Interfacial Thickness of Liposomes Containing Poly(ethylene glycol)-Cholesterol from Electrophoresis

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ABSTRACT The electrophoretic mobilities of liposomes incorporating a polyethylene glycol (PEG) headgroup coupled to cholesterol for PEG of average chain index 3.0, 13.2, and 22.3 have been determined as a function of PEG-cholesterol mole fraction between 5% and 40% and ionic strength between 2 and 200 mM. The liposome compositions were 40 mole % cholesterol plus PEG-cholesterol, 10 mole % 1,2-dipalmitoyl-*sn*-glyerco-3-phosphoglycerol, and 50 mole % egg phosphatidylcholine. The mobilities were fit to a model in which the PEG forms a surface layer of polymer subject to viscous drag arising from electroosmotic flow within this layer. The model provides estimates of the average layer thickness that are comparable to those determined from contemporary models of surface-attached polymer.

INTRODUCTION

The properties of polymer surface layers are of great interest from both a fundamental and applied perspective, as they are present on particles as diverse as biological cells (Levine et al., 1983) and latex beads in paint (Napper, 1983). Tethered layers can both stabilize and destabilize colloidal particles, depending on their chemical characteristics, surface concentration, thickness, and the presence of free macromolecules in solution (Napper, 1983; Fleer et al., 1993; Israelachvili, 1991). One polymer that is proving of particular interest as a surface component is poly(ethylene glycol), as it seems not only to be able to stabilize particle suspensions against aggregation but can drastically reduce the adsorption of proteins and macromolecules in solution to the surface on which it is anchored (Harris, 1992). This activity is proving valuable in the field of drug delivery by liposomes, where incorporation of lipids with PEG headgroups, usually derivatives of phosphatidyl ethanolamine (Parr et al., 1994), has been shown to prolong the circulation time of the particles when injected in animal models (Allen et al., 1991).

One of the properties of the tethered layer that is of importance in understanding the behavior of such systems is its average thickness. Recent studies have utilized direct force-distance measurements between mica sheets coated with PEG-lipids (Israelachvili, 1991) and x-ray diffraction of multilayers of PEG-lipids subjected to osmotic stress (Parsegian et al., 1986) to determine measurements of the distance at which two opposing surfaces carrying PEG surface layers interact with and repel each other. These lipid systems are convenient because the area per molecule can be varied by controlling composition and charge effects can be studied by including phospholipids bearing net charges.

There is also considerable theoretical interest in tethered layers (de Gennes, 1980; Cosgrove et al., 1987; Milner et al., 1988; Zhulina and Vilgis, 1995). Estimates of PEG headgroup layer thicknesses are available from a number of works, particularly those dealing with the properties of surfactants carrying PEG headgroups (Sarmoria and Blankschtein, 1992; Kuhl et al., 1994; Kenworthy et al., 1995a). It is widely expected that the dimensions of such layers should change as the area per headgroup decreases, from a "mushroom" in the dilute, unhindered state at low surface concentration to an extended "brush" at high surface concentration, when interactions between adjacent headgroups force each coil to extend farther from the surface to which the tether is attached (Fleer et al., 1993). These effects have been observed with PEG headgroups of molecular masses in the range 750 to 5000 Da (Kuhl et al., 1994; Kenworthy et al., 1995a).

The methods by which thicknesses of PEG layers on lipids have been examined to date require ordered surface layers in two dimensions in either multilayers or spread on mica, although thickness information for nonlipid surfaces carrying PEG grafts has been obtained from small-angle neutron scattering (Cosgrove and Ryan, 1990) and ellipsometry (Lin et al., 1994). The question of interpenetration of opposing layers can complicate the interpretation of thicknesses determined from force-distance measurements for systems with dilute surface concentrations of polymer, however (Kenworthy et al., 1995a).

It would be useful to have a method that could be applied to less specialized surfaces or large particulates carrying polymer layers. One approach that suggests itself is the analysis of electrokinetic effects of surface-attached polymers (Yoshioka, 1991; Woodle et al., 1992). If a neutral layer of tethered polymer is present adjacent to a charged surface in an electric field the electroosmotic motion of the electrical double layer through the surface polymer will be retarded. The degree to which this occurs will depend on the

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hydrodynamic properties of the polymers, their surface concentration, and molecular weight, and the thickness of the double layer as determined by the ionic strength of the bathing medium. If the surface carrying the layer is undergoing electrophoresis, the effect translates into increased drag on the particle and a reduction in electrophoretic mobility. This effect has been analyzed independently by several groups (Jones, 1979; Donath and Pastushenko, 1979; Wunderlich, 1982; Levine et al., 1983; Ohshima and Kondo, 1989).

