Examination of the Electrophoretic Behavior of Liposomes

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The electromigration of liposomes is a complex process resulting in many unexpected behaviors that are difficult to address with existing theories. In this study, the electrophoretic behaviors of liposome populations under various conditions were examined through the use of capillary electrophoresis and the results compared to classical electrokinetic, colloid, and spheroid theories. To elucidate the possible effects of applied field strength, bilayer rigidity, and surface charge on these behaviors, the electrophoretic mobilities of liposome populations were monitored while varying the applied potential, ionic strength of the medium, and the surface charge and cholesterol content of the liposomes. On the basis of comparisons made to the theoretical predictions, our results suggest that liposomal deformation and field-induced polarization may occur during electrophoresis and these mechanisms help to describe many of the observed behaviors.

Introduction

Liposomes are artificial phospholipid vesicles that are formed by the self-assembly of lipids into spherical or quasispherical structures. These vesicles range in size from nanometers to micrometers and can consist of a single bilayer shell, unilamellar, or multiple concentric bilayer shells, containing an aqueous central cavity. The relative ease at which their basic properties such as size, lamellarity, and composition are manipulated makes them ideal for numerous uses in research and industry. Liposomes have been used to mimic biological membranes in the study of membrane-bound proteins, ion transport, energy transduction, and cellular functions. Their ability to act as versatile containers has been utilized for drug delivery and targeting, for the transfer of genetic materials to cells, and for use as food and cosmetics ingredients.[1−9]

Numerous properties and behaviors of liposome systems can be studied by capillary electrophoresis (CE). Recently, determination of the size and aqueous central cavity volume of individual liposomes was demonstrated through the use of CE coupled with postcolumn laser-induced fluorescence.[10] Differences in electrophoretic mobilities have been utilized to monitor the effects of compositional variation on membrane fluidity[11] and permeability,[12] as well as the effects of transbilayer pH gradients on the

apparent surface charges of liposomes.[13] CE is also useful for separation and purification. For example, the size-dependent separation of liposome populations was demonstrated through the manipulation of the ionic strength of the medium.[14]

In the past, attempts have been made to explain some of the electrophoretic behaviors of liposomes through comparisons to classical electrokinetic as well as colloidal theories; however, due to their unique properties, their behaviors remain poorly characterized and not well understood.[14−16] To take full advantage of the information obtained by CE, a better understanding of the unique behaviors of liposomes in electric fields is necessary. This will not only help to more accurately characterize the behaviors of liposomes but may also be applicable to the electrophoretic behaviors of other closely related systems such as organelles and cells.

In this study, the electrokinetic behavior of liposomes was examined under various conditions in order to characterize and compare their behaviors to the established theories. The apparent effects of surface charge, applied field strength, and bilayer rigidity were observed through variation of the surface charge density of the populations, ionic strength of the medium, magnitude of the applied potential, and cholesterol content of the liposomes.

Theory

Within the framework of classical electrokinetic theory, the electrophoretic mobility (μ) of a particle is predicted to be proportional to its charge density. If simple electrophoretic actions are assumed, then the expected mobility of the particle can be estimated from the basic equation for mobility

$$\mu = q/6\pi\eta R \tag{1}$$

where q is the charge, R is the radius of the particle, and

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η is the viscosity of the medium. On the basis of this equation, a particle with a high charge is expected to have a high mobility, whereas one with a lower charge is expected to have a correspondingly lower mobility. This equation, however, is only valid for a limited number of systems.

For large particles, such as colloids and liposomes, more exact solutions to the series of electrokinetic transport equations are necessary. Numerous colloidal theories, such as those developed by Overbeek,[17,18] Booth,[19,20] O'Brien,[21-23] and White,[21] have been developed to model the behavior of these systems. These colloidal models describe the behavior of solid spherical particles with homogeneous surface charges in the presence of a low potential gradient. Such models differ from the classical theories in that they take into account the effects of relaxation and retardation, both of which work to reduce the electrophoretic migration rates of large analytes. Relaxation is the direct result of distortions of the ionic atmosphere due to the inability of the diffusion-controlled re-forming of the ionic cloud to keep up with the movement of the particles. Retardation is the increased hydrodynamic drag due to the movement of the counterions in the opposite direction of the particle in response to the applied field.

