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Electrokinetic Properties of Lipid and Sarcoplasmic Reticulum Membranes in Aqueous
Electrolyte and in the Presence of Lipophilic Ions

by

Laura Elizabeth Satterfield

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Applied Physics

Dissertation Committee:
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Abstract

The purpose of this study is the characterization of the membrane-water interfaces of both sarcoplasmic reticulum membrane (SR) and charged lipid bilayers under varied properties of the surrounding aqueous solution. In this work we studied the electrokinetic properties of liposomes and SR vesicles as well as the interaction of lipophilic ions with these membranes.

The study of electrokinetic properties is based on the measurements of electrophoretic mobility of SR membrane vesicles and PC/PG liposomes. Electrophoretic mobility of SR vesicles was measured as a function of ionic strength for six pH values (pH 4.0, 4.7, 5.0, 6.0, 7.5, and 9.0). Electrophoretic mobility of single-layered and multi-layered PC/PG liposomes was measured at neutral pH as a function of ionic strength. For interpretation of electrophoretic mobility studies, SR vesicles (at pH 4, 7, and 9) and multi-layered and single-layered liposome sizes were determined using photoelectron microscopy. The study of the interaction of lipophilic ions with these membranes is based on (1) measurements of their partition coefficients described in terms of an ion partition model based on the Langmuir adsorption model and (2) electrophoretic mobility measurements of SR vesicles and PC liposomes in suspension with varied concentration of lipophilic ions.

SR-water and PC-water partition coefficients were measured as a function of concentration for two anions tetraphenylborate (TePB^-) and pentabromophenol (PBP^-) and two cations (Imipramine⁺, and Clomipramine⁺). The anions belong to a class of

pesticides and the cations are drugs once prescribed as anti-depressants. Partition into the SR membrane was shown to be significantly greater for all lipophilic ions except TePB^- , which only showed this effect at the higher lipophilic ion range of the data. The PC-water partition coefficient was also measured for TePP^+ . Since the lipid bilayer of SR is not significantly different than that of PC liposomes, we believe the differences in partition are due to excess lipophilic ions being absorbed to the proteins of SR.

The electrokinetics of charged PCPG liposomes, and PC liposomes with absorbed lipophilic ions could be understood in terms of the charge being located below their surface and screened by counter-ions inside the polar head-group region. We call this model the “permeable surface model.” The assumptions of this model are that (1) the charge exists on a plane at a depth, d , below the surface of the liposome within the lipid head-group region and (2) small ions (Na^+ , K^+ , Cl^-) are able to penetrate the lipid head-group region with a molar membrane-water partition coefficient of 0.4.

Using this model we were able to obtain the depth of sorption of lipophilic ions in PC liposomes. We found values of 0.13 nm for TePB^- , 0.5 nm for PBP, 0.12 nm for Imipramine⁺, 0.17 nm for Clomipramine and 0.25 nm for TePP^+ . The depth of lipophilic ions in PC is a valuable quantity for the study of the effect of lipophilic ions on membrane function.

For PCPG mobility we found the charged plane due to PG lipids was 0.2 nm for single-layered liposomes and 0.1 nm for multi-layered liposomes. This is consistent with the relative size of PC and PG head groups

The dependence of SR mobility on pH was found to be directly correlated with the total charge of the A, P, and N domains of the Ca^{2+} -ATPase as determined by the amino acid residues and their corresponding pKa values in water.

We found that detached charged plane model, a new model developed in our group, could be fit to the mobility of SR as a function of ionic strength while other soft particle models failed. The assumptions of this model are that (1) the friction caused by protruding proteins on the surface of SR can be represented by a homogeneous retardation layer of thickness D and softness parameter λ_{RL} , and (2) the charge of the APN domain can be represented as a plane of charge embedded in the retardation layer at a distance s from the membrane surface. The best-fit values for λ_{RL} and s were not consistent for different pH value studies.

The detached charged plane model was unable to predict the mobility of SR vesicles in the presence of lipophilic ions if we assumed that the lipophilic ions were sorbing to the detached charged plane that represents the native charge of the APN domains of SR. At high lipophilic ion concentration the experimental mobilities consistently were greater in magnitude than the values predicted by the model. We concluded that there is significant absorption of lipophilic ions to the proteins in SR membrane, and that the lipophilic ion sorption sites are not the same as the detached plane of charge that represents the native charge of the APN domain.

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Table of Contents

Abstract	i
Acknowledgements	iv
List of Tables	vii
List of Figures	ix
List of Symbols	xvi
Chapter 1: Introduction	1
Chapter 2: Sarcoplasmic Reticulum	7
Section 1: The role of sarcoplasmic reticulum in muscle contraction and relaxation .	7
Section 2: Detailed surface properties of SR vesicles.....	10
Chapter 3: Electrostatic Description of Simple Membrane-Water Interfaces	21
Section 1: The linearized Poisson-Boltzmann equation	22
Section 2: The non-linearized Poisson-Boltzmann equation	27
Section 3: Applicability of linearized Poisson-Boltzmann equation	32
Chapter 4: Electrophoretic Mobility of Smooth-surfaced Colloids.....	35
Section 1: Electrophoretic mobility and the zeta potential	35
Section 2: Overview of electrophoretic mobility models	37
Section 3: Forces on a colloid during electrophoresis	39
Section 4: The Electric Field Around the Particle and the Retardation Effect	42
Section 5: Momentum transfer and the hydrostatic force	46
Section 6: Important analytic models.....	47
Section 7: Comparison of models	54
Chapter 5: Electrophoretic Mobility of Colloids with Surface Structure	59
Section 1: Levine's model for a charged retardation layer	60
Section 2: Ohshima's model for a charged spherical colloid with an uncharged retardation layer	65
Section 3: The detached charged plane model	66
Chapter 6: Partition of Lipophilic Ions	71
Section 1: The partition coefficient.....	71
Section 2: Molecular models of lipophilic ions	73
Section 3: The thermodynamics of partition.....	76
Section 4: The modified Langmuir isotherm model of partitioning	77
Chapter 7: Materials and Methods: Electrophoretic Mobility Measurements.....	83
Section 1: Preparation of liposome suspensions	83
Section 2: Preparation of suspensions of SR vesicles.....	87
Section 3: Particle electrophoresis	88
Section 4: Electrophoresis of SR suspensions of varying ionic strength and consideration of buffer pKa and pH	88
Section 5: The optical bench of the electrophoretic mobility analyzer.....	91
Section 6: Electro-osmotic flow and the electrophoretic cell	95
Section 7: Sample treatment, and injection.....	97
Section 8: Electrophoretic mobility measurements	98
Chapter 8: Materials and Methods: Photoelectron Microscopy	99
Chapter 9: Materials and Methods: Partition of Lipophilic Ion Measurements	100

Section 1:	Spectrophotometry and extinction coefficients	100
Section 2:	Partition coefficients and dialysis experimental design.....	101
Chapter 10:	Results: Photoelectron Microscopy	111
Chapter 11:	Results: The Dependence of Electrophoretic Mobility of Liposomes on Ionic Strength	114
Section 1:	Mobility model fits	115
Chapter 12:	Results: The Dependence of Electrophoretic Mobility of SR on Ionic Strength	121
Section 1:	Performance of mobility models.....	121
Chapter 13:	Results: Absorbance Spectra and Extinction Coefficients of Lipophilic Ions	130
Chapter 14:	Results: Partition of Lipophilic Ions	145
Chapter 15:	Results: Electrophoretic Mobility of SR and Liposomes in the Presence of Lipophilic Ions	165
Chapter 16:	Discussion: Electrophoretic mobility of liposomes	177
Section 1:	Background information and overview of results.....	177
Section 2:	Electrophoretic mobility of liposomes in the presence of lipophilic ions	179
Section 3:	Electrophoretic mobility of PC/PG liposomes.....	190
Section 4:	Conclusions to electrophoretic mobility studies of liposomes	195
Chapter 17:	Discussion: Partition of Lipophilic Ions into PC and SR	200
Chapter 18:	Discussion: Electrophoretic mobility of SR vesicles.....	210
Section 1:	Effect of ionic strength on SR mobility of SR vesicles	210
Section 2:	The effect of lipophilic ions on mobility of SR vesicles	234
Chapter 19:	Final Overview and Conclusions	261
Section 1:	Overview	261
Section 2:	Conclusions.....	263
Section 3:	Accomplishments.....	266
Chapter 20:	References	269

List of Tables

Table 2.1: Frequencies and pKa values of amino acids in the Ca^{2+} -ATPase along with protonated and deprotonated forms.	18
Table 2.2: Ionizable sites on lipid head groups.....	18
Table 10.1: Size distributions of single layered and multilayer liposomes.	111
Table 10.2: Size distributions of SR vesicles at different pH values.....	112
Table 12.1: Parameters used in Ohshima 2004 model least squares fit to SR mobility versus ionic strength..	128
Table 12.2: Parameters used in Levine's model least squares fit to SR mobility versus ionic strength.....	129
Table 13.1: Concentration and absorbance data used for calculating extinction coefficients for TePB^-	139
Table 13.2: Calculated extinction coefficients for tetraphenylborate.....	139
Table 13.3: Concentration and absorbance data used for calculating extinction coefficients for TePP^+	141
Table 13.4: Calculated extinction coefficients for TePP^+	141
Table 13.5: Absorbance versus concentration for Clomipramine	142
Table 13.6: Absorbance versus concentration for Imipramine.....	143
Table 14.1: PC-water partition of TePB^- weighted average of all five wavelengths.....	147
Table 14.2: SR-water partition of TePB^- weighted average of all five wavelengths.....	149
Table 14.3: SR-water partition of TePP^+ weighted average of all five wavelengths.....	151
Table 14.4: SR-water partition of PBP^- pH 9.4 analyzed at $\lambda=314.5$ nm.....	153
Table 14.5: SR-water partition of PBP^- pH 9.4 analyzed at $\lambda=314.5$ nm.....	155
Table 14.6: PC-water partition of Imipramine analyzed at $\lambda=250.5$ nm.	157
Table 14.7: SR-water partition of Imipramine analyzed at $\lambda=250.5$ nm.	159
Table 14.8: PC-water partition of Clomipramine analyzed at $\lambda=251.5$ nm.....	161

Table 14.9: SR-water partition of Clomipramine analyzed at $\lambda=251.5$ nm.....	163
Table 14.10: Summary of partition experiment results.	164
Table 15.1: Initial PBP ⁻ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [PBP ⁻]..	167
Table 15.2: Initial PBP ⁻ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [PBP ⁻].....	167
Table 15.3: Initial TePB ⁻ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [TePB ⁻]..	169
Table 15.4: Initial TePB concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePB].....	170
Table 15.5: Initial TePB concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePB].....	170
Table 15.6: Initial Imipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [Imip ⁺].....	172
Table 15.7: Initial Imipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [Imip ⁺]..	172
Table 15.8: Initial Clomipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [Clom ⁺].....	173
Table 15.9: Initial Clomipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [Clom ⁺].....	174
Table 15.10: Initial TePP ⁺ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [TePP ⁺]..	175
Table 15.11: Initial TePP ⁺ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePP ⁺].....	176
Table 16.1: Reduced χ^2 values for model predictions	193
Table 18.1: Fit parameters for detached charged plane model fit to data of mobility as a function of pH.	217
Table 18.2: Sum of deviations squared for fits of detached charged plane model fits...	224
Table 18.3: Fit parameters for least square fit of detached charged plane model to SR mobility as a function of ionic strength for various pH values.....	232

List of Figures

Figure 2.1: Two myofibrils of a muscle fiber.	10
Figure 2.2: Two views of the Ca^{2+} -ATPase.....	14
Figure 2.3: The total charge per Ca^{2+} -ATPase, the APN domain of the Ca^{2+} -ATPase, and the lipid matrix of one unit cell of SR membrane.....	19
Figure 3.1: Potential profile of the diffuse layer.....	26
Figure 3.2: Potential as a function of distance from the membrane surface. Calculations are made with surface charge density of 10 mC/m^2	33
Figure 3.3: Potential as a function of distance from the membrane surface. Calculations are made with varying surface charge density and ionic strength of 25 mM.	34
Figure 4.1: Negatively charged colloids driven through a fluid by an applied electric field.	36
Figure 4.2: Forces on a charged colloid in an electrolyte undergoing electrophoresis.....	41
Figure 4.3: The near field (A) and far field (B) components of the electric field due to the charged surface and the counter-ions and the polarization of the diffuse layer.....	43
Figure 4.4: The effect of a non-conducting colloid on an otherwise uniform electric field.	44
Figure 4.5: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 10 \text{ mC/m}^2$ and colloid radius $a = 100\text{nm}$	54
Figure 4.6: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 10 \text{ mC/m}^2$ and colloid radius $a = 500\text{nm}$	55
Figure 4.7: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 40 \text{ mC/m}^2$ and colloid radius $a = 100\text{nm}$	57
Figure 5.1: Illustration of three mobility models.....	64
Figure 6.1 Space filling molecular models of lipophilic ions. The colors red, yellow, white, green, and blue indicate relative charge.....	74
Figure 6.2 Space filling molecular models of lipophilic ions. The colors indicate predicted lipophilicity	75

Figure 7.1: Frequency spectrum of scattered light intensity with electric field off and on.	92
Figure 7.2: Schematic representation of light rays scattering off SR particles in DELSA cell.....	93
Figure 7.3: Vector diagram representing origin of scattering vector ($\vec{K}$)	94
Figure 7.4: Representation of electro-osmotic flow in DELSA cell chamber.....	96
Figure 10.1: Log normal distributions of liposome sizes.	112
Figure 10.2: Log normal distributions of SR vesicle size.....	113
Figure 11.1: Electrophoretic mobility of single-layered liposomes with predictions using the OHW model, Henry's model, and the Smoluchowski model.....	117
Figure 11.2: Plots of k_a versus ionic strength for single-layered liposomes.	118
Figure 11.3: Electrophoretic mobility of multi-layered liposomes with predictions using the OHW model, Henry's model, and the Smoluchowski model.....	119
Figure 11.4: Plot of k_a versus ionic strength for multilayer liposomes.....	120
Figure 12.1: Electrophoretic mobility of SR vesicles at pH 4 as a function of ionic strength.....	123
Figure 12.2: Electrophoretic mobility of SR vesicles at pH 4.7 as a function of ionic strength.....	124
Figure 12.3: Electrophoretic mobility of SR vesicles at pH 5 as a function of ionic strength.....	125
Figure 12.4: Electrophoretic mobility of SR vesicles at pH 6 as a function of ionic strength.....	126
Figure 12.5: Electrophoretic mobility of SR vesicles at pH 7.5 as a function of ionic strength.....	127
Figure 12.6: Electrophoretic mobility of SR vesicles at pH 9 as a function of ionic strength.....	128
Figure 13.1: The absorbance spectrum of tetraphenylborate.....	132
Figure 13.2: The absorbance spectrum of tetraphenylphosphonium.	133
Figure 13.3: The absorbance spectrum of pentabromophenol.....	134

Figure 13.4: The absorbance spectrum of Imipramine.	135
Figure 13.5: The absorbance spectrum of Clomipramine.....	136
Figure 13.6: Concentration dependence of absorbance of TePB^- at five wavelengths... 138	
Figure 13.7: Concentration dependence of absorbance of TePP^+ at four wavelengths.. 140	
Figure 13.8: Concentration dependence of absorbance of clomipramine at 251.5 nm... 142	
Figure 13.9: Concentration dependence of absorbance of Imipramine at 250.5 nm. 143	
Figure 14.1: Partition of TePB^- between PC liposomes and water as a function of equilibrium concentration of TePB^-	147
Figure 14.2: Partition of TePB^- between SR vesicles and water as a function of equilibrium concentration of TePB^-	149
Figure 14.3: Partition of TePP^+ between PC liposomes and water as a function of equilibrium concentration of TePP^+	151
Figure 14.4: Partition of PBP^- between PC liposomes and water as a function of equilibrium concentration of PBP^- , titrated to pH 9.4.....	153
Figure 14.5: Partition of PBP^- between SR vesicles and water as a function of equilibrium concentration of PBP^- titrated to pH 9.4.....	155
Figure 14.6: Partition of Imipramine between PC liposomes and water as a function of equilibrium concentration of Imipramine, titrated to pH 7.0.....	157
Figure 14.7: Partition of Imipramine between SR vesicles and water as a function of equilibrium concentration of Imipramine, titrated to pH 7.0.....	159
Figure 14.8: Partition of Clomipramine between PC liposomes and water as a function of equilibrium concentration of Clomipramine, titrated to pH 7.0..	161
Figure 14.9: Partition of Clomipramine between SR vesicles and water as a function of equilibrium concentration of Clomipramine, titrated to pH 7.0..	163
Figure 15.1: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of PBP^-	166
Figure 15.2: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of TePB^-	169
Figure 15.3: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of imipramine ⁺	171

Figure 15.4: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of clomipramine ⁺ .	173
Figure 15.5: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of TePP ⁺ .	175
Figure 16.1: The permeable surface model.	181
Figure 16.2: The mobility of TePB ⁻ is fit with a sorption plane depth of 0.13 nm.	186
Figure 16.3: The mobility of PBP ⁻ is fit with a sorption plane depth of 0.5 nm.	187
Figure 16.4: The mobility of Imipramine ⁺ is fit with a sorption plane depth of 0.12 nm.	188
Figure 16.5: The mobility of Clomipramine ⁺ is fit with a sorption plane depth of 0.17 nm.	189
Figure 16.6: The mobility of TePP ⁺ is fit with a sorption plane depth of 0.25 nm.	190
Figure 16.7: The graph shows model predictions of the electrophoretic mobility of single layered PCPG liposomes for various depths of charge plane and experimental results.	192
Figure 16.8: The graph shows model predictions of the electrophoretic mobility of multilayer PCPG liposomes for various depths of charge plane and experimental results.	193
Figure 16.9: The best fit of the OHW mobility model to our single layered PCPG liposome mobility as a function of ionic strength.	194
Figure 16.10: Schematic representation of placement of lipophilic ions in the lipid head region of PC liposomes, drawn to scale.	199
Figure 17.1: Partition of TePB ⁻ between two membranes (PC liposomes and SR vesicles) and water.	202
Figure 17.2: Partition of Imipramine ⁺ between two membranes (PC liposomes and SR vesicles) and water.	203
Figure 17.3: Partition of Clomipramine ⁺ between two membranes (PC liposomes and SR vesicles) and water.	204
Figure 17.4: Partition of PBP ⁻ between two membranes (PC liposomes and SR vesicles) and water.	205
Figure 17.5: Number of TePB ⁻ molecules per unit cell absorbed to SR, to lipid bilayer of unit cell and to a single Ca ²⁺ -ATPase protein.	206

Figure 17.6: Number of Imipramine ⁺ molecules per unit cell absorbed to SR, to lipid bilayer of unit cell and to a single Ca ²⁺ -ATPase protein.	207
Figure 17.7: Number of Clomipramine ⁺ molecules per unit cell absorbed to SR, to lipid bilayer of unit cell and to a single Ca ²⁺ -ATPase protein.	208
Figure 17.8: Number of PBP ⁻ molecules per unit cell absorbed to SR, to lipid bilayer of unit cell and to a single Ca ²⁺ -ATPase protein.....	209
Figure 18.1: Linear least square fits to SR mobility at various pH values versus ionic strength in the range of 100 mM ionic strength.	212
Figure 18.2: Electrophoretic mobility interpolated to 100 mM ionic strength as a function of pH.	213
Figure 18.3: The charge of the entire Ca ²⁺ -ATPase, the APN-domain alone and one surface of the lipid bilayer as a function of pH.....	214
Figure 18.4: Demonstration of linear relationships between electrophoretic mobility and charge of the Ca ²⁺ -ATPase, the APN domain, and the lipid bilayer surface.....	215
Figure 18.5: Experimental mobilities interpolated to 100 mM along with mobilities calculated using the linear fit of mobility to charge of one APN domain.	216
Figure 18.6: The agreements between the predicted and measured mobility obtained for λ_{RL} values listed in Table 18.1.	218
Figure 18.7: Illustration of the performance of the detached charged plane model at pH 4.0.....	219
Figure 18.8: Illustration of the performance of the detached charged plane model at pH 4.7.....	220
Figure 18.9: Illustration of the performance of the detached charged plane model at pH 5.0.....	221
Figure 18.10: Illustration of the performance of the detached charged plane model at pH 6.0.....	222
Figure 18.11: Illustration of the performance of the detached charged plane model at pH 7.5.....	223
Figure 18.12: Illustration of the performance of the detached charged plane model at pH 9.0.....	224
Figure 18.13: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 4.0.	227

Figure 18.14: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 4.7..	228
Figure 18.15: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 5.0..	229
Figure 18.16: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 6.0..	230
Figure 18.17: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 7.5.	231
Figure 18.18: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 9.0..	232
Figure 18.19: Calculation of mobility of lipid bilayer of SR assuming the lipid charge is on the surface of the membrane.	234
Figure 18.20: Calculated number of Clomipramine ⁺ molecules sorbed to the detached charged plane of SR required to achieve measured mobilities as a function of initial Clomipramine ⁺ concentration	238
Figure 18.21: Calculated number of Imipramine ⁺ molecules sorbed to the detached charged plane of SR as a function of initial Imipramine ⁺ concentration	239
Figure 18.22: Calculated number of TePP ⁺ molecules sorbed to the detached charged plane of SR t as a function of initial TePP ⁺ concentration.	240
Figure 18.23: Calculated number of TePB ⁻ molecules sorbed to the detached charged plane of SR as a function of initial TePB ⁻ concentration.	241
Figure 18.24: Calculated number of TePB ⁻ molecules sorbed to the detached charged plane of SR as a function of initial TePB ⁻ concentration	242
Figure 18.25: Calculated number of PBP ⁻ molecules sorbed to the detached charged plane of SR as a function of initial PBP ⁻ concentration	243
Figure 18.26: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial Clomipramine ⁺ concentration	249
Figure 18.27: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial Imipramine ⁺ concentration.....	250
Figure 18.28: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePP ⁺ concentration.....	251

Figure 18.29: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePB^- concentration.....	252
Figure 18.30: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial PBP^- concentration.....	253
Figure 18.31: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePB^- concentration.....	254
Figure 18.32: Illustration of the effect of changing the location of the detached charged plane.....	259

List of Symbols

Symbol	Description
a	Colloid radius
A	Sorbate molecule; Absorbance
A_L	Surface area that one lipid occupies on the membrane
A_M	Area of the surface of the membrane
AM	Sorbate molecule bound to a membrane site
a_p	Radius of each resistance center in the retardation layer
A_S	Sorption site area
C	Molar concentration; Concentration of lipophilic ion
C_{bound}	The "missing concentration" which is absorbed
C_{eq}	Bulk equilibrium concentration of lipophilic ions
CHRG	Charge of a given ionizable domain
C_{if}	Interfacial concentration of lipophilic ions
C_W	Molar concentration of water in water
D	Retardation layer thickness (detached charged plane model); Diffusion coefficient of the particle
E	Applied electric field
e	Unit charge
F	Applied force
f	Linear frequency
F_a	Faraday's constant
$freq_i$	Number of ionizable sites of type i in the protein
I_1	Intensity of light leaving the background sample
I_2	Intensity of light leaving the compound solution
IS	Ionic strength
k	Boltzmann constant
K	Scattering vector
k_0	Wave vector of incident light
k_1	Wave vector of scattered light
$K_{1,2}$	Molar partition coefficient
K_a	Dissociation constant
k_a	Sorption rate constant

Symbol	Description
k_d	Desorption rate constant
K_m	Equilibrium constant for partition process
Kp_x	Mole fraction partition coefficient
$Kp_{x,0}$	Mole fraction partition coefficient at infinite dilution
L	Number of lipids
ℓ	Path length of light through the sample
M	Membrane site
MSF	Mobility structure factor
n	Volume density of ions; Index of refraction of the liquid suspension
N	Avogadro's number
N_m	Total number of lipid molecules
n_m	Number of lipophilic ions absorbed to the lipid vesicles
N_p	Volume density of resistance centers in the retardation layer
N_w	Total number of water molecules
n_w	Total number of lipophilic ions in the aqueous phase
pol	Polarity of the domain
q	Charge
Q_{APN}	The charge of the APN domain
R	Ideal gas constant
r_a	Rate of absorption
r_d	Rate of desorption
s	Distance of the detached charged plane from the particle core (detached charged plane model)
S	Number of molecules sorbed to the membrane
$S(\omega)$	Power function
T	Temperature
u	Velocity of a fluid element
UCA	Unit cell area
u_{EO}	Electro-osmotic fluid velocity
v	Velocity
V	Volume
v_t	Terminal velocity of colloid
X_{aq}	Mole fraction of solute in water
X_m	Mole fraction of sorbate in the membrane
z	The multiplicity of charge
β	Retardation layer thickness (Levine's model)

Symbol	Description
$\Delta G_{I \leftarrow 2}$	Gibbs free energy of transfer
ΔH	Change of enthalpy
ΔS	Change of entropy
ε	Dielectric constant; Molar decadic extinction coefficient
ε_0	Permittivity of free space
ζ	Zeta potential
η	Viscosity coefficient
Θ	Membrane fractional coverage
κ	Debye-Hückel parameter
λ	Drag coefficient
λ_0	Wavelength of the laser in vacuum
λ_{RL}	Softness parameter
μ	Electrophoretic mobility
ρ	Volume charge density
σ	Surface charge density
χ^2	Goodness of fit
ψ	Local electric potential
ω	Angular frequency
Γ	Spectral broadening at half width

Chapter 1: Introduction

The study of the terminal velocity of colloids in a uniform electric field, called the electrophoretic mobility, is an established means for gaining understanding of the colloid surface characteristics through electrostatic and hydrodynamic modeling. Specimens that have been studied in this way range from nanoparticles (Agnihotri and others 2009) to bacteria (de Kerchove and Elimelech 2004; Wilson and others 2001). This means of study is particularly well-suited for biological specimens because the structures of many biological specimens, such as membranes, are dependent on their aqueous environment. To measure electrophoretic mobility, the specimens are suspended in an aqueous solution between two electrodes, an electric field is applied, and as the specimens move in response, their terminal velocity is measured. The electrophoretic mobility is defined as the terminal velocity divided by the applied electric field ($\mu=v/E$). Electrophoretic mobility studies have been used to study the charge distribution over the surface of biological specimens (Young and others 2005), as well as the frictional characteristics of the surface structures of biological specimens, such as transmembrane proteins, gangliosides, gram positive and gram negative bacterial cell walls, and the proteic capsid of viruses (de Kerchove and Elimelech 2004; Duval and Gaboriaud 2010).

The sarcoplasmic reticulum (SR) is a membranous lumen within muscle cells that collects, stores, and releases Ca^{2+} ions, thus regulating their intracellular concentration. The intracellular concentration of Ca^{2+} governs the excitation and relaxation of muscle

cells. When removed from the muscle and kept in aqueous solution, this membrane spontaneously forms spherical vesicles (de Meis 1981).

Although electrophoretic mobility studies of SR vesicles were first measured years ago (Arrio and others 1984; Brethes and others 1986), these results not been understood in detail. This is due to the complex surface structure of SR and the relative simplicity of available analytic electrophoretic mobility models. There are only a few successful models because the size, distribution of charge, and the nature of the frictional surface of particles (biological membranes vesicles in particular) vary widely. However, several biological specimens of similar complexity have had their surface structure described by the analysis of electrophoretic mobility. Of note are human red blood cells. The mobility of red blood cells can be explained by using a model that consists of a surface layer with homogeneous frictional characteristics that is broken down into three sub-layers of defined thickness with different volume charge densities (Nakano and others 1994). In the case of the SR membrane, much is known about its surface, such as the lipid constituents of the lipid bilayer (Lau and others 1979) and the amino-acid sequence of the most predominant protein, the Ca^{2+} -ATPase (Shi and others 1998), which provide us with information about the charge density of the lipid bilayer and the protruding protein respectively. However, this information has not yet been used to elucidate electrophoretic mobility data, nor have electrophoretic mobility data been used to further our understanding of the surface of the SR membrane. We aim to gain an understanding of the relationship between the electrokinetic properties and the surface properties of SR membrane. Specifically we hope to establish which charges on the SR

membrane surface determine the electrophoretic mobility, and what physical surface model can be used to predict the electrophoretic mobility.

There are two main types of electrophoretic mobility models. The mobility models that deal with charged colloids with discrete surfaces are known as “hard sphere” models. On the other hand, models with surface structures that are attached and protruding from the hard surface are called “soft particle” models. To investigate the surface of SR we vary the conditions of the solution in which SR vesicles are suspended and measure the effect on electrophoretic mobility. Then we test the applicability of several electrophoretic mobility models that are based on assumptions about the surface structure of the membrane. For example, Ohshima developed a model that consists of a hard particle with surface charge and an attached uniform uncharged retardation layer (Ohshima 2005). This model is one of only a few soft-particle models that include curvature effects of the colloid. In addition to testing this model, we also test a model that includes a friction or retardation layer with a uniform volume charge density (Levine and others 1983), and a model that includes a detached charged plane embedded in an uncharged retardation layer.

The main structure of SR vesicles is a lipid bilayer with densely packed trans-membrane Ca^{2+} -ATPase proteins that protrude from the surface (Franzini-Armstrong and Ferguson 1985; Scales 1976) of the bilayer that we will model as a retardation layer. The protruding proteins slow the flow of water relative to the colloid near the surface of the colloid, which reduces mobility. Both the bilayer surface and the protruding proteins are charged, which we model as thin charged layers. Complicating the matter is the pH of the surrounding water phase. The charge of the lipids and the protein changes with the pH

of the suspending solution. Also, when the ionic strength of the solution is increased, the native charge of SR vesicles is increasingly screened by ions of opposite charge in the aqueous solution as they approach the location of the native charge (Lyklema 1995). This lowers the electric potential near the surface of the membrane and the electrophoretic mobility. However, the underlying surface structure of SR, the charge distribution and frictional characteristics affect the way in which the mobility changes with changing ionic strength and pH. In this work we present studies of the mobility of SR vesicles as a function of ionic strength at several pH values (4.0, 4.7, 5.0, 6.0, 7.5, and 9.0).

In order to investigate some of the hard sphere mobility models, which are the basis of the more complicated soft particle models, our studies begin with the electrophoretic mobility of simple charged lipid bilayer vesicles or liposomes. We mix uncharged phosphatidylcholine (PC) lipids with negatively charged phosphatidylglycerol (PG) lipids to make charged liposomes. We measure their electrophoretic mobility as a function of ionic strength. We then test the applicability of both the well-known Smoluchowski model, which is applicable to large particles, and other models in which the effects of particle curvature become significant such as the Ohshima-Healy-and White (OHW) model (Ohshima 2006). We seek to determine if these models can predict the electrophoretic mobility with the correct nominal surface charge density as determined by the mixture of PC and PG and the surface area per lipid. There are a number of reasons why these models would possibly not work for liposomes. For example the charged surface might not coincide with the surface of the hydrodynamic particle, the surface of lipid might not actually be discrete (it might have some

“softness”), or the shape of the liposome might deviate from spherical as it travels in the electric field.

Lipophilic ions are a class of hydrophobic molecules that include a great many drugs and toxins. Because of their importance to improving human health and the environment there are numerous studies of the partition of lipophilic ions between aqueous and organic phases (Escher and others 2000; Fisar and others 2004; Flewelling and Hubbell 1986; Word and Smejtek 2005). Liposomes carrying lipophilic drugs have recently become popular as drug delivery vehicles (Fahr and others 2005). The mechanisms for the transfer of drugs from the carrier liposomes to the target membrane or interface depend on the location and orientation of the lipophilic compounds in the liposome (Fahr and others 2005). However, in the case of biological membranes or liposomes, the location of lipophilic compounds within the organic phase is rarely known. By measuring PC-water and SR-water partition of lipophilic ions and electrophoretic mobility of PC liposomes and SR vesicles in the presence of lipophilic ions, we hope to determine the location of lipophilic ions upon sorption. Specifically in the case of PC liposomes, we would like to know if we can treat liposomes with sorbed lipophilic ions using a hard sphere model and assume that the sorbed charge is added directly to the surface of the hard sphere, or if we must consider the sorbed ions to have some depth in the hydrodynamic particle. The potential will decrease throughout this depth if ions of opposite charge (counter-ions) also exist in this region, which in turn will decrease the mobility. In the case of SR, we would like to know if there is sorption to the ubiquitous Ca^{2+} -ATPase proteins or if sorption only occurs at the lipid bilayer. If there is sorption to the Ca^{2+} -ATPase, we would like to know if it is in the trans-membrane portion

of the protein or in the protruding part. We measure the effect of changing the concentration of several lipophilic ions species on the electrophoretic mobility of both SR vesicles and simple PC liposomes to attempt to answer these questions. In conjunction with these studies we do dialysis experiments to study the partitioning of lipophilic ions between the aqueous solution and PC liposomes or SR vesicles.

Chapter 2: Sarcoplasmic Reticulum

The sarcoplasmic reticulum is an ideal model biological membrane because it is both critically important for muscle function and simple in composition. Simple, here, is a relative term since in comparison to a lipid bilayer, modeling the physical properties of SR will be no small task. Let us begin with a description of the physiological properties of SR.

Section 1: The role of sarcoplasmic reticulum in muscle contraction and relaxation

The regulation of Ca^{2+} ions in a muscle cell is essential to its contraction and relaxation. The sarcoplasmic reticulum (SR) is an organelle whose sole function is the storage and release of Ca^{2+} . The SR membrane is simple. It has two major species of transmembrane proteins, which are responsible for regulating the concentration of Ca^{2+} in the muscle fiber cytoplasm. The first is the calcium release channel called the ryanodine receptor (RyR) which opens during the muscle contraction cycle, releasing Ca^{2+} into the sarcoplasm of the muscle fiber by diffusion (Stokes and Wagenknecht 2000). The RyR is a large protein, but not numerous since it plays the role of a gatekeeper against the Ca^{2+} ion gradient. The second important, and far more numerous, protein is the Ca^{2+} -ATPase, which consumes ATP to pump Ca^{2+} back into the lumen of the SR membrane upon muscle relaxation (Stokes and Wagenknecht 2000). For the purposes of understanding the electrophoretic mobility of SR the task is to understand the properties of the Ca^{2+} -ATPase.

The mechanism of muscle contraction begins with the absence of Ca^{2+} in the sarcoplasm. At this time tropomyosin sterically obstructs myosin binding sites on actin. When the Ca^{2+} permeates the sarcoplasm it bonds to troponin C. This releases tropomyosin from actin binding sites (Shier and others 2010). This allows the myosin to form bonds with actin. The ratchet theory of muscle contraction suggests that, once the actin and myosin are bound, a crossbridge can bend slightly, pulling on the actin, then the bond can break and a new bond can be made with an active site further down the actin filament(Shier and others 2010). This process repeats and the actin and myosin slide along each other, the muscle shortens, and the tension within the muscle increases.

During muscular relaxation, the Ca^{2+} -ATPase protein pumps Ca^{2+} from the sarcoplasm back into the SR membrane lumen. This process requires chemical energy via the hydrolysis of ATP. For each ATP molecule hydrolyzed, a pump transfers two Ca^{2+} ions from the sarcoplasm to the inner lumen of the SR membrane (Stokes and Wagenknecht 2000). This pumping takes 10^5 times as long as it takes for the RyR protein to release the same number of ions through diffusion (Stokes and Wagenknecht 2000). To be able to reduce the sarcoplasmic concentration of Ca^{2+} to a one one-thousandth of the concentration inside the SR lumen, it is necessary for there to be a high concentration of Ca^{2+} -ATPase proteins on the surface of the membrane. These pumps make up 70% to 80% of the total protein mass of the SR membrane (de Meis 1981).

From a larger perspective, while SR is a simple biomembrane in composition, it is one component of a complex system. It may be instructive to put SR in context. Vertebrate skeletal muscle is composed of long, cylindrical multinucleate muscle fibers 20 to 100 μm in width (Squire 1981) that are connected to each other in bundles called

fascicles (Shier and others 2010). A muscle fiber contains many parallel cylindrical contractile units, called myofibrils that run the length of the fiber and have diameters of 1 to 2 μm . These units have a repeating pattern of alternating bundles of actin and myosin filaments that overlap at places along the length of the myofibril. One repeating unit of the pattern along a myofibril is called a sarcomere. Adjacent myofibrils have their sarcomeres roughly aligned, giving vertebrate skeletal muscle tissue its striated appearance (Squire 1981).

The larger structure of sarcoplasmic reticulum (SR) is more complex than its composition might suggest (Figure 2.1). It forms a web-like system of enclosed lumen surrounding the myofibrils. The transverse tubules or t-tubules are membranous channels that are continuous with the sarcolemma (the cell membrane of a muscle fiber) and wrap around myofibrils (Shier and others 2010)). Two enlarged portions of SR called the terminal cisternae of SR sit on either side of each t-tubule. The signal for muscle contraction, the action potential, permeates the muscle fiber by means of the t-tubules. Figure 2.1 shows the SR lumen and t-tubules surrounding two myofibrils. The terminal cisternae of SR and t-tubules run perpendicular to the actin and myosin filaments and run through the muscle fiber near the sections of the sarcomeres where the actin and myosin filaments overlap (Shier and others 2010).