Another approach to interpreting the reduction in electrophoretic mobility associated with the presence of neutral polymer layers is to assume that the layer shifts the hydrodynamic surface of the particle away from the charged surface and reduces the zeta potential (Fleer et al., 1993, pp. 73–76). This is analogous to estimating layer thickness from the apparent increase in particle hydrodynamic radius measured by quasi-elastic light scattering or intrinsic viscosity measurements. In both cases the apparent dimension from which flow is excluded is taken to be the layer thickness. However, for electrokinetic phenomena the assumption that flow is totally excluded from the polymer layer is clearly incorrect for dilute layers and is likely incorrect for even reasonably high concentrations. Neutral polymers have only a small effect on the solution conductivity of electrolyte solutions (Brooks and Seaman, 1973), for instance, so the ion movement that drives electroosmosis cannot be affected much by their presence. Moreover, electroosmosis is routinely observed through polymeric membranes of high polymer content (Barragán et al., 1994). The present approach uses the Brinkman equation for flow within the layer and interprets the permeability of the polymer solution in this region in terms of Stokes drag of the segments (Levine et al., 1983).

We have applied the electrophoretic method to liposomes containing PEG-cholesterol with headgroups of mean molecular weights of 150, 600 and 1000. Measurements of electrophoretic mobility as a function of ionic strength, mole %, and headgroup molecular weight of PEG-lipid have been analyzed through application of our nonlinear theory of the above effect, which assumes that creeping flow occurs within the surface layer (Sharp and Brooks, 1985). The data have been interpreted to provide values for the thicknesses of the headgroup region that are in substantial agreement with theoretical estimates. These results open the application of this method to the analysis of a wide variety of surfaces on which polymer layers are present.

MATERIALS AND METHODS

Materials

Cholesterol *p*-toluene sulfonate and cholesterol (CHOL), chromatography standard grade, were from Sigma (St. Louis, MO). Triethylene glycol (PEG150) and poly(ethylene glycols) of 600 (PEG600) and 1000 (PEG1000) average molecular weight were from Aldrich (Milwaukee, WI). Egg phosphatidylcholine (EPC) and 1,2-dipalmitoyl-*sn*-glyerco-3-phosphoglycerol, sodium salt (PG), were from Northern Lipids (Vancouver, BC). Sodium chloride, 1,4-dioxane, and isopropanol, all ACS grade, were from Fisher Scientific (Edmonton, AB). Chloroform, ethylacetate, and methanol were either Omnisolve from BDH (Toronto, ON) or HPLC-grade Fisher Scientific. Thin-layer chromatography (TLC) plates were Baker-flex silica gel IB2-F from J. T. Baker Chemical Co. (Phillipsburg, NJ) and KC18 (reverse-phase) silica gel 60A, 200-μm thickness from Whatman (Maidstone, England). EDTA and 3-(*N*-morpholino)propanesulfonic acid (MOPS) were from Eastman (Rochester, NY) and BDH, respectively. Water was glass distilled and further deionized using a MilliQ+ filtration system (Millipore Canada, Mississauga, ON).

Synthesis of PEG cholesterol

The PEG derivatives of cholesterol (PEG-CHOL) were prepared as described by Patel (1984), with the addition of a preliminary drying of the PEG stock by azeotropic distillation from 1,4-dioxane. Silica TLC in chloroform:ethyl acetate:isopropanol (5:5:1) indicated the presence of some molecular weight polydispersity in the PEG stocks. Reverse-phase C18 TLC in methanol:water (20:1) showed that the products were substantially (<5%) free of unreacted PEG. The products were dried to constant weight over P_2O_5 and stored as chloroform solutions under N_2 at −20°C. Evaporative loss of solvent was observed and corrected gravimetrically.

Preparation of liposomes

Except for PG, solutes were dissolved in chloroform; PG was dissolved in chloroform:methanol (1:1). Lipid mixtures were prepared from solutions gravimetrically. Aliquots containing approximately 1 mg of total lipids were transferred to surfactant-free 250-ml round bottom flasks, and the solvent was removed by rotary evaporation followed by drying at less than 0.25 mm Hg for 60 min. The lipids were rehydrated in 3 ml of the solution used for electrophoresis by continuous swirling agitation for 50 min at 41°C in an orbital shaker. The electrophoresis solutions were prepared from a stock of 200 mM NaCl, 2 mM MOPS, and 1 mM EDTA (pH 7.0 ± 0.2) diluted to the desired ionic strength with water by volume. Liposome suspensions were stored at 4°C before use, and measurements were made within 4 days.

Electrophoretic mobility measurements

The electrophoretic mobilities of liposomes were determined in a Rank Mark I apparatus with a cylindrical chamber at 25.0°C. Measurements were made at the stationary level on the largest in-focus particles. There was no size dependence observed. Each particle was timed twice with reversal of the electric field between timings; measurements were rejected when the motion due to drift exceeded 0.05 μm-cm/V-s or 25% of the mean mobility. Generally, ten or more particles were timed per experimental condition.

