Effects of Deformability, Uneven Surface Charge Distributions, and Multipole Moments on Biocolloid Electrophoretic Migration

Michele D. Pysher and Mark A. Hayes*

Department of Chemistry and Biochemistry, Arizona Applied NanoSensors and The Center for Solid State Electronics Research, Arizona State University, P.O. Box 871604, Tempe, Arizona 85287-1604

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Liposomes have been widely used as cellular and bioparticle mimics due to their lipid bilayer structure and relative ease of production and manipulation. Such biocolloids are frequently characterized by capillary electrophoresis (CE), which promises a wealth of information about such properties as surface charge, composition, and rigidity. The applicability of this information is somewhat limited, however, since it is interpreted with colloidal theories that do not account for the unique properties of biocolloids. In this work, the effects of deformability, mobile surface charges, intrinsic polarizability, and uneven surface charge distributions are incorporated into colloidal theories in order to better model the electrophoretic behaviors of liposomes.

Introduction

The term biocolloid encompasses a broad range of biologically significant particles ranging from relatively small viruses to aggregates and much larger cells, bacteria, and spores. The manipulation, functions, and separation of these bioparticles are governed by numerous complex processes that obscure their analysis and characterization. The characterization process is further complicated by the intrinsic properties of softness, deformability, mobile surface charges, and polarizability.

Capillary electrophoresis (CE) can be used to investigate many basic biocolloidal properties. While this technique can be applied directly to bioparticles, for convenience, they are often modeled using liposomes because their basic properties, including size, lamellarity, and composition, are similar and, in liposomes, can be easily manipulated. Recently, determination of the size and aqueous central cavity volume of individual liposomes was demonstrated with CE coupled to postcolumn laser-induced fluorescence.[1] Additionally, characteristic particle size, in combination with manipulation of the ionic strength of the suspending medium, has been exploited for the separation of liposome populations.[2] The effects of compositional variation on membrane fluidity[3] and permeability[4] as well as the effects of transbilayer pH gradients on the apparent surface charges of liposomes have also been related to differences in liposomal electrophoretic mobilities.[5−7] CE is, by nature, extremely versatile, and its usefulness extends across a broad spectrum of other biological particles; for example, it has proven to be a rapid separation and purification technique for such analytes as proteins,[8] cells,[9,10] and organelles.[11, 12]

A major drawback in the application of CE to biocolloidal and liposomal analysis is that the interpretation of electrophoretic mobilities and the modeling of these structures is typically based on traditional colloidal theories such as those developed by Overbeek,[13,14] Booth,[15,16] O'Brien,[17−19] and Dukhin,[20−22] which do not take into account the unique properties of these populations. This has led to difficulties in the interpretation of data and limited the usefulness and applicability of the information obtained. This study presents a qualitative and quantitative analysis of liposome behavior that accounts for possible electrokinetic effects due to particle deformability, mobile surface charges, intrinsic polarizability, and uneven surface charge distribution.

Theory

One of the fundamental assumptions of most classical electrokinetic and colloidal theories is that the particle is

* To whom correspondence should be addressed. E-mail: MHayes@asu.edu.

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a rigid sphere with uniform charge distribution. This allows its surface potential and electrophoretic mobility to be approximated using the monopole moment of its area-averaged ζ-potential (ζ). The ζ-potential is a measure of the potential at the surface of the shear of the particle and is related to the surface charge density of the particle and buffer properties. Essentially, the particle is represented as a point charge and the distribution of the charge on the surface and its possible effects on electrokinetic behavior are not taken into consideration. While these models have been successfully used to explain the behaviors of numerous large particles, they do not adequately depict biological particles. Charge distributions within these particles are seldom symmetrical, and this lack of symmetry can be accentuated by the fluidity of the membrane and the mobility of charged species of their surfaces. These inherent and field-induced deviations from the idealized rigid sphere with a uniform charge distribution can negate the use of a monopole moment for approximating their apparent surface potential and electrophoretic mobility.

