

Electrohydrodynamics of Simple and Complex Interfaces

by

Paul F. Salipante

B.A., Dartmouth College; Hanover, NH, 2007

M.S., Dartmouth College; Hanover, NH, 2009

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in the School of Engineering at Brown University

PROVIDENCE, RHODE ISLAND

May 2013

© Copyright 2013 by Paul F. Salipante

This dissertation by Paul F. Salipante is accepted in its present form
by the School of Engineering as satisfying the
dissertation requirement for the degree of Doctor of Philosophy.

Date_____

Petia M. Vlahovska, Ph.D., Advisor

Recommended to the Graduate Council

Date_____

Kenneth Breuer, Ph.D., Reader

Date_____

Thomas Powers, Ph.D., Reader

Approved by the Graduate Council

Date_____

Peter Weber, Dean of the Graduate School

Acknowledgements

I would like to thank my advisor, Petia Vlahovska, who has been instrumental in developing my abilities as a scientist. Her passion for science and her dedication to teaching has been a motivation from my first course in fluid dynamics up to today. This work would not have been possible without her excellent teaching and her encouragement.

I would also like to thank the members of the theory department at the Max Planck Institute for Colloids and Interfaces in Golm, Germany, who were extremely supportive during my time there. I especially thank Rumiana Dimova and Roland Knorr for their assistance with my experimental work on biomembranes.

I would also like to thank the group members with whom I have worked and had fruitful discussions. In particular, James Hanna, Jacopo Seiwert, Tatiana Kuriabova, Malika Ouriemi, Jonathan Schwalbe, Adam Musial, Scott Decker, Marc Shapiro, Hui He, Fernanda Lugo-Bolanos, and Lynn Della Grotta.

Finally, the encouragement of my family and friends throughout my graduate studies has been invaluable. In particular, Alex Dahlman, for her persistent support and my parents, Bob and Cathie Salipante, for supporting my entire education and cultivating my interest in science.

PAUL F. SALIPANTE

182 HOPE STREET · PROVIDENCE, RI · 02912
paul.salipante@brown.edu (781) 454-6751

EDUCATION

- 2009 to 2013** **Doctor of Philosophy in Engineering (Ph.D.)**
Brown University School of Engineering
THESIS Electrohydrodynamics of Simple and Complex Interfaces
- 2007 to 2009** **Master of Science / Master of Engineering Management (M.S./M.E.M.)**
Dartmouth College Thayer School of Engineering
THESIS Deformation and Rotation of a Drop in a Uniform DC Electric Field
- 2007 to 2008** **Bachelor of Engineering (B.E.)**
Dartmouth College Thayer School of Engineering
ABET accredited Bachelor of Engineering degree
- 2003 to 2007** **Bachelor of Arts (B.A.)**
Dartmouth College
MAJOR Engineering Sciences (High Honors)
MINOR Government

PUBLICATIONS

- 2013** **P. Salipante and P.M. Vlahovska** “Nonlinear electrohydrodynamics of a viscous droplet.” (submitted)
- 2013** **H. He, P. Salipante, and P.M. Vlahovska** “Electrorotation of a viscous droplet in a uniform DC electric field.” *Physics of Fluids*, **25**, 2013, 032106.
- 2012** **P. Salipante, R. Knorr, R. Dimova, P.M. Vlahovska** “Electrodeformation method for measuring the capacitance of bilayer membranes.” *Soft Matter*, **8**, 2012, 3810-3816
- 2010** **P. Salipante and P.M. Vlahovska** “Rotation of a deformable drop in a uniform electric field.” *Physics of Fluids*, **22**, 2010, 112110

AWARDS AND CERTIFICATIONS

- 2013** NIST NRC Postdoctoral Research Associateship, National Institute of Standards and Technology
- 2012** Simon Ostrach Fellowship, Brown University
- 2012** Sigma Xi Scientific Research Society Member
- 2010** NSF Dissertation Enhancement Grant
- 2009** Citation for Engineering Sciences 156 Teaching Assistant
- 2008** Tau Beta Pi National Engineering Honors Society
- 2007** Engineering in Training (EIT) Certification

TEACHING ASSISTANT

- Spring 2012** **ENGN 2760 - Brown University**
Heat and Mass Transfer (Graduate)
- Fall 2011** **ENGN 0810 - Brown University**
Fluid Mechanics (Undergraduate)
- Spring 2010** **ENGS 156 - Dartmouth College**
Heat, Mass, and Momentum Transfer (Graduate)
- Winter 2010** **ENGS 34 - Dartmouth College**
Fluid Dynamics (Undergraduate)

Fall 2009 **ENGS 177 - Dartmouth College**
 Decision-Making Under Risk and Uncertainty (Graduate)

EMPLOYMENT

2010 - 2011	Max Planck Institute for Colloids and Interfaces	Potsdam, Germany
POSITION	Visiting Researcher	
Summer 2008	Town of Wolfeboro, NH	Wolfeboro, NH
POSITION	Summer Energy Intern	
Summer 2007	Savin Engineering, P.C.	Pleasantville, NY
POSITION	Summer Intern	
Summer 2006	MFS Investment Management	Boston, MA
POSITION	Summer Intern for Fixed Income Quantitative Team	

PRESENTATIONS

June 2012	National Institute for Standards and Technology (NIST)
GAITHERSBURG, MD	Invited Speaker
August 2011	Gordon-Kenan Research Seminar - Condensed Matter Physics
NEW LONDON, NH	Invited Speaker
May 2011	Max Planck Institute for Colloids and Interfaces - Theory Department Seminar
POTSDAM, GERMANY	Invited Speaker
September 2009	Master's Thesis - Electrohydrodynamics of Drops in Strong Fields
HANOVER, NH	Master's Thesis Defense
August 2009	Gordon Research Conference - Soft Condensed Matter Physics
NEW LONDON, NH	Poster Presenter - 1 of 4 poster award presenter selected from pool of 70
June 2007	Senior Thesis - Measurement of Thermal Conductivity of Human Nail Plate
HANOVER, NH	Undergraduate Senior Thesis Defense

CONTRIBUTED TALKS

2009 - 2012	APS - Division of Fluid Dynamics November Meeting
2012	AICHE Annual Meeting
2012	ICREA Symposium 2012 - "Nanofluidics, Colloids, and Membranes"
2012	APS March Meeting

COMPUTER SKILLS

Engineering Software	Matlab, Mathematica, Comsol, Abaqus, Solidworks, ProE
Other Software	Microsoft Office, Adobe Illustrator, $\text{\LaTeX}$
Systems	Mac, Windows

Contents

1	Introduction and motivation	1
2	Background	8
2.1	Introduction	8
2.2	Governing equations and Boundary Conditions	15
2.3	List of Symbols	19
3	Nonlinear electrohydrodynamics of a viscous drop	21
3.1	Introduction	21
3.1.1	Charge accumulation	24
3.1.2	Drop deformation	25
3.2	Quincke rotation	29
3.3	Summary of previous work	32
3.4	Timescales and dimensionless numbers	34
3.5	Experimental methods	37
3.5.1	Fluid system	37
3.5.2	Experimental setup and procedure	40
3.6	Experimental results	43
3.6.1	Types of behaviors	43
3.6.2	Phase diagram	48
3.6.3	Transition to unsteady behavior	52
3.6.4	Unsteady drop deformation	58
3.7	Fluid ellipsoid model	67
3.7.1	Rigid ellipsoid	67
3.7.2	Fluid ellipsoid	69
3.7.3	Model results	76
3.7.4	Model phase diagram	80
3.7.5	Rotation rate: comparison with Quincke	84
3.7.6	Phase behavior: comparison to experiment	86
3.7.7	Lyapunov exponents	88
3.8	Conclusions	91
3.9	Future directions	92
4	Measurement of membrane capacitance and membrane viscosity in AC electric fields	99

4.1	Introduction	99
4.2	Complex interfaces: membranes	102
4.2.1	Physical Model	103
4.2.2	Vesicle Model	104
4.2.3	Dimensionless variables and timescales	106
4.2.4	Governing Equations	108
4.2.5	Solution approach	111
4.2.6	Hydrodynamic field	114
4.2.7	Evolution equation	118
4.2.8	Transition frequency	125
4.2.9	Model Results	127
4.3	Materials and experimental procedures	133
4.3.1	Preparation of giant vesicles	133
4.3.2	Electrodeformation set-up	135
4.3.3	Optical microscopy and imaging	136
4.4	Measurement of membrane capacitance	137
4.4.1	Effect of membrane thickness and vesicle radius	140
4.4.2	Effect of salt concentration (solutions conductivity)	142
4.4.3	Effect of field strength	145
4.4.4	Time dependent deformation	149
4.5	Conclusions	158
4.6	Future Directions	159
5	Vesicle dynamics in DC electric pulses	162
5.1	Introduction	162
5.2	Physical system	164
5.2.1	Timescales and Nondimensionalization	166
5.2.2	Governing equations	170
5.2.3	Discharging	179
5.2.4	Model results	182
5.3	Experimental methods	189
5.4	Results: experiment	193
5.4.1	Weak fields: prolate deformation	193
5.4.2	Strong fields: oblate deformation and collapse	197
5.4.3	Two-step pulse	198
5.4.4	Phase diagram	203
5.5	Conclusions	204
5.6	Future Directions	206
6	Cylindrical deformation and vesicle collapse in DC electric pulses	208
6.1	Introduction	208
6.2	Vesicle collapse: observations	210
6.2.1	Rate of deformation	213
6.2.2	Comparison with simple interfaces	216
6.3	Cylindrical deformation from membrane poration	223

6.3.1	Experiment	226
6.4	Conclusions	231
6.5	Future Directions	232
7	Membrane fluctuations in AC electric fields	234
7.1	Introduction	234
7.2	Membrane fluctuations	236
7.3	Experimental procedure	241
7.4	Results	245
7.5	Conclusions	249
7.6	Future directions	250
8	Conclusion	252
A	Vesicle deformation in an external field: general equations	256
A.1	Hydrodynamic stresses	256
A.2	Membrane stresses	257
A.3	Results	258
B	Higher order vesicle deformation	260
B.1	Physical model	260
B.2	Stresses	263
C	Membrane fluctuations	265
C.1	Seifert-Langer theory	265
C.2	Values of material properties	269
C.3	Relaxation rates: dependence on membrane properties	271

List of Tables

3.1	Material properties for drop experiments.	38
3.2	Characteristic timescales for the experimental fluid systems and values of the dimensionless parameters estimated at a field strength where unsteady behavior is observed $E_0 = 1\text{kV/cm}$ and $E_0 = 10\text{kV/cm}$ and drop radius 3mm.	38
3.3	Capillary numbers, Ca , for typical ranges of drop diameter and electric field strengths.	39
3.4	Capillary numbers, Ca_ω , for typical ranges of drop diameter and electric field strengths.	39
4.1	Material properties of the amphiphiles used in this study. In all polymer systems, the 1,2-butadiene content is about 90%.	134
4.2	Capacitance values for $\text{PBd}_n\text{-b-PEO}_m$ measured with the electrodeformation method C_{mm} and corrected for the effect of the Debye layers. The uncertainty is determined using error propagation and weighted sample variance for uncertainties associated with conductivity measurements and transition frequency.	143
6.1	Values for membrane capacitance, critical transmembrane voltage, and lysis tension estimated from the Eq.[6.11].	231
C.1	Material values for various membrane types. Citations: 1) [68], 2) [48], 3) [160], 4) [57], 5) [136]	269
C.2	Cutoff wave vectors for different membranes.	269
C.3	Wave vector dependence for γ_1 for various membrane types	270
C.4	Wave vector dependence for γ_2 for various membrane types	270

List of Figures

1.1	Schematic representation of the cell membrane [1]	2
1.2	Sketch of a lipid membrane vesicle.	4
2.1	Sketch of a fluctuating membrane [146].	10
2.2	Examples of the charging of a a) vesicle and b) drop.	13
2.3	Due to differences in solution conductivities, ions accumulate at different rates at membrane physical surfaces. This gives rise to an imbalance in the charge densities on the inner and outer surface, which in the zero-thickness limit appears as induced surface charge, similar to simple fluid-fluid interfaces [125, 172]. In the depicted case, $\lambda_{\text{ex}} < \lambda_{\text{in}}$. The surface charge is negative in the opposite case.	14
3.1	Definition of axes where $a_{\parallel}$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right). An aspect ratio from the projected image is defined as $\beta = a_{\parallel}/a_{\perp}$	22
3.2	Charge distribution for drops with a) $R/S > 1$ and b) $R/S < 1$	25
3.3	Surface charge distribution and direction of surface electric tractions for a drop with $R/S < 1$ (a) and $R/S > 1$ (b).	26
3.4	R-S diagram of prolate and oblate deformation determined by Taylor's discriminating function. When $R/S < 1$ the flow direction is from pole to equator for both oblate and prolate drop shapes, denoted by OB and PR_B . When $R/S > 1$ the direction of flow is from the drop equator to pole and the shape is always prolate, denoted by PR_A .	29
3.5	Charge distribution and induced dipole $\mathbf{P}$ in unstable equilibrium for a sphere with $R/S < 1$. Above a critical field strength $E_0 > E_Q$, where $E_0 = \mathbf{E} $ and E_Q is given by Eq.[3.16], constant rotation around an axis perpendicular to the electric field is induced by the misaligned dipole moment of the particle (right). The rotation can be either clockwise or counter-clockwise.	30
3.6	Exposure to a uniform direct current (DC) electric field with increasing strength excites a variety of drop responses [168]. a) A silicone oil drop suspended in castor oil is spherical in the absence of electric field. b). Weak fields induce axisymmetric oblate deformation. c) In strong fields, the drop is tilted with respect to the applied field direction and the flow is rotational. The sketches illustrate the drop shape and flow streamlines.	32
3.7	Critical field strength for initiation of drop rotation for different viscosity ratios and drop diameters [168].	33
3.8	(top) Drops at increasing and then decreasing field strength. Drop remains tilted as field strength is decreased. (bottom) Hysteresis in the tilt angle observed for 2.5mm diameter, $\Lambda=0.14$ drop [168].	34
3.9	Plot of $\frac{\omega_Q^{-1}}{t_{\text{hd}}}$ for different viscosity ratios.	36
3.10	Sketch of the experimental setup.	40

3.11	Experimental setup in laboratory. The amplifier is connected to the power supply (left) and chamber with camera (right).	41
3.12	Model for drop electrorotation. Drop asphericity is characterized by the aspect ratio $\beta=a_{\parallel}/a_{\perp}$. The rigid body (Quincke) rotation is related to the tilt angle of the deformed shape, θ , by $\omega = d\theta/dt$. The difference between the interface velocity and the Quincke rotation speed quantified by the rotation rate ν	44
3.13	Examples of one period of unsteady drop behavior for three viscosity ratios. Low viscosity drops deform and oscillate while high viscosity drops tumble. The parameters for the drops shown are a) $\Lambda=1.1$, $E_0=9.9\text{kV/cm}$, $2a=3.6\text{mm}$, b) $\Lambda=4.5$, $E_0=8.1\text{kV/cm}$, $2a=5.4\text{mm}$, and c) $\Lambda=14$, $E_0=9.7\text{kV/cm}$, $2a=6.0\text{mm}$. . .	45
3.14	Polar plots of orientation angle (angular axis) compared to aspect ratio (radial axis) during unsteady behavior for three different viscosity ratios $\Lambda = 1.1, 4.5, 14$ (left to right). The parameters for the $\Lambda = 1.1$ drop (left) are $2a = 3.7\text{mm}$, $E_0 = 3.2E_Q$. The parameters for the $\Lambda = 4.5$ drop (center) are $2a = 3.1\text{mm}$, $E_0 = 3.2E_Q$. The parameters for the $\Lambda = 14$ drop (right) are $2a = 5.4\text{mm}$, $E_0 = 3.6E_Q$	46
3.15	Drop breakup for different viscosity drops: (top) Breakup for 5.8mm , $\Lambda = 1.1$ drop in 10.2 kV/cm . (middle) Breakup for 5.8mm , $\Lambda = 4.5$ drop in 10.2 kV/cm . (bottom) Breakup for $\Lambda = 14$ in 10.7 kV/cm	47
3.16	Phase diagrams for different viscosity ratios: (top) $\Lambda = 0.7$ and 1.1 , (middle) $\Lambda = 2.9, 4.5$ (bottom) $\Lambda = 14$. The electric field is slowly increased and steady-state behavior is observed. Taylor (Ta) denotes axisymmetric flow and oblate deformation. Tilted (Ti) denotes a tilted drop orientation with rotational flow. Unsteady (Un) denotes time dependent drop shape and orientation. Breakup (Br) denotes regions where drop breakup is observed.	49
3.17	Aspect ratio as a function of electric field strength: (top) $\Lambda=1$, (bottom) $\Lambda = 14$	53
3.18	Field strength for the transition from steady to unsteady behavior for different viscosity ratios. $\square$ denotes drops that have initial diameter in range of $5.3\text{-}5.7\text{mm}$ and $\circ$ denotes drops that have an initial diameter $3.5\text{-}3.8\text{mm}$	54
3.19	Drop aspect ratio is compared to initial drop diameter at the transition to unsteady drop shape. The data show that for a given viscosity ratio, the transition to unsteady rotation will occur at a similar aspect ratio.	55
3.20	Drop aspect ratio at the transition from steady to unsteady for different viscosity ratios as a function of initial drop diameter $2a$	56
3.21	Aspect ratio at the transition from steady to unsteady for different viscosity ratios as a function of Ca_{ω}	56
3.22	Drop orientation angle for a $\Lambda = 1.1$, $a=3.6\text{mm}$, drop in a field with strength $E_0 = 9.9\text{ kV/cm}$ ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).	59
3.23	Aspect ratio β^{-1} for a $\Lambda = 1.1$, $2a=3.6\text{mm}$ drop in an electric field with strength $E_0 = 9.9\text{ kV/cm}$ ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).	59
3.24	Drop orientation angle for a $\Lambda = 1$, $2a=5.4\text{mm}$ drop in an electric field with strength $E_0 = 9.9\text{ kV/cm}$ ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).	60
3.25	Aspect ratio β^{-1} for a $\Lambda = 1.1$, $2a=5.4\text{mm}$ drop in an electric field with strength $E_0 = 9.9\text{ kV/cm}$ ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).	60
3.26	Drop orientation angle for a $\Lambda = 4.5$, $2a=3.5\text{mm}$ drop in an electric field with strength $E_0 = 9.1\text{ kV/cm}$ ($3.1 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).	62

3.27	Aspect ratio β^{-1} for a $\Lambda = 4.5$, $2a=3.5\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).	62
3.28	Drop orientation angle for a $\Lambda = 4.5$, $2a=5.6\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).	63
3.29	Aspect ratio β^{-1} for a $\Lambda = 4.5$, $2a=5.6\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).	63
3.30	Drop orientation angle for a $\Lambda = 14$, $2a=6.9\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).	64
3.31	Aspect ratio β^{-1} for a $\Lambda = 14$, $2a=6.9\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).	65
3.32	Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 4$ $E_0 = 1.2E_Q$, $\beta^{-1} = 1.4$. The initial condition is $\theta = \pi/2 - \theta'$, $\theta' = 0.02$	77
3.33	Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 4$ $E_0 = 2.3E_Q$, $\beta^{-1} = 2.8$. The initial condition is $\theta' = 0.02$	77
3.34	Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 14$ $E_0 = 2E_Q$, $\beta^{-1} = 1.3$. The initial condition is $\theta' = 0.02$	78
3.35	Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 14$ $E_0 = 2.5E_Q \text{ kV/cm}$, $\beta^{-1} = 1.8$. The initial conditions are $\theta' = 0.02$ and $\theta' = 0.02 + \pi/10^5$	79
3.36	Phase diagram for a fluid ellipsoid with viscosity ratio $\Lambda = 14$. The lines represent the boundaries between various behaviors computed from the model. These are denoted by the regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic. The symbols correspond to experimental data: $\square$ - Taylor (steady axisymmetrically oriented ellipsoid), $\diamond$ - steady tilted ellipsoid, $\triangle$ - chaotic tumbling. The lines crossing the symbols reflect the variations in the aspect ratio of the drop during the rotation.	80
3.37	Phase diagram from tank treading ellipsoid model $\Lambda = 4$. Regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic.	81
3.38	Phase diagram from tank treading ellipsoid model $\Lambda = 1$. Regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic.	82
3.39	Comparison of the Quincke rotation rate ω with the rotational flow rate computed from model, ν , for $\beta^{-1} = 1.2$ for viscosity ratios $\Lambda = 1, 4, 14$	84
3.40	Comparison of the Quincke rotation rate ω with the rotational flow rate computed from model, ν , for $\Lambda = 1$ for viscosity for aspect ratios $\beta^{-1} = 1.2, 2, 3$	85
3.41	Swinging behavior for fluid ellipsoid model with $\Lambda = 4$, $\beta^{-1} = 3$, electric field strength $E_0 = 3E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).	86
3.42	Swinging behavior for fluid ellipsoid model with $\Lambda = 4$, $\beta^{-1} = 3$, electric field strength $E_0 = 4E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).	87
3.43	Swinging behavior for fluid ellipsoid model with $\Lambda = 14$, $\beta^{-1} = 1.4$, electric field strength $E_0 = 1.8E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).	88

3.44	Contour plot of Lyapunov exponents for $\Lambda = 14, 4, 1$ from top left to bottom center. Positive values indicate regions of chaotic behavior (red and orange). Values close to 0 indicate regions of oscillatory solutions including swinging and tumbling (green). Negative values indicate stable solutions, tilted or aligned (blue and white).	90
3.45	Silicone oil drop suspended in castor oil subjected to a uniform DC electric field at $t=0$ s. The drop deforms into an oblate ellipsoid and then breaks up at ~ 2 s. . .	94
3.46	Silicone oil in PS-DMF mix showing prolate deformation.	95
3.47	Prototypes of a coil (top) and ribbon (bottom) "swimmer". The coil is made from a curing silicone clay and the ribbon is cut from a cylinder made from cured silicone.	96
4.1	The steady electric field around a vesicle immersed in an electrolyte solution, following the imposition of a background uniform AC field (a) $f > f_c$ (b) $f < f_c$. Over a charging time t_{mm} Eq.[4.3] dipolar charge distribution forms in response to currents from the bulk.	104
4.2	Definition of axes where $a_{\parallel}$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right) ellipsoid. The deformation parameter is defined as $D = a_{\parallel}/a_{\perp}$	106
4.3	Typical values of isotropic membrane tension as function of applied electric field for different values of initial tension. The isotropic tension increases nonlinearly with electric field strength, but only reaches values of $\sigma_h \sim 10^{-5} \text{N/m}$ for typical electric field strengths.	123
4.4	Frequency sweep showing the effect of membrane capacitance of membrane capacitance. $C_m = 0.7 \mu\text{F}/\text{cm}^2$ (solid line), $C_m = 0.3 \mu\text{F}/\text{cm}^2$ (dashed line), $C_m = 0.7 \mu\text{F}/\text{cm}^2$ (dash-dot line). $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 30 \mu\text{S}/\text{cm}$, $R = 0.17$, $a = 20 \mu\text{m}$, $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$, $\kappa = 15 k_B T$	128
4.5	Frequency sweep showing the effect of conductivity ratio for a typical range of conductivities used in experiments. The external conductivity is set at $\lambda_{\text{ex}} = 10 \mu\text{S}/\text{cm}$, Internal conductivity is varied from $\lambda_{\text{in}} = 100 \mu\text{S}/\text{cm}$ ($R = 10$) (solid line), $\lambda_{\text{in}} = 20 \mu\text{S}/\text{cm}$ ($R = 2$) (dashed line), $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$ ($R = 0.5$) (dash-dot line), $\lambda_{\text{in}} = 1 \mu\text{S}/\text{cm}$ ($R = 0.1$) (dotted line). $C_m = 0.7 \mu\text{F}/\text{cm}^2$, $a = 20 \mu\text{m}$, $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$, $\kappa = 15 k_B T$	129
4.6	Frequency sweep showing the effect of electric field strength for two values of bending rigidity, $\kappa = 20 k_B T$ (left) and $\kappa = 5 k_B T$ (right). The electric field is varied from $E_0 = 2 \text{kV/m}$ (solid line), $E_0 = 2 \text{kV/m}$ (dashed line), $E_0 = 2 \text{kV/m}$ (dash-dot line). Other parameters are set at $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 30 \mu\text{S}/\text{cm}$, $R = 0.17$, $C_m = 0.7 \mu\text{F}/\text{cm}^2$, $a = 20 \mu\text{m}$, $\sigma_{\text{eq}} = 4 \times 10^{-7} \text{N/m}$	130
4.7	Time dependent deformation for different values of initial tension. The initial tension is varied from $\sigma_{\text{eq}} = 10^{-8} \text{N/m}$ (solid line), $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$ (dashed line), $\sigma_{\text{eq}} = 10^{-6} \text{N/m}$ (dash-dot line). Other parameters are set at $E_0 = 2 \text{kV/m}$, $\lambda_{\text{in}} = 10 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 3 \mu\text{S}/\text{cm}$ ($R = 3.3$), $C_m = 0.27 \mu\text{F}/\text{cm}^2$, $a = 20 \mu\text{m}$, $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$, $\kappa = 15 k_B T$	130
4.8	Time dependent deformation for different values of membrane viscosity, the field is turned on at $t = 0$ and turned off at $t = 50 \text{s}$. The full shape evolution (left) and the relaxation after the field is turned off (right) are plotted. Values for membrane viscosity is varied from $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$ (solid line), $\mu_{\text{mm}} = 10 \times 10^{-7} \text{Ns/m}$ (dashed line), $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$ (dash-dot line). Other parameters are set at $E_0 = 4 \text{kV/m}$, $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$, $\lambda_{\text{in}} = 10 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 3 \mu\text{S}/\text{cm}$ ($R = 3.3$), $C_m = 0.27 \mu\text{F}/\text{cm}^2$, $a = 20 \mu\text{m}$, $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$, $\kappa = 15 k_B T$	131

4.9	Time dependent deformation for different values of electric field strength and bending rigidity, the field is turned on at $t = 0$ and turned off at $t = 50$ s. The shape evolution for two values of bending rigidity, $\kappa = 5k_B T$ (left) is compared to $\kappa = 20k_B T$ (right). The electric field is varied from $E_0 = 6kV/m$ (solid line), $E_0 = 4kV/m$ (dashed line), $E_0 = 2kV/m$ (dash-dot line). Other parameters are set at $\sigma_{eq} = 10^{-7} N/m$, $\lambda_{in} = 10\mu S/cm$, $\lambda_{ex} = 3\mu S/cm$ ($R = 3.3$), $C_m = 0.27\mu F/cm^2$, $a = 20\mu m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$	132
4.10	Method for vesicle preparation using electroformation technique. The lipids or polymers are spread to create a film on an ITO slide (left). After all solvent has been evaporated in a desiccator, another clean ITO glass is used with conducting side facing inward to create a chamber with a 1mm teflon spacer. The chamber is filled with sucrose solution and held together with clips. The chamber is connected to a 10 Hz AC field after 1-2 hours and the vesicle solution is removed with a pipette.	133
4.11	Formation of vesicles using electroformation method.	134
4.12	Electrodeformation chamber.	135
4.13	Microscope setup.	136
4.14	Frequency dependent deformation of polymersomes with $R = 40$ ($\square$) and $R = 0.5$ ($\blacksquare$) exposed to a 6kV/m AC field. For $R = 40$, an increase in elongation is observed in the 10-100kHz range, while at these frequencies for $R = 0.5$ the vesicle changes shape from a prolate to an oblate ellipsoid. Aspect ratio $D > 1$ indicates prolate shape (vesicle elongation in the field direction), while $D < 1$ corresponds to oblate shape. In both cases, the vesicle shape becomes spherical in the MHz frequency range. Increasing or decreasing frequency sweep shows no hysteresis. Polymersome radii are $41\mu m$ ($\square$) and $18\mu m$ ($\blacksquare$).	138
4.15	Comparison of frequency scans for $R = 0.6$ (left) and $R = 40$. The electric conductivities are varied with NaCl, $\sigma_{in} = 25\mu S/cm$, $\sigma_{ex} = 40\mu S/cm$ (left) and $\sigma_{in} = 100\mu S/cm$, $\sigma_{ex} = 2\mu S/cm$ (right). Three frequency sweeps are taken with electric field values of $E_0 = 2kV/m$ (solid line), $E_0 = 4kV/m$ (dashed line), $E_0 = 6kV/m$ (dash-dot line). The values are measured as $a = 18\mu m$ (left) and $a = 41\mu m$ (right). Parameters for the $R = 0.6$ fit are $\sigma_{eq} = 0.2 \times 10^{-7} N/m$, $C_m = 0.27\mu F/cm^2$, $\kappa = 2k_B T$. Parameters for the $R = 40$ fit are $\sigma_{eq} = 40 \times 10^{-7} N/m$, $C_m = 0.27\mu F/cm^2$, $\kappa = 5k_B T$	139
4.16	Critical frequency, f_c , for the oblate-prolate transition as a function of inverse radius of vesicles made of POPC and polymersomes PS1, PS3 and PS4. The solution conductivities are $\lambda_{in} = 130\mu S/cm$ and $\lambda_{ex} = 520\mu S/cm$ ($\Lambda = 0.25$). The data is fit to theoretical transition values with $C_{mm}=0.71\mu F/cm^2$ for POPC, $C_{mm}=0.26\mu F/cm^2$ for PS1, $C_m=0.16\mu F/cm^2$ for PS3, and $C_m=0.065\mu F/cm^2$ for PS4. The relative errors of all experimental systems are comparable. The axis scale obscures the error in systems PS1 and LS1.	141
4.17	Membrane capacitance as a function of polymer molecular weight. Error bar indicate the standard deviation of capacitance values determined from a set of measurements. The theoretical capacitance is based on a thickness to molecular weight relationship $d \sim M_w^b$, where $0.5 < b < 1.0$ and the dielectric constant is assumed to be independent of molecular weight. The solid lines represent the power-law relationships for $b=0.5$ and $b=1.0$	142
4.18	Total specific membrane capacitance at different interior solution conductivities (salt concentrations) for POPC vesicles (LS1). The bilayer capacitance is obtained by subtracting the contribution of the double layers according to Eq.[4.77]. The exterior conductivity was varied to produce a conductivity ratio within $R=0.1-0.3$. The lines are plotted to guide the eye. The error bars represent standard deviation.	144

4.19	Transition frequency for PS2 polymersomes with interior conductivity $\lambda_{\text{in}} = 18 \mu\text{S}/\text{cm}$ in external solutions of different conductivity. All data are fit with $C_m = 0.22 \mu\text{F}/\text{cm}^2$ using Eq.[4.76].	145
4.20	Shape elongation D variation with frequency at different field strengths $E_0 = 6, 4, 2 \text{ kV}/\text{m}$ for a $18 \mu\text{m}$ polymersome (PS1) and conductivity conditions $\lambda_{\text{in}} = 20 \mu\text{S}/\text{cm}$ and $\lambda_{\text{ex}} = 40 \mu\text{S}/\text{cm}$. The transition between prolate and oblate ellipsoids occurs between 7-8 kHz for all field strengths, where $D=1$ corresponds to a spherical shape. There is no observable field strength dependence for the transition frequency (i.e. all transitions occur at the same approximate value). The greatest deformation observed, aspect ratio $D = 1.3$, corresponds to an areal strain $\Delta A/A = 7\%$	146
4.21	(A) $E_0 = 5 \text{ kV}/\text{m}$: upon increase of frequency (from 10 to 50 kHz) a polymersome evolves towards a steady tilted orientation with respect to the applied field radius (B) $E_0 = 20 \text{ kV}/\text{m}$ frequency 100 kHz: Tumbling polymersome, $a = 15 \mu\text{m}$	148
4.22	Time dependent deformation of a polymer (PS2) vesicle at different field strengths. A 100 kHz electric field is turned on at $t = 0\text{s}$ and turned off at approximately $t = 50\text{s}$. The entire experiment is shown with time normalized by t_{hd} (top) and the relaxation is shown normalized by t_{σ} (bottom). The experiment is repeated on the same vesicle for electric field strengths $E_0 = 5 \text{ kV}/\text{m}$ (solid line), $E_0 = 10 \text{ kV}/\text{m}$ (dashed line). The vesicle size is measured to $a = 20.3 \mu\text{m}$, $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 3 \mu\text{S}/\text{cm}$ ($R = 1.6$). Parameters for the fit are $\sigma_{\text{eq}} = 3 \times 10^{-7} \text{ N}/\text{m}$, $C_m = 0.22 \mu\text{F}/\text{cm}^2$, $\kappa = 12 k_B T$, $\mu_{\text{mm}} = 120 \times 10^{-8} \text{ N s}/\text{m}$	150
4.23	Time dependent deformation of a polymer (PS2) vesicle at different field strengths. A 100 kHz electric field is turned on at $t = 0\text{s}$ and turned off at approximately $t = 50\text{s}$. The entire experiment is shown with time normalized by t_{hd} (top) and the relaxation is shown normalized by t_{σ} (bottom). The experiment is repeated on the same vesicle for electric field strengths $E_0 = 4 \text{ kV}/\text{m}$ (solid line), $E_0 = 6 \text{ kV}/\text{m}$ (dash-dot line), $E_0 = 8 \text{ kV}/\text{m}$ (dashed line). The vesicle size is measured to $a = 27 \mu\text{m}$, and the conductivities are $\lambda_{\text{in}} = 7 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 28 \mu\text{S}/\text{cm}$ ($R = 0.25$). Parameters for the fit are $\sigma_{\text{eq}} = 4 \times 10^{-7} \text{ N}/\text{m}$, $C_m = 0.22 \mu\text{F}/\text{cm}^2$, $\kappa = 4 k_B T$, $\mu_{\text{mm}} = 100 \times 10^{-8} \text{ N s}/\text{m}$	151
4.24	The time dependent deformation of a polymer (PS2) from Figure 4.23. Time is normalized by $t_{\sigma h} = a\mu(1 + \Lambda)/\sigma_h$. The magnitude of deformation is normalized by D_{max}	152
4.25	Time dependent deformation of a polymer (PS2) vesicle with different initial tensions. A 100 kHz 10 kV/m electric field is turned on at $t = 0\text{s}$ and turned off at approximately $t = 50\text{s}$. The entire experiment is shown with time normalized by t_{hd} . In this case the field strengths are equal and t_{hd} is identical for both experiments. The vesicles are fit with different initial tension $\sigma_{\text{eq}} = 4 \times 10^{-7} \text{ N}/\text{m}$ (solid line), $\sigma_{\text{eq}} = 1.3 \times 10^{-7} \text{ N}/\text{m}$ (dash-dot line). The tense vesicle size is measured to $a = 20.3 \mu\text{m}$ and the less tense is $a = 12.8 \mu\text{m}$. The conductivity for both vesicles are $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 3 \mu\text{S}/\text{cm}$ ($R = 1.6$). Parameters for both vesicles are fit with $C_m = 0.22 \mu\text{F}/\text{cm}^2$, $\kappa = 13 k_B T$, $\mu_{\text{mm}} = 120 \times 10^{-8} \text{ N s}/\text{m}$	153

4.26	Time dependent deformation of a POPC vesicle with different initial tensions. A 100 kHz $4kV/m$ electric field is turned on at $t = 0s$ and turned off at approximately $t = 30s$. The entire experiment is shown with time normalized by t_{hd} (left) and the relaxation is shown normalized by t_{σ} (right). The vesicles are fit with different initial tension $\sigma_{eq} = 6 \times 10^{-7} N/m$ (solid line), $\sigma_{eq} = 1.2 \times 10^{-7} N/m$ (dash-dot line). The tense vesicle size is measured to $a = 20.3\mu m$ and the less tense is $a = 12.8\mu m$. The conductivity for both vesicles are $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 28\mu S/cm$ ($R = 0.18$). Parameters for both vesicles are fit with $C_m = 0.71\mu F/cm^2$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$. The bending rigidity is set at $\kappa = 20k_B T$ for the tense vesicle, and $\kappa = 3.5k_B T$ for the low tension vesicle.	154
4.27	Time dependent deformation of DOPC, POPC, and PS2 vesicles. A 100 kHz $4kV/m$ electric field is turned on at $t = 0s$ and turned off at approximately $t = 30s$. The entire experiment is shown with time normalized by t_{hd} (left) and the relaxation is shown normalized by t_{σ} (right). The vesicles are fit with different membrane viscosities, the PS2 vesicle is fit with $\mu_{mm} = 100 \times 10^{-8} Ns/m$ (solid line), POPC with $\mu_{mm} = 10 \times 10^{-8} Ns/m$ (dashed line), and DOPC with $\mu_{mm} = 1 \times 10^{-8} Ns/m$ (dash-dot line). The PS2 is measured to $a = 27.0\mu m$, POPC is $a = 21.2\mu m$, and DOPC is $a = 19\mu m$. The conductivity for both vesicles are $\lambda_{in} = 7\mu S/cm$, $\lambda_{ex} = 28\mu S/cm$ ($R = 0.25$). Parameters for PS2 are fit with $C_m = 0.22\mu F/cm^2$, $\sigma_{eq} = 8.5 \times 10^{-7} N/m$, $\kappa = 40k_B T$. Parameters for POPC are fit with $C_m = 0.71\mu F/cm^2$, $\sigma_{eq} = 6.5 \times 10^{-7} N/m$, $\kappa = 24k_B T$. Parameters for DOPC are fit with $C_m = 0.71\mu F/cm^2$, $\sigma_{eq} = 5.5 \times 10^{-7} N/m$, $\kappa = 20k_B T$	156
4.28	Transition to a two-dumbbell shape for a deflated vesicle in a 100 Hz 20 kV/m electric field. The vesicles transitions between a connected vesicle and a dumbbell shaped vesicle (top). Other vesicles remain in the two-dumbbell shape with two equally sized daughter vesicles (bottom).	161
5.1	Examples of vesicle behavior: poration in a short strong DC pulse and collapse in a long weaker pulse. a) DOPC vesicle subjected to a $E_0 = 2kV/cm$, 300 μs pulse. A pore persists after the pulse and is visible due to the loss of contrast at the interface. The pore is fully resealed after approximately 60 ms . b) DOPC vesicle subjected to a $E_0 = 1kV/cm$, 50 ms pulse. Although weaker, the longer pulse leads to collapse.	163
5.2	The steady electric field around a vesicle immersed in an electrolyte solution, following the imposition of a background uniform DC field (a) $t < t_{mm}$ $R > 1$ (b) $t < t_{mm}$ $R < 1$ (c) $t > t_{mm}$. Over a charging time t_{mm} Eq.[5.2] dipolar charge distribution forms in response to currents from the bulk.	165
5.3	Transition from oblate to prolate for DOPC vesicle. A lipid membrane with capacitance $C_m = 0.71\mu F/cm^2$, initial radius $a=24.5 \mu m$, $\lambda_{in}=3 \mu S/cm$, $\lambda_{ex}=10 \mu S/cm$. The charging time is $t_{mm}=0.95$ ms. A two pulse field is applied, pulse 1 is $E_0=400$ kV/m with duration 20 μs pulse followed by $E_0=20$ kV/m strength with duration 50 ms.	171
5.4	(left) The electric forcing term, $C_{20}(t)$, for an initial uncharged vesicle with a field turned on at $t = 0$. (right) The electric forcing term, $C_{20}(t)$, for an initially fully charged vesicle after the field is turned off. The relevant parameters are $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 50\mu S/cm$ ($R = 0.1$), $a = 20\mu m$, $\mu_{mm} = 1 \times 10^{-8} Ns/m$	182
5.5	Effect of field strength by changing magnitude of the first step (left) and second step (right). The duration of the first pulse step is 20 μs and the second is 50 ms . The other vesicle parameters are constant. $\lambda_{in} = 3\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\kappa = 20k_B T$, $\mu_{mm} = 10^{-8} Ns/m$	183

5.6	Effect of membrane tension on vesicle evolution (left) and relaxation (right). The membrane tension is varied from $\sigma_{eq} = 0.1, 1, 10 \times 10^{-7} Nsm$. The first pulse step is $400kV/m$ for $20\mu s$ and then $40kV/m$ for $50ms$. The other vesicle parameters are constant. $\lambda_{in} = 3\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\kappa = 20k_B T$, $\mu_{mm} = 10^{-8} Ns/m$	184
5.7	Effect of bending rigidity on shape evolution. Larger bending rigidity limits resisting transfer of excess area from fluctuations to systematic deformation. Bending rigidity is varied from $\kappa = 20, 50, 100k_B T$. The first pulse step is $400kV/m$ for $20\mu s$ and then $40kV/m$ for $50ms$. The other vesicle parameters are constant. $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\sigma_{eq} = 10^{-7} N/m$, $\mu_{mm} = 10^{-8} Ns/m$	185
5.8	Membrane viscosity slows the rate of deformation, but does not change the equilibrium shape. Membrane viscosity is varied from $\mu_{mm} = 1, 10, 50 \times 10^{-8} Ns/m$. The first pulse step is $400kV/m$ for $20\mu s$ and then $40kV/m$ for $50ms$. The other vesicle parameters are constant: $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\sigma_{eq} = 10^{-7} N/m$	186
5.9	Effect of membrane capacitance. A greater capacitance leads to slower charging. This causes greater deformation into oblate and a greater effect of membrane discharging. Membrane capacitance is varied from $C_m = 0.1, 0.3, 0.7\mu F/cm^2$. The other vesicle parameters are constant: $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $a = 20\mu S/cm$, $\sigma_{eq} = 10^{-7} N/m$, $\mu_{mm} = 1 \times 10^{-8} Ns/m$	187
5.10	Shape evolution for different solution conductivities. An increase in exterior conductivity mostly changes conductivity ratio, R , leading to slightly faster charging. The internal conductivity affects both conductivity ratio and capacitor charging time. A lower internal conductivity leads to slower charging and greater deformation. (left) The external conductivity is varied $\lambda_{ex} = 10, 20, 100\mu S/cm$ and is constant at $\lambda_{in} = 5\mu S/cm$. (right) The internal conductivity is varied $\lambda_{in} = 1, 5, 25\mu S/cm$ and is constant at $\lambda_{ex} = 50\mu S/cm$. The other vesicle parameters are constant: $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\sigma_{eq} = 10^{-7} N/m$, $\mu_{mm} = 1 \times 10^{-8} Ns/m$	188
5.11	Example of the voltage profile 2-step DC pulse.	191
5.12	Definition of axes where $a_{ }$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right). The deformation parameter is defined as $D = a_{ }/a_{\perp}$	192
5.13	DOPC vesicle behavior in a single DC pulse. The electric field is turned on at $t = 0$ for a duration of $50ms$. The field strength is $E_0 = 20kV/m$. The vesicle is fit with $\sigma_{eq} = 5 \times 10^{-7} N/m$, membrane viscosity $\mu_{mm} = 0.1 \times 10^{-8} Ns/m$, or $\Lambda_s \sim 0.04$, $C_m = 0.71\mu F/cm^2$, $a = 24\mu m$, $\kappa = 13k_B T$, $\lambda_{in} = 5.5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$	194
5.14	Prolate deformation for a single vesicle subjected to 10,15, and 20 kV/m 50 μs pulse. The vesicle parameters are $a = 18\mu m$, $C_m = 0.71 \mu F/cm$, $\lambda_{in} = 3 \mu S/cm$, $\lambda_{ex} = 10 \mu S/cm$, $\mu_{mm} = 0.3 \times 10^{-8} Ns/m$, $\sigma_{eq} = 1.7 \times 10^{-7} N/m$, $\kappa = 14 k_B T$	196
5.15	Example of vesicle collapse in a DC pulse. DOPC vesicle subjected to a 1 kV/cm, 50 ms pulse.	197
5.16	Oblate prolate deformation for a vesicle. An initial field of strength 400 kV/m for 20 μs is applied, the field strength is decreased to 20 kV/m for 50 ms. (top) The DOPC vesicle parameters are $a = 20.2\mu m$, $C_m = 0.71 \mu F/cm$, $\lambda_{in} = 2 \mu S/cm$, $\lambda_{ex} = 10 \mu S/cm$, $\mu_{mm} = 0.3 \times 10^{-8} Ns/m$, $\sigma_{eq} = 4 \times 10^{-7} N/m$, $\kappa = 23 k_B T$. (bottom) The polymer vesicle parameters are $a = 27.2\mu m$, $C_m = 0.27 \mu F/cm$, $\lambda_{in} = 2 \mu S/cm$, $\lambda_{ex} = 10 \mu S/cm$, $\mu_{mm} = 30 \times 10^{-8} Ns/m$, $\sigma_{eq} = 2.5 \times 10^{-7} N/m$, $\kappa = 40 k_B T$	199

