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Investigation of ionic stability criteria for ion-permeable charged membranes

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The flocculation criteria in the DLVO theory of colloid stability are applied to ion-permeable membranes containing ionizable fixed groups. These groups are not restricted to the membrane surface but are uniformly distributed throughout a thick surface layer. The flocculation concentrations for such membranes are calculated by using a numerical method to solve the nonlinear Poisson-Boltzmann equation. Results are compared with calculations previously carried out for more restrictive models of biological membranes. The flocculation concentrations are shown to depend on the density of ionizable groups, the dissociation constant of these groups, and the pH of the bulk solution.

1. Introduction

The formation of a number of stable biological structures has previously been explained theoretically 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 opposing membrane surfaces. The formation of regularly spaced arrays of grana thylakoid membranes has been explained in this way [1]. The van der Waals and electrostatic forces were determined for negatively charged surfaces with a specified pK_a (dissociation constant) and bulk pH, and a range of monovalent and divalent cation concentrations. After taking account of specific binding of divalent cations to the membrane surface, it was found that experimental measurements of granal membrane equilibrium separations correspond to a minimum in the curve of total potential energy vs distance. The balance between attractive van der Waals forces and repulsive electrostatic forces due to membrane charges is also thought to play a major role in cell adhesion. The presently accepted view of cell adhesion is that the total interaction force between cell membranes is the sum of a number of forces and that all of these forces except for the van der Waals force and the electrostatic force only act at very short distances between the cells [2,3]. Curtis [4] has carried out an extensive review of the literature on cell adhesion and has come to the conclusion that the experimental evidence strongly suggests that cell adhesion is predominantly due to the interaction of van der Waals attractive forces and electrostatic repulsive forces. Weiss and Harlos [5] have also carried out a review of the experimental literature on cell adhesion. They concluded that the stability of cell suspensions is again due to the balance between van der Waals and electrostatic forces.

A membrane array or a suspension of biological cells which is in equilibrium due to such a balance of forces is not thermodynamically stable, since the total free energy of such a system can

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always be lowered by a reduction in the interfacial area. The high degree of stability which is observed in such systems is a kinetic phenomenon; that is the coagulation rate is practically zero. Reduction in the rate of coagulation occurs when the electrostatic double layer forces causing repulsion between the surfaces increase. Increasing the ionic strength of the solution reduces the extent of the double layers and hence enhances coagulation. At a high enough ionic strength the system changes from a state of slow coagulation to a state of fast coagulation and becomes unstable. The ionic concentration at which this transition occurs is known as the flocculation concentration.

A number of experimental studies have been carried out on the stability of suspensions of biological cells. The results of these experiments have been analysed using the Derjaguin-Landau-Verwey-Overbeek theory of colloid stability. Wilkins et al. [6] examined the rate of flocculation of sheep polymorphonucleocytes by different cations. They found that DLVO theory provided a good description of their flocculation data and estimated the Hamaker constant for the van der Waals force. Brooks et al. [7] studied the aggregation of a number of different types of cells at various ionic strengths. They concluded that colloid stability theory was applicable to cellular interactions and were also able to estimate the Hamaker constant. Curtis [4] examined the flocculation kinetics of embryonic chick cells and again found evidence supporting the applicability of colloid stability theory to cell adhesion. In summary, it can be stated that the relevance of colloid stability theory to the problem of cellular adhesiveness is supported by a large amount of experimental data. Many theoretical studies have also investigated biological membrane interactions using colloid stability theory. Refs 8–10 are examples of these.

Applications of DLVO theory to the analysis of the stability of lyophobic colloids have assumed constant charge or constant potential boundary conditions [11,12]. Should surface charge be derived from the adsorption of solute ions, as in lyophobic colloids, and if the free energy of adsorption is not such as to render the system effectively irreversible, the approach of two such surfaces will be accompanied by desorption of surface charges into solution and the maintenance of a constant surface potential; as the distance separating the surfaces approaches zero, their surface charge densities also approach zero [13]. The constant potential condition assumes the equilibrium relation $\Delta\mu_i + e_i\psi = 0$, where e_i is the ionic charge, ψ the boundary potential, and $\Delta\mu_i$ the change in the potential-independent part of the free energy on adsorption of the potential determining ion, species i, from bulk solution onto the surface.