Effects of Charge Distribution on Mobility. The concept of nonsymmetrical charge distributions and its potential effects on electrophoretic behavior is not new, and models have been developed for a limited number of systems. One electrophoretic model, which incorporates the first three multipole moments of the charge distribution, was developed by Anderson and applied to both spherical and ellipsoidal particles.[23,24] This model was derived according to many of the same basic assumptions of classical electrokinetic and traditional colloidal theories except the surface charge density and resulting ζ-potential of the particle is allowed to vary along its surface. The applications of this model toward ellipsoidal particles are reiterated here, as they are likely the most relevant for predicting the electrophoretic behaviors of biological particles.

According to the model, the electrophoretic mobility (μ) of an ellipsoid of revolution can be calculated using the first three moments of the multipole expansion of the ζ-potential using the following equation:

$$\mu = \frac{\epsilon}{4\pi\eta}\left[\beta_0 I - \frac{1}{2}\beta_2\right] \qquad (1)$$

where β_0 is the monopole moment, β_2 is the quadrupole moment, I is the identity tensor, ϵ is the dielectric constant, and η is the viscosity of the medium. The ζ-potential is assumed to be an axisymmetrical distribution that can be expressed as a function of z, the axis of rotational symmetry of the ellipsoid. The most essential conclusion that can be drawn from this model is that the electrophoretic mobility of a particle is dependent on all three moments of the charge distribution rather than just the monopole moment. Translational mobility is determined by, in this case, the monopole and quadrupole moments, while orientation is a function of the dipole moment of the particle. The existence of even a small dipole moment in a particle will result in alignment of the particle with the applied field, while a large quadrupole moment can significantly alter the observed mobility of a particle in comparison to that predicted on the basis of the area-averaged ζ-potential, or monopole moment. In contrast to the monopole moment, the quadrupole is determined by both the magnitude and distribution of surface charge; because of this, it is possible for two particles that are equivalent in all aspects except the spatial distribution of charge on their surfaces to exhibit markedly different electrophoretic mobilities.

Effects of Particle Size. Size-dependent effects are seen for the electrophoresis of particles in an electrolyte solution. The models developed by Anderson described above (eq 1) do not address these effects.[23,24] To examine the behavior of relatively large liposomes and biological particles, it is certainly necessary to consider the effects of relaxation and retardation. Relaxation is the direct result of distortions of the ionic atmosphere due to the inability of the diffusion controlled reforming of the ionic cloud to keep up with the movement of the particles. Retardation is a measure of hydrodynamic drag caused by the movement of the counterion cloud in the opposite direction of the particle in response to the applied field.

To investigate effects of particle size in this study, the spherical colloid theory developed by O'Brien was applied. This model indicates that the mobility of a spherical particle can be predicted by the following equation:

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

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.[17−19] The first term in eq 2, the Smoluchowski relation, is the approximate solution of the electrokinetic equations assuming no distortion of the ionic cloud. The second term is a correction for relaxation and retardation effects. There is no existing direct way to include charge distribution effects with the size of particles, since there is some as yet undefined relationship between higher order moments and size, deformability, and surface group mobility.

Integration of the Effects of Charge Distribution and Liposome Deformation. For a given size of liposomes, direct examination of the effects of charge distribution is possible. A particularly useful model was developed by O'Brien and Ward.[25] According to this model, the electrophoretic mobility of a population of randomly oriented spheroids can be calculated from the following 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 component of those ellipsoids with their major axis aligned with the applied field and $g^1(\beta)$ pertains to the velocity component of those with their major axis transverse to the field. This includes factors for shape and relaxation effects. In this particular system, the spheroids would all be aligned with the field; therefore, the transverse velocity component was removed for our calculations. To include the effects of uneven charge distributions, liposomal deformation, relaxation, and retardation for our systems, eqs 1 and 3 were combined to yield

$$\mu = \frac{\epsilon\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{\eta} - \frac{\epsilon kT}{3\eta e}\left(\frac{e\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{kT} - \gamma\right)[g^0(\beta)] \qquad (4)$$

(A more detailed explanation of this equation and further definitions of variables can be found in the appendix and reference material.[25])

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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 extrusion or reverse phase evaporation[26] and consisted of PC, PA, and chol in varying amounts. Samples were stored at 4 °C and used within 4 days.

Buffers. Buffers contained 2 mM tricine and were adjusted to the correct ionic strength with the appropriate amounts of K_2SO_4 and then titrated to pH 8.8.