According to these theories, the extent of relaxation effects is dependent on the inverse of the electric double layer (EDL) thickness and the radius of the particle. This is usually described with the value κR, which is the inverse Debye length (or the Debye–Huckel approximation, κ) times the radius of the particle (R). Relaxation is also dependent on the potential at the surface of the shear of the particle (the so-called zeta potential, ζ). The ζ potential is a measure of the surface charge density and the thickness of the EDL. While the potential is a function of κ and vice versa, they are treated as separate parameters in these theories.

An accepted colloidal theory that is frequently applied to the electrophoretic behavior of large particles, including liposomes, was developed by O'Brien.[23] This method involves the solution of the coupled differential transport equations for spherical colloidal particles whose dimensions are much greater than the double-layer thickness. By this method, the electrophoretic mobility of a sphere in a general electrolyte can be calculated from the following equation

$$\mu = \frac{3e\zeta}{2kT} + \frac{3M\lambda}{2(1+\lambda M)}\left(1 - \exp\left(\frac{-e|z_i\zeta|}{2kT}\right)\right)\left(\gamma - \frac{e\zeta}{kT}\right) \quad (2)$$

where k is the Boltzmann constant, e is the elementary charge, z_i is the valence of the counterion, and the other variables are defined in the reference material. The first term in eq 2, the Smoluchowski relation, is the approximate solution of the electrokinetic equations assuming no distortion of the ionic cloud. In this limit, the double layer is considered to be approximately flat; therefore the ions within it only move parallel to the surface of the particle so there is no variation in ion densities. With increased ζ potential this assumption is no longer valid
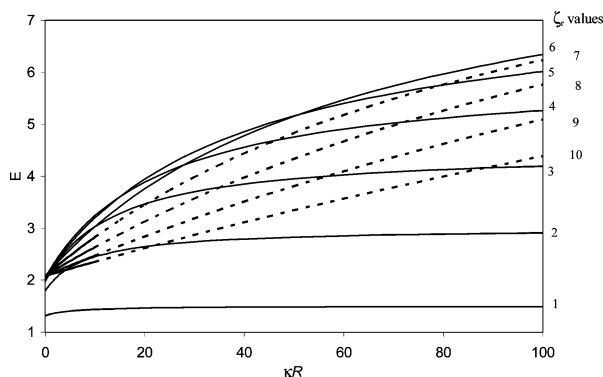
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Figure 1. Representative graph of reduced mobility (E) vs κR based on the spherical colloid theory developed by O'Brien.[23] Each curve represents a series of particles with constant ζ potential and varying κR values.

due to the high concentration of counterions present in the atmosphere. On the basis of the governing equations for ion densities and potential, variations in these quantities normal to the particle surface are of the order κ^{-1} while those tangential are of the order R, which result in the development of gradients in ion density and electrical potential within the ionic atmosphere. While the effects of these gradients are considered small and can be ignored when the surface potential and, hence, the concentration of charged counterions in the ionic atmosphere are small, their effects become significant at larger potentials and counterion concentrations. The second term in eq 2 is an adjustment for relaxation effects due to these ionic and electrical gradients within the ionic cloud.

The mobilities predicted by this and similar colloidal theories are typically presented as a graph of reduced mobilities (E) vs κR (Figure 1). Each of the series represents particles with consistent ζ potentials but varying κR values. If relaxation and retardation effects are ignored, as in the Smoluchowski approximation, each series would exhibit a constant E. Deviations from linearity, as well as the maxima and minima observed in the mobilities of each series, are the result of relaxation and retardation effects.

Building on this work, theories for nonspherical particles have also been developed and may help to describe some electrophoretic behaviors of liposomes. The electrokinetic theories for nonspherical particles must address the overall loss of symmetry in the system. While the spherical colloid theories account for relatively minor losses of symmetry through corrections for relaxation effects, they assume that the atmosphere is only slightly asymmetrical. This is the basis for the linearization of the governing equations, which greatly simplifies the mathematical modeling of the system by allowing the potential and charge distribution to be represented by superposition of their symmetrical components with a small asymmetrical correction added to account for distortion of the ionic atmosphere.

