ELECTROPHORETIC MOBILITY OF HUMAN ERYTHROCYTES
On the Applicability of the Charged Layer Model

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ABSTRACT The limitations of previous linear electrokinetic theories are discussed. A special model of the surface charge distribution, based on the minimum condition of the interfacial electrostatic free energy, is introduced. The model describes the electrophoretic mobility, taking into account the electroosmotic flow through the surface macromolecular layer and the surface conductivity. This nonlinear electrophoretic theory describes experimental data obtained with human erythrocytes. Numerical results for an uniformly distributed space charge are also presented.

INTRODUCTION
There is a discrepancy between the observed electrophoretic mobility of human erythrocytes and the mobility calculated using Smoluchowski's formula and biochemical data of the net surface charge density. This discrepancy has been the subject of several papers (Vassar et al., 1969; see also the references listed by Levine et al., 1983).

Two other major problems in applying the Smoluchowski equation to erythrocytes are the observed dependency of zero-mobility on ionic strength (Heard and Seaman, 1961; Nordt et al., 1978; Donath and Steidel, 1980) and the calculated increase in charge density with increasing ionic strength. Both these observations are inconsistent with a Gouy-Chapman diffuse double layer adjacent to a smooth surface. These inconsistencies have been observed with other surfaces. There have been several attempts to explain these discrepancies. Lyklema and Overbeek (1961) discussed the influence of a changed viscosity near the interface. Hunter (1960) accounted for the nonconstant surface charge by taking into account the finite size of the counter ions. Heard and Seaman (1960) explained the increase in charge density in terms of an adsorption of co-ions using a Langmuir isotherm. This interpretation was criticized by Haydon (1961a) because of the lack of specificity. Haydon (1961b) pointed out that a permeable surface would increase the calculated surface charge density, and discussed the dispersion of ionogenic groups in large regions of nonionogenic surface material. Haydon and Seaman (1962) published a qualitative treatment of the porosity of the surface region.

Parsegian and Ninham (1970) introduced a fuzzy layer of considerable thickness adjacent to the surface. Their quantitative calculations showed that the electrokinetic potential does not reflect the true charge density when using the classical surface potential-surface charge density relationship. They used a model of surface charge distribution that discussed this paper within the framework of a nonlinear theory. But they did not allow for an electroosmotic flow inside the fuzzy layer. Dukhin and Deryaguin (1976) discussed hydrodynamic flow properties near an interface under different conditions. The effect of a porous surface region on the electrokinetic properties, however, was discussed only in qualitative terms.

It was then that new quantitative theories of the hydrodynamic flow near an interface started to appear. Thus Pastushenko and Donath (1976) derived an expression for the electrophoretic mobility for a surface region with constant friction, thereby allowing for an electro-osmotic flow through this layer. This problem was solved for the case of arbitrary distributions of fixed charges inside this layer. However, this theory was only valid within the limitations of the linearized Poisson-Boltzmann equation. Later Donath and Pastushenko (1979, 1980) investigated several special cases of charge distributions. Donath and Lerche (1980) then compared this theory with the electrophoretic mobility of human erythrocytes and determined the surface-charge density, the layer thickness, and the friction coefficient inside the layer in relation to the degree of neuraminidase treatment. They also recognized the molecular origin of the friction and made a comparison between the friction coefficient obtained by fitting and a

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Theoretical estimate, taking the layer molecules as cylinders. Jones (1979) independently derived an identical expression for electrophoretic mobility with constant space charge density throughout the layer. Later Wunderlich (1982) again derived an identical expression applicable for arbitrary fixed charge distributions inside the layer. He assumed an independent flow inside the layer and could calculate the friction coefficient regarding the macromolecules as chains of spheres. He also recognized the failure of the previous papers to predict zero-mobility at very high ionic strength. For this reason he used a Padé-approximation to combine the classical expression and the new theory. Another paper in this sequence was published by Levine et al. (1983) who again arrived at an identical expression for three different charge distributions that they compared with experimental data concerning human erythrocytes, and they found practically identical parameters, as was the case in the paper of Donath and Lerche (1980). They also regarded the macromolecules as chains of spheres.

Quite recently, McDaniel et al. (1984, 1985) applied the linear and nonlinear theoretical approaches to electrophoretic data of liposomes containing glycolipids.