The constant charge boundary condition is appropriate for a different type of surface. If surface charges are derived not from the adsorption of solute ions but due to the ionization of fixed surface groups, with a fixed degree of dissociation, the approach of similar surfaces will be accompanied by a rise in surface potential at constant charge density [13].

In studying the behaviour of biological membranes under a range of bulk solution conditions, neither the constant charge or constant potential boundary condition should be used. 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 in the membrane [14]. The corresponding charge density due to dissociation must then be determined as a self-consistent function of the potential. As the membranes approach each other, the fraction of dissociated groups and hence the membrane charge density changes; a boundary condition known as charge regulation. This mechanism of the generation of charge in biological membranes has been experimentally verified in the case of plant thylakoid membranes [15]. The applicability of the charge regulation model to biological cells has also been demonstrated experimentally. Prieve and Ruckenstein [9] analysed data on the dependence of the cell surface potential on bulk solution pH and solution ionic strength for human erythrocytes and sheep leucocytes. They found that the experimental data were best fitted by a charge regulation model of the cell surface. Using the charge regulation model with the parameters applicable to the human erythrocyte, Prieve and Ruckenstein

have shown that in low ionic strength solutions, the interaction force at large cell separations predicted by the charge regulation model differs by a factor of two from the force predicted by the constant charge or constant potential models of the cell surface. This result shows that under certain conditions charge regulation can have a significant effect on the force between biological cells.

Since biological membranes obey this third type of boundary condition, it is of interest to investigate theoretically the stability conditions which apply to such structures by extending the DLVO theory of colloid stability. Accordingly, in ref. 16 a numerical solution of the non-linear Poisson-Boltzmann equation was used in the calculation of the ionic flocculation concentrations for two interacting membranes undergoing surface charge regulation. The dependency of the flocculation concentration on the surface density of ionizable groups, the pK_a of the surface groups and the pH of the bulk solution was investigated.

In ref. 16 the membrane was treated as a solid surface with charges located only on the surface and which was impermeable to electrolyte ions. The exterior surface of many biological cells is coated with a layer of extended macromolecules which is permeable to the external solution, known as the glycocalyx [17]. An important example of a cell with a glycocalyx is the human erythrocyte. There is good evidence from biochemical [18] and electrokinetic studies [19] that the surface charge of the human erythrocyte is located in the glycocalyx and is due to the dissociation of the carboxyl group of sialic acid. A number of studies on human electrophoresis data have been made [20–22]. These studies conclude that the glycocalyx is realistically modelled as a permeable charged layer which is much thicker than the Debye length. For example, in ref. 21 the thickness of the glycocalyx was found to be 75 Å compared with a Debye length under physiological conditions of about 10 Å. This model of the membrane surface has been investigated in a number of theoretical works [23–25]. The purpose of this paper is to investigate theoretically the stability conditions which apply to ion permeable membranes with membrane charges distributed through a thick surface layer, as the ionic strength of the solution is changed. This will be done by using a numerical solution of the non-linear Poisson-Boltzmann equation together with DLVO theory, and charge regulation boundary conditions.

Other authors have considered the interaction between surfaces which contain charge in a thick permeable layer. Miklavic et al. [26] have calculated the force between membranes with bound polyelectrolytes which have a fixed charge. The theory developed in ref. 26 does not apply to biological cells where charge regulation is important, and stability conditions are not considered.

Lyklema [27] has investigated the interaction between porous metal oxides. His work is related to that described here, but there are significant differences. Biological membranes are not considered and coagulation conditions are not worked out for two interacting surfaces obeying charge regulation. Ohshima and Kondo [28] have investigated the repulsive interaction between ion-penetrable membranes. In their work the membrane charge is located in a thick surface layer and the charge regulation model is used; however stability conditions are not considered by these authors. The main area of interest in this paper is the investigation of stability conditions, and hence it differs significantly from the work of previous authors.