More rigorous nonspherical theoretical models based on spheroidal particles have appeared as well. The mobility of a spheroid can be calculated from its aspect ratio (ratio of the length of the short axis to the length of the long axis) and is predicted to be larger than a spherical particle with the same volume.[17,24,25] The average electrophoretic mobility of a population of spheroids is typically calculated based on the assumption of a random orientation of the

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population with respect to the applied field. Mathematically this is accomplished by considering one-third of the population to be aligned with and two-thirds transverse to the applied field. While most spheroidal theories that have been developed involve solving complicated systems of coupled differential equations, several simplified models have been described. According to the spheroidal theory developed by O'Brien and Ward,[24] the average mobility of a randomly oriented prolate spheroid population can be calculated from the equation

$$\mu = \frac{\epsilon\zeta}{\eta} - \frac{\epsilon kT}{3\eta e}\left(\frac{e\zeta}{kT} - \gamma\right)[g^0(\beta) + 2g^1(\beta)] \qquad (3)$$

where $g^0(\beta)$ pertains to the velocity components of those ellipsoids with their major axis aligned with the applied field and $g^1(\beta)$ pertains to those with their major axis transverse to the field. The second and subsequent terms are corrections for relaxation effects and the aspect ratio of the spheroid. (A more detailed explanation of this equation can be found in the Appendix and reference material.[24]) When this model was utilized, great care was used to be cognizant of the numerous assumptions, in the form of boundary conditions, that were made in order to arrive at this equation.

Experimental Section

Materials. Phosphatidylcholine (PC), phosphatidic acid (PA), and cholesterol (chol) were obtained from Avanti Polar Lipids, Inc. (Alabaster, Al). Tricine, BRIJ 35, and octadecyltrichlorosilane were obtained from Sigma-Aldrich (St. Louis, MO), K_2SO_4 was obtained from EM Science (Gibbstown, NJ), and fused silica capillaries were obtained from Polymicro Technologies (Phoenix, Az).

Methods. Liposomes. The liposome samples were prepared by reverse phase evaporation[26] and consisted of 90% phosphatidylcholine, 10% phosphatidic acid, and varying amounts of cholesterol. This technique produces large, unilamellar liposomes. The diameter of these populations is approximately 160 nm as previously determined by laser light scattering.[13] Samples were stored at 4 °C and used within 1 week. The pH gradients were created by titration of the exterior buffer to the desired pH, and all pH gradient samples were used before equilibration of the gradient occurred.[13]

Buffers. Buffers used contained 2 mM tricine and were adjusted to the correct ionic strength with the appropriate amounts of K_2SO_4 then titrated to either pH 7.4 or 8.8. 0.001% (w/v) BRIJ 35 was added to the running buffers to minimize leaching of the surfactant capillary coating.

Instrumentation. Capillary electrophoresis was performed on a machine designed in-house. A Spectra 100 UV–vis spectrometer (ThermoSeparation Products, Fremont, CA) set at 214 nm was utilized for detection of the liposome samples. Data collection and processing were accomplished with a personal computer running a 4880 data-handling system (ATI Unicam, Cambridge, U.K.) and Excel.

Fused silica capillaries with a 50 μm inner diameter, 360 μm outer diameter, and 75 cm total length were utilized for all experiments. The capillaries were coated with BRIJ 35 according to the method described by Towns and Regnier.[27] Electroosmotic flow was measured using the neutral marker mesityl oxide, and all samples were pressure injected for 1 s at 30 psi.

Data Analysis. All experimental data points are representative of a minimum of four independent electrophoretic runs. Additionally, all samples were remade and experiments were repeated multiple times (samples made and tested for various experiments in excess of 25 times) in order to verify the reproducibility of the results. The electroosmotic flow was measured via the neutral marker mesityl oxide after every

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electrophoretic run in order to monitor coating stability and adsorption issues. The experiments have also been run utilizing alternative coatings to assess possible adsorption issues (data not shown). The liposome migration rates were found to be independent of coating technique. All runs were performed such that the current did not exceed 40 μA in order to avoid possible adverse effects due to Joule heating.

Results and Discussion

The electromigration of liposomes in high potential fields is a complex process that results in many counterintuitive and seemingly confounding results compared to traditional electrophoretic behaviors.[11,13–16] While a fundamental basis for migration is established from colloids and can help to qualitatively describe some behaviors, others cannot be addressed with existing models. In this study, the behavior of liposomes in the presence of an electric field under a variety of conditions is examined and several unusual behaviors are presented and discussed. First, the general electrokinetic behaviors and trends exhibited by liposome populations were examined by observing the electrophoretic mobilities exhibited as the surface charge of the liposomes and ionic strength of the medium was varied. Subsequently, the effects of bilayer rigidity on the electrophoretic behavior of the populations were monitored through variation of liposome cholesterol content. Finally, the effects of the applied field were examined by varying the magnitude of the applied potential while the surface charge of the populations and ionic strength of the medium remained constant.

