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Effects of fixed-charge distribution and pH on the electrophoretic mobility of biological cells

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Abstract: The electrophoretic mobility of biological cells is investigated theoretically. In particular, the effects of the distribution of the charges in the surface layer and the pH of bulk liquid phase on the mobility of cells are examined. The former includes the fixed charges due to the dissociation of the functional groups and the charges due to the penetrated electrolyte ions. The present analysis extends previous results in that the fixed charges are distributed nonuniformly across the surface layer of a cell. It is found that the distribution of the fixed charges in the surface layer has a significant effect on its electrophoretic mobility. Thus, assuming that the fixed charges are homogeneously distributed in the surface layer of a cell may lead to a significant deviation.

Key words: Electrophoretic mobility – cells – fixed charge – Donnan potential

Introduction

The electrophoretic behavior of biological cells in an electric field is a subject of interest in both fundamental research and practical applications. For instance, electrophoresis is one of the basic analytical tools to characterize both qualitatively and quantitatively the parameters of biocolloids. In this case, the simplest way to obtain a quantitative result is to treat colloids as solid particles, and apply Smoluchowski's theory [1]. This theory is based on the following major assumptions: 1) a particle is spherical, rigid, and nonconducting, 2) its radius is larger than the Debye length, 3) the surrounding fluid is unbounded, and 4) the zeta potential is uniform over the particle surface [2]. For a nonconducting rigid particle, the electric charges are located on its surface. It is often assumed that either the potential or the charge density on the surface remains unchanged. In the case of a biological cell, the assumptions leading to Smoluchowski's result become unrealistic. This is because the surface of a cell comprises various protein molecules appendage and dissociable functional groups. Therefore, rather than being a rigid wall, the surface of a cell is often penetrable to electrolytes. Furthermore, it is capable of adjusting its charge density through regulating the degree of dissociation of functional groups [3–8]. Clearly, the classic theory on electrophoresis needs to be modified to take these characteristics into account. However, this fact is often overlooked [9], presumably for an easier mathematical treatment.

Several attempts have been made to improve the classic rigid-wall model [9–12] for biological cells. In these studies, a surface layer comprises a charged polymer attached to the surface of a solid particle and is used to simulate the structure of a cell. The results obtained reveal that the electrophoretic mobility of a cell depends not only on the nature of the surface layer, but also on that of the solid-fluid interface. In a study of the electrostatic interactions between two membranes, Ohshima and coworkers [13–16] pointed out that the ions in the liquid phase may penetrate into the membrane-liquid interface. Furthermore, the dissociation of the functional groups in the membrane yields a fixed-charge distribution within the membrane [13]. The conventional

Poisson–Boltzmann equation has been modified to take these effects into account. The electrophoretic mobility of a large colloidal particle with a surface layer having a fixed uniformly distributed charge density is examined by Ohshima and Kondo [17]. In their investigation, the fixed charges are not due to the dissociation of functional groups.

In the studies of Ohshima and coworkers discussed above, the fixed charges distributed in the surface layer are assumed to be uniform for simplicity. Although the relevant studies are ample, a general rule for the description of the exact distribution for biological cells and membranes has not been established. Intuitively, the distribution of fixed charges is highly dependent upon the nature of a cell or membrane. In particular, various types of fixed-charge distribution can exist in synthetic membranes. For example, Mafe [18] found that some surface layers exhibit a local fixed-charge concentration that decreases from the solid-liquid interface to the interior of the surface layer. Conversely, other surface layers have a fixed charge increasing from the interface to the interior. It is found that the inhomogeneity of the distribution of fixed charges play an important role in determining the physicochemical properties, such as conductivity [18], current efficiency [19, 20], and transport phenomena [21] of a membrane. Ohshima et al. [22], for instance, examined the electrophoretic mobility of colloidal particles coated with a layer of charged polymers. This is a special case for non-uniform fixed-charge distribution. In an attempt to simulate the inhomogenity of fixed-charge distribution, Ohshima et al. [23] assume that a membrane can be divided into two sections, and each has a uniform fixed-charge distribution. The mathematical treatment is generalized to the case of an arbitrary continuous function by Hsu et al. [24]. It is concluded that the type of fixed-charge distribution has a significant effect on the electrostatic repulsion, potential, and pH distribution of two interacting membranes. In a recent study, Ohshima et al. [25] derived a general expression which is applicable for arbitrary non-uniform fixed-charge distribution.

In the present study, the electrophoretic mobility of an ion-penetrable cell having an arbitrary continuous fixed-charge distribution is examined. The fixed charges may arise from the dissociation of the functional groups in the surface layer.

Modeling

By referring to Fig. 1, we consider a large flat cell located in the space confined by two electrodes. The solution between the electrodes contains univalent electrolytes (i.e., $v = 1$, v being the valence of electrolytes) of concentration n_0. The cell is charge penetrable within a surface layer of width d. Due to the interaction of the electric field and the cell, the latter is moving parallel to the electric field.