This paper will discuss the obtained fits of human erythrocyte electrophoretic mobility data, using the above discussed linear models, to demonstrate their limitations and to show that a nonlinear theory and a better understanding of the surface charge distribution does indeed give theoretical predictions that closely correspond to the experimental data.

**GLOSSARY**

Key of symbols:

- $a$: friction coefficient
- $b$, $b^{exr}$: electrophoretic mobilities
- $cosh$, $sinh$, $tanh$: hyperbolic functions
- $e_0$: elementary charge
- $f$: current
- $J$: volume flow
- $K$, $K_1$, $K_2$, $K_3$: conductivities
- $k$: Boltzmann constant
- $L_q$: Onsager's coefficients
- $n_i^*$, $n_0$: ionic concentrations
- $p$: pressure
- $r$: particle radius
- $R$: ionic mobility
- $x$: coordinate
- $z_i$, $z$: charge numbers

Greek symbols:

- $\Delta$: layer thickness
- $\delta$: relative dielectric
- $\varepsilon$: constant and absolute permittivity of vacuum
- $\eta$: viscosity
- $\kappa$: Debye-Hückel parameter
- $\rho$: space charge density
- $\sigma$: surface charge density

**THEORY**

Recently, we have been able to extend the above-discussed linear theory of electrophoretic mobility to cover nonlinear treatment (Donath and Voigt, 1985). Originally, we were compelled to do this because streaming current and streaming potential measurements can be achieved successfully only at low ionic concentrations. This is exactly the reason why the Padé-approximation of Wunderlich (1982) is of lesser practical importance, as most electrophoretic measurements could be performed only in low ionic concentrations. Unfortunately, under these conditions linearization of the Poisson-Boltzmann equation would yield erroneous results.

If one makes streaming current measurements, a pressure gradient instead of an electric field is applied. This gives a completely different flow velocity profile near the interface, as compared with electrophoretic or electroosmotic measurements. But if we apply linear irreversible thermodynamics, it immediately becomes clear that the final theoretical result is not influenced when the electroosmotic flow, induced by an electric potential gradient, is calculated or the electric current induced by a pressure gradient

$$I = L_{11} \Delta \phi + L_{12} \Delta \rho \quad (1)$$

$$J = L_{21} \Delta \phi + L_{22} \Delta \rho. \quad (2)$$

Here $I$ is the electric current, $J$ the volume flow, $\Delta \rho$ the pressure difference and $\Delta \phi$ the electric potential difference. The kinetic coefficients $L_{11}$ and $L_{21}$ should be identical according to Onsager's law. So all the above-discussed linear theories are equally applicable to other inner electrokinetic phenomena. Only the respective coefficients should be specified according to Eqs. 1–2. The special equivalent relation between the electroosmotic and the electrophoretic phenomena makes possible the bridging of the gap between the inner and outer electrokinetic (Dukhin and Deryaguin, 1976).

Unfortunately, we have not been able to find an analytical expression for the electrophoretic mobility for arbitrary charge distributions without linearization of the Poisson-Boltzmann equation. However, there is one special distribution of charges for which an analytical expression of the electrokinetic effects can be derived. In the case of linear treatment we have discussed this distribution extensively elsewhere (Donath and Voigt, 1983). It consists of a constant space-charge distribution inside the macromolecular layer and a surface charge located at the macromolecular layer-bulk liquid interface. The sum of both is the net surface charge, which is constant. But the ratio of space and outer surface-charge density essentially depends on ionic strength. The lower the ionic strength, the greater the charge that is located at the outer edge of the surface layer.
This distribution is characterized by a zero electric field inside the surface layer. And this is the reason why this sort of surface charge distribution has the lowest interfacial electrostatic free energy. Parsegian and Ninham (1970, unpublished manuscript: A major artifact in cell surface charge measurements by electrophoresis due to the presence of cell peripheral material) worked with the same distribution of fixed surface charges, minimizing the electrostatic energy.

In fact, a zero electric field inside the layer and a consequently constant electric potential make the above-described distribution very similar to a Donnan equilibrium, the only difference being the existence of one finite phase—the surface macromolecular layer.