The mobility was calculated from the averaged velocities, the applied voltage, and the chamber electrical length. The latter was determined in separate conductivity measurements. The electric field strength was about 3.5 V/cm, and fixed human red cells were found to have a mobility of 1.07 ± 0.06 μm-cm/V-s in 0.15 M NaCl (pH 7.2).

Model calculations

Particle mobilities were calculated using a model developed for red cells that considers a surface region containing distributed fixed charges, mobile charges forming an extended electrical double layer, and resistive elements representing polymers anchored to the particle surface that transmit viscous drag to the particle in the presence of electroosmotic motion. The full Poisson-Boltzmann and Stokes equations are solved numerically throughout the surface region to provide an expression for the electrophoretic mobility as a function of the effective size of polymer elements in the

surface layer, the concentration of polymer in the surface region, and the depth of the layer (Sharp and Brooks, 1985).

In the present case the model has been simplified to a surface bearing a uniform surface charge density due to charged lipid headgroups located at $x = 0$ and a superficial layer of anchored neutral polymer elements occupying a region of thickness h (Fig. 1). The shear plane is taken either at $x = 0$ or at some distance exterior to the charge plane (Eisenberg et al., 1979). The electrical double layer forms within the region occupied by neutral polymer and extends beyond it to varying degrees, depending on the ionic strength. In an external electric field, electroosmotic motion driven by migration of ions in the double layer produces the drag exerted on the polymer segments, which is transmitted to the particle. The electrophoretic velocity of the liposomes therefore is predicted to be retarded to different degrees, depending on the double layer extension relative to h. Calculation of particle mobilities with this model, given values for charge density, ionic strength, polymer mass, area per polymer molecule, and segment size, allows the predicted values to be fit to experimental data and as a function of h to provide an estimate of layer thickness on the basis of best fit. The nature of the dependence may be appreciated from an approximate expression for the mobility as a function of the above parameters, based on the linearized Poisson-Boltzmann equation, given as equation 23 in Levine et al. (1983).

The Stokes drag exerted by the layer of anchored polymer is characterized by the volume density N_v and hydrodynamic radius of the PEG monomer segments. This radius is taken to be equal to one-half of the statistical segment length for PEG, $a_m = 4.3$ Å (Evans et al., manuscript submitted for publication). The polymer index N is the number of segments per chain,

$$N = (M_{peg} - 18)/44,$$

where M_{peg} is the average molecular weight of the PEG head group. The PEG is assumed to be uniformly distributed in a region of depth h. Thus the volume density of PEG segments N_v is given by

$$N_v = N/A_{peg}h = NX_{peg}/A_{lip}h,$$

where A_{peg} and X_{peg} are, respectively, the average area per molecule and mole fraction of PEG-cholesterol in the liposomes, and A_{lip} is the average area per lipid in the bilayer, 68.5 Å^2 (Small, 1967). The PEG segment volume v_m is 61.4 Å^3 (Evans et al., manuscript submitted for publication); thus the volume fraction of the PEG chain ϕ_{peg} in the layer h is

$$\phi_{peg} = v_m N_v.$$

The fit of the data to the theoretical calculations was determined visually.

RESULTS

Formation of liposomes

Fewer liposomes formed at 40 mole % in the case of PEG600-CHOL and at mole fractions higher than 10% in the case of PEG1000-CHOL than at lower concentrations and molecular weights. This was evident in that the liposome suspensions were less opaque and that fewer particles were visible microscopically.

Mobility as a function of composition

Fig. 2 shows the mobilities at an ionic strength of 0.100 M of liposomes composed of 50 mole % EPC, 10 mole % PG, between 5 and 40 mole % PEG-cholesterol, and a complementary amount of cholesterol (EPC:PG:CHOL:PEG-CHOL, 50:10:40-x:x). Data for three different average molecular weight PEGs are shown. In addition, the data for liposomes without PEG, without PG, and without PEG and PG are shown. The nonzero mobilities of the latter two indicate that there is a small amount of charge associated with components other than PG. The mobility of EPC (Table 1) shows that the charge is associated primarily with cholesterol, presumably because of oxidation products (Smith, 1981). For cholesterol at 40 mole %, the charge is estimated to be between 1400 and 1900 esu/cm^2 (Table 1), depending on the shear plane location assumed in the electrophoretic model calculations. The estimates are interpolated from the mobility assuming it is a linear function of charge density at high ionic strengths. Fig. 3 shows exact calculations of mobility for smooth particles, using the nonlinear solution to the Poisson-Boltzmann equation (Levine et al., 1983) as a function of surface charge density at different ionic

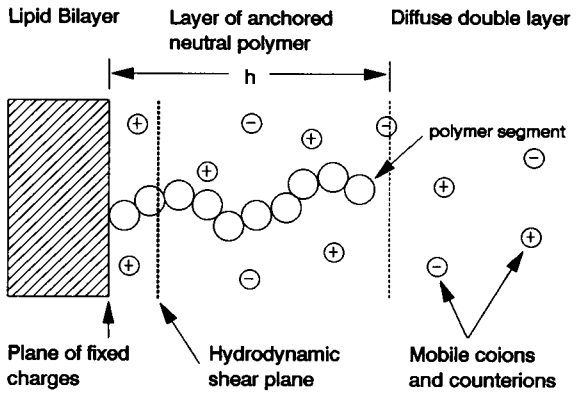

FIGURE 1 Model of the PEG-cholesterol liposome surface region. The fixed charges are due to PG. The anchored PEG head group is represented by N segments of average radius a uniformly distributed over a depth h extending from the charge plane. A region of zero flow extends from the charge plane to the hydrodynamic shear plane.