2. Calculation of electrostatic force

In order to simplify calculations, a number of idealizations of a real biological membrane will be made. In the analysis that follows, the charge in the membrane is assumed to arise from the dissociation of membrane-fixed ionizable groups of valence 1 according to the reaction

$$AH \leftrightarrow A^- + H^+ \tag{1}$$

where A represents a charged anionic group. In a biological membrane such as a plant thylakoid membrane a number of different types of such groups occur; each with a different dissociation constant, given by pK_a [29].

In the model to be considered, all of these groups will be replaced by a single type of group with a composite pK_a value. These will be distributed at uniform density N through a thick surface layer $d \geq 1/\kappa$, κ being the Debye-Hückel parameter.

The electrostatic repulsive force between two membranes bearing acidic ionizable groups depends on membrane separation, the density of electric charges in the membranes and the concentration of screening ions in the aqueous region separating the membranes. This force can be calculated using a previously derived method [30] which makes the following assumptions. Firstly, the membranes are treated as infinite flat plates. The membrane can be considered infinitely flat if the radius of curvature of the surface is much larger than the characteristic length of the double layer; as is the case for plant thylakoid membranes. Secondly, the possibility of the membrane reaction

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

where C^+ is a bulk ionic species is excluded. Thirdly, the surface layer is considered to be completely permeable to free mobile ions from the bulk solution.

Let n_i^0 be the bulk concentration of ionic species i in the solution and v_i the valence number with appropriate sign. Choose the x-axis of a spatial co-ordinate system perpendicular to the membrane surfaces and let $x = \pm a$ be the positions of the membrane surfaces. The Poisson-Boltzmann equation:

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

describes the potential ψ at any point between the membranes [13]. Here, ϵ is the dielectric constant of the solution, k Boltzmann's constant, T the temperature and e the electron charge. Since the membranes are identical, the system is symmetrical and it is only necessary to consider one membrane. Let α equal the fraction of ionized groups at a distance x inside the membrane. These groups have a known dissociation constant for the reaction shown in eq. 1 given by

$$K_a = \frac{[H^+]_x[A^-]_x}{[AH]_x} = [H^+]_x\frac{\alpha}{1-\alpha} \tag{4}$$

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

$$[H^+]_x = n_{H^+}^0 \exp(-e\psi(x)/kT) \quad x < -a \tag{5}$$

where $n_{H^+}^0$ is the bulk H^+ concentration and $\psi(x)$ is the potential at distance x inside the membrane. The membrane fixed charge density is related to K_a and the density of anionic groups per unit volume, N, and is obtained by combining eqs 4 and 5.

$$\rho_x = -eN/\left[1 + \left(n_{H^+}^0/K_a\right)\exp(-e\psi(x)/kT)\right] \tag{6}$$

Assuming the dielectric constant of the membrane is the same as that of the solution, the Poisson-Boltzmann equation inside the membrane can be written.

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi e}{\epsilon}\left[\sum_i v_i n_i^0 \exp(-ev_i\psi(x)/kT) - N/\left[1 + \left(n_{H^+}^0/K_a\right)\exp(-e\psi(x)/kT)\right]\right]$$
$$x < -a \tag{7}$$

Eqs 3 and 7 are subject to the following boundary conditions.

$$\left.\frac{d\psi}{dx}\right|_{x=0} = 0 \tag{8}$$

$$\left.\frac{d\psi}{dx}\right|_{x=-a^+} = \left.\frac{d\psi}{dx}\right|_{x=-a^-} \tag{8b}$$

$$\psi(-a^+) = \psi(-a^-) \tag{8c}$$

$$\psi \to \psi_D \text{ as } x \to -\infty \tag{8d}$$

Eq. 8 follows from the symmetry of the system. Eqs 8b and 8c are the conditions for the continuity of the electric field and the potential at the membrane-solution interface. Eq. 8d follows from the assumption that the potential far inside the

membrane; the Donnan potential, is a constant, independent of membrane separation. This assumption is equivalent to the assumption of electroneutrality in the membrane bulk phase and has been justified by theoretical analysis [31]. Eq. 8d is also based on the assumption that the surface charged layer can be assumed infinitely thick compared with the screening length of the solution, as discussed above. Eqs 3 and 7 with boundary conditions, eq. 8, can be solved numerically to give the separation between the membranes

$$H = 2\int_{\psi_m}^{\psi_s} \frac{d\psi}{g(\psi,c)} \tag{9}$$

where

$$g^2(\psi,c) = \left(\frac{8\pi kT}{\epsilon}\right)\sum_i n_i^0 \exp\left[\left(\frac{-v_i e\psi}{kT}\right) + C\right]$$