Instrumentation. Capillary electrophoresis was performed on a machine designed and fabricated 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 was 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 a lipid coating (PC) according to the method described by Cunliffe et al.[27] or were coated with BRIJ 35 by the method of Towns and Regnier[28] as noted. Electroosmotic flow was measured using the neutral marker mesityl oxide, and all samples were pressure injected for 1 s at 20 psi.

Results and Discussion

Model of Liposome Behavior in an Applied Field. Liposomes have unique properties that are accentuated in the presence of an electrical field. The electrophoretic behaviors of biocolloids such as liposomes have been modeled as classical colloidal systems; however, due to these unique properties, their behaviors remain poorly described and predicted.[2,8,29] This is a direct result of these models not accounting for the intrinsic deformability and generally complex structures of liposomes. Contrary to the idealized rigid, nondeformable colloidal particle, liposomes and many biological structures consist of complex fluid bilayer exteriors surrounding internal aqueous cavities. These bilayers can contain numerous and varied charged species, which, in the presence of an applied field, have been shown to migrate within the bilayer, leading to polarization of the particle.[6,30−37] These structures are also prone to shape distortions due to shear forces as well as field-induced polarization, and these distortions are likely manifested as an elongation in the direction of the applied field. We have previously addressed these issues, and a discussion of these phenomena can be found in the reference material.[6,30−38]

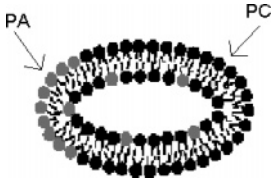

Figure 1. Cartoon representation of field-induced polarization of the model liposome system. The gray headgroups are representative of the negatively charged phosphatidic acid (PA), and the black ones represent the phosphatidylcholine (PC).

For biological structures with such complex surface properties, a better approximation of the effective potential may be obtained by performing a multipole expansion of the particle's surface charge distribution. The multipole expansion involves representing the charge distribution as a finite number of point charges which produce a field equivalent to the true surface charge. To perform a multipole expansion, it is first necessary to construct a model of a representative liposome in the presence of an applied field. The previously described liposomal properties such as deformability, membrane fluidity, and polarizability were all considered in constructing this model. A basic representation of the shape and charge characteristics can be seen in Figure 1. The liposomes were modeled as prolate ellipsoids aligned with the applied field. It was predicted that the negatively charged surface components, in this study phosphatidic acid, would migrate to one end of the structure; conversely, any positive components (phosphatidylcholine) would move to the opposite end. It should be noted that, while, in theory, PC is considered to be neutral in this pH range, it has been shown in practice to exhibit a positive surface potential.[37,39] For that reason, it has been assigned a slight positive potential throughout this study.

Application to Experimental Data: Effect of Size and Charge Variation. Particle size is a significant attribute to consider when modeling colloidal systems. Several series of liposome populations with varying total charges and sizes were used to investigate electrophoretic behavior. To help understand how liposome size, or radius, is important in migration, a spherical colloid theory was calculated and plotted (eq 2; solid lines, Figure 2).[19] Migration data were collected from liposomes with diameters of 50, 100, and 200 nm and surface charge densities ranging from −1 to −11 μC/cm^2. The observed reduced mobilities (E) of the various populations were also plotted against surface charge density (dashed lines, Figure 2) and compared to theory. Note, at this point in the develpoment of this discussion, that the effects of charge distribution are not included. Although they do agree in trend, there is a striking deviation from the theoretically predicted mobilities for most of the populations studied. A qualitative arguement can support the use of charge and shape to help explain these deviations, since analytical equations connecting particle size and change to charge distribution and shape are presently lacking.

On the basis of the multipole expansion of a charge distribution, a more asymmetrically shaped particle (a more elongated ellipsoid in this case) will have a larger quadrupole moment than a less asymmetrical particle and, hence, should exhibit a greater deviation from the standard electrokinetic theory. The smaller liposomes (50