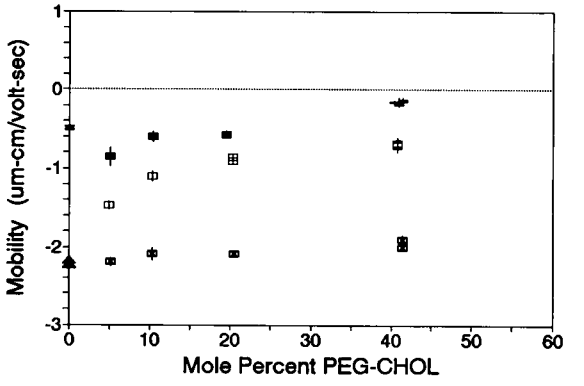

FIGURE 2 The electrophoretic mobility in 0.1 M NaCl of PEG-cholesterol liposomes as a function of PEG-cholesterol mole fraction and head group molecular weight. The liposome compositions are EPC:PG:CHOL:PEG-CHOL (50:10:40-x:x) for PEG150 ⊠, PEG600 □, PEG1000 ■, and EPC:PG:CHOL (50:10:40) ▲. The PG free liposomes (▬) are EPC:CHOL (60:40) and EPC:PEG-CHOL (60:40). The mobilities of the latter liposomes are nearly indistinguishable for the three PEG molecular weights.

TABLE 1 Charge of liposome lipids

Liposome composition	Ionic strength (M)	Mobility u (um-cm/V-s)	Charge density* σ (esu/cm^2)	Charge fraction Q (moles/mole)
EPC (100)	0.002	−0.21 ± 0.18	70 ± 70	0.001
EPC:CHOL (60:40)‡	0.100	−0.50 ± 0.03	1405 ± 90	
EPC (60)			42 ± 42	0.001
CHOL (40)			1400 ± 100	0.050
EPC:CHOL (60:40)§	0.100	−0.50 ± 0.03	1920 ± 120	
EPC (60)			42 ± 42	0.001
CHOL (40)			1900 ± 130	0.068
EPC:PG:CHOL (50:10:40)§				
EPC (50)			35 ± 35	0.001
PG (10)			7007	1.0
CHOL (40)			1900 ± 130	0.068
			8942 ± 135	
EPC:PG:CHOL:PEG-CHOL (50:10:20:20)§				
EPC (50)			35 ± 35	0.001
PG (10)			7007	1.0
CHOL (20)			950 ± 65	0.068
PEG-CHOL (20)				NA‖
			7992 ± 70	

*The charge density is the sum of the component charge densities. $\sigma = \Sigma\sigma_i = \Sigma\, Q_i\,(X_i/\Sigma X_i)/A_{lip}$, where Q_i is the moles charge/mole component i, X_i is the mole % component i, and A_{lip} is 68.5 × 10^{-16} cm^2/molecule.
‡Estimated with the shear plane at 0 Å.
§Estimated with the shear plane at 2.8 Å.
‖No estimate available.

strengths. It shows that the calculated points are reasonably well fit by straight lines over the ionic strengths of interest here.

For the PEG derivatives at 40 mole %, charges are also implied; however, to estimate these the mobility reduction due to drag must be known. As independent estimates of the PEG-cholesterol charge densities are not available, a small uncertainty is introduced from this source.

As an approximation we assume that the mobilities of the liposomes with zero PG are not significantly altered by the drag factor. Thus, Fig. 4 shows the mobilities corrected for non-PG mobility, i.e., with the non-PG charge effect subtracted and the mobility curves determined for the theoretical model. The model curves are fit by varying two parameters. First, the location of the shear plane was set at 2.8 Å so that the calculated mobilities matched the mobility of the (EPC:PG:CHOL, 50:10:40) liposomes. This value compares to the 2.0 Å found by Eisenberg et al. (1979) to rationalize their data for phospholipid mobilities. The fitting was carried out using 7007 esu/cm^2 for the liposome charge density, calculated from the charge per mole PG, the mole fraction of PG in the liposomes, and the average area per lipid. If the last of these was allowed to increase to 97 Å^2, the (EPC:PG:CHOL, 50:10:40) could have been fit with the shear plane at zero angstroms.