In these studies, the mobilities of liposome populations demonstrate unique behaviors that appear to contradict basic electrokinetic relations (eq 1). For instance the population with the highest charge did not always exhibit the highest mobility (Figure 2A). Somewhat amazingly, the highest charged population instead exhibited the lowest mobility under some conditions, as exhibited when the ionic strength of the medium is 45 mM. The order with respect to surface charge, and therefore the overall charge, of these liposome populations is pH $7.4_{in}/8.8_{out}$ > pH 8.8 > pH $8.8_{in}/7.4_{out}$ > pH 7.4. (A more detailed explanation of these populations and their charges can be found in the Appendix and reference material.[13])

In addition, unique behaviors of elution orders were demonstrated in that the order of elution of the various populations changed dramatically when the ionic strength of the buffer was varied (Figure 2). Certainly from a classic electrophoresis standpoint the mere changing of the ionic strength should not alter the order of elution, yet this was observed in several of the data sets. For instance, the elution order completely changes from pH $7.4_{in}/8.8_{out}$, pH 7.4, pH 8.8, pH $8.8_{in}/7.4_{out}$ at an ionic strength of 9 mM to pH 8.8, pH 7.4, pH $8.8_{in}/7.4_{out}$, pH $7.4_{in}/8.8_{out}$ at an ionic strength of 45 mM.

While this was a surprising result in the context of classical electrokinetic theory, colloidal models can provide some insight into this behavior. In the present study, the electrophoretic mobilities of liposome populations with varying ζ potential and κR values were measured. Since the ζ potential and κR values of these populations fall well within the range where relaxation and retardation effects are predicted to be significant, their observed mobilities were compared to those predicted by the colloidal theories. To effectively compare the results obtained to the theoretical predictions, methods detailed by Hunter[20] were utilized to convert the surface charge densities of the liposomes to reduced ζ potentials and their mobilities to reduced mobilities (details of these calculations are shown in the Appendix).
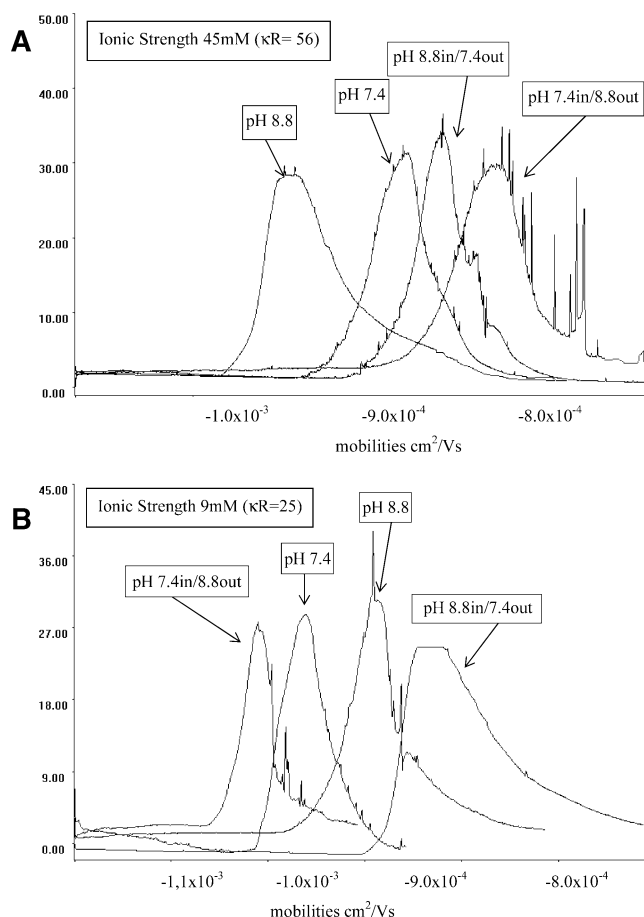
Figure 2. Composite electropherograms of liposome populations with varying surface charges. The populations depicted in A have an ionic strength of 45 mM and those in B have an ionic strength of 9 mM. The order with respect to surface charge densities is pH $7.4_{in}/8.8_{out}$ > pH 8.8 > pH 7.4 > pH $8.8_{in}/7.4_{out}$. The spikes seen in these electropherograms are characteristic of liposome populations and have been detailed elsewhere.[15,16]