Of course, this distribution is only a rough approximation of reality, whereas for the state of minimum free energy, chemical and mechanical contributions are just as important as electrical contributions. However, at present it is virtually impossible to take into account all of these factors to determine the charge distribution within a glycoplycalyx, because most of the necessary parameters are unknown. Recently, Feudel and Ebeling (1982) tried to consider elastic deformations together with electric forces to describe energetically, in numerical terms, a macromolecular layer. Van der Schee (1984) was also able to describe the distribution of charges in model systems in numerical terms based on the simultaneous assessment of electrical and entropic factors.

We are aware of the limitations connected with our approach. Nevertheless, we will discuss below this special distribution of charges in detail, because it has the great advantage of yielding an analytical expression for the electrophoretic mobility. As such, it allows one to analyze the influence of different parameters quite easily. In particular, the main qualitative features of the influence of the space charge on the electrophoretic mobility can be demonstrated very clearly. For the real system, however, we will also look at a constant charge distribution in numerical terms, since it describes the behavior of the human erythrocyte over the whole ionic strength region more adequately (Fig. 2).

Let us now briefly discuss the quantitative results of the model of minimum-free electric energy. A more comprehensive analysis in the context of streaming current and streaming potential is given elsewhere (Donath and Voigt, 1985).

The first question to be answered is that of the value of the potential difference between the surface ($0 < x < \delta$) and infinity $\psi_o$. We solve the following set of three equations:

$$\rho = -\varepsilon_0 \sum_n n^0_i z_i e^{-\alpha n_i \rho T}$$  \hspace{1cm} (3)

$$\sigma = \left[2kT\varepsilon_0 \sum n^0_i (e^{-\alpha n_i \rho T} - 1)\right]^{1/2}$$  \hspace{1cm} (4)

$$\sigma_o = \rho \delta + \sigma,$$  \hspace{1cm} (5)

where $\delta$ is the thickness of the macromolecular layer, $\rho$ is the space charge density, $\sigma$ the outer surface charge density, $\sigma_o$ the total surface charge density and $n^0_i$ are the ionic concentrations in the bulk of the electrolyte. $\varepsilon_0$, $z_i$, $k$, $T$, $\varepsilon$, $\epsilon$ have their usual meaning.

If there is a symmetrical $\pm z$-valent electrolyte an explicit expression connecting the inverse of the Debye-Hückel length $\kappa^{-1}$ and the potential difference $\psi_o$ can be found

$$\kappa = \frac{1}{2\delta \cosh \left(\frac{\varepsilon_0 kT}{2e^0 z_o}\right)} \left(1 + \frac{2e_0 \delta z_o}{e_0 kT \tanh \left(\frac{2e_0 z_o}{2kT}\right)}\right)^{1/2} - 1.$$  \hspace{1cm} (6)

Outside the macromolecular layer the electric potential as a function of distance can be given implicitly

$$x - \delta + \left(\frac{\varepsilon_0 kT}{2z^2 e_0 ^2 \rho_o}\right)^{1/2} \ln \left(\frac{\tanh \left(\frac{z e_0 \psi_o}{4kT}\right)}{\tanh \left(\frac{z e_0 \psi(x)}{4kT}\right)}\right).$$  \hspace{1cm} (7)

where the coordinate $x$ has its origin immediately at the membrane surface. So, we easily derive the expression for the electrophoretic mobility $b$, analytically taking into account the electroosmotic flow through the layer

$$b = \frac{1}{\eta} \left(\varepsilon_0 \psi_o + \sinh \left(\frac{z e_0 \psi_o}{2kT}\right) \left(\frac{1}{2} \tanh \left(\frac{a \delta}{T}\right) \right) \right) + \frac{4e_0 \rho_o \cosh \left(\frac{z e_0 \psi_o}{2kT}\right)}{a^2} \left(1 - \frac{1}{\cosh \left(\frac{a \delta}{T}\right)}\right).$$  \hspace{1cm} (8)

Here, $a$ denotes the friction coefficient as in the linear theories and $\eta$ denotes the viscosity.