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 is given by a standard thermodynamic argument [13] as

$$F_R = kT\sum_i n_i^0(C+1) \tag{10}$$

The corresponding free energy at any separation H is given by Bell and Peterson [32] as

$$V_R = -F_R\kappa H - 2\int_{\psi_m}^{\psi_s} g(\psi,C)\,d\psi + 2\int_0^{\psi_\infty} g(\psi,C)\,d\psi + 2\int_{\psi_\infty}^{\psi_s} g(\psi,C)\,d\psi \tag{11}$$

where ψ_∞ is the potential on an isolated surface and the Debye-Hückel parameter is given by

$$\kappa = \left(\frac{8\pi ne^2v^2}{\epsilon kT}\right)^{1/2} \tag{12}$$

Here n is the ionic concentration for a binary v-v type electrolyte. The computational procedure for calculating the force and free energy at any given separation has been described in detail previously [30].

By integration of eq. 7 and application of boundary condition, eq. 8d an implicit equation for the Donnan potential, ψ_D can be written,

$$\sum_i v_i n_i^0 \exp(-ev_i\psi_D/kT) - N \Big/ \left(1 + \left(n_{H^+}^0/K_a\right)\exp\left(\frac{-e\psi_D}{kT}\right)\right) = 0 \tag{13}$$

By further manipulation of the initial equations we can also write

$$\sum_i n_i^0\left[\exp\left(\frac{-ev_i\psi_D}{kT}\right) - 1\right] + N\left[\frac{e}{kT}(\psi_D - \psi_\infty) - \log\left|1 + \frac{n_{H^+}^0}{K_a}\exp\left(\frac{-e\psi_\infty}{kT}\right)\right| + \log\left|1 + \frac{n_{H^+}^0}{K_a}\exp\left(\frac{-e\psi_D}{kT}\right)\right|\right] = 0 \tag{14}$$

where ψ_∞ is the potential of an isolated membrane surface. Eqs 13 and 14 can be solved numerically to give values of ψ_D and ψ_∞ for particular values of the model parameters.

Fig. 1 shows the result of this calculation for a

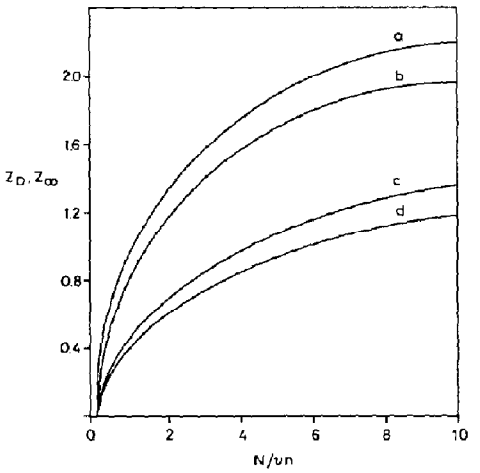

Fig. 1. Normalized Donnan potential Z_D and normalised surface potential Z_∞ vs ratio of ionizable site density to total solution charge density $N/\upsilon n$. Curves for an isolated surface: (a) Z_D, $\beta = 0.01$; (b) Z_D: $\beta = 0.1$; (c) Z_∞, $\beta = 0.01$; (d) Z_∞, $\beta = 0.1$.

binary v-v type electrolyte with ionic concentration n. The normalised potentials are defined by

$$Z_D = ve\psi_D/kT \tag{15}$$

$$Z_\infty = ve\psi_\infty/kT \tag{16}$$

and β is given by

$$\beta = n^0_{H^+}/K_a \tag{17}$$

Fig. 1 shows that as the ratio of ionizable membrane groups to total charge concentration, N/vn, rises, both Z_D and Z_∞ also rise. An increase in β leads to a decreased sensitivity of both Z_D and Z_∞ to N/vn.