Second, the thickness of the PEG surface layer was varied. Data fits to the calculations based on our model imply that differences in h can be distinguished over the range of PEG head group molecular weights used here (Table 2).

Mobility as a function of ionic strength

Fig. 5 shows the mobilities of (EPC:PG:CHOL:PEG-CHOL, 50:10:20,20) liposomes for three PEG-cholesterol derivatives and (EPC:PG:CHOL, 50:10:40) liposomes as functions of ionic strength. In addition, calculations are shown for a range of charge densities assuming that there is no drag and that the shear plane is at zero angstroms (*solid*

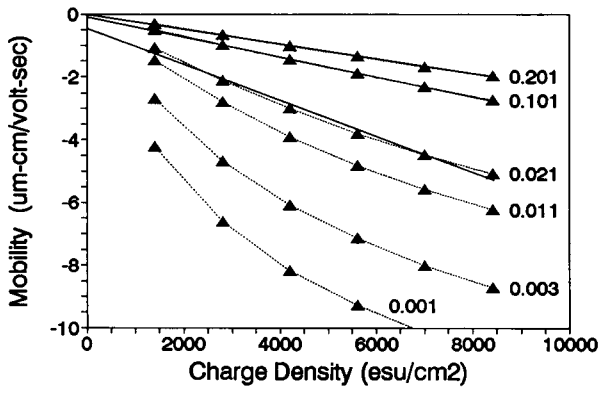

FIGURE 3 The calculated electrophoretic mobility ("▲") as a function of charge density at the ionic strengths indicated with the shear plane set at zero Å. For ionic strengths greater than about 0.1 M the mobility is proportional to the charge density independent of the position of the shear plane to within the precision of the data. Linear regression (——) of the mobility points at 0.1 M in the figure are described by intercept, slope, and regression fit parameter values of −0.08 ± 0.03, −(32 ± 0.5) × 10^{-5}, and 0.9995, respectively.

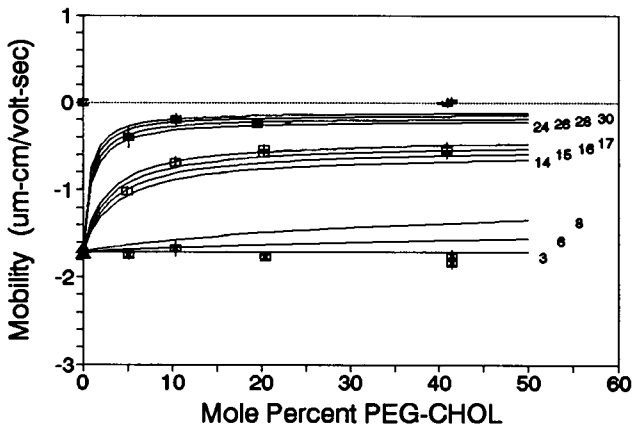

FIGURE 4 The mobility data shown in Fig. 2 corrected for non-PG charge. The mobilities in Fig. 2 were reduced by an amount given by the linear regression fit of the mobilities of liposomes without PG. This approximate correction is discussed in the text. The solid lines show model calculations in which the charge density is constant at 7007 esu/cm^2 and the shear plane is located 2.8 Å from the charge plane. The PEG head group is represented by segments of radius 2.15 Å distributed uniformly in a layer of thickness h angstroms given in the figure and extending out from the charge plane.

TABLE 2 PEG layer thickness from liposome electrophoretic mobility

PEG headgroup molecular mass (Da)	Shear plane location (Å)	Layer thickness h (Å)			
		as function of mole % PEG at 0.100 M			
		5%	10%	20%	40%
150	2.8	<3	<6	<3	<3
600	2.8	15 ± 1	17 ± 1	17 ± 1	16 ± 2
1000	2.8	25 ± 5	30 ± 3	25 ± 2	—
		as function of ionic strength at 20 mole % PEG			
		0.002 M	0.020 M	0.100 M	0.200 M
150	0.0	12 ± 2	8 ± 2	6 ± 1	7 ± 1
	2.8	11 ± 3	<8	—	<2
600	0.0	22 ± 3	19 ± 1	14 ± 1	13 ± 1
	2.8	22 ± 3	19 ± 1	14 ± 1	13 ± 1
1000	0.0	28 ± 3	28 ± 2	17 ± 2	17 ± 2
	2.8	28 ± 3	28 ± 2	17 ± 2	17 ± 2

lines). It is evident that if headgroup drag is not explicitly taken into account an unreasonably low charge density would have to be assumed to explain the observed mobilities. Over the parameter range in this figure, the relation between mobility and charge density is nonlinear, so that subtraction of a control mobility would be incorrect, unlike the analysis used for the data of Fig. 2. Rather, where the charge densities of non-PG components are known, they have been added to that due to PG. The solid curve labeled 8942 esu/cm^2 corresponds to the estimated charge density of EPC:PG:CHOL (50:10:40) liposomes, as delineated in Table 1.