5.17	Oblate to prolate deformation for two different DOPC vesicle exposed to the same two-step pulse with different initial tensions. The shape deformation for vesicle with a higher initial tension is denoted by squares ($\square$) and the vesicle with a lower initial tension is denoted by circles ($\circ$). An initial field of strength 400 kV/m for 20 μ s is applied, the field strength is decreased to 20 kV/m for 50 ms. The high tension DOPC vesicle parameters, radius $a = 19.9 \mu\text{m}$, $C_m = 0.71 \mu\text{F/cm}$, $\lambda_{\text{in}} = 2.5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 0.1 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 5 \times 10^{-7} \text{ N/m}$, $\kappa = 22 k_B T$. The low tension $\circ$ DOPC vesicle parameters, radius $a = 20.9 \mu\text{m}$, $C_m = 0.71 \mu\text{F/cm}$, $\lambda_{\text{in}} = 2.5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 0.1 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 0.4 \times 10^{-7} \text{ N/m}$, $\kappa = 24 k_B T$	201
5.18	Oblate to prolate deformation for two polymer vesicles. An initial field of strength 400 kV/m for 20 μ s is applied, the field strength is decreased to 20 kV/m or 40 kV/m for 50 ms. The polymer vesicle (PS1) parameters are radius 20.9 μm , $C_m = 0.27 \mu\text{F/cm}$, $\lambda_{\text{in}} = 0.8 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 20 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 1.5 \times 10^{-7} \text{ N/m}$, $\kappa = 4 k_B T$. The polymer vesicle (PS1) parameters are radius 27.2 μm , $C_m = 0.27 \mu\text{F/cm}$, $\lambda_{\text{in}} = 1.5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 30 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 2.1 \times 10^{-7} \text{ N/m}$, $\kappa = 30 k_B T$	202
5.19	Vesicle behavior as a function of the duration and field strength of the first pulse, the duration of the first pulse is normalized by the capacitor charging time t_{mm} . The second pulse is set at $E_0 = 20 \text{ kV/m}$ for 50 μ s. Three outcomes are observed, only prolate deformation, the transition from oblate-to prolate, and only oblate deformation. The oblate only deformation occurs when the vesicle has porated the membrane capacitor is short circuited. The comparison with the timescale t_{crit} is shown with $E_c = 1 \text{ kV/cm}$. The comparison with t_{hd} is shown normalized with $t_{\text{mm}} = 0.5 \text{ ms}$	203
6.1	POPC vesicle in a $E_0 = 2 \text{ kV/cm}$ 2ms DC pulse. The vesicle fully collapses but is able to reform into a vesicle. The phase contrast decreases after it reforms. . . .	211
6.2	Examples of lipid vesicle collapse in a DC pulse. The vesicle radius is $a = 18.4 \mu\text{m}$ and the applied DC electric pulse is $E_0 = 8 \text{ kV/cm}$ and duration 150 μ s.	212
6.3	Polymer vesicle pausing between 266 μ s and 667 μ s (PS1 radius 18 μm) in $E_0 = 4 \text{ kV/cm}$ 5ms DC pulse.	212
6.4	Rotation of a PS3 vesicle rotating in a 6 kV/cm DC field.	213
6.5	Deformation as a function for t/t_{hd} for POPC and polymer (PS1) vesicles. The rate of deformation scales as E_0^2	214
6.6	Deformation as a function for t/t_{hd} for lipid and polymer vesicles. The rate of deformation scales as E_0^2	215
6.7	Collapse of a POPC vesicle (top) and Polymer-PS1 vesicle (bottom) in a single DC pulse. The symbols are experimental data. The solid line corresponds to the model with capacitance, the dashed line is the model of a highly conducting membrane, the dash-dot line is the model with a conducting membrane and no membrane tension. The electric field is turned on at $t = 0$ for a duration of 10ms. The strength of the field for both experiments is 3 kV/cm . The POPC vesicle is fit with $\sigma_{\text{eq}} = 1 \times 10^{-7} \text{ N/m}$, $\mu_{\text{mm}} = 10 \times 10^{-8} \text{ Ns/m}$, $C_m = 0.71 \mu\text{F/cm}^2$, $a = 15.2 \mu\text{m}$, $\kappa = 20 k_B T$, $\sigma_{\text{in}} = 5.0 \mu\text{S/cm}$, $\sigma_{\text{ex}} = 68 \mu\text{S/cm}$. The PS1 vesicle is fit with $\sigma_{\text{eq}} = 1 \times 10^{-7} \text{ N/m}$, $\mu_{\text{mm}} = 30 \times 10^{-8} \text{ Ns/m}$, $C_m = 0.27 \mu\text{F/cm}^2$, $a = 13.4 \mu\text{m}$, $\kappa = 30 k_B T$, $\sigma_{\text{in}} = 5.0 \mu\text{S/cm}$, $\sigma_{\text{ex}} = 68 \mu\text{S/cm}$	221

6.8	Collapse of a POPC (top) and polymer (PS1) (bottom) vesicles at different field strengths. The lines show the results from the evolution equation Eq.[6.8] for different field strengths. (left) The solid line corresponds 2 kV/cm, the dashed line is 3 kV/cm, the dash-dot line is 4 kV/cm. The vesicle is fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$ membrane viscosity $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $a = 14.1, 15.2, 13.2 \mu m$. (right) The solid line corresponds 3 kV/cm, the dashed line is 5 kV/cm, the dash-dot line is 6 kV/cm. The PS1 vesicles are fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $a = 13.7, 17.2, 12.9 \mu m$. The conductivities for both experiments are $\sigma_{in} = 5.0 \mu S/cm$, $\sigma_{ex} = 68 \mu S/cm$	222
6.9	(left) Charges on a vesicle with porated regions at poles. (right) Deformation and direction of forces for $R < 1$ with porated regions near poles.	224
6.10	Sequence of deformation for in a $E_0 = 1 kV/cm$ DC pulse. The angle of the edge is measured from the field axis.	226
6.11	The location of θ_c compared t/t_{mm} for DOPC vesicles. The data is fit for different field strength values, but with the same parameters: $V_c = 2.0V$, $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 70 \mu S/cm$	227
6.12	Critical angle measurements for polymer systems PS1. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=4.2$. The systems all have conductivities $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 70 \mu S/cm$	228
6.13	Critical angle measurements for polymer systems PS2. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=6.5$. The systems all have conductivities $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 70 \mu S/cm$	228
6.14	Critical angle measurements for polymer systems PS3. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=7$. The systems all have conductivities $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 70 \mu S/cm$	229
6.15	Critical angle measurements for polymer systems PS4. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=16$. The systems all have conductivities $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 70 \mu S/cm$	229
7.1	Normalized correlation function for a DOPC vesicle. Average radius of the vesicle is $a=14.3 \mu m$. The symbols represent experimental data calculated using Eq.[7.24] and the lines are the fits of the data for each mode number using Eq.[7.20]. . . .	245
7.2	Relaxation rates as a function of modes n . Theoretical curves show the effect of higher bending rigidity (dashed) and lower tension (dotted). The relaxation rates for a $14.3 \mu m$ DOPC vesicle with and without electric field are compared. . . .	247
7.3	Bending rigidity (top) and membrane tension (bottom) as a function of field strength for one vesicle. The effective bending rigidity given by Eq.[7.1] is plotted for $\kappa^{el} = 1.8[k_B T/(kV/m)^2]E_0^2$, where E_0 is the electric field in kV/m. The effective tension is plotted for $\sigma^{el} = -0.08 \times 10^{-7}[N/m/(kV/m)^2]E_0^2$, where E_0 is the electric field in kV/m.	248
7.4	Normalized correlation function for PEO-Pbd (0.6-b-1.2) membrane compared to normalized Eq.[C.17]. The parameter for the fit are $\kappa = 40k_b T$, $\sigma = 0.3 \times 10^{-7} N/m$, $d = 2.5 \times 10^{-9} m$, $K_a = 0.01 N/m$, $b = 50 \times 10^8 Ns/m^3$, $\mu_{mm} = 1 \times 10^{-6} Ns/m$	251

C.1	The effect of intermonolayer slip, b , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed line shows relaxation rates for identical parameters except for $b = 10 \times 10^8Ns/m^3$.	271
C.2	The effect of membrane thickness, d , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed line shows relaxation rates for identical parameters except for $d = 2.5 \times 10^8Ns/m^3$.	272
C.3	The effect of membrane stretching modulus, K_a , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed line shows relaxation rates for identical parameters except for $K_a = 0.04N/m$.	272
C.4	The effect of membrane bending rigidity, κ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$.	273
C.5	The effect of membrane viscosity, μ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\mu_s = 1 \times 10^{-6}$.	274
C.6	The effect of membrane tension, σ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\sigma = 1 \times 10^{-7}N/m$.	275
C.7	The effect of all parameters excluding tension, on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$, $\sigma = 0$, $d = 2.5 \times 10^{-9}m$, $K_a = 0.04N/m$, $\mu_s = 1 \times 10^{-6}$, $b = 10 \times 10^8Ns/m^3$.	275
C.8	The effect of all parameters, including tension, on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data for with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$, $\sigma = 1 \times 10^{-7}N/m$, $d = 2.5 \times 10^{-9}m$, $K_a = 0.04N/m$, $\mu_s = 1 \times 10^{-6}$, $b = 10 \times 10^8Ns/m^3$.	276

CHAPTER ONE

Introduction and motivation

Living cells depend on electrochemical potentials across membranes for many crucial cell functions. Changes in the transmembrane electric field are necessary for such processes as signal propagation in neurons, directed cell migration, and ciliary motility in cell swimming [36, 15, 123, 124, 118, 24]. The transmembrane electric field is also coupled with the mechanical properties of the membrane. This coupling is utilized by cochlear hair cells to transfer acoustic vibrations into electric signals that can be interpreted by the brain [227].

Electric fields are also used in engineering and medical applications such as the electroporation and electrofusion of cells. Application of an electric field increases the membrane tension, which leads to the opening of a pore. This allows for the transport of molecules such as drugs or DNA across the plasma membrane, which is used for drug delivery and gene therapy technologies [140, 43]. The fundamental concept underlying these phenomena is the dynamic response of a cell membrane to an electric potential, which remains poorly understood [218, 195, 45].

Real biomembranes are a complex structure composed of many molecular components necessary for cell function, see Figure 1.1. The plasma membrane

regulates the electric properties of the cell through the transport of ions across the membrane [152]. The main structural component of the plasma membrane is the lipid bilayer. The lipid bilayer has two main functions for the cell. First, the membrane physically protects the interior of the cell by limiting the transfer of material into the cell to very small molecules such as water [23]. Second, the lipid bilayer is an insulator, shielding the interior from electrical signals and acting as a capacitor [150]. The transmission of molecules, such as DNA, as well as electric conduction through the membrane is regulated by proteins within the lipid bilayer structure.

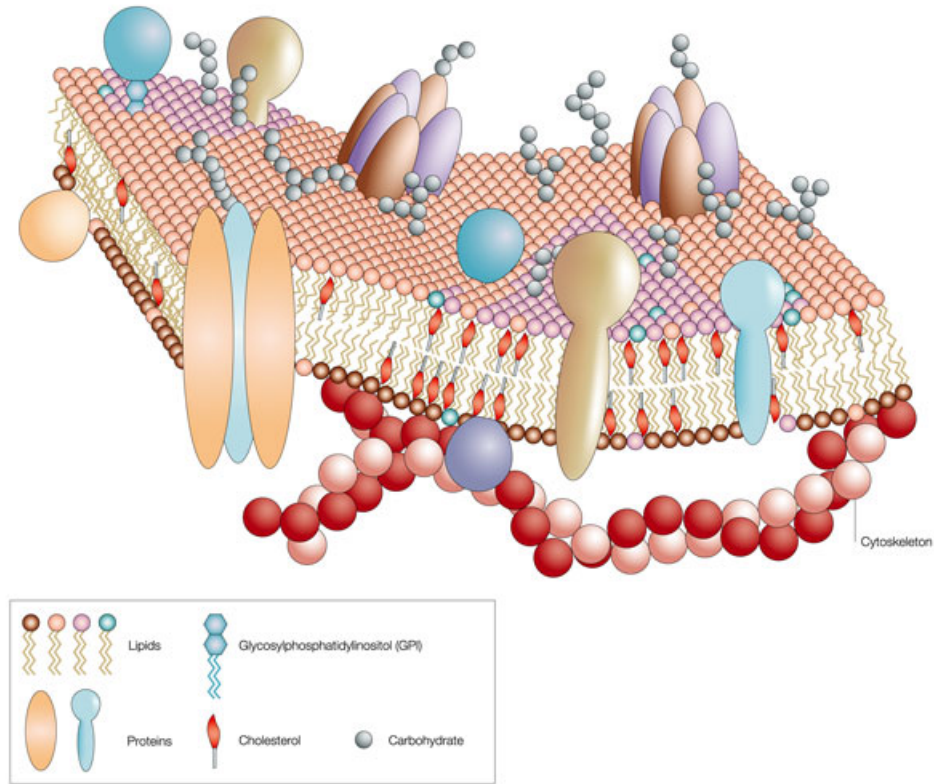


Figure 1.1: Schematic representation of the cell membrane [1]

Fundamental understanding of cell membrane electromechanics necessitates a reduction in the layers of complexity. The approach taken in this thesis is to study interfacial dynamics in electric fields by using the foundations of fluid-fluid interfaces to guide the study of complex behavior of bilayer membranes [125]. Artificial

cell-sized membrane envelopes, called vesicles, are commonly used as a simplified model system for cells. Vesicle membranes are composed only of the lipid bilayer, which allows the study of bilayer membranes in isolation, see Figure 1.2. Giant unilamellar vesicles (GUVs), will be used in this thesis because their large size of 10-100 microns in radius allows visualization of interfacial dynamics with light microscopy, which can then be compared to analytical theories [51].

The dynamics of vesicles under various types of stress, such as an applied flow, have been studied both theoretically and experimentally for over two decades [102, 94, 204, 213, 51]. The steady state configurations of vesicles in an applied electric field have also been investigated [77, 104, 84, 148, 8]. Studies have noticed that due to the finite thickness of the interface, bilayer capacitance is critical to membrane dynamics [207]. Recently, a model incorporating the influence of membrane capacitance on the behavior in AC field was developed [211]. Experimental observations of the dynamic response of vesicles in electric fields have primarily focused on short DC pulses, i.e. less than 1 ms [163, 161, 162]. There are no known observations of vesicle dynamics for longer pulses, where the membrane capacitor is fully charged. One goal of this thesis is to experimentally observe the dynamics of vesicles in both AC and pulsed DC electric fields and provide a quantitative comparison with theoretical models [177, 207, 211].

Electric fields are also used to create pores in membranes [196, 223, 218, 197, 186]. The dynamics of an individual pore, which is created by the application of a uniform stress, such as an osmotic pressure, has been studied theoretically and experimentally [97, 226, 133, 170, 28, 95]. The dynamics of pore resealing have been of particular interest; the timescale for pore closure determines the duration where material can be exchanged across the interface [56, 154, 155]. A physical model for the dynamics of pore opening in an electric field remains a challenge due to the finite time for

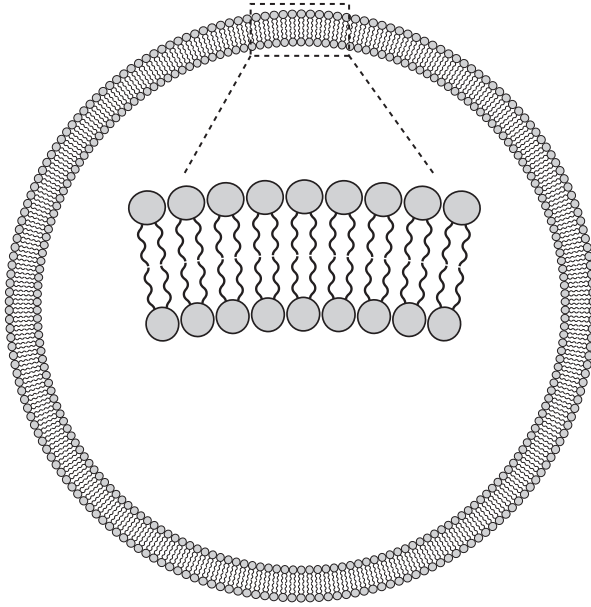


Figure 1.2: Sketch of a lipid membrane vesicle.

interfacial charging and gradients in electric stress. The influence of membrane poration on vesicle dynamics has not been studied in detail, although poration may explain edges observed in short DC pulses [162]. The development of pores affects transmembrane conduction and the structural integrity of the membrane, both of which lead to changes in dynamics. This motivates studying the dynamics of vesicles with porated membranes.

Lipid membranes are soft and easily bent by thermal noise [76]. Physical descriptions of membrane undulations have been developed to include increasing levels of complexity [27, 129, 182, 29, 128]. These models have been used to measure membrane properties, such as bending rigidity, through the observation of membrane shape fluctuations [126, 65, 50, 145, 68, 127, 228]. Real cell membranes are surrounded by charge; lipid molecules can have nonzero charge or electric fields can exist near a cell membrane. How the presence of charges affects membrane

fluctuations has motivated both theoretical descriptions and experimental work [81, 151, 229, 230]. Experimental observations have mainly focused on membranes with charged lipids, or the effect of low frequency AC fields across a planar membrane [108]. A method utilizing the response of the vesicle in an AC field is developed to observe membrane fluctuations on a spherical vesicle in the presence of electric fields.

Vesicle response to an electric field bears similarity to that of drops, therefore the stability of drops in electric fields is also explored [207]. The dynamics of drops in electric fields has potential applications in its own right, for instance, electrorheological fluids and oil separation technologies [21, 20, 112, 143, 209, 193]. Although nonlinear phenomena under creeping flow conditions are rare, nonlinearities in drop electrohydrodynamics offers potential applications for mixing, electrorheology, and microfluidic devices [187, 222, 10, 191, 217, 216, 26]. The nonlinear response of a viscous oil drop in a uniform DC electric field is studied to better understand mechanisms for nonlinear behavior in creeping flow.

Outline

The following chapters investigate the influence of interfacial charging on the dynamics of drops and vesicles. Both drop and vesicle systems are investigated through experimental observation and, when possible, compared to theory. In both cases theoretical descriptions are either developed or adapted from existing models.

Chapter 2 introduces the physical system and governing equations used in this thesis. In particular, bilayer membrane mechanics and the capacitive model for membranes are introduced. The equations governing the electric and fluid behaviors and the assumptions underlying those equations are discussed.

Chapter 3 details the unsteady behavior of a viscous fluid drop in a DC electric field, including many new features such as oscillations and chaotic tumbling. The role of viscosity contrast is shown to be critical to drop dynamics in the combined rotational and extensional flow generated by the interfacial electric stress . A set of systematic observations of silicone oil drops suspended in castor oil is performed to characterize the effect of drop size, viscosity ratio, and field strength. The onset to unsteady behavior is observed by gradually increasing the electric field strength. Drop breakup is also observed for larger drops at high field strengths. The behavior of an individual drop is analyzed by recording the shape and orientation of the drop as a function of time. A fluid ellipsoid model is developed, which predicts features similar to those seen in experiments.

Chapter 4 investigates the steady and time-dependent deformation of vesicles in AC electric fields. A novel method is developed to measure membrane capacitance, an important parameter controlling membrane stability in electric fields [167]. The method utilizes the morphological changes of a vesicle in AC fields. The vesicle evolution in an AC field is also used to determine the viscosity of bilayer membranes.

Chapter 5 investigates the time-dependent deformation of vesicles in DC electric fields. A two-step DC pulse is used to observe vesicle dynamics before and after the membrane capacitor has charged. A theoretical description is developed to describe the behavior in during the two-step pulse and after the field is switched off.

Chapter 6 extends the work from Chapter 5 to observe the dynamics of vesicles in strong DC electric fields, capable of causing membrane poration. The formation of corners in the membrane and vesicle collapse in DC pulse is investigated and related to the formation of membrane pores.

Chapter 7 proposes a method for observing membrane fluctuations on a vesicle in the presence of an AC electric field. The theory for a fluctuating planar membrane is introduced interpret the experiments. The experimental methods for measuring the membrane fluctuations are explained and the effect of electric fields on membrane fluctuations is investigated.

CHAPTER TWO

Background

2.1 Introduction

Lipid bilayers resemble fluid-fluid interfaces in the sense that they are both fluid and deformable. The bilayer thickness, however small, gives rise to more complex features. It imparts resistance to bending and its hydrophobic core does not allow ions to cross the membrane. For a fluid drop, when an electric field is applied across an interface, the conservation of current leads to the accumulation of charges at the interface. In the case of a lipid membrane, ions accumulate on both sides of the membrane and as a result the membrane acts as a capacitor. The charges at the two interfaces create electric stresses on the membrane, which alter the response of the membrane to an applied field compared to simple interfaces. A fluid-fluid interface has interfacial tension to resist changes in area, in contrast, the membrane is an inextensible flexible interface.

In this chapter, the physical description and electro-mechanical coupling of bilayer membranes are introduced and compared to fluid-fluid interfaces. The governing equations and boundary conditions for both systems are given and the

assumptions underlying the use of these equations are discussed.

Zero-thickness electromechanical model of lipid bilayer membranes

The phospholipid (lipid) membrane is the main structural component of cell membranes. Lipid membranes are composed of two layers of lipid molecules. The lipids self-assemble from solution to form a bilayer that shields the hydrophobic core from water. The membrane closes upon itself to form a shell called a vesicle, see Figure 1.2. The physical properties of lipid bilayers and vesicle configurations are surveyed in the classical reviews [22, 115, 180], and more recently in [51]. The bilayer thickness is about 4nm, more than 1/1000 the radius of a typical giant unilamellar vesicles (GUVs) ($a = 20\mu\text{m}$). The large difference in length scales allows the membrane to be treated as a two-dimensional surface embedded in a three-dimensional space. The finite thickness of the membrane is accounted for through modifications to the electro-mechanical properties of the interface, such as bending rigidity and interfacial capacitance.

Bending and entropic elasticity of bilayer membranes

In most membranes, the lipid molecules are able to flow within the membrane; the lipid membrane behaves as a two-dimensional fluid. However, unlike fluid-fluid interfaces the finite bilayer thickness resists changes in curvature and thus also acts like an elastic sheet. Bending leads to compression and expansion of the monolayers, these internal stresses are accounted for by introducing an effective bending modulus κ . A classic result from thin shell elasticity [59, 89, 88, 76, 30, 180] derives bending

stresses in terms of membrane deformation,

$$\boldsymbol{\tau}^\kappa = -\kappa [(2H - C_0)(2H^2 - 2K_G + C_0H) + 2\nabla_s^2 H] \mathbf{n}, \quad (2.1)$$

where κ is the bending modulus, H is the mean curvature, $H = \frac{1}{2}\nabla \cdot \mathbf{n}$, K_G is the Gaussian curvature, $K_G = \frac{1}{2}\nabla \cdot [\mathbf{n}\nabla \cdot \mathbf{n} + \mathbf{n} \times (\nabla \times \mathbf{n})]$. C_0 is the bilayer spontaneous curvature, which is the intrinsic curvature due to asymmetry in packing density of the heads and tails of lipid molecules [50]. $\mathbf{n}$ is the unit normal vector to the interface, ∇_s is the surface gradient operator, and ∇_s^2 is the Laplace-Beltrami operator.

Typical values for the bending rigidity of lipid bilayers are $\kappa \sim 20k_B T$ [121, 51], where k_B is the Boltzmann constant and T is the absolute temperature. The small resistance to bending allows the membrane to be easily bent by thermal noise. As a result, part of the membrane area is stored in thermal shape undulations around a mean shape. These thermal undulations are characterized by a fluctuation spectrum characterized by a mean square displacement $\langle h(q_\perp)^2 \rangle \sim \kappa q_\perp^4 + \sigma q_\perp^2$, where h is the height difference from a mean position and $q_\perp$ is the wavenumber [27, 129, 182, 128]. In this expression, σ is an effective membrane tension, which arises from the constraint of constant surface area for an incompressible membrane [78, 179].

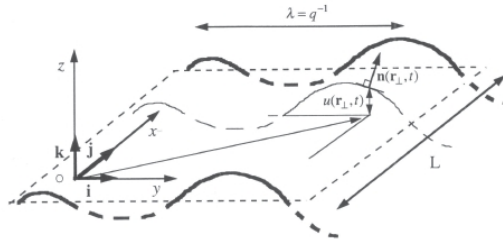


Figure 2.1: Sketch of a fluctuating membrane [146].

When the vesicle deforms due to an applied stress, such as suction pressure from a micropipette [58] or an electric stress [104, 68], area stored in fluctuations can

be pulled out without stretching the true membrane area. Stretching of true area results from increase of the area per lipid molecule. The mean membrane position determines the apparent area A , which is also the area optically observable with a microscope. In the case of a quasi-spherical vesicle, the transfer of area from fluctuations to systematic deformation leads to a change in apparent area, ΔA , which is related to a change in membrane tension by, [58],

$$\frac{\Delta A}{A} = \frac{k_B T}{8\pi\kappa} \ln \frac{\sigma}{\sigma_{\text{eq}}}, \quad (2.2)$$

where σ_{eq} is the membrane tension in the absence of applied forces. If large stresses are applied to the vesicles, all area stored in fluctuations will be pulled out and A becomes equal to the true area. The lipid membrane is assumed to be nearly incompressible due to the high energy cost of increasing the area per lipid; the area expansion modulus is $K_a \sim 200\text{mN/m}$ [159, 51]. At large tension values where stretching can occur, $\sigma \sim 1\text{ mN/m}$, the membrane tension also approaches the limit for membrane rupture, called the lysis tension, $\sigma_{\text{lys}} \sim 5\text{ mN/m}$ [51]. Once the tension exceeds this critical value, the membrane will open pores to relieve tension. If the external stress is removed, the pores may eventually reseal as a result of the edge tension along the pore [95, 155].

Depending on the nature of the applied stress, gradients in tension may occur along the membrane. Area-incompressibility also enforces that the area of a local patch of membrane will remain constant as fluctuations are transferred into systematic deformation. Accordingly, local tension adjusts to keep the local area constant [181]. A position dependent tension arises from the variations in interfacial strain along the interface. In addition, the isotropic tension needs to be determined self-consistently with membrane deformation to account for the global vesicle area stored in either fluctuations or systematic deformation.

In the dynamic response of the vesicle to an external force, the membrane area stored in systematic deformation depends on the magnitude of the external forcing rather than the instantaneous shape [181]. This subtlety has been addressed in the context of vesicle dynamics in shear flow [181, 142, 131, 212], and in this thesis the same approach is taken in the case of electric stresses. The details of this are discussed further in Chapter 4.

The surface forces associated with membrane tension σ are,

$$\boldsymbol{\tau}^\sigma = 2\sigma H\mathbf{n} - \nabla_s \sigma. \quad (2.3)$$

The tangential part of the surface force density, $\nabla_s \sigma$, arises from nonuniformities in the tension, which are needed to ensure local area incompressibility.

In addition to elastic forces, the fluid nature of the membrane leads to viscous dissipation. For a Newtonian surface, the in-plane shear viscous stress is described by [52],

$$\boldsymbol{\tau}^\mu = \mu_{\text{mm}} \nabla_s \cdot (\nabla_s \mathbf{u}_s + \nabla_s \mathbf{u}_s^\dagger), \quad (2.4)$$

where $\dagger$ denotes transpose. The membrane viscosity of lipid bilayers is relatively low, $\mu_{\text{mm}} \sim 10^{-9}\text{Ns/m}$ [44]. Surface viscous effects become important in gel-phase lipid bilayers; a dramatic increase in the surface viscosity is a signature of the fluid-gel transition [44]. Furthermore, in bilayers assembled from polymers, also called polymersomes, the membrane viscosity can be very high, $\mu_{\text{mm}} \sim 10^{-6}\text{Ns/m}$, compared to lipid membranes [49, 48].

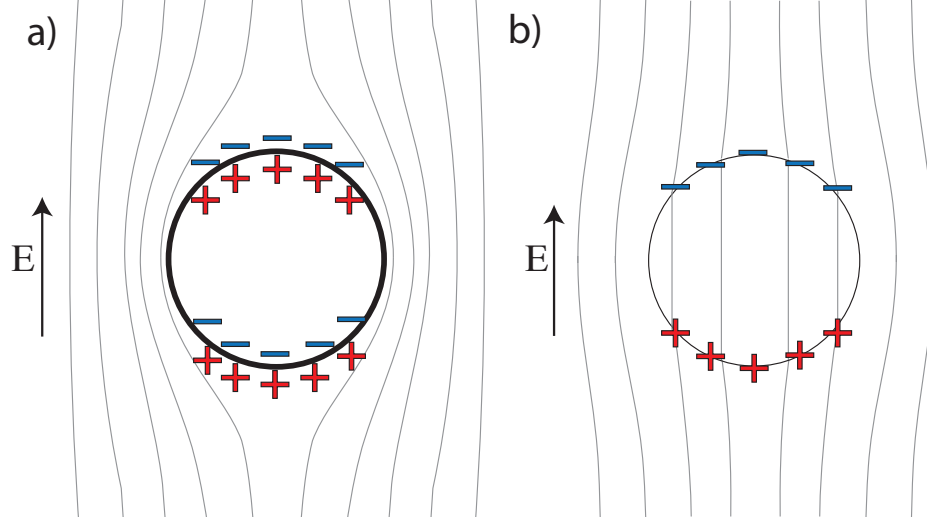


Figure 2.2: Examples of the charging of a a) vesicle and b) drop.

Charging of a spherical shell capacitor system

When an electric field is applied across an interface, charge is conducted from the bulk at a rate given by the characteristic charging timescale of the fluid,

$$t_{c,i} = \frac{\epsilon_i}{\lambda_i}, \quad (2.5)$$

where ϵ is the dielectric constant, λ is the electric conductivity, and i denotes either internal or external bulk fluid. For a fluid drop, charges accumulate at an interface separating two fluids with different electrical properties, see Figure 2.2b. The mismatch in electric properties between the interior and exterior fluids are described by,

$$R = \frac{\lambda_{\text{in}}}{\lambda_{\text{ex}}}, \quad S = \frac{\epsilon_{\text{in}}}{\epsilon_{\text{ex}}}. \quad (2.6)$$

In contrast to the fluid-fluid interface, a bilayer membrane is impermeable to ions. When an electric field is applied across the membrane, free charges will accumulate on both sides of the membrane, therefore the membrane will act as a capacitor [207].

For a spherical vesicle, the membrane will charge with the characteristic timescale for a spherical shell capacitor,

$$t_{\text{mm}} = aC_m \left(\frac{1}{\lambda_{\text{in}}} + \frac{1}{2\lambda_{\text{ex}}} \right), \quad (2.7)$$

where C_m is the capacitance of the membrane and a is the vesicle radius. The membrane capacitance is $C_m = \epsilon_{\text{mm}}/d$, where ϵ_{mm} is the dielectric constant and d is the thickness of the membrane. The finite charging time of a vesicle is controlled by t_{mm} , and as a result regulates the electric stress acting on the vesicle during charging.

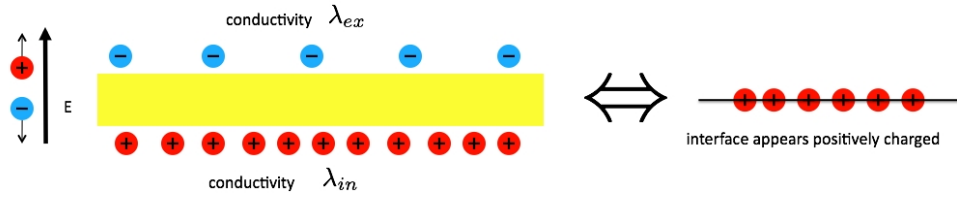


Figure 2.3: Due to differences in solution conductivities, ions accumulate at different rates at membrane physical surfaces. This gives rise to an imbalance in the charge densities on the inner and outer surface, which in the zero-thickness limit appears as induced surface charge, similar to simple fluid-fluid interfaces [125, 172]. In the depicted case, $\lambda_{\text{ex}} < \lambda_{\text{in}}$. The surface charge is negative in the opposite case.

Using fluids of equal dielectric constant, such as aqueous systems, the solution with higher conductivity will conduct charge to the membrane at a faster rate. As the membrane charges there will be an imbalance in charge on the interface, which will result in a net charge on the membrane. In the limit of zero membrane thickness, the interface will appear to have an induced surface charge, similar to a simple fluid-fluid interface, see Figure 2.3.

2.2 Governing equations and Boundary Conditions

The modeling approach for both simple and complex interfaces uses the leaky dielectric assumptions; the bulk fluid is net charge free and the coupling occurs only at the interface.

Hydrodynamic field

Hydrodynamics inside and outside the vesicle obey the Stokes equation,

$$\rho \frac{\partial \mathbf{u}}{\partial t} = \nabla \cdot \mathbf{T}^{\text{hd}}, \quad (2.8)$$

where $\mathbf{u}$ is the fluid velocity and the bulk hydrodynamic stresses is,

$$\mathbf{T}^{\text{hd}} = -p\mathbf{I} + \mu [\nabla \mathbf{u} + (\nabla \mathbf{u})^\dagger]. \quad (2.9)$$

A no-slip boundary condition is used at the interface of both drops and vesicles,

$$\mathbf{u}_{\text{ex}} = \mathbf{u}_{\text{in}} \text{ at } r = a \quad (2.10)$$

Electric field

The electric field is irrotational, $\nabla \times \mathbf{E} = 0$. The absence of magnetic fields and time varying currents in the experiments validates this assumption. Given these

assumptions, the electric field is found from the gradient of the potential,

$$\mathbf{E} = -\nabla\Phi. \quad (2.11)$$

The absence of bulk free charges determines that the electric field is solenoidal ($\nabla \cdot \mathbf{E} = 0$). From this, the electric potential satisfies the Laplace equation,

$$\nabla^2\Phi = 0. \quad (2.12)$$

The boundary conditions for the potential differ between fluid-fluid and lipid membrane interfaces and will be discussed in their respective sections.

Once the electric field has been determined from Eq.[2.12], the electric stresses are determined from the Maxwell stress tensor, written in component form as,

$$T_{ij}^{\text{el}} = \epsilon(E_i E_j - \delta_{ij} E_i E_i / 2). \quad (2.13)$$

Coupling

The hydrodynamic and electric fields are coupled through a stress balance at the interface,

$$\mathbf{n} \cdot [(\mathbf{T}_{\text{ex}}^{\text{hd}} - \mathbf{T}_{\text{in}}^{\text{hd}}) + (\mathbf{T}_{\text{ex}}^{\text{el}} - \mathbf{T}_{\text{in}}^{\text{el}})] = \boldsymbol{\tau}^{\text{int}} \quad (2.14)$$

where $\boldsymbol{\tau}^{\text{int}}$ denotes interfacial stresses. In the case of drops this term is interfacial tension, while in the case of vesicles includes contributions from membrane bending and tension stresses.

Physical Assumptions

The Stokes equation, Eq.[2.16], is used for systems with negligible inertia. The relative importance of inertia to viscous stresses is given by the Reynolds number,

$$Re = \frac{\rho a U}{\mu}, \quad (2.15)$$

where ρ is density, μ is the bulk dynamic viscosity, a is the radius of either a drop or vesicle, and U is the characteristic velocity of the system. Reynolds number must be small for the assumption of negligible inertia to hold. The oil drops satisfy this assumption due to their high viscosity. The simple interface (oil-oil) experimental system has typical values of $\rho \sim 1000 \text{ kg/m}^3$, $a \sim 0.1 \text{ cm}$, $U \sim 0.1 \text{ cm/s}$, and $\mu \sim 1 \text{ kg/(m s)}$, which gives a Reynolds number, $Re \sim 0.001$. Vesicle systems also have small Reynolds numbers, primarily due to their small size. For the a typical vesicle system, the experimental parameters are $\rho \sim 1000 \text{ kg/m}^3$, $a \sim 10 \mu\text{m}$, $U \sim 1 \mu\text{m/ms}$, and $\mu \sim 10^{-3} \text{ kg/(m s)}$. These values give a Reynolds number of $Re \sim 0.01$.

In the case of AC fields, the assumption of $Re \sim 0$ reduces the full Navier-Stokes equation to the unsteady Stokes equation, Eq.[2.16]. A comparison between timescales is needed to determine the relative importance of the unsteady term. Time is normalized by the timescale over which the boundary conditions change, t_p . The dimensionless unsteady Stokes equation becomes,

$$\frac{t_\mu}{t_p} \frac{\partial \tilde{\mathbf{u}}}{\partial t} = \nabla \cdot \tilde{\mathbf{T}}^{\text{hd}}, \quad (2.16)$$

where $t_\mu = a^2 \rho / \mu$ is the viscous timescale and $\sim$ denotes a dimensionless value. The process timescale is the inverse of the applied field frequency, $t_p = f^{-1}$ [188]. The unsteady term in Eq.[2.16] can be neglected if the viscous timescale, $t_\mu = a^2 \rho / \mu$,

is much faster than changes in the field. In this thesis, AC fields are only used for studying vesicles. Typical values for water, $\mu = 10^{-3}$ Ns/m² and $\rho = 1000$ kg/m³, and vesicle radius, $a \sim 20\mu\text{m}$, give a viscous timescale of approximately $t_\mu \sim 100\mu\text{s}$. This corresponds to an applied field frequency of $f \sim 10\text{kHz}$.

Laplace's equation is used to determine the potential, Eq.[2.12]. This assumes that there is no bulk charge. This in turn allows the bulk electric stresses to be neglected in the Stokes equation; the electromechanical coupling is confined to the interface [125].

Limiting the electro-mechanical coupling to the interface simplifies the analysis significantly. Nevertheless, complexity is introduced to the system through the coupling at the interface. In the case of drops, the balance between charge convection and interfacial charging leads to complex behavior. For biomembranes, the finite thickness of the membrane introduces complexity by the capacitive charging of the interface. The governing equations and boundary conditions specific to both drops and vesicles are described in the following chapters.

2.3 List of Symbols

λ	Electrical conductivity
ϵ	Electrical permittivity
μ	Dynamic viscosity
ρ	Density
t	Time
a	Radius
γ	Interfacial tension
κ	Bending rigidity
σ	Membrane tension
E	Applied electric field
Φ	Electrical potential
Q	Electrical charge
$\mathbf{P}$	Electrical dipole
χ^0	Low frequency electrical susceptibility
χ^∞	High frequency electrical susceptibility
$\mathbf{T}$	Stress tensor
$\boldsymbol{\tau}$	Stress vector
L	Torque
Π	Energy
$\mathbf{u}$	Fluid velocity
ν	Surface flow frequency
p	Pressure
ω	Rotation rate

Λ	Viscosity ratio
R	Conductivity ratio
S	Permittivity ratio
β	Aspect ratio
D	Deformation parameter
in	denotes interior quantity
ex	sub/super-script denotes exterior quantity
s	sub/super-script denotes surface quantity
el	sub/super-script denotes electrical quantity
hd	sub/super-script denotes hydrodynamic quantity

CHAPTER THREE

Nonlinear electrohydrodynamics of a viscous drop

3.1 Introduction

The coupling between fluid motion and electric fields is used for a wide range of applications including electrosprays, ink-jet printing, electrohydrodynamic (EHD) pumps, etc. [86, 144, 184]. Many of these EHD applications involve the interaction between an electric field and fluid drops. Exposing a drop to an electric field gives rise to a variety of drop responses, such as deformation, rotation, coalescence, or breakup, which are not completely understood [55, 53, 34].

A drop with no net charge and different physical properties from its surrounding fluid will polarize when placed in a uniform electric field. As a result, the electric stress undergoes a jump across the interface and deforms the drop [125]. If the fluids are perfect dielectrics, there are no free charges and the electric stress discontinuity has only a normal component. At steady state, where electric stresses are balanced by interfacial tension, the drop adopts an equilibrium shape of a prolate ellipsoid and

both internal and external fluids are motionless [105]. Slightly conducting materials behave dramatically differently from ideal dielectrics due to the presence of mobile charges. Charges carried by conduction accumulate at interfaces. The electric field acting on the surface charge creates a tangential stress, not present in the perfect dielectric case, which drags the fluid into motion [172]. The direction of the resulting straining flow about the drop depends on the charge distribution along the interface and the drop can deform into a prolate or oblate ellipsoid. G.I. Taylor introduced the leaky dielectric model to describe the electrohydrodynamic deformation of a drop [194]. The theory applies to weak electric fields, where drop deformation is small. Taylor’s main result is that drop shape is a spheroid with axes $a_{\parallel}$ and $a_{\perp}$ parallel and perpendicular to the direction of the applied electric field, see Figure 5.12.

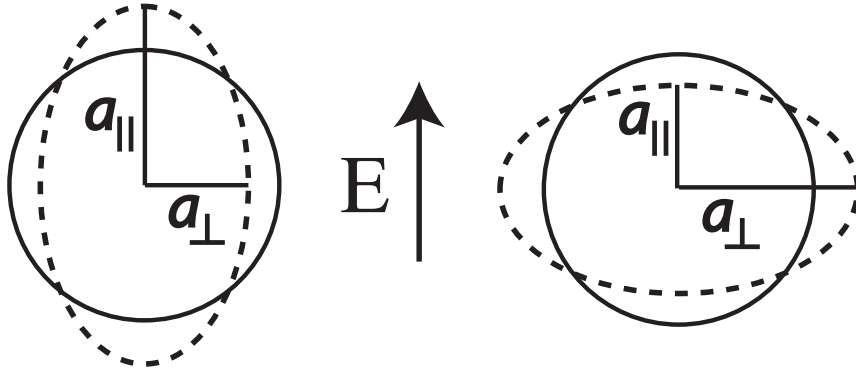


Figure 3.1: Definition of axes where $a_{\parallel}$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right). An aspect ratio from the projected image is defined as $\beta = a_{\parallel}/a_{\perp}$.

Although accurate for small deformation, Taylor’s model does not fully capture all phenomena that have been observed in experiments. Recent studies have discovered non-axisymmetric drop shapes [103, 71, 171], in which the fluids undergo rotational flow and the drop adopts a tilted orientation with respect to the electric field. The continuous fluid circulation resembles the Quincke rotation of a rigid sphere [158]. The spontaneous spinning of a rigid sphere in a uniform electrostatic field was first discovered by Quincke in 1896 [158]. The analogy between spheres and drops has

inspired recent research into drop rotation and deformation in uniform electric fields [164, 71, 61, 113, 171, 98]. The effect relies on a mismatch in charging timescales, leading to an induced surface-charge distribution, which creates an electric torque and induces rotation. The balance between charge convection by rotation and supply by conduction from the bulk fluid results in an oblique dipole orientation.

Previous studies have found that symmetry-breaking occurs only for drops satisfying $R/S < 1$, above a threshold electric field strength comparable to the critical field for Quincke rotation [71]. However, these studies also reported strong dependence of the threshold electric field on drop size and viscosity, which are both absent in the Quincke effect. The effect of convection on the transition to fluid rotation, as well as the orientation of the tilted drop shape, was quantitatively studied in previous work [168, 169]. The rotation of fluid drops in uniform electric fields has also shown transitions to unsteady shape oscillations [171]. Although this work was limited to one viscosity ratio and presented limited analysis of the unsteady behavior. Electrorotation of drops remains poorly understood, in particular the interaction between fluid flow, shape deformation, and charge convection along the interface. To gain insight into these effects, a systematic experimental study is conducted by measuring the transition from steady to unsteady behavior for different drop sizes, viscosity ratios, and electric field strengths.

This chapter discusses the deformation and rotation mechanics that cause nonlinear motion of fluid drops in uniform DC electric fields. The mechanisms of drop deformation will be discussed first, followed by the mechanism for rotation. The descriptions of deformation and rotation motivate the experimental study of drop rotation in strong fields. A theoretical model is developed to describe the nonlinear behavior.

3.1.1 Charge accumulation

Let us consider a neutrally-buoyant spherical drop with radius a , dynamic viscosity μ_{in} , electrical conductivity ϵ_{in} , and dielectric constant ϵ_{in} , freely suspended in a different fluid characterized by μ_{ex} , ϵ_{ex} , and ϵ_{ex} . The drop has no net charge and it is subjected to a uniform DC electric field with a magnitude E_0 .

