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Biophysical Chemistry 113 (2005) 115–122

Biophysical Chemistry

www.elsevier.com/locate/bpc

Size-dependent mobile surface charge model of cell electrophoresis

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Received 2 July 2004; received in revised form 21 July 2004; accepted 21 July 2004
Available online 29 September 2004

Abstract

A model that accurately predicts the effects of cellular size and electric field strength on electrophoretic mobility has been developed. Previous models have predicted that electrophoretic mobility (EPM) is dependent only on cell surface charge, bath viscosity and ionic strength of the electrolyte. However, careful analysis of experimental data from the literature shows that these models do not accurately depict the relationship between chemically determined surface charge and observed mobility. We propose a new model that accounts for electrically driven redistribution of mobile surface charge islands, such as the recently proposed lipid raft structures. This model predicts electrophoretic mobility as a function of a new dimensionless quantity, A, that incorporates the cell radius, the electric field strength, and the average diameter of charged membrane complexes.

Keywords: Electrophoretic mobility; Cell electrophoresis; Cell surface charge; Lipid rafts; Mathematical model

1. Introduction

1.1. Electrophoretic theory

Electrophoresis of living cells is a phenomenon well-studied for its potential impact on the study of cell physiology and the practice of cell separation and identification [1]. Eukaryotic cells exhibit a negative net surface charge and therefore migrate toward the positive pole of an electrophoretic chamber with a velocity, U, proportional to the applied electric field strength, E_0. The relationship between electrophoretic velocity and field strength is typically presumed to be linear, such that $U=\mu E_0$, where the proportionality constant, α, is known as the electrophoretic mobility (EPM). The EPM of mammalian cells has been observed to fall into the range of approximately 0.3–4.0 μm/s per V/cm for a variety of mammalian cell types [2].

The classical Smoluchowski expression for the EPM of spherical particles, Eq. (1), represents the EPM as a function of the particle's surface potential, ζ, as well as the viscosity, η, and permittivity, ε, of the surrounding electrolyte solution [3].

$$\alpha = \varepsilon\zeta/\eta \tag{1}$$

Huckel's correction [4] to the Smoluchowski equation takes into account the non-uniformity of the electric field around spherical particles, ultimately adding a 2/3 correction factor to Smoluchowski's formulation (Eq. (2)).

$$\alpha = 2/3\ \varepsilon\zeta/\eta \tag{2}$$

Many cell electrophoresis experiments use either Eq. (1) or Eq. (2) to calculate zeta potentials from measured EPM values, but the zeta potential changes with the surrounding electrolyte concentration and is therefore not an intrinsic parameter of the cell.

It is well known in studies of nucleic acid electrophoresis that the number of charged groups is the primary factor in explaining electrophoretic mobility differences [5]. Similarly, the surface charge density of a cell, σ, is thought to

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0301-4622/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.bpc.2004.07.034

provide a better explanation of a cell's electrophoretic mobility than the zeta potential. Since the surface charge is dependent only on the molecular composition of the cell membrane, σ is much closer to being an intrinsic property of a given cell than ζ, making it a more useful parameter for comparing the surface properties of two different cell types. It is worth noting, however, that since most of the ionizable groups that contribute to cell surface charge are organic acids or bases, the surface charge is highly dependent on the pH of the electrolyte bath in which the measurement is performed.

For these reasons, it is desirable to calculate σ from measurements of α. The simplest model is given by the Gouy-Chapman theory (Eq. (3)), which relates zeta potential to surface charge via the Debye-Huckel parameter, κ, and the solution permeability, ε.

$$\zeta = \sigma/\ \varepsilon\kappa \tag{3}$$

The net result of combining Eqs. (1) and (3) is given in Eq. (4a); the equivalent result with the Huckel formulation (Eq. (2)) is given in Eq. (4b). It should be noted that both of these models assume a fixed, even charge distribution on the cell surface.

$$\alpha = \sigma/\kappa\eta \tag{4a}$$

$$\alpha = 2/3\ \sigma/\kappa\eta \tag{4b}$$

1.2. Disagreements with literature data

As researchers began to link EPM data with biochemical analyses of cell membranes, and as additional cell types with more-complicated surface chemistries were characterized, it became apparent that Eq. (4b) underestimated cell surface charge from mobility data, perhaps by as much as an order of magnitude [1]. A recently developed quantitative colloid titration method [6] for accurately determining cell surface charge has enabled an accurate picture of this underestimation. For the red blood cells with which much of the early electrophoresis work was done, the charge predicted by Eq. (4b) agrees within a factor of 2 with the colloid titration number, but the Huckel theory significantly underestimates the charge of bovine endothelial cells [6].