Distribution of potential

Let $\psi(x)$ be the potential at position x measured relative to the bulk solution. By assuming that the linear size of a cell is much greater than the Debye length, the variation of space charge density is described by the following one-dimensional Poisson equation:

$$d^2\psi/dx^2 = -\rho(x)/\varepsilon_r\varepsilon_0 , \tag{1}$$

where $\rho(x)$ is the space charge density, and ε_r and ε_0 are the relative permittivity of the solution and the permittivity of vacuum, respectively. It can be shown that the spatial distributions of electrolyte ions are described by

$$n^- = n_0 \exp(ve\psi/kT) \tag{2}$$

$$n^+ = n_0 \exp(-ve\psi/kT) , \tag{3}$$

where $n^+(x)$ and $n^-(x)$ are the charge densities of positive ion and negative ion, respectively, e and k denote the elementary charge and the Boltzmann constant, respectively, and T represents the absolute temperature. The pH of the bulk liquid phase is fixed. The surface layer of a membrane contains univalent acidic ionizable groups. The dissociation of the functional groups in the surface layer and the resulted distribution of H^+ are described by

$$HA = H^+ + A^- \tag{4}$$

$$K = [H^+(x)][A^-(x)]/[HA(x)] \tag{4a}$$

$$[H^+(x)] = n_H \exp[-e\psi(x)/kT] . \tag{4b}$$

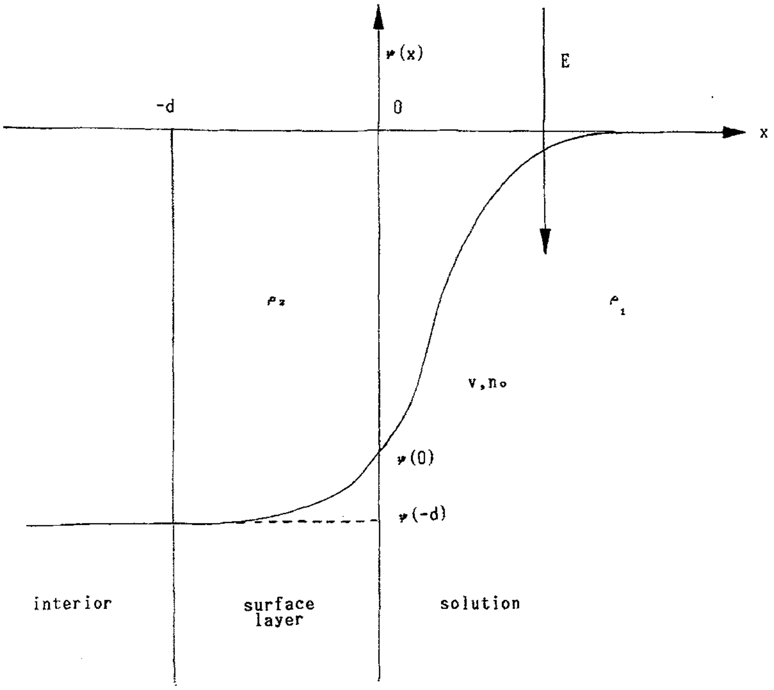

Fig. 1. A schematic representation of the system under consideration

In these expressions, n_H is the concentration of H^+ in the bulk liquid phase, K represents the equilibrium dissociation constant, $[H^+(x)]$, $[A^-(x)]$, and $[HA(x)]$ denote the concentrations of H^+, A^-, and HA in the surface layer, respectively, and $M(x)$ is the density of HA. The density of fixed charges in the surface layer, ρ_{fix}, is

$$\begin{aligned}\rho_{fix} &= -e[A^-]\\ &= -eM(x)/[1 + n_H \exp(-e\psi/kT)/K]\,,\end{aligned} \tag{4c}$$

where $M(x)$ denotes the distribution of ionizable functional groups initially present in the surface layer. The space charge density outside a cell, $\rho_1(x)$, and that inside the cell, $\rho_2(x)$, are respectively,

$$\begin{aligned}\rho_1(x) &= n_0 ev[\exp(-ve\psi/kT)\\ &\quad - \exp(ve\psi/kT)], \quad 0 < x < \infty\end{aligned} \tag{5a}$$

and

$$\begin{aligned}\rho_2(x) &= n_0 ev[\exp(-ve\psi/kT)\\ &\quad - \exp(ve\psi/kT)] + \rho_{fix}\\ &= n_0 ev[\exp(-ve\psi/kT) - \exp(ve\psi/kT)]\\ &\quad - eM(x)/[1 + n_H \exp(-e\psi/kT)/K],\\ &\quad -d < x < 0\,.\end{aligned} \tag{5b}$$

Equation (1) becomes

$$d^2\psi/dx^2 = -\rho_1(x)/\varepsilon_r\varepsilon_0, \quad 0 < x < \infty \tag{6a}$$

$$d^2\psi/dx^2 = -\rho_2(x)/\varepsilon_r\varepsilon_0, \quad -d < x < 0\,. \tag{6b}$$

The associated boundary conditions are

$$\psi \text{ is continuous at } x = 0 \tag{6c}$$

$$\psi \to 0 \text{ as } x \to \infty \tag{6d}$$

$$(d\psi/dx)_{0^-} = (d\psi/dx)_{0^+} \tag{6e}$$

$$(d\psi/dx)_{-d^-} = 0\,. \tag{6f}$$

The distribution of potential is obtained by solving Eqs. (6a) and (6b) subject to Eqs. (6c) through (6f).