In an electric field, the charging response for a fluid is characterized by the timescale,

$$t_{c,i} = \frac{\epsilon_i}{\lambda_i}, \quad (3.1)$$

where the index i represents either interior or exterior fluids. Free charges, carried by conduction, accumulate at boundaries that separate media with different electric properties. The magnitude and orientation of the induced dipole depend on the mismatch of electric properties between the drop and the suspending fluid,

$$R = \frac{\lambda_{\text{in}}}{\lambda_{\text{ex}}}, \quad S = \frac{\epsilon_{\text{in}}}{\epsilon_{\text{ex}}}. \quad (3.2)$$

The ratio of R and S compares the charge relaxation times of the media [125, 172],

$$R/S = \frac{t_{c,\text{ex}}}{t_{c,\text{in}}}. \quad (3.3)$$

If $R/S > 1$ ($t_{c,\text{in}} < t_{c,\text{ex}}$), the conduction response of the interior fluid is faster than the exterior fluid. In this case, the induced dipole is oriented in the direction of the applied electric field. If $R/S < 1$ ($t_{c,\text{in}} > t_{c,\text{ex}}$), the conduction response of the exterior fluid is faster than the drop fluid. As a result, the induced dipole is oriented opposite to the applied electric field direction. These two configurations are illustrated in Figure 3.5, for a sphere in a uniform electric field. The characteristic timescale for

interfacial charging of a sphere is given by the Maxwell-Wagner polarization time [92],

$$t_{\text{mw}} = \frac{\epsilon_{\text{in}} + 2\epsilon_{\text{ex}}}{\lambda_{\text{in}} + 2\lambda_{\text{ex}}}. \quad (3.4)$$

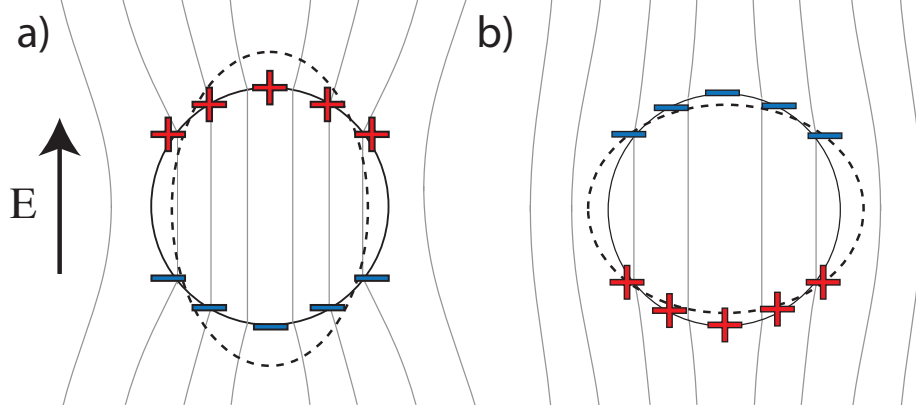


Figure 3.2: Charge distribution for drops with a) $R/S > 1$ and b) $R/S < 1$

3.1.2 Drop deformation

Reiterating the governing equations from Section 2, the static electric field is irrotational and it is assumed that the bulk fluid is net neutral. The electric field is solenoidal and the potential satisfies the Laplace equation,

$$\mathbf{E} = -\nabla\Phi, \quad \nabla^2\Phi = 0. \quad (3.5)$$

For a fluid-fluid interface, the potential is continuous across the interface

$$\Phi_{\text{ex}} - \Phi_{\text{in}} = 0, \quad \text{at } r = a, \quad (3.6)$$

where $r = a$ denotes the location of the interface in spherical coordinates.

Free charge at the interface causes a discontinuity in the normal component of

the displacement vector,

$$\mathbf{n} \cdot [\epsilon_{\text{ex}} \mathbf{E}^{\text{ex}} - \epsilon_{\text{in}} \mathbf{E}^{\text{in}}] = Q, \quad \text{at } r = a, \quad (3.7)$$

where $\mathbf{n}$ is the outward unit normal vector and Q is the interfacial free charge density.

The rate of free charge accumulation is governed by the conservation of electrical current at the interface,

$$\mathbf{n} \cdot [\lambda_{\text{ex}} \mathbf{E}^{\text{ex}} - \lambda_{\text{in}} \mathbf{E}^{\text{in}}] = -\frac{\partial Q}{\partial t} - \nabla_s \cdot (\mathbf{u}_s Q), \quad \text{at } r = a, \quad (3.8)$$

where $\mathbf{u}_s$ is the surface velocity and ∇_s is the surface gradient. Charge convection resulting from the surface velocity is described by the term, $\nabla_s \cdot (\mathbf{u}_s Q)$. The deformation of a leaky dielectric drop was first considered by Melcher and Taylor [125]. Their formulation assumes small steady deformations and negligible charge convection; the righthand side of Eq.[3.8] is zero.

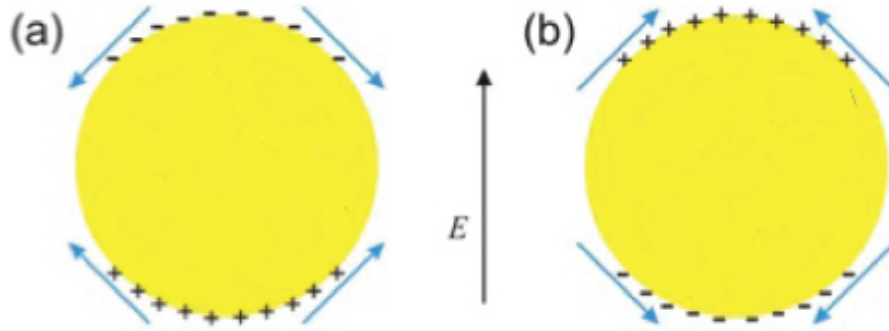


Figure 3.3: Surface charge distribution and direction of surface electric tractions for a drop with $R/S < 1$ (a) and $R/S > 1$ (b).

Taylor's leaky dielectric approach is briefly summarized. The hydrodynamic field obeys the Stokes equation and the fluid is incompressible,

$$\nabla \cdot \mathbf{T}^{\text{hd}} = 0, \quad \nabla \cdot \mathbf{u} = 0. \quad (3.9)$$

The bulk hydrodynamic stress is,

$$\mathbf{T}^{\text{hd}} = -p\mathbf{I} + \mu [\nabla\mathbf{u} + (\nabla\mathbf{u})^\dagger], \quad (3.10)$$

where the superscript $\dagger$ denotes transpose. The fluid boundary conditions are continuous across the interface,

$$\mathbf{u}_{\text{in}} = \mathbf{u}_{\text{ex}} = \mathbf{u}_s, \quad \text{at } r = a. \quad (3.11)$$

The approach taken by Melcher and Taylor is to first solve the steady state electric potential around a sphere using equation Eq.[5.24] subject to boundary conditions given by Eq.[3.7] and Eq.[3.8]. The time dependent charging and charge convection are neglected. In other words, the righthand side of Eq.[3.8] is set equal to zero. The flow field is solved using the tangential part of the stress balance, Eq.[2.14]. The final step is to solve for the equilibrium deformed shape through the radial stress balance, which includes the surface tension. The details of the theory are given in Taylor and Melcher (1969) and summarized in previous work [166].

The main result from the Taylor and Melcher theory is an equation describing drop deformation, D ,

$$D = \frac{a_{\parallel} - a_{\perp}}{a_{\parallel} + a_{\perp}} = \frac{9a\epsilon_{\text{ex}}E_0^2}{16\gamma S(2+R)^2} \left[(R^2 + 1)/S - 2 + 3(R/S - 1)\frac{2 + 3\Lambda}{5 + 5\Lambda} \right] \quad (3.12)$$

where $a_{\parallel,\perp}$ are the semi-axes of the ellipsoidal drop, and γ is interfacial tension. The viscosity ratio is defined as,

$$\Lambda = \frac{\mu_{\text{in}}}{\mu_{\text{ex}}}. \quad (3.13)$$

Oblate deformation is described by $a_{\parallel} < a_{\perp}$ ($D < 1$), while prolate deformation

occurs when $a_{\parallel} > a_{\perp}$ ($D > 1$).

Of particular interest for this thesis is how the type of deformation, prolate or oblate, depends on the system parameters. Solving Eq.[3.12] for $D = 0$ shows that the type of deformation depends on the sign of the expression in brackets. This term is commonly described as the discriminating function. The discriminating function determines the crossover between oblate, $D < 1$, and prolate, $D > 1$, deformation.

The crossover from prolate to oblate is plotted as a function of R , S , and viscosity ratio Λ , in Figure 3.4. In the situation where $R/S > 1$, the induced dipole aligns with the electric field and the drop will always deform the drop into a prolate ellipsoid. For $R/S < 1$, the induced dipole is oriented opposite to the electric field and the deformation can be either prolate or oblate, depending on the values of R , S , and Λ . It is important to note that the crossover between oblate and prolate does not follow the dependence on dipole orientation, i.e. $R/S = 1$. The transition between prolate and oblate depends on all three dimensionless parameters, although viscosity ratio does not significantly change the type of deformation.

The purpose of introducing the small deformation theory is to demonstrate the expected type of drop deformation given certain experimental parameters. This allows a physical system to be chosen that will produce either oblate or prolate deformation, and an induced dipole that aligns either with or against the electric field direction.

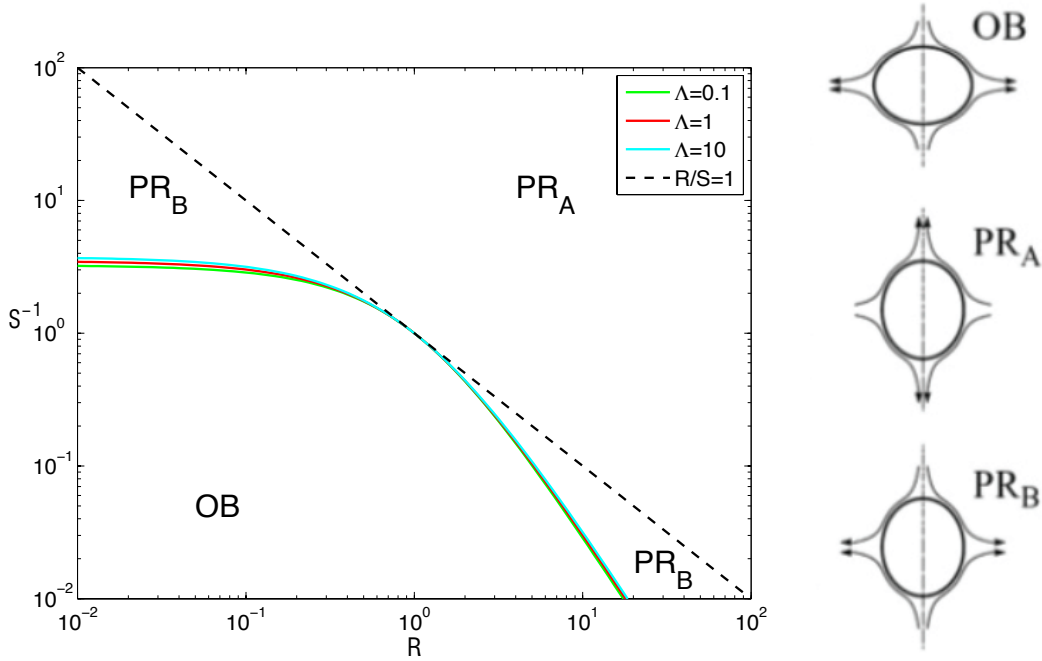


Figure 3.4: R-S diagram of prolate and oblate deformation determined by Taylor’s discriminating function. When $R/S < 1$ the flow direction is from pole to equator for both oblate and prolate drop shapes, denoted by OB and PR_B . When $R/S > 1$ the direction of flow is from the drop equator to pole and the shape is always prolate, denoted by PR_A .

3.2 Quincke rotation

Taylor’s model is limited to small deformation and assumes axisymmetric flow. Symmetry breaking is possible if the electric field produces a torque on the drop. An analogous system, the spontaneous spinning of a rigid sphere in a uniform DC electric field, has been known for over a century, first attributed to the work of Quincke in 1896 [158]. The mechanism for Quincke rotation is introduced for a rigid sphere and serves as a reference for understanding the rotation behavior of a fluid drop.

Analogous to the previous section, if $R/S < 1$ ($t_{c,in} > t_{c,ex}$), the charging response of the exterior fluid is faster than the particle material. As a result, the induced dipole on the particle is oriented opposite to the applied electric field direction. This configuration is unfavorable and becomes unstable above a critical field strength [90,

201, 111]. A perturbation in the dipole alignment gives rise to a torque, which induces physical rotation of the sphere. The induced surface-charge distribution rotates with the particle, but at the same time the exterior fluid continuously recharges the interface. The balance between charge convection by rotation and supply by conduction from the bulk results in an oblique dipole orientation shown in Figure 3.5(right). The rotation rate, ω , is determined from the conservation of angular momentum.

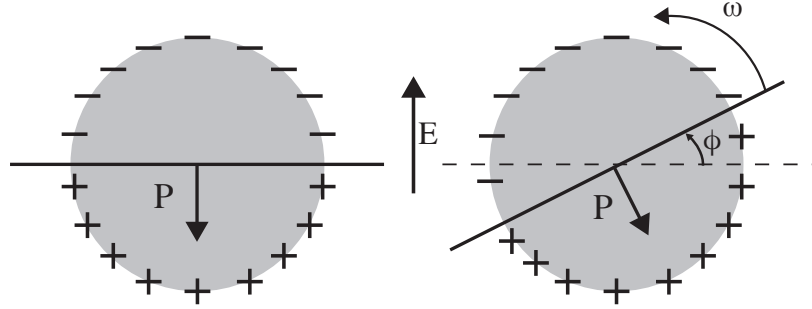


Figure 3.5: Charge distribution and induced dipole $\mathbf{P}$ in unstable equilibrium for a sphere with $R/S < 1$. Above a critical field strength $E_0 > E_Q$, where $E_0 = |\mathbf{E}|$ and E_Q is given by Eq.[3.16], constant rotation around an axis perpendicular to the electric field is induced by the misaligned dipole moment of the particle (right). The rotation can be either clockwise or counter-clockwise.

In the case of a sphere, the friction matrix is diagonal and the equation of motion is

$$I \frac{d\omega}{dt} = \mathbf{P} \times \mathbf{E} - 8\pi\mu_{\text{ex}}a^3\omega, \quad (3.14)$$

where I is the moment of inertia of the sphere. In the classic case of the Quincke sphere, a straightforward calculation assuming instantaneous polarization yields three possible solutions [90]: no rotation, $\omega = 0$, and

$$\omega_Q = \pm \frac{1}{t_{\text{mw}}} \sqrt{\frac{E_0^2}{E_Q^2} - 1}, \quad (3.15)$$

where the $\pm$ sign reflects the two possible directions of rotation and t_{mw} is the Maxwell-Wagner polarization time. Eq.[3.15] includes a parameter, E_Q , which

describes the critical field for rotation,

$$E_Q^2 = \frac{2\lambda_{\text{ex}}\mu_{\text{ex}}(R+2)^2}{3\epsilon_{\text{ex}}\epsilon_{\text{in}}(1-R/S)}. \quad (3.16)$$

The dipole “tilt” is steady and the angle between the dipole and the electric field is $\phi = \text{arccot}[(t_{\text{mw}}\omega)^{-1}]$. Eq.[3.15] shows that rotation is possible only if the electric field exceeds a critical value given by E_Q . Hence, if $E_0 \leq E_Q$, the sphere and the suspending fluid are motionless, and at $E_0 > E_Q$, the sphere rotates and drags the fluid in motion; the flow is purely rotational. A more detailed analysis shows that the transition to rotation occurs via a pitchfork bifurcation [201, 111].

This analysis neglects the finite time over which the induced dipole develops. This is taken into account by using the polarization relaxation equations, i.e., non-instantaneous charging of the interface. Taking this into account, the evolution of the induced dipole is described by [200],

$$\partial_t \mathbf{P} = \omega \times \mathbf{P} - t_{\text{mw}}^{-1} [\mathbf{P} - (\chi_0 - \chi_\infty)\mathbf{E}], \quad (3.17)$$

where χ_0 and χ_∞ are the low and high frequency susceptibilities respectively. It has been shown that the polarization relaxation equation and the torque balance, including inertia, map onto the Lorenz chaos equations [111, 149]. A second bifurcation occurs in stronger fields and the sphere exhibits chaotic reversal of the rotation direction. Nonlinear behavior can also arise in the absence of inertia from an anisotropic shape such as an ellipsoid [31, 134]. The polarization relaxation equation, Eq.[3.17], is particularly useful for considering the polarization of an ellipsoid [32], which will be discussed further in Section 3.7 .

3.3 Summary of previous work

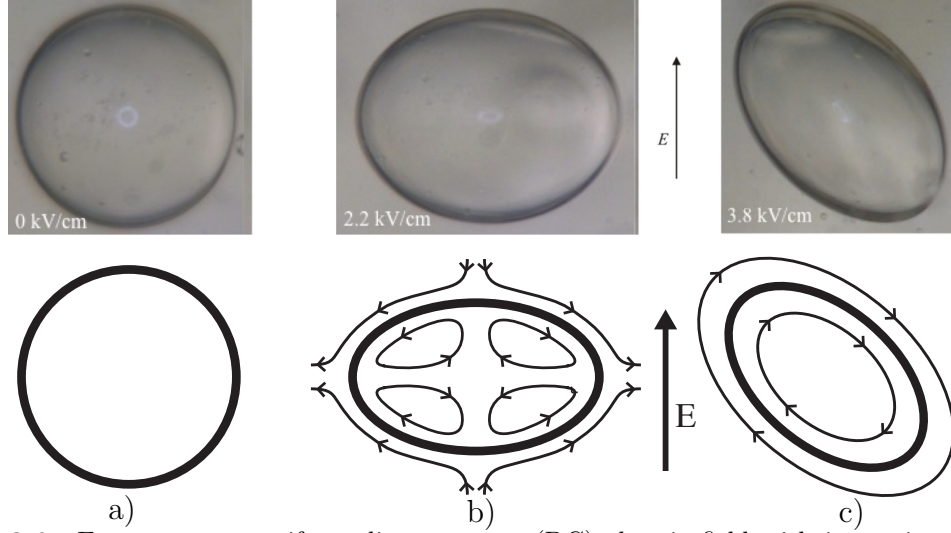


Figure 3.6: Exposure to a uniform direct current (DC) electric field with increasing strength excites a variety of drop responses [168]. a) A silicone oil drop suspended in castor oil is spherical in the absence of electric field. b). Weak fields induce axisymmetric oblate deformation. c) In strong fields, the drop is tilted with respect to the applied field direction and the flow is rotational. The sketches illustrate the drop shape and flow streamlines.

Unlike the rigid sphere, a droplet deforms and drop fluid moves at any field strength. Below a certain critical value, $E_0 \leq E_c$, the flow is axisymmetric, as described by the Taylor model, and the streamlines are sketched in Figure 3.6b). At $E_0 > E_c$, the flow acquires a rotational component in addition to the straining flow. The straining flow affects the charge convection via Eq.[3.8], and thus the critical field for drop electrorotation, E_c , is different from the critical field for Quincke rotation, E_Q . Previous studies discovered non-axisymmetric drop shapes, in which the fluids undergo rotational flow and the drop adopts a tilted orientation with respect to the electric field [103, 71, 171]. The goal in my previous work was to measure E_c for different drop sizes and viscosities, and interpret the deviations from the Quincke threshold [168, 169]. This served as a measure for the strength of the charge convection by the straining EHD flow. Experimental data was also gathered to quantify the dependence of tilt angle on viscosity ratio, drop size, and electric

field strength.

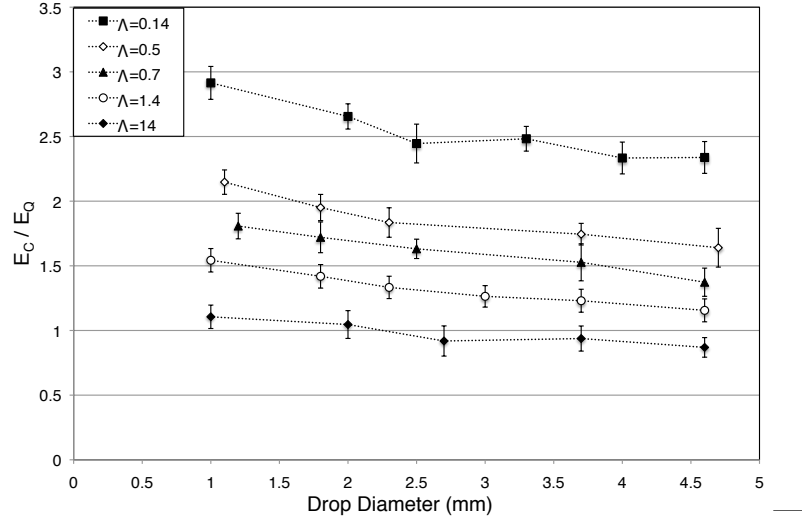


Figure 3.7: Critical field strength for initiation of drop rotation for different viscosity ratios and drop diameters [168].

My earlier work showed that lower drop viscosity corresponds to a higher critical electric field, see Figure 3.7. Larger drops were found to have a slightly lower critical electric field for a given viscosity ratio, but the effect of drop size was weaker compared to that of viscosity ratio. Once rotation begins, low viscosity drops rapidly transitioned to a large tilt angle while high viscosity drops made a gradual transition. At higher field strengths, the angle continues to slowly increase. Hysteretic behavior in the angle was observed for large, low viscosity drops, see Figure 3.8. Measurement of the rotation rate showed that once rotation was induced, the rotation rate was well approximated by the Quincke model for a rigid sphere.

It was proposed that charge convection plays a role in the hysteresis observed between axisymmetric and asymmetric drop shapes. Hysteresis implies bistability, which is absent in the simple Quincke model [16, 185, 12]. The observation of hysteresis resulting from convection also suggested that the unsteady drop shape behavior, previously observed in [171], is related to charge convection. The presence of these nonlinear behaviors motivates a more detailed investigation into the unsteady

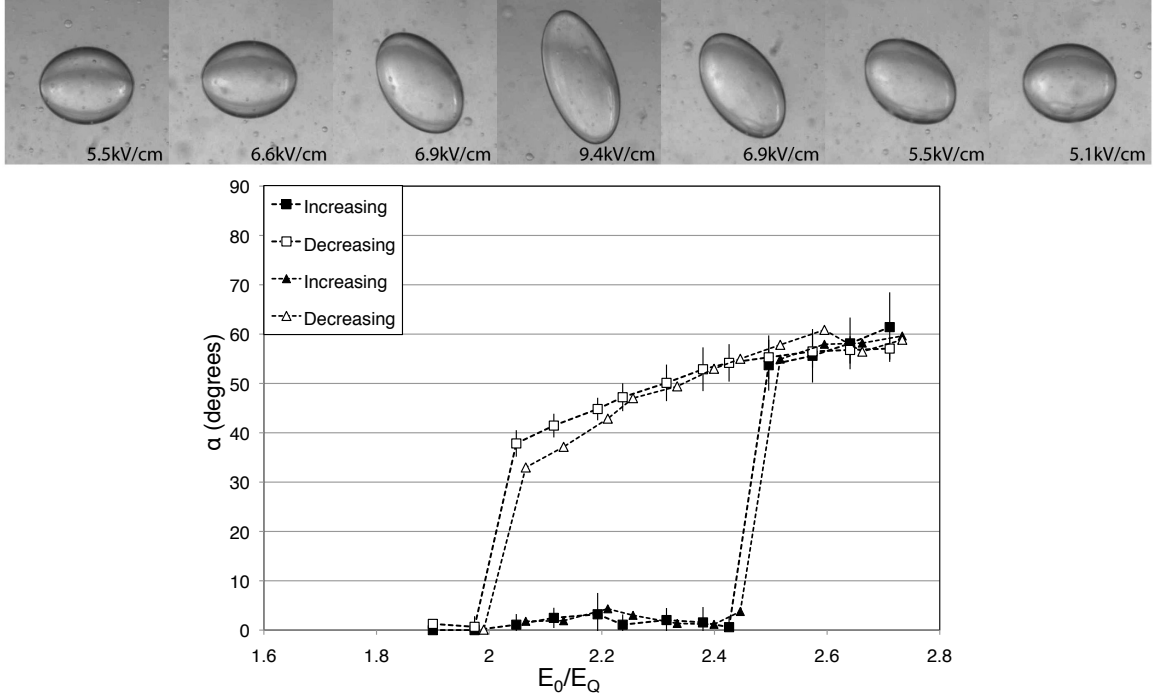


Figure 3.8: (top) Drops at increasing and then decreasing field strength. Drop remains titled as field strength is decreased. (bottom) Hysteresis in the tilt angle observed for 2.5mm diameter, $\Lambda=0.14$ drop [168].

drop behavior in strong DC fields.

3.4 Timescales and dimensionless numbers

As discussed in the previous section, a fluid drop can also rotate as a result of the Quincke effect. Unlike a rigid sphere, the drop can deform and undergo straining flow as described by Taylor. For a fluid drop, where both rotational and straining flows are present, the response depends on more compared to a solid sphere. The relevant timescales and dimensionless numbers are introduced and are used throughout the chapter to gain insight into drop behavior.

The accumulation of charge on the interface creates an electric stress, which drags

the fluids into motion. The drop deforms on a timescale characterized by the inverse shear rate,

$$t_{\text{hd}} = \frac{\mu_{\text{ex}}(1 + \Lambda)}{\epsilon_{\text{ex}} E_0^2}. \quad (3.18)$$

The interfacial tension, γ , is a restoring force acting to minimizing the interfacial area of the drop. The characteristic timescale for a deformed drop to relax to an equilibrium spherical shape is given by,

$$t_\gamma = \frac{\mu_{\text{ex}}(1 + \Lambda)a}{\gamma}. \quad (3.19)$$

Drop deformation in the Taylor regime, $E_0 < E_c$, (axisymmetric EHD flow at weak fields) is controlled by the balance of the distorting straining flow and the opposing surface tension stresses. The electric capillary number is defined as the ratio of the EHD timescale to the surface tension timescale,

$$Ca = \frac{t_\gamma}{t_{\text{hd}}} = \frac{a\epsilon_{\text{ex}}E_0^2}{\gamma}. \quad (3.20)$$

In strong fields, $E_0 > E_c$, the flow has both straining and rotational components. A comparison of timescales can be written to describe the relative strength of the EHD straining flow and rotation flow,

$$\frac{\omega_Q^{-1}}{t_{\text{hd}}} = \frac{\epsilon_{\text{ex}}E_0^2 t_{\text{mw}}}{\mu_{\text{ex}}(1 + \Lambda) \sqrt{\frac{E_0^2}{E_Q^2} - 1}}. \quad (3.21)$$

Note that the strength of the straining flow, characterized by Eq.[3.18], depends on viscosity ratio while the rotational flow, characterized by the Quincke rotation rate ω_Q , is independent of viscosity ratio. In general, the rotation rate of drops should

differ from Quincke, but previous measurements have shown that the rotation rate for drops is similar to that predicted by Quincke [168].

The dependence of the timescales in Eq.[3.21] on field strength is not straightforward. However, neither timescale depends on drop size. The ratio of these two timescales is plotted as a function of field strength for different viscosity ratios in Figure 3.9. This plot shows that the strength of the straining flow is at a minimum compared to rotational flow soon after the rotation is initiated. The straining flow is dominant for low viscosity drops, which results in significant charge convection by straining flow and larger deformation. In contrast, high viscosity drops have a weak straining flow compared to the rotational flow and there is a weak dependence on field strength.

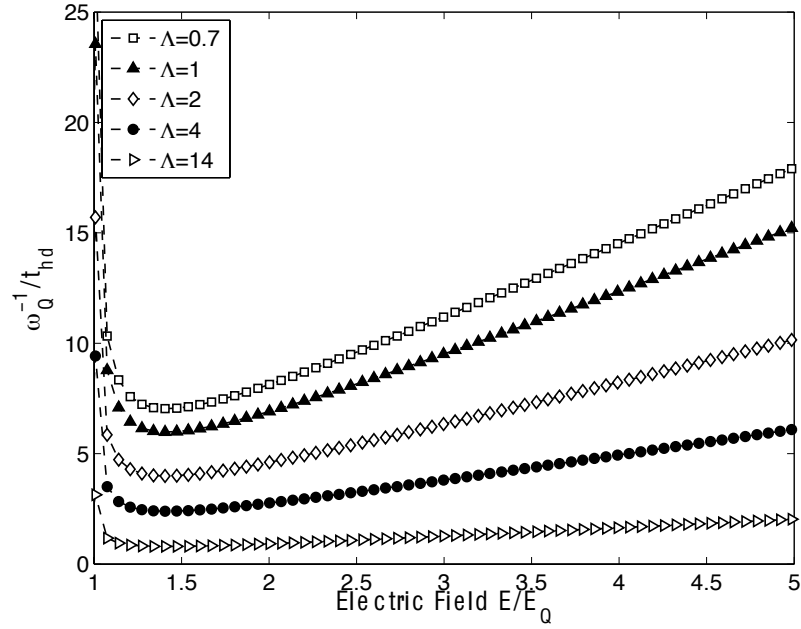


Figure 3.9: Plot of $\frac{\omega_Q^{-1}}{t_{hd}}$ for different viscosity ratios.

The ratio of the rotation rate predicted by Quincke and surface tension timescale compares the relative strength of the rotational flow to the response of the interface.

This is denoted by a capillary number,

$$Ca_\omega = \frac{t_\gamma}{\omega_Q^{-1}} = \frac{a\mu_{\text{ex}}(1 + \Lambda)}{\gamma t_{\text{mw}}} \sqrt{\frac{E_0^2}{E_Q^2} - 1}. \quad (3.22)$$

This number will be important for describing the onset of unsteady drop shape behavior.

3.5 Experimental methods

The experimental materials and methods used in this chapter are explained in detail in [166]. The following section summarizes the materials and equipment used for the experiments.

3.5.1 Fluid system

Silicone oil (SO) and castor oil (CO) (Alfa Aesar) are used as drop and suspending fluids, respectively. The material property data are listed in Table 3.1. The total electrical resistance of the fluid-filled chamber, measured with a sensitive ohmmeter, is used to determine the fluid conductivity. Similarly, the capacitance of a fluid-filled chamber is measured to determine permittivity. The surface tension is measured to be 4.5 mN/m using the pendant drop method. For this system, the conductivity and permittivity ratios are $R = 0.027$ and $S = 0.56$. According to Eq.[3.12], this system produces oblate deformation in a DC electric field for all viscosity ratios.

The viscosity is measured with a capillary viscometer. The viscosity ratio is adjusted by mixing silicone oils of different molecular weight. In the results, the

Material	Density (kg/m ³)	Relative permittivity ϵ_r	Conductivity σ (S/m)	Viscosity μ (Pa-s)
Castor Oil (CO)	961	5.3	4.5×10^{-11}	0.69
Silicone Oil (SO1)	970	3.0	1.23×10^{-12}	0.53
Silicone Oil (SO2)	971	3.0	1.23×10^{-12}	0.81
Silicone Oil (SO3)	972	3.0	1.23×10^{-12}	2.04
Silicone Oil (SO4)	973	3.0	1.23×10^{-12}	3.17
Silicone Oil (SO5)	973	3.0	1.23×10^{-12}	9.74

Table 3.1: Material properties for drop experiments.

	Viscosity ratio	Maxwell Wagner	EHD $E_0 = 1kV/cm$	EHD $E_0 = 10kV/cm$	Surface tension
System	Λ	$t_{mw}(s)$	$t_{hd}(s)$	$t_{hd}(s)$	$t_\gamma(s)$
CO/SO1	0.7	1.32	2.5	0.025	0.8
CO/SO2	1.1	1.32	3.1	0.031	1.0
CO/SO3	2.9	1.32	5.6	0.056	1.7
CO/SO4	4.5	1.32	8.1	0.081	2.5
CO/SO5	14	1.32	22.1	0.022	6.9

Table 3.2: Characteristic timescales for the experimental fluid systems and values of the dimensionless parameters estimated at a field strength where unsteady behavior is observed $E_0 = 1kV/cm$ and $E_0 = 10kV/cm$ and drop radius 3mm.

viscosity ratios are divided into three categories. The two lowest viscosity silicone oils, SO1 and SO2, are referred to as low viscosity ratios, $\Lambda \sim 1$. The two intermediate viscosity silicone oils, SO3 and SO4, are referred to as intermediate viscosity ratios, $\Lambda \sim 4$. The highest viscosity silicone oil, SO5, is referred to as high viscosity ratios, $\Lambda \sim 14$.

The critical field strength predicted by Quincke, Eq.[3.16], gives a value of $E_Q = 2.7kV/cm$ using the material properties of silicone oil and castor oil from Table 3.1. The magnitude of the relevant timescales introduced in Section 3.4 can be estimated using the material property values and typical field strengths used in the experiment, see Table 3.2

A comparison between timescales gives intuition for the relative strength of forces

System	Diameter 2a (mm)	Ca ($E_0 = 1kV/cm$)	Ca ($E_0 = 10kV/cm$)
CO/SO	1	0.05	5.2
CO/SO	5	0.26	26.1

Table 3.3: Capillary numbers, Ca , for typical ranges of drop diameter and electric field strengths.

acting on the system. The strength of the EHD (axisymmetric) flow to the restoring surface tension, given by Ca , is estimated for different drop sizes and field strengths in Table 3.3. Large Ca number implies large deformation. Capillary number scales as $Ca \sim E_0^2$, leading to a significant increase in drop deformation as E_0 increases.

System	Viscosity ratio Λ	Diameter 2a (mm)	Ca_ω ($E_0 = 1.5E_Q$)	Ca_ω ($E_0 = 3E_Q$)
CO/SO1	0.7	1	0.11	0.3
CO/SO1	0.7	5	0.55	1.4
CO/SO4	4.5	1	0.35	0.9
CO/SO4	4.5	5	1.78	4.5

Table 3.4: Capillary numbers, Ca_ω , for typical ranges of drop diameter and electric field strengths.

When rotational flow is initiated, straining flow is still present and the drop remains deformed. Ca_ω describes the ability of the surface tension to respond to the rotational flow. Ca_ω depends on field strength, drop size, and viscosity ratio. Typical values of Ca_ω are estimated in Table 3.4 for a range of parameters. High viscosity and larger drops reach large values of Ca_ω at weaker field strengths relative to small, low viscosity drops, which require very strong fields to reach $Ca_\omega \sim 1$.

3.5.2 Experimental setup and procedure

A uniform electric field is generated in a parallel-plate chamber set-up represented in Figure 3.10. The chamber base and walls are constructed from transparent polymethyl methacrylate. Two 5 x 5 cm brass plates serving as electrodes are attached to the chamber walls. Chambers with different electrode distances, 1cm and 2.5cm, are used in order to observe a wide range of drop sizes and field strengths.

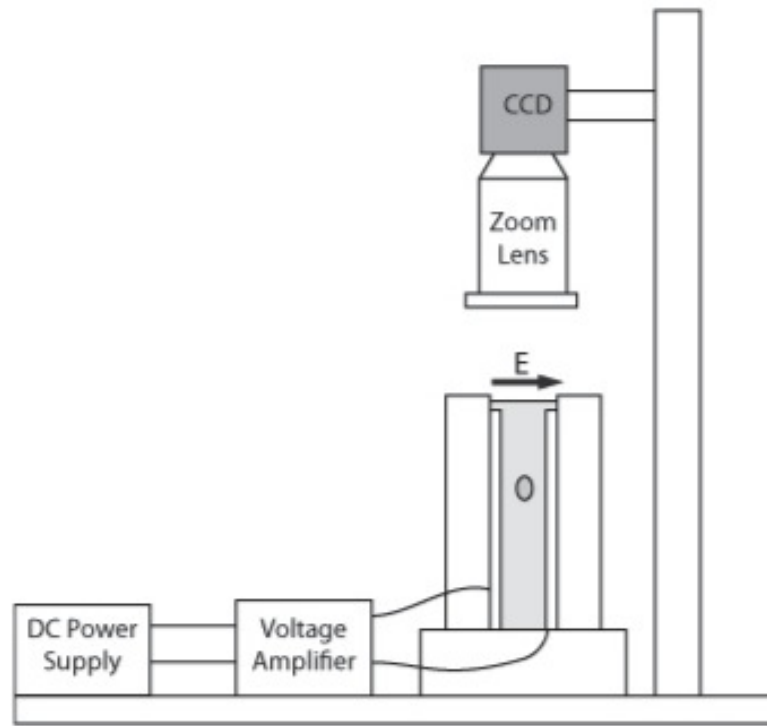


Figure 3.10: Sketch of the experimental setup.

The electric field is produced with a high voltage DC amplifier (Ultravolt 40A-12) powered by a 2-output power supply (Agilent E3646A). The amplifier sits inside a sealed plastic box and is connected to the chamber with a high voltage rated wire. The output from the amplifier was verified using a Fluke air cleaner probe (80K-15) connected to a Fluke 115 multimeter.

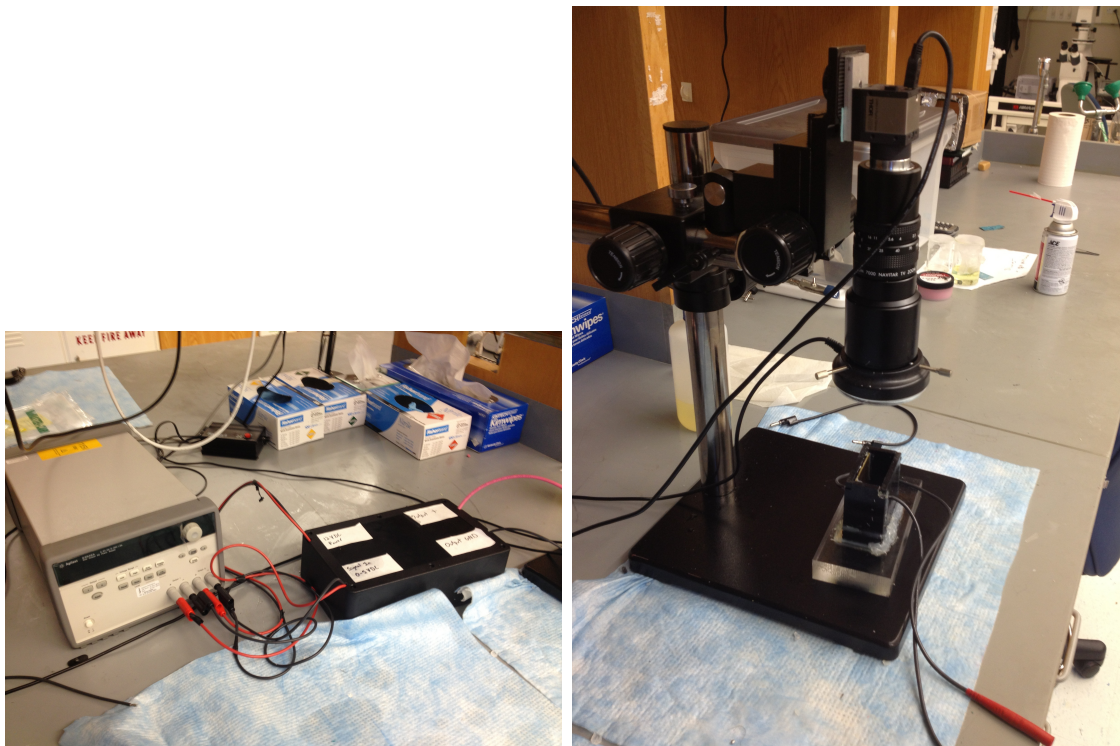


Figure 3.11: Experimental setup in laboratory. The amplifier is connected to the power supply (left) and chamber with camera (right).

The experiment is prepared by pipetting a single drop (silicone oil), in the range of 1-7 mm in diameter, into the chamber filled with castor oil. An image of the drop is taken before being exposed to an electric field. The diameter of the spherical drop is measured using calibrated imaging software.

A phase diagram of drop behavior is measured by increasing the electric field in incremental steps. The gradual increase reduces the possibility of seeing transient drop behaviors, which can occur if the field is suddenly increased to a high field strength. Gradually increasing the field strength also produces the most reproducible results; transient behavior is found to be less repeatable. If the drop interacts with the wall chamber, the electric field is turned off and the drop is moved with a glass pipette to the center of the chamber. The experiment is then continued after gradually increasing the electric field to the previous field strength.

Video recordings of unsteady drop behavior are taken with a CCD camera (ThorLabs) at 15 frames per second. The chamber is aligned with the camera such that the electric field is oriented from bottom to top in the image. Video is recorded until the drop sediments to the bottom of the chamber, approximately 2-5 minutes, depending on the size of the drop.

The images are processed using image processing software, ImageJ. A pixel intensity threshold is taken on a stack of images to provide an outline of the drop interface. The image is then converted to binary and the shape enclosed by the interface is converted to black. The shape is then analyzed using the built-in ImageJ algorithm “fit ellipse”, which determines the major and minor axes and angle of inclination of the fitted ellipse. The drop deformation is determined by the ratio of axes parallel and perpendicular to the axis of symmetry,

$$\beta = \frac{a_{\parallel}}{a_{\perp}}. \quad (3.23)$$

Once rotation begins, the true drop shape may deviates from an ellipsoid. For simplicity, it is assumed that the drop shape seen in experiments is an oblate ellipsoid, $\beta < 1$.

Note that the observation axis does not always coincide with the axis of drop rotation. The rotation axis can assume any direction perpendicular to the electric field and its orientation is not known a priori. For measurements of drop angle, recordings were made when the drop tilted within the viewing plane, i.e. at an angle that is observable from the camera.

To verify that the chamber remains at room temperature, the amount of ohmic heating resulting from electric current through the chamber is estimated. The highest

voltage used is approximately 20kV. The conductivity of the castor oil, $\lambda = 4.5 \times 10^{-11}\text{S/m}$, yields a resistance of approximately $100M\Omega$ for an area of 0.0025m^2 and a gap of 2cm. This yields a current across the chamber of $0.1\mu\text{A}$. This current gives a total energy input of 1 Joule over an estimated time of 1000 s. Using properties of castor oil, the mass of the chamber is estimated to be 0.05kg and the heat capacity is $c_p \sim 1\text{J/kgT}$. The change in temperature is estimated to be $0.01K$. The low amount of current through the chamber makes the ohmic heating negligible over the duration of an experiment.

3.6 Experimental results

Drop behavior is investigated for different drop sizes, viscosity ratios, and field strengths. First, the types of behavior observed in experiments are described and classified. Next, phase diagrams are made to show the effect of behavior on the adjustable parameters. Finally, the unsteady behavior is examined and characterized for different parameters.

3.6.1 Types of behaviors

Taylor

Below a critical electric field strength, $E_0 \leq E_c$, the drop flow is described by the Taylor flow discussed in Section 3.1.2. The drop adopts a prolate or oblate spheroidal shape with the axisymmetric flow being aligned with the applied field. The streamlines are sketched in Figure 3.6b.

Tilted with steady rotation

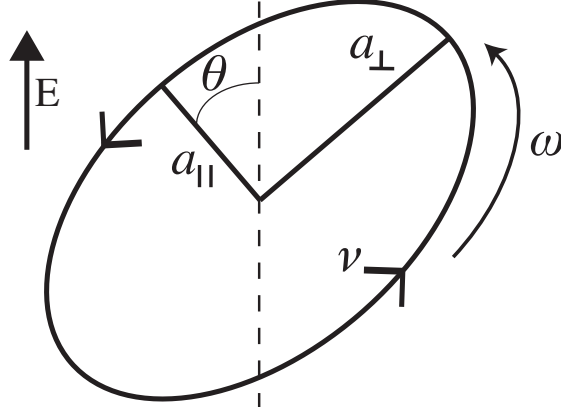


Figure 3.12: Model for drop electrorotation. Drop asphericity is characterized by the aspect ratio $\beta = a_{\parallel}/a_{\perp}$. The rigid body (Quincke) rotation is related to the tilt angle of the deformed shape, θ , by $\omega = d\theta/dt$. The difference between the interface velocity and the Quincke rotation speed quantified by the rotation rate ν .

Above a critical field strength, $E_0 > E_c$, the drop fluid begins rotating at a constant rate and the drop adopts a steady tilted shape, see Figure 3.6c. Lower viscosity drops have a higher critical electric field due to the effect of charge convection, which weakens the induced dipole. Larger drops have a slightly lower critical electric field for a given viscosity ratio, but the effect of drop size is weaker compared to that of viscosity ratio. The rotational component of the flow adds to the straining flow.

The tilt angle and drop deformation above E_c was compared to theory in [75]. The theory assumes small deformations from sphericity and charge convection only by rotation. The results show agreement with aspect ratio and orientation angle data from high viscosity drops, where convection due to straining flow is weak and drop deformation is small.

Unsteady drop shape

At field strengths above the transition to rotation, drops may exhibit unsteady

dynamics. The transition to an unsteady shape develops spontaneously from the tilted steady shape as field strength is gradually increased. Typical unsteady drop

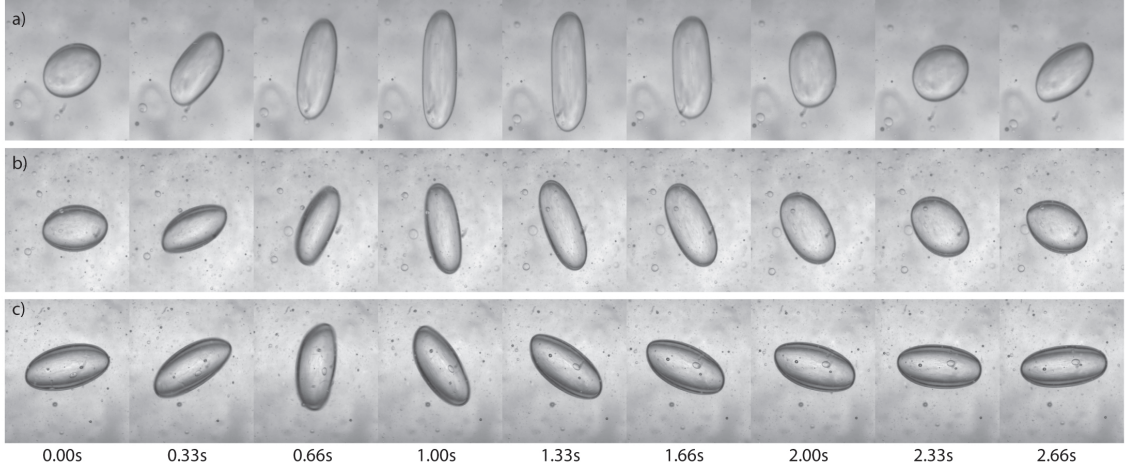


Figure 3.13: Examples of one period of unsteady drop behavior for three viscosity ratios. Low viscosity drops deform and oscillate while high viscosity drops tumble. The parameters for the drops shown are a) $\Lambda=1.1$, $E_0=9.9\text{kV/cm}$, $2a=3.6\text{mm}$, b) $\Lambda=4.5$, $E_0=8.1\text{kV/cm}$, $2a=5.4\text{mm}$, and c) $\Lambda=14$, $E_0=9.7\text{kV/cm}$, $2a=6.0\text{mm}$.

behaviors are illustrated in Figure 3.13. The drop dynamics are particularly sensitive to the viscosity ratio. Low viscosity drops ($\Lambda \sim 1$) undergo shape oscillations with steady amplitude, see Figure 3.13 (top), while high viscosity drops ($\Lambda \sim 14$) tumble and reverse direction chaotically, see Figure 3.13 (bottom). Intermediate viscosity drops ($\Lambda \sim 4$) exhibit characteristics of both behaviors, namely repeated cycles consisting of shape oscillations with increasing amplitude followed by several tumbles.