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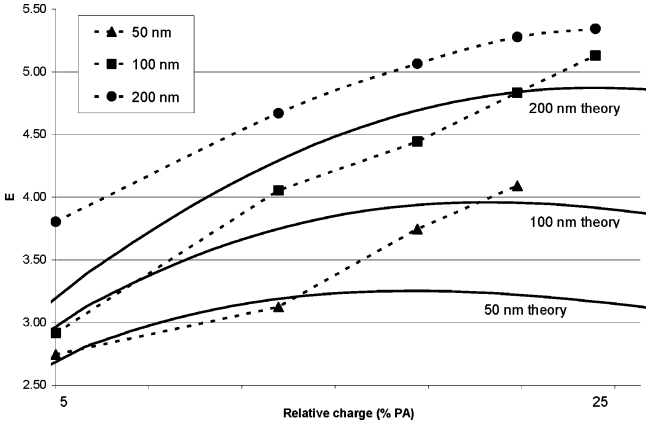


Figure 2. Comparison of liposome electrophoretic behaviors to predictions based on spherical colloid theory. The reduced mobility of the various populations is plotted vs the relative surface charge (determined by % phoshatidic acid (PA) contained in the liposomes). The dashed lines show the experimentally obtained values for the liposome populations. The solid lines show the mobilities predicted by the spherical colloid theory developed by O'Brien.[17,19]

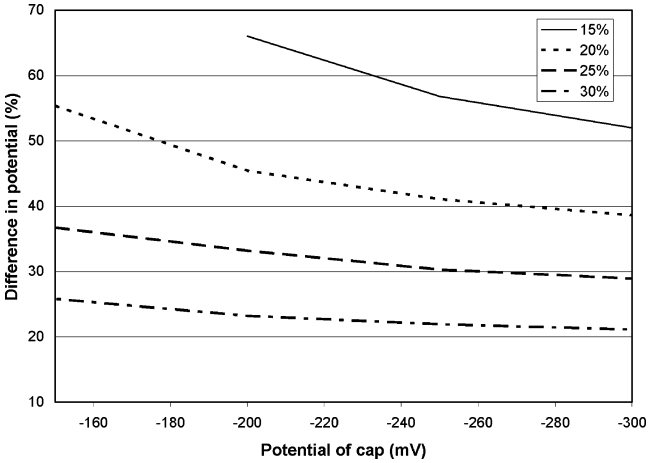


Figure 3. Percent difference in the magnitude of the multipole predicted potential and the area-averaged ζ-potential for several series of model liposome populations. The model populations represent ellipsoids with an aspect ratio of 0.5 that consist of a cap of negative potential, representative of PA, which covers a specific percentage of their surface and a small positive potential, representative of PC, assigned to the rest of the structure. For example, the 15% trend line is a series of vesicles with a negative cap that extends across 15% of their long axis and the ζ-potential of this cap of negative charge (representative of PA) varies from −150 to −300 mV along the *x*-axis.

nm diameters) are expected to be less deformable than the larger liposomes (100 and 200 nm diameters) on the basis of the length scale and bending constants of the constituent lipids and local radius of curvature of the structures. The smaller liposomes should, as a result, exhibit behavior more aligned with that predicted by the standard theories. Furthermore, a smaller number of charged lipids should result in less deformation due to polarization effects. Consequently, the liposome populations with less total surface charge should be less distorted, or remain more spherical in shape. This agrees well with the behavior of the smaller (50 nm diameters) liposomes with low charge (5−10% PA content), which exhibited mobilities consistent with the predictions. Conversely, the larger liposome populations (200 nm diameters) are expected to be more easily deformed because of both polarization and shear forces. As a result, a more significant quadrupole moment is expected for all of the 200 nm diameter populations and would result in a greater deviation from the monopole-predicted mobilities even for those populations with a low total surface charge. This also agrees well with the observed high mobilities for all of the 200 nm diameter populations studied. The 100 nm diameter populations appear to fall between the other two populations, acting more like spherical colloid particles in the low charge regime and polarized ellipsoids in the higher charge regime.

Examination of Distributed Charge Effects. To determine the potential effects of shape and charge distribution on the electrophoretic behavior of liposomes, the predicted charge distribution was integrated over the particle's surface according to the method detailed by Anderson.[23,24] On the basis of the proposed liposomal model, the dipole and quadrupole moments were calculated to be significant and, in this regime, would result in a full alignment of the structures along the applied field as well as much larger electrophoretic mobilities. The percent difference between the calculated potential based on both the monopole and quadrupole moments and that based on the area-averaged ζ-potential (or monopole moment) for several series of model liposome populations were calculated (Figure 3). Since electrophoretic mobility is a function of potential, the larger effective potential due to the inclusion of the quadrupole moment would result in an increased mobility (in the simplest case). Each line represents liposome populations with a different percent of the particle's surface covered by negative components. For instance, the 25% trend line represents a series of populations in which the negative component covers 25% of the length of the structure. The difference between the area-averaged potential and the potential which includes the quadrupole moment is significant for all of the series shown and indicates that this may be an important factor in accurately predicting the electrokinetic behaviors of liposomes.