For comparison of experimental mobilities with those predicted from known surface charges, a new quantity, $\hat{u}$, is defined by Eq. (5) as the observed mobility divided by the mobility formula defined in Eq. (4a).

$$\hat{u} = \alpha/(\sigma/\eta\kappa) \tag{5}$$

Table 1 shows $\hat{u}$ for several cell types and experimental conditions calculated from a survey of values reported in the literature.

Unless otherwise noted, electrophoretic mobilities and the associated electric field strengths and electrolyte concentrations were obtained from a seminal "database" of such values [2]. Cell radii, not often reported in electrophoresis experiments, were determined as accurately as possible from related literature, and $\hat{u}$ values were calculated with the additional assumption that all experiments took place at a temperature of 25 °C in a bath with viscosity equal to that of pure water, 0.001 Pa s. For simplicity, only values measured in neutral-pH environments were used.

Table 1
Analysis of reported values for cell surface charge and electrophoretic mobility

Cell type	Abbrev.	a (μm)	n (charge per cell$\times 10^{-9}$)	C_0 (mM)	E_0 (V/cm)	α (μm cm /V s)	$\hat{u}$[a]
Human erythrocyte	RBC01	3.0[b]	0.032[c]	145	2	1.25	0.368
	RBC02			145	3.2	1.04	0.300
	RBC03			145	3.2	1.38	0.406
	RBC04			150	3.22	0.99	0.297
	RBC05			15	3.5	2.79	0.260
	RBC06			145	4	1.14	0.336
	RBC07			145	4	1.03	0.303
	RBC08			145	4.3	1.01	0.297
	RBC09			145	4.54	1.04	0.306
	RBC10			150	5	1.11	0.333
	RBC11			145	5.2	1.10	0.324
	RBC12			145	9.4	1.05	0.309
	RBC13			15	20	0.47	0.045
Rat mast cell line	RCMC	4.85[d]	0.0772[d]	145	3.16	1.06	0.338
Rat bone marrow mast cell (primary)	BMMC	4.85[d]	0.0858[d]	145	3.16	1.06	0.304
Human pancreatic epithelial cell line	PANC-1	8.75[e]	11.3[f]	38	80	0.86	0.003
Bovine aortic endothelial cell (primary)	Endo	8.0[g]	8.4[e]	150	10	0.69	0.006

[a] Calculated as $\hat{u}=\alpha/(\sigma/\eta\kappa)$, where $\sigma=q_e n/(4\pi a^2)$ and $\kappa = \sqrt{(2F^2C_0/\varepsilon RT)}$.
[b] Ref. [26].
[c] Ref. [6].
[d] Ref. [27].
[e] Ref. [28].
[f] Ref. [29].
[g] Ref. [30].

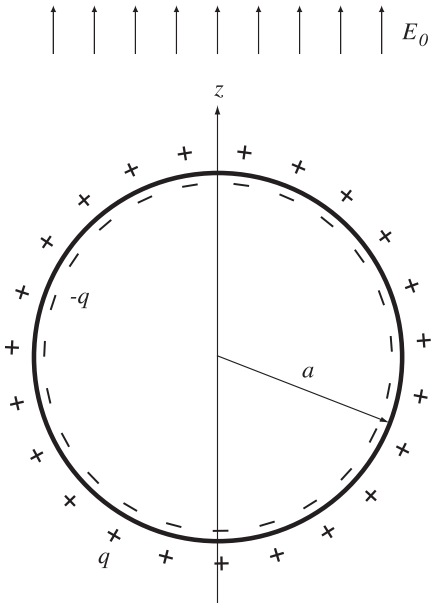

Fig. 1. Macroscopic schematic of cell electrophoresis. A cell of radius a moves in an electric field; far from the cell the electric field points evenly in the z-direction and has a magnitude of E_0. The cell is coated with charged proteins and sugar residues with a net charge of $-q$, and surrounded with a shell of solvated counterions with a net charge of q.