Figure 2.1: Two myofibrils of a muscle fiber. T-tubules and sarcoplasmic reticulum lumen wrap around the myofibrils. (Adapted from Jennett S. (1989). Human physiology. Churchill Livingston Edinburgh)

Section 2: Detailed surface properties of SR vesicles

When sarcoplasmic reticulum is removed from muscle tissue via centrifugation, and mixed into an aqueous suspension with salt and buffer, spherical vesicles of the membrane spontaneously form and are oriented in such a way that the outside of the vesicle corresponds to the sarcoplasmic side of the SR lumen in the muscle fiber. Surprisingly, the proteins held in the lipid bilayer matrix are oriented in the same way as they are in the muscle fiber. They protrude from the outside of the vesicle (Napolitano and others 1983). Therefore, we can be confident that the properties of SR removed from its natural state are relatively unchanged.

The surface of the membrane is charged net negatively or positively depending on pH. The charge is due to several sources, which are described in more detail below. Ions from the aqueous solution of opposite charge as the vesicle surface (counter-ions) form a

diffuse cloud around the vesicle, which screens the vesicle's surface charge. For the purpose of discussing what is known about SR and what we would like to learn, let us submit that several factors influence the electrophoretic mobility of a colloidal particle. In particular, the distribution of charge as well as the friction-causing molecules at the surface of the membrane.

There are three sources of electric charge on the surface of SR membrane: proteins, lipids, and sialic acid residues from gangliosides. In this chapter we show that the main contributors to charge are the trans-membrane proteins, most notably the Ca^{2+} -ATPase, and the ionizable lipids. The charge due to sialic acid on gangliosides is found to be negligible.

The Ca^{2+} -ATPase protein exists with the greatest frequency of any protein in the sarcoplasmic reticulum. The distribution of different types of protein varies, depending on the location on the SR organelle (or for a vesicle, where the SR membrane originated). For longitudinal sections, Ca^{2+} -ATPase accounts for about 90% of the total protein content while at the terminal cisternae it accounts for 55-65% as other proteins such as the calcium release channel, RyR, are more prominent in this region (Meissner 1975). The bulk of the SR membrane will come from the longitudinal portion of the SR in the muscle. For this reason we only consider the Ca^{2+} -ATPase protein when modeling the SR surface.

Because of its importance in muscle contraction and therefore cardiac function, the structure of the Ca^{2+} -ATPase has been studied extensively. It is important for this study that the protein's amino acid sequence is known. It has 994 amino acid residues in total and a molecular mass of 110,000 amu (Toyoshima and others 2000). The overall

dimensions are also known. It fits in a box with dimensions 100 Å by 80 Å by 140 Å (Toyoshima and others 2000). It has a large headpiece on the outer, cytoplasmic side of the membrane that consists of three distinct domains. The A, P, and N domains move relative to each other during active transport of Ca^{2+} into the SR lumen. The headpiece is connected by a short stalk to a trans-membrane region, that consists of ten α helices (Toyoshima and others 2000). Most of the protein's mass is on the cytoplasmic side of the membrane. It protrudes 7 to 9 nm from the lipid bilayer (Toyoshima and others 2000). This fact gives us an expectation for the model frictional thickness of the membrane's surface.

The density of Ca^{2+} -ATPase determines how much of a role the protein plays in the frictional properties of SR. If the density is low we should expect that the lipid bilayer portion of the membrane is exposed and will be important. If the density is high, the lipid membrane is buried and will be a minor player.

The density of Ca^{2+} -ATPase protein in rabbit skeletal muscle has been studied using electron microscopy of freeze-fractured vesicles giving density values of $21,000 \pm 3,900$ (Scales 1976) and $32,500 \pm 4,000$ (Franzini-Armstrong and Ferguson 1985) proteins per μm^2 . Napolitano and others found that the Ca^{2+} -ATPase protein is distributed throughout the membrane surface in either a loose tetragonal or hexagonal pattern or a random pattern and that the unit cell area per protein is $12,285 \text{ \AA}^2$ for the tetragonal array and $12,969 \text{ \AA}^2$ for the hexagonal array (Napolitano and others 1983). In their paper they conclude that the Ca^{2+} -ATPases were in dimers rather than monomers. With this they calculated a protein density of 17,300 proteins per μm^2 . Lau and others measured the

ratio of phospholipids to protein on longitudinal SR to be 0.87 μmol of phospholipid per mg of protein. For the SR membrane of the terminal cisternae, it was 0.53 μmol of phospholipid per mg of protein (Lau and others 1979). When we calculate the number of lipids per Ca^{2+} -ATPase from the longitudinal SR value, we obtain 106 phospholipids per protein:

$$\frac{8.7 \times 10^{-7} \text{ mol}}{9 \times 10^{-7} \text{ kg}} \cdot \frac{1.83 \times 10^{-22} \text{ kg}}{\text{Ca}^{2+} \text{ ATPase}} \cdot \frac{6.02 \times 10^{23}}{\text{mol}} = 106.$$

Using the average of the above Ca^{2+} -ATPase surface densities (25,000 proteins per μm^2) we calculate a surface area per protein of 40 nm^2 . This value is compatible with the area taken up by the phospholipids per Ca^{2+} -ATPase in one layer of the bilayer (53 times the area per lipid 0.72 nm^2 yields 39.2 nm^2), leaving some room for the trans-membrane helixes of the Ca^{2+} -ATPase. These are the values we will use in modeling the SR membrane surface. From this data we expect that the frictional and electrostatic properties of SR membrane will be largely determined by the Ca^{2+} -ATPase.

Figure 2.2: Two views of the Ca^{2+} -ATPase. A, P and N domains are indicated along with transmembrane, cytoplasmic and luminal regions (Toyoshima and others 2000).

To estimate the charge of the Ca^{2+} -ATPase due to ionizable amino acids we can use the amino acid sequence of rabbit Ca^{2+} -ATPase obtained by Shi and others (Shi and others 1998). Three amino acids in the Ca^{2+} -ATPase protein (arginine, histidine, and lysine) become positively charged upon protonation. As pH becomes lower, these amino acids have a higher probability of being positively charged. On the other hand, four amino acids (aspartic acid, cysteine, glutamic acid, and tyrosine) become negatively charged upon deprotonation. Their probability of being negatively charged increases with increasing pH (Langlet and others 2008).

Lipids that make up the lipid bilayer of SR also contribute to the surface charge and surface potential in a similar manner as the amino acid of the Ca^{2+} -ATPase. The constituent lipids are 68% phosphatidylcholine, 17% phosphatidylethanolamine, 7.6% phosphatidylinositol, 2% phosphatidylserine, and 4% sphingomyelin (Lau and others 1979). Of these phospholipids, phosphatidylinositol (PI) and phosphatidylserine (PS) are charged at biological pH and in the pH range of our experiments (pH 4-9). Using the unit cell areas and the average surface area taken up by one lipid on the lipid bilayer (0.72 nm^2), we can calculate that this leaves us with 1.06 PS and 4.03 PI molecules per Ca^{2+} -ATPase protein. Phosphatidylserine has three protonation/deprotonation sites with pKa values of 2.6 ($\text{R}_2\text{-HPO}_4$), 5.5 (R-COOH), and 11.55 (R-NH_3^+). The first two sites start out neutral at low pH and become negatively charged at higher pH. The third site starts out positively charged at low pH and becomes neutral at high pH. Phosphatidylinositol has only one protonation/deprotonation site with pKa 2.5 which starts out neutral at low pH and becomes negatively charged at higher pH.

To find the fraction of a particular amino acid on the Ca^{2+} -ATPase or an ionizable group on a lipid that are charged for a given bulk pH we start with the Henderson-Hasselbalch equation, which relates pH and pKa to the molar equilibrium concentrations of acid and base of the acid/base reaction of the following form:

For this chemical equation, the dissociation constant is

$$K_a = \frac{[\text{H}^+][\text{base}]}{[\text{acid}]}. \quad (2.2)$$

Applying logarithm rules and the definitions of pH and pKa ($pH = -\log_{10}[H^+]$ and $pKa = -\log_{10} K_a$), we obtain the Henderson-Hasselbalch equation

$$pH = pK_a + \log_{10} \frac{[base]}{[acid]}. \quad (2.3)$$

For amino acids that have the potential for contributing positive charge upon protonation the following version of dissociation reaction, Equation (2.1), applies:

And for amino acids that have the potential for contributing negative charge upon deprotonation the following version of dissociation reaction, Equation (2.1), applies:

Equation (2.3) leads to

$$10^{(pH-pKa)} = \frac{[base]}{[acid]}. \quad (2.6)$$

To calculate the fraction of sites ionized due to protonation that contribute positive charge to the SR membrane, we use equation (2.7).

$$\frac{[BH^+]}{[B] + [BH^+]} = \frac{1}{10^{(pH-pKa)} + 1} \quad (2.7)$$

To calculate the fraction of deprotonation sites that contribute negative charge to the surface of the SR membrane, we use equation (2.8).

$$\frac{[A^-]}{[HA] + [A^-]} = \frac{10^{(pH - pKa)}}{10^{(pH - pKa)} + 1} \quad (2.8)$$

There is another complication regarding pH to address. In the above equations we have assumed that the pH in the vicinity of the amino acids is the same as the bulk pH. This is not necessarily true because as the membrane becomes charged, hydronium ions are either attracted or repelled from the membrane, thus altering the local pH. The interfacial concentration of hydronium ions is related to the bulk concentration and the local electric potential, ψ , in the following way:

$$[H^+]_{if} = [H^+]_{bulk} \cdot \exp\left(\frac{-\psi F_a}{RT}\right). \quad (2.9)$$

From this we find that the local, or interfacial, pH is related to bulk pH by

$$pH_{if} = pH_{bulk} - \log_{10}\left(\exp\left(\frac{-\psi F_a}{RT}\right)\right). \quad (2.10)$$

Also if we consider local perturbation of pH, equations (2.7) and (2.8) respectively become:

$$\frac{[BH^+]}{[B] + [BH^+]} = \frac{1}{10^{(pH - pKa)} \exp\left(\frac{-\psi F_a}{RT}\right) + 1} \quad (2.11)$$

for the fraction of positively charged ions and

$$\frac{[A^-]}{[HA] + [A^-]} = \frac{10^{(pH - pKa)} \exp\left(\frac{-\psi F_a}{RT}\right)}{10^{(pH - pKa)} \exp\left(\frac{-\psi F_a}{RT}\right) + 1} \quad (2.12)$$

for fraction of negatively charged ions.

In Table 2.1 we summarize the number of each ionizable amino acid in each segment of the Ca^{2+} -ATPase. We also give their pKa values in water. The A, P, and N domains of the protein are defined in terms of the amino acid sequence by Reuter and others (Reuter and others 2003). We include data for the entire Ca^{2+} -ATPase, the ten trans-membrane helices and the combined A, P, and N domains which we call the APN domain. Table 2.2 we show the number of each ionizable group of the lipid matrix for each Ca^{2+} -ATPase protein, along with the associated pKa values in water, and the protonated and deprotonated forms. We will consider one Ca^{2+} -ATPase protein and its associated lipids to be one “unit cell” of SR membrane.

Table 2.1: Frequencies and pKa values of amino acids in the Ca^{2+} -ATPase along with protonated and deprotonated forms.

Amino Acid	pKa	Protonated form	Deprotonated form	# per APN	# per helices	# per Ca^{2+} -ATPase
Arginine	12.5	BH^+	B	33	8	43
Aspartic acid	3.9	HA	A^-	34	4	50
Cysteine	8.0	HA	A^-	16	6	25
Glutamic acid	4.1	HA	A^-	44	15	76
Histidine	6.1	BH^+	B	4	1	12
Lysine	10.5	BH^+	B	37	8	51
Tyrosine	10.1	HA	A^-	10	7	21

Table 2.2: Ionizable sites on lipid head groups.

Ionizable site	pKa	Protonated form	Deprotonated form	# per unit cell
PS				
R2-HPO_4^-	2.6	HA	A^-	4.03
R-COOH^-	5.5	HA	A^-	4.03
R-NH_3^+	11.55	BH^+	B	4.03
PI				
R2-HPO_4^-	2.5	HA	A^-	1.06

Figure 2.3 shows the total charge per Ca^{2+} -ATPase for the APN domain and the entire protein as well as the total charge of the associated lipids of the lipid matrix for one

side of the bilayer, calculated using the number and type of ionizable amino acids and the corresponding pKa values.

Figure 2.3: The total charge per Ca²⁺-ATPase, the APN domain of the Ca²⁺-ATPase, and the lipid matrix of one unit cell of SR membrane.

Within the pH range of our experiments, both lipids and the Ca²⁺-ATPase protein contribute substantial charge to the surface of the membrane. Phospholipids contribute -4 to -6 electric charges per unit cell, if we assume their pKa values are the same as those in pure water. Under the same assumptions, the Ca²⁺-ATPase protein contributes from 44 to -58 electric charges per unit cell.

Sialic acid residues from gangliosides of glycosphingolipids on SR also contribute to the total surface charge. Narasimhan and Murry measured sialic acid content of rabbit skeletal muscle SR to be 0.613 ± 0.043 μg of lipid-bound sialic acid per mg of total protein (Narasimhan and others 1974). If we assume an average SR vesicle has Ca^{2+} -ATPase as 75% of its total protein, then we have $0.817\mu\text{g}$ of lipid-bound sialic acid per mg of Ca^{2+} -ATPase protein. With sialic acid having a molecular mass of 309.3 amu and each Ca^{2+} -ATPase having a molecular mass of 110,000 amu, we calculate that we have 0.29 molecules of sialic acid for each molecule of Ca^{2+} -ATPase. The pKa of sialic acid is 2.6, making each molecule very likely to be negatively charged at biological pH and also the pH range we use in our experiments (pH 4-9). However, there are so few charges due to sialic acid compared to those due to proteins and lipids that the charge due to sialic acid can be considered negligible.

In summary, SR is a relatively simple biological membrane whose function is the release and sequestering of calcium ions which is crucial to the contraction and relaxation of muscle cells. As a simple and much studied membrane it is an ideal candidate for an electrophoretic mobility study. Despite the apparent simplicity of its composition, being almost entirely lipids and Ca^{2+} -ATPase protein, there is much to be learned about its surface charge and frictional properties, especially in an aqueous environment.

Chapter 3: Electrostatic Description of Simple Membrane-Water Interfaces

In this chapter we derive equations that describe the electric potential around a charged colloid suspended in an aqueous solution. Two derivations are shown. In the first, we use only the linear terms of the Taylor series expansion of the Poisson-Boltzmann equation. That is, we use the linearized Poisson-Boltzmann equation. In the second, we use the non-linearized Poisson-Boltzmann equation. The linearized solution is limited to situations where the potential at the colloid surface, ψ_0 , is low, however it is generalized for ions of any charge, while the nonlinearized solution works for any surface potential and is specific to ions of symmetrical charge. For simplicity, we make the following assumptions (1) The fixed charge on the colloid is not discrete, but continuous and uniform. (2) The ions in the aqueous phase are point charges. (3) Other than the ions, the aqueous phase has no structure. It is a homogenous medium with uniform dielectric constant (McLaughlin 1989).

In our experiments we model charged membrane vesicles or charged liposomes as spherical charged colloids. When a charged colloid is suspended in an electrolytic solution, ions of opposite charge are attracted to the surface, however, thermal energy of these ions keeps them diffuse in the aqueous phase rather than forming a sheet of equal and opposite charge at the colloid surface. When the colloid is stationary, the cloud of screening ions that accumulate around the vesicle is spherically symmetric. The concentration of screening ions is greater near the colloid surface and more diffuse further from the surface (Ohshima 2006). The charged surface and the screening ions in

the aqueous surroundings form what is called an electrical double-layer. When more ions are available in the aqueous phase, the charges that form the diffuse layer are drawn in more closely to the colloid surface. Although the entire thickness of the diffuse layer is infinite in theory, the representative thickness of the diffuse layer, termed the Debye length, is defined as the distance at which screening ions cause the electric potential to decay to $1/e$ or $\sim 37\%$ of the surface potential. If we consider only large vesicles with substantial ionic strength so that the Debye length is small compared to the radius of the vesicle, we can model the surface as a plane.

*Section 1:   **The linearized Poisson-Boltzmann equation***

The Gouy-Chapman electrostatic condition is the application of electro-neutrality to the electrical double layer. It states that the surface charge of the membrane is screened by an equal net charge of opposite polarity in the aqueous phase (Ohshima 2006). In this simplified example of a flat charged surface where x is the distance away from the surface, the Gouy-Chapman condition can be written as (Aveyard and Haydon 1973; Lyklema 1995)

$$\sigma_0 + \int_0^{\infty} \rho(x) dx = 0. \quad (3.1)$$

σ_0 is the surface charge density of the membrane and $\rho(x)$ is the volume charge density as a function of x . The second term in Equation (3.1) represents the layer of screening charges in the aqueous phase, which is often called the diffuse layer. If the diffuse layer were flattened into a plane, parallel to the membrane surface, the plane would have a

surface charge density, σ_d , equal in magnitude and opposite in polarity to that of the colloid surface. Then we have

$$\sigma_0 + \sigma_d = 0 \quad (3.2)$$

and

$$\sigma_d = \int_0^{\infty} \rho(x) dx. \quad (3.3)$$

The well-known Poisson's equation relates the electric potential, ψ , at any point to the distribution of ions at that point (Hunter 1981).

$$\nabla^2 \psi = -\frac{\rho}{\epsilon \epsilon_0} \quad (3.4)$$

In our problem, the potential only changes in the x direction, thus Poisson's equation is

$$\frac{d^2 \psi(x)}{dx^2} = -\frac{\rho(x)}{\epsilon \epsilon_0}. \quad (3.5)$$

If we integrate both sides of Eq. (3.5) with respect to x from $x=0$ to $x=\infty$ and consider Eqs. (3.2) and (3.3) we get the following

$$\left. \frac{d\psi(x)}{dx} \right|_{x=0} = \frac{\sigma_d}{\epsilon \epsilon_0} = -\frac{\sigma_0}{\epsilon \epsilon_0}. \quad (3.6)$$

This is another statement of the Gouy-Chapmann condition (Lyklema 1995; Ohshima 2006). Also when Eq. (3.7) is true, the electric field inside the membrane is zero and all the electric field lines that come from the membrane end on counter-ions in the diffuse layer, or vice versa: the electric field lines coming from the diffuse layer end on the membrane surface (Ohshima 2006).

The distribution of ions in the diffuse layer is given in terms of the position dependent potential by a Boltzmann factor

$$n'_i = n_i \exp(-z_i e \psi(x) / kT). \quad (3.7)$$

n'_i is the volume density of ion species i in a specific region in space with potential $\psi(x)$ and n_i is the average volume density of ions of species i in the bulk solution (Aveyard and Haydon 1973). In the exponent, z_i is the multiplicity of charge or valency of the i^{th} species of ion. In this case the sign of z is the same as the sign of the charge of the species of ion. e is the unit charge, and k is the Boltzmann constant. The charge density $\rho(x)$ in a the given region of the diffuse layer is expressed as

$$\rho(x) = \sum_i z_i e n'_i = \sum_i z_i e n_i \exp(-z_i e \psi(x) / kT). \quad (3.8)$$

Eqs. (3.5) and (3.8) can be combined to form the Poisson-Boltzmann equation. If we take only the linear terms of the Taylor series expansion of the exponential function in Eq. (3.8),

$$\exp(-z_i e \psi(x) / kT) = 1 - \frac{z_i e \psi(x)}{kT} + \frac{1}{2!} \left(\frac{z_i e \psi(x)}{kT} \right)^2 - \dots \quad (3.9)$$

we are making an approximation applicable to the case when the electrostatic potential energy is much less than the thermal energy, i.e. $z_i e \psi \ll kT$. With this assumption the charge density becomes

$$\rho(x) = \frac{e^2 \psi(x) \sum_i n_i z_i^2}{kT}. \quad (3.10)$$

The first term of the exponential Taylor expansion vanishes due to the net electro-neutrality of the ionic solution. Using this charge density and Poisson's equation (3.5) we arrive at the linearized Poisson-Boltzmann equation.

$$\frac{d^2\psi(x)}{dx^2} = \frac{e^2\psi(x)}{\epsilon\epsilon_0 kT} \sum_i n_i z_i^2 = \kappa^2 \psi(x) \quad (3.11)$$

Here κ is the Debye-Hückel parameter, defined as (Aveyard and Haydon 1973; Lyklema 1995; Ohshima 2006)

$$\kappa = \sqrt{\frac{e^2 \sum_i n_i z_i^2}{\epsilon\epsilon_0 kT}}. \quad (3.12)$$

It is useful to explore κ in terms of the ionic strength (IS) because that is one of our experimentally adjustable parameters. Ionic strength is defined as

$$IS \equiv \frac{1}{2} \sum_i z_i^2 C_i. \quad (3.13)$$

C_i is the molar concentration of ion i . In terms of the ionic strength, the Debye-Hückel parameter is

$$\kappa = \left[\frac{2000 N_a^2 e^2 IS}{\epsilon\epsilon_0 RT} \right]^{\frac{1}{2}} \quad (3.14)$$

where N_a is Avogadro's number and R is the gas constant.

If we solve Eq. (3.11), and apply the boundary conditions that the potential and the first derivative of the potential both approach zero as x approaches infinity

($\psi(x) = \frac{d\psi}{dx} = 0$ at $x = \infty$), and also that $\psi(x) = \psi_0$ at $x = 0$, we get

$$\psi(x) = \psi_0 \exp(-\kappa x). \quad (3.15)$$

The electric potential in the aqueous phase decays with distance as a simple exponential function with characteristic decay length $1/\kappa$. This function is shown in Figure 3.1. The membrane-water interface is located at $x=0$.

Figure 3.1: Potential profile of the diffuse layer. The Debye length, $1/\kappa$, is shown to be the distance from the membrane at which the potential is 0.37 times the surface potential. The electric potential exponentially decays with increasing distance.

A higher concentration of ions in the aqueous phase will result in a shorter Debye length and vice-versa. Notice that the Debye length is not a function of electric potential or surface charge density. It is only a function of properties of the ionic solution: ion concentration, temperature and multiplicity of charge of the ionic species.

Combining Eq. (3.15) for the electric potential and the Gouy-Chapmann condition (Eq. (3.6)) we get the following expression for the surface potential, ψ_0 , in terms of the surface charge density, σ_0 , and the Debye-Hückel parameter, κ :

$$\psi_0 = \frac{\sigma_0}{\epsilon \epsilon_0 \kappa}. \quad (3.16)$$

Eq. (3.16) is an approximation for the surface potential of a flat surfaced or large colloid with a short Debye length that works only for low potentials (Ohshima 2006). This limitation is a result of only using the linear terms of the Taylor series expansion of Eq. (3.9). Although this solution is simple and quite useful in many electrophoretic mobility models, it is also possible to analytically solve the Poisson-Boltzmann equation for this geometry without using this approximation.

Section 2: The non-linearized Poisson-Boltzmann equation

We return to the general case of arbitrary surface potential. Similar solutions to this problem are provided by Ohshima and Lyklema (Aveyard and Haydon 1973; Lyklema 1995; Ohshima 2006). In this case we use the non-linear Poisson-Boltzmann equation. We do however assume we have a symmetrical electrolyte. In the following equations z is always positive and is equal to the absolute value of the multiplicity of charge ($z = |z^+| = |z^-|$). The nonlinearized Poisson-Boltzmann equation is

$$\begin{aligned} \frac{d^2 \psi(x)}{dx^2} &= -\frac{zen}{\epsilon \epsilon_0} \left[\exp\left(-\frac{ze\psi(x)}{kT}\right) - \exp\left(\frac{ze\psi(x)}{kT}\right) \right] \\ &= \frac{2zen}{\epsilon \epsilon_0} \sinh\left(\frac{ze\psi(x)}{kT}\right). \end{aligned} \quad (3.17)$$

To simplify, we introduce the scaled potential $y(x)$.

$$y(x) = \frac{ze\psi(x)}{kT} \quad (3.18)$$

At approximately room temperature (290 K), with $z=1$, $kT/ze \sim 25$ mV. Also for a symmetrical electrolyte the Debye-Hückel parameter is

$$\kappa = \left(\frac{2z^2 e^2 n}{\epsilon \epsilon_0 kT} \right)^{\frac{1}{2}}. \quad (3.19)$$

If we multiply each side of Eq. (3.17) by ze/kT and make the above substitutions (Eqs. (3.18) and (3.19)) the Poisson-Boltzmann equation becomes

$$\frac{d^2 y(x)}{dx^2} = \kappa^2 \sinh(y(x)). \quad (3.20)$$

To obtain an analytical solution, we can integrate this equation if we first multiply each side by dy/dx as follows:

$$\frac{dy}{dx} \cdot \frac{d^2 y(x)}{dx^2} = \kappa^2 \sinh(y(x)) \cdot \frac{dy}{dx}. \quad (3.21)$$

For the right side we make the following substitution:

$$\frac{d}{dx} \cosh(y(x)) = \sinh(y(x)) \cdot \frac{dy}{dx}. \quad (3.22)$$

For the left side, we use this relationship:

$$\frac{d}{dx} \left[\left(\frac{dy}{dx} \right)^2 \right] = 2 \left(\frac{dy}{dx} \right) \frac{d}{dx} \left(\frac{dy}{dx} \right) = 2 \frac{dy}{dx} \cdot \frac{d^2 y}{dx^2}. \quad (3.23)$$

Combing the above gives us the following:

$$\frac{1}{2} \frac{d}{dx} \left[\left(\frac{dy}{dx} \right)^2 \right] = \kappa^2 \frac{d}{dx} \cosh(y). \quad (3.24)$$

After integrating Eq. (3.24) we obtain

$$\left(\frac{dy}{dx}\right)^2 = 2\kappa^2 (\cosh(y) + \text{const.}) \quad (3.25)$$

To evaluate the constant, we use the boundary condition that as $x \rightarrow \infty$ dy/dx and y approach zero. Taking this limit, we find that the constant is equal to -1. Next by using the identity

$$\cosh(x) = 1 + 2 \sinh^2\left(\frac{x}{2}\right) \quad (3.26)$$

our equation becomes

$$\left(\frac{dy}{dx}\right)^2 = 4\kappa^2 \sinh^2\left(\frac{y}{2}\right). \quad (3.27)$$

By taking the square root of both sides we obtain

$$\frac{dy}{dx} = \pm 2\kappa \sinh\left(\frac{y}{2}\right). \quad (3.28)$$

At an infinite distance from the membrane surface the potential is zero. Therefore as x increases, negative potentials are increasing to approach zero and positive potentials are decreasing. The negative sign in Eq. (3.28) is therefore necessary because when potential is positive, dy/dx is negative and when potential is negative, dy/dx is positive. Now Eq. (3.28) can be integrated further.

$$-\frac{1}{2} \int_{y_0}^y \frac{dy}{\sinh\left(\frac{y}{2}\right)} = \kappa \int_0^x dx \quad (3.29)$$

The integral on the left side is of the form

$$\int \frac{dx}{\sinh(ax)} = \frac{1}{a} \ln\left(\tanh\left(\frac{ax}{2}\right)\right). \quad (3.30)$$

After integration we obtain

$$-\ln\left(\tanh\left(\frac{y}{4}\right)\right)\Bigg|_{y_0}^y = \kappa x|_0^x + const \quad (3.31)$$

which leads to

$$\ln\left(\tanh\left(\frac{y_0}{4}\right)\right) - \ln\left(\tanh\left(\frac{y}{4}\right)\right) = \kappa x + const. \quad (3.32)$$

We obtain the constant in Eq. (3.32) by applying the condition that $y(x) \rightarrow y_0$ as $x \rightarrow 0$. We find that the constant is equal to zero, giving us

$$\ln\left(\tanh\left(\frac{y}{4}\right)\right) = \ln\left(\tanh\left(\frac{y_0}{4}\right)\right) - \kappa x. \quad (3.33)$$

We make the following substitution,

$$\gamma = \tanh\left(\frac{ze\psi_0}{4kT}\right) \quad (3.34)$$

and solve for $y(x)$ to obtain

$$\tanh\left(\frac{y}{4}\right) = \exp\left(\ln(\tanh(\gamma)) - \kappa x\right), \quad (3.35)$$

which results in

$$y(x) = 4 \tanh^{-1}(\gamma \exp(-\kappa x)) = 2 \ln\left(\frac{1 + \gamma \exp(-\kappa x)}{1 - \gamma \exp(-\kappa x)}\right). \quad (3.36)$$

To obtain the final solution, we use the identity

$$\tanh^{-1}(x) = \frac{1}{2} \ln\left(\frac{1+x}{1-x}\right). \quad (3.37)$$

The final solution is

$$\psi(x) = \frac{4kT}{ze} \tanh^{-1}(\gamma \exp(-\kappa x)) = \frac{2kT}{ze} \ln \left(\frac{1 + \gamma \exp(-\kappa x)}{1 - \gamma \exp(-\kappa x)} \right). \quad (3.38)$$

To solve for the surface charge density and the surface potential we use the Gouy-Chapman condition (Eq.(3.6)). First we take the derivative of Eq.(3.38),

$$\frac{d\psi}{dx} = -\frac{4kT\kappa}{ze} \left(\frac{\gamma}{1 - \gamma^2 \exp(-\kappa x)} \right) \exp(-\kappa x) \quad (3.39)$$

evaluate at $x=0$,

$$\left. \frac{d\psi}{dx} \right|_{x=0} = -\frac{4kT\kappa}{ze} \left(\frac{\gamma}{1 - \gamma^2} \right) \quad (3.40)$$

and apply the Gouy-Chapman condition to find

$$-\frac{\sigma_0}{\varepsilon \varepsilon_0} = -\frac{4kT\kappa}{ze} \left(\frac{\gamma}{1 - \gamma^2} \right). \quad (3.41)$$

Now we use the following identity,

$$\sinh(x) = \frac{2 \tanh\left(\frac{x}{2}\right)}{1 - \tanh^2\left(\frac{x}{2}\right)} \quad (3.42)$$

to obtain the following expression for the surface charge density at the membrane surface.

$$\sigma_0 = \frac{2\varepsilon \varepsilon_0 \kappa kT}{ze} \sinh\left(\frac{ze\psi_0}{2kT}\right) = (8n\varepsilon \varepsilon_0 kT)^{\frac{1}{2}} \sinh\left(\frac{ze\psi_0}{2kT}\right) \quad (3.43)$$

We may solve Eq. (3.43) for the surface potential ψ_0 .

$$\psi_0 = \frac{2kT}{ze} \sinh^{-1}\left(\frac{\sigma_0}{\sqrt{8n\varepsilon \varepsilon_0 kT}}\right) = \frac{2kT}{ze} \sinh^{-1}\left(\frac{ze\sigma_0}{2\varepsilon \varepsilon_0 \kappa kT}\right) \quad (3.44)$$

Eq. (3.44) relates the surface potential to the surface charge density and the Debye-Hückel parameter, κ . This expression is valid for any value of surface potential. In the case of low surface potential, we may use the relation $\sinh(x) \cong x$ and find that Eq. (3.43) is equivalent to Eq. (3.16), which was derived using the linearized Poisson-Boltzmann equation.

Section 3:   Applicability of linearized Poisson-Boltzmann equation

In the following two graphs we explore the applicability of the linearized Poisson-Boltzmann equation. In the first example (Figure 3.2) we see that as the ionic strength increases the linearized Poisson-Boltzmann equation becomes more applicable. Here the surface charge density is set to a moderate 10 mC/m^2 . In the second example (Figure 3.3) we keep the ionic strength constant at 25 mM and vary the surface charge density from 10 to 40 mC/m^2 . We see that the Poisson-Boltzmann equation becomes more applicable as the surface charge density is reduced. To summarize, the applicability of the linearized Poisson-Boltzmann equation depends on the electric potential at the surface of the membrane. If we expect the surface potential of the model system to be less than 40 mV , we may use the linearized Poisson-Boltzmann model which offers a significant computational advantage.

Figure 3.2: Potential as a function of distance from the membrane surface calculated from the linearized Poisson-Boltzmann equation and the nonlinearized Poisson Boltzmann equation. Calculations are made with surface charge density of 10 mC/m^2 .

Figure 3.3: Potential as a function of distance from the membrane surface calculated from the linearized Poisson-Boltzmann equation and the nonlinearized Poisson Boltzmann equation. Calculations are made with varying surface charge density and ionic strength of 25 mM.

Chapter 4: Electrophoretic Mobility of Smooth-surfaced Colloids

Section 1: Electrophoretic mobility and the zeta potential

When an electric field is applied to an electrolyte solution containing a charged colloid, the colloid is accelerated by the electric force until it reaches a terminal velocity due to hydrodynamic drag forces. When the applied electric field is small compared to the electric field in the diffuse layer, there is a linear relationship between the applied field and the terminal velocity of the colloid (O'Brien and White 1978; Ohshima 2006). In an otherwise static system, the electrophoretic mobility of a colloid is defined as its magnitude of the terminal velocity divided by the magnitude of the applied electric field. The sign of the mobility is negative when the velocity vector and the electric field vector oppose each other as in Figure 4.1. Thus, the direction of the mobility is negative for negatively charged colloids and positive for positively charged colloids. As defined above

$$\mu \equiv \frac{v_t}{E}. \quad (4.1)$$

Figure 4.1: Negatively charged colloids driven through a fluid by an applied electric field.

In this chapter we explore a variety of models for the electrophoretic mobility of smooth-surface spherical colloids. These models relate the electrophoretic mobility, which is measured, to properties of the colloid and solution such as colloid radius, a , the zeta potential, ζ (explained in the next section), and Debye parameter of the solution, κ .

When a colloid moves through an electrolyte, a layer of water and ions moves with it, adhered to its surface. At some distance away from the colloid, L_s , the onset of relative motion between the colloid and the surrounding solution begins. The surface of shear is the boundary between the fluid that does not have relative motion with respect to the colloid and the fluid that does. The adhered solution, together with the colloid, are often called the hydrodynamic colloid or particle.

An important parameter for the description of smooth colloids is the zeta potential, ζ . This theoretical parameter is the electric potential at the surface of shear. In Chapter 3 we described the relationship between surface potential and surface charge density of a charged colloid in an electrolyte. By fitting the electrophoretic mobility models described in this chapter to experimental data, we can find the surface charge density of our colloids when we make the assumption that the surface potential, ψ_0 , is equal to ζ .

In the two simplest electrophoretic mobility models discussed in this chapter, the Smoluchowski model and the Hückel model the zeta potential is linearly proportional to the electrophoretic mobility (Ohshima 2006). In Henry's model and the OHW model, the relationship is more complicated but the mobility is still a function of the zeta potential (Ohshima 2006). As we shall see later in this chapter, the value of the zeta potential is also important in determining the applicability of various electrophoretic mobility models.

Section 2: Overview of electrophoretic mobility models

The simplest model we present in this chapter is Hückel's model, which treats the colloid as a small particle with a potential distribution around it that is equivalent to the potential distribution of a point charge positioned at the center of the colloid. The effects of the screening ions are ignored because the thickness of the diffuse layer (κ^{-1}) is much greater than the radius of the particle. This model works only for colloids that have small radii compared to their Debye lengths ($\kappa a \ll 1$). Another simple model is the

Smoluchowski model which works for large colloids with short Debye lengths, ($\kappa a \gg 1$), such that the surface can be modeled as a plane. In this case we find an expression for the electrophoretic mobility by calculating the flow of fluid in the aqueous phase relative to the colloid. Fluid flow is driven by the electric force as fluid around the colloid contains charges of opposite polarity of the colloid surface. In this model the effects of curvature of the colloid are neglected. The Hückel model predicts a mobility that is two-thirds that predicted by Smoluchowski's model for a colloid with the same ζ , in the same electrolyte (same κ). Henry derived a mathematical function whose value varies from 1 to 2/3 that bridges the gap between these two extremes and is applicable to colloids of all sizes (Ohshima 2006). However, Henry's mobility equation only works for low zeta potential ($\zeta \ll kT/e$). This is because Henry used the linearized version of the Poisson-Boltzmann equation to describe the ion distribution around the colloid when studying the effects of curvature on mobility (Ohshima 2006). He also did not treat an important phenomenon that occurs with greater zeta potentials ($\zeta > kT/e$) due to the effects of ions in the diffuse layer on the colloid's motion, namely the relaxation effect, a force due to the polarization of the diffuse layer, which will be explained in the next section.

Overbeek and Booth independently derived equations for the electrophoretic mobility of spherical colloids with arbitrary size and zeta potential, which include the effects of polarization of the diffuse layer (Booth 1950; Overbeek 1943). These equations were solved numerically first by Wiersema and others and the solutions were improved by O'Brien and White (O'Brien and White 1978; Wiersema and others 1965). The resulting model became known as the standard electrophoretic mobility model. Ohshima later developed a very useful semi-empirical analytic solution, which agrees with O'Brien

and White's numerical solution with less than 1% error for $\kappa a \geq 10$ (Ohshima 2006). This solution is called the Ohshima-Healy-White (OHW) model. It is used in the analysis of our data.

Section 3: Forces on a colloid during electrophoresis

There are phenomena called the 'electrophoretic retardation effect' and the 'relaxation effect' that slow a colloid's motion. These effects become significant when the zeta potential is large ($\zeta > kT/e$). Henry's model includes the electrophoretic retardation effect for low ζ . The 2/3 factor difference between Hückel and Smoluchowski is a result of this effect (Lyklema 1995). At low ζ , this effect can be estimated as a function of κ and a . However, at high ζ this simple function doesn't work and the force that results from this effect is a function of ζ (Lyklema 1995).