Distribution of fluid velocity

The distribution of fluid is described by the Navier–Stokes equation [9–13, 17]

$$\eta\frac{d^2u}{dx^2} + \rho_1 E = 0, \quad 0 < x < \infty \tag{7a}$$

$$\eta\frac{d^2u}{dx^2} - \gamma u + \rho_1 E = 0, \quad -d < x < 0\,, \tag{7b}$$

where η and γ denote, respectively, the viscosity of fluid and the frictional coefficient of the surface layer, and E is the strength of electric field. The associated boundary conditions are

$$u = 0 \text{ at } x = -d \tag{7c}$$

$$u \text{ is continuous at } x = 0 \tag{7d}$$

$$(du/dx)_{0^-} = (du/dx)_{0^+} \tag{7e}$$

$$u \to -U \text{ as } x \to \infty \ , \tag{7f}$$

where U is constant. The mobility of a cell, defined by U/E, is determined by solving Eqs. (7a) and (7b) subject to Eqs. (7c) through (7f), and combining the result obtained from Eqs. (6a) through (6f).

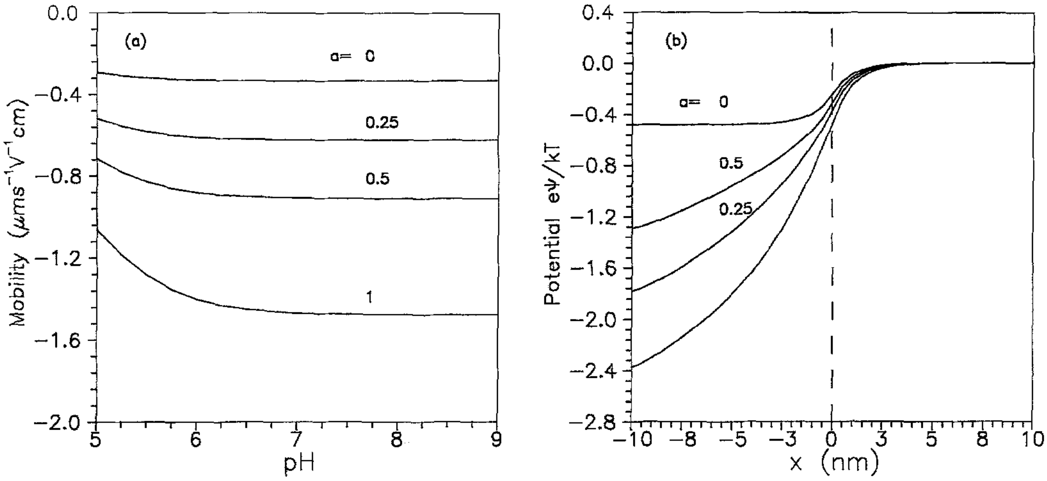

Fig. 2. (a) Variation in the mobility of a cell as a function of pH for positive values of a for the case $\lambda = 10^8\ \mathrm{m}^{-1}$ and $n_0 = 0.1$ mol/l. (b) The variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: $\eta = 10^{-8}$ g/cm·s, $E = 100$ V/m, $K = 10^4$ mol/l, $d = 10$ nm, $M_0 = 0.1$ mol/l, $v = 1$