The Huckel model (Eq. (4b)) predicts that $\hat{u}$ values should all be 0.667. Values lower than this indicate an overprediction of EPM (underprediction of surface charge) by the Huckel and Smoluchowski models. Actual values in Table 1 are typically about half the Huckel prediction, which cannot be regarded as a significant difference, but three values are at least an order of magnitude low (RBC13, PANC-1, and Endo). All of these low values occur at high field strengths (E_0=10 V/cm), and two of the three occur in very large cells (a>7.5 μm). Recent work in nucleic acid electrophoresis suggests that size plays a distinct role in determining electrophoretic mobility independent of the number of charges [5]. It is therefore possible that the underestimation of cell charges by the Huckel model may be related to that model's lack of consideration for cell size.

Many authors attribute the Smoluchowski and Huckel models' underestimation of cell surface charge from mobility measurements to an incorrect assumption that the particle surface is impermeable and has a fixed quantity of charge. Cell electrophoresis models have attempted to correct Eqs. (4a) Eqs. (4b) by accounting for ion-permeability of the surface layer and for the charge-dissociation equilibrium exhibited by cell-surface molecules [7–11]. None of these models, however, predicts a particle size or field strength dependence for EPM.

Recent models in the colloidal particle literature consider deviations of counterion distribution due to dipole moments [12], surface charge concentrations above the linear range [13], and effects of surface polymers [14]. These newer models predict a degree of size-dependence, but not the significant differences seen in the literature. In addition, all of these models require numerical solutions, rendering them much less useful than an analytical solution that would allow simple back-calculation of surface charge values from EPM data.

All of these literature models assume that the surface charges are evenly distributed across the particle as depicted in Fig. 1. It is known, however, that membrane charge distributions are effected by the presence of an electric field [15,16], producing a θ-dependent charge distribution as shown in Fig. 2. The phenomenon of charged-particle redistribution was studied extensively in *Xenopus* myoblasts by Poo et al. [16] in 1979, who observed both that charged membrane species migrate on the surface of cells in response to an electric field and that this migration did not cause any distortion of the cell shape or geometry. Intriguingly, they showed that one class of surface proteins relaxed perfectly to their initial symmetric distribution once the electric field was discontinued, but that another class maintained some asymmetry for several hours.

While this charge redistribution effect has not been demonstrated for the cell types used in this paper, it is interesting to ask whether a similar surface-charge mobility would explain the size- and field-dependence of the data shown in Table 1. It seems reasonable to assume that the charged membrane-bound molecules of mammalian cells will be similarly mobile to the *Xenopus* myoblasts, especially when the cells are in suspension for electro-

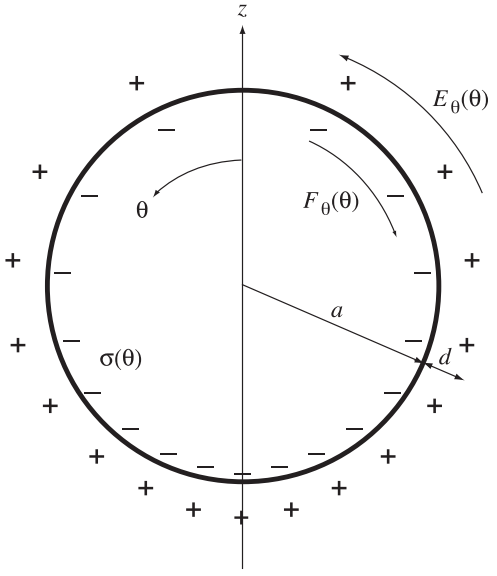

Fig. 2. Microscopic schematic of cell electrophoresis. At the cell surface, the r-component of the electric field collapses to zero, and the cell feels only a θ-directed field. The field causes a rearrangement of mobile charged species in the fluid bilayer, concentrating the negative charges toward the negative pole of the electric field. The resulting force on the cell is the sum of the forces on each charged particle, which depend on the product $E_\theta(\theta)\sigma(\theta)$. Each of these forces acts within the small space of width d between the cell membrane and the solvated counterion layer, where a net charge is present. Note that while the surface is depicted as comprising individual mobile charges, in reality the charges form mobile units, or rafts, containing many related proteins and other charged species.