The observed mobilities of the liposomes where plotted along with those predicted (eq 2) by the spherical colloid theory developed by O'Brien[23] (Figure 3). Note that the theoretically predicted values were plotted such that each series consists of particles with a consistent surface charge density, rather than a constant ζ potential. Traditionally such theories have been plotted such that each series consists of particles with consistent ζ potentials (Figure 1). In this study, the surface charge densities rather than ζ potentials remained constant while the ionic strength of the medium, and therefore the κ and κR values, was varied for each series of liposome populations. The ζ potential of a particle is a function of both surface charge density and ionic strength; if the surface charge density of a particle is held constant as the ionic strength of the medium is varied, the particle's calculated ζ potential will decrease with an increase in the ionic strength. For this reason, it is more appropriate to calculate theoretical values based on consistent surface charge densities to clarify the comparison to the experimental results. The theoretical curves shown (Figure 3) are those for particles with surface charge densities that bracket those calculated for the liposome samples.

While the general trends exhibited by the data are similar to those predicted by the colloid model, there are striking deviations; the most notable is the considerably larger mobilities compared to those calculated from the estimated surface charges of the populations. This observation is consistent with an overall reduction in the
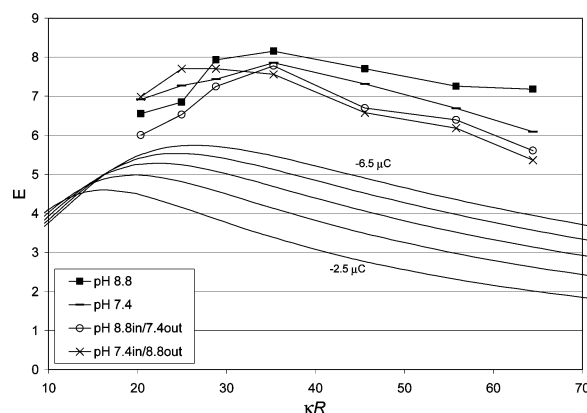

Figure 3. Plot of reduced mobility (E) vs κR for liposome populations (upper curves) and the theoretically predicted mobilities based on colloidal theory (lower curves). Each of the theoretical curves represents a series of particles with constant surface charge densities and varying κR values. The surface charge densities for the theoretical curves are −6.5, −5.5, −4.5, −3.5, and −2.5 μC/cm² from top to bottom. Error bars have been omitted from the experimental data for clarity of presentation. The error bars were found to be indiscernible from the data points when the figure size was reduced. Each data point represents a minimum of four independent electrophoretic runs.

effects of retardation and relaxation, whose effects always cause a decrease in the observed mobility. On the basis of the size of the liposomes, the ionic strengths of the medium, and the calculated ζ potentials, this theoretical model does not accurately describe their behavior.

It may be possible to explain some of these behaviors through consideration of the unique properties of liposome populations. An assumption made in the colloidal models is that the particle is rigid and nondeformable. In contrast to this, liposome structures have been shown to be squishy and capable of undergoing a variety of shape deformations in response to externally applied pressure and shear forces.[28−30] To ascertain the effects of liposome rigidity on their electrophoretic behavior, an additional study involving the variation of the cholesterol content of the liposome populations was conducted. One method of altering the overall rigidity of vesicles and their resistance to deformations is through the incorporation of cholesterol. For lipid bilayer structures above their phase transition temperature, cholesterol incorporation has been shown to increase mechanical stiffness and resistance to externally applied forces. Since the ionic strengths and surface charges were kept constant, any changes in the mobility were attributed to the mechanical rigidity, or ability of the liposomes to deform from a spherical shape. The observed results (Figure 4) show that the softer liposomes, those containing less cholesterol, exhibited higher mobilities. It is worth mentioning that incorporation of cholesterol into bilayers may cause a slight change in surface charge density and, thus, ζ potential;[30] however, the relatively minor changes in mobilities this would cause do not account for the significant changes observed (approximately 10%).