The computational method described above has also been used to calculate free energy versus separation curves for plates interacting under boundary conditions of constant charge, constant potential and surface charge regulation [16]. In all these cases the membranes are impermeable to free ions from solution, and all membrane charge is located in a surface layer of zero depth. It is of interest to compare the stability conditions for the generalized membrane model with those for the more restrictive models. This is carried out in the following sections. 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 of interaction, V_T, is given by the sum of the energy of interaction of the double layers and the interaction energy of the membranes due to van der Waals forces. Assuming for ease of calculation infinitely thick, homogeneous membranes, and neglecting retardation effects, the van der Waals energy per unit area of the surfaces is given by

$$V_A(\delta) = -A/48\pi\delta^2 \tag{18}$$

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

$$V_T = V_R + V_A \tag{19}$$

where V_R and V_A are defined by eqs 11 and 18. In essence, the attractive force follows an inverse cubic law while the repulsive force varies as an inverse exponential 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 must overcome a potential barrier before coagulation can take place. It is reasonable [33] 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 [11], a dimensionless repulsive force

$$F = F_R/64nkT \tag{20}$$

and a dimensionless interaction energy

$$W = \kappa V_R/64nkT \tag{21}$$

can be defined. The flocculation conditions are

$$V_T(\delta) = 0 \tag{22}$$

$$\frac{dV_T}{d\delta}(\delta) = 0 \tag{23}$$

Applying these conditions to eqs 18 and 19 leads to

$$V_R = A/48\pi\delta^2 = 64nkTW/\kappa \tag{24}$$

$$F_R = A/24\pi\delta^3 = 64nkTF \tag{25}$$

since $F_R = -dV_R/d\delta$. Combining eqs 24 and 25 leads to

$$\kappa\delta = W/F \tag{26}$$

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. 26 states that the distance at which the transition from slow to rapid coagulation takes place is equal to the ratio of normalized interaction energy to normalized repulsive force.

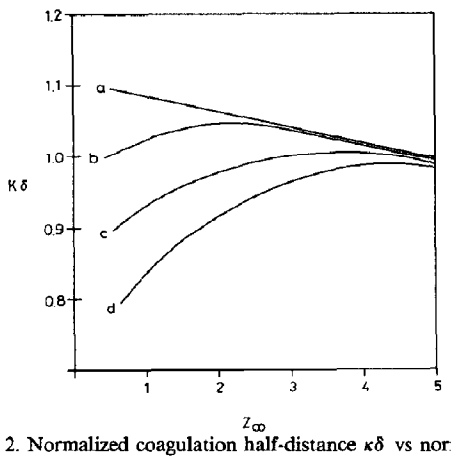

Fig. 2. Normalized coagulation half-distance $\kappa\delta$ vs normalised surface potential at infinite separation, Z_∞. Curves: (a) constant potential; (b) generalized membrane model: $\beta = 10$; (c) surface charge regulation model: $\beta = 10$; (d) constant charge.

4. Results

The computational method of section 2 has been used to calculate curves of force and free energy versus plate separation, and hence to find the coagulation separations which satisfy eq. 26. In fig 2, the coagulation half-distance $\kappa\delta$ is plotted vs Z_∞.

The definition of Z_∞ allows fig. 2 to be used for a binary symmetrical electrolyte of any valence v. In fig. 2 curves are also shown for membranes interacting under constant charge, constant potential and surface charge-regulated boundary conditions.

In the surface charge-regulated case, discussed in detail in ref. 16, all membrane charge is confined to the membrane surface and is given by

$$\sigma = -e\Gamma/\left[1 + \left(n^0_{H^+}/K_a\right)\exp(-e\psi_s/kT)\right] \quad (27)$$

where Γ is the surface density of ionizable groups, ψ_s the surface potential of the membrane and K_a the dissociation constant of the surface groups. This equation is analogous to eq. 6 for the generalised model, which has charge distributed through a thick layer.