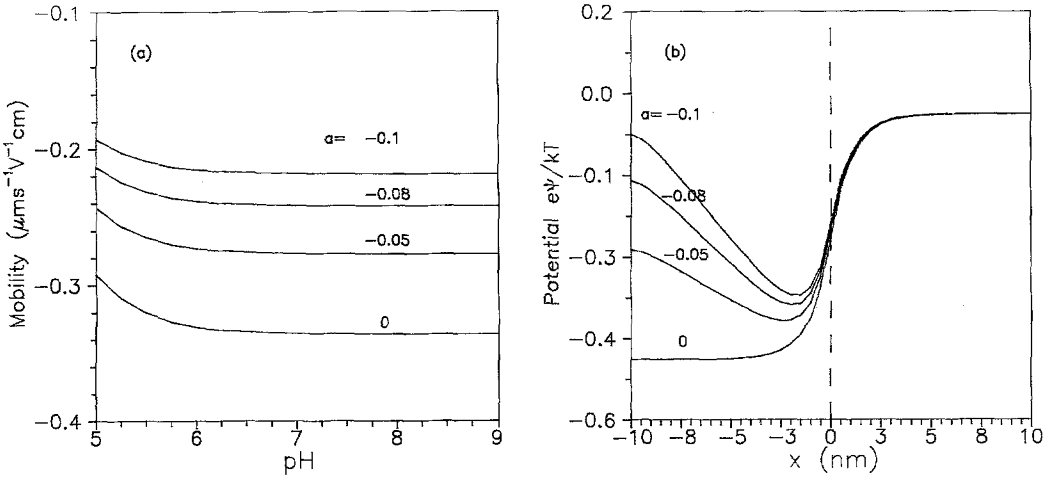

Fig. 3. (a) Variation in the mobility of a cell as a function of pH for negative values of a for the case $\lambda = 10^8\ \mathrm{m}^{-1}$ and $n_0 = 0.1$ mol/l. (b) Variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: same as that of Fig. 2

Results and discussions

Depending upon the nature of membranes, various types of distribution for fixed charge are possible [18]. Here, linear and nonlinear distributions for functional groups are chosen as an illustration. Since the width of surface layer is usually small, these distributions simulate a large class of possible smooth distributions.

Case 1. $M(x) = M_0(-ax + 1)$, $(-ax + 1) > 0$, a is constant

Here, M_0 is the value of $M(x)$ at $x = 0$. Figure 2(a) shows the variation in the mobility of a cell as a function of pH for positive values of a. For convenience, we define $\lambda = (\gamma/\eta)^{1/2}$. In the numerical simulation, we assume $T = 298\,^{\circ}\mathrm{K}$, $\varepsilon_r = 78.5$, and $K = 10^{-4}$ M. These values were suggested for carboxyl groups [15]. Figure 2(a) reveals that, for a fixed value of a, the mobility (absolute value) of a cell increases with pH. This is because the lower the pH, the higher the value of $[\mathrm{H}^+]$ in the solution. This results in a lower degree of dissociation of AH, and both A^- and ρ_{fix} in the surface layer are reduced. Therefore, the influence of electric field on a cell is less significant, and the mobility of the latter is lowered. On the other hand, the higher the pH, the higher the degree of dissociation of AH, and both A^- and ρ_{fix} are increased. This leads to a higher mobility. The mobility increases with pH and approaches to an asymptotic value. This is because as pH increases, both $[\mathrm{H}^+]$ and n_{H} decrease. The definition of ρ_{fix}, Eq. (4c), suggests that it approaches to $-eM(x)$, which is almost independent of n_{H}. Also, as will be discussed later, the variation in the distribution of potential is very limited. The parameter a is a measure of the degree of deviation of fixed charge in the surface layer from the uniform distribution ($a = 0$). The greater the absolute value of a, the greater the deviation. In the present case, since a is positive, the greater its value, the greater the amount of negative charge in the surface layer (referring to Eq. (4c)), and the greater the mobility of a cell. Figure 2(b) illustrates the variation in the distribution of potential as a function of distance for the case pH = 7. For a fixed x, the greater the value of a, the greater the negative value of ψ. This is because the greater the value of a, the greater the amount of fixed negative charge in the surface layer. For a fixed a, the potential decreases with the decrease of x, as expected.

The variation in the mobility of a cell as a function of pH for negative values of a is illustrated in Fig. 3(a). The qualitative behavior of the curves shown in this figure is similar to that of Fig. 2(a) for the same reasons discussed above. Note that, however, since a is negative in this case, the amount of negative fixed charge decreases with the decrease of x. The smaller the value of a, the less the amount of fixed negative charge in the surface layer, and the smaller the mobility. The variation in the distribution of potential as a function of distance for the case pH = 7 is shown in Fig. 3(b). The value of $M(x)$ decreases with the decrease of x. For a fixed value of a, the amount of fixed negative charge decreases with the distance towards the interior of a cell. In other words, the amount of positive charge increases with the decrease of x. Therefore, the potential decreases with the decrease of x first, passes through a minimum, and then increases with a further decrease in x. The smaller the absolute value of a, the less the deviation of $M(x)$ from a uniform distribution ($a = 0$), and the lower the potential. On the other hand, the larger the absolute value of a, the greater the deviation of $M(x)$ from a uniform distribution, the greater the amount of positive charge, and the higher the potential.

Figure 4 shows the variation in the mobility of a cell as a function of pH for different values of λ.