Impact of Charge Distribution on Shaped Particles. Multipole effects can be directly incorporated into spheroidal theory which accounts for the relaxation and retardation effects (eqs 1 and 3). The liposomes in this study consisted of four series of populations containing 10% phosphatidic acid (PA) and 90% phosphatidylcholine (PC). The order with respect to surface charge and therefore the overall charge of these liposomes is population D > C > B > A (a more detailed explanation of these populations and their charges appears in the Appendix and reference material[5−7]). The ionic strength of the medium was varied for each series of populations in order to probe the effects of charge distribution over a broad range of double layer thicknesses. The ionic strength and resulting electric double layer thickness is arguably one of the most important factors in evaluating colloidal electrophoretic behavior. The thickness of the double layer determines, to a large extent, the magnitude of relaxation and retardation and, therefore, the extent of deviation from classical electrophoretic theory. The observed reduced mobilities of the liposomes (data points (lines for clarity only), Figure 4) along with the theoretically predicted mobilities (continuous curves, Figure 4) were plotted versus κ (κ is the Debye−Huckel approximation and is related to ionic strength). The lines shown in Figure 4A are those predicted for a prolate ellipsoid with an aspect ratio of 0.5 based on the area-averaged ζ-potential, or monopole moment, while the theoretical curves shown in Figure 4B are those predicted by including both the monopole and quadrupole moments. As can be seen in Figure 4A, the predictions based on the monopole moment

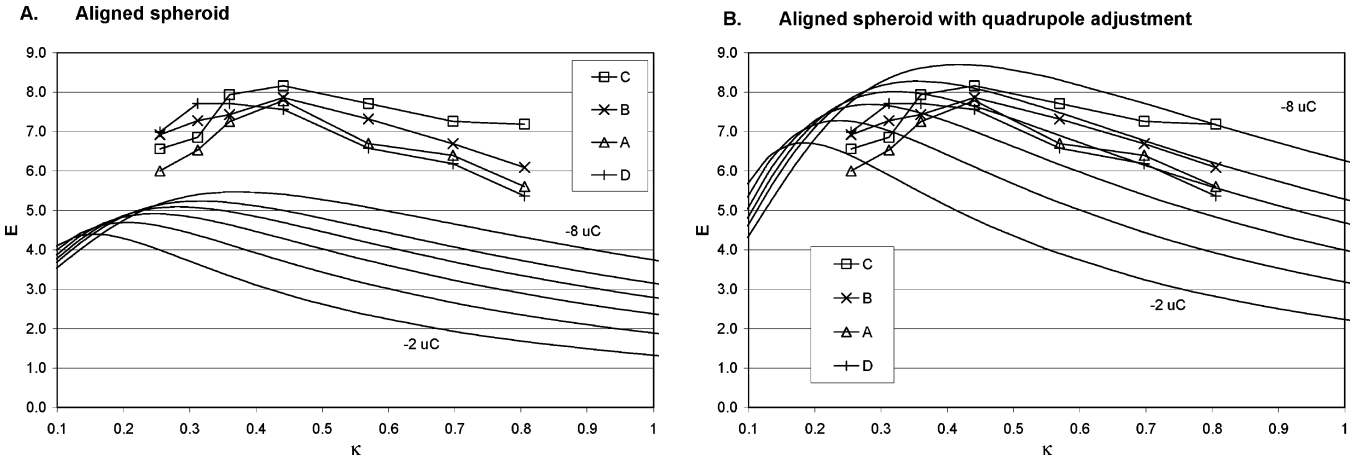


Figure 4. Plots of reduced mobilities (E, data points; lines for clarity only) vs κ for four series of liposome populations along with predicted values (continuous curves) for aligned spheroids with an aspect ratio of 0.5. The data points are the experimentally obtained values for the liposomes, and the curves are the theoretical predictions for series of populations with surface charge densities that bracket those calculated for the liposomes. Part A is based on the area-averaged ζ-potential, while part B is the same theoretical series adjusted to account for multipole effects.

alone are inconsistent with liposome behavior. The observed mobilities were nearly twice as large as those predicted over the entire range of double layer thicknesses studied. When the theoretical values are adjusted to include the quadrupole moment of the charge distribution (Figure 4B), a much closer fit is obtained. The close fit of the observed behavior with these predictions suggests that the liposomal model for charge distribution and elongation is applicable to these systems.