The orientation and deformation of drops with different viscosity ratios are plotted on a polar plot in Figure 3.14, where the radial axis is the inverse aspect ratio, β^{-1} . An angle between the axis of symmetry, $a_{\parallel}$, and the applied field direction is defined as shown in Figure 3.12. Videos demonstrating the unsteady behavior observed at each viscosity ratio are also provided. The three videos have the following parameters:

$\Lambda=1.1$ at $E_0=10.2\text{kV/cm}$, $2a=3.6\text{mm}$

$\Lambda=4.5$ at $E_0=9.2\text{kV/cm}$, $2a=3.6\text{mm}$

$\Lambda=14$ at $E_0=9.2\text{kV/cm}$, $2a=6.9\text{mm}$.

Low viscosity drops, $\Lambda \sim 1$, exhibit steady oscillations between a prolate and a tilted oblate shape, see Figures 3.13(top) and 3.14(left). These shape oscillations can be explained by considering the dynamics of the induced dipole and drop shape. The lower fluid viscosity allows the drop to be easily deformed by the electric stress. As the interface charges, the drop is squeezed into an oblate shape. The accumulated surface charge rotates due to the electric torque until the induced dipole has flipped and is nearly aligned with the field. In this dipole configuration, the drop is pulled into a prolate shape until the surface charge relaxes. The cycle repeats by the interface recharging and deforming the drop back into an oblate shape. In contrast to the high viscosity drops, the drop can easily deform during the rotation and oscillations in the drop shape are observed. As the field strength is increased further, the frequency of drop shape oscillations can vary. This occurs for drops well above the transition to unsteady behavior and with large drops.

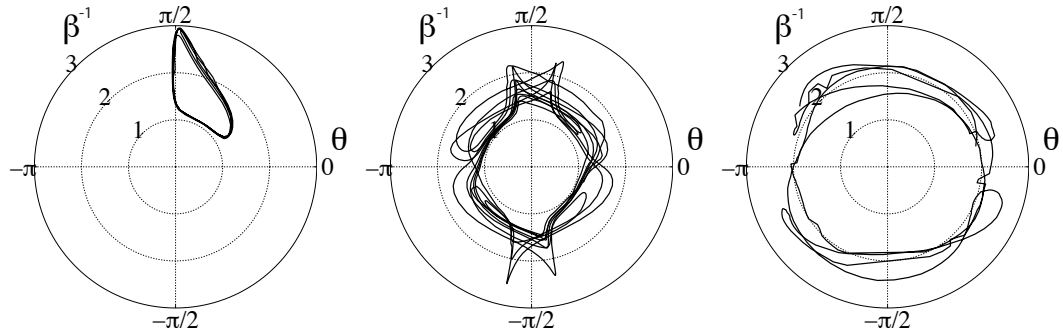


Figure 3.14: Polar plots of orientation angle (angular axis) compared to aspect ratio (radial axis) during unsteady behavior for three different viscosity ratios $\Lambda = 1.1, 4.5, 14$ (left to right). The parameters for the $\Lambda = 1.1$ drop (left) are $2a = 3.7\text{mm}$, $E_0 = 3.2E_Q$. The parameters for the $\Lambda = 4.5$ drop (center) are $2a = 3.1\text{mm}$, $E_0 = 3.2E_Q$. The parameters for the $\Lambda = 14$ drop (right) are $2a = 5.4\text{mm}$, $E_0 = 3.6E_Q$.

For high viscosity drops, $\Lambda \sim 14$, a perturbation to the oblate drop shape grows until the drop reaches a chaotic tumbling state. The behavior is described as chaotic because the rotation direction changes aperiodically, see Figure 3.14(right). The largest deformation occurs while the short axis of the oblate drop is aligned with the field. At this point, the drop can either break up or continue to rotate.

Shape oscillations that vary in magnitude and frequency are observed for intermediate viscosity drops, see Figure 3.14(middle). Similar to the $\Lambda \sim 1$ drops, the $\Lambda \sim 4$ drops oscillate between a tilted oblate and extended prolate shape. The degree of deformation changes with each period, usually increasing until the drop reverses direction, and begins oscillating again at a lesser deformed shape. The number of oscillations in any given direction was not observed to repeat. As the field strength is increased, the number of oscillations in one direction tends to decrease.

Breakup

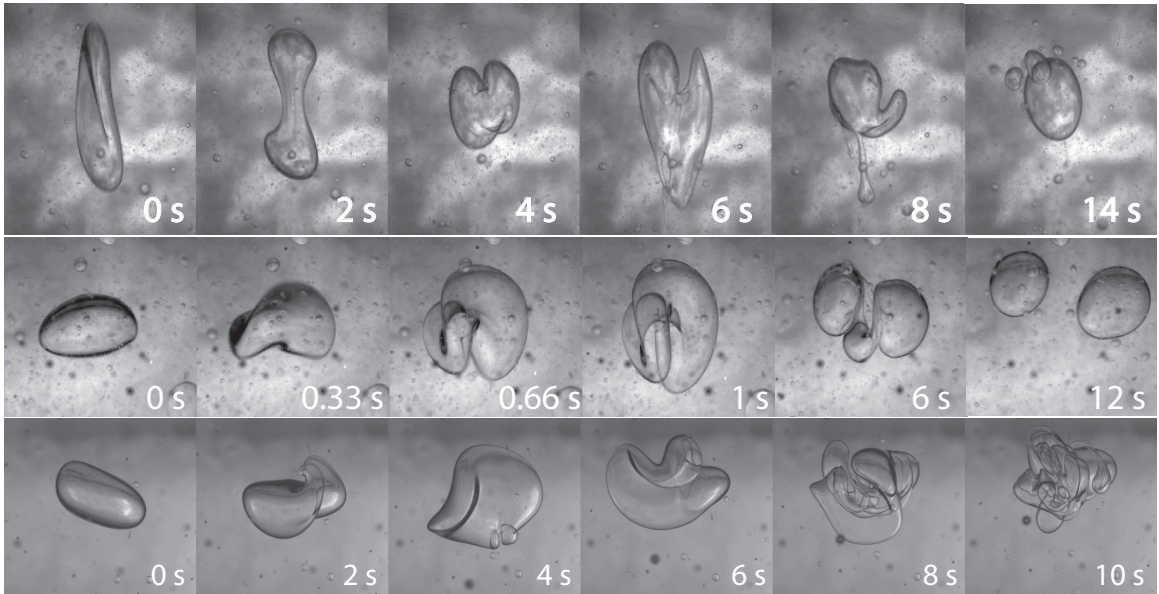


Figure 3.15: Drop breakup for different viscosity drops: (top) Breakup for 5.8mm, $\Lambda = 1.1$ drop in 10.2 kV/cm. (middle) Breakup for 5.8mm, $\Lambda = 4.5$ drop in 10.2 kV/cm. (bottom) Breakup for $\Lambda = 14$ in 10.7 kV/cm.

Drop breakup is observed at field strengths above the transition to unsteady

behavior. The characteristics of drop breakup depend on viscosity ratio. Low viscosity drops, $\Lambda \sim 1$, tend to break during elongation when the drop is pulled into a prolate shape. Breakup occurs when oscillations in deformation amplitude increase and eventually result in the drop stretching into a highly deformed prolate “dumbbell” shape, see Figure 3.15(top). The two main “dumbbells” will each begin rotating independently and the fluid connecting the two main drops will breakup into daughter droplets. The daughter drops rarely break up into two equally sized drops, but usually form many small drops as shown in Figure 3.15.

Intermediate and high viscosity drops, $\Lambda \sim 4 - 14$, tend to break up when the short axis of the oblate shaped drop is aligned with the field and rotation temporarily pauses. The magnitude of oblate deformation continues to increase until the drop folds upon itself. The “folding” occurs when the two sides of the oblate begin rotating towards each other or twist against the rest of the drop shape. For the $\Lambda = 14$ drops, once a fold is created, the irregular drop shape often continues folding upon itself as seen in Figure 3.15(bottom). The folded drops usually do not immediately separate into daughter drops, but remain attached as the entire folded structure continues rotating. Eventually the daughter drops will separate. Intermediate viscosity drops, $\Lambda = 4$, show similar folding behavior, but will separate into daughter droplets more quickly, see Figure 3.15(middle).

3.6.2 Phase diagram

A phase diagram for each viscosity ratio is recorded to demonstrate the transitions between the behaviors described in Section 3.6.1. For a given viscosity ratio, the drop size and field strength is varied.

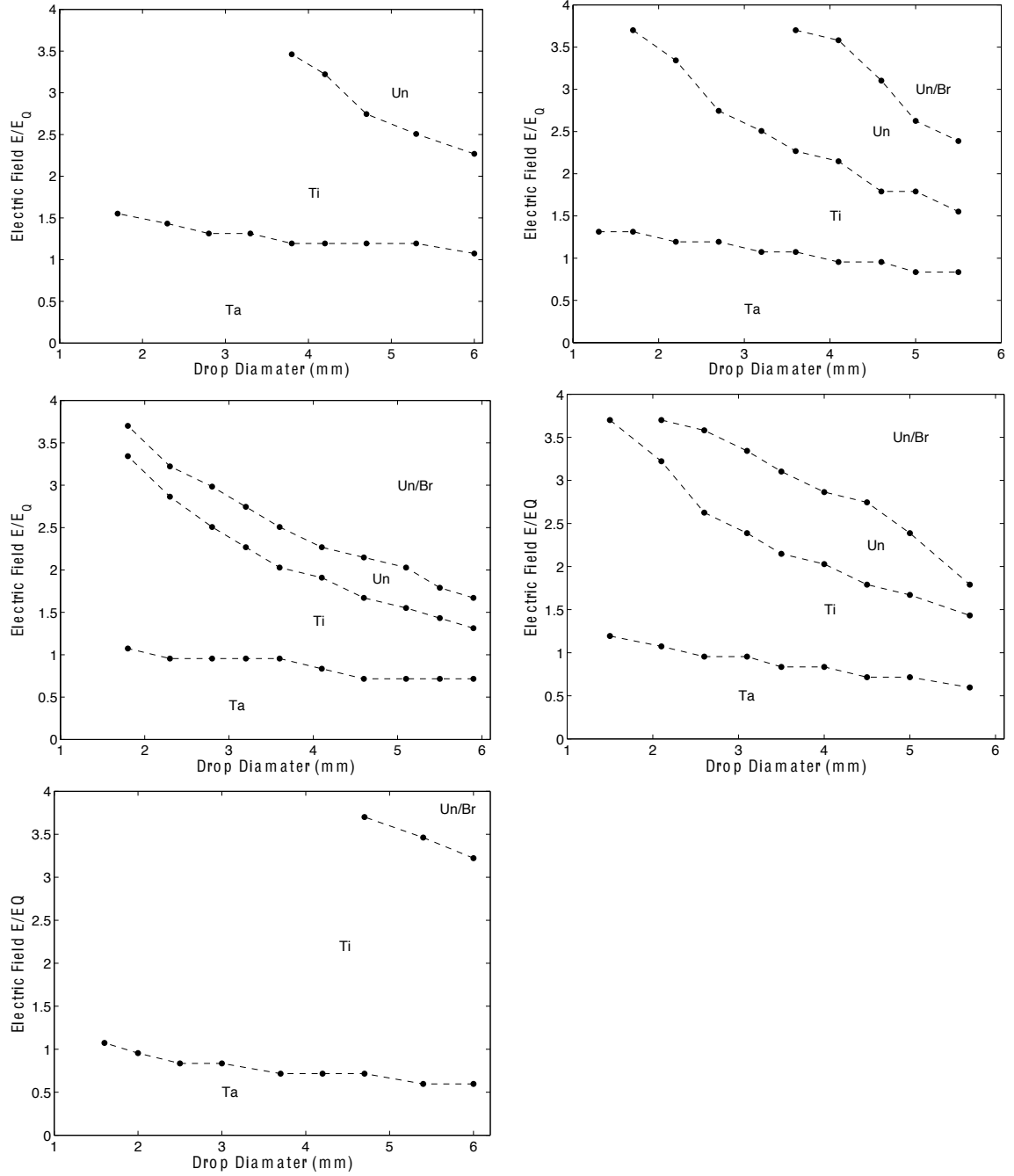


Figure 3.16: Phase diagrams for different viscosity ratios: (top) $\Lambda = 0.7$ and 1.1 , (middle) $\Lambda = 2.9$, 4.5 (bottom) $\Lambda = 14$. The electric field is slowly increased and steady-state behavior is observed. Taylor (Ta) denotes axisymmetric flow and oblate deformation. Tilted (Ti) denotes a tilted drop orientation with rotational flow. Unsteady (Un) denotes time dependent drop shape and orientation. Breakup (Br) denotes regions where drop breakup is observed.

The drop behavior changes in the following sequence as field strength is increased:
Taylor flow, steady tilted orientation with fluid rotation, unsteady drop shape, and

finally a mix of breakup and unsteady drop shape. Breakup behavior is usually observed for large drops at the highest field strength. A breakup was defined as an event at which any volume of the drop separates. Because the field strengths were gradually increased, the drop did not break up from an initially spherical drop as has been observed in [171, 166]. Instead, all of the observed drop breakup was observed after the drop began shape oscillations.

As was shown in [168], the critical field for the transition from Taylor flow to tilted orientation is higher for low viscosity drops. The transition also decreases slightly with larger drops. Lower viscosity drops have stronger charge convection, which weakens the induced dipole and therefore a stronger field is required to produce enough torque for rotation. The slight decrease for larger drops is due to larger deformation, which increases the dipole strength as the axis parallel to the field direction becomes shorter. This will be discussed further in Section 3.7.4.

The transition from tilted to unsteady drop shape typically occurs within the range of $E_0 = 1.5 - 3.5E_Q$. The viscosity ratios, $\Lambda = 1.1, 2.9, 4.5$, require the lowest fields for unsteady behavior and the transition occurs at similar field strengths for these viscosity ratios. The lowest viscosity ratio, $\Lambda = 0.7$, and highest viscosity ratio, $\Lambda = 14$, require strong fields and large drops, $E_0 \sim 3E_Q$ and $2a > 4\text{mm}$, for unsteady behavior. Another noticeable feature is that for each viscosity ratio, the transition to unsteady behavior occurs at a lower field strength as drop size increases. The effect of viscosity ratio and drop size on the transition to unsteady behavior is discussed in the next section.

Drop breakup is most prevalent at intermediate viscosity ratios, particularly $\Lambda = 2.9$. The propensity for drops with viscosity ratios in the range $\Lambda \sim 2 - 4$ to break up under a weaker applied stress has also been observed for drops in shear flow [17].

The drops in the (Un/Br) region may not break up immediately, but can have long periods of unsteady behavior before breakup. Predicting breakup is difficult due to the chaotic behavior of the intermediate and high viscosity drops.

3.6.3 Transition to unsteady behavior

Previous measurements focused on the drop orientation after the onset of rotation [168, 166]. Drop deformation was not measured as a function of field strength. It will be shown in the theoretical model that aspect ratio is an important parameter for the onset of unsteady shape behavior.

The aspect ratio of the drop is more difficult to measure because of the degeneracy in the axis of rotation of the tilted drop. In other words, the drop can tilt towards the camera as easily as it can tilt within the plane of view. Options such as multiple cameras were considered, but the drops can be gently manipulated with a pipette once rotation is initiated to an orientation within the plane of view. Care must be taken to avoid disrupting the drop, and observations are only taken after the drop has stabilized in a given orientation. Once rotation begins in one direction, drops with a steady state do not usually precess out of plane.

In the Taylor regime, an increase in the electric field strength produces a monotonic increase in drop deformation. Above the critical field for rotation, the rotational flow creates an additional torque that can act to keep the drop spherical. As a result, there is a competition between the rotational flow and an extensional flow described by the Taylor flow. Depending on the relative strength of these two flows, the drop can either increase or decrease in deformation. Considering the dependence of viscosity ratio in Eq.[3.21], adjusting viscosity ratio will change the relative strength of the straining flow to the rotational flow. Low viscosity ratios will experience greater deformation because the straining flow is significant while high viscosity ratios will have a slow EHD timescale and a dominant rotational flow.

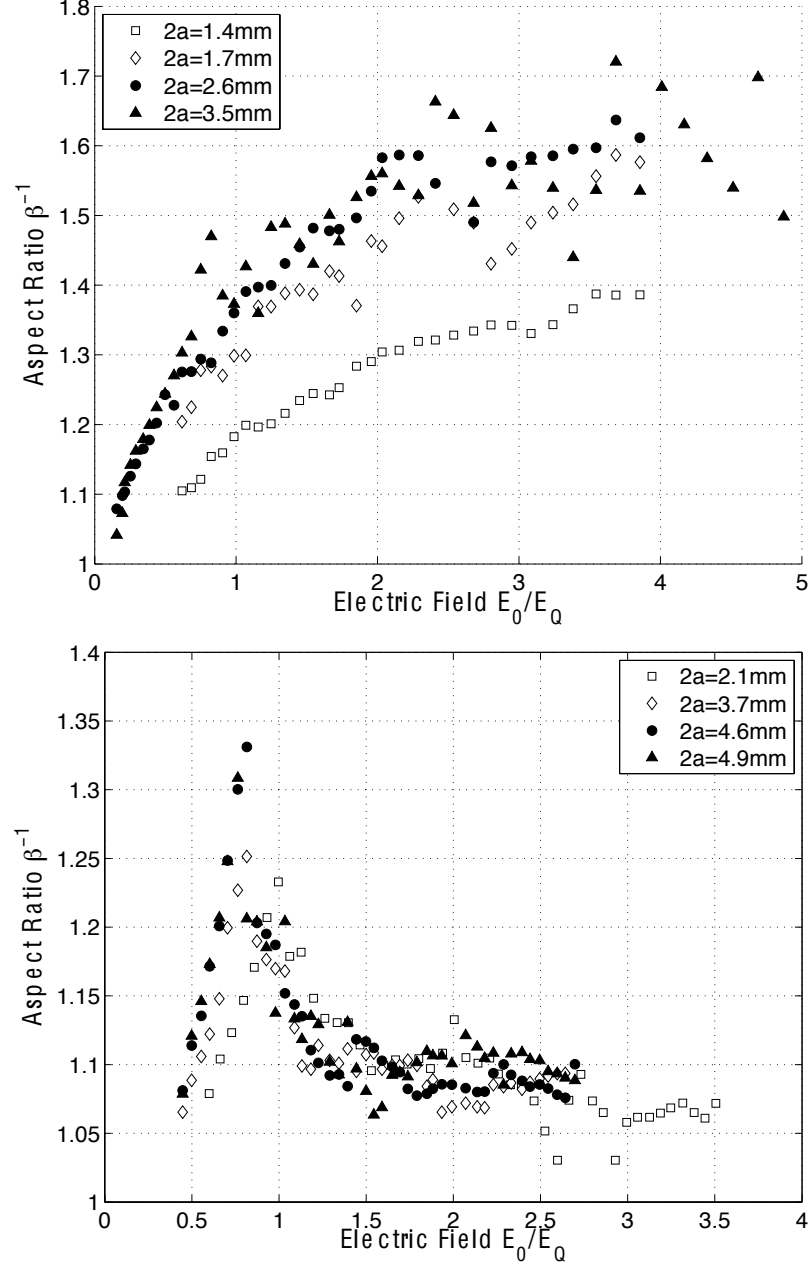


Figure 3.17: Aspect ratio as a function of electric field strength: (top) $\Lambda=1$, (bottom) $\Lambda = 14$.

Figure 3.17 shows the steady state aspect ratio for various size drops as a function of electric field strength. The onset of rotation acts to keep the high viscosity drops spherical. As a result, the transition from “Taylor” to “Tilted” behavior is apparent by the decrease in aspect ratio corresponding to the onset of rotation. For lower viscosity ratios, $\Lambda \sim 1$, drop deformation increases monotonically with electric field

strength.

Toroidal flow and rotational flow are represented by the timescales, t_{hd} and ω_Q^{-1} respectively. The comparison of these two flow timescales relative to surface tension demonstrates their strength to deform the interface. For all viscosity ratios except $\Lambda = 14$, the t_{hd} timescale is faster than ω_Q^{-1} . At high field strengths, t_{hd} is also faster than the restoring timescale and drop deformation is significant, i.e. $Ca > 1$.

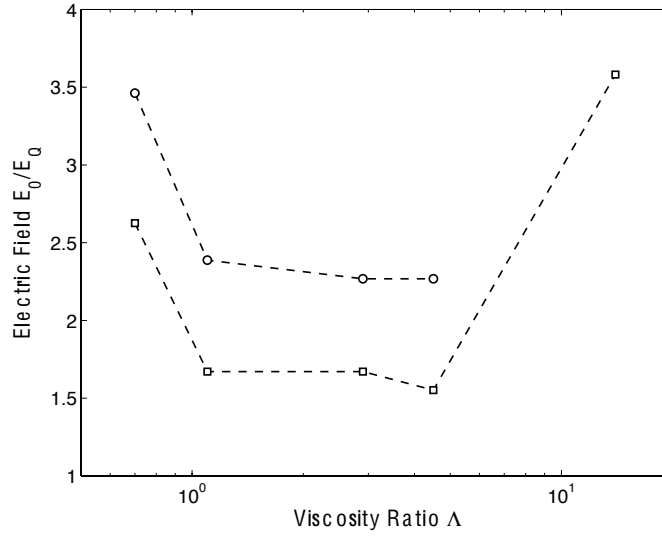


Figure 3.18: Field strength for the transition from steady to unsteady behavior for different viscosity ratios. $\square$ denotes drops that have initial diameter in range of 5.3-5.7mm and $\circ$ denotes drops that have an initial diameter 3.5-3.8mm.

Drop aspect ratio was measured for all viscosity ratios immediately before the transition to unsteady behavior. This corresponds to the points separating “Tilted” and “Unsteady” behaviors plotted in Figure 3.16. Figure 3.19 shows that aspect ratio at the unsteady transition decreases with viscosity ratio. The transition occurs at a similar aspect ratio for a given viscosity ratio, with the exception of $\Lambda = 0.7$, which shows greater variability. This suggests that once the drop reaches a critical aspect ratio, the drop shape can become unstable. For smaller drops, a stronger field is required to overcome surface tension forces and reach the critical aspect ratio, see Figure 3.16.

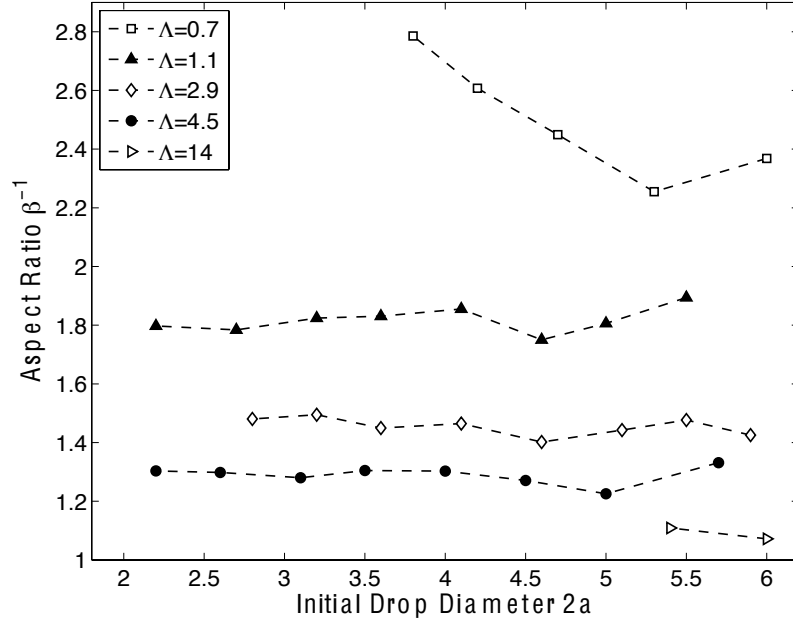


Figure 3.19: Drop aspect ratio is compared to initial drop diameter at the transition to unsteady drop shape. The data show that for a given viscosity ratio, the transition to unsteady rotation will occur at a similar aspect ratio.

The dependence on viscosity on the steady to unsteady transition in Figure 3.19 can be explained by considering the ratio of rotation rate to the surface tension timescale given by Ca_ω in Eq.[3.22]

For a drop that is already deformed, a rotational flow will deform the interface faster than surface tension can respond if $Ca_\omega \gtrsim 1$. When the surface cannot respond to distortions from fluid rotation, the transition from steady to unsteady is likely to occur. This can be seen in Figure 3.20, where the transition from steady to unsteady is plotted for each viscosity ratio. With the exception of $\Lambda = 14$, the transition to unsteady behavior occurs at $Ca_\omega \sim 1 - 2$. The dependence on aspect ratio and Ca_ω allows the data to be reduced to one graph showing the aspect ratio and Ca_ω number where the transition to unsteady behavior can occur, see Figure 3.21. The clustering for a given viscosity ratio shows that the drop transitions to unsteady behavior at similar aspect ratios and $Ca_\omega \sim 1 - 2$.

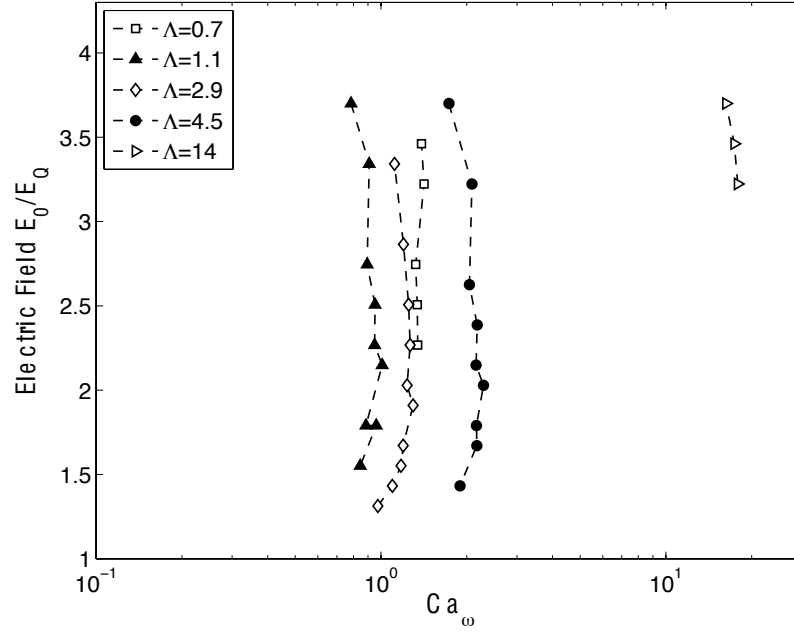


Figure 3.20: Drop aspect ratio at the transition from steady to unsteady for different viscosity ratios as a function of initial drop diameter $2a$.

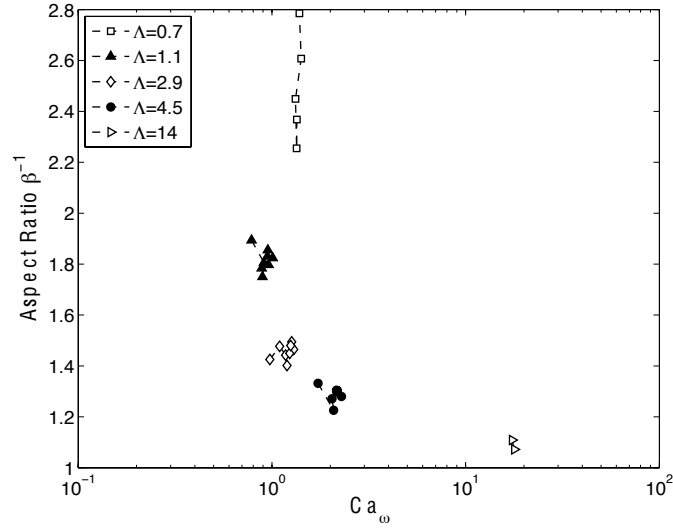


Figure 3.21: Aspect ratio at the transition from steady to unsteady for different viscosity ratios as a function of Ca_ω .

As electric field is increased, the rotation increases relative to the fixed surface tension timescale. For low viscosity drops, it takes a stronger field to reach greater deformation and $Ca_\omega \sim 1$. This explains the higher critical field required for the lowest viscosity drops, $\Lambda = 0.7$. The higher variability in the transition for $\Lambda = 0.7$

drops may be due to charge convection resulting from the axisymmetric flow or the effects of the chamber walls on a highly deformed drop.

The transition to unsteady behavior can also be understood by considering a perturbation in the drop shape. If $Ca_\omega \gtrsim 1$, the perturbation is convected by rotation before surface tension can act to restore the drop shape. The perturbation may grow if the new shape leads to greater stress on the drop. The distorted shape will alter the induced dipole and disrupt the rotation rate. A decrease in rotation rate will limit charge convection, leading to a buildup of charge and return to oblate shape. Eventually, the buildup of charge will lead to an increase in electric torque and reinitiate rotation. The coupling between shape and induced charge results in the oscillatory motion observed at low to intermediate viscosity ratios.

For high viscosity drops, the rotational flow is the dominant flow, $t_{\text{hd}} > \omega_Q^{-1}$. In contrast to lower viscosity drops, rotational flow limits drop deformation and stabilizes the shape. The comparison between t_{hd} and ω_Q^{-1} in Figure 3.9 shows that drop shapes are significantly inhibited by the rotational flow. This can also be seen in the data for aspect ratio in Figure 3.17, where the high viscosity drops become less deformed after rotation begins.

The weak straining flow implies that the source of unsteady behavior is different for high viscosity drops. The tumbling behavior observed in experiments indicates that the high viscosity drops behave similarly to rigid ellipsoids in an electric field, where anisotropic charging can lead to unsteady behavior even without inertia [31, 134]. The experiments in Figure 3.17 show that aspect ratio stays at a relatively constant value once rotation is initiated. Similar to an ellipsoid in an electric field, the deformed drop can reach a critical strength where tumbling occurs due to the anisotropic shape. The threshold for unsteady behavior in high viscosity drops is

better estimated by the stability of an ellipsoid with a nonzero surface velocity. This will be examined theoretically in Section 3.7.

3.6.4 Unsteady drop deformation

The characteristics of the unsteady behavior described in Section 3.6.1 are investigated in further detail. The drop angle and aspect ratio are analyzed from recordings of unsteady drop behavior. The data for drop orientation angle demonstrates the range of orientations the drop takes during unsteady behavior, i.e. oscillations or tumbling. The aspect ratio shows the variability in deformation as a function of time.

The data is plotted as a function of time as well as with delay plots, which compares a value recorded at time, t , with the same value recorded after a time step, $t+t'$ [3]. Delay plots are a useful method for characterizing experimental observations of dynamical systems. In essence, they can reproduce part of the state space of the system. For a periodic orbit, the delay plot should show a predictable path in phase space. For chaotic systems, which have a sensitive dependence to initial conditions, the delay plot will show an unpredictable path. A chaotic system may only access certain regions of state space, but will be chaotic within that region. The purpose of the delay plots is to give information about the regions of state space the system explores. Delay plots also allow for useful comparisons between experimental data and a theoretical model. The following analysis will be repeated using the model developed in Section 3.7.

$\Lambda = 1.1$

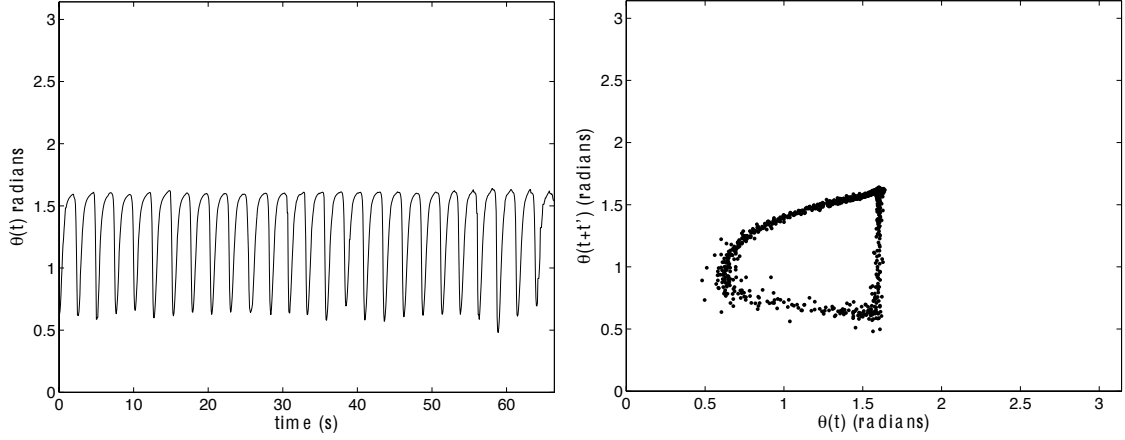


Figure 3.22: Drop orientation angle for a $\Lambda = 1.1$, $a=3.6\text{mm}$, drop in a field with strength $E_0 = 9.9$ kV/cm ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).

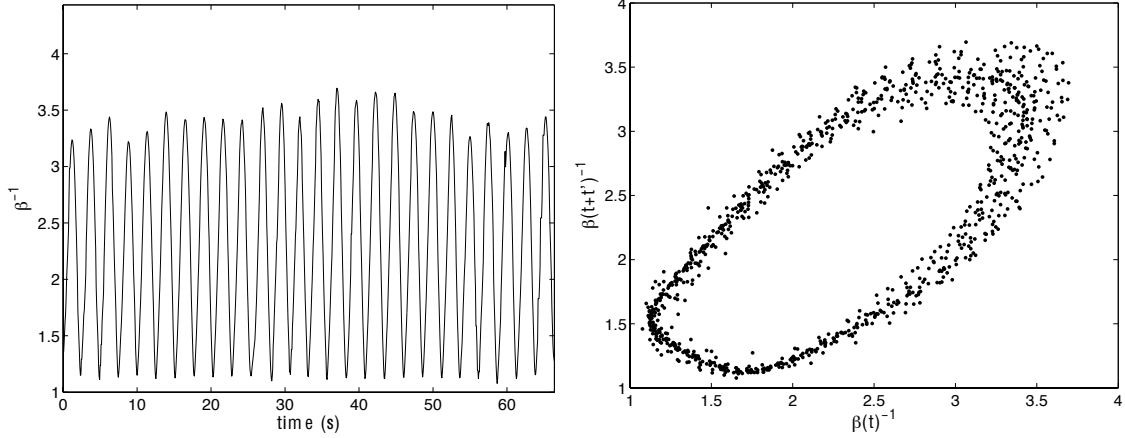


Figure 3.23: Aspect ratio β^{-1} for a $\Lambda = 1.1$, $2a=3.6\text{mm}$ drop in an electric field with strength $E_0 = 9.9$ kV/cm ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).

The orientation angle and aspect ratio for a $\Lambda = 1.1$ drop are shown as a function of time and in delay plots in Figures 3.22 and 3.23. The oscillations are steady and the delay plot for angle is confined to a well defined path. Figure 3.22 shows that the oscillations are limited to one rotational direction, which is evident from the confinement of the angle data to one quadrant. The scatter is limited to regions of shallow angles and large deformations. The scatter may be due to small variations in the oscillations or variations in the orientation of the drop with respect to the camera. Errors may also originate from image processing, particularly in fitting a

slightly irregularly shaped drop shape to an ellipse.

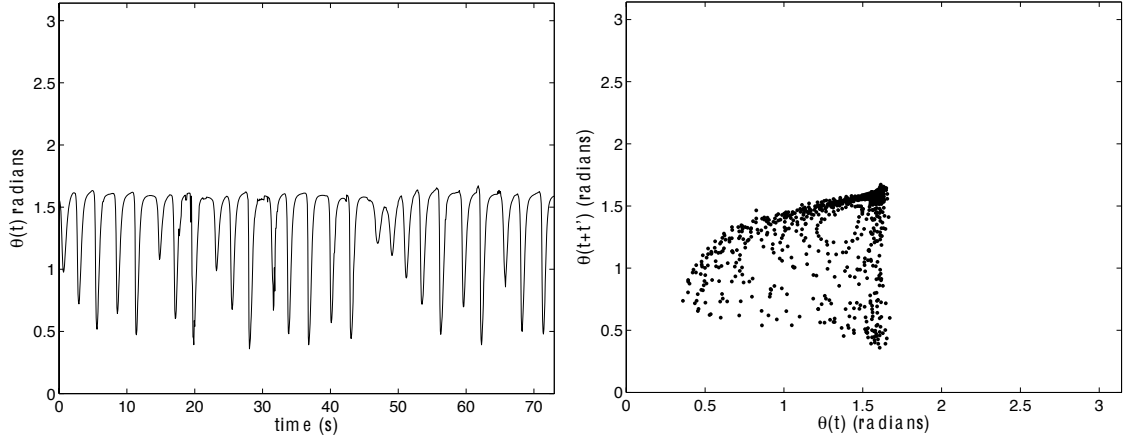


Figure 3.24: Drop orientation angle for a $\Lambda = 1$, $2a=5.4\text{mm}$ drop in an electric field with strength $E_0 = 9.9 \text{ kV/cm}$ ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).

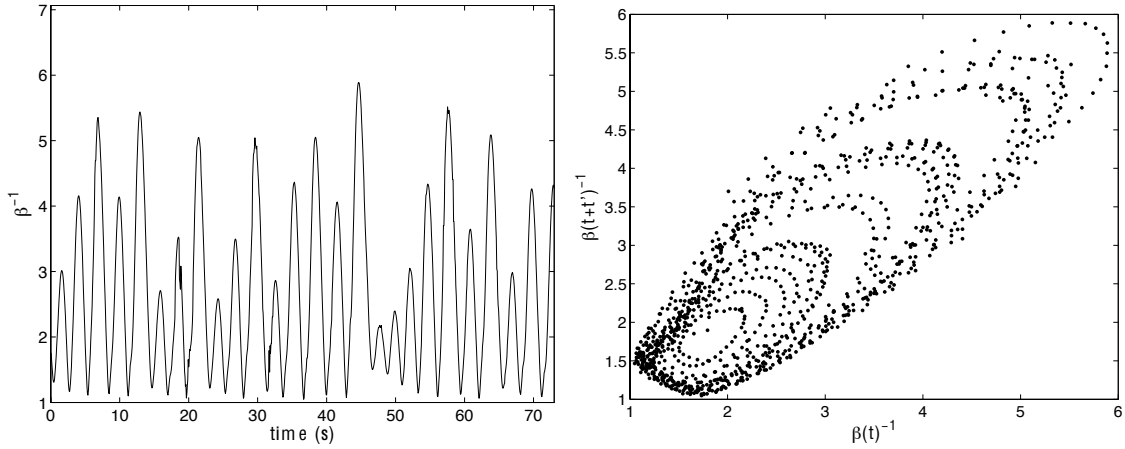


Figure 3.25: Aspect ratio β^{-1} for a $\Lambda = 1.1$, $2a=5.4\text{mm}$ drop in an electric field with strength $E_0 = 9.9 \text{ kV/cm}$ ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).

The same analysis is repeated for a larger $\Lambda = 1.1$ drop at an increased field strength, see Figures 3.24 and 3.25. The angle and aspect ratio data show growing and decaying oscillations as well as greater variability for both parameters. The drop deformation will grow until a large deformation disrupts the rotational flow and the drop retracts in shape. The drop continues to rotate in the same direction and the shape oscillations begin to grow from the less deformed shape. This is most clearly seen in the angle data in Figure 3.24 at 40-55 seconds where the drop experiences a

long pause at $\theta \sim \pi/2$. Over this same period of time, the aspect ratio experiences a large decrease and then builds from approximately 45-55 seconds. The growing and decaying oscillations are apparent in the larger regions explored in the delay plots. Figure 3.25 shows that a common sequence in drop shape is from a small, to a medium, and then finally a large deformation. This may indicate that certain orbits in the drop aspect ratio are more likely than others, even though the trajectory is aperiodic. This demonstrates that there can be chaotic behavior for low viscosity ratios, but that large drops and strong fields are required. The chaotic behavior is confined to the same direction of motion and a limited range of aspect ratios.

Viscosity ratios lower than $\Lambda < 1$ show qualitatively similar behavior to $\Lambda = 1.1$. The $\Lambda = 0.7$ drops deform more, but also oscillate in only one direction. The main difference is that stronger fields and larger drops are required to see unsteady behavior in behavior in $\Lambda = 0.7$ drops, see Figure 3.16.

$\Lambda = 4.5$

For $\Lambda = 4.5$ drops of all sizes, growing oscillations and changes in rotation direction are observed at any field strength where unsteady behavior is observed. The magnitude of deformation, oscillation frequency, and frequency of rotation direction reversal all change with drop size and field strength.

Figure 3.26 shows the growing oscillations and reversal of rotation direction in the orientation angle. The aspect ratio, seen in Figure 3.27, also oscillates and increases in amplitude until direction reversal, when the drop retracts and the oscillations restart from a less deformed shape. The drop reverses direction after about 7-9 periods of oscillation and the drop shape increases with each period until a reversal in direction. The orientation angles appear in both the upper right and

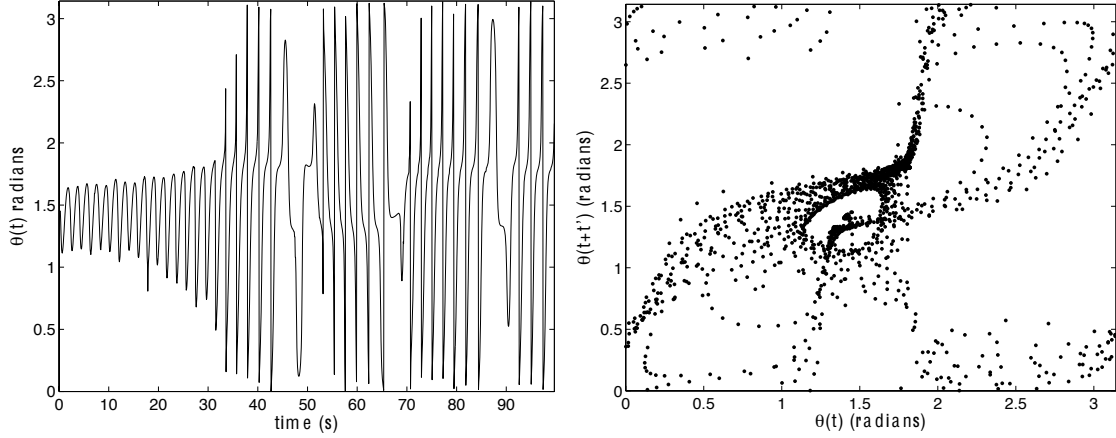


Figure 3.26: Drop orientation angle for a $\Lambda = 4.5$, $2a=3.5\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). Angle data as a function of time (left) and delay plot for angle with $\tau'=1/3$ second delay (right).

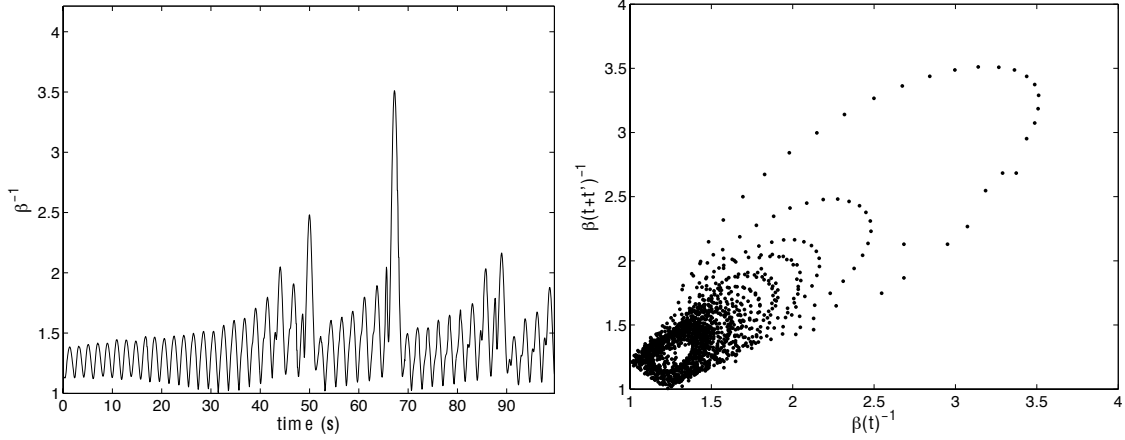


Figure 3.27: Aspect ratio β^{-1} for a $\Lambda = 4.5$, $2a=3.5\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $\tau'=1/3$ second delay (right).

lower left quadrants of the delay plot, which represent oscillations in clockwise and counterclockwise directions.