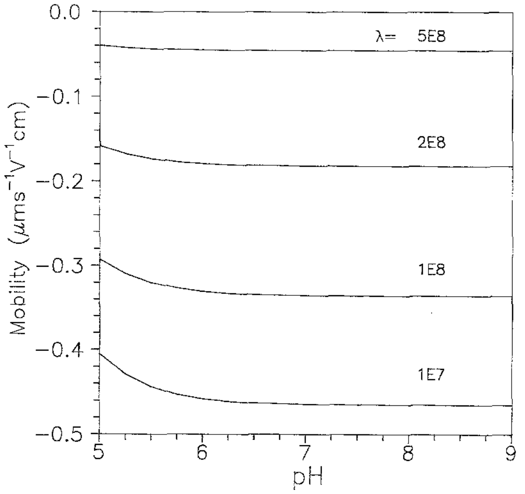

Fig. 4. Variation in the mobility of a cell as a function of pH for different values of λ for the case $a = 0$, and $n_0 = 0.1$ mol/l. Key: same as that of Fig. 2

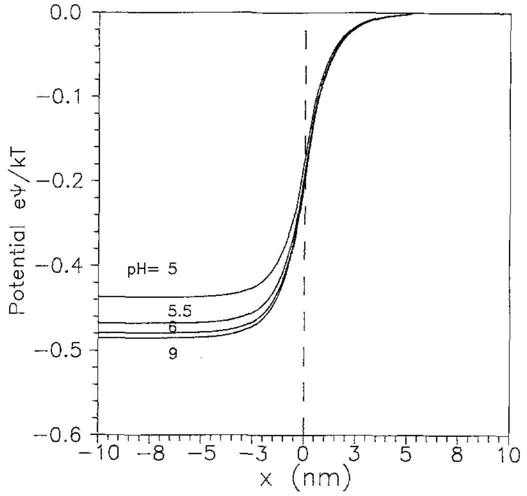

Fig. 5. Variation in the distribution of potential as a function of distance for different values of pH for the case $a = 0$, $\lambda = 10^7\ \mathrm{m}^{-1}$, and $n_0 = 0.1$ mol/l. Key: same as that of Fig. 2

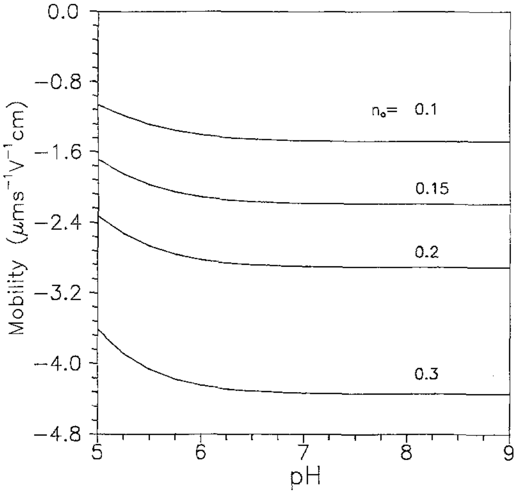

Fig. 7. Variation in the mobility of a cell as a function of pH for different values of n_0 for the case $a = 1$ and $\lambda = 10^8\ \mathrm{m}^{-1}$. Key: same as that of Fig. 2

The definition of parameter λ suggests that it is a measure of relative resistance for electrolytes in the surface layer and in the bulk liquid phase. For a fixed pH, the greater the value of λ, the smaller the mobility of a cell, as expected.

The variation in the distribution of potential as a function of distance for different values of pH for the case $a = 0$ (uniform distribution), $\lambda = 10^7\ \mathrm{m}^{-1}$, and $n_0 = 0.1$ mol/l is illustrated in Fig. 5. For a fixed x, the potential approaches to an asymptotic value as pH increases. This can be deduced by referring to the definition of ρ_{fix}, Eq. (4c).

Figure 6(a) shows the variation in the distribution of potential as a function of distance for different values of pH for the case $a = -0.05$, $\lambda = 10^8\ \mathrm{m}^{-1}$, and $n_0 = 0.1$ mol/l. In this case, since a is negative, ρ_{fix} decreases (or the amount of

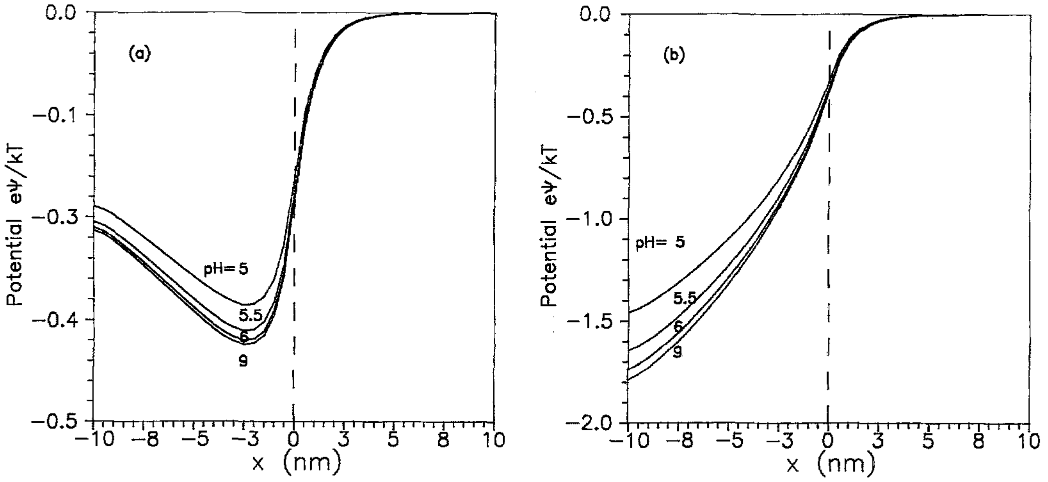

Fig. 6. (a) Variation in the distribution of potential as a function of distance for different values of pH for the case $a = -0.05$, $\lambda = 10^8\ \mathrm{m}^{-1}$, and $n_0 = 0.1$ mol/l. (b) Variation in the potential distribution as a function of distance for the case of part (a) except that $a = 0.5$. Key: same as that of Fig. 2

