as being due to a balance of attractive and repulsive forces between constituent parts of the structure.

A number of theoretical analyses have been carried out which explain the formation of these structures as due to the opposing effects of long-range attractive van der Waals forces and long-range repulsive electrostatic forces due to the presence of like charges on the membrane surface. Parsegian and Gingell [1] have computed the balance of long-range forces as it may occur between the membrane layers surrounding biological cells. They concluded that under physiological conditions, energy minima in the total potential energy versus distance curve can easily be deep enough to hold the two cells together in close proximity. In an earlier investigation [2], an analysis was made of the van der Waals attractive forces and electrostatic repulsive forces between the grana thylakoid membranes of chloroplasts. These forces were determined for negatively charged surfaces with a specified pK_a and bulk pH, and a range of mono- and divalent cation concentrations. It was concluded that the formation of regularly spaced membrane arrays could be explained as being due to the balance of attractive and repulsive forces, once the specific binding of divalent cations to the membrane surface was taken into account.

Having explained the formation of these structures in terms of Derjaguin-Landau-Verwey-Overbeek theory, it is of interest to investigate the effect of varying the solution ionic strength on the stability of the structure. Such a membrane system or a suspension of biological cells is not thermodynamically stable. The total free energy of a dispersed system can be always be lowered by reduction in the interfacial area. This reduction in area can occur by coalescence. The high degree of stability which is frequently observed in such systems is a

kinetic phenomenon in that the rate of coagulation may be practically zero. Reduction in the rate of coagulation can be due to the formation of an electrical double layer at the fluid-surface interface. When two surfaces approach one another, their double layers interact, giving rise to repulsive forces which oppose coagulation. Increasing the ionic strength of the solution reduces the extent of the double layers and thus enhances coagulation. In this work, a system is called stable if it has not changed from a state of slow to fast coagulation. The ionic concentration at which this transition occurs is known as the flocculation concentration. The DLVO theory of colloid stability can be used to investigate the stability of biological membrane structures. Previous applications of this theory to calculate the stability of lyophobic colloids have assumed constant charge or constant potential boundary conditions, [3,12]. The constant potential boundary condition assumes that the potential at the boundary of the diffuse charged layer adjacent to the membrane is independent of the distance between the membranes. This condition can be physically justified as follows. It is assumed that the surface potential arises due to the adsorption of ions of species i, 'potential determining ions', onto the surface. For ions of species i adsorbed onto a surface and immersed in bulk solution the equilibrium relation can be written $\Delta\mu_i + e_i\psi = 0$, where e_i is the ionic charge, ψ the boundary potential, and $\Delta\mu_i$ the difference between the part of the chemical potential of an adsorbed ion which is not dependent on ψ and the chemical potential of a bulk ion. The constant potential condition thus implies that $\Delta\mu_i$ is independent of the surface density of adsorbed ions [9].

In the case of colloidal particles, Frens [14] has suggested that the equilibrium between adsorbed ions and bulk solution cannot be maintained during rapid changes in particle separation due to Brownian motion. Frens thus proposed that the constant potential condition should be replaced by that of constant surface charge density.

In the case of biological membranes, equilibrium should have time to be established because these structures are far larger than colloidal particles, and hence move more slowly. It has been suggested [8] that neither constant charge nor constant potential boundary conditions should be applied in the case of biological membranes. Rather than the assignment of a fixed surface charge density or surface potential as a basis for calculation of the electrostatic field, the important parameter is the density of ionizable groups on the surface. The corresponding surface charge density due to dissociation must then be determined as a self-consistent functional of the potential. As the membranes approach each other, the fraction of dissociated groups, and hence the surface charge density changes. This is known as surface charge regulation.

This mechanism of the generation of charge on the surface of biological membranes has been experimentally verified in the case of plant thylakoid membranes [16]. As far as is known no experimental investigation has been made of the stability of biological membrane structures with which the following theory can be verified. However, since it is known by experiment that biological membranes obey this third type of boundary condition it has been considered worthwhile to calculate theoretically the stability conditions which apply. It is hoped that this will lead to experimental testing of this theory. The purpose of this paper is to investigate the stability of membranes undergoing surface charge regulation in a binary symmetrical electrolyte, as the ionic strength of the solution is changed, by using DLVO theory together with a numerical computation method.