If the liposomes change shape as a result of their intrinsic deformability, they could change from the assumed spherical symmetry to that of a prolate spheroid. In this system, the aspect ratio of the resulting spheroid

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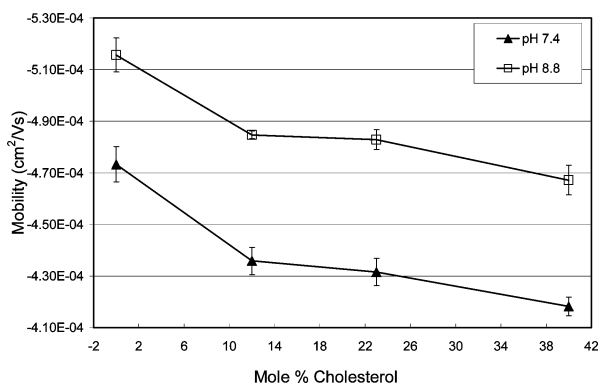
Figure 4. Observed mobilities of liposome populations vs cholesterol content. The ionic strength of these populations is 18 mM.

would be determined by the softness of the liposome. A softer, more easily deformed liposome (one with lower cholesterol content in this study) would result in a smaller minor axis measurement, a decrease in friction, and a larger observed mobility.

The model developed by O'Brien and Ward[24] for the electrophoretic mobility of spheroidal particles was utilized to compare the observed liposome behaviors to those predicted for prolate spheroids. In this particular system, the ellipsoids would all be aligned with the field (the reason for this will be addressed later), which would require the alteration of the mobility equation for a randomly aligned population of spheroids (eq 3) as follows:

$$\mu = \frac{\epsilon\zeta}{\eta} - \frac{\epsilon kT}{3\eta e}\left(\frac{e\zeta}{kT} - \gamma\right)[3g^0(\beta)] \qquad (4)$$

In this equation, the velocity component due to the transverse particles has been omitted.

The observed mobilities of the liposome populations were graphed along with the mobilities predicted for aligned spheroids with aspect ratios of 0.20 and 0.80 (Figure 5). For reasons discussed earlier, the series shown consists of particles with constant surface charge densities rather than ζ potentials. A comparison of the theoretical curves for the two different aspect ratios shows that the effects of a decreased aspect ratio, or more elongated particle, are an increase in the predicted mobilities and a faster decrease in mobility after the maxima for each series are reached. On the basis of a visual comparison, the theoretical predictions for spheroids with an aspect ratio of 0.20 (Figure 5A) appear to more closely model the observed mobilities of the liposome populations. The theoretically predicted mobilities are still lower than those observed; however they are much closer than those predicted by the colloidal theory for spherical particles. This suggests that a shape distortion to that of an elongated spheroid may be a contributing factor in the observed migration rates of the liposome populations.

To further compare liposome behaviors to those predicted for colloidal particles, a subsequent study was conducted in which the magnitude of the applied potential was varied while the surface charges of the populations and ionic strengths of the mediums were kept constant. Classical electrophoretic theory, as well as the colloidal models, assumes that the electrophoretic velocity of a particle is linearly related to the applied field strength.[14−19,21,31] Bearing this in mind, if all other factors remain constant, the electrophoretic mobility of a given particle should be constant regardless of the applied field strength. Contrary to this, the observed mobilities of the

liposome populations were shown to decrease with an increase in the applied potential (Figure 6).

To begin to understand the observed behaviors, a careful assessment of the assumptions made during the development of the electrophoresis theories is necessary. The established theories assume that there is a uniform charge distribution on the surface of the particle and that the ionic atmosphere around the particle is only slightly distorted by the applied field. This model does not appear adequate for liposome populations, which consist of fluid lipid bilayers with varying charge distributions that may behave more like point charges on a two-dimensional spherical surface.[32,33] Moreover, an applied potential field can result in the migration of the charged species within such bilayers.[34,35] This type of migration has been shown to lead to polarization and an accompanying elongation of vesicles along the direction of the applied field.[36−42] This field-induced alignment is the basis for the adjustments made in developing eq 4. The extent of this shape deformation would be fundamentally dependent on the intrinsic deformability of the particle. Additionally, field-induced polarization may help to explain the increase in relative mobilities observed with decreased applied field strength (Figure 6).

As mentioned earlier, the physical basis for relaxation effects is the overall symmetry of the ionic atmosphere around the particle and its ability to remain symmetrical during the electrophoretic motion of the particle. The corrections for relaxation effects that are built into the colloidal models do not consider the effects of dipole formation due to polarization of the particle itself. These corrections were also designed assuming a low applied potential (<100 V/cm) and relatively low particle velocity. The field strengths, and resulting buffer and particle velocities, in this study are much larger than this. It is reasonable to speculate that distortions of the ionic atmosphere due to particle polarization and dipole character increased field strength, and larger velocities may alter the extent of relaxation effects. It should be noted that all of the observed mobilities in this study were larger than those predicted based on the colloidal theories and the increased mobilities that were observed in the field strength study are relative to identical liposome populations at the lower applied field, not the predictions of colloidal theory.