positive charge increases) with the decrease of x. The potential decreases with the decrease of x first, passes through a minimum, and then increases with a further decrease in x. For a fixed x, the effect of pH on potential is less significant for higher pH than that for lower pH. The reason for this is elaborated in the discussion of Fig. 5. Figure 6(b) illustrates the variation in the distribution of potential as a function of distance for the case of Fig. 6(a) except that $a = 0.5$. Since a is positive, the negative fixed charge increases linearly with the decrease of x, and the potential decreases accordingly.

The variation in the mobility of a cell as a function of pH for different values of n_0 is shown in Fig. 7. This figure reveals that, for a fixed value of pH, the mobility of a cell increases with the increase of n_0. This is because the thickness of

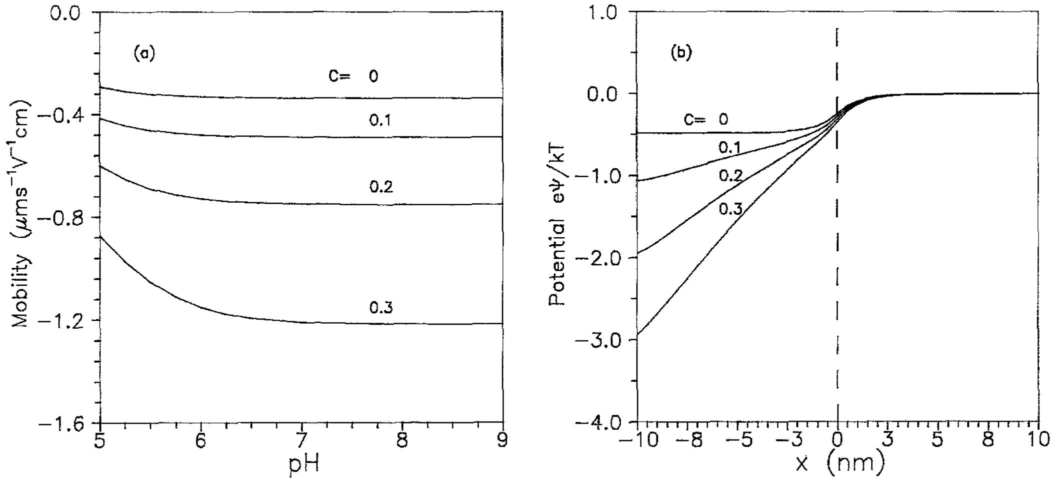

Fig. 8. (a) Variation in the mobility of a cell as a function of pH for positive values of c for the case $\lambda = 10^8\ \mathrm{m}^{-1}$ and $n_0 = 0.1$ mol/l. (b) Variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: same as that of Fig. 2

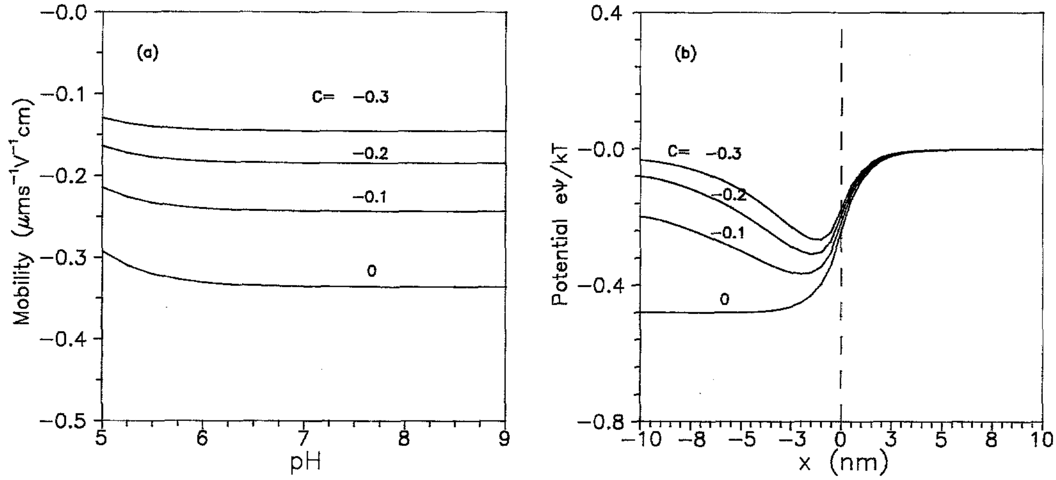

Fig. 9. (a) Variation in the mobility of a cell as a function of pH for negative values of c for the case $\lambda = 10^8\ \mathrm{m}^{-1}$ and $n_0 = 0.1$ mol/l. (b) Variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: same as that of Fig. 2