The charge density estimated for cholesterol depended upon the location assumed for the shear plane (Table 1). The higher charge per mole estimated with the shear plane at 2.8 Å was used in the charge density calculation above. However, small increases in the shear plane and charge density produce opposing changes in the mobility. The broken curves adjacent to that for 8942 esu/cm^2 are for the same charge density with the shear plane at 2.0, 2.8, and 4.0 Å. It is evident that given a charge density, mobility data may be fit by adjusting the shear plane without changing the shape of the mobility-ionic strength curve a great deal. However, where a surface layer provides resistance the location of the shear plane has a differential effect, which obscures thinner layers more than thicker ones.

Fig. 6 shows the mobility data and calculated mobility curves for EPC:PG:CHOL:PEG-CHOL (50:10:20:20) liposomes for a charge density of 7992 esu/cm^2 (Table 1) and the layer thicknesses indicated in the figure. The shear plane is at 2.8 Å; however, except for PEG150 the fit to the data

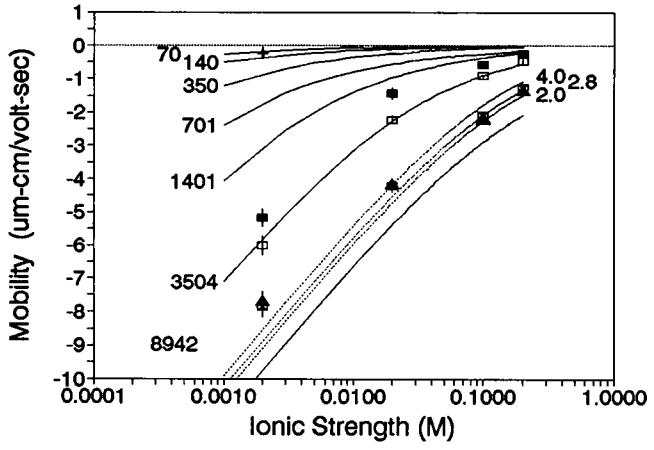

FIGURE 5 The electrophoretic mobility of 20 mole % PEG-cholesterol liposomes as a function of ionic strength. The liposome compositions are EPC:PG:CHOL:PEG-CHOL (50:10:20:20) for PEG150 ⊠, PEG600 □, and PEG1000 ■, and EPC:PG:CHOL (50:10:40) ▲. Pure EPC liposomes are shown at 0.002 M ionic strength (✚). Mobility model calculations are shown for the indicated surface charge densities in esu/cm^2, assuming no PEG and a shear plane at zero (*solid line*), or the indicated displacement in angstroms from the charge plane (*broken lines*). The charge density of the EPC:PG:CHOL (50:10:40) liposomes is estimated to be 8942 esu/cm^2.

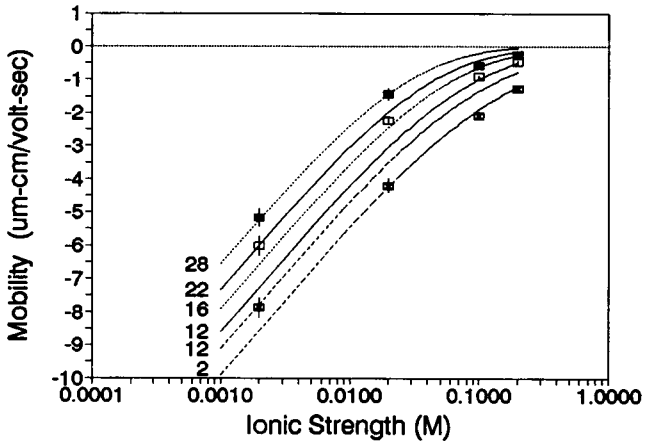

FIGURE 6 Mobility model calculations assuming a constant charge density and shear plane location and varied layer thickness h and PEG head group molecular weight compared with the mobilities of the 20 mole % PEG-cholesterol liposomes shown in Fig. 5. The charge density of the liposomes is estimated to be 7992 esu/cm^2, the shear plane is displaced 2.8 Å from the charge plane, and the layer thicknesses are indicated in the figure in angstroms. The PEG head group average molecular masses are 150 (– – –), 600 (——), and 1000 (·····) Da.

is unchanged if the shear plane is reduced to zero. The layer thickness for PEG150 appears to be about the magnitude of the shear plane displacement from the charge plane. The same fitting analysis assuming a lower charge density of 7007 esu/cm^2, i.e., that of 10 mole % PG, shifts all the curves to slightly lower mobilities and thus reduces the estimated layer thicknesses by 2 to 3 Å.