phoresis. While some cells do maintain polarity in vivo, they typically do so by means of selective sorting of membrane proteins to one pole of a cell or the other. Epithelial cells maintain polarity by means of tight junctions that form only when in place as a member of an epithelial sheet [17]. Selective sorting of membrane proteins should not effect the large-scale redistribution of charged membrane species by electrophoresis, and epithelial cells are known to lose their polarity when dissociated from their neighbors.

This paper aims to explain the size- and field-dependence of observed values of $\hat{u}$ using a θ-dependent model of net surface charge. A simple drift-diffusion model for membrane surface charge distribution has already been developed by Jaffe [18]. This paper modifies the Jaffe model slightly to account for new evidence that charged particles are grouped on the cell surface into such structures as lipid rafts [19,20]. The resulting charge distribution model will be combined with a force balance over the cell to determine its effect on electrophoretic mobility, and the model's predictions will be compared to the literature data from Table 1.

2. Theory

2.1. Electrophoretic force balance

The electrophoretic velocity of a spherical particle can be calculated from the balance of forces on the particle. The drag on a sphere of radius a moving at a velocity U in an unconfined fluid of viscosity η is estimated by the Stokes "creeping flow" equation [21] to be

$$F_{\mathrm{D}} = 6\pi a\eta U \tag{6}$$

The steady-state velocity in the z-direction can be calculated by setting the drag force equal to the electric force, $F_{\mathrm{E},z}$, and solving for U as shown in Eq. (7).

$$U = F_{\mathrm{E},z}/6\pi a\eta \tag{7}$$

The electric force for a charge particle in isolation is given by the expression $F_{\mathrm{E},z}=qE_z$, where q is the charge of the particle and E_z is the z-component of the electric field. This simple form of the electromotive force is not adequate for the electrophoresis of particles in electrolyte solutions, however, because the negatively charged particle carries with it a boundary-layer of positive charges in order to maintain electroneutrality. At this macroscopic level, the net charge of the mobile unit—the particle and its surrounding counterion layer—is zero (see Fig. 1).

Inspecting the cell membrane at a more microscopic level (Fig. 2), however, allows the force on the cell to be examined independently from forces on the boundary-layer charges, which can be considered as moving independently as separate bodies. The r-component is identically zero because the cell membrane is assumed to be a perfect insulator [22]. In the θ-direction, however, there exists both a nonzero electric field and a space of thickness d in which there is a net charge (Fig. 2). The θ-component of the electric force, therefore, is expected to be proportional to the surface charge, σ, multiplied by a differential area to obtain a differential q, multiplied by the θ-component of the electric field, E_θ, as shown in Eq. (8). Since the force is applied over the length d but must act over the entire cell radius, a, Eq. (8) includes a "leverage coefficient" of d/a. For a cell in an electrolyte solution, $d\sim 1=\kappa$.

$$\mathrm{d}F_{\mathrm{E},\theta} = \frac{d}{a}E_\theta(a,\theta)\sigma(\theta)2\pi a\sin\theta a d\theta \tag{8}$$

To obtain a differential force in the z-direction, the θ-component of the electric force is converted to cylindrical coordinates: $\mathrm{d}F_{\mathrm{E},z}=-\mathrm{d}F_{\mathrm{E},\theta}\sin\theta$. The θ-component of the electric field around a nonconducting sphere, as decribed by Cole [22], is 3/2 E_0 $\sin\theta$. The resulting differential force must be integrated from the front to the back of the cell, as shown in Eq. (9).

$$F_{\mathrm{E},z} = \int_0^\pi \frac{1}{a\kappa}\left(-\frac{3}{2}E_0\sin^2\theta\right)\sigma(\theta)2\pi a\sin\theta a d\theta \tag{9}$$