In contrast to $\Lambda \sim 1$ drops, the intermediate viscosity drops explore a wide range of phase space, indicating that the orientation angle is difficult to predict based on previous orientations. The most frequent orientation is close to $\theta \sim \pi/2$, which corresponds to the long axis aligning with the field. The aspect ratio varies significantly for the $\Lambda = 4.5$ drops, but is usually smaller than the $\Lambda = 1.1$ drops.

The $\Lambda = 4.5$ drop is primarily within $\beta^{-1} < 2$, but during direction reversals the aspect ratio swings to large values around $\beta^{-1} \sim 3$.

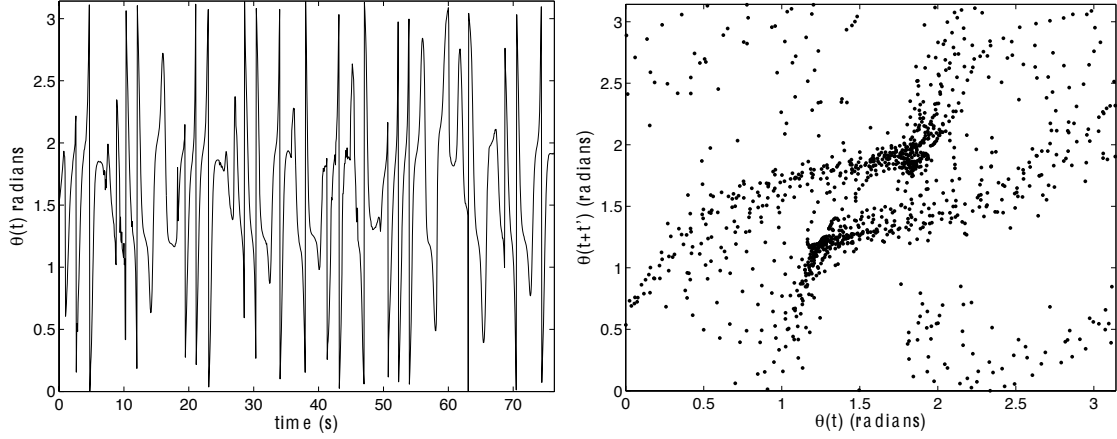


Figure 3.28: Drop orientation angle for a $\Lambda = 4.5$, $2a=5.6\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.4 E_Q$). Angle data as a function of time (left) and delay plot for angle with $t'=1/3$ second delay (right).

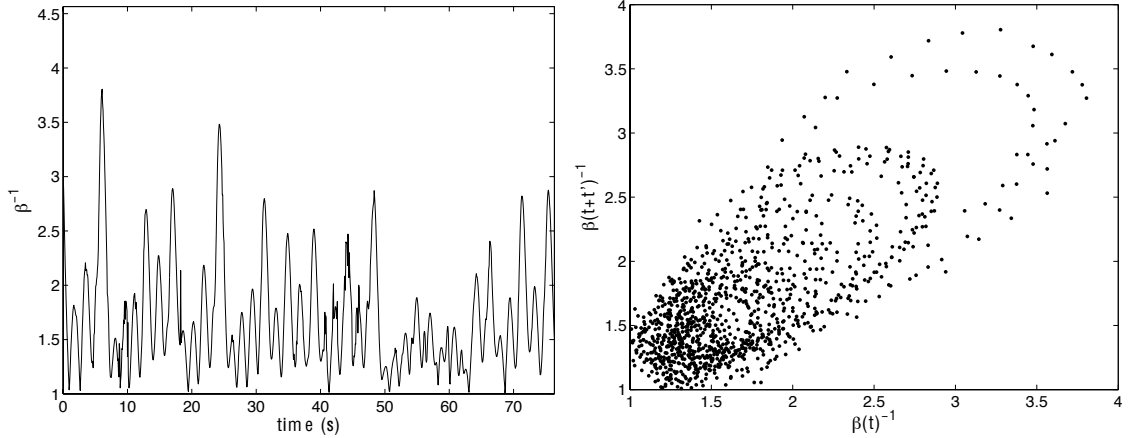


Figure 3.29: Aspect ratio β^{-1} for a $\Lambda = 4.5$, $2a=5.6\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.4 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $t'=1/3$ second delay (right).

Figures 3.28 and 3.29 show the evolution of the drop angle and aspect ratio for a larger $\Lambda = 4.5$ drop at the same field strength. The larger drop reverses direction more frequently, about 2-4 periods before reversing. The more frequent direction reversals of the larger drop are apparent in the variations in aspect ratio. The drop experiences large deformation more frequently and shows a wider shape variation, see Figure 3.29. Delay plots of the orientation angle and aspect ratio for the larger

drop shows that the drop explores a wider range of phase space, indicating a more chaotic behavior. This drop did not break up during this period of observation, but drops of similar size were observed to break up at this field strength. Breakup usually occurs after an unusually large deformation, the drop subsequently folds upon itself as shown in Figure 3.15.

Viscosity ratio $\Lambda = 2.9$ drops show similar behavior to $\Lambda = 4.5$ drops. As a result, the analysis is not repeated for this viscosity ratio. The similarity indicates that the behavior is not sensitive to relatively small variations in viscosity ratio within the $\Lambda = 2 - 5$ range.

$\Lambda = 14$

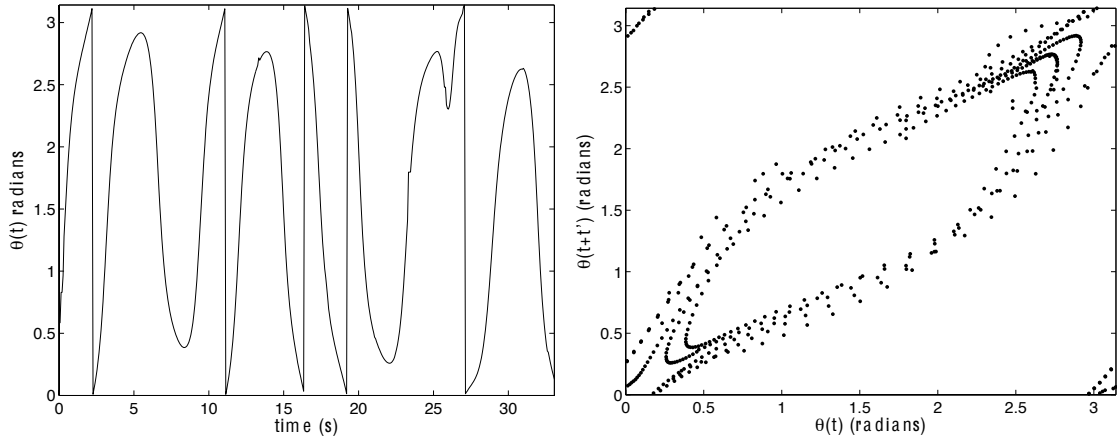


Figure 3.30: Drop orientation angle for a $\Lambda = 14$, $2a=6.9\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). Angle data as a function of time (left) and delay plot for angle with $\tau'=1/3$ second delay (right).

The behavior of high viscosity drops differs significantly from the behavior shown for lower viscosity drops. $\Lambda = 14$ drops predominantly exhibit tumbling behavior. At the onset of unsteady behavior, the drop shape will tumble in the same direction and the deformation will grow. Once the drop reaches a large deformation, the drop will reverse direction and resume tumbling.

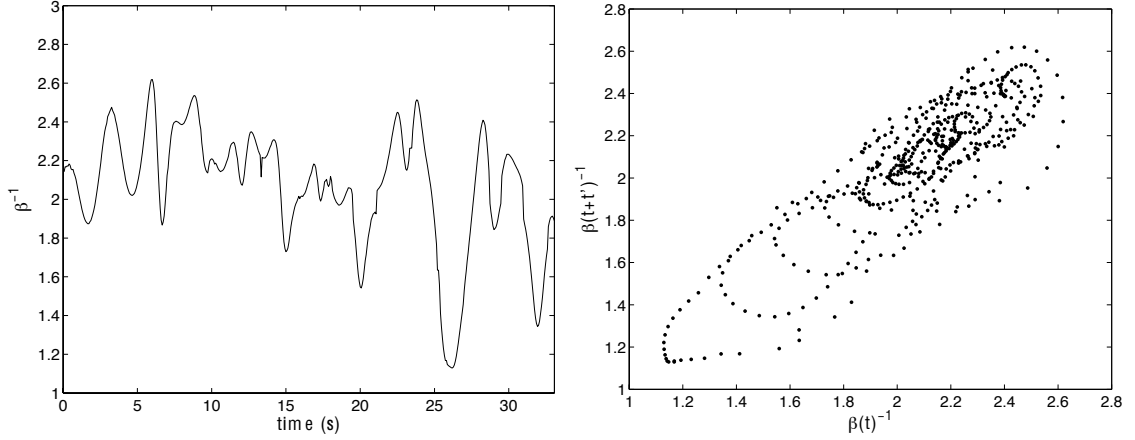


Figure 3.31: Aspect ratio β^{-1} for a $\Lambda = 14$, $2a=6.9\text{mm}$ drop in an electric field with strength $E_0 = 9.1 \text{ kV/cm}$ ($3.1 E_Q$). β^{-1} as a function of time (left) and delay plot for β^{-1} with $\tau'=1/3$ second delay (right).

Figures 3.30 and 3.31 show angle and aspect ratio data during tumbling and reversals of rotation direction for a $\Lambda = 14$ drop. The drop can reverse direction after as little as 1 period, or tumble in the same direction for over 4 periods. The drop shape oscillates slightly with each period; the largest deformation coincides with the short axis aligning with the field direction. In this orientation, the drop is squeezed in a flatter oblate and then recovers while rotating. The slower surface tension timescale decreases the variability in aspect ratio compared to lower viscosity ratios.

The rotation axis is degenerate within the plane perpendicular to the field. Due to the chaotic behavior of the drop, the shape can become obscured if the drop rotates towards the camera. The drop shape appears to be spherical while the drop is in this orientation. This is reflected in Figures 3.30 and 3.31 at $t = 25\text{s}$, where aspect ratio approaches $\beta \sim 1$. Before $t = 25\text{s}$, the drop is tumbling in the plane of view and the drop aspect ratio varies little, usually within $\beta^{-1} = 1.8 - 2.5$.

Delay plots of the orientation angle and aspect ratio are shown in Figures 3.22 and 3.23. The tumbling behavior occupies a different phase space than the lower

viscosity drops. The longest pauses are around $\theta = 0, \pi$, corresponding to the short axis of the drop aligning with the field. The pauses in orientation occur after the drop flips. While in the paused state, the induced dipole recharges until the electric torque is strong enough to reinitiate rotation. The drop can either continue or reverse in rotation direction.

3.7 Fluid ellipsoid model

To capture the effect of fluid flow and anisotropic shape, a fixed shape fluid ellipsoid model is proposed to describe the behavior of fluid drops in electric fields. The theoretical model is derived by expanding on previous work on rigid ellipsoids in electric fields. The model is analyzed and compared to experimental data. Finally, the parameters leading to chaotic behavior are explored.

3.7.1 Rigid ellipsoid

The Quincke model developed in Section 3.2 can be generalized to a rigid ellipsoid by accounting for the polarization relaxation equations in a co-rotating frame of reference [200, 31, 32, 134]. Rotation is around the axis perpendicular to the symmetry axis of the ellipsoid. In this model inertia is neglected because, as discussed in Chapter 2, Reynolds number is much less than one for an oil system. Similar to Eq.[3.14], but in co-rotating reference frame, the torque balance for an ellipsoid is,

$$\alpha_{\perp} \frac{d\theta}{dt} = P_{\parallel} E_{\perp} - P_{\perp} E_{\parallel} + (\chi_{\parallel}^{\infty} - \chi_{\perp}^{\infty}) E_{\perp} E_{\parallel}, \quad (3.24)$$

where $\alpha_{\perp}$ is the friction coefficient, χ^{∞} is the high-frequency susceptibility, and $\parallel, \perp$ denote components parallel and perpendicular to the axis of symmetry, see Figure 3.12. The electric torque, i.e. the righthand side of Eq.[3.24], is abbreviated by,

$$L_E = P_{\parallel} E_{\perp} - P_{\perp} E_{\parallel} + (\chi_{\parallel}^{\infty} - \chi_{\perp}^{\infty}) E_{\perp} E_{\parallel}. \quad (3.25)$$

The evolution of the induced dipole is described by the polarization relaxation

equations [200]. In the case of an ellipsoid, the dipole can be decomposed into components parallel and perpendicular to the symmetry axis of the ellipsoid,

$$\frac{\partial P_{\parallel}}{\partial t} = -\frac{1}{t_{\parallel,\text{mw}}} [P_{\parallel} - (\chi_{\parallel}^0 - \chi_{\parallel}^{\infty})E_{\parallel}], \quad (3.26a)$$

$$\frac{\partial P_{\perp}}{\partial t} = -\frac{1}{t_{\perp,\text{mw}}} [P_{\perp} - (\chi_{\perp}^0 - \chi_{\perp}^{\infty})E_{\perp}], \quad (3.26b)$$

where $t_{\parallel,\text{mw}}, t_{\perp,\text{mw}}$ and $\chi_{\parallel,\perp}^0$ are directional Maxwell-Wagner relaxation timescales and low-frequency susceptibility respectively. These parameters are,

$$\chi_{\parallel,\perp}^0 = \frac{\epsilon_{\text{ex}} V (\lambda_{\text{in}} - \lambda_{\text{ex}})}{(\lambda_{\text{in}} - \lambda_{\text{ex}}) n_{\parallel,\perp} + \lambda_{\text{ex}}}, \quad \chi_{\parallel,\perp}^{\infty} = \frac{\epsilon_{\text{ex}} V (\epsilon_{\text{in}} - \epsilon_{\text{ex}})}{(\epsilon_{\text{in}} - \epsilon_{\text{ex}}) n_{\parallel,\perp} + \epsilon_{\text{ex}}}, \quad (3.27)$$

$$t_{\parallel,\text{mw}} = \frac{(\epsilon_{\text{in}} - \epsilon_{\text{ex}}) n_{\parallel} + \epsilon_{\text{ex}}}{(\lambda_{\text{in}} - \lambda_{\text{ex}}) n_{\parallel} + \lambda_{\text{ex}}}, \quad t_{\perp,\text{mw}} = \frac{(\epsilon_{\text{in}} - \epsilon_{\text{ex}}) n_{\perp} + \epsilon_{\text{ex}}}{(\lambda_{\text{in}} - \lambda_{\text{ex}}) n_{\perp} + \lambda_{\text{ex}}}, \quad (3.28)$$

where V is the volume of the ellipsoid. In Eq.[3.27] and Eq.[3.28], the depolarization factor is denoted by $n_{\parallel,\perp}$. For prolate ellipsoids ($\beta > 1$),

$$n_{\parallel} = \frac{1 - e^2}{e^3} (\text{arctanh}(e) - e), \quad \text{where } e = \sqrt{1 - 1/\beta^2}, \quad (3.29)$$

while for oblate ellipsoids ($\beta < 1$),

$$n_{\perp} = \frac{1 + e^2}{e^3} (e - \text{arctanh}(e)), \quad \text{where } e = \sqrt{1/\beta^2 - 1}. \quad (3.30)$$

The second shape factor is determined by $n_{\perp} = (1 - n_{\parallel})/2$ for both oblate and prolate ellipsoids. Note that the shape factors for oblate and prolate can be related by a transformation of the principle axes. A prolate with axes $a_1 > a_2 = a_3$ corresponds to an oblate, $\hat{a}_1 = \hat{a}_2 > \hat{a}_3$, where $\hat{a}_1 = a_1$, $\hat{a}_2 = a_1 a_3 / a_2$, $\hat{a}_3 = a_3$ [54].

The solution of Eq.[3.24] and Eq.[3.26] results in three types of behavior [134]. In weak fields, the long axis of the ellipsoid aligns with the field. As the field

strength is increased, the ellipsoid either swings around this aligned position or tumbles. Some sets of parameters show alternating between swinging and tumbling. The alternating behavior can be shown to be chaotic, i.e. the switching between swinging and tumbling is unpredictable and sensitive to initial parameters.

This ellipsoid model does not capture the behavior of drops seen in experiments. Specifically, the ellipsoid is modeled as rigid and there is no dependence on drop viscosity. There are no solutions that result in a steady tilted orientation or swinging around a tilted orientation, similar to that seen in drop experiments. To capture the behaviors seen in experiment, the fluidity of the drops must be considered.

3.7.2 Fluid ellipsoid

The ellipsoid model is modified through the addition of a tangential surface velocity at the interface. The velocity of the surface flow is described by a flow frequency, ν , see Figure 3.12. The surface velocity affects both the torque balance and the polarization relaxation equations. An additional torque arises from the flow field created by the surface velocity. This torque is identical to that developed for the tank-treading interface on a red blood cell in shear flow [96]. The surface flow also contributes to charge convection, which modifies the polarization relaxation equations.

A tangential fluid flow is considered around a fixed shape ellipsoid, i.e. no normal interface velocity. Following the approach originally derived by Jeffrey [87], the fluid flow inside the ellipsoid is given by the equation,

$$\mathbf{u}^{\text{in}} = \nu \left(-y\beta, \frac{x}{\beta}, 0 \right). \quad (3.31)$$

At distances far from the fluid drop, the external flow behaves as a rotational flow with amplitude $\Omega = \dot{\theta}$,

$$\mathbf{u}^{\text{ex}} = -\boldsymbol{\Omega} \times \mathbf{r} = (y\Omega, -x\Omega, 0). \quad (3.32)$$

The external field can be written in terms of the internal field plus a disturbance field, $\mathbf{u}^D$, as,

$$\mathbf{u}^{\text{ex}} = \mathbf{u}^{\text{in}} + \mathbf{u}^D. \quad (3.33)$$

At $r \rightarrow \infty$, the velocity fields must match, this gives,

$$\mathbf{u}^D = \left(y(\Omega + \nu\beta), -x\left(\Omega + \nu\frac{1}{\beta}\right), 0 \right). \quad (3.34)$$

The velocity-gradient tensor can be decomposed as,

$$\lim_{r \rightarrow \infty} \frac{\partial \mathbf{u}_i^D}{\partial x_j} = \gamma_{ij} + \omega_{ij}, \quad (3.35)$$

with symmetric and antisymmetric components γ_{ij} and ω_{ij} respectively. For the case of an ellipsoid, the tensor components are,

$$\gamma_{12} = \gamma_{21} = \frac{\nu}{2} \left(\beta - \frac{1}{\beta} \right), \quad \omega_{12} = -\omega_{21} = \Omega + \frac{\nu}{2} \left(\beta + \frac{1}{\beta} \right). \quad (3.36)$$

A torque from the flow field is produced by the combined effects of surface flow and rigid body rotation. This torque is given by [54],

$$L_{TT+TB} = V\mu_{\text{ex}} (A_{21} - A_{12}), \quad (3.37)$$

where,

$$A_{12} = 2 \frac{n_{\parallel} \gamma_{12} + n'_3 \omega_{12}}{(\beta^2 n_{\parallel} + n_{\perp}) n'_3}, A_{21} = 2 \frac{n_{\perp} \gamma_{21} + \beta^2 n'_3 \omega_{21}}{(\beta^2 n_{\parallel} + n_{\perp}) n'_3}, \quad n'_3 = -\frac{n_{\parallel} - n_{\perp}}{\beta^2 - 1}. \quad (3.38)$$

Combining the expressions in Eq.[3.36] into Eq.[3.37], the torque simplifies to the expression,

$$L_{TT+TB} = -\alpha_{\perp} \left(\dot{\theta} + \frac{2\beta}{1 + \beta^2} \nu \right) \quad (3.39)$$

where $\alpha_{\perp} = 2\mu V M_{\perp}$ is the friction coefficient and

$$M_{\perp} = \frac{1 + \beta^2}{\frac{1-n_{\parallel}}{2} + n_{\parallel} \beta^2}. \quad (3.40)$$

As already introduced in Eq.[3.25], the electric torque on an ellipsoid in a uniform electric fields is given by,

$$L_E = P_T \times E. \quad (3.41)$$

Note that total polarization is $P_T = P + P_{\infty}$, and $P_{\infty} = \chi^{\infty} E$ is the instantaneous polarization.

The torque balance between viscous and electric components, $L_{TT+TB} + L_E = 0$, gives the expression,

$$-\alpha_{\perp} \left(\dot{\theta} + \frac{2\beta}{1 + \beta^2} \nu \right) - P_{\perp} E_{\parallel} + P_{\parallel} E_{\perp} + (\chi_{\parallel}^{\infty} - \chi_{\perp}^{\infty}) E_{\perp} E_{\parallel} = 0, \quad (3.42)$$

where $E_{\parallel} = E_0 \cos \theta$ and $E_{\perp} = -E_0 \sin \theta$ are introduced for convenience.

As discussed in Section 3.2, the polarization relaxation equations describe time dependent interfacial charging. In a frame of reference co-rotating with the ellipsoid,

the convection due to rigid body rotation is captured by the relation between $P_{\parallel}$ and $P_{\perp}$; there is no explicit dependence on ω . The convection due to surface flow remains because the interfacial charge can move relative to the rigid body rotation. The polarization relaxation equations in the co-rotating frame are,

$$\frac{\partial P_{\parallel}}{\partial t} = -\nu P_{\perp} \beta - \frac{1}{t_{\parallel, \text{mw}}} [P_{\parallel} - (\chi_{\parallel}^0 - \chi_{\parallel}^{\infty}) E_{\parallel}], \quad (3.43a)$$

$$\frac{\partial P_{\perp}}{\partial t} = \frac{\nu P_{\parallel}}{\beta} - \frac{1}{t_{\perp, \text{mw}}} [P_{\perp} - (\chi_{\perp}^0 - \chi_{\perp}^{\infty}) E_{\perp}]. \quad (3.43b)$$

Equations Eq.[3.42] and Eq.[3.43] are nondimensionalized before solving. The variables are normalized by,

$$\tilde{t} = \frac{t}{t_{\text{mw}}}, \quad \tilde{P} = \frac{P}{P_c}, \quad P_c = \Delta\chi_s \epsilon_{\text{ex}} E_0 V, \quad \Delta\chi_s = (\chi_s^0 - \chi_s^{\infty}) / \epsilon_{\text{ex}} = \frac{9(R-S)}{(R+2)(S+2)} \quad (3.44)$$

$$\chi_s^0 = \epsilon_{\text{in}} \frac{R-1}{R+2}, \quad \chi_s^{\infty} = \epsilon_{\text{in}} \frac{S-1}{S+2}, \quad \tilde{L}_E = \frac{L_E}{P_c E_0}, \quad \tilde{\nu} = \nu t_{\text{mw}} \quad (3.45)$$

In dimensionless form, the torque balance reduces to,

$$\dot{\theta} = -\frac{2\beta}{1+\beta^2} \tilde{\nu} + \frac{BC_1}{2M_{\perp}} \left[\left(-\tilde{P}_{\perp} \cos \theta - \tilde{P}_{\parallel} \sin \theta \right) - C_2 \cos \theta \sin \theta \right], \quad (3.46)$$

with the values,

$$B = \frac{\epsilon_{\text{ex}}^2 E_0^2}{\mu_{\text{ex}} \sigma_{\text{ex}}}, \quad C_1 = \frac{9(R-S)}{(R+2)^2}, \quad C_2 = \left[\frac{(S-1)}{(S-1)n_{\parallel} + 1} - \frac{(S-1)}{(S-1)n_{\perp} + 1} \right] \left(\frac{S+2}{R+2} \right). \quad (3.47)$$

The polarization relaxation equations are nondimensionalized using the variables

in Eq.[3.44] and Eq.[3.45].

$$\frac{\partial P_{\parallel}}{\partial t} = -\tilde{\nu}\tilde{P}_{\perp}\beta - T_{\parallel} \left(\tilde{P}_{\parallel} - X_{\parallel} \cos \theta \right), \quad (3.48a)$$

$$\frac{\partial P_{\perp}}{\partial t} = \frac{\tilde{\nu}\tilde{P}_{\parallel}}{\beta} - T_{\perp} \left(\tilde{P}_{\perp} + X_{\perp} \sin \theta \right), \quad (3.48b)$$

where,

$$T_{\parallel,\perp} = \left(\frac{t_{\text{mw}}}{t_{\parallel,\perp \text{mw}}} \right) = \left(\frac{S+2}{R+2} \right) \left(\frac{(R-1)n_{\parallel,\perp} + 1}{(S-1)n_{\parallel,\perp} + 1} \right) \quad (3.49)$$

and,

$$X_{\parallel,\perp} = \frac{(R+2)(S+2)}{9(1+n_{\parallel,\perp}(R-1))(1+n_{\parallel,\perp}(S-1))}. \quad (3.50)$$

The final variable to be determined is the surface flow frequency, ν , which is found by balancing the rate of electrical energy input with viscous dissipation. The viscous dissipation, first derived by Keller and Skalak [96], is given by,

$$\Pi_{TT+TB} = (\mu_{\text{in}} - \mu_{\text{ex}}) \nu^2 V \left(\frac{1}{\beta} - \beta \right)^2 + \mu_{\text{ex}} \nu V \left(\beta A_{12} - \frac{1}{\beta} A_{21} \right) \quad (3.51)$$

The equation reduces by utilizing the torque balance, Eq.[3.39],

$$\Pi_{TT+TB} = \mu_{\text{ex}} \nu^2 V (\Lambda f_1 - f_2) + \frac{4\nu L_E}{M_{\perp}(\beta n_{\parallel} + \frac{1}{\beta} n_{\perp})}, \quad (3.52)$$

where,

$$f_1 = (\beta - 1/\beta)^2, \quad f_2 = f_1 \left[1 - \left(\frac{\beta^2 - 1}{(n_{\parallel} - n_{\perp})(\beta^2 + 1)} \right) \right] \quad (3.53)$$

The energy input from the electric field, Π_E , is determined by the surface integral,

$$\Pi_E = \int \mathbf{u} \cdot \left(\underline{\underline{\mathbf{T}}}^{\text{el}} \cdot \hat{\mathbf{n}} \right) dS, \quad (3.54)$$

$$\mathbf{u} \cdot (\underline{\mathbf{T}}^{\text{el}} \cdot \hat{\mathbf{n}}) = \nu(\mathbf{E}^d \cdot \hat{\mathbf{t}})(\mathbf{P} \cdot \hat{\mathbf{n}}), \quad (3.55)$$

where $\mathbf{E}^d$ is the electric field inside the drop. The electric field in an ellipsoid is given by [92, 114],

$$E_{\parallel,\perp}^d = E_{\parallel,\perp}^\infty - \frac{n_{\parallel,\perp}}{\epsilon_{\text{ex}}}(P_{\parallel,\perp} + P_{\parallel,\perp}^\infty), \quad (3.56)$$

$E_{\parallel,\perp}^\infty$ is the electric field at infinity and $P_{\parallel,\perp} + P_{\parallel,\perp}^\infty$ is the total polarization in component form. The exact expressions in the integral, Eq.[3.55], are,

$$\mathbf{E}^d \cdot \hat{\mathbf{t}} = -\beta E_{\parallel}^d y + \frac{1}{\beta} E_{\perp}^d x, \quad \mathbf{P} \cdot \hat{\mathbf{n}} = P_{\parallel} \hat{\mathbf{n}}_{\parallel} + P_{\perp} \hat{\mathbf{n}}_{\perp}. \quad (3.57)$$

Eq.[3.55] is integrated using Eq.[3.56] and Eq.[3.57], note that the calculation is simplified with the relation $\int dS r_i n_k = V \delta_{ik}$. The final energy is found to be,

$$\Pi_E = \nu V \left(-\beta E_{\parallel}^d P_{\perp} + \frac{1}{\beta} E_{\perp}^d P_{\parallel} \right). \quad (3.58)$$

Introducing this into Eq.[3.58] gives the expression for electric energy input in terms of known quantities E_0 , $P_{\parallel,\perp}$, and $P_{\parallel,\perp}^\infty$. Nondimensionalizing using the same parameters from Eq.[3.44] and Eq.[3.45] yields,

$$\begin{aligned} \Pi_E = \Delta\chi_s E_0^2 \tilde{\nu} V & \left(-\beta \left(\cos \theta - n_{\parallel} \Delta\chi_s (\tilde{P}_{\parallel} + C_3 \cos \theta) \right) \tilde{P}_{\perp} + \right. \\ & \left. + \frac{1}{\beta} \left(-\sin \theta - n_{\perp} \Delta\chi_s (\tilde{P}_{\perp} - C_4 \sin \theta) \right) \tilde{P}_{\parallel} \right) \end{aligned} \quad (3.59)$$

where,

$$C_3 = \frac{\chi_{\parallel}^\infty}{\Delta\chi_s}, \quad C_4 = \frac{\chi_{\perp}^\infty}{\Delta\chi_s}. \quad (3.60)$$

Setting this expression equal to Π_{TT+TB} and solving for $\tilde{\nu}$ gives a lengthy

expression for the surface flow frequency,

$$\begin{aligned} \tilde{\nu} = & \frac{BC_1}{\Lambda f_1 - f_2} \left[\frac{-2}{(\beta n_{\parallel} + \frac{n_{\perp}}{\beta}) M_{\perp}} \left(\tilde{P}_{\parallel} \sin \theta + \tilde{P}_{\perp} \cos \theta + C_2 \cos \theta \sin \theta \right) + \right. \\ & \left. + \left(-\beta \left(\cos \theta - n_{\parallel} \Delta \chi_s (\tilde{P}_{\parallel} + C_3 \cos \theta) \right) \tilde{P}_{\perp} + \frac{1}{\beta} \left(-\sin \theta - n_{\perp} \Delta \chi_s (\tilde{P}_{\perp} - C_4 \sin \theta) \right) \tilde{P}_{\parallel} \right) \right] \end{aligned} \quad (3.61)$$

The final equations describing the tank treading ellipsoid in an electric field are given in dimensionless form,

$$\frac{\partial \theta}{\partial t} = -\frac{2\beta}{1 + \beta^2} \tilde{\nu} + \frac{B}{2M_{\perp}} \left[C_1 \left(-\tilde{P}_{\perp} \cos \theta - \tilde{P}_{\parallel} \sin \theta \right) - C_2 \cos \theta \sin \theta \right], \quad (3.62a)$$

$$\frac{\partial \tilde{P}_{\parallel}}{\partial t} = -\tilde{\nu} \tilde{P}_{\perp} \beta - T_{\parallel} \left(\tilde{P}_{\parallel} - X_{\parallel} \cos \theta \right), \quad (3.62b)$$

$$\frac{\partial \tilde{P}_{\perp}}{\partial t} = \frac{\tilde{\nu} \tilde{P}_{\parallel}}{\beta} - T_{\perp} \left(\tilde{P}_{\perp} + X_{\perp} \sin \theta \right). \quad (3.62c)$$

The convection from surface flow allows for a steady oblique dipole orientation. This is apparent from the coupling between Eq.[3.62b] and Eq.[3.62c]. The tilted dipole implies there can be a steady tilted orientation of the ellipsoid as well. A transition from alignment to oblique orientation with respect to the electric field occurs above a critical electric field. Due to the presence of aspect ratio in Eq.[3.43], the critical electric field will differ from that of a sphere as β changes.

If $\nu = 0$, Eq.[3.62a-3.62c] reduce to the equations of motion for a rigid ellipsoid [32]. As discussed, these equations produce three types of behavior: alignment with the electric field, swinging around the field direction, and continuous tumbling [134]. The additional torque associated with the interface fluidity, characterized by ν , modifies these behaviors to: steady axisymmetric orientation (Taylor regime), steady tilted orientation, swinging around a non-zero tilt angle with respect to the electric field, and tumbling. Moreover, chaotic swinging and tumbling states are also

found. The phase diagram resulting from the numerical solution of Eq.[3.62a-3.62c] is shown in the next section.

3.7.3 Model results

The equations given in Eq.[3.62a-3.62c] are numerically integrated using the *ode45* solver in MATLAB. Aspect ratio, viscosity ratio, and electric field are varied and the time dependent behavior of the orientation is plotted. An oblate ellipsoid is assumed for all model results. Initial values of zero polarization ($P_{\parallel} = P_{\perp} = 0$) are used. The initial ellipsoid orientation, θ_0 , must be misaligned with the field ($\theta_0 \neq 0$, $\theta_0 \neq \pi/2$) to find nontrivial results. A value $\theta_0 = \pi/2 - \theta'$ is chosen, where θ' is a small value. The choice of θ' was not found to alter the type of behavior. These initial conditions simulate the initial charging of an ellipsoid after an electric field is applied. The same parameters from the experimental system are used for the model values, see Table 4.1.

Examples of the four types of behaviors are shown in Figures 3.32-3.34. The results show qualitatively similar behavior to those seen in experiments. At weak field strengths, the ellipsoid orients with the axis of symmetry parallel to the electric field. The surface flow at steady state is zero. This is analogous to the Taylor flow in the drop, which deforms into an oblate ellipsoid shape with the long axes perpendicular to the electric field. For the oblate fluid ellipsoid case, the stable orientation is $\theta = 0$. This differs from the rigid ellipsoid, where the long axis aligns with the electric field.

At higher field strengths the ellipsoid adopts an oblique orientation with respect to the electric field, see Figure 3.32. The rotational surface velocity reaches a

steady nonzero value. In this case, charge is convected by the surface flow and both components of the dipole reach nonzero steady values.

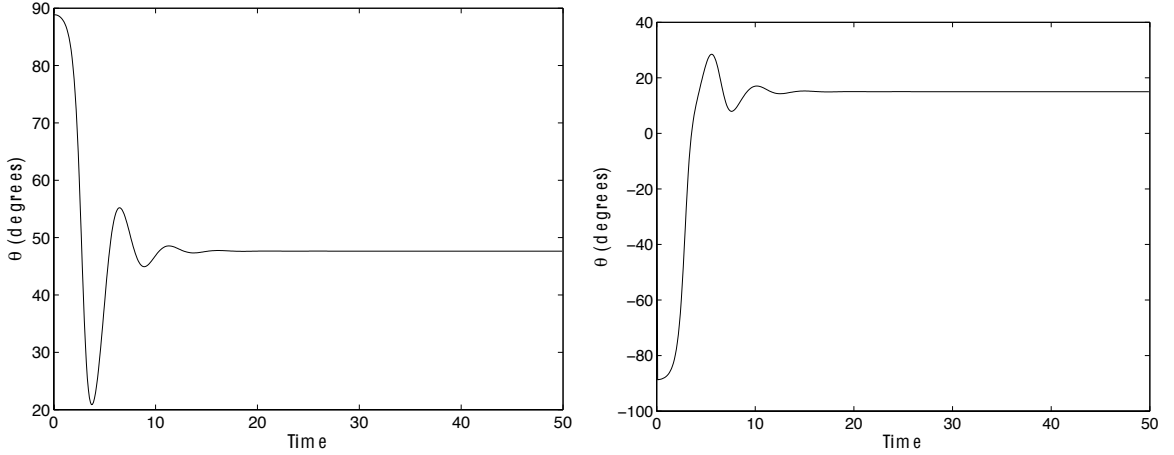


Figure 3.32: Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 4$ $E_0 = 1.2E_Q$, $\beta^{-1} = 1.4$. The initial condition is $\theta = \pi/2 - \theta'$, $\theta' = 0.02$.

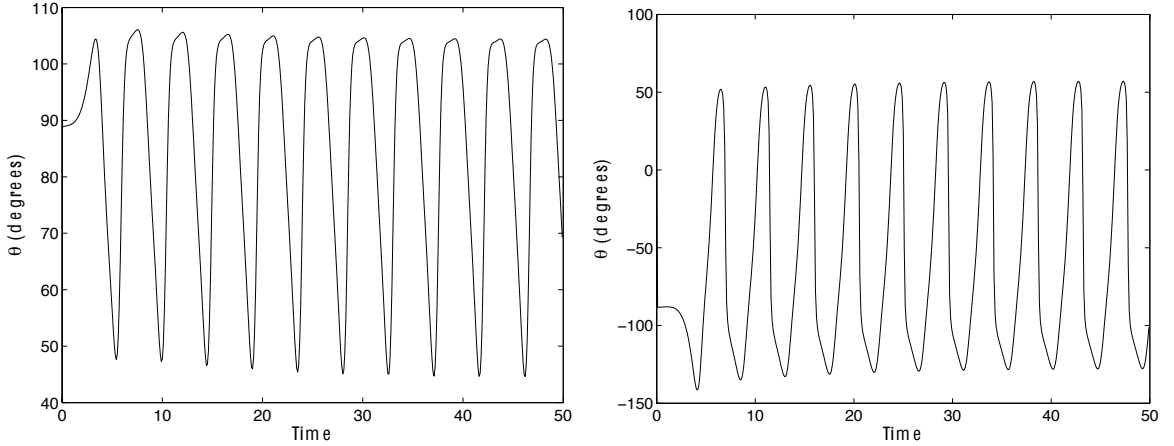


Figure 3.33: Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 4$ $E_0 = 2.3E_Q$, $\beta^{-1} = 2.8$. The initial condition is $\theta' = 0.02$.

At higher field strengths, the model predicts unsteady behavior. The unsteady behavior is characterized into three distinct categories: swinging, tumbling, and chaotic behavior. Swinging, shown in Figure 3.33, shows a bounded oscillation around a tilted orientation, i.e not $\theta = 0, \pi/2$. The oscillations are persistent, but do not change direction. Tumbling is characterized by a complete and persistent rotation in the same direction, see Figure 3.34.

Chaotic behavior is characterized by reversals in rotation direction at irregular periods. The ellipsoid never adopts a predictable trajectory. For chaotic behavior, a small difference between two initial conditions will result in diverging trajectories. The chaotic behavior is demonstrated in Figure 3.35 by comparing two trajectories started at an angular distance of $\theta = \pi/10^5$ apart. This sensitivity to initial conditions is used to quantify chaotic behavior using Lyapunov exponents in Section 3.7.7.

In all cases except for the chaotic behavior, the motion of the dipole mirrors that of the ellipsoid. The chaotic motion stems from the lack of synchronization between the ellipsoid and dipole orientations.

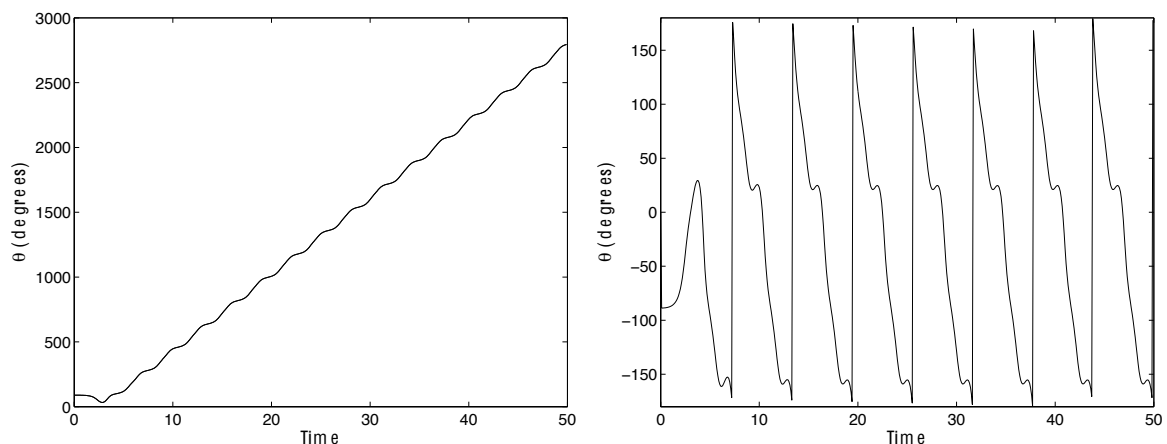


Figure 3.34: Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 14$, $E_0 = 2E_Q$, $\beta^{-1} = 1.3$. The initial condition is $\theta' = 0.02$.

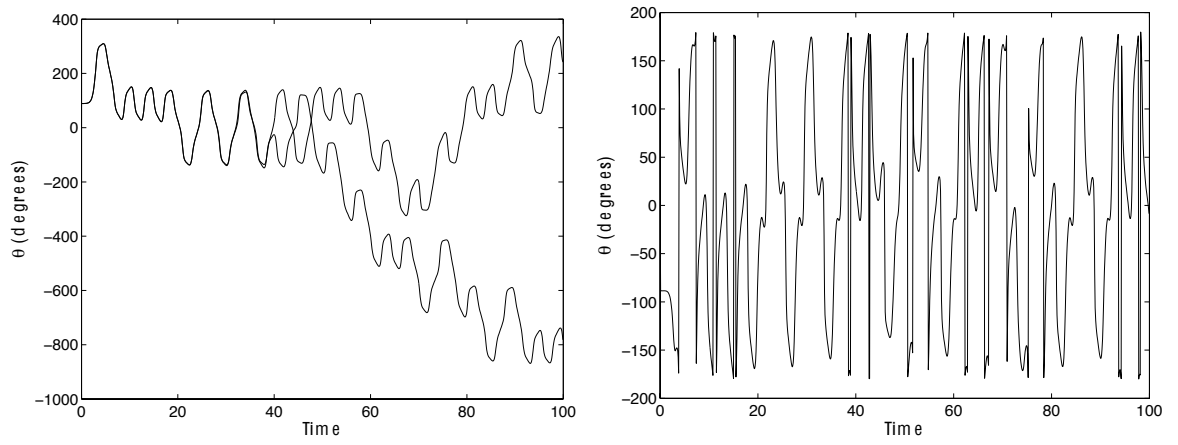


Figure 3.35: Ellipsoid orientation (left) and dipole orientation (right) in degrees for $\Lambda = 14$ $E_0 = 2.5E_Q$ kV/cm, $\beta^{-1} = 1.8$. The initial conditions are $\theta' = 0.02$ and $\theta' = 0.02 + \pi/10^5$.

3.7.4 Model phase diagram

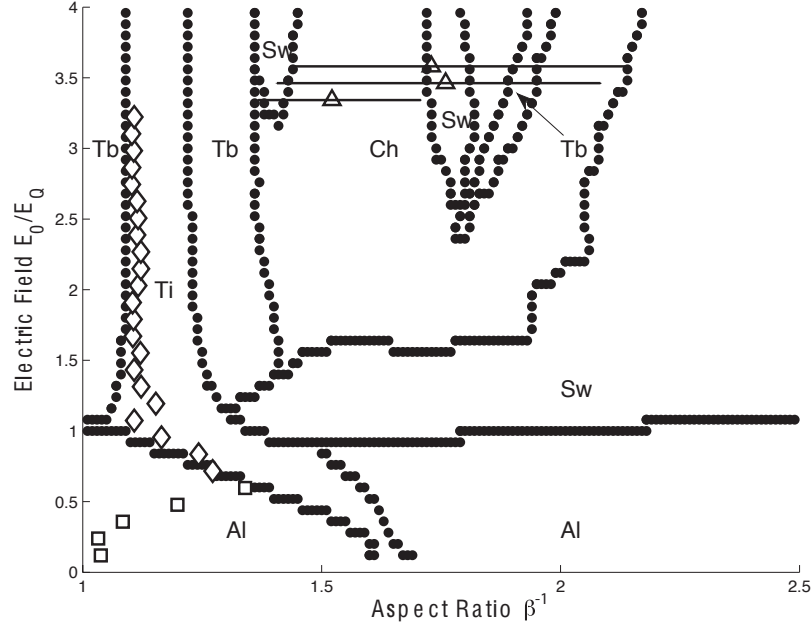


Figure 3.36: Phase diagram for a fluid ellipsoid with viscosity ratio $\Lambda = 14$. The lines represent the boundaries between various behaviors computed from the model. These are denoted by the regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic. The symbols correspond to experimental data: $\square$ - Taylor (steady axisymmetrically oriented ellipsoid), $\diamond$ - steady tilted ellipsoid, $\triangle$ - chaotic tumbling. The lines crossing the symbols reflect the variations in the aspect ratio of the drop during the rotation.