DISCUSSION

The low liposome yields at higher mole fractions of the PEG 600 and PEG 1000 cholesterol derivatives suggest that a substantial amount of the material has formed micelles. Under these conditions the composition of the liposomes is unlikely to be that of the bulk lipid solutions. On this account it follows that our estimates of the PEG layer thicknesses could be low for these liposomes. Kenworthy et al. (1995b) have reported phase diagrams for liposomes formed from PEG derivatives of distearoylphosphatidylcholine. Above the gel to liquid-crystalline phase transition temperature, the L_α phase of the bilayer was observed up to about 40 mole % for PEG 600 and about 25 mole % for PEG 1000; micelles began to be observed at higher concentrations. For derivatives of headgroups greater than 1000 the surface concentration at which micelles begin to form progressively decreases. This is generally in accord with our incidental observations, notwithstanding the difference in the lipid anchors used.

The presence of charge associated with non-PG components of the liposomes complicated the analysis of mobilities in terms of a PEG interfacial model. As explained above, we had to correct for the charged impurities by two different methods, depending on whether the data set was taken at constant ionic strength or constant mole fraction of PEG derivative. For the modeling at constant ionic strength as a function of PEG mole fraction, the variation in charge density with composition required a correction for the resulting variable driving force applied to the particles. Our approach was to directly subtract mobilities associated with the excess charge densities. The essentially linear relationship between mobility and charge density over the relevant range of ionic strengths suggested that this was a reasonable approach. When the data set taken at constant mole fraction of PEG-CHOL as a function of ionic strength was analyzed, this approach was not open, as the mobility-charge density relationship was nonlinear in the range of interest. Instead, we estimated the total charge density for each composition, given in Table 1, and fit the data assuming this charge was all located in the charge plane.

The data in Table 2 summarize the parameter values that gave the best fits to our calculations. It is seen that quite consistent values are obtained at 0.1 M ionic strength as the molecular weight and mole fraction of the PEG headgroup are varied. The data obtained by fitting the data at constant mole fraction as a function of ionic strength show a variation as a function of ionic strength, as discussed below. They also indicate that the two fitting procedures lead to different values for the thickness of 20 mole % PEG 1000 at 0.1 M ionic strength, however, with the latter value being the lower.

The discrepancy may lie in the two different methods used for correcting for charge impurities. One problem is the nonlinear sensitivity of the mobility to layer thickness at 0.1 M ionic strength. Subtracting "impurity" mobilities results in very low mobilities, which poorly resolve the layer thickness at 0.1 M ionic strength. For instance, for PEG 1000 liposomes the correction reduces the mobility to -0.25 from -0.57 μm-cm/V-s. From Fig. 4 this mobility reduction is associated with an increase in the layer thickness on the order of 10 Å. Hence, a small error in the correction procedure could produce a significant effect on layer thickness, possibly causing the discrepancy noted.

Although no estimates for any charge associated with the PEG 150, PEG 600, and PEG 1000 cholesterol derivatives are available, these components remain at constant composition, so the charge densities remain constant. Thus analysis of the ionic strength dependence of the mobilities does not require correction for variation in charge densities due to composition.

The data in Table 2 suggest that the layer thicknesses determined by fitting the data to our model increase with decreasing ionic strength. This feature was found in our earlier work interpreting red cell mobilities in terms of glycocalyx dimensions, as well (Levine et al., 1983), although use of the nonlinear theory significantly reduced the dependence (Sharp and Brooks, 1985). In that case the polymer layer was assumed to carry a fixed charge density, so a conceptual swelling of the polyelectrolyte layer at low ionic strengths seemed reasonable. However, assuming the PEG chains are in fact neutral, against which there is no contrary evidence, it is less clear physically why the layer thickness should be ionic strength dependent. At less than 0.100 M ionic strength the differences between the mobilities could be explained if a) the PEG layer thickness was in fact ionic strength dependent; b) the charge density of the liposomes decreases with ionic strength; c) the shear plane moves farther from the surface as the ionic strength decreases; or d) there are features of the PEG layer not incorporated in our model. At present we cannot eliminate any of the above possibilities.

Table 3 compares our estimates of PEG layer thickness with model predictions for PEG in the mushroom and brush regimes proposed by de Gennes (1988). The Flory dimension R is an estimate of the unperturbed diameter of a polymer. It is given by $aN^{3/5}$, where a is the statistical monomer (segment) length and N is the polymer index. However, the scaling concepts that apply to long polymer chains are inappropriate for chains shorter than 30 bonds, i.e., PEG 458 (Sarmoria and Blankschtein, 1992). A possible model for very short chains is a rod of length aN; however, this presumably would overestimate the PEG extension. Thus we have taken as one estimate values computed for an anchored chain attached to a wall, R_r (Sarmoria

TABLE 3 Comparison of observed PEG layer thickness with model predictions for PEG mushrooms and brushes

PEG headgroup		Layer thickness h (Å)								
		Observed		Form*	Mushrooms‡			Brushes§		
Molecular mass (Da)	mole%	h_1‖	h_2¶	D:R	R_r	R_{f1}	R_{f2}	L_r	L_{f1}	L_{f2}
150	5	<3	—	M	8	7	8			
	10	<6	—	M						
	20	<3	2–11	M						
	40	<3	—	M						
600	5	15	—	M	17	17	20			
	10	17	—	M						
	20	17	13–22	~				16	15	17
	40	16	—	B				21	19	22
1000	5	25	—	M	23	23	28			
	10	30	—	~				20	20	23
	20	25	17–28	B				26	26	29
	40	—	—	B				32	32	37

*The form of the theoretically appropriate PEG structure, mushroom (M) or brush (B), is chosen on the basis of the relative values of the Flory dimension (R) and the average separation of PEG attachment sites (D), which is calculated from A_{1ip} and the PEG-CHOL mole fraction. An intermediate condition is suggested when $D \approx R$ (~).