2.2. Nonuniform charge distribution

Typically, the surface charge is assumed to be evenly distributed across the cell. However, this assumption does not take into account the fluid-mosaic nature of the cell membrane, in which all particles present in the membrane are continually in diffusive motion. With no electric driving force, the steady-state concentrations of charged species will be uniform, but in the presence of an electric field, negatively charged species in the membrane will tend to migrate toward the positive pole of the electric field. This paper uses a model for electrically driven charge redistribution that follows a modified version of the derivations in Refs. [18,16].

The balance between electrically driven drift and concentration-driven diffusion of charged species at steady state can be determined by setting the flux equal to zero in the ion transport equation, Eq. (10).

$$\frac{z_i}{|z_i|}u_i E(a,\theta)S_i(\theta) - D_i\nabla S_i = 0 \tag{10}$$

Here, S_i is the surface concentration of negatively charged molecules, which are each assumed to be a nearly homogeneous population with similar diffusivities, D_i, and valences, z_i.

For electrolyte particles in free solution, the Nernst-Einstein equation (Eq. (11a)) defines the relationship between the ion mobility u and the diffusivity D, where $\phi=RT/F$ is the "thermal potential," equal to 25.7 mV at 25 °C.

$$u = zD/\phi \tag{11a}$$

The decades-old fluid mosaic model of cell membrane structure, on which the Jaffe model was based, assumed that membrane-bound particles diffused independently of each other. New evidence, however, indicates that membrane-bound particles form clusters, known as lipid rafts, with a diameter ranging from 50 to 100 nm. We define here a modified version of the Nernst-Einstein relation (Eq. (11b)) that incorporates a "packing factor", β, that attempts to correct for the non-independence of charged molecules in the cell membrane.

$$u = z\beta D/\phi \tag{11b}$$

The packing factor, $\beta=\pi/2\ a/\delta$, is defined as the ratio of the average diffusion path length ($\pi/2\ a$, or one-quarter of the cell circumference) to the average diameter of a charged complex (δ, taken to be 75 nm for this study) [19].

Substituting Eqs. (8) and (11b) and into Eq. (10) and assuming that the net charge of each membrane-bound molecule is -1 yields a differential equation (Eq. (12)) that is independent of diffusivity or mobility coefficients.

$$-\frac{\beta}{\phi}S_i(\theta)\frac{3}{2}E_0\sin\theta - \nabla S_i = 0 \tag{12}$$

Eq. (12) can be solved with the boundary condition that the sum of the charged particles over the cell surface must equal the initial number of particles (Eq. (13)).

$$\int_0^{\pi} S_i(\theta)2\pi a^2\sin\theta \mathrm{d}\theta = 4\pi a^2 S_{i,0} \tag{13}$$

Finally, the surface charge is given by the surface concentration multiplied by the charge per particle: $\sigma(\theta)=zFS(\theta)$; the whole-cell charge density remains $\sigma=zFS_0$.

3. Results

3.1. Analytical results

An expression for electrophoretic velocity can be obtained by substituting the result of Eq. (9) into Eq. (6). The simplest solution, where $\sigma(\theta)$ is assumed constant, gives a result identical to the Huckel model:

$$U = \frac{2}{3}\frac{\sigma}{\eta\kappa}E_0 \tag{14}$$

Solving Eq. (12) and implementing the boundary condition in Eq. (13) gives an expression for distribution of charged surface particles. Converting this equation to surface charge density gives Eq. (15).

$$\sigma(\theta) = \frac{A}{\sinh A}\sigma\varepsilon^{-A\cos\theta} \tag{15}$$

Eq. (15) shows a dependence not only on θ, but on a new dimensionless parameter that we refer to here as A.

$$A = \frac{3\pi}{4}\frac{a^2E_0}{\delta\phi} \tag{16}$$

The A parameter represents both the balance between electrically driven migration and thermally driven diffusion (3/2 aE_0=phi) and the balance between available space for diffusion and the size of a typical membrane complex ($\pi/2$ a/d). In other words, A represents the ability of an electric field to concentrate charged species at one end of a cell when balanced both by diffusive forces and by the space limitation imposed by membrane complex formation.