In total there are four governing forces acting on a colloid moving in an electric field. The first is the electric force exerted on the colloid due the applied field and the particle's charge, $F_1 = qE$. The second force is the Stokes hydrodynamic frictional force, $F_2 = -6\pi\eta av_t$, where η is the viscosity coefficient of the electrolyte, a is the radius of the hydrodynamic colloid, and v_t is the electrophoretic velocity. The Stokes drag force is applicable to any spherical colloid moving with a given velocity through a fluid. To explain the physical origins of the electrophoretic retardation effect (F_3 in Figure 4.2) and the relaxation effect (F_4) we will consider only the counter-ions in the aqueous phase, as co-ions are repelled from the colloid's surface and thus do not significantly affect the electric field or potential at the colloid surface (O'Brien and White 1978).

Electrophoretic relaxation effect

When the spherical colloid is stationary the counter-ion cloud surrounding it is spherically symmetric. However when the colloid moves, it encounters new counter-ions in the bulk phase. Counter-ions in the bulk phase are pulled in the direction opposite to that of the colloid by the external electric field. As these counter-ions flow past the colloid, their electric attraction to the colloid causes their velocities to increase as they approach and decrease once they pass. Because of their changing velocities, the counter-ions spend more time behind the colloid than in front of it and therefore the counter-ion cloud around the colloid becomes asymmetric. At any time during electrophoresis, there is a higher density of counter-ions residing directly behind the colloid than directly in front of it (O'Brien and White 1978). The colloid is then slowed by its attraction to its own ion cloud. This attractive force has been named the relaxation effect (Ohshima 2006; Wiersema and others 1965). Figure 4.2 depicts a colloid undergoing electrophoresis with an asymmetric ion cloud. The center of charge of the colloid and the ion cloud are shown. The distortion of the counter-ion cloud increases as the zeta potential increases. This is because with higher potentials around the colloid there are increased attractive forces between the ions and the colloid resulting in increased perturbations in the ions' velocities as they encounter the colloid.

Figure 4.2: Forces on a charged colloid in an electrolyte undergoing electrophoresis. E is the applied electric field. v_t indicates the terminal velocity vector of the colloid.

Electrophoretic retardation effect

The electrophoretic retardation effect is a hydrodynamic force on the colloid caused by counter-ions moving in the applied electric field. These ions drag water with them and cause the fluid around the colloid to move in the direction opposite to that of the colloid. In this case the viscous drag force on the colloid is greater than the Stokes drag of an equivalent colloid with the same velocity through the fluid in the absence of an ion cloud (O'Brien and White 1978). This retardation effect depends on the spatial distribution of ions around the colloid (κ , a , and ζ), the mobilities of the ions, and the trajectories of the ions (which are determined by the electric field lines around the colloid). At higher ζ , this effect is generally greater because the concentration of counter-ions near the colloid surface is greater and therefore more momentum transfer between the fluid (set in motion by the ions) and the colloid can take place. This is also true when

the Debye length ($1/\kappa$) is short and the ions that make up the diffuse layer are drawn in close to the colloid surface.

In summary F_3 and F_4 act against the electric force caused by the applied electric field. They slow the particles motion. They are difficult to quantify because they are dependent on both the ion distribution and the fluid flow, which are themselves dependent on each other (Lyklema 1995). To explore these forces in exhaustive detail is a theoretical endeavor that is beyond the scope of this dissertation.

Section 4:   The Electric Field Around the Particle and the Retardation Effect

To further understand the electrophoretic retardation effect (F_3) we will examine the electric field around the colloid during electrophoresis. The electric field around the colloid has three sources (Lyklema 1995). The first is the electric field caused by the charge on the colloid and the counter-ion cloud surrounding the colloid. The field lines from this source are generally oriented in the radial direction (Figure 4.3A). The second source is the field caused by the polarization of the diffuse layer (Figure 4.3B) and the third is the applied electric field (Figure 4.4). Since the electric field is a vector quantity at any point in space, we can study these three sources of the field separately knowing that the total field is the vector sum of the three components.

Figure 4.3: The near field (A) and far field (B) components of the electric field due to the charged surface and the counter-ions and the polarization of the diffuse layer respectively.

The applied field is particularly important to the retardation effect because it drives the ions around the colloid. It becomes distorted by the presence of the colloid. To examine this effect, we use a spherical coordinate system with the origin set at the center of the colloid. The external field is applied in the direction of the z -axis. θ is the angle between the z -y plane and a point. There is no variation in potential or electric field in ϕ , the angle between the x -z plane and the origin. To evaluate this component of the field we ignore the surface charge and counter charge so that Laplace's formula can be used. In reality, Laplace's equation is only valid outside the diffuse layer where there is no net space charge, however, since we are examining the applied electric field separately from the other field sources we view it in absence of space charge. From Laplace's equation

we find that the general form of the potential on the outside of a dielectric colloid is (Cook 1975)

$$\psi(r, \theta) = - \left(1 - \frac{\epsilon_2 - \epsilon_1}{\epsilon_2 + 2\epsilon_1} \frac{a^3}{r^3} \right) E_0 r \cos \theta \quad (4.2)$$

for an otherwise homogeneous field. Here a is the radius of the colloid, E_0 is the magnitude of the applied field and θ is the angle indicated on Figure 4.4.

In our situation, the aqueous solution has a dielectric constant ($\epsilon_l \cong 80$) that is much larger than that of the colloid ($\epsilon_2 \cong 2$ for a lipid bilayer), therefore the electric field lines are channeled around the colloid. The electric potential around a colloid in this case reduces to (Lyklema 1995; Ohshima 2002)

$$\psi(r, \theta) = -E_0 r \cos \theta - \frac{a^3}{2r^2} E_0 \cos \theta. \quad (4.3)$$

Since $E = -\nabla \psi$, we find that the electric field is

$$\vec{E}(r, \theta) = \left[E_0 \cos \theta - \frac{a^3}{r^3} E_0 \cos \theta \right] \hat{r} + \left[-E_0 \sin \theta - \frac{a^3}{2r^3} E_0 \sin \theta \right] \hat{\theta}. \quad (4.4)$$

Figure 4.4: The effect of a non-conducting colloid on an otherwise uniform electric field.

At the colloid surface ($r = a$), the first term in Eq. (4.5) is zero, and the electric field due to the applied field is $\frac{3}{2}E_0 \sin \theta$ in the negative $\hat{\theta}$ direction. In the absence of the colloid the component of this E field in the $\hat{\theta}$ direction at the colloid surface would be $E_0 \sin \theta$. When the particle is present, the electric field at $r = a$ in the direction tangent to the surface is 3/2 times greater than without the particle. This difference in electric field accounts for the fact that $\mu_{Smol} = \frac{3}{2}\mu_{Huckel}$ (Ohshima 2006).

As r increases beyond $r = a$, the component of the electric field parallel to the particle surface decreases and eventually is equal to the undistorted field. At the same time, the component in the r direction goes from 0 to $E_0 \cos \theta$. From Figure 4.4, we can see that a bigger colloid in an equal applied field would result in a greater volume of distorted field around the colloid. Therefore when a is large and the Debye length (κ^{-1}) is short ($\kappa a \gg 1$), the diffuse layer of countercharge will exist in a volume of increased electric field parallel to the colloid surface and reduced field perpendicular to the surface. In fact, at the large κa extreme, the electric field tangent to the surface is very near to $\frac{3}{2}E_0 \sin \theta$ throughout the entire diffuse layer. At the opposite extreme ($\kappa a \ll 1$), the field is completely undistorted and the field tangent to the surface is $E_0 \sin \theta$. There are simple expressions for electrophoretic mobility for these extremes, which are derived in Section 6: of this chapter. However, in the intermediate case, some portion of the diffuse layer is in a region where $\vec{E} = \frac{3}{2}E_0 \sin \theta \hat{\theta}$ while the rest is in a changing field described by Eq. (4.5). This leads to greater complexity in electrophoretic mobility models.

Section 5: Momentum transfer and the hydrostatic force

The hydrostatic force on a colloid is determined by the rate of momentum transfer from the fluid to the surface of the colloid. For a given surface element of the colloid, the momentum transfer has a component normal to the surface and one tangent to the surface. The component normal to the surface is related to the change in radial velocity with respect to radial distance (normal stress). The component tangent to the surface is related to the change in velocity tangent to the surface with respect to radial distance (shear stress). The total hydrostatic force on our colloid in the z direction can be evaluated by summing the normal and shear stress over all the surface elements. Since velocity of an ion in the double layer is linearly related to the electric field by

$$\frac{r}{v} = \frac{qE}{6\pi\eta a}, \quad (4.5)$$

lines of fluid flow will look similar to electric field lines for ions with positive charge because the fluid is dragged by ions. However, there is often a difference in the conductivity of the diffuse layer and the bulk aqueous phase. This can also affect the shape of electric field lines around the colloid. For example when the conductivity of the diffuse layer is greater the field lines will be drawn in to the particle surface (Lyklema 1995). This further complicates the flow of fluid around the colloid.

From the previous discussions it is clear that fully solving the electrophoretic mobility problem for a colloid for which the retardation and relaxation forces are substantial would prove to be quite difficult. This problem has not been solved analytically. O'Brien and White state in their 1978 paper that included a numerical

solution “It does not seem possible to obtain exact analytic solutions to these equations” (O'Brien and White 1978). Therefore instead of deriving a complete solution to the electrophoretic mobility problem, we summarize useful models from the literature, which employ simplifying assumptions.

Section 6: Important analytic models

In the context of this study, we consider the following models of electrophoretic mobility to be the most relevant:

Hückel's Model

Hückel's model deals with colloids that have very small radii compared to the thicknesses of their diffuse layers ($\kappa a \ll 1$). At this extreme, the screening effect of the counter-ions in the diffuse layer can be ignored. The electric potential around the colloid is that of a point charge located at the center of the colloid (Ohshima 2006). The electric potential due to a point charge is

$$\psi(r) = \frac{q}{4\pi\epsilon\epsilon_0 r}. \quad (4.6)$$

The zeta potential of a small colloid of hydrodynamic radius a is therefore

$$\zeta = \frac{q}{4\pi\epsilon\epsilon_0 a}. \quad (4.7)$$

With an applied electric field, the colloid reaches a terminal velocity (v_t) when the electric force $F_e = qE$ and the Stokes drag force $F_d = 6\pi\eta a v_t$ become equal. Setting these

forces equal to each other, and solving for the terminal velocity divided by the electric field, we get

$$\mu = \frac{v_t}{E} = \frac{q}{6\pi\eta a}. \quad (4.8)$$

Substituting for q from Eq. (4.7) we get Hückel's mobility equation,

$$\mu = \frac{2}{3} \frac{\varepsilon\varepsilon_0}{\eta} \zeta. \quad (4.9)$$

Smoluchowski Mobility Equation

The problem of electrophoretic mobility of large colloids can be addressed by considering the flow of free ions in the diffuse layer. The ionic solution that forms the diffuse layer flows due to the applied electric field because ions are solvated and drag the surrounding water. Due to the fluid dynamic boundary condition for a viscous fluid at a surface, the membrane and immediate layers of water molecules are immobile relative to each other. In Smoluchowski's model, at some distance L_s from the membrane surface water begins to slip relative to the membrane (Cohen and Khorosheva 2001). As mentioned before, the electrostatic potential at the shear plane is the zeta potential, ζ . The development below is applicable to cases when the radius of the particle, a , is much greater than the thickness of the diffuse layer, $1/\kappa$, ($\kappa a \gg 1$). In such cases the curvature of the vesicle can be ignored. The membrane surface can be regarded as planar and uniformly charged. The free ions in the diffuse layer cause electro-osmotic flow of the fluid with respect to the membrane surface. We invoke the one-dimensional Navier-Stokes equation to find an expression for the fluid velocity, v , as a function of distance

from the colloid surface, x . The fluid velocity relative to the colloid at infinity has a value equal in magnitude but opposite in direction to the colloid's velocity relative to the electrophoretic cell. This velocity is then used to find an expression for the electrophoretic mobility.

For incompressible fluid in the absence of pressure gradients, the forces acting on a unit volume of suspending solution are given by the Navier-Stokes equation

$$\eta \frac{d^2 v}{dx^2} + \rho(x)E = 0. \quad (4.10)$$

The velocity of flow is v , η the viscosity of aqueous phase, and E the applied electric field of the electrophoretic mobility analyzer. Each term on the left-hand side represents a force acting on the differential lamina of fluid parallel to the surface. The first term is the shear force, which is the frictional force between lamina, and the second term is the driving force caused by the applied electric field. Using the expression for $\rho(x)$ from Poisson's equation (Eq. (4.11))

$$\frac{d^2 \psi}{dx^2} = -\frac{\rho(x)}{\epsilon \epsilon_0}, \quad (4.11)$$

we obtain the following equation for the distribution of flow velocity:

$$\frac{d^2 v}{dx^2} = \left(\frac{\epsilon \epsilon_0 E}{\eta} \right) \frac{d^2 \psi}{dx^2}. \quad (4.12)$$

We integrate Eq. (4.12) and evaluate the integration constant using the boundary

conditions that $\left. \frac{dv}{dx} \right|_{x=\infty} = \left. \frac{d\psi}{dx} \right|_{x=\infty} = 0$. We find the integration constant is equal to zero and

we obtain

$$\frac{dv}{dx} = \left(\frac{\varepsilon \varepsilon_0}{\eta} \right) \frac{d\psi}{dx}. \quad (4.13)$$

We integrate a second time and apply the boundary conditions that $\psi(\infty) = 0$ and $v(\infty) = v_{EO}$, where v_{EO} is the velocity of bulk fluid relative to the surface of membrane, also called the electro-osmotic velocity, we find the integration constant is equal to v_{EO} corresponding to the velocity of flow at $x \rightarrow \infty$, giving us

$$v(x) = \left(\frac{\varepsilon \varepsilon_0 E}{\eta} \right) \psi(x) + v_{EO}. \quad (4.14)$$

At the shear plane located at $x=L_s$ the velocity $v(L_s)=0$ because the fluid adheres to the surface of membrane. Also, at $x=L_s$ the electrostatic potential $\psi=\zeta$, therefore it follows from Eq. (4.14), the electro-osmotic velocity v_{EO} is equal to

$$v_{EO} = -\frac{\varepsilon \varepsilon_0 E}{\eta} \zeta. \quad (4.15)$$

Since the electro-osmotic velocity, v_{EO} , is the velocity of fluid relative to the surface of the colloid (liposome or SR vesicle in our case), the velocity of the colloid particle relative to the stationary fluid is $v_t = -v_{EO}$. The membrane surface moves in the opposite direction with velocity v_t . It follows that the electrophoretic mobility, μ , which is velocity per unit electric field is given by (Cohen and Khorosheva 2001; Ohshima 2006)

$$\mu \equiv \frac{v_t}{E} = \frac{\varepsilon \varepsilon_0}{\eta} \zeta. \quad (4.16)$$

This equation is the well-known Smoluchowski equation relating the mobility to the zeta potential. It is valid when the particle is not too small, too charged or when the ionic

strength is not too low. We use this model with the assumption that the slipping plane is at the colloid surface.

Henry's Equation

Henry's Equation bridges the gap between the Smoluchowski limit and the Hückel limit. It is a mobility expression for a spherical particle with a concentric spherical diffuse layer. However it is applicable only when the zeta potential is low since the linearized version of the Poisson-Boltzmann equation was used in Henry's theory of mobility. Also, when the zeta potential is high, the relaxation effect becomes important, which is not included in Henry's theory.

The general form of Henry's equation is

$$\mu = \frac{\varepsilon \varepsilon_0}{\eta} \zeta f(\kappa a). \quad (4.17)$$

The function $f(\kappa a)$ varies from 2/3 to 1. The following useful approximation of the function $f(\kappa a)$ was developed by Ohshima and is valid for all values of κa with less than 1% error (Ohshima 2006).

$$f(\kappa a) = \frac{2}{3} \left\{ 1 + \frac{1}{2 \left(1 + \frac{\delta}{\kappa a} \right)^3} \right\} \quad (4.18)$$

with $\delta = 2.5 / \{1 + 2 \exp(-\kappa a)\}$.

At high ionic strength, the zeta potential is low due to adequate screening of the surface by counter-ions. The zeta potential is also low when surface charge density is low. Under these conditions, Henry's model is applicable.

The OHW Semi-empirical Model.

Ohshima has developed an analytical solution which is based on exact numerical solution of this problem by O'Brien and White (O'Brien and White 1978; Ohshima 2006). This is a semi-empirical solution which is applicable for $\kappa a \geq 10$. This model is called the Ohshima-Healy-White model (OHW). The expression for mobility is

$$\begin{aligned} \mu = & \text{sgn}(\zeta) \frac{\varepsilon \varepsilon_0}{\eta} \left\{ \left| \zeta \right| - \frac{2F}{1+F} \left(\frac{kT}{ze} \right) H \right\} \\ & + \text{sgn}(\zeta) \frac{2\varepsilon \varepsilon_0 kT}{3\eta e} \left[\frac{1}{\kappa a} \left\{ -18 \left(t + \frac{t^3}{9} \right) K + \frac{15F}{1+F} \left(t + \frac{7t^2}{20} + \frac{t^3}{9} \right) \right. \right. \\ & - 6(1+3\bar{m}) \left(1 - \exp(-\bar{\zeta}/2) \right) G + \frac{12F}{(1+F)^2} H + \frac{9\bar{\zeta}}{1+F} (\bar{m}G + mH) \\ & \left. \left. - \frac{36F}{1+F} \left(\bar{m}G^2 + \frac{m}{1+F} H^2 \right) \right\} \right] \end{aligned} \quad (4.19)$$

with

$$m = \frac{2\varepsilon \varepsilon_0 kT}{3\eta \kappa^2 e^2} \lambda_{drag}$$

$$\bar{m} = \frac{2\varepsilon \varepsilon_0 kT}{3\eta \kappa^2 e^2} \bar{\lambda}_{drag}$$

$$\bar{\zeta} = \frac{ze|\zeta|}{kT}$$

$$F = \frac{2}{\kappa a} (1 + 3m) (\exp(-\bar{\zeta}/2) - 1)$$

$$G = \ln \left(\frac{1 + \exp(-\bar{\zeta}/2)}{2} \right)$$

$$H = \ln \left(\frac{1 + \exp(\bar{\zeta}/2)}{2} \right)$$

$$K = 1 - \frac{25}{3(\kappa a + 10)} \exp \left[-\frac{\kappa a}{6(\kappa a - 6)} \bar{\zeta} \right]$$

$$t = \tanh \left(\frac{\bar{\zeta}}{4} \right).$$

Here λ_{drag} and $\bar{\lambda}_{drag}$ are drag coefficients of the counter-ions and co-ions respectively. In

this work we employ the OHW model because it provides ease of use and accuracy.

Section 7: Comparison of models

In this section we compare the electrophoretic mobility models described in Section 6: in terms of electrophoretic mobility versus ionic strength of solution for changing surface charge density and colloid size. In Figure 4.5, Figure 4.6, and Figure 4.7, we can see that in terms of absolute magnitude, the Smoluchowski model gives the largest values for electrophoretic mobility, especially towards lower ionic strength. The mobility predicted from Hückel's model is consistently smaller in magnitude than that predicted by Smoluchowski model. Predictions from Henry's model lie between these two. The OHW model yields predictions that agree with Smoluchowski's model for high ionic strength but deviate markedly at lower ionic strength.

Figure 4.5: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 10 \text{ mC/m}^2$ and colloid radius $a = 100 \text{ nm}$.

Figure 4.6: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 10 \text{ mC/m}^2$ and colloid radius $a = 500 \text{ nm}$.

The stark deviation of the OHW mobility for low ionic strength is due to increased relaxation and retardation effects. The ionic strength of the solution determines the thickness of the diffuse layer of counter ions. This thickness is equal to the Debye length (κ^{-1}) given by equations (3.12) and (3.13). At low ionic strength the Debye length (κ^{-1}) becomes large and there is less electric screening near the particle surface. Consequently the magnitude of the zeta potential becomes greater resulting in enhanced relaxation and retardation effect.

The effect of the colloid size is demonstrated in Figure 4.5 and Figure 4.6 for colloids of the same surface charge density but different sizes. Figure 4.5 is for a colloid of radius 100 nm while Figure 4.6 is for one of radius 500 nm. We can see that the

deviation of the OHW model from the Smoluchowski model is greater for colloids of small radii. In Figure 4.5,

Figure 4.5: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 10 \text{ mC}/\text{m}^2$ and colloid radius $a = 100 \text{ nm}$.

for a 100-nm radius colloid the two curves diverge at about 0.01 M while in figure 3.7 for a 500-nm radius colloid the divergence does not take place until the ionic strength is reduced to about 0.003 M.

Figure 4.7: Electrophoretic mobility as a function of ionic strength for several important models for surface charge density $\sigma = 40 \text{ mC}/\text{m}^2$ and colloid radius $a = 100 \text{ nm}$.

Figure 4.5 and Figure 4.7 are for colloids of identical size but different surface charge density. The surface charge density in Fig 3.6 was $10 \text{ mC}/\text{m}^2$ and in Figure 4.7 it was $40 \text{ mC}/\text{m}^2$. We see that the difference between the OHW and Smoluchowski mobilities are greater when the surface charge density were greater. Greater surface charge density leads to greater zeta potential and in turn to greater retardation and relaxation effects.

The OHW model is a significant advancement over other models because the prominent deviation from Smoluchowski mobility values at low ionic strength is an

important feature of experimental data for colloids with small radii. The deviation is explained by the inclusion of retardation and relaxation effects.

Chapter 5: Electrophoretic Mobility of Colloids with Surface Structure

The surface of SR vesicles is more complex than the surface of liposomes. SR membrane has protruding trans-membrane Ca^{2+} ATPase proteins and gangliosides that slow the flow of water around the vesicles. We treat our data with several models that have water and ion penetrable frictional layers attached to the surface that cause hydrodynamic drag. These layers are called retardation layers. These retardation layers are characterized in the same manner for all three of the models in this chapter. The underlying model is of small frictional resistance centers, designed to represent polymer segments, with uniform radii, a_p , and with uniform volume density, N_p . The retardation layer is assumed to be permeable to both water and ions and the values for κ , η , and ε are assumed to be the same within the retardation layer as they are in the bulk medium. The layer has an abrupt end at a distance D from the colloid surface. Each resistance center represents a small hard sphere that undergoes a Stokes drag force as the colloid moves through the fluid. This force is in the opposite direction of the colloid's terminal velocity v_t (Levine and others 1983; Ohshima 2007).

$$F_s = -6\pi\eta a_p v_t \quad (5.1)$$

A volume of fluid in within the retardation layer experiences a force of

$$\frac{F_r}{\text{volume}} = 6\pi\eta a_p N_p v_t. \quad (5.2)$$

The “softness parameter”, λ_{RL} , with dimensions of inverse length is defined to characterize the retarding action of the retardation layer:

$$\lambda_{RL} = \left(6\pi a_p N_p\right)^{\frac{1}{2}}. \quad (5.3)$$

The inverse of the softness parameter is often called the Debye-Bueche length and represents the depth of flow penetration into the retardation layer (Duval and Gaboriaud 2010; Duval and Ohshima 2006).

Much of the charge on SR is due to the amino acids of the Ca^{2+} -ATPase proteins. Because of this we will consider an electrophoretic mobility model in which there is charge distributed within the retardation layer (Levine’s model) and one in which a charged plane detached from the membrane surface is embedded in a retardation layer (the detached charged plane model), as well as a model in which the charge spread evenly on the surface of the bilayer and a retardation layer is attached to the surface (Ohshima’s model).

Section 1: Levine’s model for a charged retardation layer

In this section we describe an electrophoretic mobility model designed by Levine and others (Levine and others 1983), which they used in the analysis of electrophoretic mobility data of human erythrocytes. The model applies to colloidal particles that are large enough to be considered to have planar surfaces. They are coated with a charged retardation (frictional) layer of thickness β that consists of polyelectrolyte molecules anchored to the surface of the membrane. The model assumes that the retardation layer

has a uniform charge distribution with volume charge density ρ_{fix} , ignoring the discreteness of charge and giving total surface charge density $\sigma = \rho_{fix}\beta$.

The coordinates are set such that $z=0$ is the plane of the boundary between the retardation layer and the bulk fluid. The plane $z=-\beta$ is the shear plane and the beginning of the uniformly charged retardation layer. It is also assumed to be within molecular distances of the surface of the membrane. The E-field is applied in the x-direction, parallel to the membrane surface.

The linearized Poisson-Boltzmann equations for the polyelectrolyte layer and the diffuse layer are as follows

$$\frac{\partial^2 \psi(z)}{\partial z^2} = \kappa^2 \psi(z) - \frac{\rho_{fix}}{\epsilon \epsilon_0}, -\beta < z < 0 \quad (5.4)$$

and

$$\frac{\partial^2 \psi(z)}{\partial z^2} = \kappa^2 \psi(z), z > 0, \quad (5.5)$$

with the following boundary conditions

$$\psi(z), \frac{\partial \psi}{\partial z} \text{ continuous at } z = 0, \quad (5.6)$$

$$\frac{\partial \psi}{\partial z} = 0 \text{ at } z = -\beta, \quad (5.7)$$

and

$$\psi \rightarrow 0 \text{ at } z \rightarrow \infty. \quad (5.8)$$

The Navier-Stokes equations are for the steady state flow of fluid relative to the membrane surface

$$\eta \frac{\partial^2 u(z)}{\partial z^2} + \rho(z)E - \eta \lambda_{RL}^2 u(z) = 0, -\beta < z < 0, \quad (5.9)$$

$$\eta \frac{\partial^2 u(z)}{\partial z^2} + \rho(z)E = 0, z > 0, \quad (5.10)$$

with

$$\rho(z) = \rho_{free}(z) = -\epsilon \epsilon_0 \frac{\partial^2 \psi(z)}{\partial z^2} \quad (5.11)$$

and

$$\lambda_{RL}^2 = 6\pi aN. \quad (5.12)$$

When Equations (5.4) and (5.5) are solved for the potential as a function of distance from the membrane surface using the appropriate boundary conditions (Equations (5.6), (5.7), and (5.8)), and the corresponding charge distributions are found via (5.11), these charge distributions are substituted into Equations (5.9) and (5.10), which are subsequently integrated. The following boundary conditions are applied:

$$u = 0 \text{ at } z = -\beta \quad (5.13)$$

and

$$u, \frac{\partial u}{\partial z} \text{ continuous at } z = 0. \quad (5.14)$$

If we take u as equal to $-v_t$ (the terminal electrophoretic velocity of the vesicle) when

$z \rightarrow \infty$, for the electrophoretic mobility $\mu \equiv \frac{v_t}{E}$ we get

$$\begin{aligned} \mu = \frac{-\sigma}{2\eta\kappa(\kappa\beta)} & \left\{ -(1 - \exp(-2\kappa\beta)) \left(1 + \frac{\kappa}{\lambda_{RL}} \tanh \lambda_{RL}\beta \right) \right. \\ & - 2 \frac{\kappa^2}{\lambda_{RL}^2} \left(1 - \frac{1}{\cosh \lambda_{RL}\beta} \right) + \frac{\kappa^2}{(\kappa^2 - \lambda_{RL}^2)} \\ & \left. \left[-1 - \exp(-2\kappa\beta) + \frac{\kappa}{\lambda_{RL}} (1 - \exp(-2\kappa\beta)) \tanh \lambda_{RL}\beta + \frac{2 \exp(-\kappa\beta)}{\cosh \lambda_{RL}\beta} \right] \right\}. \end{aligned} \quad (5.15)$$

In a similar way, Levine et al derived the mobility of a colloid with all of the surface charge located at the membrane surface $z = -\beta$. The result for this case is

$$\mu = \frac{-\sigma}{\eta\kappa(\kappa^2 - \lambda_{RL}^2)} \left[\lambda_{RL}^2 \left(1 + \frac{\kappa}{\lambda_{RL}} \tanh \lambda_{RL}\beta \right) \exp(-\kappa\beta) - \frac{\kappa^2}{\cosh \lambda_{RL}\beta} \right]. \quad (5.16)$$

Finally for all of the surface charge located at the edge of the retardation layer, at $z=0$, we get

$$\begin{aligned} \mu = \frac{-\sigma}{2\eta\kappa} & \left[-(1 + \exp(-2\kappa\beta)) \left(1 + \frac{\kappa}{\lambda_{RL}} \tanh \lambda_{RL}\beta \right) \right. \\ & \left. + \frac{\kappa^2}{(\kappa^2 - \lambda_{RL}^2)} \left(-1 + \exp(-2\kappa\beta) - \frac{\kappa}{\lambda_{RL}} (1 - \exp(-2\kappa\beta)) \tanh \lambda_{RL}\beta - \frac{2 \exp(-\kappa\beta)}{\cosh \lambda_{RL}\beta} \right) \right]. \end{aligned} \quad (5.17)$$

Below is a graph of mobility versus ionic strength for Equations (5.15), (5.16), and (5.17). For this demonstration, $\beta=6.5$ nm, $\lambda_{RL}=6 \times 10^7$, and $\sigma=1$ mC/m². Curve A is from (5.15) for a homogenous volume distribution of charge within the frictional layer. Curve B is from (5.16) for a plane of charge at the membrane surface and Curve C is from (5.17) for a plane of charge at the interface between the frictional layer and the surrounding electrolyte solution. Mobilities are lowest when the charge is distributed at

the membrane surface and are larger for the case when the charge is uniformly distributed throughout the frictional layer, and largest for when the charge is distributed in a plane at the edge of the retardation layer. Note that when the charge is only at the membrane surface, at high ionic strength the mobility approaches zero. In contrast, when the charge is not isolated to the surface, but is either distributed evenly in the frictional layer or is at the edge of the frictional layer the mobility approaches a nonzero value.

Figure 5.1: Illustration of three mobility models. Curve A represents a membrane surface with a frictional layer with a homogeneous volume charge density. Curve B is for a frictional layer and a plane of charge at the membrane surface and Curve C is for a frictional layer and a plane of charge at the edge of the frictional layer.

Section 2: Ohshima's model for a charged spherical colloid with an uncharged retardation layer

There are few electrophoretic mobility models of soft particles that include relaxation effects. The following model, developed by Ohshima is one such model. It is for a charged spherical colloid with an ion-penetrable uncharged retardation layer.

Ohshima claims the following equation is correct to $\kappa a \geq 1$ (Ohshima 2005). We simply state the result. The mobility is

$$\mu = \frac{3a(L_3 + \lambda_{RL}aL_4)\epsilon\epsilon_0}{2\eta\lambda_{RL}^2b^3L_1} \left\{ \zeta - \frac{2F}{1+F} \left(\frac{kT}{ze} \right) \ln \left[\frac{1 + \exp\left(\frac{ze\zeta}{2kT}\right)}{2} \right] \right\} \quad (5.18)$$

with

$$L_1 = \left(1 + \frac{a^3}{2b^3} + \frac{3a}{2\lambda_{RL}^2b^3} - \frac{3a^2}{2\lambda_{RL}^2b^4} \right) \cosh[\lambda_{RL}(b-a)] - \left(1 - \frac{3a^2}{2b^2} + \frac{a^3}{2b^3} + \frac{3a}{2\lambda_{RL}^2b^3} \right) \frac{\sinh[\lambda_{RL}(b-a)]}{\lambda_{RL}b}, \quad (5.19)$$

$$L_3 = \cosh[\lambda_{RL}(b-a)] - \frac{\sinh[\lambda_{RL}(b-a)]}{\lambda_{RL}b} - \frac{a}{b}, \quad (5.20)$$

$$L_4 = \sinh[\lambda_{RL}(b-a)] - \frac{\cosh[\lambda_{RL}(b-a)]}{\lambda_{RL}b} + \frac{\lambda_{RL}a^2}{3b} + \frac{2\lambda_{RL}b^2}{3a} + \frac{1}{\lambda_{RL}b}, \quad (5.21)$$

$$F = \frac{2}{\kappa a} \left(1 + 3m \right) \left[\exp\left(\frac{ze\zeta}{2kT}\right) - 1 \right], \quad (5.22)$$

and

$$m = \frac{2\epsilon\epsilon_0 kT}{3\eta\zeta^2 e^2} \lambda_{drag} \quad (5.23)$$

where λ_{drag} is the drag coefficient of counterions, a is the inner radius to the colloid surface, and b is the outer radius to the edge of the retardation layer.

Section 3: The detached charged plane model

In this section we describe the derivation of the detached charged plane model, developed in our lab, for an uncharged colloidal core covered with a homogeneous retardation layer with softness parameter λ_{RL} and thickness D . Embedded in this retardation layer is a charged plane with surface charge density σ that is a distance of s from the particle core. We use the linearized Poisson-Boltzmann equation to describe the electrostatics near the surface of the colloid.

$$\frac{d^2\psi(x)}{dx^2} = \frac{e^2\psi(x)}{\epsilon\epsilon_0 kT} \sum_i n_i z_i^2 = \kappa^2 \psi(x) \quad (5.24)$$

with

$$\kappa = \sqrt{\frac{e^2 \sum_i n_i z_i^2}{\epsilon\epsilon_0 kT}} \quad (5.25)$$

The potential profile of the surface has two regions separated by the detached charged plane located at $x=s$. The general solutions to Equation (3.11) for these two regions are

$$\psi_1(x) = A \exp(\kappa x) + B \exp(-\kappa x) \quad (5.26)$$

and

$$\psi_2(x) = C \exp(-\kappa x) + D \exp(\kappa x). \quad (5.27)$$

The electrostatic boundary conditions include: (1) There is no charge at the surface of the colloid, $x=0$, therefore the electric field there is equal to zero $\left(\frac{d\psi_1}{dx}\bigg|_{x=0} = 0\right)$. (2) ψ_2 goes to zero as x goes to infinity ($D=0$). (3) The electric potential is continuous at $x=s$ ($\psi_1(s) = \psi_2(s)$). And (4) Gauss' Law applies at $x=s$,

$$\left(E_2(s) - E_1(s) = -\frac{d\psi_2}{dx}\bigg|_{x=s} - \left(-\frac{d\psi_1}{dx}\bigg|_{x=s}\right) = \frac{\sigma}{\epsilon\epsilon_0}\right). \text{ When we apply these boundary conditions}$$

to Equations (5.26) and (5.27) the electric potential profile as a function of distance from the colloid surface can be described as

$$\psi_1(x) = \frac{\sigma}{2\epsilon\epsilon_0\kappa} \left[\exp(-\kappa(s-x)) + \exp(-\kappa(s+x)) \right] \quad (5.28)$$

and

$$\psi_2(x) = \frac{\sigma}{2\epsilon\epsilon_0\kappa} \left[\exp(\kappa(s-x)) + \exp(-\kappa(s+x)) \right] \quad (5.29)$$

We use the Navier-Stokes equation to obtain the velocity distribution of the fluid surrounding the particle as a function of x . The Navier-Stokes equation is

$$\frac{d^2 u(x)}{dx^2} - \lambda_{RL}^2 u(x) + \frac{\rho_{mob}(x) E_{appl}}{\eta} = 0. \quad (5.30)$$

The hydrodynamics of the problem have three distinct regions with the following versions of the Navier-Stokes equations:

$$\frac{d^2 u_1(x)}{dx^2} - \lambda_{RL}^2 u_1(x) = \frac{\varepsilon \varepsilon_0 \kappa^2 E_{appl}}{\eta} \psi_1(x) \text{ for } 0 < x \leq s \quad (5.31)$$

$$\frac{d^2 u_2(x)}{dx^2} - \lambda_{RL}^2 u_2(x) = \frac{\varepsilon \varepsilon_0 \kappa^2 E_{appl}}{\eta} \psi_2(x) \text{ for } s \leq x \leq D \quad (5.32)$$

and

$$\frac{d^2 u_3(x)}{dx^2} = \frac{\varepsilon \varepsilon_0 \kappa^2 E_{appl}}{\eta} \psi_2(x) \text{ for } x > D. \quad (5.33)$$

The general solutions for velocity flow in these three regions are

$$u_1(x) = F \exp(\lambda_{RL} x) + G \exp(-\lambda_{RL} x) + \frac{\sigma \kappa}{2\eta(\kappa^2 - \lambda_{RL}^2)} E_{appl} \{ \exp[-\kappa(s-x)] + \exp[-\kappa(s+x)] \} \quad (5.34)$$

for $0 < x \leq s$

$$u_2(x) = K \exp(\lambda_{RL} x) + L \exp(-\lambda_{RL} x) + \frac{\sigma \kappa}{2\eta(\kappa^2 - \lambda_{RL}^2)} E_{appl} \{ \exp[-\kappa(s-x)] + \exp[-\kappa(s+x)] \} \quad (5.35)$$

for $s \leq x \leq D$, and

$$u_3(x) = u_{EO} + \frac{\sigma E_{appl}}{2\eta \kappa} \{ \exp[-\kappa(x-s)] + \exp[-\kappa(x+s)] \} \quad (5.36)$$

for $x > D$. The boundary conditions for the electro-osmotic flow are as follows (1) the no

slip boundary condition ($u_1(0) = 0$). (2) At $x = s$, fluid velocity is

continuous ($u_1(s) = u_2(s)$). (3) At $x = s$, the first derivative of fluid velocity is also

continuous $\left(\frac{du_1}{dx} \Big|_{x=s} = \frac{du_2}{dx} \Big|_{x=s} \right)$. (4) and (5) Fluid velocity and the first derivative of fluid

velocity are also both continuous at D, $(u_2(D) = u_3(D))$, and $\left(\frac{du_2}{dx}\bigg|_{x=D} = \frac{du_3}{dx}\bigg|_{x=D}\right)$. We

apply these boundary conditions to equations (5.34) to (5.36) and solve for u_{EO} .

$$u_{EO} = \frac{\sigma E_{appl}}{\eta \kappa} \left(\frac{\kappa \lambda_{RL}}{\kappa^2 - \lambda_{RL}^2} \right) \cosh(\kappa s) \exp(-\kappa D) \left[\tanh(\lambda_{RL} D) + \frac{\lambda_{RL}}{\kappa} \right] - \frac{\sigma E_{appl}}{\eta \kappa \cosh(\lambda_{RL} D)} \frac{\kappa^2}{\kappa^2 - \lambda_{RL}^2} \left[\exp(-\kappa s) + \frac{\kappa}{\lambda_{RL}} \sinh(\lambda_{RL} s) \right] \quad (5.37)$$

with

$$\mu = -\frac{u_{EO}}{E_{appl}}. \quad (5.38)$$

We obtain

$$\mu = \frac{\sigma}{\eta \kappa} \frac{\kappa^2}{\kappa^2 - \lambda_{RL}^2} \left\{ \frac{1}{\cosh(\lambda_{RL} D)} \left[\exp(-\kappa s) + \left(\frac{\kappa}{\lambda_{RL}} \right) \sinh(\lambda_{RL} s) \right] - \frac{\lambda_{RL}}{\kappa} \cosh(\kappa s) \exp(-\kappa D) \left[\tanh(\lambda_{RL} D) + \frac{\lambda_{RL}}{\kappa} \right] \right\}. \quad (5.39)$$

This is

$$\mu = \mu_0 \frac{\kappa^2}{\kappa^2 - \lambda_{RL}^2} \left\{ \frac{1}{\cosh(\lambda_{RL} D)} \left[\exp(-\kappa s) + \left(\frac{\kappa}{\lambda_{RL}} \right) \sinh(\lambda_{RL} s) \right] - \frac{\lambda_{RL}}{\kappa} \cosh(\kappa s) \exp(-\kappa D) \left[\tanh(\lambda_{RL} D) + \frac{\lambda_{RL}}{\kappa} \right] \right\} \quad (5.40)$$

Where μ_0 is the mobility of a hard sphere. Since this is the mobility of a hard sphere multiplied by a factor which accounts for the enhancement of the mobility due to the separation between the colloid surface and the detached charged plane, as well as the reduction of the mobility due to the retardation layer, we call this factor the mobility structure factor (MSF).

$$\mu_{DCPM} = \mu_{HS} \cdot MSF \quad (5.41)$$

and

$$MSF = \frac{\kappa^2}{\kappa^2 - \lambda_{RL}^2} \left\{ \frac{1}{\cosh(\lambda_{RL} D)} \left[\exp(-\kappa s) + \left(\frac{\kappa}{\lambda_{RL}} \right) \sinh(\lambda_{RL} s) \right] - \frac{\lambda_{RL}}{\kappa} \cosh(\kappa s) \exp(-\kappa D) \left[\tanh(\lambda_{RL} D) + \frac{\lambda_{RL}}{\kappa} \right] \right\}. \quad (5.42)$$

We sometimes use Equation (5.41) with Smoluchowski mobility place of μ_{HS} and other times we use the OHW mobility.