Eq. 8 gives the electrophoretic mobility in quite a managable form, and can be more easily used than the linear theories published. It has the great advantage of demonstrating the inapplicability of the Zeta-potential concept most clearly. Indeed, it shows that the mobility is related to the surface potential $\psi_o$ in a nonlinear form. If, however, the friction inside the layer is infinitely high, that is, no electroosmotic flow inside the layer occurs ($a \to \infty$), only the first term in Eq. 8 is different from zero and this is Smoluchowski's formula.

The derived charge distribution model has further important consequences. It allows us to derive an analytical expression for the surface conductivity, which is a very important correcting factor for streaming potential measurements and significantly decreases the electrophoretic mobility by short-circuiting the external potential difference at the particle surface if the ionic strength is sufficiently small.
Usually the surface conductivity correction at moderate ionic strength is negligible, but in the layer model with a significant electroosmotic flow inside the layer the convective part of the surface conductivity may exceed the migration part by orders of magnitude. In the Bikerman theory of a classic Gouy-Chapman diffuse double layer the convective part is usually not >20% of the value of the migration part. The convective surface conductivity $K_c$ becomes

$$K_c = \frac{8kTn_0\alpha_0\delta}{\eta} \sinh^2\left(\frac{ze\phi_0}{2kT}\right) \left[\frac{\tanh(ab)}{ab}\right]$$

$$ \cdot \left(1 - \frac{k^2}{a^2}\cosh\left(\frac{ze\phi_0}{2kT}\right)\right)$$

$$ + \frac{k^2}{a^2}\cosh\left(\frac{ze\phi_0}{2kT}\right) \left[\frac{\cosh\left(\frac{ze\phi_0}{2kT}\right)}{\cosh\left(\frac{ze\phi_0}{2kT}\right) + 1}\right]$$

(9)

And the migration part of the surface conductivity $K_m$ is now

$$K_m = ze\phi_0 \sum_{i=1}^{2} u_i \left(\exp\left(-\frac{ze\phi_i}{2kT}\right) - 1\right) + \frac{2}{k}\left(\exp\left(-\frac{ze\phi_0}{2kT}\right) - 1\right),$$

(10)

where in the last equation $u_i$ is the mobility of the anion and cation, respectively. $z_i$ should be equated to $-z$ and $+z$, respectively. In Eqs. 9 and 10 the last terms represent the Bikerman theory.

Levich (1952) criticized Bikerman’s approach (1933, 1934, 1935), where he added up the convective and migration part of the surface conductivity. Since he did not provide a quantitative analysis along with his criticism, Levich’s conclusion remained questionable. Deryaguin and Dukhin (1969) reexamined this problem again by means of a rigorous analysis applying the Navier-Stokes equation and came to the conclusion that Bikerman’s original approach is completely justified.

Independently, Henry (1948) and Booth (1948) derived the correction of the electrophoretic mobility for surface conductivity, $K_s = K_c + K_m$

$$b^{orr} = b \frac{K}{K + K_c/r},$$

(11)

where $K$ is the bulk electrolyte conductivity and $r$ the particle radius.

Recently, Dukhin and Deryaguin (1976) showed that a better equation is

$$b^{orr} = b \frac{1 + \text{Rel}}{1 + 2\text{Rel}},$$

(12)

where $\text{Rel} = K_d/K_r$. However, Eqs. 11 and 12 do not differ significantly if $\text{Rel} << 1$. Generally, the correction of the electrophoretic mobility given above is meaningful only if the particle radius is much greater than the Debye-Hückel length, and also if the surface conductivity is not too large. Otherwise, relaxation phenomena should be taken into account (Booth, 1950; Overbeek, 1943). However, the calculation of relaxation correction for electrophoretic mobility of particles coated with a macromolecular layer until now remains an unsolved problem.

Before starting to compare the theory with experimental results, we shall describe two further features of the special surface charge distribution. The first point is that at low ionic strengths when $1/k$ is much larger than $\delta$ Eqs. 8 to 10 equally well describe any given charge distribution inside the layer. At high ionic strengths Eqs. 8–10 approach the solution for uniform charge distribution inside the layer, that is, the model used by Levine et al. (1983).

Comparison with Experimental Results

Instead of plotting the mobility directly against the concentration, we prefer to plot the quotient of the mobility and the Debye-Hückel length $b \times k$ against the inverse of the Debye-Hückel length.