The behavior of the fluid ellipsoid model in a DC electric field is explored for three different viscosity ratios, $\Lambda = 1, 4, 14$, see Figures 3.36, 3.37, and 3.38. These values are representative of the range of viscosity ratios used in experiments. For a given viscosity ratio, the aspect ratio and electric field are varied. In terms of adjustable parameters, the aspect ratio replaces the initial drop diameter in comparison with the experimental phase diagram. In the case of drops, aspect ratio varies with field strength, while in the model aspect ratio is fixed. During unsteady behavior, the drop's aspect ratio also changes in time. The comparison between drop size and aspect ratio is qualitative, but in general a larger drop will correspond to a larger aspect ratio. Additionally, low viscosity drops deform more than high viscosity, hence, a higher range of aspect ratios is explored in the phase diagram for low

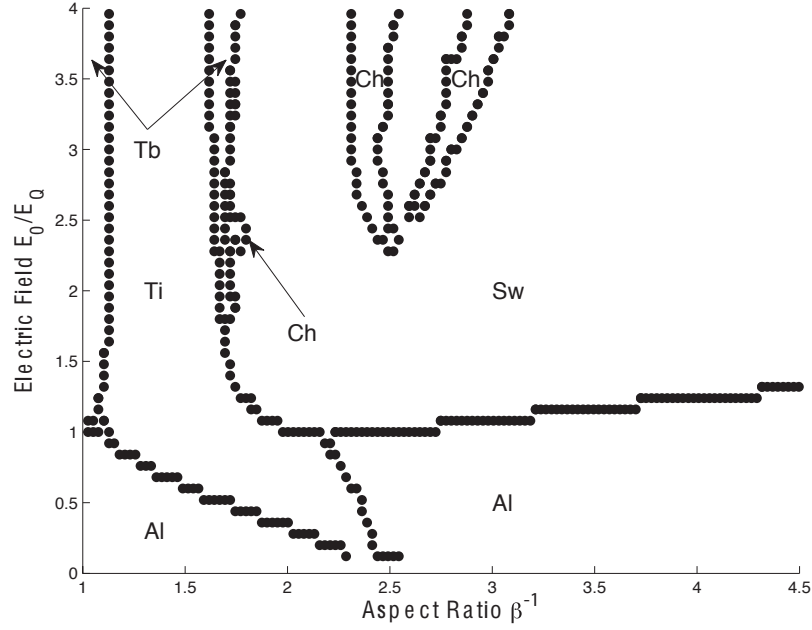


Figure 3.37: Phase diagram from tank treading ellipsoid model $\Lambda = 4$. Regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic.

viscosity drops.

For $\beta = 1$, the ellipsoid transitions from aligned to tilted at the electric field strength predicted by the Quincke theory, $E_Q = 2.7$ kV/cm. For all viscosity ratios, the model predicts a decrease in critical electric field strength as aspect ratio increases. The decrease is more pronounced at high viscosity ratios, where the effect of the surface flow is less significant and the fluid ellipsoid behaves more like a rigid ellipsoid.

Unsteady behavior is observed at all viscosity ratios, predominantly at high aspect ratios. Low viscosity ratios, $\Lambda = 1$ and $\Lambda = 4$, exhibit mostly swinging motions, with relatively small regions of chaotic behavior. The effect of the surface flow is most pronounced for low viscosity ratios, which acts to stabilize the ellipsoid in a tilted orientation.

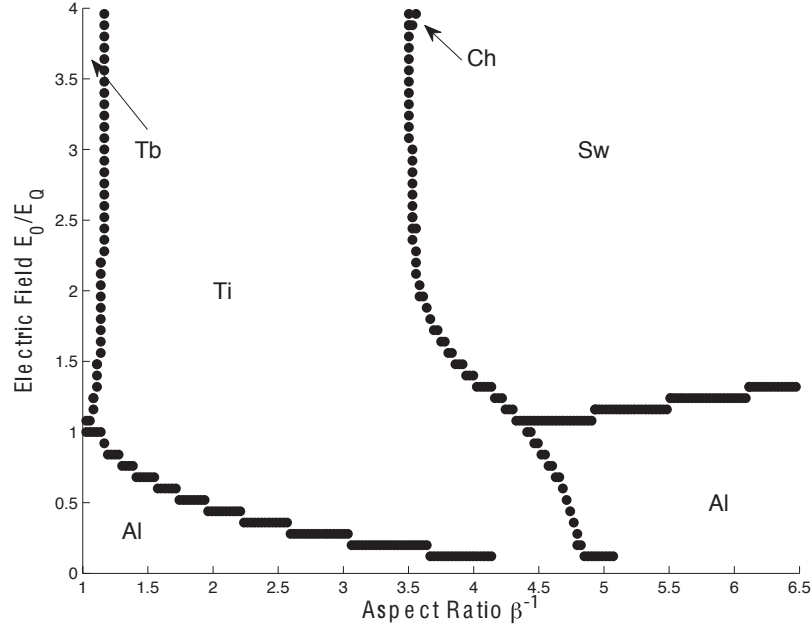


Figure 3.38: Phase diagram from tank treading ellipsoid model $\Lambda = 1$. Regimes: Al - alignment; Ti - tilted; Tb - tumbling; Sw -Swinging; Ch - Chaotic.

Unsteady behavior is more prevalent for high viscosity ratios, i.e. $\Lambda = 14$. The fluid ellipsoid tumbles at low aspect ratios and has large regions of chaotic behavior at high aspect ratios. Regions of swinging behavior are limited compared to lower viscosity ratios. The tendency for swinging motions in lower viscosity ratios and tumbling in higher viscosity ratios agrees with experiments.

Experimental results are compared to the model phase diagram in Figure 3.36. The aspect ratio and field strength data for a $\Lambda = 14$ drop are plotted. The transition from aligned to tilted orientation predicted in the model corresponds to the transition to rotational flow seen in experiments. The aspect ratios seen during tilted behavior in experiment also agree with the model. Although the drop shape does not remain constant in time during unsteady behavior, the range of aspect ratios observed during drop tumbling correspond to the tumbling and chaotic regions from the model.

The low variability in drop shape for high viscosity drops allows for the best

comparison with the fluid ellipsoid model. Although this comparison is qualitative, it captures the effect of aspect ratio on the tumbling dynamics seen in experiments. The shape oscillations observed in intermediate and low viscosity drops limits the applicability of the current model for these viscosity ratios. A more refined model that includes changes in shape could capture these dynamics.

3.7.5 Rotation rate: comparison with Quincke

The steady tilted behavior predicted by the fluid ellipsoid model provides insight into the effect of aspect ratio and viscosity ratio on the fluid rotation rate. The frequency of the surface flow, ν , is calculated from the model for a fixed aspect ratio and viscosity ratio as electric field strength is increased. This is compared to the rotation rate for a sphere predicted by Quincke, see Figures 3.39 and 3.40.

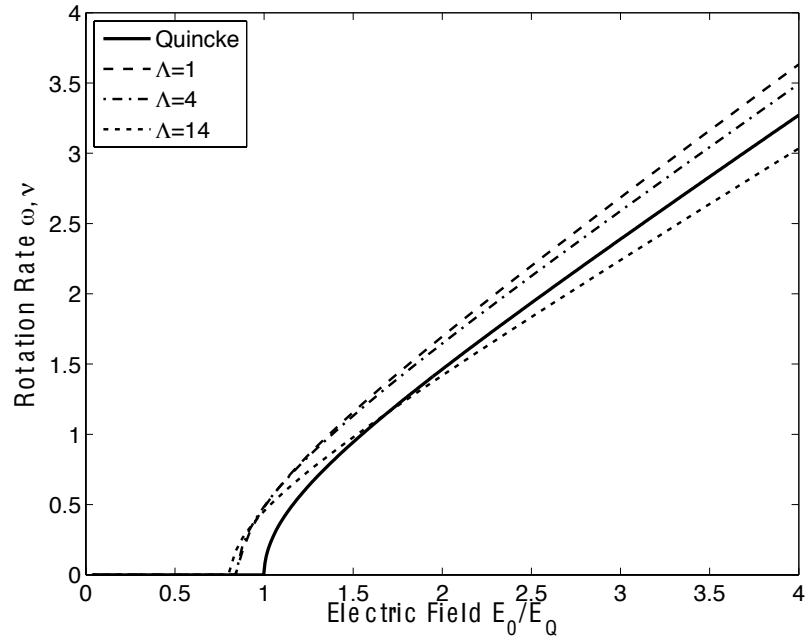


Figure 3.39: Comparison of the Quincke rotation rate ω with the rotational flow rate computed from model, ν , for $\beta^{-1} = 1.2$ for viscosity ratios $\Lambda = 1, 4, 14$.

The critical field for rotation is similar for different viscosity ratios and slightly lower than E_Q . This differs from experiments, where the axisymmetric toroidal flow convects charge away from the induced dipole, weakening its strength and delaying the onset of rotation to higher field strengths. In the fluid ellipsoid model this effect is not captured because there is no toroidal flow included. When rotation has begun, the slope of the rotation rate is smaller for higher viscosity ratios. This is caused by the increase in viscous dissipation due to the increase in drop viscosity.

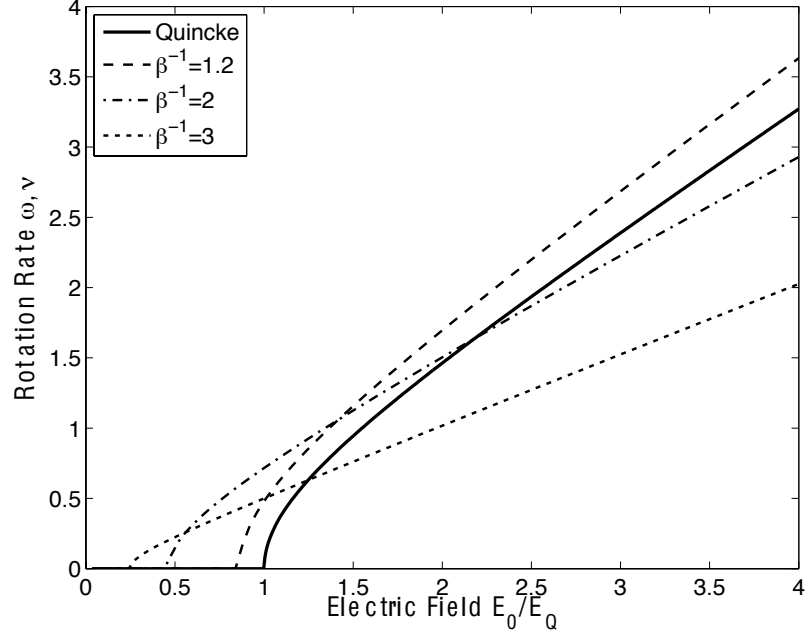


Figure 3.40: Comparison of the Quincke rotation rate ω with the rotational flow rate computed from model, ν , for $\Lambda = 1$ for viscosity for aspect ratios $\beta^{-1} = 1.2, 2, 3$.

The amount of viscous dissipation also depends on aspect ratio. This affects both the critical field for rotation and the slope of rotation rate as a function of electric field. As aspect ratio is increased, the critical field for rotation decreases. This can be understood by considering the short axis of the ellipsoid, which corresponds to the strongest dipole direction. As field strength is increased, the dipole associated with the short axis is the first to produce the torque necessary for rotation. As the short axis is decreased, the dipole is enhanced and a weaker field is needed for rotation. Once rotation is initiated, the slope of the rotation rate with electric field strength is lower for higher aspect ratios. Similar to the effect of high viscosity, the surface flow around a highly deformed ellipsoid will dissipate more energy and lead to slower rotation.

3.7.6 Phase behavior: comparison to experiment

The phase behavior of the model can be compared to experiment by constructing delay plots from the model. The orientation of the fluid ellipsoid can be compared for different values of aspect ratio, electric field strength, and viscosity ratio.

Swinging

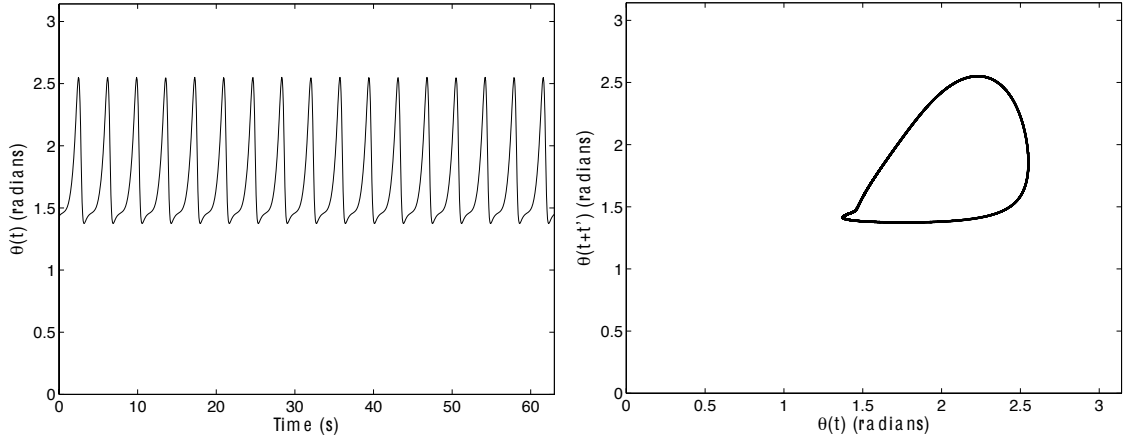


Figure 3.41: Swinging behavior for fluid ellipsoid model with $\Lambda = 4$, $\beta^{-1} = 3$, electric field strength $E_0 = 3E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).

Figure 3.41 shows an example of a swinging motion around a tilted orientation from the model. The delay plot of the swinging motion is qualitatively similar to the oscillations of $\Lambda = 1$ drops seen in experiments, see Figure 3.22. Notably, the swinging is confined within one quadrant and follows a repeated trajectory.

Chaotic swinging

The model also shows trajectories qualitatively similar to the changes in oscillation direction seen in $\Lambda = 4.5$ drops. The changes in orientation angle in time and delay plots are shown in Figure 3.42. The ellipsoid may swing around a tilted orientation before reversing direction and begin swinging in the opposite

direction. The alternating between swinging directions is most dramatically shown in the delay plot, which shows the swinging as orbits in the lower left and upper right quadrant. This delay plot qualitatively resembles the drop behavior plotted in Figures 3.26 and 3.28. The similarity to the chaotic oscillations seen in drops is surprising given the constraint of fixed shape in the model.

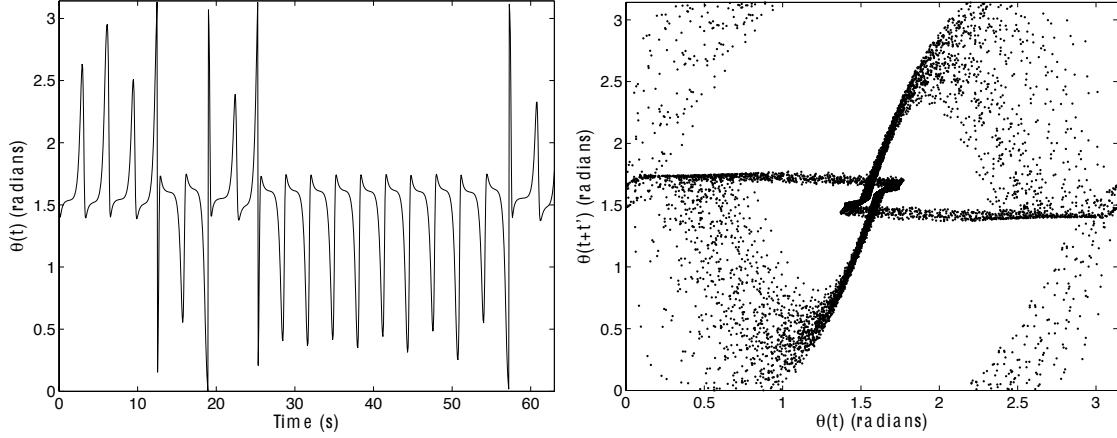


Figure 3.42: Swinging behavior for fluid ellipsoid model with $\Lambda = 4$, $\beta^{-1} = 3$, electric field strength $E_0 = 4E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).

Tumbling

The phase diagrams, shown in Figure 3.36, have regions where the ellipsoid tumbles in one direction. In the regions where chaotic behavior is observed, the ellipsoid can switch tumbling directions. An example of this behavior is shown in Figure 3.43. The phase diagram is similar to the tumbling behavior seen in experiments for $\Lambda = 14$ drops. The ellipsoid may tumble in the same direction for a few periods, but then may reverse direction. The similarity between experiment and model is also apparent in the delay plot, which is qualitatively similar to Figure 3.30.

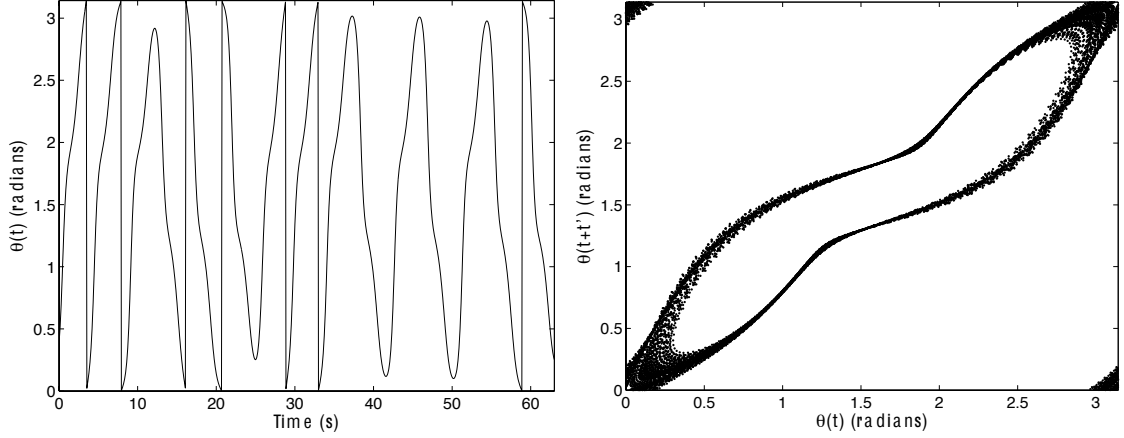


Figure 3.43: Swinging behavior for fluid ellipsoid model with $\Lambda = 14$, $\beta^{-1} = 1.4$, electric field strength $E_0 = 1.8E_Q$. Time dependent behavior is plotted as a function of time (left) and delay plot with $t' = 1/3s$ (right).

3.7.7 Lyapunov exponents

Lyapunov exponents are commonly used to measure the chaotic behavior in a dynamical system [39]. The largest Lyapunov exponent for the fluid ellipsoid model can be defined as

$$\lim_{t \rightarrow +\infty} \frac{1}{t} \ln \frac{|\theta(t) - \theta^*(t)|}{|\theta(0) - \theta^*(0)|}, \quad (3.63)$$

where $\theta(t)$ and $\theta^*(t)$ are two orientation trajectories starting at an infinitesimally close starting position. The Lyapunov exponent can be thought of as the rate of divergence between two material points. In a chaotic system, an infinitesimal perturbation will grow exponentially with time, which corresponds to a positive Lyapunov exponent.

A local Lyapunov exponent can be used to quantify the degree of chaos for different regions in the model phase diagram. This is done by considering two trajectories, $\theta(t)$ and $\theta^*(t)$, at $t = t_i$. The system is first allowed to reach an initial orientation by running a trajectory for 100 time steps. From this initial point, a second trajectory is started after the angle is changed by $\pi/10^5$. The two trajectories

are then iterated for 1000 more time steps. The distance between the two trajectories is recorded at t_i and then at a later time t_f . The local Lyapunov exponent can then be defined as,

$$\left\langle \frac{1}{(t_f - t_i)} \ln \frac{|\theta(t_f) - \theta^*(t_f)|}{|\theta(t_i) - \theta^*(t_i)|} \right\rangle \quad (3.64)$$

where the $\langle \rangle$ denotes average over t_f for 1000 time steps. The local Lyapunov exponent is calculated for a range of values of β and electric field strength.

The Lyapunov exponent can also differentiate oscillatory behavior from steady orientation. The Lyapunov exponent will be approximately zero for oscillatory trajectories. This indicates that the two initial points stay at the same distance as they follow a similar trajectory. A negative Lyapunov exponent is seen when the ellipsoid adopts a fixed orientation. The final position is the same for different initial points and therefore the distance between two initial points will decrease with time. The different behaviors are illustrated in Figure 3.44, where the red and orange regions correspond to positive Lyapunov exponents, green corresponds to Lyapunov exponents close to zero, and blue and white regions have negative Lyapunov exponents.

A positive Lyapunov exponent, indicating chaotic behavior, is seen for many parameter values for $\Lambda = 14$. As viscosity ratio decreases, the prevalence of chaotic behavior decreases as wider regions of tilted and swinging behavior are seen. $\Lambda = 4$ shows windows of chaotic behavior while $\Lambda = 1$ and show only small regions of chaotic behavior between swinging and tilted regions. The lower viscosity ellipsoids easily dissipate energy into surface flow, which stabilizes the ellipsoid and limits chaotic behavior. This trend agrees with experimental observations, where more predictable oscillatory behavior is seen at low viscosity ratios and chaotic behavior is observed in higher viscosity drops.

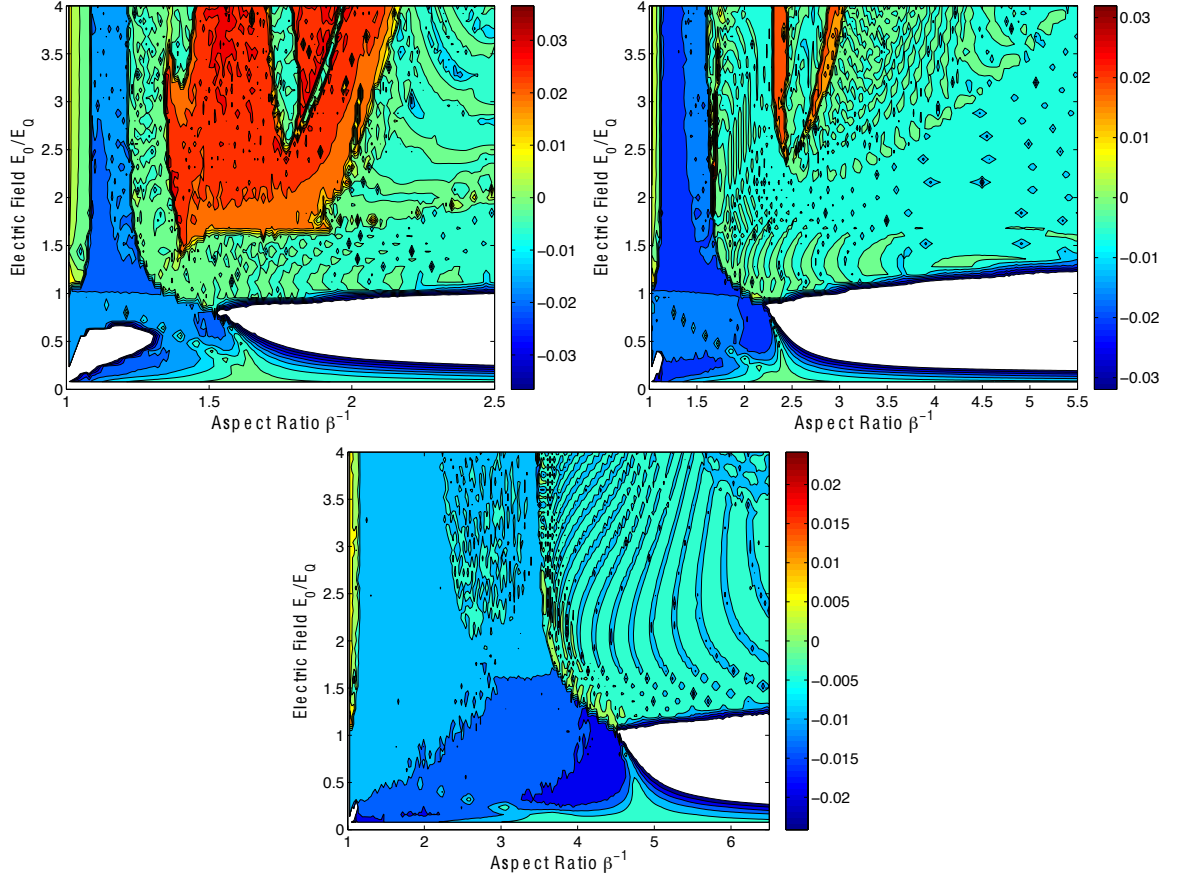


Figure 3.44: Contour plot of Lyapunov exponents for $\Lambda = 14, 4, 1$ from top left to bottom center. Positive values indicate regions of chaotic behavior (red and orange). Values close to 0 indicate regions of oscillatory solutions including swinging and tumbling (green). Negative values indicate stable solutions, tilted or aligned (blue and white).

3.8 Conclusions

The nonlinear dynamics of viscous drops are investigated in uniform DC electric fields. Drop tumbling, shape oscillations, and chaotic rotations occur under creeping flow conditions, where nonlinear phenomena are rare. In contrast to drops in shear flow, the driving electric stress acts on the interface of the drop, rather than from an external flow field. The nonlinear behavior is a complex interaction between electric and fluid stresses. Drop fluidity allows for charge convection and deformation, both of which influence the charging of the drop interface.

Previous models have only considered charge convection by rotation for rigid particles. A fluid drop differs from a rigid particle because charge can be convected by either surface flow or rigid rotation. The relative importance of interfacial velocity and rigid rotation is controlled by viscosity ratio. High viscosity drops act similarly to rigid ellipsoids and tumble because the rotation is faster than deformation. The source of the nonlinear behavior for high viscosity drops is related to the ellipsoidal shape, which creates anisotropic charging of the interface. The agreement between model and experiment is best for high viscosity ratios, where the effect of EHD flow and variations in drop shape are small. The favorable comparison is surprising given the many simplifications in the theory.

The description of low and intermediate viscosity ratio drops is more complex due to the fact that the drop shape can easily deform. The oscillating motion observed at low viscosity ratios indicates that the drop deformation follows changes in the induced dipole orientation. When the rotation rate exceeds the surface tension timescale, the drop shape begins to oscillate. The relation between drop shape and interfacial charge contributes to the nonlinearity.

The rotation of fluid drops represents an example of a spontaneous conversion from electrical energy into mechanical motion. The nonlinear drop physics could inspire new approaches to electromanipulation for small-scale fluid and particle motion in microfluidic technologies. For instance, a collection of rotating drops may reduce the viscosity of an emulsion [33, 112].

3.9 Future directions

Time dependent deformation

The fluid ellipsoid model could be extended to include time dependent shape deformation. The main difficulty is to include the entire flow field produced by the electric stresses on the interface as well as convection effects and large deformations. One possible direction for large deformations is to utilize a model based on the Eshelby solution for ellipsoidal inclusions in a stress field. The Eshelby solution was adapted to model a fluid drop in shear flow for any shape ellipsoid in Stokes flow [219, 85]. This approach computes the stress field around the ellipsoid, then advances the drop shape by a relation between stress and strain rate. To adapt this to a drop in an electric field, the electric field, surface charge, and flow field would have to be solved simultaneously and then the drop shape would be advanced in time. The model could first be attempted without surface tension, although surface tension has been added in the case of shear flow [130].

Rigid ellipsoids

An experimental study of rigid ellipsoids in electric fields is difficult due to irregularities in fabricated ellipsoids. A systematic study would require a variety

of ellipsoidal shapes made from low conducting material with limited defects. One potential method for producing ellipsoids is to make a drop from a curing silicone and apply an electric field while it cures. The field strength would be limited to below the transition to rotation in order to make ellipsoidal shapes. The ratio of charging timescales between the drop and bulk fluids could be adjusted to produce oblate or prolate shapes. Processing would be easier and more reliable if the time required for curing is short and controllable. To achieve this, UV activated silicone (Semicosil) could be used, which cures after being exposed to high intensity UV light. The electric field strength could be varied to produce a range of deformed shapes. Applying a strong UV light, where curing occurs within a few minutes, would be required. This approach could potentially be adapted to a microfluidic process if a large quantity of small ellipsoids were needed.

Interaction between drops

Interactions between drops is necessary for understanding the rheology of a collection of rotating drops in electric fields. In the case of rigid spheres and ellipsoids, both electric and hydrodynamic interactions occur between rotors [40]. The relative strength of these two interactions can result in the two spheres rotating in the same direction or in opposite directions. It is also possible for two spheres to orbit around each other.

In the case of fluid drops, both the electric and hydrodynamic interactions will change compared to rigid spheres. The fluid ellipsoid model could provide a useful model for describing the interaction between drops. The shape and anisotropic polarization of the ellipsoid may produce new features compared to spheres. For instance, a collection of ellipsoids may arrange in different configurations than spheres in an emulsion.

Drop stability

The stability of drops in electric fields is of interest for applications such as oil refining, which uses electric fields to enhance drop coalescence for separating oil from water. The study of drop stability was limited to gradually increasing field strength, see Figures 4.19. Subjecting a drop to a step increase in electric field can produce different breakup behavior. An example of drop breakup, shown in Fig 3.45, demonstrates that breakup can occur before rotation begins.

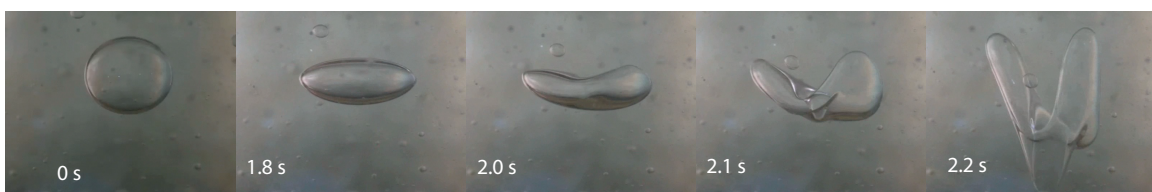


Figure 3.45: Silicone oil drop suspended in castor oil subjected to a uniform DC electric field at $t=0$ s. The drop deforms into an oblate ellipsoid and then breaks up at ~ 2 s.

Prolate - B

The electrohydrodynamic model of drop deformation developed by Taylor and Melcher predicts the type of deformation by the discriminating function Eq.[3.12], see Figure 3.4. The deformation can be oblate or prolate depending on the electrical parameters and viscosity ratio. Most experimental studies have used oils with similar dielectric constants and vary the conductivity ratio to produce either oblate or prolate deformation. Studies using prolate drops have only used PR_A systems. In order to observe PR_B experimentally, large differences in dielectric constants are necessary. PR_B are potentially interesting because the induced dipole is opposite to the electric field and Quincke rotation should be possible, i.e $R/S < 1$.

A large dielectric constant difference can be attained by using polymers dissolved in a solvent with a high dielectric constant. The conductivity of the solution can

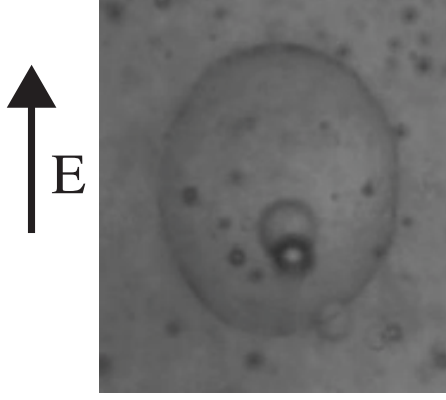


Figure 3.46: Silicone oil in PS-DMF mix showing prolate deformation.

be adjusted with the weight percent of the polymer. For example, polystyrene (PS) dissolved in dimethylformamide (DMF) has a dielectric constant, $\epsilon \sim 20$, and conductivity in the range $\lambda \sim 10^{-8}\text{S/m}$ [70]. A low conductivity oil, such as silicone oil has a low dielectric constant, $\epsilon \sim 2$, and low conductivity $\lambda \sim 10^{-12}\text{S/m}$. The large dielectric constant difference places this system in the range of PR_B . Preliminary experiments of silicone oil in a PS-DMF mix confirm prolate deformation, see Figure 3.46. Changing the weight percent of the polymer adjusts the conductivity, dielectric constant, and viscosity of the fluid simultaneously. The high viscosity and low transparency of the polymer solution also makes visualization of the flow is difficult. An estimate of the system using $\epsilon_{\text{ex}} = 20$, $\epsilon_{\text{in}} = 2$, $\lambda_{\text{ex}} = 10^{-9}\text{S/m}$, and $\lambda_{\text{in}} = 10^{-12}\text{S/m}$, and $\mu_{\text{ex}} = 1\text{Pa-s}$, yields an estimate for the critical field for rotation at $E_Q = 9.28\text{kV/cm}$. Thus, Quincke rotation is possible, but requires strong fields.

Presence of surfactant

The presence of surfactant has been studied for drops in a variety of flow conditions such as shear and parabolic flow [206]. In the case of a drop in an electric field, the presence of surfactant at the interface will create a Marangoni stress that will oppose the electric stresses. In weak fields, the Marangoni stress will replace

the viscous stress and reduce the axisymmetric toroidal EHD flow. If the drop fluid is undergoing Quincke rotation, the convection will influence the distribution of surfactant. This may lead to new drop behaviors and possibly give insight into the importance of surface tension on unsteady behavior.

Insoluble surfactant is commonly assumed in theoretical models. For this to be valid, an experimental system must have slow adsorption/desorption kinetics compared to the timescale of the experiment. This could be achieved with a surfactant that is created at the interface with a compatibilizer [83]. A nonionic surfactant will likely have the least amount of interference with the electric system.

Swimmer by Quincke rotation

There has been significant interest in the area of self propelled particles at micron length scales [82]. Most mechanisms convert energy, i.e. chemical or electrical, into mechanical work in the form of propulsion. The Quincke rotation effect is an example of a system that transfers electric energy into mechanical work. In Stokes flow, a net propulsion is possible if the the geometry lacks a mirror symmetry plane, which is the case for helical shapes.

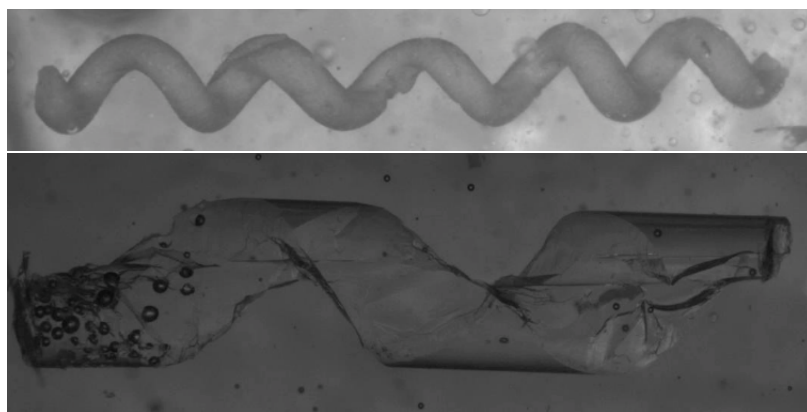


Figure 3.47: Prototypes of a coil (top) and ribbon (bottom) “swimmer”. The coil is made from a curing silicone clay and the ribbon is cut from a cylinder made from cured silicone.

This concept could be applied to a solid coil or ribbon made from a low conducting material which rotates due to Quincke rotation. An example was produced using a ribbon shape cut from a cylinder made from silicone, see Figure 3.47. The velocity of the ribbon was measured at different field strengths. Preliminary results showed that the ribbon velocity scales with a rotation rate higher than one, where hydrodynamics predicts $\mathbf{u} \sim \omega$. The interaction between the electric field and the surface charge on the angled surface of the ribbon may explain the increase in velocity. A more detailed study could be done by adjusting the shape of the ribbon or coil. Finite element simulation software such as COMSOL could be used to model the effect of the surface charge on propulsion. The critical field for rotation could be approximated by an analysis for Quincke rotation of a cylinder [149].

The lack of a length scale in the critical field for Quincke rotation allows for rotation regardless of size. A micron scale helical shape made from a low conducting material would also rotate from Quincke rotation. In general, Quincke rotation is limited to materials with low conductivities ($\lambda \sim 10^{-10} - 10^{-12} \text{S/m}$) and in high field strengths ($E_0 \sim 1 \text{kV/cm}$), although decreasing viscosity will lead to a lower critical field strength. A micron scale ribbon could be propelled in a low viscosity silicone oil ($\sim 10^{-3} \text{Pa-s}$) with a field in the range of $E_0 \sim 1 - 10 \text{kV/m}$.

Viscoelastic drop

The experimental system could be modified to use a drop fluid with viscoelastic properties. The introduction of elasticity could allow for the drop to store and release energy as it rotates. The types of fluids that experience viscoelastic behavior at the typical shear rates for Quincke rotation would need to be determined. These fluids would also require low conductivities in order to rotate at reasonable field strengths, $E_0 \sim 1 - 10 \text{kV/cm}$.

A description of a deformable elastic ellipsoid with no internal flow undergoing Quincke rotation could be developed. The internal viscous dissipation from the fluid ellipsoid model could be replaced by elastic deformation. The viscous dissipation in this case would be confined to the external fluid. The elastic ellipsoid would be able to store and release energy as the induced dipole and ellipsoid orientation change. Large ellipsoidal deformations could be described by a model such as the Eshelby solution [219, 85].

CHAPTER FOUR

Measurement of membrane capacitance and membrane viscosity in AC electric fields

4.1 Introduction

Electric properties of biomembranes play a pivotal role in cellular functions [150], for instance the synaptic efficacy and electric signal propagation in neurons [36, 66, 15]. The response of cells to electric fields also has implications for biomedical applications such as gene transfection, where transient pores are induced in cell membranes through the controlled application of electric pulses, thereby enabling the delivery of foreign DNA into the cell [140].

The main structural component of biological membranes is the lipid bilayer, which is impermeable to ions. Upon application of external voltage, charges accumulate on the two sides of the membrane establishing a potential difference, i.e., the membrane acts as a capacitor. The capacitive nature of the plasma membrane is crucial for

cell viability in external electric fields because it helps shield the cell interior. If the capacitor is fully charged, it draws zero current and the voltage drop occurs entirely across the membrane; the external electric field does not penetrate the cell. However, charging of the membrane capacitor occurs over a finite time period, on the order of a few microseconds, during which the cell interior participates in the conduction process. Thus, nanosecond electric pulses affect the intracellular content and have been used for nucleus electroporation [175, 42]. The capacitive charging can also destabilize and disrupt the plasma membrane [176], resulting in irreversible loss of the barrier function and cell death.

The charging timescale depends on the membrane capacitance [178], hence accurate knowledge of this parameter is needed in order to better control cell interactions with electric fields. Existing methods to measure plasma membrane capacitance of real cells include techniques such as patch-clamp [67], electromechanical methods based on individual cell dielectrophoresis, electrorotation and electro-orientation [92], and dielectric spectroscopy based on the frequency-dependent permittivity and conductivity of cell suspensions [64, 38, 192]. The patch-clamp method is very effective, but can be invasive and laborious. Both the electromechanical and the dielectric spectroscopy techniques are non-invasive and can be applied to measure the membrane potential of live cells [157], and structural organization of the lipid bilayer [25]. However, these techniques require complex equipment and mono disperse suspensions; probing the collective response of a polydisperse suspension of cells (or vesicles) yields only an average value of the membrane electric properties. The low-throughput and difficulties associated with the existing methods motivate the effort to establish new and facile method for measuring the membrane capacitance of lipid bilayers.

A model membrane system provides a better defined experiment and the means

of interrogating membranes of various composition in isolation. A classic example is the artificial black lipid membrane (BLM) [198, 37, 35]. Voltage- or current-clamp methods for measuring the capacitance of BLMs [11, 18, 220, 4, 137], recently reviewed in [101], have yielded a wealth of data. However these techniques are limited by BLM fragility. BLMs usually retain some of the solvent used for their formation; thus, they are thicker than the bilayer portion of a biological membrane. In the past 15 years, giant unilamellar vesicles (GUVs) have gained popularity as a model to study membrane biophysics [51]. Their large size (10-100 μm) allows for direct observation of the dynamic response of individual vesicles to electric fields using real time optical microscopy [47, 45]. Yet another advantage of giant vesicles compared to BLMs is the possibility for tension control in the system. High tensions characteristic for BLMs could result in bilayer thinning.

Vesicles aspirated in a micropipette have been employed to study the electromechanical properties and limits of lipid and polymer membranes [138, 9]. Freely suspended quasi-spherical vesicles (vesicles whose equilibrium shape is a sphere but excess area is stored in submicron fluctuations) deform into ellipsoids when subjected to a uniform AC electric field [104, 132, 8, 7]. If the inner fluid is less conducting than the suspending medium, an increase in frequency will lead to a shape transition from a prolate to oblate ellipsoid, i.e. at low frequencies the vesicle elongates in the field direction, while at higher frequencies the vesicle's long axis is perpendicular to the applied field. The transition frequency depends on the membrane charging time [211, 225, 147]. As a result, the frequency dependent deformation of a vesicle in an AC field can be utilized to infer a value for membrane capacitance, C_m .

The main purpose of this chapter is to measure membrane capacitance in a noninvasive manner. This is accomplished by exploiting the prolate-oblate transition

to infer membrane capacitance. Giant vesicles from artificial and natural lipids are relatively easy to prepare using the electroformation method. The measurement itself requires a frequency sweep that can be quickly and easily done with either home-made or commercially available electroporation chambers. Finally, the method is non-invasive as it does not require any direct contact with the membrane. The method is applied to membranes assembled from lipids and block-copolymers. The latter form shells (polymersomes) that have been subject to increasing interest as drug-delivery systems [49, 72].

The time dependent deformation of a vesicle in an AC electric field is also investigated in this chapter. A theoretical model has been developed in [208], but has not been compared to experimental data. In contrast to the steady state, the dynamics of vesicle is affected by viscous dissipation, both in the bulk fluids and within the membrane. The effect of membrane viscosity has been studied for cells in external flow [199, 141], but has not been experimentally investigated for electric fields. In addition, a theoretical description of membrane tension dynamics, originally developed for vesicles in external flow [181], is applied to vesicles in electric fields and compared to experiment.

4.2 Complex interfaces: membranes

In this section, the main features and results of the theoretical model underlying the measurement method are summarized, including the theoretical model for time dependent deformation of a vesicle in an AC field [211, 225]. This theory primarily follows [208] with a description of membrane tension dynamics from [181].

4.2.1 Physical Model

Let us consider an insulating charge-free lipid bilayer membrane with dielectric constant, ϵ_{mm} , and thickness, d . The bilayer is a spherical shell (vesicle) which separates two bulk fluids, each with their own conductivities, λ , viscosities, μ , and permittivities, ϵ . The vesicle is subjected to a uniform AC electric field,

$$\mathbf{E}^\infty = E_0 \cos(\omega t) \hat{\mathbf{z}}, \quad (4.1)$$

where ω is the angular field frequency and t is time.

The main difference with simple fluid-fluid interface is the presence of the nonconducting bilayer membrane separating the two bulk phases. The application of an electric field causes ions to move and accumulate at boundaries that separate media with different electric properties. Since the lipid membrane is impermeable to ions, charges accumulate on both the inner and outer physical surfaces and a membrane acts as a capacitor, with a capacitance $C_m = \epsilon_{\text{mm}}/d$. The capacitance quantifies the amount of charge that can be stored by the membrane. For a fully charged capacitor, the charge densities on the inner and outer membrane surfaces are equal (but with opposite sign).

The presence of the membrane alters the electrical boundary conditions compared to the drop case. Rather than a continuous potential, a jump in the potential field occurs across the membrane,

$$\Phi_{\text{in}} - \Phi_{\text{ex}} = V_m, \quad (4.2)$$

where V_m is the transmembrane potential.

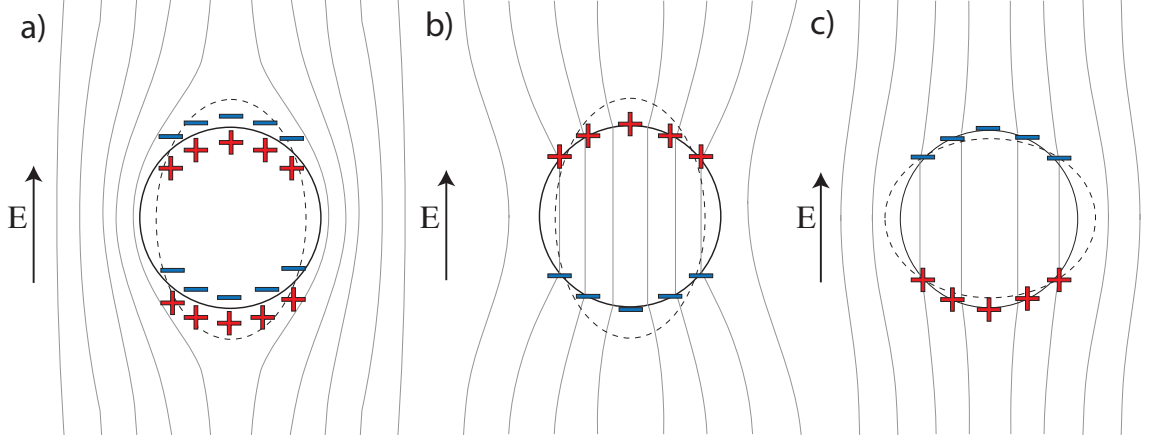


Figure 4.1: The steady electric field around a vesicle immersed in an electrolyte solution, following the imposition of a background uniform AC field (a) $f > f_c$ (b) $f < f_c$. Over a charging time t_{mm} Eq.[4.3] dipolar charge distribution forms in response to currents from the bulk.

The amount of charge that can be stored by the membrane and the time over which this charge builds up depend on the membrane capacitance. For a quasi-spherical vesicle of radius a , modeled as a spherical thin insulating shell with capacitance C_m , the characteristic charging time scale is, [69]

$$t_{\text{mm}} = aC_{\text{mm}} \left(\frac{1}{\lambda_{\text{in}}} + \frac{1}{2\lambda_{\text{ex}}} \right). \quad (4.3)$$

4.2.2 Vesicle Model

The departure of the vesicle shape from a sphere is quantified by the excess area, which is the difference between the vesicle area and the area of an equivalent-volume sphere,

$$\Delta = A/a^2 - 4\pi, \quad a = \left(\frac{3v}{4\pi} \right)^{1/3}. \quad (4.4)$$

Here A and v are the true surface area and volume inside the vesicle respectively.