Fig. 3 shows the relationship between surface charge distribution and A. For small cell sizes (A=0.1), the membrane complexes are large relative to the available surface area, and so diffusion dominates, giving a practically constant charge distribution. Diffusion and electrophoresis of charges reach a balance at A=1. When A reaches 10, the pull of the electric field clearly begins to dominate over diffusive processes, and there is ample room for packing of charges at the rear end of the cell. By the time Cc=100, the charge is concentrated almost entirely in one-tenth of the cell.

Finally, combining Eqs. (6), (9), and (15) yields a new expression for electrophoretic mobility, Eq. (17), which we will refer to as the mobile surface charge (MSC) model.

$$U = 2\frac{\sigma}{\eta\kappa}\left(\frac{1}{A\tanh A} - \frac{1}{A^2}\right)E_0 \tag{17}$$

Substituting Eq. (16) into Eq. (17) allows a plot of predicted electrophoretic velocity versus field strength and

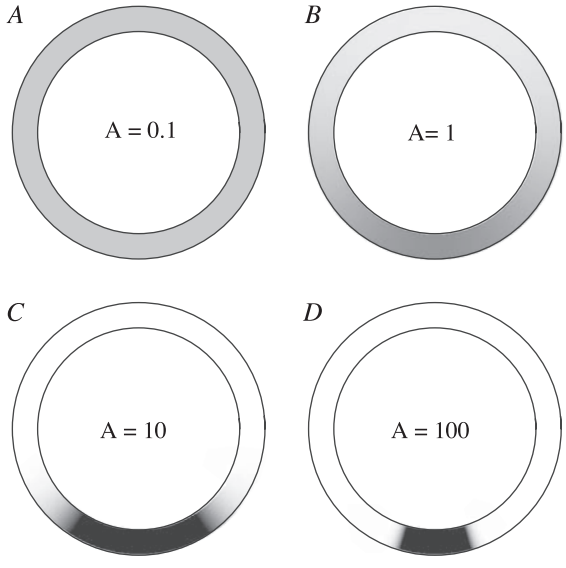

Fig. 3. Charge distributions in cell membranes resulting from different values of A. Darker areas indicate a higher concentration of negative charges. For small cells and low field strengths (A and B), little perturbation from normal is seen, while for large cells or high field strengths (C and D) the cell becomes clearly polarized.

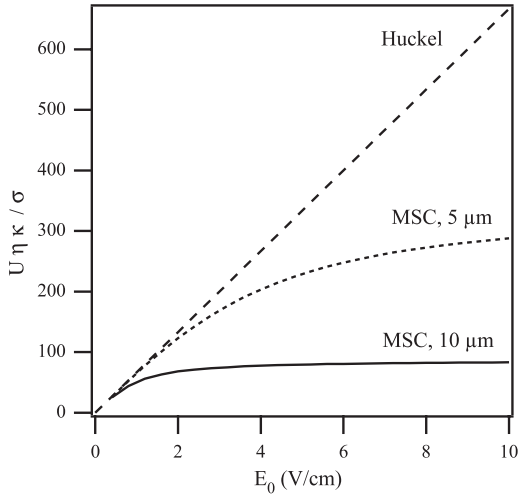

Fig. 4. Comparison of predicted velocity-versus-field curves for two different theoretical models. The traditional Huckel model (dashed line) predicts a linear response to electric field, while the new mobile-charge model (dotted and solid lines) predicts a plateau effect at high electric field strengths. This effect is more pronounced for larger cells (solid line) than for smaller cells (dotted line).

cell size. In Fig. 4, the Huckel result is compared to the MSC model predictions for limits of small (4 μm) and large (20 μm) cell sizes. The two models produce similar results at low electric field strengths, but significant deviations occur as the field strength increases. The MSC model predicts a plateau effect at high electric field strengths, and a comparison of the small- and large-diameter curves indicates that the plateau velocity decreases with increasing cell size.