In the analysis that follows, the charge on the membrane surface is assumed to arise from the dissociation of acidic surface groups according to the reaction

$$AH \leftrightarrow A^- + H^+ \qquad (1)$$

Where A represents a charged anionic group. In a biological membrane such as a plant thylakoid membrane there are a number of types of such groups and this type of membrane provides a good example of the complexity that can occur. Thylakoid membranes contain 50% protein and 50% lipid [4]. The proteins of the thylakoid membrane, such as the chlorophyll-protein complexes and the cytochromes of the photosynthetic elec-

tron transport chain, are viewed as globular particles embedded in or attached to a lipid bilayer. The glycolipids, monogalactosyl diglyceride and digalactosyl diglyceride account for 80% of the lipids of grana thylakoids, with the phospholipids and sulpholipids making up most of the remaining 20% [5]. The glycolipids have neutral hydrophilic head groups, while the phospholipids and sulpholipids each carry a single negatively charged head at neutral pH. It is characteristic of thylakoids that most of the phospholipids are singly charged, rather than being zwitterionic. The pK_a values of the charged groups on the phospholipids and sulpholipids are in the region of 2.0 in an open solution. The negatively charged groups due to the protein at the surface of the membrane at neutral pH will be due mainly to the side chain carboxyl groups of aspartic acid ($pK_a = 3.8$) and glutamic acid ($pK_a = 4.25$). Positively charged groups will be contributed by the side chain basic groups of lysine and argininc, which are protonated at neutral pH. The imidazole side chain of histidine with a pK_a of 6.0 will be mainly charged at pH 7.0. Chain-terminating amino acids may also make a small contribution to the net charge on the membrane surface. A phospholipid in a bilayer occupies an area of at least 60 Å^2 [6]. Thus, in the lipid area of the grana thylakoids the charge density is about 1 per 300 Å^2 or in the total surface area of the thylakoid about 1 per 600 Å^2, if we assume little net contribution from the proteins at neutral pH. In the model discussed below, all of these ionizable groups are replaced by a single type of group with a composite pK_a value and surface density although this restriction could easily be removed if required.

2. The electrostatic force

The electrostatic repulsive force between two membranes bearing acidic ionizable groups depends on membrane separation, the density of electric charge on the surfaces of the membrane, and the concentration of screening ions in the aqueous region separating the membranes. This force can be calculated using a previously derived method [7] which makes the following assumptions. These assumptions mean that the model is greatly simplified compared to the real system and it is worth commenting on their validity.

Firstly, the membranes are treated as infinite flat plates with chargeable head groups uniformly smeared out across the surface. In practice the membrane can be considered infinitely flat if the radius of curvature of the surface is much larger than the characteristic length of the electrical double layer. For membranes such as plant thylakoids this is the case. More serious is the fact that it is very unlikely that the charge is either smeared out or homogeneous on the membrane surface.

Secondly, since the Gouy-Chapman theory has been used, ions in solution are considered to be point charges which can approach the membrane to any distance including $x = 0$. In practice, however, the closest plane of approach will be defined by the radius of the hydrated ion. This causes an error in the potential ascribed to the plane of closest approach in the model, but this will not be serious as long as the surface charge density is small as in thylakoid membranes. Thirdly the possibility of the surface reaction

$$A^- + C^+ \leftrightarrow AC \tag{2}$$

where C^+ is a bulk ionic species is excluded. Let n_i^0 be the bulk concentration of ionic species i in the solution and ν_i the valence number with appropriate sign. Choose the x-axis of a spatial coordinate system perpendicular to the membrane surfaces and let $x = \pm a$ be the positions of the membranes. The Poisson-Boltzmann equation:

$$\frac{d^2\psi}{dx^2} = \frac{-4\pi e}{\epsilon} \sum_i \nu_i n_i^0 \exp(-e\nu_i\psi(x)/kT) \tag{3}$$

describes the electrostatic potential ψ at any point between the membranes [9]. Here, ϵ is the dielectric constant of the solution, k Boltzmann's constant, T the temperature and e the electron charge. The following boundary conditions

$$\left.\frac{d\psi}{dx}\right|_{x=\pm a} = \frac{4\pi\sigma}{\epsilon} \tag{4}$$

relate the potential to the surface charge density σ. Let α equal the fraction of ionized surface groups. These groups have a known dissociation constant

for the reaction shown in eq. 1 given by

$$Ka = \frac{[H^+]_s[A^-]}{[AH]} = [H^+]_s \frac{\alpha}{1-\alpha} \quad (5)$$

The surface H^+ concentration determined by this dissociation expression must be identical with that calculated from the Boltzmann equilibrium condition

$$[H^+]_s = n^0_{H^+} \exp(-e\psi_s/kT) \quad (6)$$

where $n^0_{H^+}$ is the bulk H^+ concentration and ψ_s the surface potential. The surface charge density is related to the dissociation reaction constant Ka and the number of surface anionic groups per unit area, Γ, and is obtained by combining eqs. 5 and 6.