Excess area is stored in suboptical fluctuations. Accordingly, we can define

an apparent area $\bar{A}$ as the area measurable within the optical resolution of the microscope. In contrast to the true area, the apparent area can change under stress (e.g. when the vesicle deforms from a sphere to an ellipsoid).

By considering a coordinate system at the center of the vesicle, the radial position of the interface is represented by,

$$r_s = a(1 + f(\theta, \phi, t)), \quad (4.5)$$

where $f(\theta, \phi, t)$ is the deviation from a sphere of equivalent volume and a is the initial vesicle radius. The deformation is expanded in terms of scalar spherical harmonics,

$$f(\theta, \phi, t) = \sum_{j=2}^{\infty} \sum_{m=-j}^j f_{jm} Y_{jm}. \quad (4.6)$$

The excess area stored in fluctuations is related to the amplitude of fluctuations by [181],

$$\sum_{j=2}^{j_{max}} \sum_{m=-j}^j |f_{jm}|^2 \frac{(j+2)(j-1)}{2} = \Delta. \quad (4.7)$$

Local incompressibility enforces that the tangential electric stress is balanced by elastic tangential stress [180]. As a result, there is no flow at steady state. The local area constraint introduces a dependence of tension on deformation resulting from the applied electric field. Although the full expansion is used to describe fluctuations, only ellipsoidal deformation will be considered for the systematic deformation of the vesicle. In the limit of small deformations, ellipsoidal deformation corresponds to $j = 2, m = 0$ spherical harmonics, which have the angular dependence,

$$Y_{20} = \frac{1}{4} \sqrt{\frac{5}{\pi}} (3 \cos^2 \theta - 1). \quad (4.8)$$

A deformation parameter is defined by the ratio of the two major axes, see Figure 4.2. The experimental data is computed from the major axes while the amplitude of the spherical harmonic that describes ellipsoid deformation, f_{20} , must be multiplied by scale factors. The equivalent expressions of D are,

$$D_{theory} = \frac{1 + f_{20}1/2\sqrt{5/\pi}}{1 - f_{20}1/4\sqrt{5/\pi}}, \quad D_{exp} = \frac{a_{\parallel}}{a_{\perp}}, \quad (4.9)$$

where $a_{\parallel}$ and $a_{\perp}$ are defined as the axes parallel and perpendicular to the axis of symmetry.

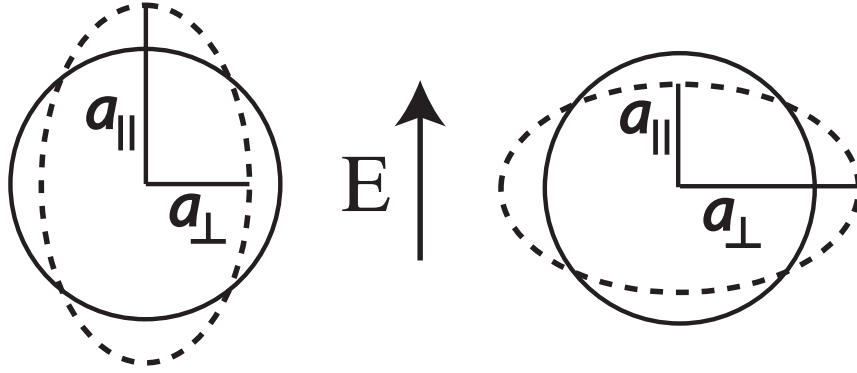


Figure 4.2: Definition of axes where $a_{\parallel}$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right) ellipsoid. The deformation parameter is defined as $D = a_{\parallel}/a_{\perp}$.

4.2.3 Dimensionless variables and timescales

The relevant dimensionless variables and timescales are introduced for the vesicle system. The vesicle is filled with a fluid of viscosity, μ_{in} , conductivity, λ_{in} , and dielectric constant, ϵ_{in} , and suspended in an external fluid characterized by μ_{ex} , λ_{ex} , and ϵ_{ex} . To characterize the mismatch in the fluid physical properties, we introduce

the ratios

$$R = \frac{\lambda_{\text{in}}}{\lambda_{\text{ex}}}, \quad S = \frac{\epsilon_{\text{in}}}{\epsilon_{\text{ex}}}, \quad \Lambda = \frac{\mu_{\text{in}}}{\mu_{\text{ex}}}. \quad (4.10)$$

The viscosity of the bilayer is given by a two-dimensional viscosity, μ_{mm} , with units of [Ns/m]. This is compared to the bulk viscosity by,

$$\Lambda_s = \frac{\mu_{\text{mm}}}{a\mu_{\text{ex}}}. \quad (4.11)$$

The membrane charging timescale for a spherical shell is rewritten in terms of conductivity ratio,

$$t_{\text{mm}} = \frac{aC_m}{\lambda_{\text{in}}} \left(1 + \frac{R}{2} \right). \quad (4.12)$$

The timescale for deformation is the electrohydrodynamic (EHD) timescale, which compares the distorting electric stresses to viscous dissipation by the bulk fluids,

$$t_{\text{hd}} = \frac{\mu_{\text{ex}}(1 + \Lambda)}{\epsilon_{\text{ex}}E_0^2}. \quad (4.13)$$

Vesicle deformation is opposed by membrane bending rigidity, κ , and tension, σ . The relaxation timescale for the bilayer membrane can be given in terms of the bending rigidity,

$$t_{\kappa} = \frac{\mu_{\text{ex}}(1 + \Lambda)a^3}{\kappa}. \quad (4.14)$$

This timescale is useful because it is based on a material property of the membrane. Although tension is primary source of resistance to deformation, the tension can vary significantly between vesicles and while the vesicle is under stress. The membrane

tension at equilibrium, σ_{eq} , can also be used for a relaxation timescale,

$$t_\sigma = \frac{\mu_{\text{ex}}(1 + \Lambda)a}{\sigma_{\text{eq}}} . \quad (4.15)$$

This timescale will be used to describe the relaxation of the vesicle back to a sphere after the field is turned off.

A comparison of the deforming and restoring timescales yields a capillary number. Using the bending timescale from Eq.[4.14], the capillary number is,

$$Ca_\kappa = \frac{a^3 \epsilon_{\text{ex}} E_0^2}{\kappa} . \quad (4.16)$$

A capillary number can also be written using the membrane tension relaxation timescale, although it is not used in the current formulation.

4.2.4 Governing Equations

As described in Chapter 2, the modeling approach uses the leaky dielectric assumptions; the bulk fluid is charge free and the coupling occurs only at the interface.

Hydrodynamic field

Hydrodynamics inside and outside the vesicle obey the Stokes equation [139, 107],

$$\nabla \cdot \mathbf{T}^{\text{hd}} = 0, \nabla \cdot \mathbf{u} = 0, \quad (4.17)$$

where the hydrodynamic stress is computed by,

$$\mathbf{T}^{\text{hd}} = -p\mathbf{I} + \mu [\nabla\mathbf{u} + (\nabla\mathbf{u})^\dagger]. \quad (4.18)$$

If it is assumed that there is no bilayer slip or membrane permeability, the hydrodynamic boundary conditions are identical to that of a drop. The flow is continuous across the interface,

$$\mathbf{u}_{\text{in}} = \mathbf{u}_{\text{ex}} = \mathbf{u}_s, \quad (4.19)$$

where $\mathbf{u}_s$ is the flow at the interface. Membrane permeability can be neglected if the vesicle is osmotically stable and the membrane is pore free. Although it is possible for the electric field to create pores in the membrane, the experiments in this chapter are limited to weak electric field strengths that do not induce poration.

Electric field

The electric field is determined by the gradient of the electric potential,

$$\mathbf{E} = -\nabla\Phi. \quad (4.20)$$

The absence of bulk free charges determines that the electric field is solenoidal ($\nabla \cdot \mathbf{E} = 0$), thus the electric potential satisfies the Laplace equation,

$$\nabla^2\Phi = 0. \quad (4.21)$$

Far from the vesicle, the electric field is a uniform AC field, $\mathbf{E}^\infty = E_0 \cos(\omega t)\hat{\mathbf{z}}$. The electric potential is solved subject to the following boundary conditions at the

interface. The electric stresses are determined from the Maxwell stress tensor,

$$\mathbf{T}^{\text{el}} = \epsilon(\mathbf{E}\mathbf{E} - \mathbf{E}^2\mathbf{I}/2), \quad (4.22)$$

where $\mathbf{I}$ is the identity tensor.

The insulating bilayer membrane causes a jump in the potential field across the membrane,

$$\Phi_{\text{in}} - \Phi_{\text{ex}} = V_m, \quad (4.23)$$

where V_m is the transmembrane potential. The charging of the interface is determined by the conservation of current across the membrane capacitor,

$$\mathbf{n} \cdot (\lambda_{\text{ex}}\mathbf{E}_{\text{ex}}) = \mathbf{n} \cdot (\lambda_{\text{in}}\mathbf{E}_{\text{in}}) = C_m \frac{dV_m}{dt}. \quad (4.24)$$

The transmembrane potential is determined as part of the solution for the potential. An effective net charge on the interface can be determined by the jump in the displacement field,

$$\mathbf{n} \cdot (\epsilon_{\text{ex}}\mathbf{E}_{\text{ex}} - \epsilon_{\text{in}}\mathbf{E}_{\text{in}}) = Q \quad (4.25)$$

Note that in the limit of zero-thickness membrane, Q is an effective charge on the membrane resulting from an imbalance in charge densities between the inner and outer surface of the membrane. A mismatch in the rate of supply of charges gives rise to the net charge while charging; when the capacitor is fully charged, $Q = 0$.

4.2.5 Solution approach

The solution approach is based on a leading order linear perturbation technique for the velocity field in spherical coordinates. This approach has primarily been utilized to study fluid drops under an external flow field [19, 205, 73]. The approach has also been used to describe vesicle electrohydrodynamics [210, 214, 177, 208]. The fluid velocity field is described using a set of fundamental solutions of the Stokes equation in spherical geometry. The flow inside and outside the drop is expanded in vector spherical harmonics,

$$\mathbf{u}_{\text{in}} = \sum_{jmq} c_{jmq} \mathbf{u}_{jmq}^+(\mathbf{r}), \quad \mathbf{u}_{\text{ex}} = \sum_{jmq} c_{jmq} \mathbf{u}_{jmq}^-(\mathbf{r}), \quad (4.26)$$

where,

$$\sum_{jmq} = \sum_{j=2}^{\infty} \sum_{m=-j}^j \sum_{q=0}^2. \quad (4.27)$$

The full expressions for the flow fields, $\mathbf{u}_{jmq}^+$ and $\mathbf{u}_{jmq}^-$, are given in Appendix A, although once the electric stresses are determined it will become clear that only a limited number of flow fields are excited. These velocity fields are limited to $j \leq 2$ and those with $q = 0, 2$, which have axial symmetry. In general, the $q = 0$ harmonic corresponds to tangential flow and the $q = 2$ harmonic corresponds to radial flow.

As a result of Eq.[5.17], the normal component of the fluid velocity is equal to the velocity of the interface. The shape evolution component corresponding to ellipsoidal deformation is related to the velocity coefficient by,

$$\frac{\partial f_{20}}{\partial t} = c_{202}. \quad (4.28)$$

The electric and membrane stresses are also written in terms of vector spherical harmonics, allowing for a comparison with the hydrodynamic stress computed from the flow fields Eq.[4.26]. The general stress balance is given by,

$$\tau_{jmq}^{\text{el}} + \tau_{jmq}^{\text{hd,ex}} - \Lambda \tau_{jmq}^{\text{hd,in}} = \tau_{jmq}^{\text{mm}}, \quad (4.29)$$

where τ_{jmq}^{el} are the electric stresses, τ_{jmq}^{hd} are the hydrodynamics stresses, and τ_{jmq}^{mm} are the membrane stresses. The expression for electric, viscous, and membrane stress are detailed in the following sections.

Electric stresses

The electric field is determined by solving for the potential field around a spherical shell with an electric field in the $\hat{\mathbf{z}}$ direction,

$$\Phi_{\text{ex}} = -E_0 \left[r + B_{\text{ex}} \frac{a^3}{r^2} \right] \exp(i\omega t) \cos \theta, \quad (4.30)$$

$$\Phi_{\text{in}} = -E_0 B_{\text{in}} r \exp(i\omega t) \cos \theta. \quad (4.31)$$

The boundary conditions for the potential across the membrane, Eq.[4.23], and the current conservation, Eq.[4.24], are used to solve for B_{in} and B_{ex} . The coefficients from Eq.[4.31] are determined in terms of the transmembrane potential, which is decomposed into a time dependent and spatial component,

$$V_m = \bar{V}(\omega) \exp(i\omega t) \cos(\theta) a E_0. \quad (4.32)$$

The spatial component of the transmembrane potential matches that from Eq.[4.31], leaving the time dependent portion to be determined. A solution is found by applying

the boundary conditions to Eq.[4.31] with an initial condition of zero transmembrane potential, $V_m = 0$. The solution gives the following expression for the coefficients,

$$B_{\text{in}} = \frac{k_{\text{ex}}(3 - 2\bar{V}(\omega))}{k_{\text{in}} + 2k_{\text{ex}}}, \quad B_{\text{ex}} = \frac{(k_{\text{ex}} - k_{\text{in}}) + k_{\text{in}}\bar{V}(\omega)}{k_{\text{in}} + 2k_{\text{ex}}}, \quad (4.33)$$

where,

$$k_{\text{in}} = R + i\omega S, \quad k_{\text{ex}} = 1 + i\omega, \quad (4.34)$$

and,

$$\bar{V}(\omega) = \frac{3}{2} \left(1 + i\omega \frac{C_m a}{\epsilon_{\text{ex}}} \left(\frac{1}{2} + \frac{1}{R} \right) \right)^{-1}. \quad (4.35)$$

The electric stresses are computed from the Maxwell stress tensor Eq.[4.22], where the electric field is determined by $\mathbf{E} = -\nabla\Phi$. The electric stress is decomposed into normal and tangential stresses,

$$\mathbf{T}^{\text{el}} = \bar{p}^{\text{el}} [1 + 3\cos(2\theta)] \hat{\mathbf{r}} + \bar{\tau}_s^{\text{el}} \sin(2\theta) \hat{\boldsymbol{\theta}}, \quad (4.36)$$

where,

$$\bar{p}^{\text{el}} = \frac{1}{2} [(E_r^{\text{ex}})^2 - (E_\theta^{\text{ex}})^2 - S((E_r^{\text{in}})^2 - (E_\theta^{\text{in}})^2)], \quad (4.37)$$

and,

$$\bar{\tau}_s^{\text{el}} = E_\theta^{\text{ex}} Q + E_r^{\text{in}} (E_\theta^{\text{ex}} - E_\theta^{\text{in}}). \quad (4.38)$$

Substituting the resulting expressions in terms of B_{ex} and B_{in} yields,

$$\bar{p}^{\text{el}} = \frac{1}{24} [2 - 2\text{Re}(B_{\text{ex}}) + 5B_{\text{ex}}B_{\text{ex}}^* - 2SB_{\text{in}}B_{\text{in}}^*], \quad (4.39)$$

$$\bar{\tau}_s^{\text{el}} = \frac{1}{4} [-1 + \text{Re}(B_{\text{ex}}) + 2B_{\text{ex}}B_{\text{ex}}^* + SB_{\text{in}}B_{\text{in}}^*], \quad (4.40)$$

where Re denotes the real part and $*$ indicates complex conjugate.

These stresses must be converted into vector spherical harmonics in order to match the other terms in the stress balance, Eq.[4.29]. The analysis is limited to the vector spherical harmonics with the appropriate angle dependence,

$$\mathbf{y}_{202} = \frac{1}{8} \sqrt{\frac{5}{\pi}} [1 + 3 \cos(2\theta)] \hat{\mathbf{r}}, \quad \mathbf{y}_{200} = -\frac{1}{4} \sqrt{\frac{15}{2\pi}} \sin(2\theta) \hat{\boldsymbol{\theta}}. \quad (4.41)$$

In order to match Eq.[5.29], the electric stresses are rewritten in vector spherical harmonics using Eq.[4.41],

$$\tau_{202}^{\text{el}} = 8 \sqrt{\frac{\pi}{5}} \bar{p}^{\text{el}} \mathbf{y}_{202}(\theta), \quad \tau_{200}^{\text{el}} = -4 \sqrt{\frac{2\pi}{15}} \bar{\tau}_s^{\text{el}} \mathbf{y}_{200}(\theta). \quad (4.42)$$

The electric stresses are limited to the $j = 2, m = 0$ spherical harmonics. This validates the assumption of only considering ellipsoidal deformation, f_{20} , and simplifies the derivation of the remaining stress terms, which only need to be computed for these harmonics. The electric stress also produces a nonzero $j = 0, m = 0$ harmonic corresponding to isotropic pressure, however this term is absorbed into the hydrostatic pressure because the fluid is incompressible.

4.2.6 Hydrodynamic field

As discussed, the hydrodynamic field is described by Eq.[4.26]. The electric stresses in Eq.[4.42] imply that the velocity field is also limited to $j = 2, m = 0$, thus only two coefficients, c_{200} and c_{202} , need to be solved. By considering an inextensible membrane, local area is conserved and the velocity field can be considered solenoidal,

Eq.[5.15]. The conservation of area enforces that [181],

$$\nabla_s \cdot \mathbf{u} = 0, \quad (4.43)$$

at the interface. This assumption leads to following relation between velocity amplitudes [177],

$$c_{jm0} = \frac{2}{\sqrt{j(j+1)}} c_{jm2}. \quad (4.44)$$

This constrains the number of velocity components that need to be solved to only one, e.g. the normal velocity component. The relation between the normal and tangential velocity components is,

$$c_{200} = \sqrt{\frac{2}{3}} c_{202}. \quad (4.45)$$

The flow fields produce a viscous stress from Eq.[4.18]. Expressions for the hydrodynamic stresses can be determined from the general flow fields, see Appendix A. Using these expressions and the relation between the flow fields, Eq.[4.45], the viscous stress can be written in terms of the normal flow coefficient, c_{202} . The internal and external stress components are,

$$\tau_{200}^{\text{hd,in}} = \frac{1}{\sqrt{6}} c_{202}, \quad \tau_{200}^{\text{hd,ex}} = -2\sqrt{\frac{2}{3}} c_{202}, \quad (4.46)$$

$$\tau_{202}^{\text{hd,in}} = -4c_{202}, \quad \tau_{202}^{\text{hd,ex}} = \frac{7}{2} c_{202}. \quad (4.47)$$

Membrane Stresses

The incompressible bilayer membrane is governed by the resistance to changes in curvature and resistance to changes in area. The free energy of the membrane is,

$$F = \int \left[\frac{\kappa}{2} (2H - C_0)^2 + \sigma \right] dA, \quad (4.48)$$

where H is the mean curvature of the interface and C_0 is the spontaneous curvature of the bilayer. The membrane stresses are determined from the variational derivative of Eq.[7.10] [181]. This stresses associated with bending are,

$$\boldsymbol{\tau}^\kappa = -\kappa \left[(2H - C_0)(2H^2 - 2K_g + C_0H) + 2\nabla_s^2 H \right] \mathbf{n}, \quad (4.49)$$

where K_g is the Gaussian curvature. The stresses associated with tension are,

$$\boldsymbol{\tau}^\sigma = 2\sigma H \mathbf{n} - \nabla_s \sigma. \quad (4.50)$$

The first term represents the isotropic tension while the second term represents the nonuniform component of the tension. Both components are needed to ensure conservation of local and global membrane area. The surface gradient operator is defined as,

$$\nabla_s = \mathbf{I}_s \cdot \nabla, \quad (4.51)$$

with, $\mathbf{I}_s = \mathbf{I} - \mathbf{n}\mathbf{n}$, the surface projection and $\mathbf{I}$ is the identity operator.

The membrane is considered a two-dimensional Newtonian fluid, i.e. the membrane molecules can move within membrane. The dissipation within the

incompressible membrane is given by the surface shear stress,

$$\boldsymbol{\tau}^\mu = \mu_{\text{mm}} \nabla_s \cdot (\nabla_s \mathbf{u}_s + (\nabla_s \mathbf{u}_s)^\dagger). \quad (4.52)$$

Note that the magnitude of the surface viscous stress can vary greatly between different types of membranes. Lipid vesicles tend to have low membrane shear viscosities $\mu_{\text{mm}} \sim 0.1 - 10 \times 10^{-8} \text{Ns/m}$, while polymer membranes have much higher viscosities, $\mu_{\text{mm}} \sim 10 - 100 \times 10^{-8} \text{Ns/m}$. It is important to include this term in the analysis because vesicle dynamics can be sensitive to membrane viscosity, particularly for high viscosity membranes.

The membrane stresses can be written in terms of vector spherical harmonics [212, 181]. The general expressions for the membrane stresses are listed in Appendix A. As discussed in Section 4.2.5, the electric stresses only have $j = 2$, $m = 0$ components. The membrane bending contributions for these harmonics are,

$$\tau_{202}^\kappa = 24f_{20}, \tau_{200}^\kappa = 0. \quad (4.53)$$

The stresses due to membrane tension include both an isotropic, σ_h , and a position dependent tensions, σ_{20} . The normal and tangential components of the tension stresses are,

$$\tau_{202}^\sigma = 2\sigma_{20} + 4\sigma_h f_{20}, \tau_{200}^\sigma = -\sqrt{6}\sigma_{20} \quad (4.54)$$

The surface viscous stresses have only in-plane contributions related to the tangential viscous stress,

$$\tau_{202}^\mu = 0, \tau_{200}^\mu = 4\mu_{\text{mm}} c_{200}. \quad (4.55)$$

The membranes used in experiments do not have significant in plane surface

elastic stresses and they are neglected in this model [51]. The expressions for surface elastic stresses are included in [215].

4.2.7 Evolution equation

The driving force for vesicle deformation and hydrodynamic flow is the electric field. The characteristic electric stress, $\epsilon_{\text{ex}} E_0^2$, is also characteristic of the viscous stress, including surface viscous stresses. Membrane bending and tension are scaled by κ/a^3 . The flow quantities are nondimensionalized using the EHD timescale, t_{hd} , vesicle radius, a , and the characteristic magnitude of the bulk electric stress, $\epsilon_{\text{ex}} E_0^2$.

The dimensionless stress balance is written in the normal and tangential directions respectively,

$$\tau_{202}^{\text{el}} + \tau_{202}^{\text{hd,ex}} - \Lambda \tau_{202}^{\text{hd,in}} = C a_{\kappa}^{-1} (\tau_{202}^{\kappa} + \tau_{202}^{\sigma}) \quad (4.56)$$

$$\tau_{200}^{\text{el}} + \tau_{200}^{\text{hd,ex}} - \Lambda \tau_{200}^{\text{hd,in}} = C a_{\kappa}^{-1} \tau_{200}^{\sigma} + \tau_{200}^{\mu} \quad (4.57)$$

where the capillary number is given by Eq.[4.16].

The tangential stress balance is solved first. This determines the non isotropic part of the membrane tension,

$$\tilde{\sigma}_{20} = C a_{\kappa} \left[\frac{-\tau_{200}^{\text{el}}}{\sqrt{6}} + c_{202} \frac{4 + (\Lambda + 8\Lambda_s)}{6} \right]. \quad (4.58)$$

Note that this tension is normalized by, $\tilde{\sigma}_{20} = \sigma_{20} a^2 / \kappa$.

Using the expression for $\tilde{\sigma}_{20}$ in the normal stress balance, the normal surface

velocity coefficient can be solved for,

$$c_{202} = C_{20} - Ca_{\kappa}^{-1} \frac{24(6 + \tilde{\sigma}_h)}{32 + 23\Lambda + 16\Lambda_s} f_{20}, \quad (4.59)$$

where C_{20} is a forcing term due to the electric stress and $\tilde{\sigma}_h = \sigma_h a^2 / \kappa$ is the normalized isotropic tension, which results from the choice of bending rigidity in the capillary number. The forcing term can be written as,

$$C_{20} = \frac{\sqrt{6} [2\tau_{200}^{\text{el}} + \sqrt{6}\tau_{202}^{\text{el}}]}{32 + 23\Lambda + 16\Lambda_s}, \text{ or } C_{20} = 16\sqrt{\frac{\pi}{5}} \frac{(3\bar{p}^{\text{el}} - \bar{\tau}_s)}{32 + 23\Lambda + 16\Lambda_s}. \quad (4.60)$$

Expanding either expression in terms of the coefficients from the potential yields,

$$C_{20} = 2\sqrt{\frac{\pi}{5}} \frac{B_{\text{ex}} B_{\text{ex}}^* - 4(\text{Re}(B_{\text{ex}}) - 1 + SB_{\text{in}} B_{\text{in}}^*)}{32 + 23\Lambda + 16\Lambda_s}. \quad (4.61)$$

The expression for C_{20} is left in terms of B_{ex} and B_{in} and computed using Eq.[4.33].

The full evolution equation is given by,

$$\frac{\partial f_{20}}{\partial t} = C_{20} - \frac{\kappa}{a^3 \epsilon_{\text{ex}} E_0^2} \frac{24(6 + \tilde{\sigma}_h)}{32 + 23\Lambda + 16\Lambda_s} f_{20}. \quad (4.62)$$

Previous work has included both bending and tension contributions to the relaxation term in Eq.[4.62]. However, for most vesicles, tension is the dominant contribution to the restoring force. Most vesicles have an isotropic tension on the order of magnitude of $\sigma_h \sim 10^{-7} \text{N/m}$ or greater when under stress. This value needs to be compared to the contribution of bending rigidity, which gives a force per length contribution of κ/a^2 . For typical values of bending rigidity, $\kappa \sim 10^{-20} J$, and vesicle radius, $a \sim 10^{-5} \text{m}$, the magnitude of bending force force per length acting on the vesicle is $\kappa/a^2 \sim 10^{-10} \text{N/m}$. Hence, the restoring mechanism is dominated by

membrane tension and the contributions from bending are negligible. This yields a simpler form for the relaxation term in the evolution equation,

$$\frac{\partial f_{20}}{\partial t} = C_{20} - \frac{\sigma_h}{a\epsilon_{\text{ex}}E_0^2} \frac{24}{32 + 23\Lambda + 16\Lambda_s} f_{20}. \quad (4.63)$$

This formulation reduces confusion concerning restoring forces in vesicles, where tension is the dominant contribution. Bending rigidity becomes important in determining the amount of excess stored in membrane fluctuations.

The stationary state of the vesicle is determined by setting Eq.[4.62] to zero and then solving for f_{20} . This gives

$$\bar{f}_{20} = Ca_\kappa \frac{16\sqrt{\frac{\pi}{5}}(3\bar{p}^{\text{el}} - \bar{\tau}_s^{\text{el}})}{24(6 + \tilde{\sigma}_h)}, \quad (4.64)$$

where $\bar{f}_{20}$ denotes a steady deformation of the vesicle. Notice that the viscous terms from Eq.[4.62] cancel in the expression above, making $\bar{f}_{20}$ independent of viscous properties.

The tension is the remaining variable to be determined. As the vesicle deforms, the excess area stored in fluctuations, Δ_f , is transferred to systematic deformation [181]. The area stored in the deformed vesicle shape, $\bar{\Delta}$, is determined by setting Eq (4.62) to zero and solving for f_{20} .

Isotropic Tension

The isotropic tension term, $\tilde{\sigma}_h$, must be determined from the constraint on area as the vesicle deforms. In order to capture the tension created by the electric forcing, an approach originally developed for an applied flow field is adapted for the case of

applied electric field [181].

The deformation of the vesicle consumes part of the membrane's excess area. This excess area is quantified using Eq.[4.7],

$$\bar{\Delta} = 2|\bar{f}_{20}|^2 = 2 \left(Ca_\kappa \frac{16\sqrt{\frac{\pi}{5}}(3\bar{p}^{\text{el}} - \bar{\tau}_s^{\text{el}})}{24(6 + \tilde{\sigma}_h)} \right)^2. \quad (4.65)$$

Unless the applied stress is very strong, it cannot be assumed that all membrane area is transferred to the systematic deformation, $\bar{\Delta}$. The remaining excess area is stored in fluctuations, which is determined by,

$$\Delta_f = \sum_{j,m} |f_{j,m}|^2 \frac{(j+2)(j-1)}{2} = \frac{k_B T}{2\kappa} \sum_{j,m} \frac{(j+2)(j-1)}{2E_j(\sigma_h)}, \quad (4.66)$$

where E_j is the partition function,

$$E_j(\tilde{\sigma}_h) = (j+2)(j-1)(j(j+1) + \tilde{\sigma}_h). \quad (4.67)$$

Eq.[4.66] lacks any dependence on m , allowing the equation to be simplified,

$$\Delta_f = \frac{1}{2} \sum_j \frac{2j+1}{j(j+1) + \tilde{\sigma}_h}. \quad (4.68)$$

This expression can be approximated by an integral to a finite value, which is determined by the ratio of the vesicle radius to the membrane thickness, $j_{\text{max}} = a/d$ [181],

$$\Delta_f = \frac{k_B T}{2\kappa} \ln \left(\frac{j_{\text{max}}^2 + j_{\text{max}} + \tilde{\sigma}_h}{6 + \tilde{\sigma}_h} \right). \quad (4.69)$$

With area accounted for in either systematic deformation or fluctuations, the

conservation of area enforces,

$$\Delta = \bar{\Delta} + \Delta_f = 2 \left(C a_\kappa \frac{16\sqrt{\frac{\pi}{5}}(3\bar{p}^{\text{el}} - \bar{\tau}_s^{\text{el}})}{24(6 + \tilde{\sigma}_h)} \right)^2 + \frac{k_B T}{2\kappa} \ln \left(\frac{j_{max}^2 + j_{max} + \tilde{\sigma}_h}{6 + \tilde{\sigma}_h} \right). \quad (4.70)$$

The balance between excess area stored in systematic deformation and excess area stored in fluctuations determines the isotropic tension. The equation cannot be solved explicitly, but can be solved numerically as the electric stresses vary. While the vesicle is under an applied stress, the tension is determined by a given forcing, not the instantaneous shape. It can also be seen in Eq.[4.70] that the transfer of area from fluctuations to systematic deformation is governed by membrane bending rigidity. A lower bending rigidity, or higher thermal energy, will allow more area to be stored in fluctuations.

When the vesicle reaches a steady state, the deformation will be equivalent to that determined by Eq.[4.64]. The applied stress and deformation at steady state can be related using Eq.[4.65]. In this case, the deformation will equal the steady state deformation, $f_{20} = \bar{f}_{20}$. The tension can be written explicitly in terms of the deformation from Eq.[4.70] [58],

$$\sigma_h = \sigma_{\text{eq}} \exp \left(\frac{4\kappa}{k_B T} \bar{f}_{20}^2 \right). \quad (4.71)$$

When the electric field is turned off, the excess area returns to entropic fluctuations. Eq.[4.70] shows that the relaxation is driven by the initial tension, i.e. when $C_{20} = 0$, all excess area is stored in fluctuations.

The isotropic tension increases significantly at large values of f_{20} because the tension scales as $\sigma_h \sim \exp(\bar{f}_{20}^2)$. This increase in tension is plotted in Figure 4.3 using

equation Eq.[4.71] with the equilibrium value determined for $\bar{f}_{20}$ from Eq.[4.64]. This demonstrates how the isotropic tension varies with electric field strength for different values of initial tension. Even when the equilibrium tension differs by two orders of magnitude, when a strong field is applied the isotropic tension is of a similar order of magnitude, i.e. $\sigma_h \sim 10^{-5} \text{N/m}$.

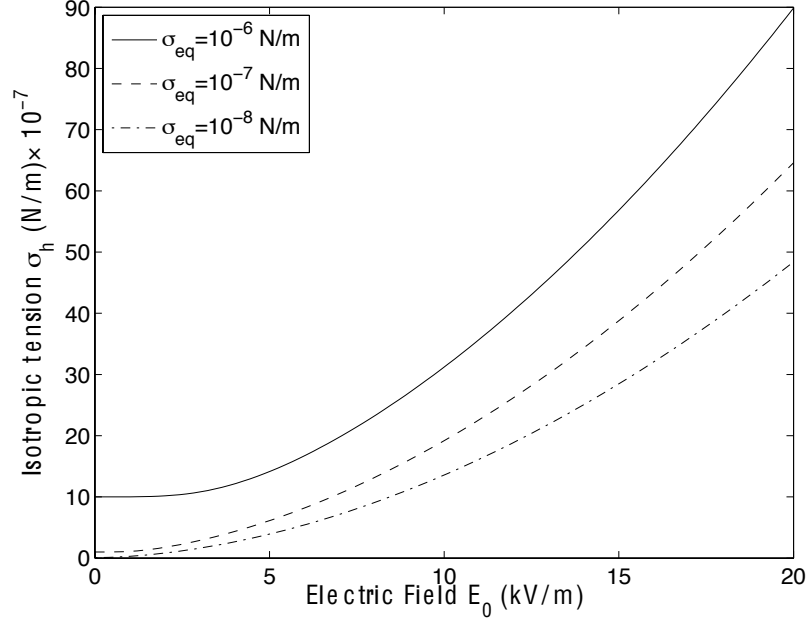


Figure 4.3: Typical values of isotropic membrane tension as function of applied electric field for different values of initial tension. The isotropic tension increases nonlinearly with electric field strength, but only reaches values of $\sigma_h \sim 10^{-5} \text{N/m}$ for typical electric field strengths.

In order to determine whether the isotropic tension values found in Figure 4.3 are strong enough to stretch the membrane, an estimate for the crossover tension where stretching can occur can be estimated. A comparison of bending and stretching moduli gives the crossover tension [48],

$$\sigma_c = \frac{K_a k_B T}{8\pi\kappa}. \quad (4.72)$$

where K_a is the stretching modulus of true membrane area. For typical values of lipid membranes, $K_a \sim 0.2 \text{N/m}$ and $\kappa/k_B T \sim 20$. This gives a crossover tension

of the order $\sigma_c \sim 10^{-3}\text{N/m}$. Comparing this to the isotropic tension of a vesicle in an electric field, a large initial tension of $\sigma_{\text{eq}} \sim 10^{-6}\text{N/m}$ will only lead to values of isotropic tension of about $\sigma_h \sim 10^{-5}\text{N/m}$. The electric field strengths necessary for isotropic tension to cause membrane stretching are much greater than typical values used in experiments.

Tension can also arise from an electro-compressive stress across the membrane capacitor. This tension is related to the transmembrane voltage by [138],

$$\sigma_{\text{el}} = \epsilon_{\text{mm}} \left(\frac{d}{2d_c^2} \right) V_m^2, \quad (4.73)$$

where d is the total thickness of the bilayer ($d \sim 4\text{nm}$ for lipids) and d_c is the thickness of the dielectric layer ($d_c \sim 2.8$ for lipids). This expressions differs from the typical expression for the Lippmann tension [116], the compressive stress on a capacitor, because the electric stress acts over the thickness of the dielectric layer, d_c , while the membrane can be considered incompressible across the entire thickness, d . An estimate for Eq.[4.73] can be made by considering $d = d_c$ and then rewriting the equation in terms of capacitance, C_m , which gives the typical expression for Lippmann tension [116],

$$\sigma_{\text{el}} = \frac{1}{2} C_m V_m^2. \quad (4.74)$$

Using either equation, an estimate for σ_{el} can be made using the transmembrane voltage at the poles where V_m is at a maximum. For a typical lipid vesicle with $a \sim 20\mu\text{m}$, an applied electric field greater than 10 kV/m will reach values comparable to the crossover tension, $\sigma_{\text{el}} \sim 10^{-3}\text{N/m}$. To avoid the effect of charging and discharging of the membrane capacitor the experiments are done at intermediate frequencies where $V_m \sim 0$.

In even stronger fields, the compressive tension can lead to lysis of the membrane, which occurs at a tension denoted by σ_{lys} . This tension has been measured to be about $\sigma_{\text{lys}} \sim 5 \times 10^{-3} \text{N/m}$ for lipid vesicles and $\sigma_{\text{lys}} \sim 20 - 30 \times 10^{-3} \text{N/m}$ for polymer vesicles [138, 48, 9]. Any initial tension, or the increase in tension caused by deformation, will add to this tension, although these values are usually small compared to the Lippman tension, σ_{el} . A critical membrane voltage for lipid vesicles has been measured at approximately $V_c \sim 1V$ [138, 48]. For polymer membranes, this critical voltage is higher due to the thicker membrane, which increases lysis tension. Experiments on the critical transmembrane voltage showed that the critical voltage is in the range of $V_c \sim 4 - 8V$ [9]. The effects of exceeding this transmembrane voltage on vesicle dynamics will be investigated in Chapter 6.

4.2.8 Transition frequency

The equilibrium deformation of the vesicle in an AC fields can be used to determine the frequency where the vesicle transitions from a prolate and oblate shape. This is an important result because the transition frequency will be compared to experimental observations and used to determine the membrane capacitance.

The transition frequency can be computed by solving for $C_{20} = 0$, corresponding to no net deforming stress. Using Eq.[4.61], this is found by considering,

$$B_{\text{ex}}B_{\text{ex}}^* - 4(\text{Re}(B_{\text{ex}}) - 1 + SB_{\text{in}}B_{\text{in}}^*) = 0. \quad (4.75)$$

Inserting the values for B_{in} and B_{ex} , this equation is solved for frequency, which gives

the final result for the transition frequency [225, 207],

$$f_c = \frac{\lambda_{\text{in}}}{2\pi a C_m} [(1 - R)(R + 3)]^{-1/2} . \quad (4.76)$$

This expression is valid if the inner and outer fluids have the same dielectric constant, which is indeed the case of vesicles, where both interior and suspending fluids are aqueous solutions. Note that the transition frequency is not equal to the inverse of the capacitor charging time, Eq.[5.2], and that the critical frequency exists only for $R < 1$.

With the exception of C_m , the parameters in Eq.[4.76] can be measured experimentally by other means. The fluid conductivities can be determined during preparation and the vesicle radius can be measured with a calibrated image. The frequency at which the vesicle is a sphere can be determined from a frequency scan. With these variables known, Eq.[4.76] will be used to determine the membrane capacitance.

Eq.[4.76] shows that the oblate–prolate transition frequency depends on membrane capacitance, vesicle size, and conductivities of the inner and outer solutions. Moreover, electric compressive pressure (electrostriction) may cause membrane thinning thereby shifting the transition to higher frequencies. In the results, the effects of each of these parameters are examined and membrane capacitance is extracted from data using the dependence of f_c with vesicle size.

4.2.9 Model Results

In an AC field, the net charge on the membrane depends on the relative magnitude of the field frequency and the capacitor charging time. At low frequencies, the capacitor charging time is shorter than the period of the electric field oscillations; the capacitor is fully charged and the charge densities on the inner and outer surfaces are exactly balanced. Hence, in this frequency range the vesicle responds as an ideally-polarizable particle with vanishing electric field in the vesicle interior.

At higher frequencies, the capacitor becomes short-circuited. Displacement current flows through the membrane and the vesicle interior participates in the conduction process. In this case, vesicle polarization and its shape depend on the conductivity ratio R . If $R > 1$, the conduction in the vesicle interior is faster than the suspending liquid. As a result, the surface charge densities become imbalanced; the interface acquires apparent charge dominated by ions brought from the interior fluid and the induced dipole is aligned with the electric field. In this case, charges at the poles are attracted by the electrodes, pulling the vesicle into a prolate shape. In the opposite case, $R < 1$, there is an excess of ions at the exterior interface and the polarization is reversed. In this induced-charge configuration, the vesicle becomes an oblate ellipsoid.

Steady deformation

The dependence of equilibrium vesicle shape is investigated for different system parameters. The effect of membrane capacitance, fluid conductivities, and bending rigidity are investigated using Eq.[4.64] as function of field frequency.

The effect of membrane capacitance on the frequency response of vesicle shape

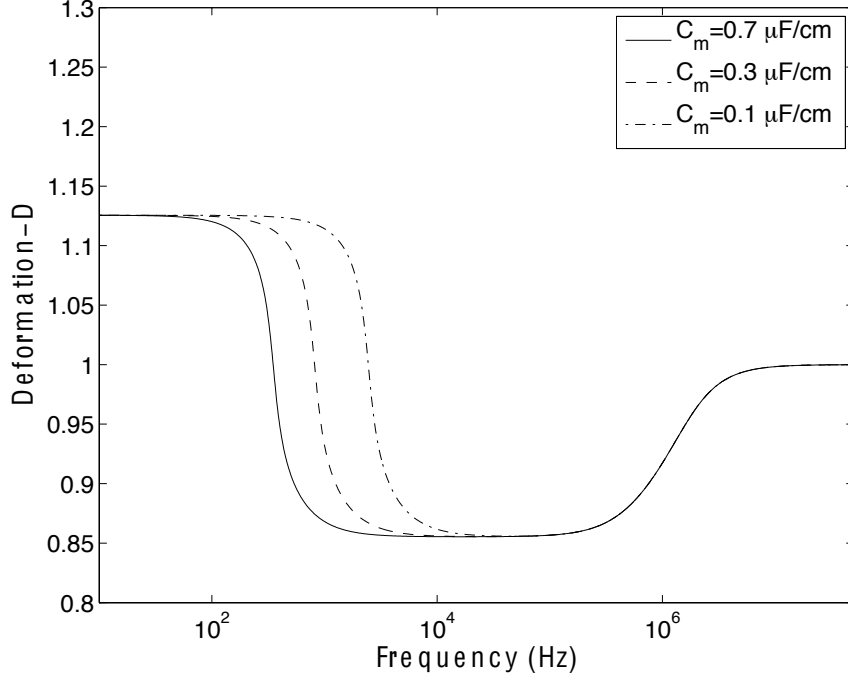


Figure 4.4: Frequency sweep showing the effect of membrane capacitance of membrane capacitance. $C_m = 0.7\mu F/cm^2$ (solid line), $C_m = 0.3\mu F/cm^2$ (dashed line), $C_m = 0.1\mu F/cm^2$ (dash-dot line). $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 30\mu S/cm$, $R = 0.17$, $a = 20\mu m$, $\sigma_{eq} = 10^{-7} N/m$, $\kappa = 15k_B T$.

is important for determining a value for C_m . Figure 4.13 shows that membrane capacitance only changes the transition frequency. Greater membrane capacitance leads to a higher transition frequency.

The fluid conductivities affect both the type of deformation and magnitude in the intermediate frequency range, see Figure 4.5. When the field frequency exceeds the inverse membrane charging time, $f > t_{mm}^{-1}$, the membrane capacitor becomes short circuited and deformation depends on conductivity ratio. The short circuiting of the membrane capacitor changes the electric stresses for all conductivity ratios. This leads to an increase in deformation for $R \gg 1$ at frequencies above t_{mm}^{-1} . Little change in deformation is observed when $R \gtrsim 1$. As discussed, the transition from oblate to prolate only occurs if $R < 1$. Lower conductivities will result in a lower f_c , particularly if the internal conductivity is small, see Eq.[4.76]. In general, as conductivity ratio differs from $R \sim 1$, the magnitude of deformation increases in the

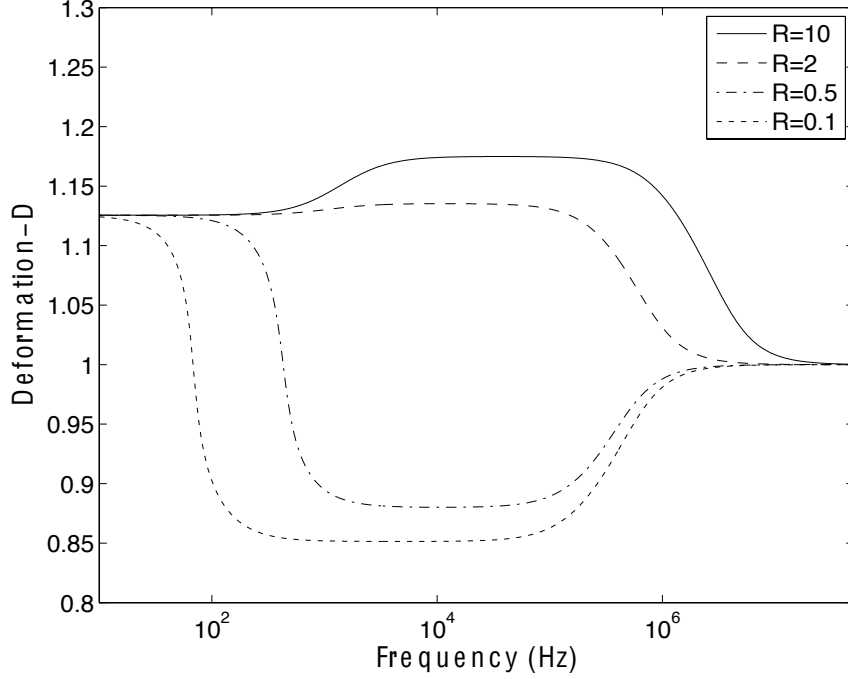


Figure 4.5: Frequency sweep showing the effect of conductivity ratio for a typical range of conductivities used in experiments. The external conductivity is set at $\lambda_{\text{ex}} = 10\mu\text{S}/\text{cm}$, Internal conductivity is varied from $\lambda_{\text{in}} = 100\mu\text{S}/\text{cm}$ ($R = 10$) (solid line), $\lambda_{\text{in}} = 20\mu\text{S}/\text{cm}$ ($R = 2$) (dashed line), $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$ ($R = 0.5$) (dash-dot line), $\lambda_{\text{in}} = 1\mu\text{S}/\text{cm}$ ($R = 0.1$) (dotted line). $C_m = 0.7\mu\text{F}/\text{cm}^2$, $a = 20\mu\text{m}$, $\sigma_{eq} = 10^{-7}\text{N}/\text{m}$, $\kappa = 15k_B T$.

intermediate frequency range.