Conclusions

This study has shown that the spherical colloid theories typically used to model the electrophoretic behavior of liposomes do not adequately depict these systems. The unique properties of liposomes such as their intrinsic deformability and susceptibility to field-induced polarization must be considered when comparing their behaviors

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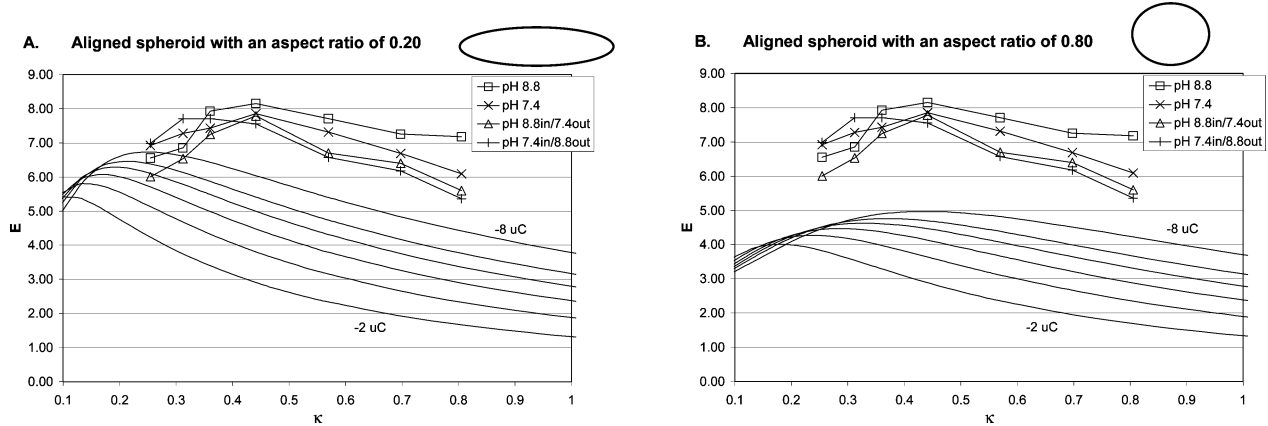
Figure 5. Plots of reduced mobilities (E) vs κ for liposome populations along with predicted values for aligned spheroids with an aspect ratio of 0.20 (A) and 0.80 (B). The lower curves are the theoretically predicted reduced mobilities for spheroids with surface charge densities of -8, -6, -4, -3, and -2 μC/cm^2.
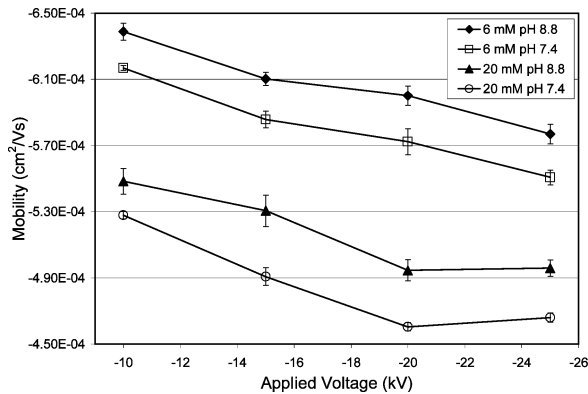

Figure 6. Observed mobility of various liposome populations vs applied potential (kV). The upper curves are for populations in a 6 mM K$_2$SO$_4$ medium (ionic strength 18 mM), and the lower curves are for populations in a 20 mM K$_2$SO$_4$ medium (ionic strength 60 mM).

to those of typical colloidal particles. The mobilities predicted for aligned spheroidal particles proved to be a better model for the observed behaviors of the liposome populations in this study. This suggests that deformation of the liposomes from their original spherical shape to that of a prolate spheroid with its major axis aligned with the applied electric field occurs during electrophoresis.