$$\sigma = -eKa\Gamma/\left(Ka + n^0_{H^+} \exp(-e\psi_s/kT)\right) \quad (7)$$

Eqs. 3 and 7 with boundary conditions (eq. 4) can be solved numerically to give the separation between the membranes

$$H = 2\int_{\psi_m}^{\psi_s} \frac{d\psi}{g(\psi,C)} \quad (8)$$

where $g(\psi,C) = ((8\pi kT/\epsilon)\Sigma_i n_i^0 \exp(-\nu_i e\psi/kT + C))^{1/2}$.

ψ_m is the potential at the midpoint between the membranes and C an integration constant. For any particular value of C, the repulsive force between the surfaces can be found by a standard thermodynamic argument [9]. It is given by

$$F_R = kT\sum_i n_i^0(C+1) \quad (9)$$

The corresponding repulsive energy at any separation H is given by Bell and Peterson [10] as

$$V_R = -F_R\kappa H - 2\int_{\psi_m}^{\psi_s} g(\psi,C)d\psi + 2\int_0^{\psi_\infty} g(\psi,C)d\psi + 2\int_{\psi_\infty}^{\psi_s} g(\psi,C)d\psi_s \quad (10)$$

where ψ_∞ is the potential on an isolated surface and

$$\kappa = \left(\frac{8\pi ne^2\nu^2}{\epsilon kT}\right)^{1/2} \quad (11)$$

Here n is the ionic concentration for a binary $\nu - \nu$ type electrolyte. The computational procedure for calculating the force and free energy at any given separation has been described in detail previously [7].

Eq. 7 relates the surface charge and surface potential for a charge-regulated surface. The computational method just described can also be used for constant charge or constant potential boundary conditions by replacing eq. 7 by either σ = constant or ψ_s = constant. Although these boundary conditions are not appropriate for biological membranes, the situations where they are applicable have been discussed earlier. In the case of the coagulation of a suspension of biological cells, the repulsive energy between two spheres is the quantity of interest. In the Derjaguin approximation [10] the repulsive force between two spheres can be derived from that between two plates. In this work, however, only the interaction between two flat surfaces will be considered. In order to simplify the discussion, calculations will only be carried out for a binary symmetrical electrolyte, although the above analysis can be applied to a general electrolyte.

3. Flocculation conditions

The total potential energy V_T of interaction between two membranes is given by the sum of the energy of repulsion of the double layers and the attractive energy of the membranes due to van der Waals forces. Assuming for simplicity infinitely thick, homogeneous membranes, and neglecting retardation effects, the van der Waals energy per unit area of the surfaces is given by

$$V_A(\delta) = -A/48\pi\delta^2 \quad (12)$$

where A is the Hamaker constant and δ the half-distance between the membranes [12]. Consequently

$$V_T = V_R + V_A \quad (13)$$

where V_R and V_A are defined by eqs. 10 and 12, respectively. In essence, the attractive force follows an inverse cubic law while the repulsive force increases exponentially with distance, which means

that the attractive force dominates at small separations. This leads to a form of V_T vs. δ curve which has a maximum. Physically, this means that the membranes have to overcome a potential barrier before coagulation can take place. It is reasonable [13] to assume that the transition from slow to rapid flocculation occurs when this barrier is reduced to zero, i.e., when the total energy of interaction and its derivative with respect to intermembrane separation are both zero.