3.2. Fit to literature data

In order to validate the mobile surface charge (MSC) model, the predicted mobility was compared to the reduced mobility data collected from experimental literature (Table 1). The experimental points were plotted alongside the predicted mobility curve as a function of A (Fig. 5).

4. Discussion

Our analysis of literature data (Table 1) illustrates that electrophoretic mobility is clearly dependent on both cell size and electric field strength. Prior models have not predicted this size dependence, nor have they taken into account the experimentally demonstrated mobility of surface proteins within the cell membrane [16]. By accounting for these mobile surface charges, this paper derives a simple correction to the classical Huckel theory that completely describes the literature data as a function of a new dimensionless group, A. This correction should enable researchers to obtain much more accurate estimates of cell surface charge than were previously possible from electrophoretic data.

Although the MSC model agrees with the Huckel model at low field strengths and for small cell sizes, substantial disagreements occur as the values of these parameters increase. As illustrated in Fig. 4, the Huckel model's prediction of solely linear dependence on electric field strength will accurately predict the mobility at low field strengths for cells less than ~10 μm diameter, such as red blood cells. However, the mobility is underpredicted for larger cells by at least 50% at all experimentally relevant field strengths. Previous examinations of electrophoretic mobility may have failed to observe this phenomenon because these studies are typically conducted over a small range of field strengths, and most often on small cells such as red blood cells. Only through the examination of the mobilites of several cells of different sizes, examined over a range of field strengths (Table 1, Fig. 5) do these deviations become evident.

Because the mobile surface charge model posits a nonlinearity in the response of cells to electric fields, it predicts that the electrophoretic mobility will not be a characteristic constant for a given cell type, but rather will decline as the applied electric field increases. The traditional definition of EPM as the ratio of velocity to applied field, $\alpha = U/E_0$, should therefore be replaced by the slope of an electrophoretic velocity curve with respect to applied field strength:

$$\alpha(\sigma, \kappa, a, E_0) = \partial U / \partial E_0 \tag{19}$$

Fig. 5 demonstrates the effectiveness of this new model in predicting the electrophoretic mobility of various types and sizes of mammalian cells. The correlation of the new model to literature data is remarkable, especially for a model with no

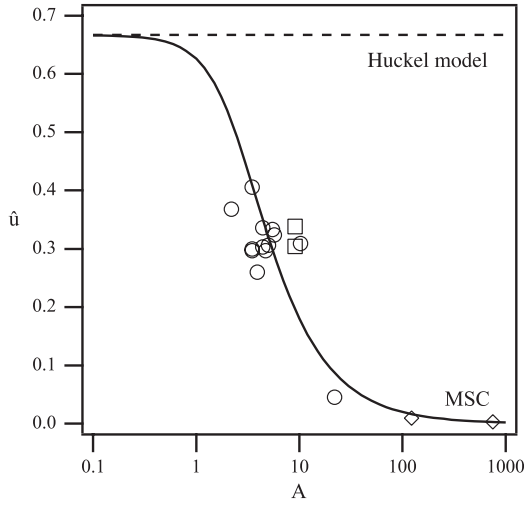

Fig. 5. Comparison of experimental data and mobile surface charge (MSC) model predictions of electrophoretic mobility (EPM). Individual data points represent nondimensionalized values calculated from EPM and surface-charge values reported in the literature, as summarized in Table 1. Circles represent red blood cells, squares represent mast cells, and diamonds represent structural cells (endothelial and epithelial). The solid line represents the prediction of the MSC model, and the dashed line represents the traditional Huckel model prediction.