Appendix

pH Gradients. This study included liposome populations with consistent inner and outer pH values, as well as ones that contained a pH gradient, or a difference in the pH of the inner compartment as opposed to the external medium. For instance, the pH 7.4$_{in}$/8.8$_{out}$ refers to a population of liposomes in which the external buffer has a pH of 8.8 and the internal compartment buffer has a pH of 7.4. The net effect of these gradients is that the pH 7.4$_{in}$/8.8$_{out}$ population possesses a higher surface charge than the regular pH 8.8 population and the pH 8.8$_{in}$/7.4$_{out}$ population possesses a lower surface charge than the regular pH 7.4 population.

Calculation of Reduced ζ Potentials and Reduced Mobilities. The surface charge density of the liposome populations, due to percent composition of PA and its pK_a-dependent charge state was calculated to be -3.4 μC/cm^2 at pH 7.4 and -4.6 μC/cm^2 at pH 8.8. This calculation is based on an average liposome radius of 80 nm with each lipid headgroup occupying 0.7 nm^2. Following the methods detailed by Hunter,[20] surface charge density was converted

to surface potential by the equation

$$\sigma_s = 11.74c^{1/2}\sinh(19.46\psi_s)$$

where σ_s is the surface charge density, ψ_s is the surface potential, and c is the concentration of ionic species. The calculated surface potential was then used to calculate the ζ potential of the particle by

$$\tanh(\psi_r) = \tanh(\psi_{rs}/4)e^{-\kappa x}$$

where ψ_{rs} is the reduced surface potential and ψ_r is the reduced potential at a distance x from the surface of the particle. ζ_r (which is related to the ζ potential by $\zeta_r = \zeta/(kT/e)$) is approximately equal to ψ_r when $x = 0.2$ nm (the distance to the surface of the shear). The observed mobilities were converted to reduced mobility (E) by the relation $E = \mu/(2\epsilon kT/3\eta e)$.

Spheroid Theory Equations. The average electrophoretic mobility of a population of prolate spheroids randomly oriented with respect to the applied field is given as a weighted average of the mobilities of transverse and aligned spheroids (eq 3). One-third of the population is assumed to be aligned with the field and is represented by the following components:

$$g^0(\beta) = \frac{3}{2}\cosh\xi_0\sinh^3\xi_0\left[\cosh\xi_0\ln\left(\frac{\cosh\xi_0+1}{\cosh\xi_0-1}\right) - 2\right]\frac{dQ_1(\cosh\xi)}{d\xi_0}\Big|_{\xi_0}[f^0(\beta)-f^0(0)]$$

$$f^0(\beta) = \frac{\left[\beta\cosh\xi_0 I_1 - \frac{2}{3}\right]}{3\sinh^2\xi_0\cosh^3\xi_0\left[\frac{2}{3}Q_1' - \beta Q_1 I_1\right]}$$

Two-thirds of the population is assumed to be transverse to the field and is represented by the following components:

$$g^1(\beta) = \frac{3}{8}\cosh\xi_0\sinh^2\xi_0\left[\sinh^2\xi_0\ln\left(\frac{\cosh\xi_0+1}{\cosh\xi_0-1}\right) - 2\cosh\xi_0\right]\frac{dQ_1^{\,1}(\cosh\xi)}{d\xi_0}\Big|_{\xi_0}[f^1(\beta)-f^1(0)]$$

$$f^1(\beta) = \frac{2\left[\frac{4}{3}\cosh\xi_0 - \beta\sinh^2\xi_0 I_2\right]}{3\cosh\xi_0\sinh^3\xi_0\left[\frac{4}{3}(Q_1^{\,1}) - \beta Q_1^{\,1}I_2\right]}$$

These components are derived from the relations for the net force that a fluid exerts on a spheroid and the hydrodynamic pressure on its surface. In the above equations

$$\tanh\xi_0 = a/b$$

where a is the length of the short axis and b is the length of the long axis of the spheroid. Given this series of equations, the mobility of a spheroid in a medium of known concentration can be calculated based on its ζ potential and aspect ratio. (Further details about these equations can be found in the reference material.[24])

The theoretical predictions based on these equations were plotted as E vs κ rather than κR due to the nature of the equations. Specifically, R is not a factor in the mobility in this series as it is based on aspect ratio rather than size. As can be seen in the above system of equations, the adjustments made for relaxation effects are primarily determined by the unitless aspect ratio rather than a radius measurement, or size of the particle. This accounts, in part, for the differences obtained when the results of this model (calculated for an aspect ratio of 1) are compared to the results of spherical colloid theory in which the radius measurement is significant in determining the relaxation corrections.

Supporting Information Available: Additional graphs and enlarged figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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