Following Honig and Mul [3], a dimensionless repulsive force

$$F = F_R/64nkT \tag{14}$$

and a dimensionless repulsive energy

$$W = \kappa V_R/64nkT \tag{15}$$

can be defined. The flocculation conditions are

$$V_T(\delta) = 0 \tag{16}$$

$$\frac{dV_T}{d\delta}(\delta) = 0 \tag{17}$$

Applying these to eqs. 12 and 13 leads to

$$V_R = A/48\pi\delta^2 = 64nkTW/\kappa \tag{18}$$

$$F_R = A/24\pi\delta^3 = 64nkTF \tag{19}$$

since $F_R = -dV_R/d\delta$. Combining eqs. 18 and 19 leads to

$$\kappa\delta = W/F \tag{20}$$

at coagulation. The ratio W/F is dimensionless since both W and F have been defined to be dimensionless. κ is the inverse of the screening length so that $\kappa\delta$ is a distance measured in multiples of the screening length. Eq. 20 states that the distance at which the transition from slow to rapid coagulation takes place is equal to the ratio of normalized repulsive energy to normalized repulsive force. The method described in section 2 has been used to calculate $\kappa\delta$ for plates approaching at constant charge, constant potential and undergoing surface charge regulation; the results are shown in fig. 1. Here, $\kappa\delta$ is plotted vs. Z_∞, which is the normalized potential of the surfaces at infinite separation, defined by

$$Z_\infty = \nu e\psi_\infty/kT \tag{21}$$

This definition allows fig. 1 to be used for a binary symmetrical electrolyte of any valence ν. Z_∞ can be interpreted in different ways depending on which boundary condition is being considered. For all three boundary conditions, the charge on an isolated plate corresponding to a particular value of ψ_∞ can be obtained by integrating eq. 3 and is given by [15],

$$\sigma_\infty^2 = (\epsilon kT/2\pi)\sum_i n_i^0(\exp(-\nu_i\psi_\infty/kT) - 1) \tag{22}$$

For surfaces approaching at constant potential, ψ_∞ is the relevant independent parameter, since we are interested in how the stability of the membranes is affected by this quantity. In the case of surfaces approaching at constant charge, σ_∞ is the parameter of interest, and this is related to Z_∞ through eqs. 21 and 22. Thus, increasing values of Z_∞ correspond to increasing values of charge on the surface. For a surface undergoing charge regulation the main parameter determining membrane stability is the density of ionizable groups on the surface, Γ. This is related to Z_∞ since for a charge-regulated surface, eqs. 7 and 22 must be satisfied simultaneously. This means that ψ_∞ and σ_∞ are not independent variables but are determined by the values of Ka, $n^0_{H^+}$ and Γ. Thus, in the case of charge-regulated surfaces, the stability of the system depends on the density and pK_a of

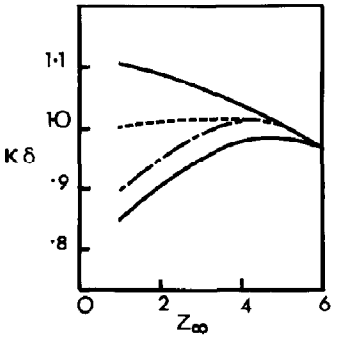

Fig. 1. Normalized coagulation half-distance $\kappa\delta$ vs. normalized surface potential at infinite separation, Z_∞. Curves (from top to bottom): constant-potential case, $\beta = n^0_{H^+}/Ka = 1000$, $\beta = 0.01$, constant-charge case.

the ionizable groups, and the pH of the bulk solution. The bulk H^+ concentration has a direct effect on the surface charge density as can be seen from eq. 1. The curves in fig. 1 for a charge-regulated surface were generated by choosing particular values of β, where $\beta = n^0_{H^+}/Ka$, and then varying Γ. Thus, the increasing value of Z_∞ can be seen as a measure of the increasing density of groups on the surface.

The significance of $\kappa\delta$ in fig. 1 is that since it is the normalized distance at which rapid coagulation begins to take place, then larger values of $\kappa\delta$ indicate less stable systems. Thus, for the constant-potential case, a lower value of Z_∞, i.e., a lower surface potential, means that the surfaces repel each other less strongly, indicated by the increasing value of $\kappa\delta$. It can also be seen from fig. 1 that surfaces approaching under constant charge are always more stable than those approaching under constant potential for the same value of Z_∞. This is related to the fact that the repulsive energy between two surfaces approaching at constant charge is always greater than that between two surfaces approaching at constant potential for the same value of Z_∞ [1].

The charge-regulated surface curves always lie between those for constant charge and constant potential, and depending on the value of β may or may not have a maximum. At low values of β, the surfaces behave like constant-charge surfaces, and as β is increased the surfaces behave more like constant-potential surfaces. This can be understood by looking at eq. 7 where it can be seen that for small β the exponential term in the denominator will have no effect and σ is effectively constant. As β increases, the system becomes more like the constant-potential case and less stable for a given value of Z_∞.