Conclusion

This study demonstrates that the use of a multipole expansion of the surface potential distribution of liposome populations can result in qualitative predictive calculations for the electrophoretic mobility. By incorporating multipole effects into colloidal theories, it is possible to take into account deformability, mobile surface charges, and polarizability and better model the electrokinetic behavior of these structures. This model appears to better describe the electrophoretic mobilities of liposomal systems compared to the traditional colloidal models that are typically used.

Acknowledgment. This work was supported by Arizona Applied NanoSensors.

Appendix

Liposome Populations. This study included liposome populations with consistent inner and outer pH values, as well as ones that contained a transbilayer pH gradient, or a difference in the pH of the inner compartment as opposed to the external medium. The order of these populations with respect to surface charge density is population D > C > B > A. The pH of the buffer used for population C is 8.8, and for population B, it is 7.4. The surface charge density of the liposome populations, due to the 10% 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. Populations A and D contained transbilayer pH gradients. For population D, the external buffer has a pH of 8.8 and the internal compartment buffer has a pH of 7.4. For population A, the external buffer has a pH of 7.4 and the internal compartment buffer has a pH of 8.8. The net effect of these gradients is that population D possesses a higher effective surface charge than population C (the regular pH 8.8 population) and population A possesses a lower effective surface charge than population B (the regular pH 7.4 population).

Mathematical Derivation of eq 4. Equation 4 was derived from eq 3 in a manner similar to that used in the derivation of eq 1. The ζ-potential was replaced with the multipole expansion of the ζ-potential distribution to produce

$$\mu = \frac{\epsilon\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{\eta} - \frac{\epsilon kT}{3\eta e}\left(\frac{e\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{kT} - \gamma\right)[g^0(\beta) + 2g^1(\beta)]$$

For reasons discussed in the text, the component due to transverse ellipsoids was removed to produce eq 4:

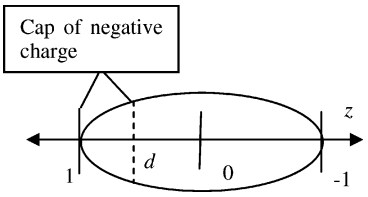
$$\mu = \frac{\epsilon\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{\eta} - \frac{\epsilon kT}{3\eta e}\left(\frac{e\left[\beta_0 I - \frac{1}{2}\beta_2\right]}{kT} - \gamma\right)[g^0(\beta)]$$

Charge Distributions Used to Produce Figures. The liposomes were modeled as prolate ellipsoids with an aspect ratio of 0.5 and a cap of high negative charge covering part of the surface and a small positive charge covering the remaining surface. The aspect ratio was chosen on the basis of preliminary imaging work performed on this system. The sensitivity of the mathematical model is such that it is only necessary to estimate the aspect ratio to one place. The surface potential was modeled as a function of z, the axis of the ellipsoid aligned with the applied field. The integral of the ζ-potential used for Figure 3 was as follows:

$$\int_1^d x\,dz + \int_d^0 -0.025\,dz + \int_0^{-1} 0.025\,dz$$

The size of the cap of high negative charge was varied along z, the axis of rotational symmetry of the ellipsoid,

and is represented by the variable d in the above equation. The potential of the cap of negative charge is represented by x in the above equation and was allowed to vary from −150 to −300 mV.



For Figure 4, we predicted that the liposome populations would have a multipole-predicted potential approximately 70% higher than their area-averaged potentials. This was done using the data from Figure 3 and was based on the phosphatidic acid (PA) content (10%) and a predicted potential for the cap of PA that would lie in the range −150 to −250 mV. The new effective potential which included the contribution from the quadrupole moment was incorporated into the spheroidal model (eq 3).

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