In fig. 2 an increasing value of Z_∞ has a different meaning depending on which membrane model is being considered. For surfaces approaching at constant potential, ψ_∞ is the relevant independent parameter, since we are interest 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. This is related to ψ_∞ by [33],

$$\sigma^2_\infty = \left(\frac{\epsilon kT}{2\pi}\right)\sum_i n^0_i(\exp(-v_i\psi_\infty/kT) - 1) \quad (28)$$

Thus, from eqs 16 and 28 increasing values of Z_∞ correspond to increasing values of charge on the surface. In the surface charge regulated case 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 27 and 28 must be satisfied simultaneously. Thus, an increasing value of Z_∞ can be seen as a measure of the increasing density of groups on the surface. In the case of the gener-

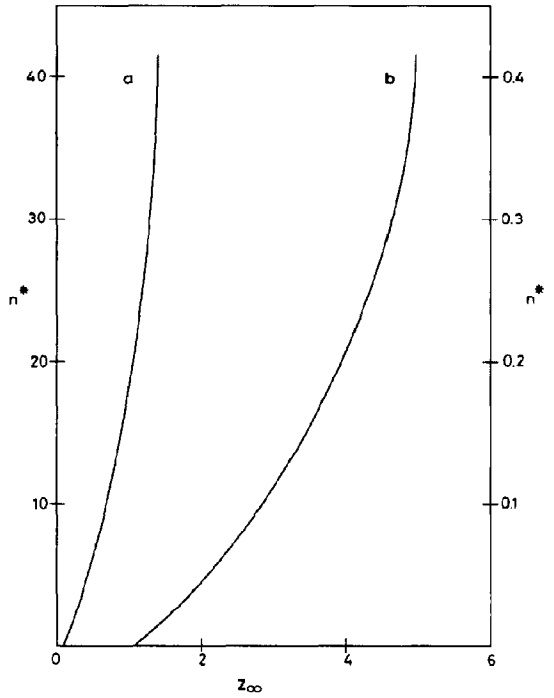

Fig. 3. Normalized coagulation concentration for generalized membrane, n^*, vs surface potential at infinite separation, Z_∞, for $\beta = 1$. Curve a shows curve b on the magnified scale shown on the right-hand axis.

alized membrane model, it can be seen from fig. 1 that an increasing value of Z_∞ corresponds to an increasing value of N/vn, the ratio of ionizable fixed membrane site density to the total charge density in the solution.

Once the value of $\kappa\delta$ at coagulation is known, the corresponding flocculation concentration n can be obtained [12]. It is convenient to introduce a dimensionless concentration

$$n^* = A^2(ve)^6 n/\epsilon^3(kT)^5 \tag{29}$$

From eq. 24

$$\kappa^2\delta^2 W = \frac{A}{48\pi} \cdot \frac{\kappa^3}{64nkT} \tag{30}$$

From eq. 12

$$n^* = \frac{18432}{\pi} \cdot W^2(\kappa\delta)^4 \tag{31}$$

This definition of n^* means that the calculated value of flocculation concentration is valid for any valence binary symmetrical electrolyte.

In fig. 3, eq. 31 has been used to plot the flocculation concentration for the generalized model versus Z_∞, for $\beta = 1$. As discussed above, an increasing value of Z_∞ corresponds to an increasing value of N/vn. Fig. 3 shows that as the ratio of fixed membrane sites to total solution charge density increases, the system becomes more stable and requires a higher flocculating concentration.

In fig. 4a and b ratios of flocculation concentrations for different membrane models are plotted as functions of Z_∞. In fig. 4a curves for the surface charge regulation model [16] are shown, together with the constant potential case. It can be seen that n_σ^* (constant charge) is always larger than n_ψ^* (constant potential) but that n_ψ^*/n_σ^* approaches 1 as Z_∞ becomes large. From this curve it can be seen that for a given value of Z_∞ the constant charge case is always more stable than the constant potential case; but that the two cases approach the same flocculation concentration as Z_∞ becomes large. The flocculation concentrations for surface charge regulated membranes lie between those for the constant charge and constant potential cases.