In the linear electrophoretic theory this immediately gives the charge density if we take into account the viscosity. The classical theory is thus represented by a parallel to the $x$-axis. Below we use the data from Levine et al. (1983) when referring to the electrophoretic mobility of human erythrocytes. The shape of the replotted curve is very similar to our values (Donath and Lerche, 1980). However, the absolute values we obtained previously were slightly smaller, probably due to differences in the measuring system.

The solid curve in Fig. 1 represents the experimental results of Levine et al. (1983) translated onto our graph. This plot gives a much better idea of the characteristic behavior of the erythrocyte mobility as a function of ionic strength. Instead of a steeply rising mobility with decreasing electrolyte concentration, we observe a characteristic sigmoidal shape if physically appropriate axes are used. The dashed line represents calculated values according to Eq. 8 and corrected for NaCl (Dobos, 1975) according to Eq. 11. The thickness $\delta$ was assumed to be 10 nm.

The decrease in the mobility due to the surface conductivity was 7% at 10 mM using both Eqs. 11 and 12. At 1 mM this decrease was 16% using Eq. 11, and 14% using Eq. 12. We tried also to take into account the effect of surface pH on the dissociation equilibrium of the ionogenic groups. However, since the physiological pH is by ~4 pH units greater than the $pK$ of sialic acid, we did not observe any significant decrease in mobility for salt concentrations as low as 1 mM.

The dashed line in Fig. 1 broadly predicts the shape of the experimental curve correctly. However, there is still a
significant difference at low ionic strengths. In contrast, the linear theory shows a steep increase of the theoretical curve (dotted line), beginning at \(5 \times 10^8 \text{ m}^{-1}\), reaching the ordinate value of \(3.55 \times 10^{-2} \text{ As/m}^2\) at zero ionic strength, which is far above the plotted area. The dashed curve was plotted using a friction coefficient \(a = 8.75/\delta\), which is higher than the values found by fitting previously, with the use of linear constant space charge models (Donath and Lerche, 1980; Levine et al., 1983).

This difference can be explained by the presence of
surface charges at the outer edge of the macromolecular layer that more effectively contribute to electrophoretic mobility within the present model. We were not able to overcome the persistently occurring difference at low ionic strengths, even when we assumed a change in thickness and in the friction coefficient within the framework of the special model applied. The reason for this is that at low ionic strengths the model of charge distribution used results in a high surface charge density at the surface layer-bulk electrolyte interface. The fact that the experiment gives lower values can be taken into account using a charge distribution with a higher amount of space charge within the macromolecular layer and less surface charge at $x = \delta$, as was the case in Fig. 2 (dashed line), namely, $\rho(x) = \text{const.}, \sigma = 0$. Since we could not find an analytical solution for this charge distribution the calculations in Fig. 2 were done numerically. We also assumed that there is a transition in the structure of the surface layer induced by the increasing repulsion of the fixed charges at low electrolyte concentrations (Donath and Lerche, 1980). The total charge of the glycocalyx, of course, was unchanged and had the same value as in Fig. 1. Now the dotted line represents the effect of a constant charge distribution with a compressed surface layer (thickness 5.5 nm) and a friction coefficient of $5.3/\delta$, which is very close to our previously determined values using the linear theory. The dashed line represents the effect of an increase in the thickness ($\delta \sim 12$ nm), leaving the constant charge distribution (Donath and Lerche, 1980) unchanged. The friction coefficient in this case is $5.5/\delta$.

The failure of the special model observed in Fig. 1 at low ionic strengths can be understood if one considers the real physical constraints that exist. As the surface ionogenic groups are attached to macromolecules, they cannot cluster together unrestrained at the outer border of the layer. Such a tendency could, however, occur in a certain form if there is a macromolecular reorientation.

There is another point that should be discussed here. If this macromolecular reorientation indeed takes place then it should also have effects on the friction parameter. The two curves in Fig. 2 suggest an inverse linear dependence of the friction coefficient $a$ on the thickness $\delta$ ($a \times \delta \sim \text{const.}$). Levine et al. (1983), however, suggest that there is an inverse linear relationship between the square root and the thickness. This assumption is based on consideration of independent hydrodynamic friction at the segments of the surface macromolecules. This would be true only in the case of very diluted segment densities. The validity of this assumption under the conditions used needs to be proved.


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