Chapter 6: Partition of Lipophilic Ions

Section 1: The partition coefficient

In this project we studied the equilibrium distribution of lipophilic ions between water and two types of membranes: (a) uncharged phosphatidylcholine (egg PC) membranes, and (b) sarcoplasmic reticulum (SR). The distribution ratio is known in the literature as the partition coefficient. It is a quantity of both theoretical and practical significance. The partition coefficient provides information on the energetics of transfer of a molecule between two media. Typically one medium is water and the other, some organic phase. The practical significance of partition coefficients is that they are used to predict concentrations of biologically active molecules in water, biological membranes, tissues, organisms, soils and other phases.

The partition coefficient of a specified compound between two different phases is defined as the equilibrium concentration of the compound in Phase 1, divided by the equilibrium concentration in Phase 2. Commonly, molar concentrations are used (Lausanne 2001). In heterogeneous systems where one of the phases is a membrane surface, it is difficult to quantify the volume associated with the membrane where the molecules of interest are located. In these cases mole fractions rather than molar concentrations, are more appropriate because they are precisely quantifiable.

The molar partition coefficient is defined by the ratio of molar concentrations of solute in each phase.

$$K_{1,2} = \frac{[solute]_1}{[solute]_2}. \quad (6.1)$$

Here we use the common bracket notation that means molar concentration of the solute in the phase. The mole fraction partition coefficient is

$$\begin{aligned} Kp_x &= \frac{X_1}{X_2} = \frac{n_1/N_1}{n_2/N_2} \\ &= \frac{\frac{V_1[solute]_1}{V_1[solute]_1 + V_1[solvent]_1}}{\frac{V_2[solute]_2}{V_2[solute]_2 + V_2[solvent]_2}} = \frac{\frac{[solute]_1}{[solute]_1 + [solvent]_1}}{\frac{[solute]_2}{[solute]_2 + [solvent]_2}}. \end{aligned} \quad (6.2)$$

n_1 is the number of solute molecules in Phase 1, N_1 is the number of all types of molecules in Phase 1, n_2 is the number of solute molecules in Phase 2, and N_2 is the number of all types of molecules in Phase 2 (Word 2002).

Often the concentration of solute is several orders of magnitude smaller than the concentration of solvent in which case

$$Kp_x \cong \frac{[solute]_1}{[solute]_2} \cdot \frac{[solvent]_2}{[solvent]_1} = \frac{[solute]_1}{[solute]_2} \cdot \frac{v_1}{v_2}. \quad (6.3)$$

v_1 and v_2 are equal to the molar volumes of the solvents. Equation (6.4) provides an approximate relationship between the molar and mole fraction partition coefficients.

$$Kp_x \cong K_{1,2} \cdot \frac{v_1}{v_2} \quad (6.4)$$

The partition coefficients found in this study have two purposes. The first is to compare partition coefficients for lipophilic ions between water and SR and between

water and egg PC liposomes. More importantly, through the use of the partition coefficient and the careful measured addition of lipophilic ions we may change the surface charge of liposomes and SR vesicles in a quantifiable way. This is a key method for manipulating the electrophoretic mobility of liposomes and SR vesicles.

Section 2:   Molecular models of lipophilic ions

In this section we present molecular models of the lipophilic ions used in this study. Two types of properties are shown. The first illustrates the distribution of charge. The colors red, yellow, white, green, and blue indicate relative charge. Red is most negative and blue most positive. White is neutral. The second model type illustrates distribution of lipophilicity with dark blue most hydrophobic and dark red most hydrophilic. The properties were calculated using Molecular Modeling Pro 6.3.3 (Chem SW). We include models for Tetraphenylphosphonium, Tetraphenylborate, Pentabromophenolate, Imipramine, and Clomipramine.

Tetraphenylphosphonium

Tetraphenylborate

Clomipramine

Pentabromophenolate

Imipramine

Figure 6.1 Space filling molecular models of lipophilic ions. The colors red, yellow, white, green, and blue indicate relative charge. Red is most negative and blue most positive. White is neutral. Conformation is least strain. Calculations by Molecular Modeling Pro 6.3.3 (Chem SW).

Tetraphenylphosphonium

Tetraphenylborate

Clomipramine

Pentabromophenolate

Imipramine

Figure 6.2 Space filling molecular models of lipophilic ions. The colors indicate predicted lipophilicity with dark blue most hydrophobic and dark red most hydrophilic. Conformation is least strain. Calculations by Molecular Modeling Pro 6.3.3 (Chem SW).

Section 3: The thermodynamics of partition

Thermodynamics is essential for understanding of the energetics of partition. The partition coefficient is the ratio of the probability of finding a solute molecule in one phase versus the probability of finding it in another. It is related to the difference in solvation Gibbs energies, which is the work of transfer of the solute molecule from phase 2 to phase 1 (Smejtek and Word 2004).

$$K_{1,2} = \exp(\Delta G_{1 \leftarrow 2} / RT) \quad (6.5)$$

We relate this to $Kp_{x,0}$ using Eq. (6.4).

$$Kp_{x,0} \cong \frac{v_1}{v_2} \exp(\Delta G_{1 \leftarrow 2} / RT) \quad (6.6)$$

The meaning of Gibbs free energy of transfer $\Delta G_{1 \leftarrow 2}$ is the work required to transfer a solute from Medium 2 to Medium 1. It is equally the difference in solvation Gibbs free energies. The solvation Gibbs free energy itself is a function of the change of enthalpy (H) and entropy (S) upon transfer from vacuum into a medium via the Gibbs-Helmholtz equation:

$$\Delta G_1 = \Delta H_1 - T\Delta S_1. \quad (6.7)$$

$T\Delta S$ is the change of organizational energy while ΔH is the energy of molecular interaction. The solvation of a molecule into a phase can be modeled as a two-step process. Step one is the formation of a cavity, which changes the organizational energy of the system and is the source of the entropy change. The second is the insertion of the molecule and activation of interactions between the molecule and the solvent. This step is

responsible for the enthalpy change (Escher and others 2000; Smejtek and Word 2004). If the transfer of a solute molecule from water to a membrane causes a net gain in entropy (positive ΔS), the organizational energy of a lipophilic ion within the lipid bilayer is less than the organizational energy of one within the aqueous surrounding. For intermolecular organization of water to be at the lowest energy state it must “push” the solute out of the aqueous phase. This is the origin of the classical hydrophobic effect (Smejtek and Word 2004). If the transfer of a solute molecule from water to a membrane causes a net decrease in enthalpy (negative ΔH) then it indicates lower binding energy states within the membrane. We know from earlier studies (Word 2002) on ionized 2,3,4,6-tetrachlorophenol (TeCP^-) that both entropy and enthalpy play important roles in partitioning of lipophilic ions. The above approach can be used in the thermodynamic analysis of measured partition coefficients $K_{p,x0}$ based on Eqs. (6.5) and (6.6).

Section 4: The modified Langmuir isotherm model of partitioning

It has been observed that the density of membrane-bound lipophilic ions increases with their aqueous concentration sub-linearly (Ruthven 1984). In a report from our laboratory (Word and Smejtek 2005) we found that this also takes place when partitioning molecules are not charged. This effect was also discussed by Escher (Escher and others 2000). It was shown that membrane-water partition coefficient was not constant but decreased with increasing concentration of partitioning solute. The membrane saturates due to a limited number of sorption sites. Thus the experimentally determined partition coefficient is an apparent one. For that reason we shall develop and

use a more thermodynamically relevant “infinite dilution partition coefficient” (Word and Smejtek 2005).

In the case of lipophilic ions, there is an additional aspect of the decrease of the partition coefficient. It is due to the electrostatic repulsion between the membrane, which has become charged by the sorption of lipophilic ions, and the free lipophilic ions on the aqueous side of the membrane-water interface. This is evident in a partition experiment as a kind of ‘electrostatic saturation’ of the membrane, to use a similar term as used above. Due to this effect, the concentration of lipophilic ions at the interface is decreased below their bulk concentration resulting in a reduced rate of sorption. The reduction of interfacial concentration, C_{if} , can be accounted for by a Boltzmann factor.

$$C_{if} = C_{eq} \exp(-ze\psi_0/kT) \quad (6.8)$$

C_{eq} is the bulk equilibrium concentration of lipophilic ions.

The Langmuir model of sorption treats the saturation associated with the reduction of the number of free sorption sites available to accept a solute molecule from the aqueous phase. As sites fill up, the probability of a solute molecule sorbing is reduced. The partition model used in this work is based on the Langmuir model of sorption, however, we modify it to include electrostatics saturation effects (Word and Smejtek 2005).

The Langmuir Isotherm model is a model for sorption of molecules onto a surface with independent, localized sorption sites. The model is based on the following assumptions (Ruthven 1984):

1. There are a fixed number of sites, N , that can be populated by a solute molecule or ion.
2. Each site holds exactly one molecule of sorbate.
3. All sites are energetically equivalent.
4. Molecules present in the membrane do not interact with each other.

The total number of sorption sites is equal to

$$N = \frac{A_M}{A_S} = \frac{LA_L}{A_S}. \quad (6.9)$$

A_M is the area of the surface of the membrane, A_S is the sorption site area, L is the number of lipids present, and A_L is the surface area that one lipid occupies on the membrane. We introduce a parameter called the membrane fractional coverage defined as

$$\Theta \equiv \frac{S}{N} = \frac{S}{L} \cdot \frac{A_S}{A_L}. \quad (6.10)$$

S is the number of molecules sorbed to the membrane.

The simultaneous sorption and desorption of sorbate is occurring by the following scheme:

A is the sorbate molecule, M is a membrane site, and AM is the sorbate molecule bound to a membrane site. The sorption and desorption rates are defined as follows:

$$\begin{aligned} r_a &= k_a X_{aq} (N - S), \\ r_d &= k_d S. \end{aligned} \quad (6.12)$$

k_a and k_d are the sorption and desorption rate constants respectively and X_{aq} is the mole fraction of solute in water. The equilibrium constant for the partition process, K_m is

$$K_m \equiv \frac{[AM]}{[A][M]} = \frac{k_a}{k_d}. \quad (6.13)$$

The equilibrium condition is that $r_a = r_d$, resulting in the following equation

$$S = K_m X_{aq} (N - S). \quad (6.14)$$

Eq. (6.10) and (6.14) yield

$$\Theta = \frac{K_m X_{aq}}{1 + K_m X_{aq}}. \quad (6.15)$$

We now need to relate the fractional coverage to the infinite dilution partition coefficient.

We do this by first relating K_m to $Kp_{x,0}$. If we solve Eq. (6.15) for K_m we obtain

$$K_m = \frac{\Theta}{X_{aq}(1 - \Theta)}. \quad (6.16)$$

As we approach infinite dilution, this becomes

$$K_m \cong \frac{\Theta}{X_{aq}}. \quad (6.17)$$

The mole fraction of sorbate in the membrane is

$$X_m = \frac{S}{L + S}. \quad (6.18)$$

Again as we approach infinite dilution X_m goes to

$$X_m \cong \frac{S}{L}, \quad (6.19)$$

therefore using Equation (6.10) we get

$$K_m \cong \frac{\Theta}{X_{aq}} \cong \frac{X_m}{X_{aq}} \cdot \frac{A_s}{A_L} = Kp_{x,0} \cdot \frac{A_s}{A_L}. \quad (6.20)$$

With this, we see membrane fractional coverage expressed in terms of infinite dilution partition coefficient is given by

$$\Theta = \frac{Kp_{x,0}X_{aq}}{\frac{A_L}{A_s} + Kp_{x,0}X_{aq}}. \quad (6.21)$$

Now we note that since $C_W \sim 56 \text{ M}$, C_W will almost always be much greater than C_{eq} . We may approximate the mole fraction of solute in water as

$$X_{aq} = \frac{C_{eq}}{C_W + C_{eq}} \cong \frac{C_{eq}}{C_W}. \quad (6.22)$$

Equation (6.21) becomes

$$\Theta = \frac{Kp_{x,0}C_{eq}/C_W}{\frac{A_L}{A_s} + Kp_{x,0}C_{eq}/C_W}. \quad (6.23)$$

This is the general form of the Langmuir isotherm sorption model in terms of the infinite dilution partition coefficient.

When our sorbate is ionic, we must account for further saturation effect caused by the reduction of lipophilic ions in the vicinity of the membrane surface due to electrostatic repulsion by previously sorbed lipophilic ions. To do this we use the interfacial concentration of lipophilic ions as expressed by a Boltzmann factor. Θ becomes

$$\Theta = \frac{Kp_{x,0}C_{eq} \exp(-ze\psi_0/kT)/C_W}{A_L/A_s + Kp_{x,0}C_{eq} \exp(-ze\psi_0/kT)/C_W}. \quad (6.24)$$

We use Equation (6.24) to perform a least squares fit of the parameters $Kp_{x,0}$ and A_s to our partition data. Then we use Equation (6.24) and our fit values of $Kp_{x,0}$ and A_s again to calculate the surface concentration of lipophilic ions absorbed to a liposome or SR vesicle in our electrophoretic mobility studies.

Chapter 7: Materials and Methods: Electrophoretic Mobility Measurements

Section 1: Preparation of liposome suspensions

Liposomes composed of phosphatidylcholine (PC) and phosphatidylglycerol (PG) were prepared from a solution of standard ionic strength. These ‘stock’ suspensions were diluted later to a variety of lower ionic strengths for electrophoretic mobility experiments. Solutions of nominal ionic strength were made from the following stock solutions: (1) 300mM NaCl 10mM HEPES pH 7.5 and (2) 100mM NaCl 10mM HEPES pH 7.5. Each stock was diluted with distilled water by a factor of two and four. Each stock and its two dilutions were further diluted by a factor of ten twice, again with pure water, yielding eighteen total samples. Preparation of liposome suspensions is described below. After preparation, liposome suspensions were stored in the refrigerator. 100 μ L of liposome suspension were added to each 9-mL salt solution prior to measuring the electrophoretic mobility.

For electrophoretic mobility of PC liposomes as a function of lipophilic ion concentration the buffer solution of nominal buffer concentration, ionic strength, and pH was prepared. Lipophilic ion salt was dissolved into the solution while it was being stirred and warmed (on low setting) on a magnetic stirrer and hot plate to maximize concentration. The solution was cooled in the refrigerator and then filtered with filter paper to remove undissolved lipophilic ion salt. Dilutions were made using more of the buffer solution. Concentrations were measured using spectrophotometry. This process is

described in Chapter 9. Finally, PC was added to each solution to make suspensions as before. The suspensions were given several hours to equilibrate.

Two kinds of liposomes were prepared: multilayered and single-layered. For both preparations, 6.7% of lipids were negatively charged (PG) the rest were assumed uncharged (PC). PG and PC solutions in chloroform were purchased from Avanti Polar Lipids and stored in a freezer at -20 °C. When taking lipid solutions from the bottle, solutions were allowed to warm up to a point that the solution was transparent. This is particularly important for PG, which will form clumps at low temperatures. Lipid concentrations of PG and PC were measured out of the bottle using the phosphate determination process described below.

Liposome Preparation

Liposome preparation takes two days. On the first day appropriate volumes of each phospholipid solution were added to a round bottom flask to total about 25 mg of lipids for each experiment. The flask was vortexed for 30 seconds to assure the solution was well mixed. The flask was put under vacuum in a rotary evaporator (Buchler Instruments, Fort Lee, NJ) for 10 minutes to evaporate the chloroform and assure that the lipids uniformly coat the bottom of the flask. To speed the process of evaporation, the lipid flask was immersed in hot tap water, while the collection flask was immersed in ice-water. After 10 minutes, the lipid flask was removed from the evaporator and attached to a vacuum pump and left overnight under vacuum to assure all the chloroform was removed.

For multi-layered liposomes, the flask was removed from the vacuum pump the next day, 3-mL of the buffer solution was added to the flask and it was gently swirled by hand until all lipids were suspended and the mixture was cloudy. At this point multi-layered liposomes had formed.

To make single-layered liposomes we use a method that involves the use of Triton X-100 detergent to dissolve lipids in buffer and Biobeads to extract detergent from buffer (Holloway 1973). The removal of detergent causes the spontaneous formation of lipid vesicles. For multilayered liposomes, detergent addition and removal is not necessary. To make single-layered liposomes, Biobeads were soaked in the buffer solution for the particular experiment, in this case 10mM HEPES at pH 7.5. In general, 3 mL of SM-2 Biobeads (Bio-Rad Laboratories, Hercules, CA) are required for every 40 mg of lipids. Generally three plastic syringes (Becton Dickinson & Co., Franklin Lakes, NJ) either 10-mL or 3-mL were used. If an experiment called for more than 9 mL of Biobeads, more syringes were used so as not to have more than 3 mL of Biobeads in one syringe, however, the number of syringes was always divisible by three. Their tips were stuffed with glass wool using a straightened-out paperclip. The glass wool was pulled through to minimize the volume it occupied inside the syringe, and to ensure the tip was tightly stuffed and able to hold the Biobeads. The excess wool was trimmed from each tip. The tips were then covered with a 1-cm² piece of Parafilm and the plastic caps were replaced. The Biobeads were evenly divided between the plastic syringes. Buffer was then poured into the syringes until the Biobeads were completely immersed. The syringes were then stored in the refrigerator overnight.

On the second day the caps and Parafilm were removed from the syringes. The syringes were placed into 30-mL centrifuge tubes and centrifuged at approximately 500×g for 5 minutes to remove the buffer. The flask was removed from the vacuum pump and 1.0 mL of buffer for every 40 mg of lipids was added. The flask was covered with a glass stopper and swirled until the lipids were completely suspended in the buffer. Next, 80 µL of Triton-X 100 detergent for every 40 mg of lipids was added to the round bottom flask. Again the flask was swirled until the detergent completely dissolved and the solution turned clear. The tip of one of the three syringes was covered with Parafilm and capped. The lipid/detergent mixture was added to the syringe using a pipette. Parafilm was then used to cover the top of the syringe. The syringe was tapped several times to make sure the solution was mixed evenly with the Biobeads. The syringe was rocked gently on a rocking platform shaker for one hour. The Biobeads from a second syringe were added to the first syringe. The syringe was again covered, tapped, and rocked for another hour. Then, the cap and Parafilm were removed from the tip of the syringe and it was placed in a 30-mL centrifuge tube and centrifuged again at approximately 500 g for 5 minutes to extract the suspension. The third syringe's tip was covered with Parafilm and capped. The lipid suspension was added to the third syringe and rocked gently on the rocker for 30 minutes. The cap and Parafilm were then removed and it was centrifuged again at approximately 500 g for 5 minutes. The lipid suspension was then diluted with buffer to the desired volume.

Section 2: Preparation of suspensions of SR vesicles

SR vesicles, kindly provided by Jon Abramson were isolated from rabbit fast twitch skeletal muscle by the method of MacLennan (MacLennan). Samples were stored in liquid N₂. The protein concentration was determined by absorption spectroscopy (Kalcker 1947). For mobility measurements, SR vesicles were diluted to about 0.08 g/L in buffer solutions of different ionic strength and pH.

Electrophoretic mobilities of vesicles suspended in aqueous solution were measured as a function of ionic strength at six different pH values (4.0, 4.7, 5.0, 6.0, 7.5, and 9.0). For each pH value, a stock solution was made with buffer concentration equal to 10 mM. Sodium acetate was used for pH 4, 4.7, and 5. MES was used for pH 6, HEPES sodium salt for pH 7.5, and CHES for pH 9. Buffer solutions were titrated to the appropriate pH using HCl or KOH. (NaOH was used in the case where TePB⁻ was to be added because we found that K⁺ precipitates TePB⁻.) Salt stock solutions were made using NaCl dissolved in the buffer solution. pH of the salt stocks was monitored and adjusted if needed using NaOH, KOH or HCl. Salt dilutions were then made by mixing buffer solution and salt stocks.

Measurements of electrophoretic mobility of SR vesicles were also performed as a function of lipophilic ion concentration. For these studies, the buffer solution of nominal buffer concentration, ionic strength, and pH was prepared and lipophilic ion salt was dissolved into the solution while it was being stirred and warmed (on low setting) on a magnetic stirrer and hot plate to maximize concentration. The solution was cooled in the refrigerator and then filtered with filter paper to remove undissolved lipophilic ion

salt. Dilutions were made using more of the buffer solution. Concentrations were measured using spectrophotometry. This process is described in Chapter 9.

Finally, an equal amount of SR was added to each solution to make the SR suspensions. We typically used 100 - 300 μL of concentrated SR per 9 mL of solution. The suspensions were given several hours to equilibrate.

Section 3: Particle electrophoresis

Electrophoretic mobility of SR vesicles was measured at 25 °C using a DELSA 440 electrophoretic mobility analyzer (Beckman-Coulter, Fullerton, CA, USA) using the manufacturers recommended settings. Nine to twelve measurements were taken for each sample divided equally between the upper and lower stationary layer of the mobility cell (see Section 6 and Figure 7.4).

Section 4: Electrophoresis of SR suspensions of varying ionic strength and consideration of buffer pKa and pH

As introduced in Chapter 3, the electrophoretic mobility is defined as

$$\mu \equiv \frac{v_t}{E}. \quad (7.1)$$

where v_t is the terminal velocity of the particle in the suspension and E is the applied electric field strength. Because native SR has a charged surface, counter ions will accumulate in the aqueous phase at the surface of the membrane. Due to electrostatic

attraction, these ions screen the surface of SR and change the mobility of SR in a way that depends on ionic strength. One obvious way of varying ionic strength is to change the salt concentration of suspending solution. We must also recognize that buffer ions contribute to ionic strength. The concentration of buffer ions that exist at equilibrium depends on the pH of the solution. pH is defined as

$$pH \equiv \log \left\{ \frac{1}{[H_3O^+]} \right\}. \quad (7.2)$$

The general rule is that a buffer is effective when the pH of the solution is within $pK_a \pm 1$ of the buffer. A buffer consists of approximately equal portions of a weak acid (HA) and its conjugate base (A^-), or a weak base (B) and its conjugate acid (BH^+). We used sodium acetate, MES, HEPES, and CHES, which are weak acids.

When dissolved in water, the following reaction describes the buffering process

The acid ionization constant, which describes the equilibrium condition, is defined according to

$$K_a \equiv \frac{[H_3O^+][A^-]}{[HA]}. \quad (7.4)$$

Using the definitions of pH Eq. (7.2) and pK_a ,

$$pK_a \equiv -\log K_a, \quad (7.5)$$

gives an expression relating pH of solution, pK_a of the buffer, and concentrations of ionized and unionized buffer components $[A^-]$ and $[HA]$.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}. \quad (7.6)$$

Concentration of ionized buffer is calculated using Eq. (7.6). Concentration of ionized buffer contributes to the total ionic strength of the SR suspension.

The electrostatic screening effect at the membrane-water interface is not only dependent on the bulk concentration of ions, but also the temperature and dielectric constant of the solution (See equation (3.14)).

The final parameter determining the mobility is the viscosity of the suspending liquid. Viscosity reduces the mobility. For large particles, $R \gg 1/\kappa$, with smooth surfaces, the mobility is given by the Smoluchowski equation,

$$\mu = \frac{\varepsilon \varepsilon_0}{\eta} \zeta, \quad (7.7)$$

where, ζ is the zeta potential, defined as the electrokinetic potential at the shear surface (where the aqueous solution starts to move relative to the membrane).

Because of the considerations described above, we specify pH , IS , η , T , and ε for each mobility measurement. However η , T , and ε are the same for all experiments: $T = 25 \pm 1^\circ\text{C}$, and η and ε are equal to that of water.

Section 5:   The optical bench of the electrophoretic mobility analyzer

The COULTER DELSA (Doppler Electrophoretic Light Scattering Analyzer) 440SX (Beckman Coulter Instruments, Inc., Fullerton, CA) uses light scattering and laser Doppler velocimetry to determine the electrophoretic mobility of particles in solution. Within the DELSA, a 5-mW Helium-Neon laser beam ($\lambda_0 = 632.8$ nm) is split into a main beam and four reference beams by a diffraction grating in such a way that the main beam has an intensity of approximately twenty times that of any one of the reference beams (Electronics 1988). All five beams then pass through the same set of collimating and focusing optics. The main beam also passes through a frequency shifter that shifts the frequency with respect to the reference beams by a controlled amount. The frequency shifter is composed of a pair of prisms that change the path length of the main beam. The frequency shifter is used to determine the direction of particle movement, and therefore the charge polarity of the particle. The main beam then passed through the sample volume in the channel of the cell. The reference beams are directed at the angles 7.5° , 15° , 22.5° , and 30° relative to the main beam to four different photodiode detectors.

Scattered light from the main beam leaves the sample volume in all directions. Some of this light reaches each of the photodiode detectors. In the absence of an electric field, the suspended particles move about randomly in Brownian motion causing the intensity of the scattered light to fluctuate around an average value (Wilson et al 2001). Taking the Fourier transform of the intensity, a power spectrum with respect to angular frequency (ω) is obtained with the following Lorentzian form

$$S(\omega) \propto \frac{\Gamma}{\Gamma^2 + \omega^2}. \quad (7.8)$$

Γ is the spectral broadening at half width, half height due to Brownian motion (Electronics 1988; Wilson and others 2001). The spectra of scattered light in the absence and presence of applied electric field are depicted in Figure 7.1.

Figure 7.1: Frequency spectrum of scattered light intensity with electric field off and on. (adapted from Wilson, 2001)

The spectral broadening is given by

$$\Gamma = DK^2. \quad (7.9)$$

D is the diffusion coefficient of the particle and K is the magnitude of the scattering vector for a particular angle. The scattering vector is defined as

$$\vec{K} \equiv \vec{k}_0 - \vec{k}_s. \quad (7.10)$$

$\vec{k}_0$ is the wave vector of incident light and $\vec{k}_s$ is the wave vector of scattered light at angle θ (Wilson and others 2001). The scattering vector has a magnitude of

$$|K| = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2}. \quad (7.11)$$

with n the index of refraction of the liquid suspension and λ_0 the wavelength of the laser in vacuum. The geometry of these vectors is shown in Figure 7.2 and Figure 7.3.

Figure 7.2: Schematic representation of light rays scattering off SR particles in DELSA cell.
(adapted from Wilson, 2001)

Figure 7.3: Vector diagram representing origin of scattering vector ($\vec{K}$)

When an electric field is applied to the suspension, in addition to the random Brownian motion, the charged particles will accelerate toward the electrode of opposite charge until they reach a terminal velocity (which occurs within microseconds (Electronics 1988)). At steady-state their drag forces equal the coulomb force. With this added motion, the power function with respect to angular frequency becomes

$$S(\omega) \propto \frac{\Gamma}{\Gamma^2 + (\omega + \vec{K} \cdot \vec{v}_t)^2}. \quad (7.12)$$

The added shift in angular frequency is a Doppler shift due to the particles' velocities and given by

$$\Delta\omega = K \cdot \frac{v}{v_t} = K v_t \cos \frac{\theta}{2}. \quad (7.13)$$

With $\omega = 2\pi f$, the terminal velocity is thus

$$v_t = \frac{2\pi\Delta f}{K \cos(\theta/2)}. \quad (7.14)$$

From this, mobility is calculated as

$$\mu = \frac{2\pi\Delta f}{EK \cos(\theta/2)}. \quad (7.15)$$

Section 6: Electro-osmotic flow and the electrophoretic cell

The sample-containing section of the electrophoretic cell, through which the laser beam passes, is made of a glass insert with a channel. The channel dimensions are 1 mm in height and 5 mm in length. When filled with a sample of ions in suspension, the suspension generally has a different electric potential than the walls of the glass surface due to the structure and type of the glass. This difference in potential causes electro-osmosis. Ions of opposite charge in relation to the glass collect near the surface of the glass leaving their counter ions in the center of the chamber. When the electric field is applied, these ions migrate toward the electrode of opposite charge in unison, dragging the bulk liquid with them. Because the chamber is closed, fluid flows in the opposite direction in the center. In between the bulk liquid flow in one direction and the bulk liquid flow in the opposite direction, on the top and the bottom of the chamber, two

stationary planes exist where there is no net flow of liquid (Figure 9.4). They are at 160 μm from the top and the bottom of the channel. In our experiments, the main beam was directed through the sample at the top or bottom stationary layer.

Figure 7.4: Representation of electro-osmotic flow in DELSA cell chamber. (adapted from Electronics, 1988)

Two concave metallic hemispheres on either side of the channel are also filled with the sample. The total sample volume of the cell is about 1 mL. The two hemispheres are coated with silver on the inside and act as electrodes. Their function is to apply homogeneous electric field to the sample in the channel. Three tubes protrude from the top of the cell: one for filling and two vent tubes. One vent tube is also used to extract the sample.

Section 7: Sample treatment, and injection

All measurements were completed within three days of sample preparation. This was important because over time the electrophoretic properties of the sample change. The degradation of samples was observed in our initial studies. Typically, the absolute magnitude of the mobility of SR preparations decreases. The effect is noticeable about 5 days after the SR suspensions are made. To minimize the degradation, samples were stored in the refrigerator.

A 10-mL plastic syringe was used to fill the electrophoretic cell. About 3 mL of suspension were drawn into the syringe and slowly injected into the fill tube of the electrophoretic cell starting with the suspension with the lowest concentration of lipophilic ions and proceeding to the one with the highest concentration. The cell was rinsed with each new suspension three times and fluid was removed from the cell through the vent tube on the opposite side of the fill tube using an aspirator. It is important to avoid air bubbles in the cell for they will cause inaccuracies. It helps to warm the suspensions by hand to about 25 °C (the temperature at which measurements are taken inside the DELSA) before filling the cell. The cell interior was washed three times with each suspension. Four mobility measurements were performed from which the average mobility and standard deviation were calculated.

Section 8: Electrophoretic mobility measurements

For each measurement the laser had to be positioned at the stationary layer of the electrophoretic mobility chamber. A position meter (Mitutoyo Corporation, Japan) was used to gauge the position of the stationary layer within the sample channel. First, we observed the signal level on the light detector as we adjusted the vertical position of the cell. At the point where the detector signal reached a minimum, we zeroed the position meter. This minimum in signal indicated that the beam was hitting the top (or bottom) edge of the channel. The cell was then raised (or lowered) 160 μm to position the laser beam where the flow velocity in the channel is zero.

The cell temperature was set to 25° C. The current was set to approximately the square root of the conductivity measured by the DELSA. Next the heterodyne ratios for each detector were checked to be within acceptable range. The heterodyne ratio is the ratio of main beam intensity to reference beam intensity. For minimizing error, this ratio should be between 2 and 30 (Electronics 1988). Heterodyne ratios were checked before each measurement. Data obtained from beam analyzer with erroneous values of heterodyne ratios were omitted.

Chapter 8: Materials and Methods: Photoelectron Microscopy

We determined the size distribution of multilayer and single-layered liposomes and sarcoplasmic reticulum vesicles by UV-PEEM using our home-built instrument at Portland State University. The aberration-corrected photoelectron microscope (CPEM) is capable of 5-nm lateral resolution under best conditions. Resolutions of ~10-15 nm are routinely achieved (Koenenkamp and others 2010). In PEEM, images are produced by electrons emitted via the photoelectric effect from a surface when it is illuminated by UV light, which in this case was from a 244-nm argon laser. PEEM offers particular advantages over SEM in the imaging of soft thin specimens such as liposomes and SR vesicles. First, there is high work function and surface layer contrast available in PEEM. Secondly, specimens are not exposed to a destructive high energy electron beam (Goldstein and others 2003).

Liposomes and SR vesicles were prepared as described in the previous chapter and diluted by a factor of 10,000 with distilled water. The liposomes consisted solely of Egg PC. The specimens were deposited on chromium (Cr oxide after exposure to air) - coated glass to generate high contrast images (the work function of Cr oxide is greater than 5.1 eV). The depositions were allowed to dry in air. To calculate the diameters of the vesicles, we assumed that they were originally spheres that collapsed when dried into flattened ellipses. Major and minor axes were measured using SXM software. The image scale was previously determined by TedPella calibration standard. The surface area of the original spheres was assumed to be twice the area of their flattened ellipses.

Chapter 9: Materials and Methods: Partition of Lipophilic Ion Measurements

Section 1: Spectrophotometry and extinction coefficients

We measured the concentration of lipophilic ions by UV spectrophotometry. If a solute absorbs light when in solution, its absorbance is linearly related to its concentration. The relationship is described by the Beer-Lambert Law (Skoog and others 2004).

$$A(\lambda) = \varepsilon(\lambda)Cl \quad (9.1)$$

$A(\lambda)$ is the absorbance for a particular wavelength defined as,

$$A(\lambda) \equiv -\log \left[\frac{I_1(\lambda)}{I_2(\lambda)} \right] \quad (9.2)$$

I_1 is the intensity of light leaving the background sample, and I_2 is the intensity leaving the compound solution. $\varepsilon(\lambda)$ is the molar decadic extinction coefficient for that wavelength, C is the concentration of the lipophilic ion and l is the path length of light through the sample. If we plot measured absorbances versus measured concentrations, we can calculate the extinction coefficient from the slope of the graph. The spectrophotometer measures the intensity of light transmitted through a solution as a function of wavelength. To isolate the absorbance of one compound, a background solution is needed that contains equal concentrations of all other solutes present in the

sample and zero concentration of the compound of interest. For this part of the experiment, we used distilled water as the background, and solutions of the lipophilic ions in distilled water as our samples.

Three stock solutions for the compounds tetraphenylborate (TePB^-), and tetraphenylphosphonium (TePP^+), were prepared in distilled water. The extinction coefficient for pentabromophenol (PBP^-) at 314.5 nm, was obtained in an earlier study (Word 2002). Compound masses were measured using an analytical balance to accuracy of $\pm 50 \mu\text{g}$ (Mettler Instrument Corporation, Hightstown, NJ) and volumes were measured using volumetric flasks. From each stock solution, two additional solutions were then made, by diluting volumes of stock solutions with distilled water. The samples were put into 1.00 ± 0.01 -cm quartz cells: one for the samples and one for distilled water to be used as a background. The intensities of light leaving the samples were measured from 200 nm to 450 nm against the background of water and absorbances were calculated automatically by the spectrophotometer. Linear fits of A and C were used to obtain ϵ .