As the value of β is increased the system becomes less like the constant charge case and more like the constant potential case. For any particular

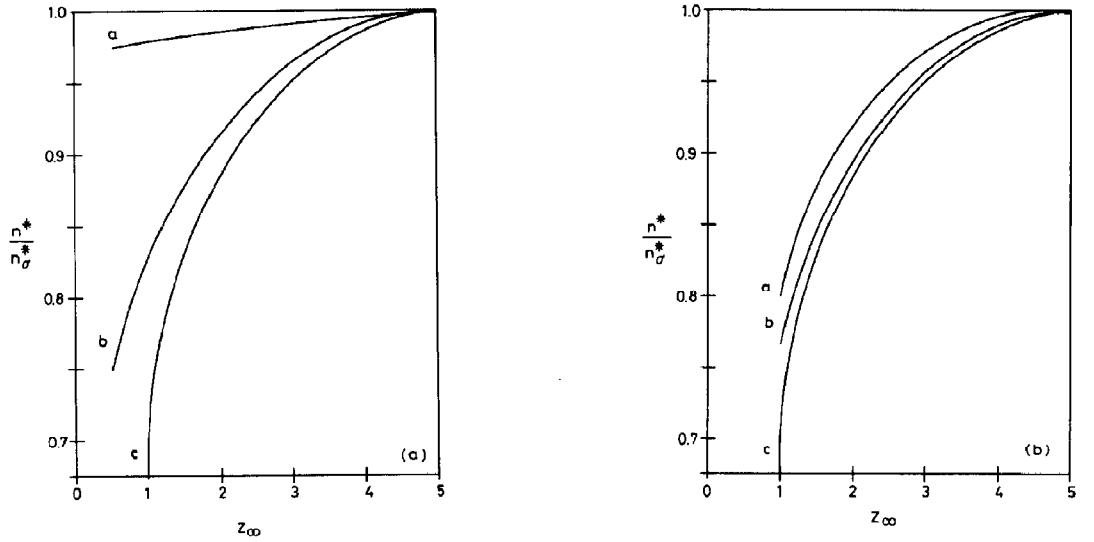

Fig. 4. (a) Ratio of coagulation concentration to constant charge coagulation concentration, n^*/n_σ^*, vs surface potential at infinite separation, Z_∞, for surface charge regulated membrane. Curves: (a) $\beta = 0.01$; (b) $\beta = 10$; (c) constant potential. (b) Ratio of coagulation concentration to constant charge coagulation concentration, n^*/n_σ^*, vs surface potential at infinite separation, Z_∞, for generalized membrane model. Curves: (a) $\beta = 0.01$; (b) $\beta = 10$; (c) constant potential.

value of β, the system is relatively less stable, compared to the constant charge case, at low values of Z_∞ than at high values. As defined in eq. 17, β is the ratio of bulk hydrogen ion concentration to the dissociation constant of the ionizable groups. In fig. 4b the corresponding curves for the generalized model are shown. It can be seen that these curves show much less variation with β than the curves for the surface charge regulated model. At large values of Z_∞, the flocculating concentrations for all models become identical.

5. Conclusion

The purpose of this work has been to investigate how the stability of biological membrane arrays or suspensions of biological cells depends on the electrolyte concentration of the suspension medium. The membrane has been modelled as an ion permeable surface containing a thick layer of ionizable groups and the DLVO theory of colloid stability has been used, together with a numerical solution of the nonlinear Poisson-Boltzmann equation to study the behaviour of the system.

The flocculation concentration has been calculated as a function of the density of ionizable groups, the ionizable group dissociation constant, and the bulk H^+ concentration. It has not been possible to compare these calculations with quantitative experimental results, since the appropriate experiments have not been carried out. However the results from this model have been compared with those from more restrictive theoretical models. It has been found that the flocculation concentration depends less sensitively on the ionizable group dissociation constant and the bulk H^+ concentration in the generalized model than in the surface charge regulated model, which restricts all membrane charge to a zero thickness surface layer.