4. Flocculating ion concentrations

If the value of $\kappa\delta$ is known, the flocculation concentration n can be obtained. Following Jones and Levine [12] it is convenient to introduce a dimensionless concentration

$$n^* = A^2(\nu e)^6 n/\epsilon^3(kT)^5 \tag{23}$$

From eq. 18

$$\kappa^2\delta^2 W = \frac{A}{48\pi}\cdot\frac{\kappa^3}{64nkT} \tag{24}$$

Therefore using eq. 11

$$n^* = \frac{18432}{\pi}\cdot W^2(\kappa\delta)^4 \tag{25}$$

This definition of n^* means that the calculated value of flocculation concentration can be used for any valence binary symmetrical electrolyte. According to eq. 25 the coagulating concentration for any type of boundary condition can be calculated by substituting the appropriate value of W and $\kappa\delta$. This has been done in fig. 2 for the constant-charge and constant-potential cases. It can be seen that n^*_δ (constant charge) is always larger than n^*_ψ (constant potential), reflecting the fact that at given value of Z_∞, the constant-charge case is more stable than the constant-potential case. The ratio n^*_σ/n^*_ψ decreases with increasing Z_∞ from 1.47 at $Z_\infty = 1$ to a limiting value of 1 as $Z_\infty \to \infty$.

The effect of the variation of β on the coagulation concentration for the charge-regulated surface is shown in fig. 3. It can be seen that as β increases, the system becomes less like the constant-charge case and more like the constant-potential case, and hence less stable. For any particular value of β, the system is relatively less

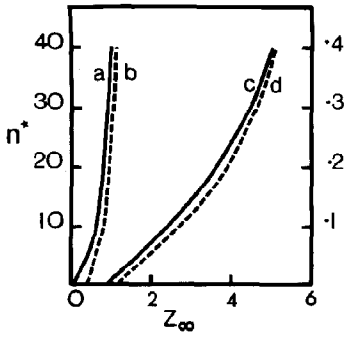

Fig. 2. Plot of normalized coagulating concentration vs. surface potential at infinite separation, Z_∞, for constant-charge and constant-potential cases. Curves: a, n^*_σ; b, n^*_ψ. Curves c and d show a and b, respectively, on the magnified scale shown on the right-hand axis.

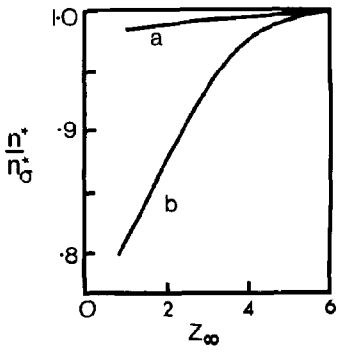

Fig. 3. Plot of ratio of charge-regulated coagulation concentration n^* to constant charge coagulation concentration n_σ^* vs. surface potential at infinite separation Z_∞. Curves: a, $\beta = n_{H^+}^0/Ka = 0.01$; b, $\beta = 1000$.

stable, compared to the constant-charge case, at low values of Z_∞ and hence Γ, than at high values. Thus, for the $\beta = 1000$ case, $n^*/n_\sigma^* = 0.8$ at $Z_\infty = 1$ and approaches an asymptotic value of 1 as $Z_\infty \to \infty$.

5. Conclusion

The purpose of this work has been to investigate the effect of electrolyte concentration on the stability of biological membrane structures or suspensions of biological cells. By calculating the electrostatic repulsive force and van der Waals attractive force between the surfaces, the total interactive potential has been found as a function of distance. This curve enables flocculation criteria from the DLVO theory of colloid stability to be applied to biological membranes. It has been shown that the flocculation concentration for membranes bearing ionizable surface groups differs from that for constant-charge or constant-potential surfaces and lies between the two extremes.

The value of the flocculation concentration has been shown to depend on the density of ionizable surface groups, and on the ratio of bulk H^+ concentration to the value of the ionizable group dissociation constant. The value of this ratio determines the extent to which charge regulation can take place as the surface potential varies, as shown by eq. 7, and hence the extent to which the system becomes less like the constant-charge case and more like the constant-potential case.

Although as far as is known no experimental measurements exist to verify this theory, it is hoped that the preceeding calculations will lead to the carrying out of such experiments.

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