Generally the points on the spectra selected for a particular compound were chosen to represent some easily recognizable feature in the absorbance spectrum like a peak, valley or saddle-point. However, the spectrum of tetraphenylborate has no distinct features, therefore five distributed points on the spectra were chosen.

Section 2: Partition coefficients and dialysis experimental design

The purpose of the partition experiments is to obtain the partition coefficient as a function of equilibrium concentration of several lipophilic ions between the aqueous

phase and lipid phase, as well as between the aqueous phase and the SR phase. As stated in the theory section (Chapter 6), the partition coefficient is defined as:

$$Kp_x \equiv \frac{X_1}{X_2}. \quad (9.3)$$

where, Kp_x is the mole fraction partition coefficient, X_1 is the mole fraction concentration of the lipophilic ion in the lipid phase at equilibrium, and X_2 is the mole fraction concentration of the lipophilic ion in the aqueous phase at equilibrium.

Due to saturation effects on the membrane that occur with some, but not all of the chosen compounds, the partition coefficient is a function of equilibrium concentration. It is not constant. By extrapolating to zero equilibrium concentration, we attempted to find the thermodynamically relevant parameter, the infinite dilution partition coefficient ($Kp_{x,0}$). This is done via a least squares fit of the sorption model to the data.

We find the partition coefficients by measuring the initial and equilibrium lipophilic ion concentrations of liposome or SR suspensions. The apparatus used is a dialysis cell with two chambers, separated by a semi-permeable cellulose membrane with permeability cut-off (6,000-8,000 MW) such that it allows the lipophilic ions to transverse through, but not the liposomes or SR vesicles. In one chamber we inject a suspension of liposomes or SR vesicles in solution; on the other, lipophilic ion solution. Thus our measured quantities are the initial concentration of the lipophilic ion (C_o), the final concentration of the lipophilic ion (C_{eq}), and concentration of lipids ($[lipid]$). Concentrations of lipophilic ions were measured via absorbance spectroscopy using a Beckman DU-7 Spectrophotometer (Beckman Coulter Instruments, Inc., Fullerton, CA).

Choosing Lipid Concentration

To minimize experimental error it is best to choose the liposome concentration of the lipid phase in such a way that the initial concentration of compound in the aqueous phase will be approximately twice the equilibrium concentration of compound in the aqueous phase. In order to do this, we must have an estimate of the infinite dilution partition coefficient, $Kp_{x,0}$, before we start. Some were found in the literature, while others were educated guesses made by examining the partition coefficients of similar compounds. Once an estimate was obtained, the following calculation was done to find the desired concentration of lipids:

$$[lipid] \cong \frac{(56M)(V_1 + V_2)}{Kp_{x,0}} \cdot \frac{C_0 - C_{eq}}{C_{eq}}. \quad (9.4)$$

C_0 is the initial aqueous concentration of compound in the aqueous phase and C_{eq} is the equilibrium concentration of compound in the aqueous phase. V_1 is the volume of the lipid-free chamber of the dialysis cell, while V_2 is the volume of the lipid-containing chamber. 56M is the molar concentration of water in water. In all the experiments in this study, V_1 has been equal to V_2 . With V_1 equal to V_2 and C_{eq} equal to $\frac{1}{2} C_0$, Equation (9.4) reduces to

$$[lipid] \cong \frac{(112M)}{Kp_{x,0}}. \quad (9.5)$$

Liposome Preparation

The method used for preparing single-layered lipid vesicles, (SLV's) is described in Chapter 7. It involves the use of Triton X-100 detergent to dissolve lipids in buffer and Biobeads to extract detergent from buffer. The removal of detergent causes the spontaneous formation of lipid vesicles. Three samples between 100 μ L and 4 mL of solution were stored in 30-mL test tubes for the determination of lipid concentration.

Preparation of SR Vesicles

White rabbit skeletal muscle sarcoplasmic reticulum vesicles were provided by Professor Jon Abramson's laboratory. The procedure used was published in 1970 by MacLennan (MacLennan 1970). The skeletal muscle from the hind legs of the rabbit was removed, mixed with buffer and ground into a slushy-like consistency. Through a series of centrifugations the soluble proteins, mitochondria, actin and myosin were isolated and removed, leaving only the SR and buffer. The SR was then stored in small containers in liquid nitrogen until needed, at which time they were warmed by hand and diluted to the desired volume.

When sarcoplasmic reticulum is isolated from rabbit skeletal muscle, prepared by freeze fracture, and viewed with an electron microscope, the surfaces show concave and convex areas (Scales 1976). The convex areas have many 90-Å wide particles ($5,730 \pm 520$ per μm^2) that are identified as the calcium pumps. Only 5% of the concave surfaces have pumps (Scales 1976). It is assumed that the concave and convex surfaces represent the inside and outside of the sarcoplasmic reticulum in the living rabbit respectively. It is

not known what mechanism maintains the asymmetry of the inner and outer surfaces of SR.

Partition Experiments

For most partitioning experiments, eight dialysis cells were used. It was sometimes necessary to use fewer when high concentrations of lipids or SR were needed and limited supplies were available.

The home-built cells consisted of two Teflon blocks held together with screws and wing-nuts. Each block had a 5-mL oval compartment. A small hole from the top of the block to the compartment was used to inject solutions. When the two blocks are put together, their compartments are separated by a semi-permeable cellulose membrane with permeability molecular weight cutoff of 6000-8000 (Cellu-Sep T2 from Membrane Filtration Products, Inc., Texas) to allow the compound molecules to pass through freely while the lipids were confined to one side.

The cellulose membranes were prepared as follows: The tubular cellulose membranes were cut into single-layered strips about 0.5 cm longer than the height of the cells. They were rinsed and soaked in distilled water in a 500-mL beaker. The water was stirred using a magnetic stirring rod for at least 3 hours to remove fine particles. Then each cellulose membrane was rinsed and shaken to remove excess water. The cellulose membrane was sandwiched between the two blocks. The screws were inserted into the screw holes and the wing-nuts were loosely attached to the back. At this time, the cellulose membrane was pulled lightly from the top until it was no longer visible at the

bottom. This step assures that the cellulose membrane is taught and wrinkle-free. The wing-nuts were then tightened.

Of the eight dialysis cells, six were sample cells, containing lipophilic ion solution on one side and lipid solution on the other. A seventh cell (the lipid reference cell) contained lipid solution on one side and buffer solution on the other. The final cell, the background cell, contained buffer solution on both sides. Prior to dialysis, solution concentrations were measured using the UV-VIS spectrophotometer with the buffer solution as a background. These are the reference concentrations. Using a 5-mL glass syringe, the chambers of the eight cells were filled with 5.00 mL of the appropriate solution. A different syringe was used to fill the lipid solution chambers. Once all the solutions were injected into the appropriate sides of the cells, screws were used to close the solution injection holes and the cells were rocked overnight on the rocking platform shaker. The next day, the solutions were extracted using pipettes. The buffer solution from the background (buffer/buffer) cell was used as the background for all the spectrophotometer measurements of lipophilic ion solutions including the buffer from the lipid reference (buffer/lipid) cell, which was used to measure the spectrum light scattered from particles (lipid micelles or cellulose particles) that may have passed through or broken away from the cellulose membrane. The spectrum from the lipid light scattering measurement was subtracted from the spectrum of the lipophilic ion.

Determination of Lipid Concentration in Suspension of SR Vesicles

In order to find the partition coefficient, it is also necessary to know the concentration of lipids in the liposome solution. We used the well-known procedure adapted from Chen (Chen and others 1956).

The reasoning is that lipids in the study contain one phosphate group each. Chen's method determines the amount of phosphate in solution, thus giving us the corresponding amount of lipids.

Recall that from the lipid suspension made for the partition experiment, three equal samples, ranging by experiment from 100 μL to 4 mL, were placed into separate 30-mL test tubes and stored in the refrigerator. The volume of lipid suspension needed for phosphate determination depends on the lipid concentration in the suspension. Approximately 1 μg of lipids is needed for each sample.

The samples were dried in a sand bath and heated to 100-200 $^{\circ}\text{C}$ under a moderate air stream. After the lipids were completely dried and the test tubes were allowed to cool, four drops of concentrated sulfuric acid were added to each tube. The dried lipids were thoroughly coated with the sulfuric acid by tapping the test tubes on a soft surface. The samples were then heated in the sand bath (100-200 $^{\circ}\text{C}$) for an hour, during which they turned into a thick dark tar. After allowing the samples to cool once more, four drops of 30% H_2O_2 were added to each tube. The samples were heated over a blue Bunsen flame until the H_2O_2 boiled away. If the remaining liquid was not clear an additional two drops of H_2O_2 was added to the liquid and the liquid heated it over the flame again until the H_2O_2 boiled away again. After allowing the test tubes to cool, we added about 8 mL of

distilled water to each and vortexed them for a few seconds. The solutions were then diluted to 25.00 mL using volumetric flasks, and stored in glass bottles.

The following solution was made: 6.00 mL of 2.5% ammonium molybdate, 6.00 mL of 10% ascorbic acid, 4.00 mL of 9 N H₂SO₄, and 14.00 mL of distilled water. This solution will be referred to as “Reagent C”. 2.00 mL of each of the liposome samples were measured into three 30-mL test tubes with 2.00 mL of water. For a fourth sample, which was to be the control, 4.00-mL of water were measured into a 30-mL test tube. Next 4.00 mL of Reagent C were measured into each test tube and the test tubes were vortexed for a few seconds. In an aluminum block heater, the four samples were heated at 37 °C for 90 minutes with glass marbles on top to minimize evaporation losses. After the samples were allowed to cool, their absorbances were measured with the control sample (no phosphate) as the background from 750-900 nm. The absorbances at 820.0 nm were used to calculate the concentration of PO₄.

From Beer’s law (with volume dilution corrections), the PO₄ concentration in each sample is:

$$[PO_4] = \frac{A}{\epsilon l} \cdot \frac{8.00ml}{2.00ml} \cdot \frac{25.00ml}{V_0} \quad (9.6)$$

A is the absorbance, l is the light path, ϵ at 820 nm is $2.71 \times 10^6 \pm 1000 \text{ M}^{-1} \cdot \text{m}^{-1}$ (Word 2002), and V₀ is the original volume of lipids.

Calculating Apparent Partition Coefficients

The molar fraction partition coefficients are calculated using the following equation:

$$Kp_x = \frac{\left(\frac{n_m}{N_m + n_m} \right)}{\left(\frac{n_w}{N_w + n_w} \right)}. \quad (9.7)$$

where N_m is the total number of lipid molecules, N_w is the total number of water molecules, n_m is the number of lipophilic ions absorbed to the lipid vesicles, and n_w is the total number of lipophilic ions in the aqueous phase. The following conversions put these variables in terms of experimentally obtained quantities:

$$\begin{aligned} N_w &= (56M)(V_1 + V_2), \\ N_m &= [lipid](V_2), \\ n_w &= C_{eq}(V_1 + V_2), \\ n_m &= (C_0 - C_{eq})(V_1 + V_2). \end{aligned} \quad (9.8)$$

V_1 is the volume of the sample chamber of the dialysis cell, V_2 is the volume of the lipid chamber of the dialysis cell, C_0 is the initial concentration of compound in the compound chamber divided by two so it is equal to the initial concentration of the compound in the entire dialysis cell, and C_{eq} is the final, or equilibrium concentration of lipophilic ion in the sample chamber.

In Equation (9.8), the term $C_0 - C_{eq}$ represents a ‘missing concentration,’ also called C_{bound} , which is the change in aqueous concentration due specifically to sorption of lipophilic ions to the membrane, not to be confused with the molar concentration of lipophilic ions within the membrane. We do not quantify the volume of the membrane and therefore do not know the molar concentration in the membrane. In terms of n_m ,

$$C_{bound} = \frac{n_m}{V} \quad (9.9)$$

where V is the total volume of the dialysis cell ($V_1 + V_2$).

Chapter 10: Results: Photoelectron Microscopy

The size distribution of multilayer and single layered liposomes was obtained by UV-PEEM as described in Chapter 8. We assumed that imaged liposomes or SR vesicles were flattened ellipses and that their original shapes were spheres. To handle the statistics of the results, we assumed a log normal distribution applied to the data (Limpert and others 2001).

The median diameter of the single layered liposomes was found to be 285 nm with a standard deviation of 203 nm (N=376). For multilayered liposomes the median diameter of 548 liposomes was found to be 218 nm with a standard deviation of 130nm. For SR we prepared three suspensions at pH values 4, 7, and 9 for PEEM. The median diameter of 545 vesicles at pH 4 was 119 nm with a standard deviation of 40 nm. For the vesicles at pH 7 the median diameter was 258 nm with a standard deviation of 106 nm and for pH 9 the median diameter was 234 nm with a standard deviation of 47 nm. The sizes of liposomes are summarized in Table 10.1 and the sizes of SR vesicles in Table 10.2. The distribution of SR and liposome sizes are displayed in histogram form (bin=50 nm) in Figure 10.1 and Figure 10.2 with least-squares best fit log normal distribution.

Table 10.1: Size distributions of single layered and multilayer liposomes.

Liposome type	Number of liposomes	Mean (nm)	Median (nm)	Mode (nm)	Standard deviation
Single layered	376	334	285	209	203
Multilayered	548	247	218	170	130

Figure 10.1: Log normal distributions of liposome sizes.

Table 10.2: Size distributions of SR vesicles at different pH values.

pH	Number of vesicles	Mean(nm)	Median (nm)	Mode (nm)	Standard deviation (nm)
9	141	238	234	225	47
7	93	276	258	225	106
4	545	125	119	108	40

Figure 10.2: Log normal distributions of SR vesicle size.

Chapter 11: Results: The Dependence of Electrophoretic Mobility of Liposomes on Ionic Strength

Electrophoresis is a common way to study the surface properties of colloidal particles in aqueous solution. In particular, from analysis of mobility we can obtain information on surface charge distribution and frictional properties. The present chapter covers the results of electrophoretic mobility data of charged liposomes. Results of SR vesicles follow in Chapter 12. Understanding the electrophoretic mobility data of charged liposomes serves as a starting place for understanding the electrophoretic mobility of the more complicated SR vesicles. Liposomes have a smooth surface and it has been shown that one can treat liposome mobility with simple models that lack frictional surface layers (Cohen and Khorosheva 2001).

We measure electrophoretic mobility as a function of ionic strength for two different kinds of preparations of (PC/PG) liposomes: single-layered liposomes and multi-layered liposomes. For each experiment, the ionic strength range is 50 μM to 0.3 M and the mole fraction of charged lipids is 0.067.

We examine the suitability of several mobility models introduced in Chapters 4 to the data and discuss the limitations of each. The first model is the classical Smoluchowski model, based on the assumptions that the colloid is sufficiently large compared to the width of the diffuse layer of screening ions in the electrolyte solution and that the surface lacks frictional surface layers (Ohshima 2006). We show that this model is applicable in the high ionic strength range of our experiments and we use this

model to find the least squares fit value for surface charge density. As the ionic strength decreases, the width of the diffuse layer of screening ions increases relative to the liposome size and the Smoluchowski model is no longer applicable. For this range we use an analytical formula obtained by Hiroyuki Ohshima using numerical results from O'Brian and White called the Ohshima-Healy-White (OHW) model (O'Brien and White 1978; Ohshima 2006). This model takes into account effects due to the curvature of the liposomes and the asymmetry of the diffuse layer that occurs with sufficient zeta potential (the potential at the shear surface between the liposome and the aqueous surrounding). Using the surface charge density obtained from the Smoluchowski model, we find the best fit for liposome size using the OHW model and show that our data fit the OHW model.

Section 1:   Mobility model fits

The Smoluchowski model was used to fit mobility data at high ionic strength. A least-squares-fit was done to solve for the surface charge density under the assumption that the surface potential is equal to the zeta potential. We used the seven highest ionic strength data points for single layer and multilayer liposomes. For single layer liposomes the surface charge density was found to be -9.56 mC/m^2 and for the multilayer liposomes it was found to be -12.0 mC/m^2 . However the calculated surface charge density according to the mole fraction of charged liposomes and the area per lipid on the surface of the liposome is -14.9 mC/m^2 . The Smoluchowski model fits are included in Figure 11.1 and Figure 11.3.

Using these values for the surface charge density, we found the best fit for particle radius using the OHW model which is applicable for $\kappa a \geq 10$. For single layer liposomes we found that the best fit for the radius was 141 nm. This radius is in agreement with the median value found for single layer liposomes via photoelectron microscopy of 140 nm. κa is plotted versus ionic strength for the experimental data points (Figure 11.2). With this radius κa is less than 10 for the 4 data points with lowest ionic strength. The model is not applicable to this range so these data points were not used in the fitting process. For multilayer liposomes the best fit for the radius was 194 nm. This value is larger than the value found via photoelectron microscopy of 110 nm. Again κa is plotted versus ionic strength for the experimental data points (Figure 11.4). This time only highest two ionic strength points were left out due to the limit of applicability of the OHW model.

Figure 11.1: Electrophoretic mobility of single-layered liposomes with predictions using the OHW model, Henry's model, and the Smoluchowski model.

Figure 11.2: Plots of κa versus ionic strength for single-layered liposomes.

Figure 11.3: Electrophoretic mobility of multi-layered liposomes with predictions using the OHW model, Henry's model, and the Smoluchowski model.

Figure 11.4: Plot of κa versus ionic strength for multilayer liposomes.

Chapter 12: Results: The Dependence of Electrophoretic Mobility of SR on Ionic Strength

The electrophoretic mobility of SR vesicles was measured as a function of ionic strength at pH values 4.0, 4.7, 5.0, 6.0, 7.5, and 9.0. Using the amino acid sequence of the APN domain of the Ca^{2+} ATPase and pKa values for these amino acids, we calculate the charge per APN domain (Chapter 2 Section 2), which we treat as the major driving force of electrophoretic mobility of SR. Since the surface of SR is complex, we invoke mobility models that use the concept of a retardation layer for the understanding of the SR surface frictional properties.

Section 1: Performance of mobility models

The data from all six pH values is graphed with the best-fit parameters for Levine's model of a large vesicle with a charged retardation layer (no relaxation effect) (Levine and others 1983) and also Ohshima's model of a vesicle with an uncharged retardation layer including relaxation effects (Ohshima 2005) in Figure 12.1 through Figure 12.6.

For both models the thickness of the retardation layer was set at 8 nm. The surface charge density was also set using data on the amino acid sequence of the APN domain of the Ca^{2+} -ATPase and the unit cell area of the Ca^{2+} ATPase. Frequency and pKa value of amino acids were used to calculate the charge of the APN domains. Levine's model was

solved for the best fit of the parameter λ_{RL} . Table 12.1 displays the parameters used in the fit and the found values of λ_{RL} for each fit.

For Ohshima's model for a charged membrane with a neutral retardation layer, we set the particle radius to 62.5 nm for pH 4.0, 4.7, and 5.0; we used 138 nm for pH 6.0 and 7.5, and 119 nm for pH 9.0 (these are the value for the radii of SR vesicles measured using the photoelectron microscopy at pH 4, 7, and 9 respectively). We found the best fit for the parameter λ_{RL} . The results are documented in Table 12.2. We can see that neither model is successful at consistently predicting the experimental results.

Figure 12.1: Electrophoretic mobility of SR vesicles at pH 4 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Figure 12.2: Electrophoretic mobility of SR vesicles at pH 4.7 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Figure 12.3: Electrophoretic mobility of SR vesicles at pH 5 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Figure 12.4: Electrophoretic mobility of SR vesicles at pH 6 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Figure 12.5: Electrophoretic mobility of SR vesicles at pH 7.5 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Figure 12.6: Electrophoretic mobility of SR vesicles at pH 9 as a function of ionic strength. Fits included are Levine's model of a colloid with an uncharged surface and a uniformly charged retardation layer, and Ohshima's model of a charged surface and an uncharged retardation layer with relaxation effects.

Table 12.1: Parameters used in Ohshima 2004 model least squares fit to SR mobility versus ionic strength. Radius, retardation layer thickness and surface charge density were independent variables, while λ_{RL} was obtained by the fit.

pH	Inner radius (nm)	Thickness of ret. layer (nm)	λ_{RL}	Surface charge density (mC/m ²)
4	62.5	8	2.854×10^8	142
4.7	62.5	8	2.991×10^8	37
5	62.5	8	2.909×10^8	12
6	138	8	3.087×10^8	-2
7.5	138	8	3.225×10^8	-47
9	119	8	3.168×10^8	-95

Table 12.2: Parameters used in Levine's model least squares fit to SR mobility versus ionic strength. Radius, retardation layer thickness and surface charge density were independent variables, while λ was obtained by the fit.

pH	Thickness of ret. Layer (nm)	λ_{RL}	Surface charge density (mC/m ²)
4	8	1.60×10^{10}	142
4.7	8	1.74×10^{10}	37
5	8	9.16×10^9	12
6	8	2.97×10^9	-2
7.5	8	2.13×10^{10}	-47
9	8	5.42×10^{10}	-95

Chapter 13: Results: Absorbance Spectra and Extinction Coefficients of Lipophilic Ions

To measure the concentrations of lipophilic ions in aqueous solution, we used UV-vis spectrophotometry and Beer's Law (Chapter 9). Here we present results of the determination of the extinction coefficient ϵ for the various ions, which is the absorption constant in Beer's Law. In the experiment we made solutions of lipophilic ions in water from dry compounds weighed by an analytical balance of accuracy $\pm 50 \mu\text{g}$ (Mettler Instrument Corporation, Hightown, NJ) and prepared in volumetric flasks. The absorbance spectra were recorded using a Beckman DU-7 spectrophotometer (Beckman Coulter Instruments, Inc. Fullerton, CA.). When applying Beer's Law it is customary to select a convenient wavelength where one evaluates an absorbance spectrum (either to evaluate the extinction coefficient or given ϵ , to determine concentration) Here we chose wavelengths that corresponded to clearly identifiable characteristic peaks or saddle points on the spectra when possible. For tetraphenylborate and tetraphenylphosphonium we used multiple wavelengths due to a lack of such characteristic features in the case of TePB^- and due to multiple features close to each other in the case of TePP^+ .

The following are typical absorbance spectra for the lipophilic ions in this study. Analysis for TePP^+ was done at 250.5 nm, 267.5 nm, 273 nm, and 275.5 nm. For TePB^- , analysis was done at 230 nm, 240 nm, 250 nm 254.5 nm, and 264.5 nm. For imipramine analysis was done at 250.5 nm and for Clomipramine, which has a similar spectrum, analysis was done at 251.5 nm. For PBP^- extinction coefficient data collection and

analysis was done in a previous study. The molar extinction coefficient of PBP^- was $5,760 \pm 50 \text{ M}^{-1}\text{cm}^{-1}$ at 314.5 nm (Word 2002).

The absorbance spectra are shown in Figure 13.1 through Figure 13.5. For PBP^- it was necessary to specify the pH and because it is a weak acid, therefore the pH of the solution determines the degree of ionization. The spectra of the ionized and neutral forms are different. The pH of the sample used in Fig 13.3 is $\text{pH}=9.4\pm 0.1$ and in Fig 13.4 is $\text{pH}=10$.

Figure 13.1: The absorbance spectrum of tetraphenylborate. The wavelengths for which extinction coefficients were measured are indicated.

Figure 13.2: The absorbance spectrum of tetraphenylphosphonium. The wavelengths for which extinction coefficients were measured are indicated.

Figure 13.3: The absorbance spectrum of pentabromophenol. The extinction coefficient at 314.5 nm was previously measured.

Figure 13.4: The absorbance spectrum of Imipramine. Extinction coefficient was obtained for 250.5 nm.

Figure 13.5: The absorbance spectrum of Clomipramine. Extinction coefficient was evaluated at 251.5 nm.

Absorbances of TePB^- , TePP^+ , imipramine and clomipramine were measured as a function of concentration to obtain their extinction coefficients. As expected, the dependence of absorbance on concentration is linear for a given light path. The slope changes with wavelength.

According to Beer's law the dependence of absorbance on the wavelength is given by

$$A(\lambda) = \varepsilon(\lambda)C\lambda \quad (13.1)$$

Thus the slopes of absorbance verses concentration are equal to $\varepsilon(\lambda)\ell$. The value for the light path for TePB^- , TePP^+ and imipramine was 1.00 ± 0.01 cm. In the case of clomipramine the light path was 0.1 ± 0.001 cm for the four highest concentration points and 1.00 ± 0.01 cm for all other points. The slopes of graphs of absorbance divided by wavelength versus concentration were calculated. The slopes are the extinction coefficients $\varepsilon(\lambda)$. The slopes were obtained by using a linear least squares fit regression to the data. The precision of the extinction coefficients is limited to three significant figures by the 1.00-cm light path. The results of absorbance divided by light path versus concentration for TePB^- , TePP^+ , imipramine and clomipramine are shown in Figure 13.7 though Figure 13.9 and the corresponding data is in Tables 13.1 through 13.6.

Figure 13.6: Concentration dependence of absorbance of TePB^- at five wavelengths. The straight lines illustrate the linear least-squares fits from which extinction coefficients were obtained.

Table 13.1: Concentration and Absorbance data used for calculating extinction coefficients for TePB^- .

Concentration (μM)	Absorbance $\lambda=230\text{nm}$	Absorbance $\lambda=240\text{nm}$	Absorbance $\lambda=250\text{nm}$	Absorbance $\lambda=254.5\text{nm}$	Absorbance $\lambda=264.5\text{nm}$
586 ± 0.306	--	--	--	--	2.0401 ± 0.0043
391 ± 0.907	--	--	--	2.1437 ± 0.0152	1.3838 ± 0.0044
237 ± 0.148	--	--	1.7339 ± 0.0169	1.2977 ± 0.0104	0.8365 ± 0.0030
195 ± 0.572	--	2.4894 ± 0.0042	1.4444 ± 0.0052	1.0797 ± 0.0036	0.6921 ± 0.0002
158 ± 0.371	2.5314 ± 0.0012	2.0564 ± 0.0076	1.1637 ± 0.0070	0.8670 ± 0.0044	0.5570 ± 0.0013
92.0 ± 0.099	1.5165 ± 0.0027	1.1701 ± 0.0054	0.6672 ± 0.0060	0.4997 ± 0.0038	0.3256 ± 0.0009
79.1 ± 0.233	1.3316 ± 0.0008	1.0309 ± 0.0031	0.5824 ± 0.0034	0.4335 ± 0.0023	0.2787 ± 0.0003
61.4 ± 0.154	1.0086 ± 0.0014	0.7775 ± 0.0035	0.4418 ± 0.0033	0.3306 ± 0.0023	0.2141 ± 0.0003
30.7 ± 0.094	0.5079 ± 0.0002	0.3926 ± 0.0009	0.2264 ± 0.0011	0.1702 ± 0.0008	0.1104 ± 0.0001

Table 13.2: Calculated extinction coefficients for tetraphenylborate.

Wavelength (nm)	Extinction coefficient ($\text{M}^{-1}\text{cm}^{-1}$)
230.0 nm	16100 ± 100
240.0 nm	12800 ± 100
250.0 nm	7360 ± 30
254.5 nm	5480 ± 20
264.5 nm	3530 ± 10

Figure 13.7: Concentration dependence of absorbance of TePP^+ at four wavelengths. The straight lines illustrate the linear least-squares fits from which extinction coefficients were obtained.

Table 13.3: Concentration and Absorbance data used for calculating extinction coefficients for TePP⁺.

Concentration (μM)	Absorbance $\lambda=250.5\text{nm}$	Absorbance $\lambda=267.5\text{nm}$	Absorbance $\lambda=273\text{nm}$	Absorbance $\lambda=275.5\text{nm}$
238 ± 0.617	0.4050 ± 0.0007	0.9771 ± 0.0015	0.7228 ± 0.0003	0.7904 ± 0.0003
206 ± 0.439	0.3315 ± 0.0012	0.8214 ± 0.0009	0.6049 ± 0.0007	0.6667 ± 0.0007
166 ± 0.358	0.2828 ± 0.0011	0.6822 ± 0.0016	0.5056 ± 0.0007	0.5554 ± 0.0010
149 ± 0.315	0.2493 ± 0.0007	0.6019 ± 0.0008	0.4461 ± 0.0001	0.4905 ± 0.0002
103 ± 0.220	0.1632 ± 0.0008	0.4111 ± 0.0010	0.3030 ± 0.0005	0.3373 ± 0.0003
83.2 ± 0.179	0.1443 ± 0.0005	0.3436 ± 0.0007	0.2557 ± 0.0004	0.2815 ± 0.0006
59.4 ± 0.300	0.1068 ± 0.0003	0.2466 ± 0.0003	0.1839 ± 0.0002	0.2016 ± 0.0001
41.1 ± 0.208	0.0617 ± 0.0007	0.1632 ± 0.0004	0.1204 ± 0.0005	0.1346 ± 0.0005
33.3 ± 0.168	0.0610 ± 0.0001	0.1408 ± 0.0002	0.1040 ± 0.0002	0.1120 ± 0.0002

Table 13.4: Calculated extinction coefficients for TePP⁺.

Wavelength (nm)	Extinction coefficient ($\text{M}^{-1}\text{cm}^{-1}$)
250.5 nm	1660 ± 10
267.5 nm	4010 ± 10
273.0 nm	2990 ± 10
275.5 nm	3290 ± 10

Figure 13.8: Concentration dependence of absorbance of clomipramine at 251.5 nm. The straight line illustrates the linear least-squares fit from which the extinction coefficient was obtained. We obtained a value of $7,690.00 \pm 10.00$ for the extinction coefficient. The highest concentration points have their absorbances multiplied by ten for graphing purposes.

Table 13.5: Absorbance versus concentration for Clomipramine

Concentration (μM)	Absorbance $\lambda=251.5\text{nm}$	Path length (cm)
504 ± 4.57	0.3860 ± 0.0001	0.100 ± 0.001
336 ± 3.11	0.2547 ± 0.0001	0.100 ± 0.001
168 ± 1.31	1.3103 ± 0.0012	1.00 ± 0.01
396 ± 3.63	0.3065 ± 0.0008	0.100 ± 0.001
264 ± 2.47	0.1967 ± 0.0003	0.100 ± 0.001
132 ± 0.718	1.0385 ± 0.0010	1.00 ± 0.01
197 ± 1.27	1.5504 ± 0.0014	1.00 ± 0.01
98.3 ± 0.725	0.778 ± 0.0006	1.00 ± 0.01
49.2 ± 0.267	0.378 ± 0.001	1.00 ± 0.01

Figure 13.9: Concentration dependence of absorbance of Imipramine at 250.5 nm. The straight line illustrates the linear least-squares fit from which extinction coefficients were obtained. We obtained a value of 8042.00 ± 5.00 for the extinction coefficient.

Table 13.6: Absorbance versus concentration for Imipramine. Pathlength 1.00 ± 0.01 cm.

Concentration (μM)	Absorbance $\lambda=250.5\text{nm}$	Absorbance $\lambda=230.5\text{nm}$
249.59 ± 0.575	2.0173 ± 0.0030	1.3330 ± 0.0014
124.79 ± 0.338	0.9983 ± 0.0002	0.6655 ± 0.0009
62.397 ± 0.114	0.4983 ± 0.0007	0.3314 ± 0.0010
199.84 ± 0.464	1.5901 ± 0.0013	1.0513 ± 0.0013
99.920 ± 0.272	0.7936 ± 0.0010	0.5290 ± 0.0007
49.960 ± 0.0344	0.3950 ± 0.0004	0.2641 ± 0.0003
150.64 ± 0.355	1.2046 ± 0.0010	0.8014 ± 0.0005
75.320 ± 0.208	0.6019 ± 0.0004	0.4015 ± 0.0003
37.660 ± 0.0715	0.2954 ± 0.0010	0.1974 ± 0.0004

We obtained values for extinction coefficients for four lipophilic ions (TePB^- , TePP^+ , imipramine and clomipramine) by performing linear least square fit to data of

absorbance divided by path length versus concentration. Values for TePB^- and TePP^+ for multiple wavelengths are summarized in Table 13.2 and Table 13.4 respectively. For imipramine at 250.5 nm the extinction coefficient was found to be 8042.00 ± 5.00 and for Clomipramine at 251.5 nm we obtained a value of 7690.00 ± 10.00 .

Chapter 14: Results: Partition of Lipophilic Ions

Results of the partition experiments are given as apparent partition coefficients versus equilibrium concentration of lipophilic ions. In the liposome and SR studies, saturation effects were present for Imipramine, Clomipramine, TePB^- , and PBP^- . However, no saturation effects were observed for TePP^+ sorbed to Egg PC (an SR study was not done with this compound due to the large amount of SR that would be needed) and average $Kp_x = Kp_{x,0}$ is assumed for this compound. We used the modified Langmuir isotherm model that accounts for space and electrostatic saturation effects to analyze those systems that exhibited saturation effects. Using this model, we found the infinite dilution partition coefficient $Kp_{x,0}$ and the binding site area A_s .

For TePB^- and TePP^+ , the partition data were analyzed for several wavelengths of the spectra. This was due to a lack of characteristic features in the absorbance spectrum in the case of TePB^- and due to multiple such features close to one other in the absorbance spectrum of TePP^+ (as discussed in the absorbance spectrum results). The weighted averages of concentrations and partition coefficients are shown for these compounds and the corresponding data for the individual wavelengths is given in Appendix A.

For each of experiments, we provided measured values for C_o , C_{eq} , and lipid concentrations as well as the calculated values of Kp_x . Standard deviations were calculated and the error bars on the plots represent these standard deviations.

We begin first with the results for PC-water partition of TePB^- analyzed at five wavelengths: 230, 240, 250, 254.5, and 264.5 nm. The weighted averages of the values

are shown in the graph and figure. The following fit parameters were obtained: $Kp_{x,0} = 3.22 \times 10^7 \pm 0.37 \times 10^7$ and $A_s = 1.22 \times 10^{-18} \pm 0.06 \times 10^{-18} \text{ m}^2$. $\chi^2/n-f$ value for the fit is 0.4. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to $\text{pH} = 7.0 \pm 0.1$. PC concentration of this experiment was found to be $3.78 \times 10^{-5} \pm 1.7 \times 10^{-6} \text{ M}$.

Figure 14.1: Partition of TePB⁻ between PC liposomes and water as a function of equilibrium concentration of TePB⁻. The solid line represents the best fit of the modified Langmuir isotherm model to the data.

Table 14.1: PC-water partition of TePB⁻ weighted average of all five wavelengths.

Cell	C ₀ (M)	C _{eq} (M)	K _{p_x}
1	5.36×10 ⁻⁵ ± 3.4×10 ⁻⁶	4.65×10 ⁻⁵ ± 3.6×10 ⁻⁶	3.27×10 ⁵ ± 6.7×10 ⁴
2	2.71×10 ⁻⁵ ± 1.9×10 ⁻⁶	1.98×10 ⁻⁵ ± 2.1×10 ⁻⁶	7.72×10 ⁵ ± 1.2×10 ⁵
3	1.67×10 ⁻⁵ ± 1.5×10 ⁻⁶	1.23×10 ⁻⁵ ± 2.2×10 ⁻⁶	8.39×10 ⁵ ± 1.8×10 ⁵
4	1.29×10 ⁻⁴ ± 2.5×10 ⁻⁶	1.21×10 ⁻⁴ ± 2.3×10 ⁻⁶	1.35×10 ⁵ ± 1.8×10 ⁴
5	1.01×10 ⁻⁴ ± 1.5×10 ⁻⁶	9.14×10 ⁻⁵ ± 2.3×10 ⁻⁶	1.98×10 ⁵ ± 2.1×10 ⁴

Below are the data for SR-water partition of TePB⁻ analyzed at five wavelengths: 230, 240, 250, 254.5, and 264.5 nm. The weighted averages of the values are shown in the graph and figure. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to pH = 7.0 ± 0.1.

The solid line represents the Langmuir model fit for a native surface charge density of zero. For this fit we obtained the values $Kp_{x,0} = 1.75 \times 10^7 \pm 1.30 \times 10^6$ and $Ps = 5.55 \times 10^{-20} \pm 1.07 \times 10^{-19} \text{ m}^2$ with $\chi^2=26.1$. Total phospholipid concentration of this experiment was found to be $1.39 \times 10^{-4} \pm 2.2 \times 10^{-6} \text{ M}$.

Figure 14.2: Partition of TePB⁻ between SR vesicles and water as a function of equilibrium concentration of TePB⁻. The solid line represents best fit of the modified Langmuir isotherm model to the data when the surface charge density is set to zero.

Table 14.2: SR-water partition of TePB⁻ weighted average of all five wavelengths.