‡The Flory dimension (R) is given by aN^{ν} (de Gennes, 1988), where a is the statistical segment length, N is the polymer index, and ν is usually 3/5. R_r, R_{f1}, and R_{f2} are based on parameters used by Sarmoria and Blankschtein (1992), a = 2.65, ν = 0.514; Kenworthy et al. (1995a), a = 3.5, ν = 0.6; and Evans et al. (manuscript submitted for publication) a = 4.3, ν = 0.6, respectively.

§The brush length (L) is given by $aN(a/D)^{2/3}$, or its equivalent $D(R/D)^{5/3}$, in the case of L_r. $L_{f2} = aN(\nu_e(a/D)^2)^{1/3}$ with ν_e = 0.51.

‖Estimated from analysis of concentration dependence of h.

¶Estimated from analysis of ionic strength dependence of h.

and Blankschtein, 1992). A second estimate, R_{f1}, uses the monomer length assumed by Kenworthy et al. (1995a), a = 3.5. The last estimate, R_{f2}, uses the monomer length assumed in the analysis of the electrophoretic mobilities in this paper. The estimates of the brush lengths L_r and L_{f1} are derived from the de Gennes (1988) analysis, $L = aN(a/D)^{2/3}$, and the assumptions used in the corresponding mushroom diameter calculations. The estimate L_{f2} due to Milner et al. (1988) is similar but includes the excluded volume parameter for the polymer estimated to be ~0.51 for PEG (Evans et al., manuscript submitted for publication). The choice of which model is appropriate follows the de Gennes (1988) criteria and is described in Table 3.

That the mobilities of the PEG 150 liposomes are independent of the PEG concentration and not different from those of the corresponding composition without the headgroup reflects the fact that even at 0.2 M ionic strength the decay length of the electrical double layer is 7 Å. Even with this shortest decay length, for placement of the hydrodynamic shear plane at 2.8 Å, the sensitivity of the mobility to short chains is not sufficient to estimate their length.

However, for the PEG 600 and 1000 liposomes the PEG layers are thick enough for differences to be observed. The values obtained agree with the range exhibited by the various theoretical estimates, as illustrated in Table 3. For these relatively low-molecular-weight polymers the transition from mushroom to brush is not predicted to be dramatic. The uncertainties in the electrokinetic model and its fitting are such that we cannot distinguish such effects, assuming they are present. Given the reasonably narrow range of the values of best fit obtained when the mole fraction of PEG-lipid was varied at constant ionic strength, it seems possible that such effects could be detected if higher-molecular-weight headgroups were studied, however.

The present analysis has assumed a surface layer of uniform segment density. However, it has been shown that this, or equivalently, the segment volume fraction, varies with distance from the surface for tethered layers. For a nonadsorbing, very long, monodisperse polymer a parabolic density profile is predicted at equilibrium (Milner et al., 1988). In cases where adsorption is significant the profile decreases more rapidly but may show a long tail (Cosgrove and Ryan, 1990). Molecular weight polydispersity is also a factor (Milner et al., 1989). Thus a prescription for the segment density profile depends at least upon the interaction of the polymer segments with the surface and the molecular weight polydispersity of the tethered chains. Considering the resolution of our results and the lack of independent estimates of surface interaction or PEG headgroup molecular weight polydispersity in our system, a more sophisticated analysis was not felt to be justified at present.

The reasonable agreement between the thickness estimates determined from our electrophoretic model and the theoretical calculations suggests this method will prove useful for examining a variety of polymer-bearing interfaces. Because electrokinetic measurements are not limited to particles, the surfaces of both particulate material and cylinders of various cross sections could be examined utilizing electrophoresis, electroosmosis, or streaming potential measurements. By including a fixed charge distribution

in the surface model it is possible to perform similar calculations on surfaces carrying charged polymers (Levine et al., 1983; Sharp and Brooks, 1985), allowing the method to be applied to a variety of interfacial types.

As has been discussed elsewhere (Levine et al., 1983), the assumptions of our model are those known to lead to good descriptions of electrokinetic and polymer frictional behavior. Given the present results, it would be interesting to compare this method with the results of force-distance measurements or neutron diffraction studies to further validate the approach.

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