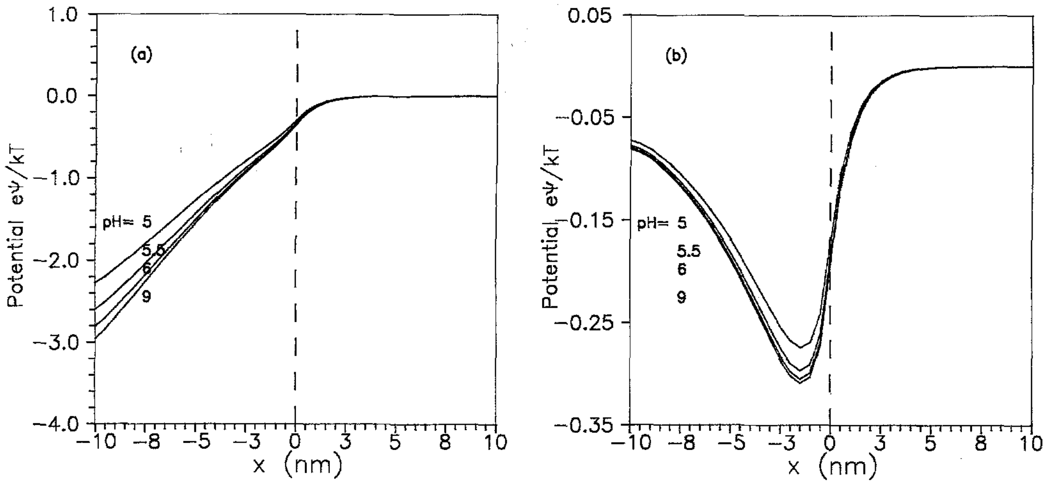

Fig. 10. (a) Variation in the potential distribution as a function of distance for different values of pH for the case $c = 0.3$, $\lambda = 10^8\ \mathrm{m}^{-1}$, and $n_0 = 0.1$ mol/l. (b) Variation in the distribution of potential as a function of distance for the case of part (a) except that $c = -0.2$. Key: same as that of Fig. 2

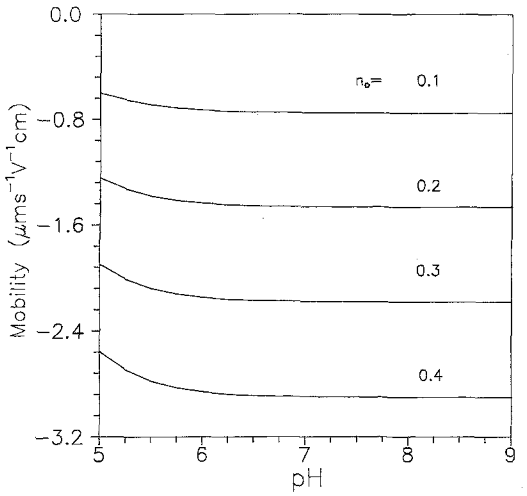

Fig. 11. Variation in the mobility of a cell as a function of pH for different values of n_0 for the case $c = 0.2$ and $\lambda = 10^8\ \mathrm{m}^{-1}$. Key: same as that of Fig. 2

electrical double layer decreases with the increase of n_0, the potential decreases accordingly, and the absolute mobility increases. The absolute mobility increases with the increase of pH, and approaches to an asymptotic value as pH increases for the same reason as that discussed in Fig. 2(a).

Case 2. $M(x) = M_0 \exp(-cx)$, c is constant

Figure 8(a) shows the variation in the mobility of a cell as a function of pH for positive values of c. The variation in the distribution of potential as a function of distance for the case pH = 7 is illustrated in Fig. 8(b). The variation in the mobility of a cell as a function of pH for negative values of c is shown in Fig. 9(a). The variation in the distribution of potential as a function of distance for the case pH = 7 is presented in Fig. 9(b). The qualitative behavior and its interpretation of Figs. 8(a), 8(b), 9(a), and 9(b) are similar to those of Figs. 2(a), 2(b), 3(a), and 3(b), respectively.