Cell	C ₀ (M)	C _{eq} (M)	K _{px}
1	$1.89 \times 10^{-5} \pm 4.0 \times 10^{-7}$	$6.10 \times 10^{-6} \pm 6.4 \times 10^{-7}$	$1.42 \times 10^6 \pm 1.5 \times 10^5$
2	$2.79 \times 10^{-5} \pm 6.5 \times 10^{-7}$	$1.13 \times 10^{-5} \pm 6.2 \times 10^{-7}$	$9.46 \times 10^5 \pm 5.6 \times 10^4$
3	$5.88 \times 10^{-5} \pm 1.7 \times 10^{-6}$	$3.31 \times 10^{-5} \pm 1.5 \times 10^{-6}$	$4.53 \times 10^5 \pm 2.4 \times 10^4$
4	$8.18 \times 10^{-5} \pm 1.9 \times 10^{-6}$	$5.65 \times 10^{-5} \pm 2.9 \times 10^{-6}$	$2.62 \times 10^5 \pm 1.7 \times 10^4$
5	$1.14 \times 10^{-4} \pm 2.1 \times 10^{-6}$	$7.55 \times 10^{-5} \pm 1.9 \times 10^{-6}$	$2.61 \times 10^5 \pm 1.0 \times 10^4$
6	$1.50 \times 10^{-4} \pm 5.8 \times 10^{-7}$	$1.04 \times 10^{-4} \pm 1.5 \times 10^{-6}$	$2.14 \times 10^5 \pm 5.5 \times 10^3$

The second set of results include PC-water partition of TePP^+ . Similar to TePB^- , the absorbance spectrum was analyzed at four different wavelengths: 250.5, 267.5, 273, and 275.5 nm. The weighted averages of the values are shown in Figure 14.3 and Table 14.3. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM KCl titrated to $\text{pH} = 7.0 \pm 0.1$. Notice that no saturation effects are observed. The apparent Kp_x values do not increase with decreasing concentration as was seen with TePB^- . Because of this, the fit shown in this graph is simply a horizontal line. We obtained an average Kp_x value of 748 ± 29 . Because the partition coefficients for this compound are so small compared to our other compounds, the experiment required a relatively high concentration of lipids. For this reason the experiment was not performed on SR, which is a precious resource. For analysis of mobility studies in the presence of TePP^+ , we will assume that $Kp_{x,PC} \approx Kp_{x,SR}$ for TePP^+ . PC concentration of this experiment was found to be $2.95 \times 10^{-2} \pm 1.9 \times 10^{-3}$ M.

Figure 14.3: Partition of TePP⁺ between PC liposomes and water as a function of equilibrium concentration of TePP⁺. The solid line represents the average of the apparent Kp_x values.

Table 14.3: SR-water partition of TePP⁺ weighted average of all five wavelengths.

Cell	C ₀ (M)	C _{eq} (M)	Kp _x
1	$3.49 \times 10^{-4} \pm 6.0 \times 10^{-6}$	$2.91 \times 10^{-4} \pm 6.9 \times 10^{-6}$	752 ± 102
2	$4.51 \times 10^{-4} \pm 8.2 \times 10^{-6}$	$3.74 \times 10^{-4} \pm 7.6 \times 10^{-6}$	766 ± 91
3	$6.99 \times 10^{-4} \pm 1.3 \times 10^{-5}$	$5.92 \times 10^{-4} \pm 1.3 \times 10^{-5}$	675 ± 95
4	$8.93 \times 10^{-4} \pm 1.7 \times 10^{-5}$	$7.48 \times 10^{-4} \pm 1.4 \times 10^{-5}$	722 ± 84
5	$1.04 \times 10^{-3} \pm 1.8 \times 10^{-5}$	$8.66 \times 10^{-4} \pm 1.5 \times 10^{-5}$	764 ± 82
6	$1.33 \times 10^{-3} \pm 2.3 \times 10^{-5}$	$1.11 \times 10^{-3} \pm 1.9 \times 10^{-5}$	726 ± 79

Sorption of PBP^- to Egg PC and SR membrane was measured pH 9.4. However at each of this pH values, the great majority of pentabromophenol is ionized because the pKa of pentabromophenol is known to be 4.6 (Word 2002). The following results are for PBP partition between PC liposomes and water. The results were obtained in two separate experiments: a and b. The buffer solution for both experiments consisted of 10 mM CHES and 100 mM NaCl titrated to $\text{pH} = 9.4 \pm 0.1$. The solid line represents the Langmuir model fit for a native surface charge density of zero. For this fit we obtained the values $Kp_{x,0} = 3.75 \times 10^6 \pm 1.9 \times 10^5$ and $A_s = 9.8 \times 10^{-19} \pm 2.6 \times 10^{-19} \text{ m}^2$ with $\chi^2/\text{n-f} = 0.16$. PC concentration for experiment a was found to be $3.09 \times 10^{-4} \pm 4.1 \times 10^{-6} \text{ M}$; for experiment b it was $1.74 \times 10^{-4} \pm 5.1 \times 10^{-6} \text{ M}$.

Figure 14.4: Partition of PBP⁻ between PC liposomes and water as a function of equilibrium concentration of PBP⁻, titrated to pH 9.4. The solid line represents the best fit of the modified Langmuir isotherm model to the data.

Table 14.4: SR-water partition of PBP⁻ pH 9.4 analyzed at $\lambda=314.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	K_{p_x}
1a	$1.78 \times 10^{-5} \pm 1.6 \times 10^{-7}$	$4.01 \times 10^{-6} \pm 1.1 \times 10^{-7}$	$1.13 \times 10^6 \pm 4.0 \times 10^4$
1b	$1.49 \times 10^{-5} \pm 1.8 \times 10^{-7}$	$5.30 \times 10^{-6} \pm 2.5 \times 10^{-7}$	$1.04 \times 10^6 \pm 6.6 \times 10^4$
2a	$3.61 \times 10^{-5} \pm 3.2 \times 10^{-7}$	$1.33 \times 10^{-5} \pm 1.8 \times 10^{-7}$	$5.33 \times 10^5 \pm 1.4 \times 10^4$
2b	$4.30 \times 10^{-5} \pm 3.7 \times 10^{-7}$	$2.74 \times 10^{-5} \pm 3.7 \times 10^{-7}$	$3.08 \times 10^5 \pm 1.3 \times 10^4$
3a	$7.37 \times 10^{-5} \pm 7.5 \times 10^{-7}$	$3.96 \times 10^{-5} \pm 4.1 \times 10^{-7}$	$2.52 \times 10^5 \pm 7.5 \times 10^3$
3b	$1.13 \times 10^{-4} \pm 1.2 \times 10^{-6}$	$8.61 \times 10^{-5} \pm 8.8 \times 10^{-7}$	$1.50 \times 10^5 \pm 6.6 \times 10^3$

Below are data for the SR-water partition of PBP⁻ pH = 9.4. The buffer solution for the experiment consisted of 10 mM CHES and 100 mM NaCl titrated to pH = 9.4 ± 0.1. The solid line represents the Langmuir model fit for a native surface charge density of zero. For this fit we obtained the values $Kp_{x,0} = 1.02 \times 10^7 \pm 6.4 \times 10^5$ and $A_s = 1.68 \times 10^{-19} \pm 1.44 \times 10^{-19} \text{ m}^2$ with $\chi^2=27.5$. Total phospholipid concentration of this experiment was found to be $5.19 \times 10^{-5} \pm 1.6 \times 10^{-6} \text{ M}$.

Figure 14.5: Partition of PBP⁻ between SR vesicles and water as a function of equilibrium concentration of PBP⁻ titrated to pH 9.4. The solid line represents best fit of the modified Langmuir isotherm model to the data when the surface charge density is set to zero.

Table 14.5: SR-water partition of PBP⁻ pH 9.4 analyzed at $\lambda=314.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	Kp_x
1	$9.51 \times 10^{-6} \pm 1.4 \times 10^{-7}$	$5.03 \times 10^{-6} \pm 1.3 \times 10^{-7}$	$1.29 \times 10^6 \pm 7.4 \times 10^4$
2	$1.85 \times 10^{-5} \pm 2.0 \times 10^{-7}$	$1.09 \times 10^{-5} \pm 2.3 \times 10^{-7}$	$8.63 \times 10^5 \pm 4.7 \times 10^4$
3	$2.82 \times 10^{-5} \pm 3.6 \times 10^{-7}$	$1.77 \times 10^{-5} \pm 2.1 \times 10^{-7}$	$6.47 \times 10^5 \pm 2.6 \times 10^4$
4	$4.59 \times 10^{-5} \pm 8.0 \times 10^{-7}$	$3.04 \times 10^{-5} \pm 3.2 \times 10^{-7}$	$4.71 \times 10^5 \pm 1.7 \times 10^4$
5	$6.79 \times 10^{-5} \pm 6.8 \times 10^{-7}$	$4.67 \times 10^{-5} \pm 4.4 \times 10^{-7}$	$3.59 \times 10^5 \pm 1.2 \times 10^4$
6	$1.00 \times 10^{-4} \pm 8.8 \times 10^{-7}$	$7.15 \times 10^{-5} \pm 1.8 \times 10^{-6}$	$2.62 \times 10^5 \pm 1.5 \times 10^4$

Below are data for the PC-water partition of Imipramine⁺. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to pH = 7.0 ± 0.1. For the best fit we get $Kp_{x,0} = 41.6 \times 10^3 \pm 3.8 \times 10^3$ and $A_s = 1.1 \times 10^{-18} \pm 0.60 \times 10^{-18}$ m² with $\chi^2/\text{n-f} = 0.41$. PC concentration of this experiment was found to be $1.27 \times 10^{-3} \pm 2.0 \times 10^{-5}$ M. The neutral form of Imipramine was found to have a $Kp_{x,0}$ of $421 \times 10^3 \pm 61 \times 10^2$.

Figure 14.6: Partition of Imipramine between PC liposomes and water as a function of equilibrium concentration of Imipramine, titrated to pH 7.0. The solid line represents the best fit of the modified Langmuir isotherm model to the data.

Table 14.6: PC-water partition of Imipramine analyzed at $\lambda=250.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	K_{px}
1	$1.83 \times 10^{-5} \pm 6.9 \times 10^{-8}$	$9.92 \times 10^{-6} \pm 3.1 \times 10^{-7}$	$7.25 \times 10^4 \pm 3.7 \times 10^3$
2	$2.70 \times 10^{-5} \pm 7.7 \times 10^{-8}$	$1.69 \times 10^{-5} \pm 3.0 \times 10^{-7}$	$5.12 \times 10^4 \pm 1.9 \times 10^3$
3	$3.64 \times 10^{-5} \pm 1.1 \times 10^{-7}$	$2.52 \times 10^{-5} \pm 3.2 \times 10^{-7}$	$3.82 \times 10^4 \pm 1.3 \times 10^3$
4	$5.42 \times 10^{-5} \pm 1.7 \times 10^{-7}$	$3.57 \times 10^{-5} \pm 3.2 \times 10^{-7}$	$4.38 \times 10^4 \pm 1.1 \times 10^3$
5	$7.26 \times 10^{-5} \pm 1.5 \times 10^{-7}$	$4.84 \times 10^{-5} \pm 3.1 \times 10^{-7}$	$4.20 \times 10^4 \pm 8.6 \times 10^2$
6	$1.09 \times 10^{-4} \pm 1.4 \times 10^{-7}$	$7.46 \times 10^{-5} \pm 3.5 \times 10^{-7}$	$3.83 \times 10^4 \pm 6.9 \times 10^2$

Below are data for the SR-water partition of Imipramine hydrochloride. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to $\text{pH} = 7.0 \pm 0.1$. The solid line represents the Langmuir model fit for a native surface charge density of zero. For this fit we obtained the values $Kp_{x,0} = 8.40 \times 10^5 \pm 1.6 \times 10^4$ and $A_s = 4.70 \times 10^{-19} \pm 7.7 \times 10^{-20} \text{ m}^2$ with $\chi^2=614.4$. Total phospholipid concentration of this experiment was found to be $7.04 \times 10^{-4} \pm 1.3 \times 10^{-5} \text{ M}$.

Figure 14.7: Partition of Imipramine between SR vesicles and water as a function of equilibrium concentration of Imipramine, titrated to pH 7.0. The solid line represents best fit of the modified Langmuir isotherm model to the data when the surface charge density is set to zero.

Table 14.7: SR-water partition of Imipramine analyzed at $\lambda=250.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	K_{px}
1	$4.55 \times 10^{-5} \pm 5.7 \times 10^{-8}$	$2.88 \times 10^{-5} \pm 8.9 \times 10^{-8}$	$1.66 \times 10^5 \pm 3.1 \times 10^3$
2	$6.73 \times 10^{-5} \pm 8.0 \times 10^{-8}$	$4.61 \times 10^{-5} \pm 1.0 \times 10^{-7}$	$1.29 \times 10^5 \pm 2.3 \times 10^3$
3	$9.08 \times 10^{-5} \pm 1.3 \times 10^{-7}$	$6.35 \times 10^{-5} \pm 9.6 \times 10^{-8}$	$1.18 \times 10^5 \pm 2.0 \times 10^3$
4	$1.42 \times 10^{-4} \pm 1.5 \times 10^{-7}$	$1.00 \times 10^{-4} \pm 1.4 \times 10^{-7}$	$1.07 \times 10^5 \pm 1.7 \times 10^3$
5	$1.88 \times 10^{-4} \pm 6.9 \times 10^{-7}$	$1.38 \times 10^{-4} \pm 1.1 \times 10^{-7}$	$8.81 \times 10^4 \pm 1.3 \times 10^2$
6	$2.79 \times 10^{-4} \pm 2.1 \times 10^{-7}$	$2.17 \times 10^{-4} \pm 1.9 \times 10^{-7}$	$6.68 \times 10^4 \pm 9.6 \times 10^2$

Below are data for the PC-water partition of Clomipramine⁺. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to pH = 7.0 ± 0.1. For the best fit we get $Kp_{x,0} = 2.12 \times 10^5 \pm 0.09 \times 10^5$ and $A_s = 0.89 \times 10^{-18} \pm 0.05 \times 10^{-18} \text{ m}^2$ with $\chi^2/\text{n}-3 = 0.2$. PC concentration of this experiment was found to be $6.31 \times 10^{-4} \pm 2.6 \times 10^{-5} \text{ M}$. The neutral form of Clomipramine was found to have a $Kp_{x,0} = 1.71 \times 10^6 \pm 0.08 \times 10^6$.

Figure 14.8: Partition of Clomipramine between PC liposomes and water as a function of equilibrium concentration of Clomipramine, titrated to pH 7.0. The solid line represents the best fit of the modified Langmuir isotherm model to the data.

Table 14.8: PC-water partition of Clomipramine analyzed at $\lambda=251.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	K_{px}
1	$1.44 \times 10^{-4} \pm 3.2 \times 10^{-7}$	$8.65 \times 10^{-5} \pm 3.9 \times 10^{-7}$	$9.85 \times 10^4 \pm 3.5 \times 10^3$
2	$1.01 \times 10^{-4} \pm 1.4 \times 10^{-7}$	$5.86 \times 10^{-5} \pm 4.0 \times 10^{-7}$	$1.12 \times 10^5 \pm 4.3 \times 10^3$
3	$8.55 \times 10^{-5} \pm 1.7 \times 10^{-7}$	$4.89 \times 10^{-5} \pm 4.1 \times 10^{-7}$	$1.18 \times 10^5 \pm 4.7 \times 10^3$
4	$7.17 \times 10^{-5} \pm 1.1 \times 10^{-7}$	$3.82 \times 10^{-5} \pm 3.9 \times 10^{-7}$	$1.39 \times 10^5 \pm 5.6 \times 10^3$
5	$4.98 \times 10^{-5} \pm 6.6 \times 10^{-8}$	$2.55 \times 10^{-5} \pm 3.8 \times 10^{-7}$	$1.56 \times 10^5 \pm 6.8 \times 10^3$
6	$2.60 \times 10^{-5} \pm 4.7 \times 10^{-8}$	$1.23 \times 10^{-5} \pm 3.9 \times 10^{-7}$	$1.88 \times 10^5 \pm 1.1 \times 10^4$

Below are data for the SR-water partition of Clomipramine hydrochloride. The buffer solution for the experiment consisted of 10 mM HEPES and 100 mM NaCl titrated to $\text{pH} = 7.0 \pm 0.1$. The solid line represents the Langmuir model fit for a native surface charge density of zero. For this fit we obtained the values $Kp_{x,0} = 1.91 \times 10^6 \pm 1.33 \times 10^5$; $A_s = 1.95 \times 10^{-19} \pm 2.31 \times 10^{-19} \text{ m}^2$ and $\chi^2 = 8.9$. We found that for this experiment, $[\text{PO}_4] = 1.44 \times 10^{-4} \pm 9.7 \times 10^{-6} \text{ M}$.

Figure 14.9: Partition of Clomipramine between SR vesicles and water as a function of equilibrium concentration of Clomipramine, titrated to pH 7.0. The solid line represents best fit of the modified Langmuir isotherm model to the data when the surface charge density is set to zero.

Table 14.9: SR-water partition of Clomipramine analyzed at $\lambda=251.5$ nm.

Cell	C_0 (M)	C_{eq} (M)	K_{px}
1	$1.32 \times 10^{-4} \pm 5.5 \times 10^{-7}$	$1.08 \times 10^{-4} \pm 1.5 \times 10^{-7}$	$1.27 \times 10^5 \pm 6.4 \times 10^3$
2	$9.87 \times 10^{-5} \pm 1.4 \times 10^{-7}$	$8.06 \times 10^{-5} \pm 1.4 \times 10^{-7}$	$1.38 \times 10^5 \pm 7.4 \times 10^3$
3	$8.23 \times 10^{-5} \pm 1.3 \times 10^{-7}$	$6.53 \times 10^{-5} \pm 1.0 \times 10^{-7}$	$1.62 \times 10^5 \pm 8.8 \times 10^3$
4	$6.59 \times 10^{-5} \pm 8.7 \times 10^{-8}$	$5.10 \times 10^{-5} \pm 7.7 \times 10^{-8}$	$1.86 \times 10^5 \pm 1.0 \times 10^4$
5	$4.83 \times 10^{-5} \pm 6.4 \times 10^{-8}$	$3.57 \times 10^{-5} \pm 1.0 \times 10^{-7}$	$2.32 \times 10^5 \pm 1.3 \times 10^4$
6	$2.58 \times 10^{-5} \pm 6.8 \times 10^{-8}$	$1.72 \times 10^{-5} \pm 4.5 \times 10^{-8}$	$3.47 \times 10^5 \pm 2.1 \times 10^4$

In this section we have presented results for the PC-water partition of TePB⁻, TePP⁺, PBP⁻, Clomipramine and Imipramine and SR-water partition of TePB⁻, PBP⁻, Clomipramine and Imipramine. Saturation effects were seen in all experiments except partition of TePP⁺ between PC and water. The modified Langmuir isotherm model was fit to the data. Table 14.11 summarizes the results shown in this chapter.

Table 14.10: Summary of partition experiment results.

Ion	pH	Membrane	n	$K_{p,x,0}$	A_s (m ²)	$\frac{\chi^2}{n-f}$
TePB ⁻	7.0	PC	5	$3.22 \times 10^7 \pm 3.7 \times 10^6$	$1.22 \times 10^{-18} \pm 6 \times 10^{-20}$	0.4
TePB ⁻	7.0	SR	6	$1.75 \times 10^7 \pm 1.30 \times 10^6$	$5.55 \times 10^{-20} \pm 1.1 \times 10^{-19}$	8.7
TePP ⁺	7.0	PC	6	748 ± 29	(no saturation)	-
PBP ⁻	9.4	PC	6	$3.75 \times 10^6 \pm 1.9 \times 10^5$	$9.8 \times 10^{-19} \pm 2.6 \times 10^{-19}$	0.16
PBP ⁻	9.4	SR	6	$1.02 \times 10^7 \pm 6.4 \times 10^5$	$1.68 \times 10^{-19} \pm 1.44 \times 10^{-19}$	9.17
Clom ⁺	7.0	PC	6	$2.12 \times 10^5 \pm 9 \times 10^3$	$8.9 \times 10^{-19} \pm 5 \times 10^{-20}$	0.2
Clom	7.0	PC	6	$1.71 \times 10^6 \pm 8 \times 10^4$		
Clom ⁺	7.0	SR	6	$1.91 \times 10^6 \pm 1.33 \times 10^5$	$1.95 \times 10^{-19} \pm 2.31 \times 10^{-19}$	2.97
Imip ⁺	7.0	PC	4	$4.16 \times 10^4 \pm 3.8 \times 10^3$	$1.1 \times 10^{-18} \pm 6 \times 10^{-19}$	0.41
Imip	7.0	PC	4	$4.21 \times 10^5 \pm 6.1 \times 10^3$		
Imip ⁺	7.0	SR	6	$8.40 \times 10^5 \pm 1.6 \times 10^4$	$4.70 \times 10^{-19} \pm 7.7 \times 10^{-20}$	204

Chapter 15: Results: Electrophoretic Mobility of SR and Liposomes in the Presence of Lipophilic Ions

Results of the effect of the presence of lipophilic ions on the electrophoretic mobility of liposomes and SR vesicles are given as mobility versus initial aqueous concentration of lipophilic ions (as opposed to aqueous concentration, which is dependent on the accuracy of the partition coefficient). We find that for SR in the presence of the lipophilic anions PBP^- and TePB^- at pH 7.0-7.5 the change in mobility in the presence of lipophilic anion is very small or nonexistent in our concentration range, while the same lipophilic anions change the mobility of PC liposomes substantially. By contrast, the lipophilic cations TePP^+ , clomipramine⁺ and imipramine⁺ change the electrophoretic mobility of SR vesicles and PC liposomes at pH 7.0 by comparable amounts over the concentration ranges of the experiments, however the onset of mobility change is at higher lipophilic cation concentration for SR vesicles than for PC liposomes. These observations will be discussed in detail in Chapter 18.

Below are results for SR vesicles and PC liposomes in the presence of PBP^- at pH 7.0. Figure 15.1 shows the results of four experiments. In this chapter we have stuck to the convention that data points of SR mobility are black and those of PC are white. The experimental values of mobility, standard deviation of mobility and initial aqueous concentration of lipophilic ion are given in Table 15.1-Table 15.2. The suspending solutions used in the experiment were 10mM HEPES, 5 mM NaCl, pH 7.0 for PC and 3 mM HEPES, 5 mM KCl, pH 7.5 for SR.

Figure 15.1: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of PBP⁻.

Table 15.1: Initial PBP⁻ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [PBP⁻]. Lipid concentration was 60 μ M. Liposomes were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

Conc PBP (M)	Ave mobility	st.dev mob
0	-0.853	0.213
1.30×10^{-9}	-0.729	0.411
2.59×10^{-9}	-0.592	0.101
5.18×10^{-9}	-0.649	0.241
1.30×10^{-8}	-0.738	0.328
2.59×10^{-8}	-0.617	0.129
5.18×10^{-8}	-0.828	0.203
1.30×10^{-7}	-0.833	0.131
2.59×10^{-7}	-1.045	0.122
5.18×10^{-7}	-1.044	0.074
1.30×10^{-6}	-1.426	0.219
2.59×10^{-6}	-1.416	0.195
5.18×10^{-6}	-1.924	0.187
1.30×10^{-5}	-3.014	0.138
2.59×10^{-5}	-3.526	0.054
5.18×10^{-5}	-3.690	0.068

Table 15.2: Initial PBP⁻ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [PBP⁻]. Lipid concentration was 92 μ M. SR vesicles were suspended in 3 mM HEPES, 5 mM KCl, pH 7.5.

Conc PBP (M)	Ave mobility	st.dev mob
6.84×10^{-8}	-1.327	0.091
1.38×10^{-7}	-1.378	0.057
2.76×10^{-7}	-1.339	0.105
6.84×10^{-7}	-1.413	0.070
1.39×10^{-6}	-1.364	0.057
2.76×10^{-6}	-1.347	0.079
6.84×10^{-6}	-1.404	0.069
1.39×10^{-5}	-1.339	0.099
2.76×10^{-5}	-1.557	0.044
6.84×10^{-5}	-1.621	0.036
1.39×10^{-4}	-1.728	0.026
2.07×10^{-4}	-2.060	0.091
2.76×10^{-4}	-2.148	0.115

Below are results for SR vesicles and PC liposomes in the presence of TePB^- at pH 7.0. Figure 15.2 shows results of three experiments. One of our experiments with TePB^- are with PC liposomes and two are with SR vesicles. PC liposomes (white circles) and SR vesicles (black circles) in Figure 15.2 were suspended in the same solution: 100 mM NaCl, 10mM HEPES, pH 7.0. By comparing these we can see that even at high salt concentration the effect of TePB^- on the mobility of PC liposomes is much greater than that of SR vesicles. The black squares represent mobility of SR vesicles in the suspending solution 5 mM NaCl, 10mM HEPES, pH 7.5. The salt concentration in this experiment was much lower which means the screening effect of counter ions is reduced, making the absolute value of mobility greater. The small difference in pH may also contribute to this difference. The experimental values of mobility, standard deviation of mobility and initial aqueous concentration of lipophilic ion for these experiments are given in Table 15.3-Table 15.5.

Figure 15.2: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of TePB^- .

Table 15.3: Initial TePB^- concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on $[\text{TePB}^-]$. Lipid concentration was $35\mu\text{M}$. Liposomes were suspended in 10 mM HEPES, 100 mM NaCl, pH 7.0.

Conc PBP (M)	Ave mobility	st.dev mob
2.36×10^{-7}	-0.334	0.141
4.36×10^{-7}	-0.253	0.201
7.84×10^{-7}	-0.201	0.029
2.36×10^{-6}	-0.935	0.188
4.36×10^{-6}	-1.691	0.128
7.84×10^{-6}	-2.037	0.196
1.98×10^{-5}	-2.765	0.222
3.82×10^{-5}	-3.051	0.210
7.84×10^{-5}	-3.191	0.236
1.91×10^{-4}	-3.345	0.329
3.75×10^{-4}	-3.316	0.187
7.78×10^{-4}	-3.724	0.165
1.93×10^{-3}	-3.969	0.155
3.83×10^{-3}	-3.830	0.116
7.66×10^{-3}	-3.746	0.101

Table 15.4: Initial TePB concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePB]. Lipid concentration was 18 μ M. SR vesicles were suspended in 10 mM HEPES, 100 mM NaCl, pH 7.0.

[TePB]	Ave mobility	st.dev mob
1.49×10^{-7}	-0.870	0.132
2.84×10^{-7}	-0.818	0.089
5.79×10^{-7}	-0.783	0.139
1.49×10^{-6}	-0.916	0.094
2.84×10^{-6}	-0.924	0.128
5.79×10^{-6}	-0.936	0.108
1.49×10^{-5}	-0.974	0.073
2.84×10^{-5}	-0.897	0.126
5.17×10^{-5}	-1.010	0.139
1.27×10^{-4}	-1.103	0.249
2.26×10^{-4}	-1.187	0.074
4.97×10^{-4}	-1.282	0.129
1.24×10^{-3}	-1.637	0.101
2.33×10^{-3}	-1.740	0.073
4.66×10^{-3}	-1.784	0.107

Table 15.5: Initial TePB concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePB]. Lipid concentration was 92 μ M. SR vesicles were suspended in 3 mM HEPES, 5 mM NaCl, pH 7.5.

[TePB]	Ave mobility	st.dev mob
4.46×10^{-7}	-1.321	0.062
9.27×10^{-7}	-1.368	0.084
1.85×10^{-6}	-1.361	0.072
4.64×10^{-6}	-1.473	0.096
9.27×10^{-6}	-1.391	0.108
1.85×10^{-5}	-1.499	0.062
4.64×10^{-5}	-1.632	0.083
9.27×10^{-5}	-1.639	0.064
1.85×10^{-4}	-1.847	0.026
4.64×10^{-4}	-2.001	0.086
9.27×10^{-4}	-2.067	0.062
1.39×10^{-3}	-2.160	0.115
1.85×10^{-3}	-2.256	0.146

Below are results for SR vesicles and PC liposomes in the presence of imipramine⁺ at pH 7.0. Figure 15.3 shows results of two experiments. The white circles are the mobility of PC liposomes as a function of concentration of imipramine⁺ and the dark circles are mobility of SR vesicles as a function of concentration of imipramine⁺.

Both experiments were done in the same suspending solution: 5 mM NaCl, 10 mM HEPES, pH 7.0. The experimental values of mobility, standard deviation of mobility and initial aqueous concentration of lipophilic ion are given in Table 15.6 and Table 15.7.

Figure 15.3: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of imipramine⁺.

Table 15.6: Initial Imipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [Imip⁺]. Lipid concentration was 60 μ M. Liposomes were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

[Imip ⁺]	Ave mobility	st.dev mob
9.20 $\times 10^{-7}$	0.005	0.435
1.78 $\times 10^{-6}$	0.073	0.291
9.20 $\times 10^{-6}$	0.154	0.613
1.73 $\times 10^{-5}$	0.368	0.154
9.10 $\times 10^{-5}$	1.475	0.125
1.78 $\times 10^{-4}$	1.870	0.168
9.18 $\times 10^{-4}$	2.883	0.099
1.82 $\times 10^{-3}$	3.533	0.274
9.18 $\times 10^{-3}$	4.729	0.130
1.82 $\times 10^{-3}$	4.652	0.038

Table 15.7: Initial Imipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [Imip⁺]. Lipid concentration was 30 μ M. SR vesicles were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

[Imip ⁺]	Ave mobility	st.dev mob
1.00 $\times 10^{-6}$	-1.543	0.125
2.00 $\times 10^{-6}$	-1.437	0.118
4.00 $\times 10^{-6}$	-1.404	0.084
1.00 $\times 10^{-5}$	-1.375	0.099
2.00 $\times 10^{-5}$	-1.222	0.137
4.00 $\times 10^{-5}$	-1.090	0.093
1.00 $\times 10^{-4}$	-1.088	0.075
2.00 $\times 10^{-4}$	-1.237	0.150
4.00 $\times 10^{-4}$	-1.207	0.127
1.00 $\times 10^{-3}$	-0.635	0.101
2.00 $\times 10^{-3}$	-0.356	0.022
4.00 $\times 10^{-3}$	-0.029	0.011
1.00 $\times 10^{-2}$	0.975	0.073
2.00 $\times 10^{-2}$	2.189	0.103
4.00 $\times 10^{-2}$	2.779	0.146

Below are results for SR vesicles and PC liposomes in the presence of clomipramine⁺ at pH 7.0. Figure 15.4 shows results of two experiments. The white circles are the mobility of PC liposomes as a function of concentration of clomipramine⁺ and the dark circles are mobility of SR vesicles as a function of concentration of clomipramine⁺. Both experiments were done in the same suspending solution: 5 mM NaCl, 10 mM

HEPES, pH 7.0. The experimental values of mobility, standard deviation of mobility and initial aqueous concentration of lipophilic ion are given in Table 15.8 and Table 15.9.

Figure 15.4: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of clomipramine⁺.

Table 15.8: Initial Clomipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on [Clom⁺]. Lipid concentration was 60μM. Liposomes were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

[Clom ⁺]	Ave mobility	st.dev mob
2.51×10 ⁻⁶	-0.044	0.270
3.03×10 ⁻⁶	0.120	0.281
1.58×10 ⁻⁵	1.246	0.316
3.33×10 ⁻⁵	1.902	0.319
1.64×10 ⁻⁴	2.947	0.138
3.37×10 ⁻⁴	3.613	0.172
1.67×10 ⁻³	4.695	0.251
3.37×10 ⁻³	4.806	0.560

Table 15.9: Initial Clomipramine concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [Clom⁺]. Lipid concentration was 30 μ M. SR vesicles were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

[Clom⁺]	Ave mobility	st.dev mob
1.11 $\times 10^{-6}$	-1.440	0.232
2.12 $\times 10^{-6}$	-1.386	0.164
4.35 $\times 10^{-6}$	-1.344	0.168
1.11 $\times 10^{-5}$	-1.384	0.205
2.12 $\times 10^{-5}$	-1.289	0.193
4.35 $\times 10^{-5}$	-1.133	0.205
1.11 $\times 10^{-4}$	-1.437	0.131
2.12 $\times 10^{-4}$	-0.988	0.113
4.35 $\times 10^{-4}$	-0.617	0.046
1.11 $\times 10^{-3}$	-0.118	0.140
2.12 $\times 10^{-3}$	1.259	0.203
4.35 $\times 10^{-3}$	2.145	0.140
1.11 $\times 10^{-2}$	2.683	0.149
2.45 $\times 10^{-2}$	2.762	0.150
4.35 $\times 10^{-2}$	1.771	0.478

Below are results for SR vesicles and PC liposomes in the presence of TePP⁺.

Figure 15.5 shows two experiments. The white circles are the mobility of PC liposomes as a function of concentration of TePP⁺ in the suspending solution 5 mM NaCl, 10 mM HEPES, pH 7.0 and the dark circles are mobility of SR vesicles as a function of concentration of TePP⁺ in the suspending solution 5 mM KCl, 3 mM HEPES, pH 7.5. The experimental values of mobility, standard deviation of mobility and initial aqueous concentration of lipophilic ion are given in Table 15.10-Table 15.11.

Figure 15.5: Electrophoretic mobility of PC liposomes and SR vesicles as a function of initial aqueous concentration of TePP^+ .

Table 15.10: Initial TePP^+ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of PC liposomes on $[\text{TePP}^+]$. Lipid concentration was $60\mu\text{M}$. Liposomes were suspended in 10 mM HEPES, 5 mM NaCl, pH 7.0.

$[\text{TePP}^+]$	Ave mobility	st.dev mob
4.05×10^{-6}	0.269	0.153
1.01×10^{-5}	-0.017	0.095
2.02×10^{-5}	-0.062	0.133
4.05×10^{-5}	0.119	0.077
1.01×10^{-4}	0.058	0.078
2.02×10^{-4}	-0.168	0.129
4.05×10^{-4}	0.140	0.074
1.01×10^{-3}	0.265	0.027
2.02×10^{-3}	0.602	0.029
4.05×10^{-3}	0.937	0.075
1.01×10^{-2}	1.560	0.073
2.02×10^{-2}	1.636	0.022
4.05×10^{-2}	1.809	0.102

Table 15.11: Initial TePP⁺ concentration, average mobility and standard deviation for the dependence of electrophoretic mobility of SR vesicles on [TePP⁺]. Lipid concentration was 92.3 μ M. SR vesicles were suspended in 3 mM HEPES, 5 mM KCl, pH 7.5.

[TePP ⁺]	Ave mobility	st.dev mob
5.81×10^{-6}	-1.359	0.121
1.16×10^{-5}	-1.314	0.047
2.97×10^{-5}	-1.294	0.030
5.81×10^{-5}	-1.296	0.076
1.16×10^{-4}	-1.328	0.071
2.97×10^{-4}	-1.374	0.105
5.81×10^{-4}	-1.348	0.050
1.16×10^{-3}	-1.313	0.058
2.97×10^{-3}	-1.281	0.042
5.81×10^{-3}	-1.143	0.026
1.16×10^{-2}	-0.514	0.017
2.97×10^{-2}	0.260	0.044
5.81×10^{-2}	0.491	0.057
8.71×10^{-2}	0.555	0.066
1.16×10^{-1}	0.517	0.076

In this chapter we have presented measurements of electrophoretic mobility of SR vesicles and PC liposomes in the presences of lipophilic ions PBP⁻, TePB⁻, imipramine⁺, clomipramine⁺, and TePP⁺. At pH values around 7, we find that lipophilic anions have very little effect on the measured mobility of SR vesicles while they do substantially affect PC mobility. Also, lipophilic cations seem to have a substantial effect on both PC liposomes and SR vesicle mobility. However, the effect on PC mobility is seen at smaller lipophilic cation concentration than that for SR.

Chapter 16: Discussion: Electrophoretic mobility of liposomes

Section 1: Background information and overview of results

In Chapter 11 we presented results of the electrophoretic mobility of charged single-layered and multi-layered liposomes made from a mixture of phosphatidylcholine (PC) and phosphatidylglycerol (PG) containing 0.067 mole fraction of negatively charged (PG) lipids. This mole fraction of charged lipid results in a surface charge density of -14.9 mC/m^2 . We find that although the OHW model can successfully reproduce the measured data, the values of surface charge density of the best fits of this model to the data are -9.56 mC/m^2 for single-layered liposomes and -12.0 mC/m^2 for multi-layered liposomes. This represents 35.8 and 19.5 percent error respectively. The Smoluchowski and Henry models were not successfully fit to the data. The Smoluchowski model fails because the model only works for colloids with radii that are sufficiently large compared to the Debye length so that the colloid surface can be modeled as flat and the electrophoretic velocity is set equal to the opposite of the fluid velocity relative to the colloid surface infinitely far from the colloid. Our liposomes have substantial curvature as shown in Chapter 10. Best fit of the superior OHW model provides a radius of 140 nm for single-layered liposomes and 190 nm for multi-layered liposomes.

The Henry model fails because it does not account for relaxation or retardation effects, both of which become more prominent at higher zeta potentials. This is seen in the data because at high ionic strength, where the zeta potentials are low, Henry's model

is able to explain the data. The discrepancies grow at lower ionic strength and at higher zeta potentials. Smoluchowski's model is also only applicable in this range of the data because the Debye length is substantially shorter than the colloid radius and the colloid can be modeled as flat.

From images taken with the photoelectron microscope we measured the size of dehydrated single-layered and multi-layered phosphatidylcholine (PC) liposomes (Chapter 10). Under the assumption that the dehydrated liposomes were flat, and that their original shape was spherical we calculated mean radii for their original spherical shape of 167 ± 102 nm for single layered liposomes and 124 ± 65 nm for multi-layered liposomes. We find that these values are consistent with the OHW model fit results for PC/PG liposomes. The agreement between microscopy and fit results validates the OHW model for mobility of PC/PG liposomes.