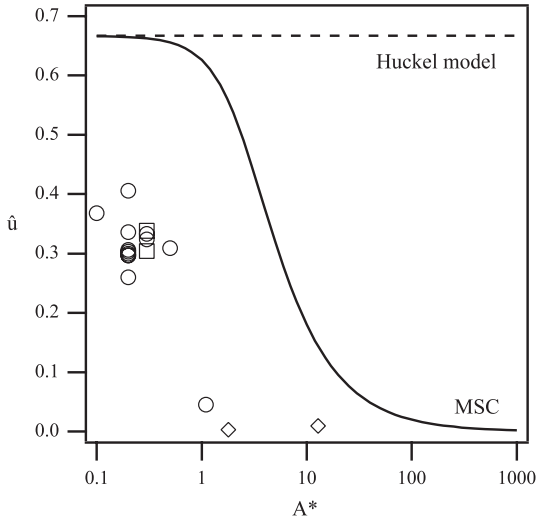

Fig. 6. Comparison of experimental data and mobile surface charge (MSC) model without incorporating the packing factor β in the calculation of the dimensionless number A^*. Comparison of this graph to Fig. 5 demonstrates the importance of the lipid raft phenomenon to the predictive value of the MSC model.

fit parameters. It is worth noting that the fit data include cells of similar types measured over a sufficient range of electric field strengths that they do not simply represent "high-EPM" and "low-EPM" classes of cells: at least one erythrocyte data point is shown, for example, that exhibits a low EPM.

Fig. 6 shows the fit that would be achieved if A values were recalculated without accounting for the packing factor, β. The new dimensionless number, A^*, is defined as:

$$A^* = 3/2aE_0/\phi \tag{20}$$

Comparison of Fig. 6 to Fig. 5 clearly shows the importance of accounting for the finite size of charged-particle groups as they move in the membrane. Eq. (20) assumes both that each charged moiety moves independently and that all proteins and glycolipids could be collapsed down to a collection of point charges at one end of the cell. One conclusion, that charged particles in the membrane cannot all be collapsed to a point, is reasonably obvious. The other conclusion, that charged species must move collectively in island-like structures, bears more discussion. The notion of organized islands of related surface proteins, glycolipids, and other species is slowly gaining acceptance in the cell biology world. The excellent fit to the data obtained by assuming the existence of lipid rafts that average 75 nm in diameter provides indirect evidence not only for the existence of these structures but for their persistence in the face of electrophoretic stress.

5. Conclusions

The new mobile surface-charge (MSC) model for cell electrophoresis appears to be an excellent predictor of experimental data. While the MSC model does not incorporate a full physical understanding of the membrane charge phenomena that are included in many advanced models published in the last decade, this first-principles approach provides a much better correlation to experimental data than other analytical models available today. This model should be a powerful tool for biochemists, cell biologists, and biophysicists attempting to determine cell surface charges in a rapid and reliable way. To do so will require the addition of only one additional measurement to typical electrophoresis protocols: accurate measurement of mean cell diameter. Such measurements have been employed recently in the characterization of liposomes [23–25], but to our knowledge no similar measurements have been performed on mammalian cells. The accuracy of surface charge calculations is also predicted to increase significantly when EPM measurements are performed over a broad range of applied field strengths.

Further, this model predicts two intriguing effects that will be of interest to designers of electrophoretic apparatus. First, there appears to be a "speed limit" for cell electrophoresis, as shown in Fig. 4, that depends only on the diameter of the cell. A 10 μm diameter cell, for example, will move faster in response to an increase in electric field only up to about 10 V/cm, above which the velocity appears to be essentially independent of electric field strength. Second, the MSC model predicts that cells of similar surface charge density but different effective diameters can be separated by exploiting this speed-limiting effect. By choosing a field strength at which the larger cell's electrophoretic velocity curve has already reached a plateau, but at which the smaller cell still responds to the electric field, the two cells can easily be separated. The two cell types in Fig. 4, for example, could easily be separated at a field strength of 10 V/cm.

This paper also provides some interesting insights into the fluid nature of cell membranes. The mobility of charged moieties on the cell surface makes a significant contribution to electrophoretic mobility that has so far been neglected in electrophoretic models. While the phenomenon of electrophoresis within the cell membrane has been observed and modeled before, never has its impact on whole-cell electrophoresis been taken into account. Perhaps just as important, the mobility of surface charges fails to properly predict the data when charges are assumed to move independently; only when charges are treated as moving groups of substantial size does the model correctly map to experimental data. This paper may therefore be one of the first mathematical models to demonstrate the importance of the emerging lipid raft model of cell membrane organization to the prediction of cellular properties, and it is certainly a novel addition to the literature surrounding electrophoresis of cells.

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