For ease of calculation the model is simplified in a number of ways. The membrane is assumed to have the same dielectric constant as the solution and to be completely permeable to it. Secondly the membrane is assumed to have a charged layer which is very thick compared to the solution screening length, and which contains a single type of ionizable group. The numerical computation method described above can be easily adapted to remove any of these restrictions if required. It is hoped that this theoretical investigation into the stability of biological membranes leads to an experimental study of such a system, in order that the theoretical results can be tested.

References

1 M.J. Sculley, J.T. Duniec, J.W. Thorne, W.S. Chow and N.K. Boardman, Arch. Biochem. Biophys. 201 (1980) 339.
2 Y. Chang, Colloids Surfaces. 41 (1989) 245.
3 D. Gingell, J. Theor. Biol. 17 (1967) 451.
4 A.S.G. Curtis, Sci. Prog. Oxf. 54 (1966) 61.
5 L. Weiss and J.P. Harlos, Prog. Surface Sci. 1 (1972) 355.
6 D.J. Wilkins, R.H. Ottewill and A.D. Bangham, J. Theor. Biol. 2 (1962) 165.
7 D.E. Brooks, J.S. Millar, G.V.F. Seaman and P.S. Vassar, J. Cell. Physiol. 69 (1967) 155.
8 A.D. Bangham and B.A. Pethica, Proc. Roy. Phys. Soc. (Edinb.) 28 (1960) 43.
9 D.C. Prieve and E. Ruckenstein, J. Theor. Biol. 56 (1976) 205.
10 V.A. Parsegian and D. Gingell, J. Adhes. 4 (1972) 283.
11 E.P. Honig and P.M. Mul, J. Colloid Interface Sci. 36 (1971) 258.
12 J.E. Jones and S. Levine, J. Colloid Interface Sci. 30 (1969) 241.
13 E.J.W. Verwey and J.T.G. Overbeek, Theory of the stability of lyophobic colloids (Elsevier, Amsterdam, 1948).
14 B.W. Ninham and V.A. Parsegian, J. Theor. Biol. 38 (1973) 101.
15 H.Y. Nakatani and J. Barber, Biochim. Biophys. Acta 591 (1980) 82.
16 M.J. Sculley, Biophys. Chem. 24 (1986) 33.
17 E. Schnepf, in: Biophysics, eds W. Hoppe, W. Lohmann, H. Markl and H. Ziegler (Springer, Berlin, 1983) p. 170.
18 M. Tomita and V.T. Marchesi, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 2964.
19 D.H. Heard and G.V.F. Seaman, J. Gen. Physiol. 43 (1960) 635.
20 S. Levine, K. Levine, A. Sharp and D.E. Brooks, Biophys. J. 42 (1983) 127.
21 R.W. Wunderlich, J. Colloid Interface Sci. 88 (1982) 385.
22 K. Sharp and D.E. Brooks, Biophys. J. 47 (1985) 563.
23 H. Oshima, K. Makino and T. Kondo, J. Colloid Interface Sci. 116 (1987) 196.
24 H. Oshima and T. Kondo, J. Theor. Biol. 124 (1987) 191.
25 H. Oshima and T. Kondo, J. Theor. Biol. 128 (1987) 187.

26 S.J. Miklavic and S. Marcelja, J. Phys. Chem. 92 (1988) 6718.
27 L. Lyklema, J. Electroanal. Chem. 18 (1968) 341.
28 H. Oshima and T. Kondo, Biophys. Chem. 32 (1988) 161.
29 C.F. Allen, P. Good, T. Trosper and R.B. Park, Biochem. Biophys. Res. Commun. 48 (1972) 907.
30 D.G. Hall and M.J. Sculley, J. Chem. Soc. Faraday II. 73 (1977) 869.
31 H. Oshima and S. Ohki, Biophys. J. 47 (1985) 673.
32 G.M. Bell and G.C. Peterson, J. Colloid Interface Sci. 41 (1972) 542.
33 R. Hogg, T.W. Healy and D.W. Fuerstenau, Trans. Faraday Soc. 62 (1966) 1638.