Chapter 14 contains results of experiments of partition of lipophilic ions between PC liposomes and water. We find that the modified Langmuir isotherm model of partition is able to explain the partition of TePB^- , PBP^- , imipramine⁺, and clomipramine⁺ between PC liposomes and water. For these lipophilic ions, both space saturation (limitation of the number of free sites) and charge saturation (electrostatic repulsion of lipophilic ions from the surface) effects are evident. Although the concentration of the neutral form of imipramine and clomipramine in the aqueous phase is low, its partition coefficient to liposomes is about an order of magnitude greater than the ionized form, and therefore it takes up significant space in the liposome phase. Because of the nontrivial quantity of the neutral species it was necessary to include its sorption in order to fit the partition model to the data. We also measured the partition of TePP^+ between PC liposomes and water. In

this case there were no apparent saturation effects in the concentration range of our experiments. The partition coefficient did not change with concentration of TePP^+ because it is relatively low. The partition coefficients were important for understanding the mobility of liposomes in the presence of lipophilic ions, the results of which were introduced in Chapter 15. These experiments show that as the initial concentration of lipophilic ions increases, the mobility becomes more positive (for cations) and more negative (for anions). In our model of the system we place the sorption plane at the surface of the liposome and use the Langmuir sorption model to calculate the expected surface charge density. As a first approximation we assume that all sorbed charge is at the liposome surface and calculate the surface potential and, from the mobility from the OHW model. As will be seen in the next section, we find that the expected mobility is substantially greater than the experimental mobility for all lipophilic ions in the study.

In the next sections we discuss propose our insight on of the nature of electrophoretic mobility of PC liposomes in the presence of lipophilic ions and the mobility of PC/PG liposomes. In the final section we discuss our conclusions in terms of the broader area of research of liposomes and the implications these results will have for SR mobility studies.

Section 2: Electrophoretic mobility of liposomes in the presence of lipophilic ions

Here we discuss the consequences of two different hypotheses on the properties of the liposome interfacial region. According to the first one the lipophilic ions add only their electric charge directly to the surface, without any additional changes at the interface. It is assumed that no ions in general penetrate the liposome surface. This

hypothesis defines the impermeable surface model. We use Equation (3.44), which follows from the nonlinearized Poisson-Boltzmann equation, to obtain the liposome surface potential from the surface charge density and subsequently solve the OHW model to obtain the prediction for the electrophoretic mobility.

The second hypothesis defines the permeable surface model. Two characteristic properties are: (1) the lipophilic ion sorption plane is embedded at some depth in the lipid headgroup region and (2) Na^+ and Cl^- ions from the aqueous phase are assumed to be also present in the lipid headgroup region, although these ions are excluded from the region of alkyl chains. In the permeable surface model the spatial distribution of Na^+ and Cl^- ions in the lipid headgroup region is determined by the Poisson-Boltzmann distribution function multiplied by a molar partition coefficient, K_m , of these ions between the liposome phase and water. Figure 16.1 is a schematic representation of the permeable surface model. The physical equations that govern the enumerated regions and boundaries are listed below. We found it necessary to include a small surface charge density, σ_0 at the liposome surface to represent the ‘native’ or ‘intrinsic’ surface charge in order to fit this model to the data. The native charge is likely due to lipid impurities. The native charge is notable at low concentration of lipophilic ions.

Figure 16.1: The permeable surface model. The dashed line represents the lipophilic ion sorption plane. It is embedded some distance below the liposome surface. Ions from the salt in the water phase are able to penetrate the entire lipid head region. Physical equations that govern the enumerated regions and boundaries are listed below.

$$1. \frac{d\psi(x)}{dx} = 0$$

$$2. \rho(x) = 0$$

$$3. \frac{d\psi(x)}{dx} = 0$$

$$4. \frac{d^2\psi(x)}{dx^2} = \frac{2zenK_m}{\epsilon_2\epsilon_0} \sinh\left(\frac{ze\psi(x)}{kT}\right)$$

$$5. \overset{\vee}{E}_r(d) - \overset{\vee}{E}_l(d) = -\frac{d\psi_r(x)}{dx}\bigg|_{d+} - \left(-\frac{d\psi_l(x)}{dx}\bigg|_{d-}\right) = \frac{\sigma_d}{\epsilon_2\epsilon_0}$$

$$6. \overset{\vee}{E}_r(0) - \overset{\vee}{E}_l(0) = -\epsilon_1 \frac{d\psi_r(x)}{dx}\bigg|_{0+} - \left(-\epsilon_2 \frac{d\psi_l(x)}{dx}\bigg|_{0-}\right) = \frac{\sigma_0}{\epsilon_0}$$

$$7. \frac{d^2\psi(x)}{dx^2} = \frac{2zen}{\varepsilon_1\varepsilon_0} \sinh\left(\frac{ze\psi(x)}{kT}\right)$$

$$8. \psi(x) = 0$$

$\vec{E}_r$ represents the electric field on the right side of the boundary while $\vec{E}_l$ represents the electric field on the left side. ε_l is the dielectric constant of the water phase and ε_2 is the dielectric constant of the lipid head region. σ_0 is the surface charge density and σ_d is the charge density of the liposome sorption plane.

For both the impenetrable surface model and the penetrable surface model we use our Langmuir sorption model to determine the charge density of the lipophilic ion sorption plane as a function of initial lipophilic ion concentration, C_0 . Our modified Langmuir sorption model is built with the same assumptions as the impermeable surface model. Although this may seem inconsistent with the permeable model, we submit that the error to the apparent partition coefficient and therefore the total absorbed change is small.

Using $Kp_{x,0}$ and A_s from our Langmuir model fits, and molar concentration of lipids, initial concentration of lipophilic ions (C_0), and ionic strength of solution from the mobility experiments, we use the following system of equations to solve for the equilibrium concentration of lipophilic ion C_{eq} in the mobility sample and the surface potential, ψ_0 .

$$C_0 = \Theta \frac{A_L}{A_s} \cdot [lipid] + C_{eq} \quad (16.1)$$

Where

$$\Theta = \frac{Kp_{x,0}C_{eq} \exp(-ze\psi_0/kT)/C_w}{A_L/A_S + Kp_{x,0}C_{eq} \exp(-ze\psi_0/kT)/C_w} \quad (16.2)$$

And

$$\psi_0 = \frac{2kT}{ze} \sinh^{-1} \left(\frac{ze\sigma_0}{2\epsilon\epsilon_0\kappa kT} \right) \quad (16.3)$$

where

$$\sigma_0 = \frac{(C_0 - C_{eq}) \cdot ez}{[lipid] \cdot A_L} \quad (16.4)$$

For the impermeable surface model we can simply use our solution for ψ_0 and κ , which are functions of the ionic strength, to find the predicted electrophoretic mobility using the OHW model. For the permeable surface model we use our values of σ_0 as a function of C_0 that we get from solving the above system of equations as the charge density of the embedded lipophilic sorption plane, σ_d .

For our permeable surface model fits we used a value of 0.4 for K_m based on the mobility of PBP^- , which due to its structure, we assume is located as deep in the head group region as possible. This values is consistent with the K^+ and Na^+ lipid membrane-water partition coefficients reported in the impressive survey made by Hyman (Hyman 1966). With K_m assumed a constant value, the depth of the sorption plane, d , becomes the adjustable parameter in the model.

Molecular dynamic simulation have been used to show that the dielectric constant within the lipid head region is a non-monotonically decreasing function and that water penetrates the lipid head region (Stern and Feller 2003). However for simplicity we use a constant value for ϵ_2 of 7 as is commonly done (Manzanares and others 2000; Samec

1988), which is an intermediate value of the dielectric constant of water and the alkyl chain region of the lipid bilayer.

We calculated the electrostatic potential and electrophoretic mobility (from the hydrodynamic flow) of the system using the finite element analysis software COMSOL Multiphysics 4.2. The model system consisted of three dielectric regions. The water phase was a 200-nm wide square. The headgroup region was 0.5 nm thick and the alkyl region was 1.0 nm thick. The surface potential was solved for various values of C_0 within the range of the experiment and the corresponding ionic strength values. The depth, d of the lipophilic ion sorption plane was adjusted to fit the mobility data. The native surface charge density, σ_0 , was found by fitting the model at the low concentration limit of the experiment. We believe that the native charge is due either to the degradation of the phosphatidylcholine or the zwitterionic dipole nature of the lipid head. In the case of degradation, a portion of the lipids may have their choline group neutralized leaving them negatively charged. If it is due to the polar nature of the lipid heads then we must assume that the negative end of the dipole protrudes from the liposome surface while the positive end does not. It has been suggested that this surprising re-orientation occurs for natural polar lipids in liposomes (Makino and others 1991). The orientation of polar lipids is a function of ionic strength and temperature. Notably it is shown that at low ionic strengths the negatively charged phosphatidyl group is protruding above the positively charged choline group and that they undergo gradual transition so that at room temperature at 90 mM ionic strength the two charges are side by side. At higher ionic strength they are inverted (Makino and others 1991). Because the negative shift in

mobility is seen at very low concentrations of lipophilic ions we know that it is a feature of the native liposome.

The results of impermeable and permeable models are displayed for PC liposomes in the presence of TePB^- , PBP^- , imipramine⁺, clomipramine⁺, and TePP^+ in Figure 16.2 through Figure 16.6. For both the impermeable and the permeable surface models we used the Smoluchowski and the OHW mobility models. The difference between these illustrates the effect of liposome curvature.

The impermeable surface model fails miserably, it does not predict the mobility of PC liposomes in the presence of lipophilic ions. We conclude from this that lipophilic ions cannot be simply assumed to add charge to the surface of a lipid membrane. On the other hand the permeable surface model successfully explains the data. One valuable feature of the permeable model is that it predicts the location of the sorption plane, which varies considerably within the membrane. The sorption plane depths obtained by best fit of the permeable surface model are 0.13 nm for TePB^- , 0.5 nm for PBP^- , 0.12 nm for Imipramine⁺, 0.17 nm for Clomipramine⁺, and 0.25 nm for TePP^+ . For the native surface charge density we used -0.001 C/m^2 for TePB^- , -0.0018 C/m^2 for PBP^- , -0.001 C/m^2 for Imipramine⁺, -0.002 C/m^2 for Clomipramine⁺, and -0.0003 C/m^2 for TePP^+ .

Figure 16.2: The mobility of TePB^- is fit with a sorption plane depth of 0.13 nm. A native surface charge density of $-0.001 \text{ mC}/\text{m}^2$ was also used. The Na^+ partition coefficient was 0.4.

Figure 16.3: The mobility of PBP^- is fit with a sorption plane depth of 0.5 nm. A native surface charge density of $-0.0018 \text{ mC}/\text{m}^2$ was also used. The Na^+ partition coefficient was 0.4.

Figure 16.4: The mobility of Imipramine⁺ is fit with a sorption plane depth of 0.12 nm. A native surface charge density of -0.001 mC/m² was also used. The Na⁺ partition coefficient was 0.4.

Figure 16.5: The mobility of Clomipramine⁺ is fit with a sorption plane depth of 0.17 nm. A native surface charge density of -0.002 mC/m² was also used. The Na⁺ partition coefficient was 0.4.

Figure 16.6: The mobility of TePP^+ is fit with a sorption plane depth of 0.25 nm. A native surface charge density of $-0.0003 \text{ mC}/\text{m}^2$ was also used. The Na^+ partition coefficient was 0.4.

Section 3: *Electrophoretic mobility of PC/PG liposomes*

We will now address the discrepancy between the expected surface charge density of our PC/PG single-layered and multi-layered liposomes due to the 0.067 mole fraction of negatively charged (PG) lipids and the surface charge density found from the best fit of OHW model to the data. The expected surface charge density was $-14.9 \text{ mC}/\text{m}^2$. The values of surface charge density corresponding to the best fits of the OHW model to the

data are -9.56 mC/m^2 for single-layered liposomes and -12.0 mC/m^2 for multi-layered liposomes. This represents 35.8 and 19.5 percent error respectively. We present computed results that include counter-ion absorption into the lipid head region with molar partition coefficient K_m equal to 0.4 and with the charged plane due PG lipids at the surface of the liposome. We also show results that include displacement of the negatively charged plane caused by the PG lipids to some depth beneath the surface of the liposome. This adjustment in depth of the charged plane may be justified because the lipid heads of phosphatidylcholine are larger than those of phosphatidylglycerol and so the plane of charge due to the PG may be below the surface of the liposome. Also, as mentioned before, phosphatidylcholine is a polar lipid. The positively charged choline group may be oriented above the negatively charged phosphatidyl group. This separation of charge should effectively reduce the electric potential at the surface of the liposome. This dipole is able to change angle according to the electrostatics of the surface of the lipid bilayer (Makino and others 1991; Mbamala and others 2006).

Below in Figure 16.7 and Figure 16.8 we show model predictions of mobility associated with changes of depth of the charged plane in the liposome. For each line the charge density of the plane was set equal to -14.9 C/m^2 . The black curve shows the prediction of the model with the charge plane on the surface of the liposome and in the absence of counterion absorption into the liposome. The green curve also represents the prediction of the model with the charged plane at the liposome surface, however, counterions are allowed to be present in the lipid head region. The dark blue, light blue, purple and red curves represent increasing depth of charged plane from 0.1 to 0.4 nm. For single-layered liposomes, the thick light blue curve, representing a depth of 0.2 nm is the

best fit. For multi-layered liposomes the thick dark blue curve is for the depth of 0.1 nm and represents the best fit. In the following table the reduced χ^2 values are given for each of these fits.

Figure 16.7: The graph shows model predictions of the electrophoretic mobility of single layered PCPG liposomes for various depths of charge plane and experimental results. All model predictions shown, except the black line, include the presence of counter-ions in the lipid head region. The best of these fits is the thick light blue line representing a depth of 0.2 nm.

Figure 16.8: The graph shows model predictions of the electrophoretic mobility of multilayer PCPG liposomes for various depths of charge plane and experimental results. All model predictions shown, except the black line, include the presence of counter-ions in the lipid head region. The best of these fits is the thick dark blue line representing a depth of 0.1 nm.

Table 16.1: Reduced χ^2 values for model predictions in Figure 16.7 and Figure 16.8

Charged plane depth	0.4 nm	0.3 nm	0.2 nm	0.1 nm	0 nm	0 nm; no CI abs.
Multi-layered	6.542	4.593	3.307	3.258	4.56	7.354
Single-layered	4.784	3.164	2.094	2.601	5.493	10.423

For single-layered liposomes it appears that we need to adjust the value used for the liposome radius because the fit diverges from most of the data points at lower ionic strength and the curvature of the fit in this region is determined by the radius fit value. Below we show our best fit for radius using the charged plane depth of 0.2 nm and K_m

equal to 0.4. The fit value of the radius is 70 nm. As the radius fit value is decreased, the range of applicability of the OHW model is also decreased. The OHW model is valid for $\kappa a \geq 10$. The dashed line is the ionic strength limit of the OHW model for $\kappa a = 10$. The reduced χ^2 value is 1.2.

Figure 16.9: The best fit of the OHW mobility model to our single layered PCPG liposome mobility as a function of ionic strength. To minimize the reduced χ^2 value we decreased the fit value of a to 70 nm. The depth of the charged plane is 0.2 nm and K_m is 0.4. The range of applicability of the OHW model decreases with decreasing radius. Reduced χ^2 is 1.2.

The best-fit value for the radius of single-layered liposomes of 70 nm is within one standard deviation of the radius value of 170 ± 100 nm that we obtained using the photoelectron microscope. We know from our photoelectron microscope studies that SR

vesicles come in a wide range of sizes. In the DELSA mobility analyzer the signal from light scattered from liposomes may be preferentially coming from vesicles of a certain size for two reasons. First, larger vesicles may be settling to the bottom of the electrophoretic cell leaving smaller ones toward the top where the measurement is taking place. Second, the light signal from larger vesicles is more intense. We believe these factors may explain the size discrepancy we have observed between photoelectron microscope data and mobility measurements for single-layered liposomes.

We find that we cannot treat mixed zwitterionic and anionic liposomes the same way as if their net charge is located at the surface of the liposome. We must instead model the system as if the negatively charged plane is some distance below the surface of the liposome. Furthermore, we must assume that ions from the water phase can penetrate the surface of the liposome. If this were not true then changing the depth of the charged plane would not change the surface potential because no ions would exist in this region to screen the charge of the charged plane.

Section 4: Conclusions to electrophoretic mobility studies of liposomes

From our mobility studies we have concluded that liposomes do not behave as if all surface layer charge is distributed directly on the hydrodynamic surface without penetration to ions below the surface. In many mobility studies of charged liposomes, researchers have found a discrepancy between the mobility predicted given these assumptions and the experimental mobility (Cohen and Khorosheva 2001; Roy and others 1998; Woodle and others 1992). They generally explained this discrepancy by considering a displacement of the plane of shear, at which water starts to move relative to

the liposome surface. Roy used a distance of 0.2 nm for this displacement (Roy and others 1998) which is approximately the thickness of the first two molecular layers of water. Cohen calculates the displacement of the plane of shear that changes with ionic strength for PC/PG and PEG-PE/PC liposomes (Cohen and Khorosheva 2001).

Results of our studies of PC mobility as a function of lipophilic ion structure and concentration can be understood if the charge associated with the lipophilic ions resides somewhere below the surface of the liposome. If there were no counter-ions present below the surface (neglecting the polarization of the polar headgroup region) there would be no electric potential drop between the sorption plane and the liposome surface. In order to achieve reduction of surface potential, we must allow small ions from the solution to penetrate the lipid head region in our models. In our studies we used molar partition coefficient of small ions equal to 0.4 (Hyman 1966) and found that different sorption plane depths were required for different lipophilic ion. Figure 16.10, which is drawn to scale, shows likely position and orientation of the absorbed lipophilic ions. If we were to explain our surface potential discrepancies using a shear plane distance as Roy and Cohen did, the shear plane distance would have to be different for each lipophilic ion. There is no apparent reason for the external water structure to be altered in this way by the species of lipophilic ion sorbed to the liposome. Because of this our current model is physically more meaningful for interpreting the mobility of PC liposomes in the presence of lipophilic ions.

In our studies of charged PC/PG liposomes in the absence of lipophilic ions we have used the same basic surface model with an adjustable depth of charged plane and penetration of small ions from the aqueous phase. The same value of molar partition

coefficient of small ions, equal to 0.4 was used as before. This model explained well the discrepancy between the mobility predicted by the conventional mobility model for impenetrable liposome surface and our experimental mobility results. Based on the success of the permeable surface model, we predict that the average location of lipophilic ions within the membrane is as shown in Figure 16.10.

Figure 16.10: Schematic representation of placement of lipophilic ions in the lipid head region of PC liposomes, drawn to scale. The depths of the charges of the lipophilic ions were determined using the permeable surface model.

Chapter 17: Discussion: Partition of Lipophilic Ions into PC and SR

In Chapter 14 we presented data for partition of lipophilic ions between two membrane phases and the aqueous phase. The two membrane phases are egg phosphatidylcholine liposomes and SR vesicles. We have used the Langmuir model and obtained fits of this model for experimental results for PBP⁻, TePB⁻, Imipramine⁺, and Clomipramine⁺. For PC liposomes the modified Langmuir model successfully fits the data. The sorption site area values are reasonable for the size of our lipophilic ions and lipids, and the fits have relatively low reduced χ^2 ($\chi^2/(n-f) \gg 1$) values. In contrast, the Langmuir model does not work as well for SR membrane-water partition. The lipophilic ion sorption site areas obtained from fit of the model were unreasonably small, smaller than the membrane surface area of one lipid, and the reduced χ^2 values are large.

As a first approximation, we assumed that sorption sites of SR are located primarily in the phospholipid bilayer region of the membrane (i.e., that sorption to proteins is insignificant). However, we found that the apparent partition coefficients for SR are much larger than those for PC membrane for most lipophilic ions. If we assume that the partition coefficients for PC liposomes and the lipid bilayer of SR are similar, the results imply that there is a significant sorption to proteins. In other words, in order for the apparent partition coefficients of lipophilic ions for PC and SR to be the same, we need another large source of sorption sites in the SR membrane. The obvious unaccounted for source are the proteins.

Figures 17.1 to 17.4 show the apparent PC and SR membrane-water partition coefficients for lipophilic ions. SR and PC are shown together for comparison along with the best fits of the Langmuir model for PC membrane-water experiments. For PBP⁻, Imipramine⁺, and Clomipramine⁺, the apparent partition coefficients between SR membrane and water are greater than those between PC membrane and water. For TePB⁻ the differences are small. Although small differences in the partition coefficient for different types of membranes are not unexpected, we believe that large increases are unphysical. The likely error is due to the assumption that proteins do not play a role in partition.

Assuming further that the PC lipid membrane-water partition model is applicable to the lipid portion of SR, we can calculate the amount of lipophilic ions that would be sorbed into the SR proteins. In Figures 17.5 to 17.8, we plot the number of lipophilic ions per unit cell of SR membrane, defined by one Ca²⁺-ATPase protein and 106 phospholipids. These curves were calculated using our apparent partition coefficients for SR vesicles. We also plot the calculated number of ions that are left over (the excess) that must be assigned as sorbing to the Ca²⁺-ATPase proteins. We find that a comparable number of lipophilic ions absorbs to the lipid membrane and to proteins.

The consequence of the results of this section is that a successful model for the mobility of SR vesicles in the presence of lipophilic ions will be more complicated than expected. The model will have to include absorption of lipophilic ions to both the lipid bilayer and the proteins of SR. These absorbed ions will add to the native charge of the lipid bilayer and the proteins.

Figure 17.1: Partition of TePB^- between two membranes (PC liposomes-black markers, and SR vesicles-white markers) and water. The solid line represents the best fit of the modified Langmuir isotherm model to partition of TePB^- between PC liposomes and water.

Figure 17.2: Partition of Imipramine⁺ between two membranes (PC liposomes-black markers, and SR vesicles-white markers) and water. The solid line represents the best fit of the modified Langmuir isotherm model to partition of Imipramine⁺ between PC liposomes and water.

Figure 17.3: Partition of Clomipramine⁺ between two membranes (PC liposomes-black markers, and SR vesicles-white markers) and water. The solid line represents the best fit of the modified Langmuir isotherm model to partition of Clomipramine⁺ between PC liposomes and water.

Figure 17.4: Partition of PBP^- between two membranes (PC liposomes-black markers, and SR vesicles-white markers) and water. The solid line represents the best fit of the modified Langmuir isotherm model to partition of PBP^- between PC liposomes and water.

Figure 17.5: Number of TePB^- molecules per unit cell absorbed to SR (black), to lipid bilayer of unit cell (green) and to a single Ca^{2+} -ATPase protein (purple).

Figure 17.6: Number of Imipramine⁺ molecules per unit cell absorbed to SR (black), to lipid bilayer of unit cell (green) and to a single Ca²⁺-ATPase protein (purple).

Figure 17.7: Number of Clomipramine⁺ molecules per unit cell absorbed to SR (black), to lipid bilayer of unit cell (green) and to a single Ca²⁺-ATPase protein (purple).

Figure 17.8: Number of PBP^- molecules per unit cell absorbed to SR (black), to lipid bilayer of unit cell (green) and to a single Ca^{2+} -ATPase protein (purple).

Chapter 18: Discussion: Electrophoretic mobility of SR vesicles

Section 1: Effect of ionic strength on SR mobility of SR vesicles

In Chapter 12 we presented data on the electrophoretic mobility of SR vesicles as a function of ionic strength at various pH values. We attempted to fit two “soft particle” models from the literature to these data. The first was Ohshima’s 2005 model for a soft particle with a charged core (Ohshima 2005). This model includes retardation and relaxation effect. The second model was Levine’s model for a soft particle with a retardation layer with homogeneous volume charge density (Levine and others 1983). These models are discussed in Chapter 5. We find that for the native charge of SR, as calculated using the sequence of amino acid residues of the Ca^{2+} -ATPase and their pKa values in water, neither of these models could predict the experimental mobilities.

There is quite a lot of scatter in the data. The reasons for this are multifold. For small mobilities the frequency shifts in the DELSA are small, which reduces the signal to noise ratio of the measurement. For the case of pH 4.0, 9.0, and 7.5, the data shown include multiple experiments with multiple preparations of SR vesicles. These vesicles may vary slightly in size distribution as well as density of Ca^{2+} -ATPase, both of which will affect mobility.

From Section 2 of Chapter 2 it follows that the electrostatic properties of the surface of SR membrane are complicated by the presence of ionizable amino acid residues of the Ca^{2+} -ATPase, in addition to a charged lipid bilayer matrix. Their

ionization state changes with pH and ionic strength. The charge of a given ionizable domain is

$$CHRG = \frac{pol}{1 + 10^{pol \cdot (pH - pKa)} \cdot \exp\left(pol \frac{F_a \psi}{RT}\right)}. \quad (18.1)$$

where pol is the polarity of the domain, and ψ is the local electric potential. The local electric potential is a function of ionic strength of suspending solution because the presence of counter-ions reduces the local potential.

To simplify our studies of electrophoretic mobility of SR vesicles we first seek to discover the source of charge on the surface of SR that determines the electrophoretic mobility. We consider the charge of the entire Ca^{2+} -ATPase protein, the charge of the lipid bilayer surface and the charge of the protruding domains of the Ca^{2+} -ATPase, the A, P, and N domains, which together we call the APN domain. Because the polarization state of ionizable groups changes with ionic strength, we will study the pH dependence of mobility at one ionic strength value. We choose to study the mobility at intermediate ionic strength of 100 mM, at which the experimental errors are small. When the ionic strength is too low, the curvature of the vesicles, which may vary, greatly affects mobility. When it is too high, the mobility values are low and the measurements have low signal to noise ratios. For this reason, we interpolate our data for each pH value to 100 mM ionic strength. Because of the scatter of our mobility data we use large data sets to obtain the representative mobility value at 100 mM using the linear least square fit. We find that the linear least squares fits in the range from 50 to 200 mM work well. Figure 18.1 shows the mobility data in this range and the linear least squares fits we obtained.

Figure 18.1: Linear least square fits to SR mobility at various pH values versus ionic strength in the range of 100 mM ionic strength.

Figure 18.2 shows the interpolated mobility values at 100 mM ionic strength. The error in mobility of these values is determined by the variance of the fit, s^2 .

$$s^2 = \frac{1}{N-2} \sum_i (y_i - a - bx_i)^2. \quad (18.2)$$

The error in pH is the uncertainty in our pH measurement.

Figure 18.2: Electrophoretic mobility interpolated to 100 mM ionic strength as a function of pH.

Next we quantify the charge of a single Ca^{2+} -ATPase protein, its APN domain, and the surface charge of one side of the lipid bilayer that are associated with one protein molecule (as calculated in Chapter 2). We will calculate the charge using the following simplified approach that does not incorporate the local potential: it is assumed that the local pH is equal to the bulk pH. Calculating the local potential is dependent on the spatial distribution of charge, for which we lack adequate information. Equation (18.3) is used to calculate the total charge of the Ca^{2+} -ATPase protein, its APN domain only, and the associated lipid of one side of the lipid bilayer as a function of bulk pH, the results are

displayed in Figure 18.3. $freq_i$ is the number of ionizable sites of type i in the Ca^{2+} -ATPase protein, its APN domain, or the associated lipids.

$$TotalCHRG = \sum_i freq_i \cdot \frac{pol_i}{1 + 10^{pol_i \cdot (pH - pKa_i)}} \cdot \quad (18.3)$$

Figure 18.3: The charge of the entire Ca^{2+} -ATPase, the APN-domain alone and one surface of the lipid bilayer as a function of pH.

In Figure 18.4, we show the representative electrophoretic mobility values for 100 mM ionic strength obtained from the linear fits of Figure 18.1 as a function of the total calculated charge of the Ca^{2+} -ATPase protein, the APN domain, and the lipid bilayer surface. Each of these structure's charge profiles exhibits a linear relationship with the

electrophoretic mobility, however, for the APN domain, the zero charge point corresponds to the zero mobility point. When the predicted charge of the APN domain is positive the mobility is positive, when it is negative the mobility is negative. This demonstrates that the APN domain is the dominant structure influencing the mobility of SR vesicles.

Figure 18.4: Demonstration of linear relationships between electrophoretic mobility and charge of the Ca^{2+} -ATPase, the APN domain, and the lipid bilayer surface. Zero mobility corresponds with zero charge for APN domain.

The linear fit formula for the APN domain is

$$\mu = 0.0355 \cdot Q_{APN} - 0.0113. \quad (18.4)$$

Using this formula we graph the predicted mobility of SR vesicles at 100 mM ionic strength as a function of pH along with our experimental results, as shown in Figure 18.5.

Figure 18.5: Experimental mobilities interpolated to 100 mM along with mobilities calculated using the linear fit of mobility to charge of one APN domain.

We have applied the detached charged plane model described in Chapter 5 to the measured dependence of mobility of SR vesicles on ionic strength. The origin of complexity of SR membrane for electrophoretic mobility studies are the dense arrays of the Ca^{2+} -ATPases with charges detached from the surface of SR bilayer, and the hydrodynamic friction they introduce. The applicability of the detached charged plane

model rests on the applicability of assumptions that (1) the charges on APN domains can be represented by the homogeneously charged plane placed at distance s above the surface of the SR lipid bilayer, and (2) that the hydrodynamic friction of the Ca^{2+} -ATPases protruding from the SR lipid bilayer can be represented by a surface coating of thickness D . The flow retardation properties of the surface layer are characterized by the frictional parameter λ_{RL} . The detached plane associated with the charges of the APN domains is assumed to be embedded in the frictional layer accounting for the hydrodynamic friction of the Ca^{2+} -ATPases.

Figure 18.6 illustrates the agreement between the measured pH dependence of mobility of SR vesicles and the mobility predicted from the detached charged plane model. In this study we considered detached charged plane located at $s = 2, 3, 4, 5$, and 6 nm inside the 8 nm thick surface frictional layer. The value of frictional parameter λ_{RL} was obtained from the least squares fit of the theoretical mobility to the measured one. The results are presented in Table 18.1. As shown in Figure 18.6, the mobility predicted from the detached charged plane model overlay one another.

Table 18.1: Fit parameters for detached charged plane model fit to data of mobility as a function of pH.

s (nm)	λ_{RL}	SSQ
2	4.7×10^8	0.021
3	5.65×10^8	0.02
4	7.0×10^8	0.021
5	9.55×10^8	0.02
6	1.71×10^9	0.02

Figure 18.6: The agreements between the predicted and measured mobility was obtained for λ_{RL} values listed in Table 18.1.

Although these predicted mobility values overly each other when looking only at a single ionic strength, Figure 18.7 through Figure 18.12 illustrate the quite divergent results they produce when applied to the entire range of ionic strengths.

Figure 18.7: Illustration of the performance of the detached charged plane model at pH 4.0 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. In this case $s=6$ nm best represents the data.

Figure 18.8: Illustration of the performance of the detached charged plane model at pH 4.7 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. Here $s=2-3$ nm best fits the data.

Figure 18.9: Illustration of the performance of the detached charged plane model at pH 5.0 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. At this pH, where the net charge is lower, the data is too scattered to make a firm selection of s .

Figure 18.10: Illustration of the performance of the detached charged plane model at pH 6.0 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. In this case $s=2-3$ nm appears the best.

Figure 18.11: Illustration of the performance of the detached charged plane model at pH 7.5 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. Here the model does not adequately explain the data for any of our paired values of s and λ_{RL} over the whole range.

Figure 18.12: Illustration of the performance of the detached charged plane model at pH 9.0 by comparing the dependence of the measured and the theoretical mobility of SR vesicles on ionic strength for parameters listed in Table 18.1. The high end of pH $s=5-6$ nm appears best.

Table 18.2: Sum of deviations squared for fits of detached charged plane model fits. The stars indicate superior fits.

s (nm)	λ_{RL}	pH 4	pH 4.7	pH 5	pH 6	pH 7.5	pH 9
2	4.7×10^8	21.52	1.09*	2.06	1.06*	3.16	23.96
3	5.65×10^8	19.17	1.10*	2.00*	1.08*	2.98	21.97
4	7.0×10^8	12.75	1.60	2.13	1.47	2.73*	15.42
5	9.55×10^8	5.44	2.91	2.56	2.46	3.65	8.29
6	1.71×10^9	1.29*	5.41	3.39	4.32	7.07	4.72*

In Table 18.2 the sums of deviations squared are given for each of the fits in Figure 18.7 through Figure 18.12. For the extreme pH values, 4.0 and 9.0, it is clear that

the detached charged plane model works better for larger detached plane distances (s). However, although the differences are not as striking, for the intermediate pH values, smaller detached plane distances produce better fits. For pH 4.7 and pH 6.0 our best of these fits is for $s = 2$ nm. For pH 5.0 it is 3 nm and for pH 7.5 it is 4 nm.

For most of our pH studies, it is apparent that even the most suitable fits of the detached charged plane model are not adequate to describe the data for the entire ionic strength range. Perhaps this is not surprising because the detached plane model was found to have a limited applicability range. At lower ionic strength the surface potential of SR vesicles may become too large to be represented by the solution of the linearized Poisson-Boltzmann equation. At high ionic strength the measured mobility values are less reliable due to the small frequency shifts in the DELSA mobility analyzer, and low signal to noise ratios. Also the ionic strength can indirectly affect the electric charge density by changing the degree of ionization of APN domains. In our studies we have assumed that, in the vicinity of ionization sites in the APN domains, the local pH is the same as that in the bulk aqueous phase. This assumption is supported by the observed linear relationship between the mobility (interpolated to 100 mM ionic strength) and the net charge of the APN domains.

Figure 18.13 through 18.18 are least squares fits of the detached charged plane model to SR mobility data as a function of ionic strength for each pH value. The fits are summarized in Table 18.3. The fits were optimized for the higher ionic strength data to avoid the limits of applicability of the model. We found that by doing this we could empirically estimate the approximate ionic strength where the model begins to deviate from the measured data and we took this ionic strength as the limit of applicability of the

model for that pH. These apparent limiting ionic strength values can be given for pH 4.0, 4.7, 6.0, 7.5, and 9.0. For pH 5.0, the applicability limit could not be determined. The least square fits only apply to the data points with ionic strengths higher than the apparent limiting value. We note that as the surface charge density increases, λ_{RL} and s obtained from the least squares fits also increase. One possible reason is that there may be conformational changes in the protein that occur at extreme pH values when the protein becomes highly charged. In Chapter 10 we obtained mean values of SR vesicle radii from photoelectron microscopy studies for pH 4.0, 7.0, and 9.0. We used these values in the calculation of detached charged plane model mobility. Those data can be compared with radii obtained from analysis of mobility data. For pH 4.0 we obtained a mean radius value of 62.5 nm. This value was used in the detached charged plane model fits for experiments done at pH 4.0, 4.7, and 5.0. For pH 7.0 we obtained a mean radius value of 138 nm. This value was used for experiments done at pH 6.0 and 7.5. And for pH 9.0 we obtained a mean radius of 119 nm, which was used for the experiment done at pH 9.0. The effect of the curvature of the vesicle is greatest at low ionic strength, where κa is large. Because this is also the range where our mobility model becomes inapplicable and the mobility data at low ionic strength are not used in the analysis, any uncertainty or variation in vesicle radius does not affect the reliability of our fit values much.

Figure 18.13: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 4.0. The dashed line represents the apparent limit of applicability of the DCP model. The fit values are $s=6.46$ nm and $\lambda_{RL}=31.8 \times 10^8$.

Figure 18.14: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 4.7. The dashed line represents the apparent limit of applicability of the DCP model. The fit values are $s=4.22\text{nm}$ and $\lambda_{\text{RL}}=7.70\times 10^8$.

Figure 18.15: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 5.0. There is no clear limit of applicability of the DCP model for this experiment. The fit values are $s=3.36$ nm and $\lambda_{\text{RL}}=6.21 \times 10^8$.

Figure 18.16: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 6.0. The dashed line represents the apparent limit of applicability of the DCP model. The fit values are $s=2.18$ nm and $\lambda_{RL}=4.77 \times 10^8$.

Figure 18.17: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 7.5. The dashed line represents the apparent limit of applicability of the DCP model. The fit values are $s=3.87$ nm and $\lambda_{RL}=7.62 \times 10^8$.

Figure 18.18: Least squares fit of the detached charged plane model to SR mobility as a function of ionic strength at pH 9.0. The dashed line represents the apparent limit of applicability of the DCP model. The fit values are $s=5.62$ nm and $\lambda_{RL}=17.5 \times 10^8$.

Table 18.3: Fit parameters for least square fit of detached charged plane model to SR mobility as a function of ionic strength for various pH values.

pH	a (nm)	D (nm)	$\lambda_{RL} (\times 10^8)$	s (nm)	SSQ	n	IS min (M)
4.0	62.5	8	31.8	6.46	0.195	35	0.028
4.7	62.5	8	7.70	4.22	0.0513	14	0.017
5.0	62.5	8	6.21	3.36	1.702	35	none
6.0	138	8	4.77	2.18	0.299	36	0.014
7.5	138	8	7.62	3.87	0.24	35	0.02
9.0	119	8	17.5	5.62	2.146	42	0.028

In the above tests of the detached charged plane model we have assumed that the influence of the lipid bilayer on the electrophoretic mobility of SR is negligible. In the next step we now evaluate this assumption using the Ohshima 2005 model for a colloid

with charged surface covered with a uniform retardation layer (including mobility relaxation effects). This model is summarized in Chapter 5. We assume the charge of the APN domain of SR is not present so that we are only left with the charge of the lipid bilayer. We used the values of λ_{RL} and D of the retardation layer obtained using the detached plane model presented in Table 18.3. The surface charge density was set to that of the lipid bilayer, which ranges from -15.9 mC/m^2 for pH 4.0 to -20.4 mC/m^2 for pH 9.0. The results are presented in Figure 18.19. We see that for pH 4.0, 4.7, 5.0, 7.5, and 9.0 for our entire ionic strength range, the mobility of these model SR vesicles containing the lipid bilayer covered by the retardation layer is less than -0.2 mobility units. However for pH 6.0 for ionic strength less than about 30 mM, the mobility due to lipids, predicted by this model is be greater, almost reaching -0.6 mobility units at 1 mM ionic strength. The reason for the anomalous pH 6.0 results stand out in this way is that the softness parameter, λ_{RL} , is small compared to the values used form the other pH values.