Figure 10(a) illustrates the variation in the distribution of potential as a function of distance for different values of pH. In this case, since c is positive, the negative fixed charge in the surface layer increases exponentially with the distance towards the interior of a cell, and the potential decreases accordingly. For a fixed x, the effect of pH on potential is pronounced at a lower pH for the same reason as that discussed in Fig. 5. The variation in the distribution of potential as a function of distance for the case of Fig. 10(a) except that $c = -0.2$ is shown in Fig. 10(b). Since c is negative, the negative fixed charge decreases with the decrease of x or the positive charge increases with the decrease of x. Therefore, the potential decreases with the decrease of x, passes through a minimum, and then increases with a further decrease in x. Again, the effect of pH on potential

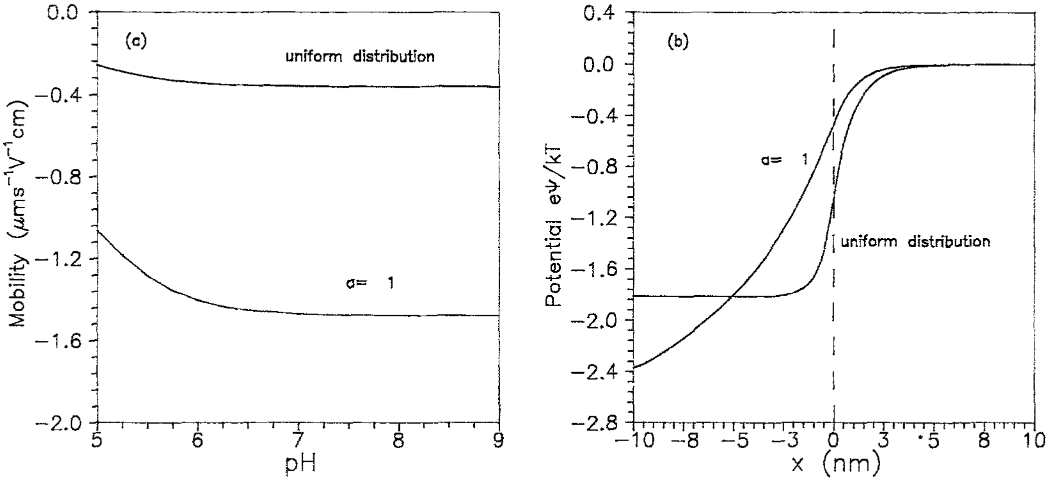

Fig. 12. (a) Variation in the mobility of a cell as a function of pH for both uniformly distributed fixed charges and linearly varied fixed charges for the case $\lambda = 10^8$ m^{-1} and $n_0 = 0.1$ mol/l. The average density of the fixed charges is the same for two cases. (b) The variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: same as that of Fig. 2

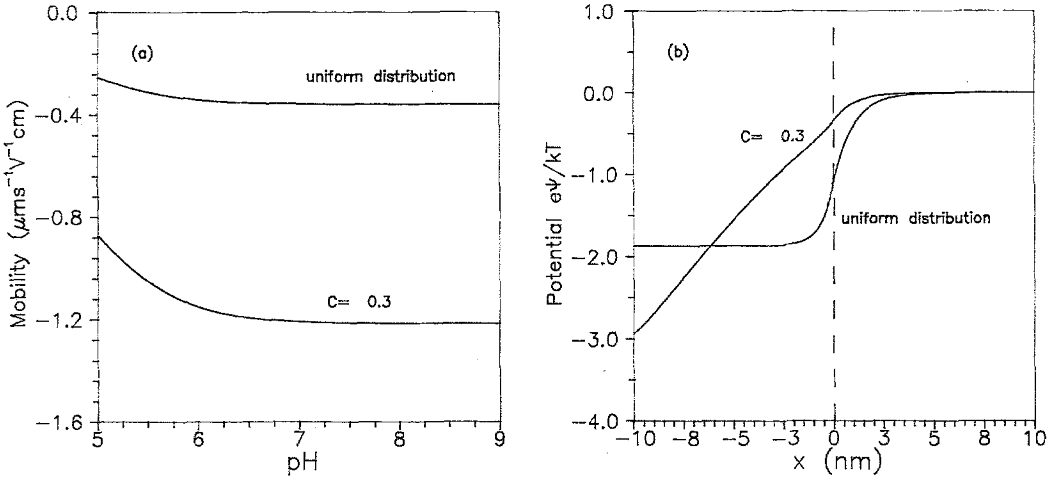

Fig. 13. (a) Variation in the mobility of a cell as a function of pH for both uniformly distributed fixed charges and exponentially varied fixed charges for the case $\lambda = 10^8$ m^{-1} and $n_0 = 0.1$ mol/l. The average density of the fixed charges is the same for two cases. (b) The variation in the distribution of potential as a function of distance for the case of part (a) at pH = 7. Key: same as that of Fig. 2

is pronounced at a lower pH. The variation in the mobility of a cell as a function of pH for different values of n_0 is illustrated in Fig. 11. A similar conclusion as that obtained from Fig. 7 can be drawn.

It should be pointed out that using an average value of a non-uniform fixed-charge distribution to simulate its effect on the relevant physical properties may lead to a significant deviation, as illustrated in Figs. 12 and 13. In these figures, the density of the fixed charges for the case of uniform fixed-charge distribution is the same as the average density of the fixed charges for the case of non-uniform fixed-charge distribution. As can be seen from these figures, knowing the exact fixed-charge distribution is essential.

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