Recall from Chapter 17, that we found for the lipid bilayer of PC liposomes that the permeable surface model was applicable to the lipid headgroup region of the lipid bilayer. Small ions can penetrate the lipid headgroup region and therefore reduce the electric potential at the liposome surface. It is reasonable to assume that this is also the case with the lipid bilayer of SR. Because of this we can assume that the mobility values obtained here are higher than the actual values. We conclude that for pH 6.0 it is possible that the lipid bilayer charge would affect the mobility of SR as a function of ionic strength, especially at low ionic strength, but the effect at other pH values is negligible.

Figure 18.19: Calculation of mobility of lipid bilayer of SR assuming the lipid charge is on the surface of the membrane, the retardation layer has the same parameters as found by the detached plane model, and the detached charged plane is missing.

Section 2: The effect of lipophilic ions on mobility of SR vesicles

In Chapter 15 we presented our findings regarding the electrophoretic mobility of SR vesicles and PC liposomes in the presence of lipophilic ions. We discussed our findings for PC liposomes in Chapter 16. In this chapter we discuss the mobility of SR vesicles in the presence of lipophilic ions. Previously, in our application of the detached charged plane model to the mobility of SR as a function of ionic strength (Section 1 of this chapter), we assumed that the native charge of the APN domain of SR could be

represented by a plane of charge, detached from the membrane surface. This plane of charge is embedded in a homogeneous retardation layer, which was used to represent the surface structure of SR that causes hydrodynamic frictional forces between the fluid and the protruding proteins during electrophoresis. We found that the charge associated with the APN domain determines the mobility and that the charge below the APN domain has a negligible effect on the mobility for all pH values studied except pH 6.0.

In this section we additionally assume that lipophilic ions can sorb to three locations (1) the detached charged plane that represents the APN domain (2) the lipid bilayer and (3) the remaining regions of the Ca^{2+} -ATPase (the stalk and trans-membrane regions). When calculating the model predictions of mobility, we only consider the native and sorbed charge associated with the APN portion of the Ca^{2+} -ATPase. We use the values we obtained from the least-squares-fit of the detached charged plane model to mobility of SR as a function of ionic strength (Table 18.3) for s and λ_{RL} . Most of these experiments were done at pH 7.0 or pH 7.5, for which we use $\lambda_{RL} = 7.62 \times 10^8$ and $s = 3.87$ nm (obtained for pH 7.5). Two experiments (TePB^- and TePP^+) were done at pH 4.0. For these we use $\lambda_{RL} = 31.8 \times 10^8$ and $s = 6.46$ nm (obtained for pH 4.0).

Calculation of number of lipophilic ions sorbed to each APN domain

We examine the data in two ways. Here we describe the first way. For each mobility measurement, we calculate the number of lipophilic ions sorbed to each APN domain as predicted by the detached charged plane model. We also calculate the percent volume of the APN domain occupied by these sorbed lipophilic ions. This gives us

insight into the physical consequences of our model. First we calculate the mobility structure factor of the detached charged plane model.

$$MSF = \frac{\kappa^2}{\kappa^2 - \lambda_{RL}^2} \left\{ \frac{1}{\cosh(\lambda_{RL} D)} \left[\exp(-\kappa s) + \left(\frac{\kappa}{\lambda_{RL}} \right) \sinh(\lambda_{RL} s) \right] - \frac{\lambda_{RL}}{\kappa} \cosh(\kappa s) \exp(-\kappa D) \left[\tanh(\lambda_{RL} D) + \frac{\lambda_{RL}}{\kappa} \right] \right\}. \quad (18.5)$$

Then we use the following equation to calculate the mobility of a hard sphere, μ_{HS} , with a surface charge density equal to the charge density of the detached plane. We assume the measured mobility is μ_{DCPM} .

$$\mu_{HS} = \frac{\mu_{DCPM}}{MSF}. \quad (18.6)$$

We then use the Smoluchowski equation and the hard sphere mobility value, μ_{HS} , to calculate the corresponding zeta potential and ultimately the surface charge density (Equations (18.7) and (18.8)). We use the Smoluchowski model rather than the OHW model because the OHW equation cannot easily be solved for ζ . Note that the OHW mobility is always less than or equal to the Smoluchowski mobility for a given surface charge density and ionic strength. Because of this, the OHW model would predict equal or greater numbers of calculated sorbed lipophilic ions.

$$\zeta_{HS} = \frac{\mu_{HS} \eta}{\epsilon \epsilon_0} \quad (18.7)$$

The surface charge density of the equivalent hard sphere containing the sorbed lipophilic ions is

$$\sigma_{0,HS} = \frac{2\varepsilon\varepsilon_0 kT}{ze} \sinh\left(\frac{ze\zeta_{HS}}{2kT}\right) \quad (18.8)$$

After we obtain the surface charge density, it is a simple matter to calculate the number of lipophilic ions sorbed. First we calculate the surface charge density due to sorbed lipophilic ions:

$$\sigma_{sorbed} = \sigma_{0,HS} - \sigma_{native} \quad (18.9)$$

Then we calculate the number of lipophilic ions sorbed, $N_{Lsorbed}$ using the following equation:

$$N_{Lsorbed} = \frac{\sigma_{sorbed} UCA}{ez} \quad (18.10)$$

where UCA is the unit cell area of the SR membrane (40 nm^2).

Figure 18.20 through Figure 18.25 show the calculated number of lipophilic ions sorbed to the detached charged plane per APN domain according to our model. On the right axis we graph the fraction of the volume of the APN domain occupied by the sorbed lipophilic ions per APN domain. The following volumes of lipophilic ions were used:

TePB⁻ $3.24 \times 10^{-28} \text{ m}^3$, TePP⁺ $3.31 \times 10^{-28} \text{ m}^3$, Clomipramine⁺ $3.12 \times 10^{-28} \text{ m}^3$, Imipramine⁺ $2.99 \times 10^{-28} \text{ m}^3$ and PBP⁻ $1.96 \times 10^{-28} \text{ m}^3$. The plots are discussed later in the section.

Figure 18.20: On the left axis the calculated number of Clomipramine⁺ molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial Clomipramine⁺ concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed Clomipramine⁺ molecules. Buffer solution is 10 mM HEPES, 5 mM NaCl titrated to pH 7.0. The important feature of this plot is that the model used requires an enormous sorption of Clomipramine⁺ at high concentration.

Figure 18.21: On the left axis the calculated number of Imipramine⁺ molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial Imipramine⁺ concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed Imipramine⁺ molecules. Buffer solution is 10 mM HEPES, 5 mM NaCl titrated to pH 7.0. As with Clomipramine⁺, the model predicts an enormous amount of absorbed Imipramine⁺ at high concentration.

Figure 18.22: On the left axis the calculated number of TePP⁺ molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial TePP⁺ concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed TePP⁺ molecules. Buffer solution is 3 mM HEPES, 5 mM NaCl titrated to pH 7.5.

Figure 18.23: On the left axis the calculated number of TePB⁻ molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial TePB⁻ concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed TePB⁻ molecules. Buffer solution is 10 mM HEPES, 100 mM NaCl titrated to pH 7.0. Again with TePB⁻ the model predicts a very large number of ions absorbed to the APN domain at high concentration.

Figure 18.24: On the left axis the calculated number of TePB^- molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial TePB^- concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed TePB^- molecules. Buffer solution is 3 mM HEPES, 5 mM NaCl titrated to pH 7.5. Here the model predicts negative sorption, which is a non-physical result although the magnitude of the negative volume is insignificant in view of approximations and assumptions used in the analysis of data.

Figure 18.25: On the left axis the calculated number of PBP⁻ molecules that are sorbed to the detached charged plane of SR required to achieve measured mobilities is graphed as a function of initial PBP⁻ concentration in the electrophoretic mobility experiment. The right axis indicates the percent volume of the APN domain that is equal to the volume of the sorbed PBP⁻ molecules. Buffer solution is 3 mM HEPES, 5 mM NaCl titrated to pH 7.5. Again the model predicts negative sorption, which is a non-physical result although the magnitude of the negative volume is insignificant in view of approximations and assumptions used in the analysis of data.

Description of the combined Langmuir detached charged plane model

In this section we describe a model that combines sorption of lipophilic ions to the lipid bilayer and Ca²⁺-ATPase protein with the detached-charge-plane model to predict the electrophoretic mobility of SR vesicles in the presence of lipophilic ions. First we use

the Langmuir sorption model to express the simultaneous sorption of lipophilic ions to the lipid bilayer and the Ca^{2+} -ATPase protein as a function of initial concentration of lipophilic ion. We assume that the lipid bilayer of SR has the same sorption parameters as the lipid bilayer of PC liposomes obtained in Chapter 14. We do not know the sorption parameters to the protein. These will be our adjustable parameters. After the sorption is calculated, we calculate the detached-charged-plane model mobility as a function of lipophilic ion concentration.

For the mobility calculations, we only consider charge sorbed or native to the APN domain. We assume that only half of the lipophilic ions absorbed to the Ca^{2+} -ATPase absorb to the APN domains (adding their charge to the detached charged plane of the model) and that the other half absorb below the APN domain where they have a negligible effect on the mobility. We made this assumption because we estimated that the lipophilic ions absorb to the Ca^{2+} -ATPase with an even spatial distribution. Since the volume of the APN domain is about equal to the volume of the remainder of the protein, we can therefore assume a 50/50 distribution of absorbed lipophilic ions between those two regions. For these relative-volume estimates, the stalk and trans-membrane portion of the Ca^{2+} -ATPase together were treated as a cylinder with a height of 7.56 nm and a radius of 2.05 nm, which results in a volume of $\sim 1 \times 10^{-25} \text{ m}^3$ (Toyoshima and others 2000). To calculate the total volume of the APN domain, each of the A, P, and N domains were treated as spheres with radii estimated from Toyoshima's scaled illustrations (Figure 2.2) (Toyoshima and others 2000). We used a radius of 1.56 nm for the A domain, 1.77 nm for the P domain and 2.47 nm for the N domain. This gives us a total volume of $1 \times 10^{-25} \text{ m}^3$ for the APN domain thus justifying the 50/50 distribution.

Using the modified Langmuir model we calculate the lipophilic ion concentration that is bound to both the lipid bilayer, C_{bm} , and the Ca^{2+} -ATPase protein, C_{bp} . Note that in the sorption part of the model we evaluate the electric potential at the lipid bilayer surface as well as the detached charged plane. To do this, we solve the following set of equations for the potential at the membrane surface, ψ_0 , the potential at the detached plane, ψ_s and the equilibrium concentration of lipophilic ions, C_{eq} , for a range of values of initial lipophilic ion concentration, C_0 .

$$C_{eq} = C_0 - C_{bm} - C_{bp} \quad (18.11)$$

$$\psi_0 = \frac{\sigma_0}{2\kappa\epsilon\epsilon_0} + \frac{\sigma_s}{\kappa\epsilon\epsilon_0 \exp(\kappa s)} \quad (18.12)$$

$$\psi_s = \frac{\sigma_s}{2\kappa\epsilon\epsilon_0} + \left[\frac{\sigma_0}{\kappa\epsilon\epsilon_0} + \left(\frac{\sigma_s}{2\kappa\epsilon\epsilon_0 \exp(\kappa s)} \right) \right] \cdot \exp(\kappa s) \quad (18.13)$$

with

$$C_{bm} = \Theta_m \cdot \frac{A_L}{A_{S(PC)}} \cdot [\text{lipid}] \quad (18.14)$$

$$\Theta_m = \frac{Kp_{x,0(PC)} C_{eq} \cdot \frac{\exp\left(\frac{-ze\psi_0}{kT}\right)}{C_w}}{\frac{A_L}{A_{S(PC)}} + Kp_{x,0(PC)} C_{eq} \cdot \frac{\exp\left(\frac{-ze\psi_0}{kT}\right)}{C_w}} \quad (18.15)$$

$$C_{bp} = \Theta_p \cdot \frac{UCA}{A_{S(protein)}} \cdot [protein] \quad (18.16)$$

$$\Theta_p = \frac{Kp_{x,0(protein)} C_{eq} \cdot \frac{\exp\left(\frac{-ze\psi_s}{kT}\right)}{C_w}}{\frac{UCA}{A_{S(protein)}} + Kp_{x,0(protein)} C_{eq} \cdot \frac{\exp\left(\frac{-ze\psi_s}{kT}\right)}{C_w}} \quad (18.17)$$

$$\sigma_0 = \frac{C_{bm}ez}{[lipid] \cdot A_L + 2[protein] \cdot A_p} + \sigma_{0,native} \quad (18.18)$$

$$\sigma_s = \frac{\frac{C_{bp}}{2}ez}{[[lipid] \cdot A_L + 2[protein] \cdot A_p] \cdot \frac{1}{2}} + \sigma_{s,native} \quad (18.19)$$

Equations (18.12) and (18.13) are the results of applying the linearized Poisson-Boltzmann equation to the two planes of charge, the membrane surface charged plane due to charged lipids and the detached charged plane of the APN domains. Equations (18.16) and (18.17) are versions of the modified Langmuir function for the bilayer and the protein respectively. Equations (18.18) and (18.19) are the total surface charge density of the two planes of charge. The first term is due to sorbed charge from lipophilic ions and

the second is the native charge. A_P is the surface area on the membrane taken up by the trans-membrane portion of the Ca^{2+} -ATPase. Notice that for Equation (18.19) the concentration of bound lipophilic ion is divided by two. This is because we assume only half of it sorbs to the APN domain of the protein. The $\frac{1}{2}$ in the denominator is because there is only one APN plane of sorption for two lipid planes of sorption (one for each side of the bilayer).

After we have determined the concentrations of lipophilic ions bound to the protein, we calculate predictions for the mobility, using the detached charged plane model. In this case, since we are able to use the OHW model for hard sphere mobility. This minimizes the error due to relaxation and retardation effects.

$$\mu_{DCPM(OHW)} = \mu_{OHW} \cdot MSF \quad (18.20)$$

For experiments in which there is mobility reversal, when we adjusted parameters to fit our model to the experimental data, we prioritized matching the data in the vicinity of the mobility reversal over other regions. At the mobility reversal our model would predict that the charge of the APN domain is zero. At this point, any errors in λ_{RL} or s will be minimized.

The solid lines in Figure 18.26 through Figure 18.28 are the detached charged plane model predictions for sorption to both the lipid bilayer and the detached charged plane. The dashed lines represent the lipid bilayer's contribution to the mobility. These are the predicted using Ohshima's 2005 model for a particle with a retardation layer and charged particle core (Chapter 5). The surface charge density is set equal to the native lipid surface charge density plus the contribution of surface charge density due to sorbed

lipophilic ions in the lipid bilayer. The dotted line shows the mobility if the partition coefficient to the protein is set to zero. This shows the effect of screening the native charge of the APN domain. As the concentration of lipophilic ion in the solution increases, ionic strength increases, electrostatic screening increases and interfacial potential as well as mobility decrease. The dashed/dotted line represents the mobility if the partition coefficient of the bilayer is set to zero. From this curve we can see the effect that the bilayer has on the sorption at the APN domain. It affects both the electric potential at the APN domain and the concentration of lipophilic ions in the aqueous phase.

The values for $Kp_{x,0(protein)}$ and $A_{s(protein)}$ used are given in the captions of each graph. $Kp_{x,0(protein)}$ values tend to be greater than $Kp_{x,0(PC)}$ values for similar numbers of sorbed lipophilic ions per unit cell. This is because of the small number of proteins (and large size) of the proteins increases the mole-fraction partition coefficient. In other words, comparing the PC and SR partition coefficients is not sensible without conversion to volume fraction partition coefficients of something more practical. Also we chose $A_{s(protein)}$ values that were smaller than those for the lipid membrane because we assume only half of the sorbed lipophilic ions sorb to the APN domains detached charged plane, the others are below the APN domain and do not decrease the number of sites on the detached charged plane. This decreases the effect of saturation at the detached charged plane. One way to adjust for this is to make the sorption site area smaller.

Figure 18.26: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial Clomipramine⁺ concentration (solid line). Fit parameters are $K_{p,x,0(\text{protein})} = 5 \times 10^6$ and $A_{S(\text{protein})} = 2 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption occurs only to the bilayer (dotted line) and when sorption occurs only to the protein (dotted/dashed line). Suspending solution was 10 mM HEPES, 5 mM NaCl, pH 7.0. Lipid concentration was 30 μM .

Figure 18.27: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial Imipramine⁺ concentration (solid line). Fit parameters are $K_{p,x,0(\text{protein})} = 8 \times 10^5$ and $A_{S(\text{protein})} = 2 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption occurs only to the bilayer (dotted line) and when sorption occurs only to the protein (dotted/dashed line). Suspending solution was 10 mM HEPES, 5 mM NaCl, pH 7.0. Lipid concentration was 30 μM .

Figure 18.28: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePP^+ concentration (solid line). Fit parameter are $K_{p,x,0(\text{protein})} = 1 \times 10^5$ and $A_{s(\text{protein})} = 2 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption happens only to the bilayer (dotted line) and when sorption happens only to the protein (dotted/dashed line). Suspending solution was 3 mM HEPES, 5 mM NaCl, pH 7.5. Lipid concentration was 90 μM .

Figure 18.29: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePB^- concentration (solid line). Fit parameters are $K_{p,x,0(\text{protein})} = 4 \times 10^8$ and $A_{S(\text{protein})} = 6.5 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption occurs only to the bilayer (dotted line) and when sorption occurs only to the protein (dotted/dashed line). Suspending solution was 10 mM HEPES, 100 mM NaCl, pH 7.0. Lipid concentration was 18 μM .

Figure 18.30: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial PBP⁻ concentration (solid line). Fit parameters are $K_{p,x,0(\text{protein})} = 3 \times 10^9$ and $A_{S(\text{protein})} = 5 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption occurs only to the bilayer (dotted line) and when sorption occurs only to the protein (dotted/dashed line). Suspending solution was 3 mM HEPES, 5 mM NaCl, pH 7.5. Lipid concentration was 90 μM .

Figure 18.31: The detached charged plane model prediction for the mobility of SR vesicles as a function of initial TePB^- concentration (solid line). Fit parameters are $K_{p,x,0(\text{protein})} = 3 \times 10^9$ and $A_{S(\text{protein})} = 5 \times 10^{-19} \text{ m}^2$. Also shown are the mobility due to the lipid bilayer charge only (dashed line) (Ohshima's 2005 model, Chapter 5), and the detached charged plane model for the case when sorption occurs only at the bilayer (dotted line) and when sorption occurs only at the protein (dotted/dashed line). Suspending solution was 3 mM HEPES, 5 mM NaCl, pH 7.5. Lipid concentration was 90 μM .

The detached charged plane model and mobility of SR vesicles in the presence of lipophilic ions.

Here we discuss the consequences of applying the detached charged plane model to mobility of SR vesicles in the presence of lipophilic ions. The assumptions are that (1)

the only charges (native or absorbed) that affect the mobility are associated with the APN domain of SR, (2) all of the charge associated with the APN domain is evenly distributed on a plane of charge located a distance s from the lipid bilayer matrix of SR, (3) the hydrodynamic friction of the protruding Ca^{2+} -ATPase proteins can be modeled by a homogeneous retardation layer with softness parameter λ_{RL} and thickness D ($=8$ nm), and (4) s and λ_{RL} are not altered by the presence of lipophilic ions. The first three assumptions were shown to be applicable to native SR (Section 1 of this chapter). In that section we acquired best fit values of s and λ_{RL} which are given in Table 18.3. We use these values in this section as well.

In the previous section (see Figure 18.17) we found that the detached charged plane model is not applicable below an ionic strength of 20 mM for native SR at pH 7.5. Our studies of SR mobility as a function of concentration of lipophilic ions were performed using buffer solutions that had ionic strengths of 6.5 mM for studies performed at pH 7.5 and 7.4 mM for those performed at pH 7.0, except TePB⁻ pH 7.0 which was performed with 100 mM NaCl (Figure 18.23 and Figure 18.29).

The electrophoretic mobility of our cations (TePP⁺ Figure 18.28, Imipramine⁺ Figure 18.27, and Clomipramine⁺ Figure 18.26) was measured at pH 7.0 in a low ionic strength suspension (7.4 mM). Although the starting ionic strength is out of range of applicability, as lipophilic ion concentration increases the detached charge plane model becomes applicable for two reasons. First, as lipophilic ion concentration is increased, ionic strength increases. Second, as positively charged lipophilic ions sorb to the negatively charged proteins the magnitude of the charge of the APN domain decreases. One consequence is that we observed mobility polarity reversal in these experiments. As

lipophilic cation concentrations increase, mobility becomes less negative and then becomes positive. This would create a range around the polarity reversal where the surface charge density is decreased and the detached charged plane model is applicable. We have indicated the lipophilic ion concentration at which mobility reversal takes place by vertical lines in Figure 18.20 through Figure 18.25.

We notice in Figure 18.20 through Figure 18.25 that when ionic strength is high, as in the high lipophilic ion concentration range in the Clomipramine⁺, Imipramine⁺, and TePP⁺ studies or the TePB⁻ study done in 100 mM NaCl, we see that the calculated volume of lipophilic ions sorbed to the APN domain becomes very large. For Clomipramine⁺ it nearly reaches 100% of the volume of the APN domain. For Imipramine⁺ it reaches 230%. For TePP⁺, which has a very low partition coefficient to PC it only reaches 9% whereas for TePB⁻ in high salt solution the volume fraction reaches 96%. (This is in contrast with our other experiment performed with TePB⁻. Although each experiment is performed at slightly different pH values, the main reason for the discrepancy in the calculated number of lipophilic ions sorbed is the ionic strength of the experiment. At high ionic strength, ions in the aqueous phase decrease the potential of the APN domains allowing more sorption.) These values for Imipramine⁺, Clomipramine⁺ and TePB⁻ are extremely high. Without significant expansion of the APN domain, this much absorption is unphysical. It suggests that the combined model of electrophoretic mobility and lipophilic ion sorption to SR membrane is breaking down. The most likely explanation for the observed breakdown effect is that the location of lipophilic sorption is not the same as that for the native charge. Within the framework of

our models, it follows that if lipophilic ions sorbed closer to the edge of the retardation layer of our model, their effect on mobility would be much greater.

Figure 18.26 through Figure 18.28 show attempts to fit the detached charged plane model to mobility of SR in the presence of lipophilic cations assuming that they sorb to the same detached plane as the native charge. The dashed curves show the mobility due to the lipid bilayer charge only, but with the retardation layer intact. These curves are all very close to zero for the entire lipophilic ion range. They show that even when substantial sorption occurs at the lipid bilayer, its contribution to the mobility is small. The dotted lines show the results of the detached charged plane model if no sorption happens at the detached plane. The reduction of mobility that is observed by these curves is the effect of the native charge being screened at higher ionic strength. Finally the dashed-dotted curves show the results of the detached charged plane model when there is no sorption to the lipid bilayer. Although the lipid bilayer charged does not directly affect the mobility, sorption to the lipid bilayer does for two reasons. First, the charge at the bilayer affects the electric potential at the detached charged plane. Second, the bilayer reduces the number of lipophilic ions in the aqueous phase that are available for sorption into the APN domains.

Under the fit conditions ($\lambda_{RL} = 7.62 \times 10^8$, $s = 3.87$ nm, $Kp_{x,0}$ and A_s of the lipophilic ion for PC liposomes, $a = 138$ nm and $D = 8$ nm) it was not possible to choose a set of $Kp_{x,0}$ and A_s for the protein for which both the polarity reversal of mobility and the mobility maximum predicted by the combined model would match the measured properties. These discrepancies can also be understood if the lipophilic cations are sorbing at a greater distance from the lipid matrix of SR than the charges of native SR. If

this were the case, then the calculated numbers of lipophilic ions sorbed to the APN domain in Figure 18.20 through Figure 18.25 would be substantially smaller. The current model used in data analysis overestimates the numbers actually sorbed. The same explanation applies to the calculated volumes of sorbed lipophilic ions in APN domains.

The effect of shift of sorption plane associated with APN domains is qualitatively demonstrated in Figure 18.32. The effect of changing the location of the sorption plane is illustrated using our Clomipramine⁺ experimental data. For all other parameters kept constant each consecutive curve corresponds to a shift of the charged plane to one nanometer closer to the edge of the retardation layer (8 nm). The red curve corresponds to the position of the charged plane very near the edge of the retardation layer. The values of $Kp_{x,0(protein)}$ (5×10^6) and $A_{s(protein)}$ ($2 \times 10^{-19} \text{ m}^2$) are the same as the values chosen in Figure 18.26. It follows that small changes in s can have a large effect on the mobility, and that progressive shifts of the position of sorption plane away from the lipid matrix of SR with the increasing aqueous concentration of lipophilic ions would result in greater predicted mobilities and would qualitatively explain the observed discrepancies.

Figure 18.32: Illustration of the effect of changing the location of the detached charged plane for our Clomipramine⁺ experiment while keeping λ_{RL} the same. $K_{p,x,0(\text{protein})} = 5 \times 10^6$ and $A_{s(\text{protein})} = 2 \times 10^{-19} \text{ m}^2$. Suspending solution was 10 mM HEPES, 5 mM NaCl, pH 7.0. Lipid concentration was 30 μM .

For anions, at high lipophilic ion concentration, the experimental mobilities become greater in magnitude. They are affected by sorbed lipophilic ions. However, the detached charged plane model is not able to predict these increases even with high partition coefficients to the proteins of SR. It can be understood within the framework of the detached charged plane model if at higher concentrations of lipophilic ions, and consequently greater charge of the APN domains, the configurations of APN domains changes in such a way that the absorbed charges are located farther away from the

surface of the lipid matrix of SR. Another alternative to explain the observed discrepancy is that these lipophilic ions absorb closer to the edge of the retardation layer than s .

The differences between the calculated and measured mobility values at low concentrations of lipophilic ions, often referred to as baseline discrepancies, notable in Figs 18.29, 18.30, 18.31 may be due to the ionic strength being out of the range of applicability of the detached charged plane model or λ_{RL} and s values that were not available for the given pH.

Chapter 19: Final Overview and Conclusions

Section 1: Overview

This dissertation project on the electrophoretic mobility of sarcoplasmic reticulum (SR) membrane vesicles became an extensive one because the properties of electrophoretic mobility of SR could not be understood in terms of conventional electrophoretic mobility models. By necessity, the project included studies of electrophoretic mobility of single-layered and multi-layered liposomes prepared from lipid components similar to those present in the lipid matrix of SR membrane. Liposomes were used to develop insight into the properties of lipid matrix of SR. In addition, mobility studies of SR vesicles and liposomes were done in the presence of negatively and positively charged lipophilic ions, known for their high affinity to lipid membranes. This has the effect of altering the electric charge of the vesicles and liposomes as the ions sorb to them, and the technique was used “as a probe”, done in order to learn how changes to the membrane’s charge affect the electrophoretic mobility of SR vesicles and PC liposomes.

In order to attempt a quantitative understanding of the effect of lipophilic ions on SR vesicles and liposomes it was necessary to perform separate studies of the sorption of these ions into membranes of lipid and SR vesicles. Partition coefficients of the lipophilic ions were measured for both types of membranes. Using our experimental values of partition coefficients it became possible to estimate the amount of electric charge associated with SR and lipid membranes and to relate them to changes of electrophoretic

mobility. The experimental variables were the pH, the ionic strength of the suspending solution, the electrostatic potential of the surface of lipid and SR vesicles, and the electric charge and aqueous concentration of lipophilic ions.

When this project was designed, experimental and theoretical electrophoretic mobility studies were typically done on particles with smooth surfaces. During the project it was hypothesized that the failure of existing electrophoretic mobility models was due to the electrohydrodynamic complexity of the surface of SR membrane. The surface of SR membrane is covered by a dense array of Ca^{2+} -ATPase proteins, protruding about 8 nm from the lipid matrix of SR. These proteins carry an electric charge that contributes to the net charge of the SR vesicle and drives its electrophoresis. In addition they cause hydrodynamic drag that decreases the electrophoretic velocity of SR vesicles.

A novel electrophoretic mobility model was developed in our research group that may be applicable to the problem of mobility of SR vesicles because it takes into account that electric charges (such as those on Ca^{2+} -ATPase proteins) may be separated from its core surface. This model also accounts for the hydrodynamic friction of the surface of SR by incorporating a retardation layer at the core surface that impedes the flow of fluid around the particle and thus reduces its electrophoretic velocity. The model, identified as DCPM (detached charge plane model) provided an analytical expression for particle mobility in terms of its electric and hydrodynamic properties including the geometry of its retardation layer. A major part of the dissertation project was devoted to the experimental study of the electrophoretic mobility of SR and lipid vesicles and their analysis in terms of electrophoretic mobility models available in literature and this new model developed in our research group.

Section 2: Conclusions

The sorption of lipophilic ions by SR, as measured by partition coefficients, was found to be significantly greater than for lipid membranes alone. Since the lipid membrane of SR is not significantly different than a pure lipid membrane, the increased sorption is almost certainly due to the Ca^{2+} -ATPase proteins.

For liposomes, charged either by lipophilic ions or by charged lipids, we found that their electrophoretic mobility could only be explained by the charge being located below their surface and screened by counter-ions inside the polar head groups. We call this model the “permeable surface model”, as opposed to the “impermeable surface model”, which corresponds to more traditional thinking where ions do not penetrate the interior of the membrane. The assumptions of the penetrable surface model are that (1) the charge of the sorbed lipophilic ions exists on a plane at depth, d , below the surface of the liposome within the lipid headgroup region and (2) small ions (Na^+ , K^+ , Cl^-) are able to penetrate the lipid headgroup region with a molar membrane-water partition coefficient of 0.4. The mobility is very sensitive to the depth of the interior charged layer. For lipophilic ions, we can conclude that the depth corresponds to the average location of the charged portion of the lipophilic ion in the membrane. This is a valuable quantity for the study of the effect of lipophilic ions on membrane function.

We then applied this model to the mobility of charged liposomes as a function of ionic strength. Charged liposomes were made using a mixture of negatively charged lipids (PG) and neutral lipids (PC) with a mole fraction of charged lipids of 0.067. Two

varieties of liposomes were studied, multi-layered and single-layered. Again we found that the permeable surface model was superior to the impermeable surface model for predicting the mobility of charged liposomes. A value of 0.4 for the molar partition coefficient of small ions to lipid headgroup region was again used. The values obtained by the best fits for the depth of the charged plane due to PG lipids were found to be 0.2 nm for single-layered liposomes and 0.1 nm for multi-layered liposomes. In fact, the headgroup regions of PC lipids are larger than those of PG lipids, which is consistent with these findings. The liposome sizes used in the permeable model fits were consistent with size measurements taken from photoelectron micrographs of single-layered and multi-layered liposomes.

Electrophoretic mobility of SR vesicles was measured as a function of ionic strength for six pH values. First we analyzed mobility of SR as a function of pH interpolated to the intermediate ionic strength of 100 mM. We found that the electrophoretic mobility was directly correlated with the total charge of the APN domain of the Ca^{2+} -ATPase. A linear relationship was found between the total charge of the APN domain, calculated using the frequency and pKa values of the amino acid residues of the domain, and the electrophoretic mobility. Also, the total charge of the APN domain was equal to zero at the mobility reversal point. The effect of charge due to gangliosides on the SR lipid membrane surface was found to be negligible. We conclude from this that the charge due to the APN domain is the main determining factor of the electrophoretic mobility of SR.

The models of Levine (Levine and others 1983) and Ohshima (Ohshima 2005) are the two best available soft particle models in the literature. Neither adequately describes

the mobility of SR. Levine's model includes a homogeneous retardation layer with a homogeneous volume charge density. Ohshima's 2005 model is for a soft particle with a homogeneously charged surface and includes the advanced features of retardation and relaxation effects.

We found that we could describe the mobility of SR as a function of ionic strength using a new detached charged plane (DCP) model. The assumptions of the DCP model are that (1) the friction caused by protruding proteins on the surface of SR can be represented by a homogeneous retardation layer of thickness D and softness parameter λ_{RL} , and (2) the charge of the APN domain can be represented as a plane of charge embedded in the retardation layer at a distance s from the membrane surface. This model does not account for other sources of charge, as we found these to have a negligible effect on mobility. The adjustable parameters of our best fits of this model to the data were λ_{RL} and s . We did not find consistent values of λ_{RL} and s for our various pH values, and we suggest that this is due to conformational changes or denaturation of the protein as the pH of the solution deviates from the native pH of the interior of muscle cells (~pH 6.9).

The values we obtained for the softness parameters using the DCP model ranged from 31×10^8 to $4.8 \times 10^8 \text{ m}^{-1}$. These represent Debye-Bueche lengths (the representative distance of flow penetration into the retardation layer) of 0.32 to 2.1 nm. These values are similar to values in the literature for gram negative and gram positive bacterial cell walls which were found to range from about 0.4 to 6.5 nm (Duval and Gaboriaud 2010) and red blood cell surfaces which were found to range from 0.6 to 1 nm (Nakano and others 1994).

Electrophoretic mobility of SR was also measured as a function of lipophilic ion concentration for multiple lipophilic cations and anions. To estimate the location of absorption of the lipophilic ions as a function of lipophilic ion concentration, we used the DCP model to calculate the mobility that would result from the ions' center of charge being located at the level of the APN domain. The sorbed charge and native charge were assumed to be at the same plane because the DCP model only includes a single plane of charge. We used the detached charged plane distance s and softness parameter λ_{RL} previously found from the best fits of the DCP model for SR mobility versus ionic strength. We found for cations, that when the partition coefficient to proteins was adjusted so that the mobility reversal point was matched by the model predictions, the model also predicted significantly lower mobilities than were measured by experiment at high lipophilic ion concentrations. The model suggests that the mobility reversal point would correspond to zero charge in the APN domain. For anions, it was not possible to obtain the experimental mobilities at high lipophilic ion concentration using a reasonable partition coefficient to the proteins of SR. Also the total volumes of the absorbed lipophilic ion would in some case be greater than the estimated volume of the APN domain. This suggests that at high lipophilic ion concentration, either the ions absorb at a more distant location than the APN domain, where they cause an increase in mobility, or their absorption affects the proteins such that s and λ_{RL} are altered.

*Section 3:   **Accomplishments***

The results of this project include several notable highlights. The major result of the mobility studies of SR as a function of pH was the finding that the mobility of SR

vesicles is determined primarily by the electric charge of the A, P, and N domains of Ca^{2+} -ATPase. There is a linear relationship between the predicted total charge of the APN domains and the mobility. The pH-dependence of mobility of SR can be quantitatively reproduced by the DCP model, which suggests that the representation of APN domains by the charged plane is physically meaningful.

The DCP model provides an explanation of why the electric charge of the lipid matrix of SR is not manifested in the measured electrophoretic mobility of SR vesicles, and why the charges on APN domains determine the mobility.

The agreement between the measured dependence of mobility of SR on ionic strength at different pH values and the mobility predicted by the DCP model was found to be semiquantitative. It suggests that the electrohydrodynamic properties of the surface of SR are determined by the interdependence of the spatial distribution of electric potential of Ca^{2+} -ATPase and the flow of the aqueous phase surrounding the SR particle. This interdependence cannot be well represented by a homogeneous retardation layer, as is used by the DCP model and other “soft particle” models.

The values of the softness parameter of the retardation layer of SR vesicles, obtained from the least-squares-fit of the DCP model to experimental SR mobility data, were found to be within the range of values obtained recently for the surface of bacteria.

Results of electrophoretic mobility studies of vesicles prepared from neutral and negatively charged phospholipids and their analysis revealed that the electric charges determining their mobility do not reside at the liposome surface. Our finding is that they are located at some distance below the surface and that small ions from the aqueous phase penetrate into headgroups of phospholipid vesicles. This established a foundation

for the so-called “permeable surface model” as an alternative to the conventional empirical slip plane model used in the conventional analysis of electrophoretic mobility of liposomes.

The important innovative feature of the dissertation project is that it integrated studies of sorption of lipophilic ions to liposomes and SR vesicles with studies of their electrophoretic mobility. It was possible to develop insight into the distribution of a number of lipophilic ions between the lipid matrix of SR membrane and the Ca^{2+} -ATPase proteins. Similar studies done with phospholipid vesicles have revealed evidence that lipophilic ions penetrate below the surface of liposomes. From electrostatic models of the interface it was possible to estimate the depth of localization of electric charge in the phospholipid bilayer membrane. This information is likely to also be applicable to the lipid matrix of SR membrane.

Chapter 20: References

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