A stronger electric field strength increases vesicle deformation by pulling more excess area out of membrane fluctuations. As fluctuations are transferred into systematic deformation, tension increases according to Eq.[4.71]. An increase in bending rigidity further limits the extent of deformation because it is more difficult to pull out fluctuations. This decreases the range of deformation available as the field is increased. Comparing the two charges in Figure 4.13, the lower bending rigidity (left) has a wider range of deformation for the same field strength as the higher bending case (right).

Time Dependent Deformation

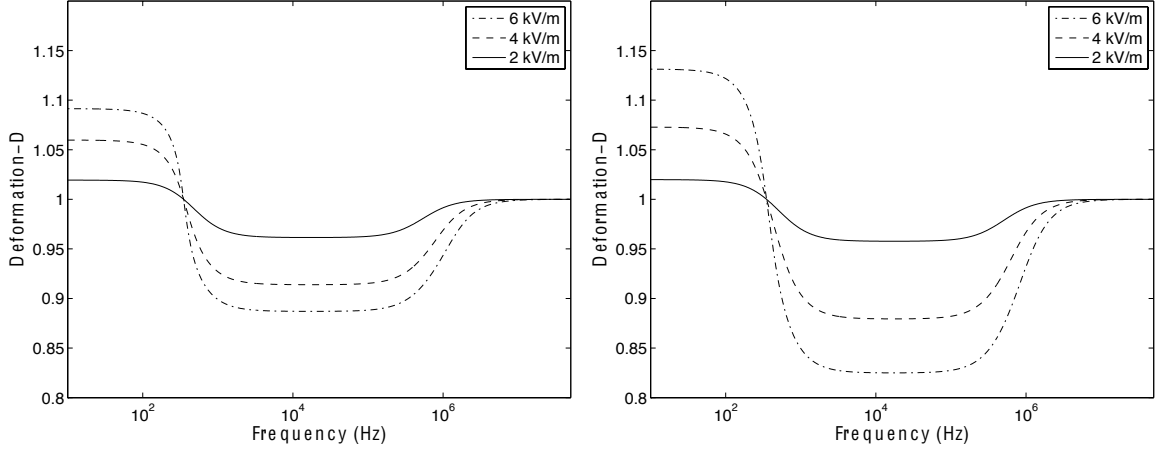


Figure 4.6: Frequency sweep showing the effect of electric field strength for two values of bending rigidity, $\kappa = 20k_B T$ (left) and $\kappa = 5k_B T$ (right). The electric field is varied from $E_0 = 2kV/m$ (solid line), $E_0 = 2kV/m$ (dashed line), $E_0 = 2kV/m$ (dash-dot line). Other parameters are set at $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 30\mu S/cm$, $R = 0.17$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu m$, $\sigma_{eq} = 4 \times 10^{-7} N/m$.

The steady state deformation does not depend on certain membrane properties such as membrane and bulk viscosities. The dynamic response of vesicle shape is investigated using Eq.[4.62] to see the influence of these parameters. The response is investigated after a step increase in the field as well as after the field is turned off. The behavior is investigate at a 100 kHz field frequency for different field strengths. Time is normalized by the EHD timescale.

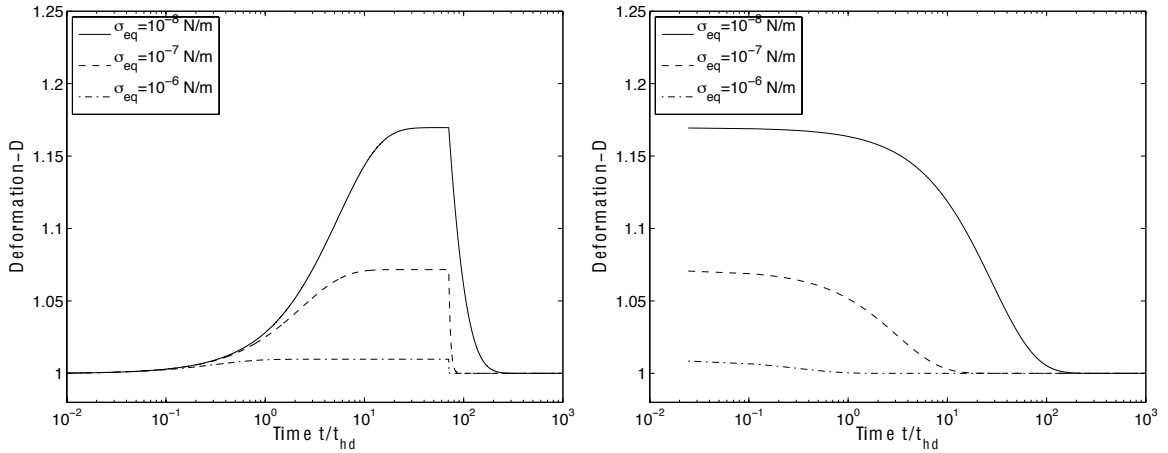


Figure 4.7: Time dependent deformation for different values of initial tension. The initial tension is varied from $\sigma_{eq} = 10^{-8} N/m$ (solid line), $\sigma_{eq} = 10^{-7} N/m$ (dashed line), $\sigma_{eq} = 10^{-6} N/m$ (dash-dot line). Other parameters are set at $E_0 = 2kV/m$, $\lambda_{in} = 10\mu S/cm$, $\lambda_{ex} = 3\mu S/cm$ ($R = 3.3$), $C_m = 0.27\mu F/cm$, $a = 20\mu m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $\kappa = 15k_B T$.

The influence of initial tension on vesicle dynamics is shown in Figure 4.7. The initial tension changes with the amount of excess area stored by the vesicle at equilibrium. This will vary the maximum deformation possible as well as the strength of the relaxation term in Eq.[4.62]. The initial tension does not significantly influence the dynamics after the field is turned on, but a vesicle with lower tension will take longer to reach a more deformed steady state. A change in initial tension is most discernible during the relaxation back to a sphere. When the field is turned off, the forcing term in Eq.[4.62] vanishes and the isotropic tension term returns to the initial tension. This causes the relaxation to be governed by the surface tension timescale, t_σ . A low tension vesicle will take longer to relax than a tense vesicle.

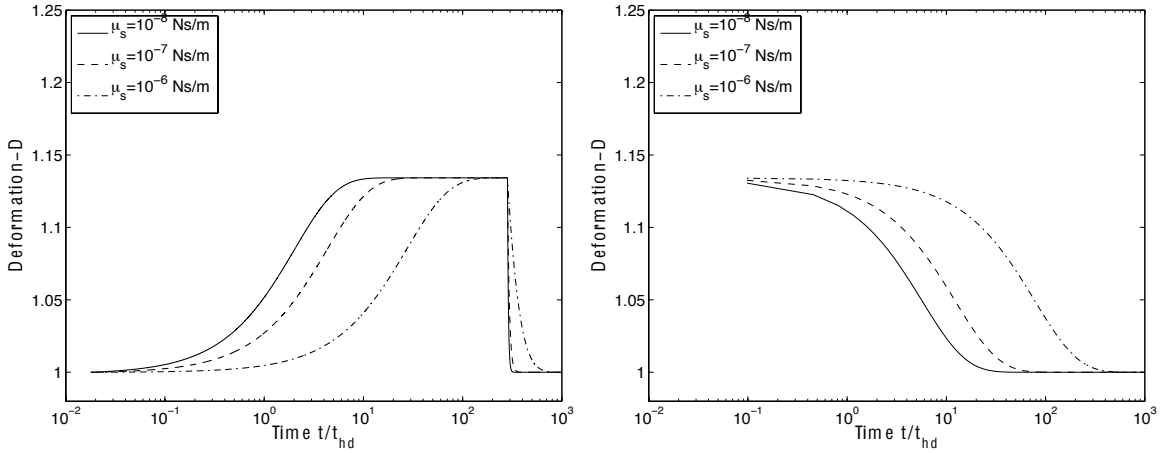


Figure 4.8: Time dependent deformation for different values of membrane viscosity, the field is turned on at $t = 0$ and turned off at $t = 50s$. The full shape evolution (left) and the relaxation after the field is turned off (right) are plotted. Values for membrane viscosity is varied from $\mu_{mm} = 10 \times 10^{-8} Ns/m$ (solid line), $\mu_{mm} = 10 \times 10^{-7} Ns/m$ (dashed line), $\mu_{mm} = 10 \times 10^{-6} Ns/m$ (dash-dot line). Other parameters are set at $E_0 = 4kV/m$, $\sigma_{eq} = 10^{-7} N/m$, $\lambda_{in} = 10\mu S/cm$, $\lambda_{ex} = 3\mu S/cm$ ($R = 3.3$), $C_m = 0.27\mu F/cm^2$, $a = 20\mu m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $\kappa = 15k_B T$.

The influence of membrane viscosity on vesicle dynamics, seen in Figure 4.8, appears only during transitions in shape. The steady state shape is unchanged because there are no viscous contributions at equilibrium. Higher membrane viscosity increases the time to reach a steady state shape, although membrane viscosity only becomes important when $\Lambda_s \gtrsim \Lambda$. For vesicles of radius $a \sim 20\mu m$, this occurs for membranes with viscosity around $\mu_{mm} = 10^{-8} Ns/m$. The membrane viscosity values

for most lipid membranes are of the order $\mu_{\text{mm}} \sim 1 - 10 \times 10^{-9} \text{Ns/m}$ and will produce only limited viscous dissipation compared to the bulk fluids. Polymer membranes have an order of magnitude or higher membrane viscosity, $\mu_{\text{mm}} \sim 10^{-7} - 10^{-6} \text{Ns/m}$ [48]. As a result the dissipation into the membrane is expected to be dominant for polymer membranes.

Stronger electric fields produce faster deformation and a more deformed steady state, see Figure 4.9. If time is scaled by t_{hd} , the deformation will occur at the same relative rate because the EHD timescale scales with the electric stress, $t_{\text{hd}} \sim E_0^{-2}$. Although, the increase in deformation with field strength remains. Figure 4.9 also

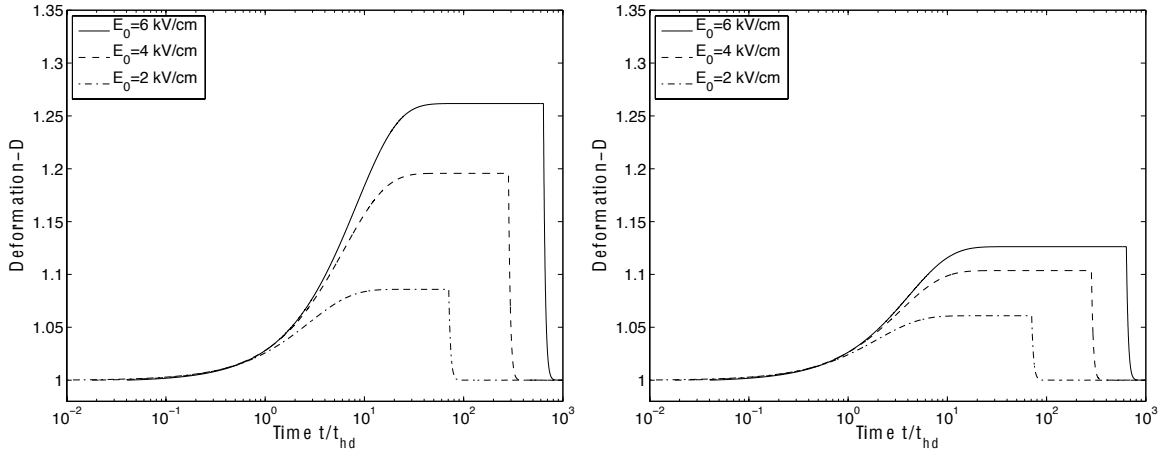


Figure 4.9: Time dependent deformation for different values of electric field strength and bending rigidity, the field is turned on at $t = 0$ and turned off at $t = 50s$. The shape evolution for two values of bending rigidity, $\kappa = 5k_B T$ (left) is compared to $\kappa = 20k_B T$ (right). The electric field is varied from $E_0 = 6kV/m$ (solid line), $E_0 = 4kV/m$ (dashed line), $E_0 = 2kV/m$ (dash-dot line). Other parameters are set at $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$, $\lambda_{\text{in}} = 10\mu\text{S/cm}$, $\lambda_{\text{ex}} = 3\mu\text{S/cm}$ ($R = 3.3$), $C_m = 0.27\mu\text{F/cm}^2$, $a = 20\mu\text{m}$, $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$.

compares the deformation for different bending rigidities. An increase in bending rigidity limits the deformation because less area is able to be stored in fluctuations and there is more resistance to pulling out fluctuations. The range of deformations at different field strengths is reduced for higher values of bending rigidity.

4.3 Materials and experimental procedures

4.3.1 Preparation of giant vesicles

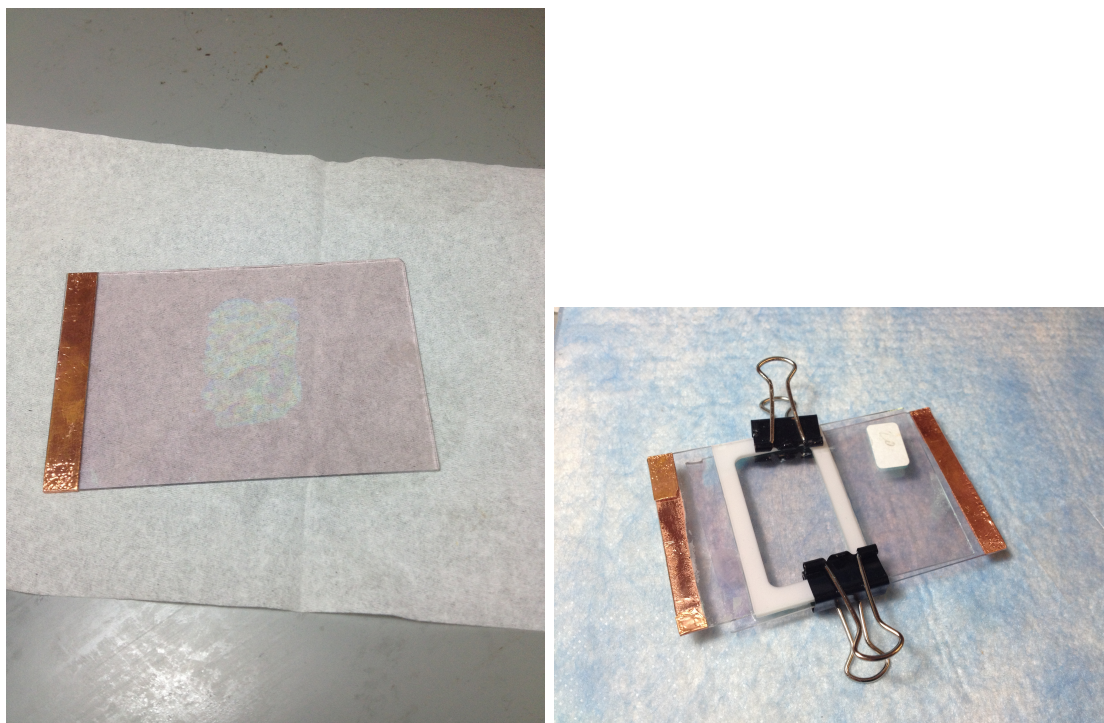


Figure 4.10: Method for vesicle preparation using electroformation technique. The lipids or polymers are spread to create a film on an ITO slide (left). After all solvent has been evaporated in a desiccator, another clean ITO glass is used with conducting side facing inward to create a chamber with a 1mm teflon spacer. The chamber is filled with sucrose solution and held together with clips. The chamber is connected to a 10 Hz AC field after 1-2 hours and the vesicle solution is removed with a pipette.

Giant vesicles are prepared from lipids (POPC) and $s\text{-Bu-}[\text{CH}_2\text{-CH}(\text{C}_2\text{H}_5)]_n\text{-}[\text{O-CH}_2\text{-CH}_2]_m\text{-OH}$ diblock copolymers (denoted as $\text{PBd}_n\text{-b-PEO}_m$) using the electro-formation method [6]. The names and properties of the polymers are listed in Table 4.1. The lipids were purchased from Avanti. The copolymers PS1, PS3 and PS4 were obtained from Polymer Source Inc. (Montreal, Canada), while PS2 was available in the lab [48], originally synthesized by Förster and Krämer [63]. The polymers were chosen to have hydrophilic fraction ϕ similar to lipids in order to

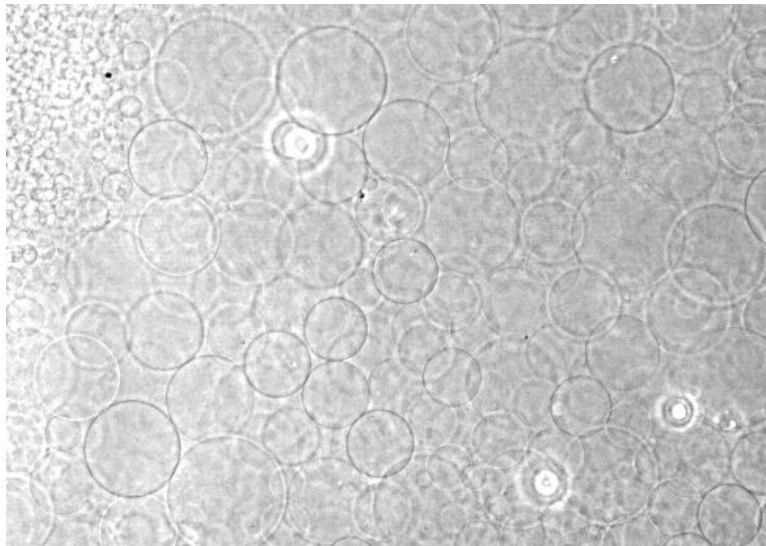


Figure 4.11: Formation of vesicles using electroformation method.

ensure formation of giant vesicles and not other structures, e.g., micelles [49]. The dry polymer melts were stored in the freezer at -20° C. All materials were used without further purification. Stock solutions with concentration 10 mg/ml were made using chloroform.

Name	Amphiphile	M_n (kDa)	M_w/M_n	$\phi(\%)$
LS1	POPC	0.760	—	30
PS1	n=21, m=14	1.2-b-0.6	1.17	33
PS2	n=32, m=20	1.8-b-0.9	1.05	33
PS3	n=47, m=34	2.5-b-1.5	1.04	37.5
PS4	n=110, m=89	6.5-b-3.9	1.10	37.5

Table 4.1: Material properties of the amphiphiles used in this study. In all polymer systems, the 1,2-butadiene content is about 90%.

In the electroformation procedure, a small volume (10 μ l) of the stock solution is spread on the conductive surfaces of two glass plates coated with indium tin oxide (ITO). After removing all traces of the organic solvent using vacuum for at least one hour, the two glasses are assembled, with the conductive sides facing inwards, forming a chamber separated by a 1 mm thick Teflon frame. The chamber is gently filled with 100 mM sucrose solution and NaCl (0.01-10mM) is added to adjust interior conductivity. The conductive sides of the ITO glass plates are connected to a function

generator and an alternating current (3.6-8.0V_{pp}, 10 Hz frequency) is applied for 1-2 h. Higher voltages within this range are used for higher M_w polymers. Vesicles with an average diameter of 50 μm and a large polydispersity are observed after about one hour. The vesicle solution is removed from the electrosweeling chamber and diluted into an isotonic glucose and salt solution. The exterior fluid conductivity is adjusted by adding NaCl solution (0.01mM to 10mM concentrations). The lipids and low M_w polymersomes were grown at room temperature, while PS4 was heated to 60° C.

4.3.2 Electrodeformation set-up

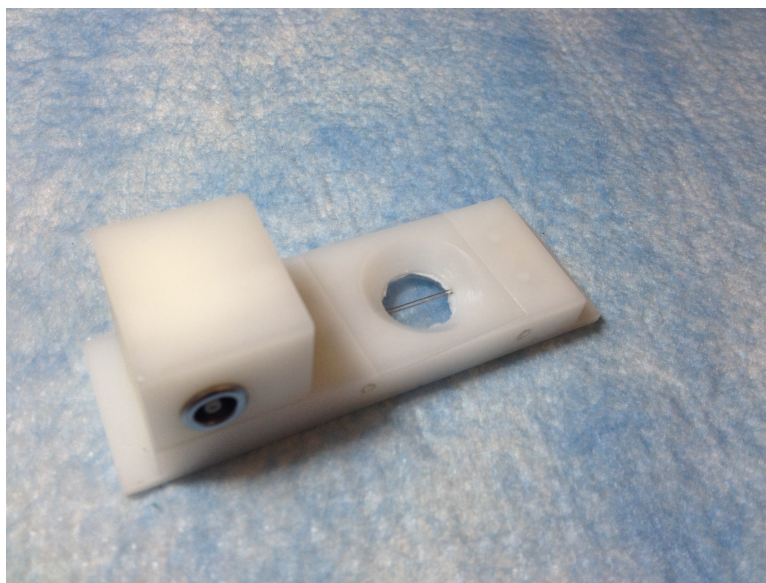


Figure 4.12: Electrodeformation chamber.

The electrodeformation experiments were conducted in a chamber (Eppendorf, Germany), which consists of a Teflon frame confined from above and below by two coverglass slides. A pair of parallel cylindrical electrodes with a radius of 92 μm and a separation of 0.5 mm are fixed to the lower glass. The chamber is connected to a AC function generator (Fluke PM5138A).

4.3.3 Optical microscopy and imaging

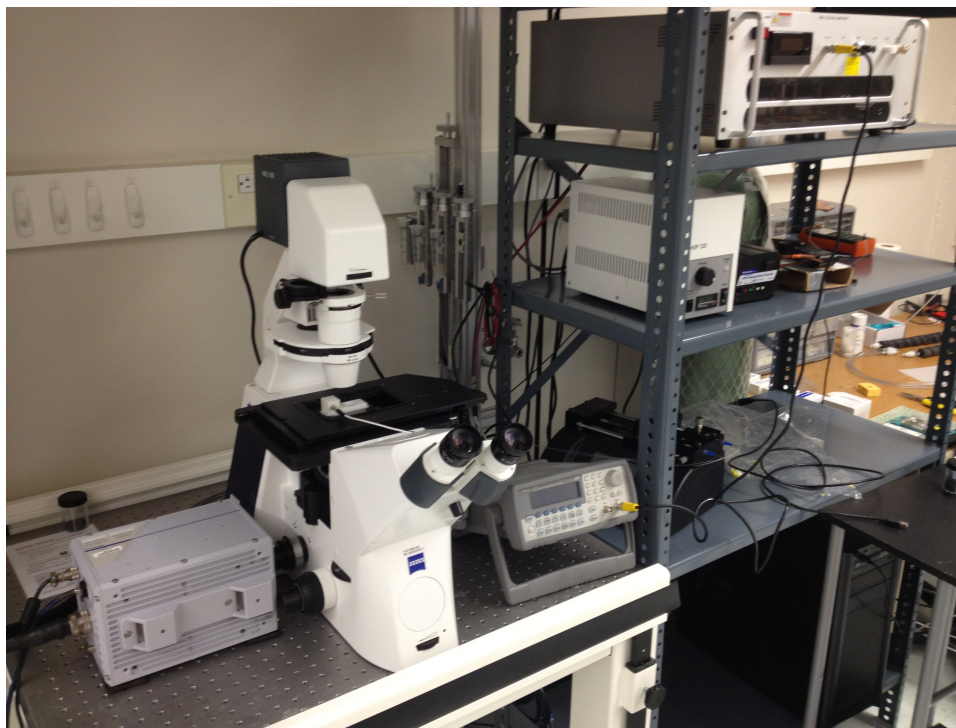


Figure 4.13: Microscope setup.

Due to the differences in density and refractive index between the sucrose and glucose solutions, the vesicles are stabilized by gravity close to the bottom of the chamber and have good contrast when observed with phase contrast microscopy. An inverted microscope Axiovert 135 (Zeiss, Germany) equipped with 20 $\times$ Ph2 objectives was used to image the vesicle under phase contrast mode. Images were recorded with a digital camera. A temperature control block was used to increase the temperature to 60 $^{\circ}$ C for the experiments with vesicles made of PS4 polymers since no appreciable deformation was detected at room temperature. At higher temperature, the fluid properties of the membrane are enhanced and the PS4 polymersomes deform more readily. The lower M_w polymer PS1-PS3 and lipid (POPC) membranes exhibited fluid behavior at room temperature, therefore all experiments with these systems were conducted at room temperature.

For routine observations, vesicle selection and electrodeformation measurements, the samples were illuminated with a halogen lamp. The vesicles were allowed sufficient time to relax to a steady state shape whenever experimental parameters (i.e. frequency or field strength) were changed. An image of the steady state shape is then taken and analyzed using in-house image analysis software. The image analysis software detects the phase contrast contour of the vesicle membrane with subpixel resolution [68] and fits the vesicle contour by a Fourier series [145] (alternatively, the shape can be fitted by an ellipse to a set of points). The second mode of the Fourier series expansion determines the two semi axes $a_{\parallel}$ and $a_{\perp}$, which are used to extract the degree of deformation D .

4.4 Measurement of membrane capacitance

Eq.[4.76] indicates that the oblate–prolate transition frequency f_c depends on membrane capacitance, vesicle size, and conductivities of the inner and outer solutions. Next the variation of the transition frequency with these parameters is examined and how to extract data for the membrane capacitance using the dependence of f_c on vesicle size is shown.

Typical experimental observations of the evolution of the vesicle shape with frequency in a uniform AC fields is illustrated in Figure 4.1. The degree of deformation is quantified by the aspect ratio $D = a_{\parallel}/a_{\perp}$, where $a_{\parallel}$ is parallel and $a_{\perp}$ is normal to the field direction; see inset of Figure 4.14. A quasi-spherical vesicle (made of neutral lipids or polymers) adopts either prolate or oblate ellipsoidal shape, depending on the ratio of the conductivities of the enclosed and suspending solutions λ_{in} and λ_{ex} respectively,

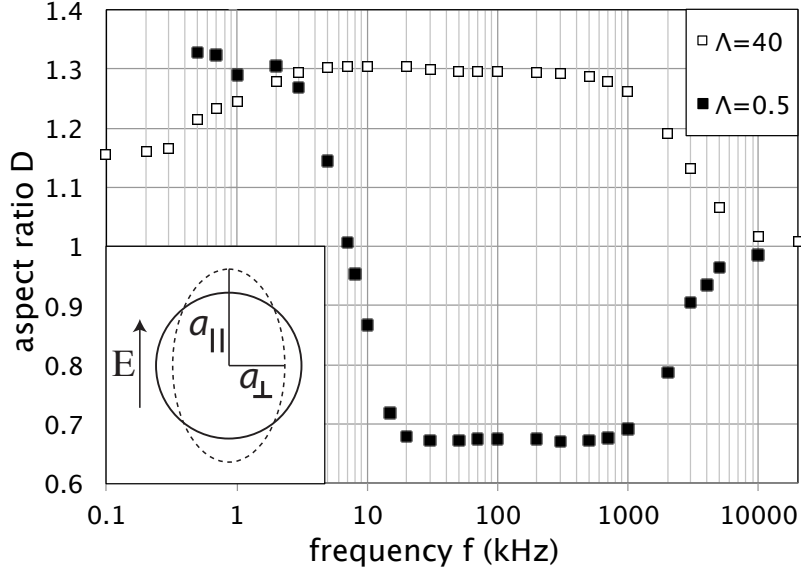


Figure 4.14: Frequency dependent deformation of polymersomes with $R = 40$ ($\square$) and $R = 0.5$ ($\blacksquare$) exposed to a 6kV/m AC field. For $R = 40$, an increase in elongation is observed in the 10-100kHz range, while at these frequencies for $R = 0.5$ the vesicle changes shape from a prolate to an oblate ellipsoid. Aspect ratio $D > 1$ indicates prolate shape (vesicle elongation in the field direction), while $D < 1$ corresponds to oblate shape. In both cases, the vesicle shape becomes spherical in the MHz frequency range. Increasing or decreasing frequency sweep shows no hysteresis. Polymersome radii are 41 μm ($\square$) and 18 μm ($\blacksquare$).

At low frequencies, in the sub-KHz range, the vesicle shape is always a prolate ellipsoid regardless of the solutions conductivities. At high frequencies (above MHz), the vesicle remains a sphere. At intermediate frequencies, a vesicle filled with a less conducting solution, $R < 1$, deforms into an oblate ellipsoid. The change in shape occurs in the 1-10 kHz range. Around this transition frequency, the prolate deformation of a vesicle characterized by $R > 1$ increases. In essence, depending on the relative magnitude of the period of the field reversal, i.e., the field frequency, and the capacitor charging time, the bilayer capacitor is either fully-charged or short-circuited. In the latter case, since the inner fluid participates in the conduction process, the vesicle polarization becomes dependent on R [211]. At high frequencies (MHz), the inverse frequency is shorter than the bulk solution charging time. Hence, the membrane does not charge and the vesicle remains spherical.

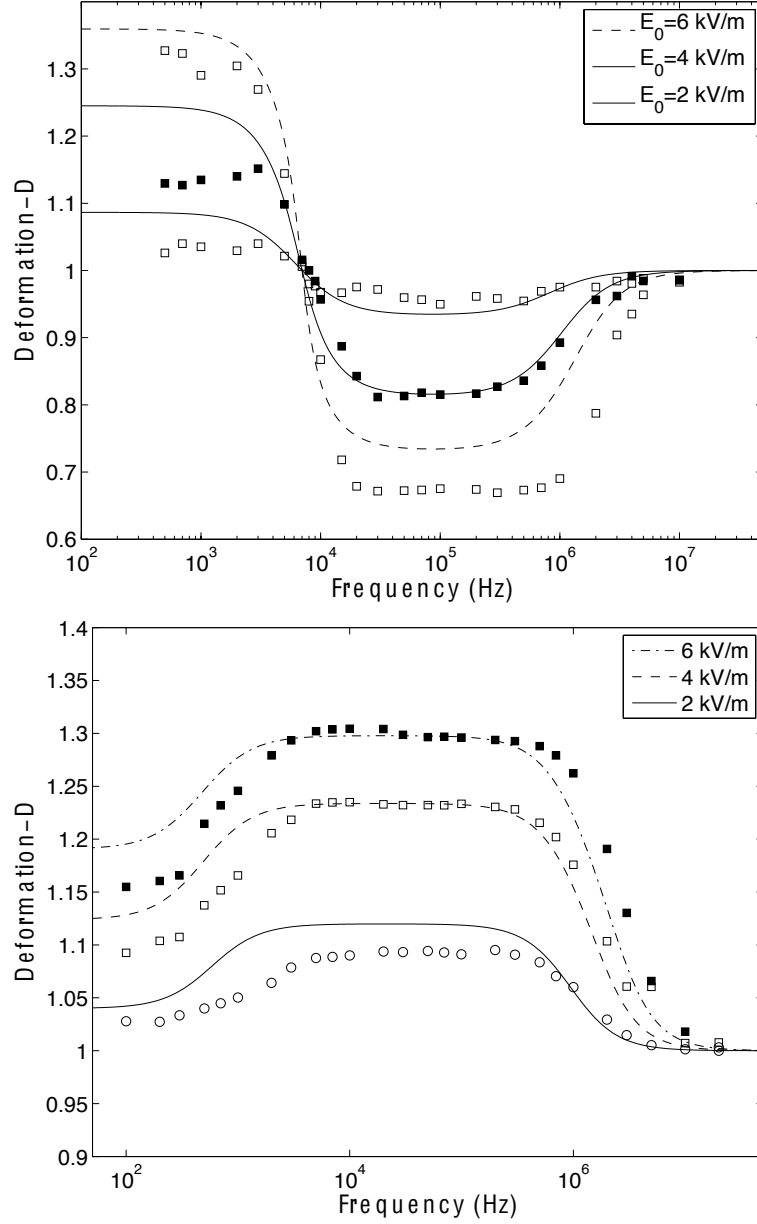


Figure 4.15: Comparison of frequency scans for $R = 0.6$ (left) and $R = 40$. The electric conductivities are varied with NaCl, $\sigma_{\text{in}} = 25\mu\text{S}/\text{cm}$, $\sigma_{\text{ex}} = 40\mu\text{S}/\text{cm}$ (left) and $\sigma_{\text{in}} = 100\mu\text{S}/\text{cm}$, $\sigma_{\text{ex}} = 2\mu\text{S}/\text{cm}$ (right). Three frequency sweeps are taken with electric field values of $E_0 = 2\text{kV}/\text{m}$ (solid line), $E_0 = 4\text{kV}/\text{m}$ (dashed line), $E_0 = 6\text{kV}/\text{m}$ (dash-dot line). The values are measured as $a = 18\mu\text{m}$ (left) and $a = 41\mu\text{m}$ (right). Parameters for the $R = 0.6$ fit are $\sigma_{\text{eq}} = 0.2 \times 10^{-7}\text{N}/\text{m}$, $C_m = 0.27\mu\text{F}/\text{cm}^2$, $\kappa = 2k_B T$. Parameters for the $R = 40$ fit are $\sigma_{\text{eq}} = 40 \times 10^{-7}\text{N}/\text{m}$, $C_m = 0.27\mu\text{F}/\text{cm}^2$, $\kappa = 5k_B T$.

Deformation for individual vesicles as a function of frequency at different field strengths is plotted in Figure 4.15. Deformation increases with field strength for both $R = 0.5$ and $R = 40$. The plots are fit according to Eq.[4.64], with the parameters

given in the caption; only electric field strength is varied. The $R = 40$ vesicle show a transition to a more deformed shape as the applied field frequency exceeds t_{mm}^{-1} as predicted by the model.

The fits are made with low values for bending rigidity. Because bending rigidity acts to regulate the amount of excess area stored in fluctuations, a lower bending rigidity membrane will fluctuate easily and store more area. The low value of bending rigidity is likely due to a surplus of excess area not accounted for by Eq.[4.69]. For instance, the vesicle may rest on the bottom cover glass in an oblate shape, but appear spherical when viewed from below. Thus, at equilibrium the vesicle already has a small systematic deformation that stores excess area. The area stored in this deformation is transferred into observable systematic deformation when the field is applied. The additional excess area would be accounted for in Eq.[4.69] by a lower value of bending rigidity, which increases the magnitude of Δ_f . The difficulty in accounting for all excess area makes using electrodeformation a less reliable measure for bending rigidity. Using a low value for bending rigidity, the theoretical model shows reasonably good agreement with experiments.

4.4.1 Effect of membrane thickness and vesicle radius

As seen from Eq.[4.76], the transition frequency is inversely proportional to vesicle radius. By sampling the prolate-oblate transition for different sized vesicles (same membrane composition and conductivity conditions) in a single experiment, the effect of radius can be isolated. For that purpose we apply the electric field to a collection of vesicles. To avoid interactions between vesicles, the vesicle suspension is sufficiently dilute so that vesicles are isolated from each other.

Figure 4.16 shows that the transition frequency increases linearly with the inverse vesicle radius. With all other parameters known (solution conductivities and vesicle equilibrium radius), the only unknown left is the membrane capacitance, which is used to fit the data. The values for the membrane capacitance obtained from the fits

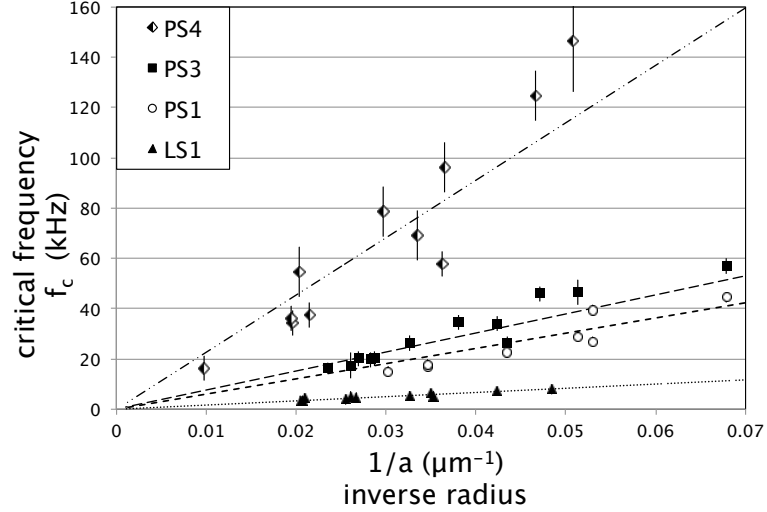


Figure 4.16: Critical frequency, f_c , for the oblate-prolate transition as a function of inverse radius of vesicles made of POPC and polymersomes PS1, PS3 and PS4. The solution conductivities are $\lambda_{in} = 130 \mu S/cm$ and $\lambda_{ex} = 520 \mu S/cm$ ($\Lambda = 0.25$). The data is fit to theoretical transition values with $C_{mm} = 0.71 \mu F/cm^2$ for POPC, $C_{mm} = 0.26 \mu F/cm^2$ for PS1, $C_m = 0.16 \mu F/cm^2$ for PS3, and $C_m = 0.065 \mu F/cm^2$ for PS4. The relative errors of all experimental systems are comparable. The axis scale obscures the error in systems PS1 and LS1.

are in the range of $0.065 - 0.71 \mu F/cm^2$, which are in agreement with data previously reported in the literature [18, 9].

The membrane capacitance is inversely proportional to the bilayer thickness, which in turn exhibits a power-law dependence on the molecular weight $d \sim M_w^b$, where the exponent b lies within the theoretical bounds of 0.5 (random Gaussian coil) and 1 (full stretch) [49, 117]. Hence, the membrane capacitance should also follow a power law $C_m \sim M_w^{-b}$ provided the membrane permittivity ϵ_m is the same. Figure 4.17 confirms this conclusion. The data show that C_m decreases with M_w complying with a power-law relationship with an exponent lying in the expected range, $0.5 < b < 1.0$. This is in agreement with the reported behavior of PBd-PEO membranes

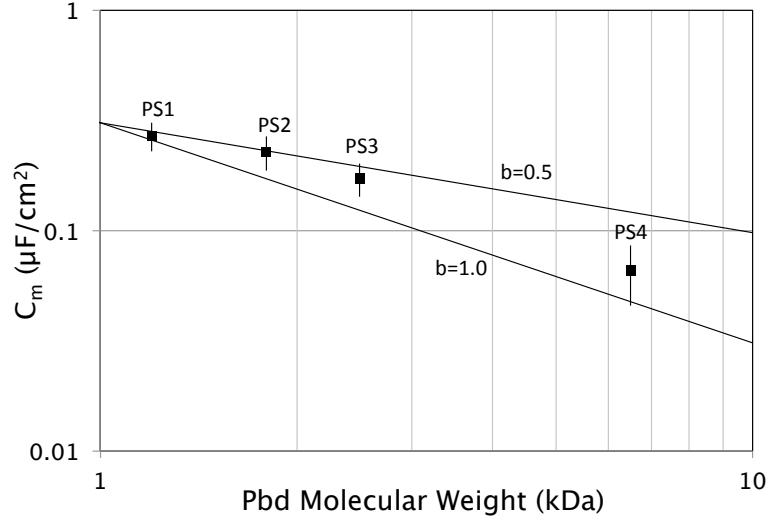


Figure 4.17: Membrane capacitance as a function of polymer molecular weight. Error bar indicate the standard deviation of capacitance values determined from a set of measurements. The theoretical capacitance is based on a thickness to molecular weight relationship $d \sim M_w^b$, where $0.5 < b < 1.0$ and the dielectric constant is assumed to be independent of molecular weight. The solid lines represent the power-law relationships for $b=0.5$ and $b=1.0$.

[122, 13]. Note that for PS4, the capacitance is measured at higher temperature, which likely influences the membrane structure and may lead to a deviation from the suggested power law.

4.4.2 Effect of salt concentration (solutions conductivity)

The charge near the membrane surfaces forms diffuse clouds. These ionic double layers have a capacitance C_D that acts in series with the bilayer capacitor [106] and increases the experimentally measured capacitance

$$\frac{1}{C_m} = \frac{1}{C_B} + \frac{1}{C_{D,\text{in}}} + \frac{1}{C_{D,\text{ex}}} \quad (4.77)$$

where C_B is the capacitance of the “bare” bilayer and the subscripts *in* and *ex* refer to inner and outer double layers at the vesicle membrane. Since the Debye time for diffusion across the double-layer thickness is of the order of nano-seconds, on the

time scale of the applied kHz AC field the electric double layers are quasi-steady and equilibrium. Assuming a linear capacitor model (appropriate for the voltages in our experiments), the double-layer capacitance is equal to the capacitance of a planar capacitor with a thickness of one Debye length κ_D and the dielectric constant of the solution [69, 172, 189, 14, 230].

Increasing the ionic strength of the solutions decreases the Debye length (for a 1:1 electrolyte $\kappa_D = 0.303c^{-1/2} \text{ nm}$, where c is the electrolyte concentration in M). Accordingly, the combined capacitance of the electrolyte-membrane system increases with ion concentration to an upper limit given by the bilayer capacitance C_B .

The effect of the diffuse layers is most significant when C_D and C_B are of comparable magnitude. This is true for low salt concentrations and/or large values of C_m . Hence, the measurements for POPC at low salt concentrations show more pronounced deviation between the effective (measured) and bilayer capacitance as seen in Figure 4.18. The measurements of PS1-PS4 polymer membranes showed minimal variation due to their lower capacitance, which is documented in Figure 4.19 and Table 4.2.

PBd _n -b-PEO _m	C_m ($\mu F/cm^2$)	C_B ($\mu F/cm^2$)	$error(SD)$
PS1	0.256	0.270	± 0.032
PS2	0.212	0.227	± 0.039
PS3	0.166	0.172	± 0.025
PS4	0.065	0.066	± 0.014

Table 4.2: Capacitance values for PBd_n-b-PEO_m measured with the electrodeformation method C_{mm} and corrected for the effect of the Debye layers. The uncertainty is determined using error propagation and weighted sample variance for uncertainties associated with conductivity measurements and transition frequency.

Bulk conductivities also influence the accuracy of the experimental data. Membrane charging depends on how fast charges are brought to the surface, which is quantified by the bulk conductivities. Hence, the transition frequency is sensitive to

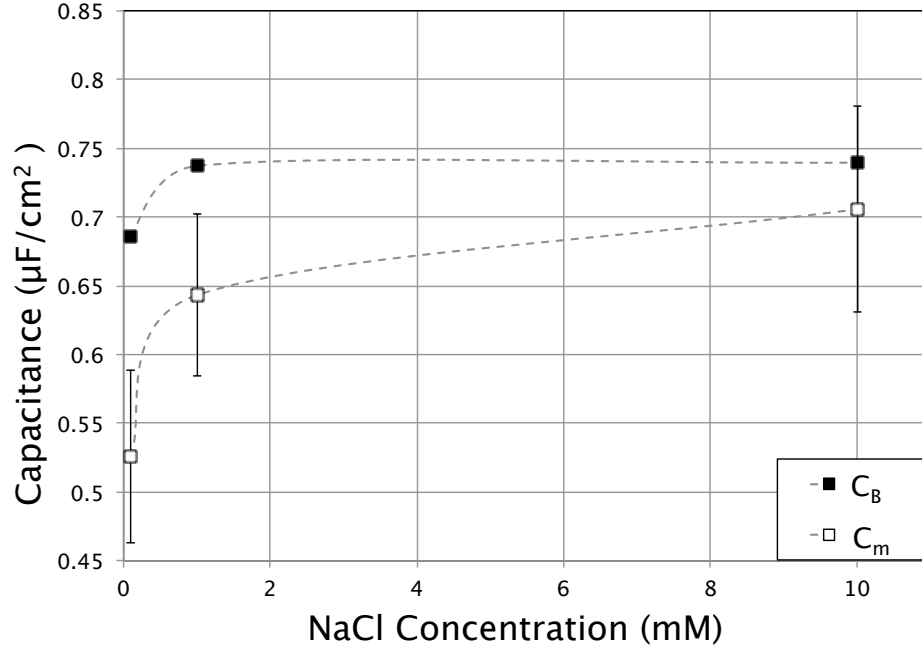


Figure 4.18: Total specific membrane capacitance at different interior solution conductivities (salt concentrations) for POPC vesicles (LS1). The bilayer capacitance is obtained by subtracting the contribution of the double layers according to Eq.[4.77]. The exterior conductivity was varied to produce a conductivity ratio within $R=0.1-0.3$. The lines are plotted to guide the eye. The error bars represent standard deviation.

errors in determining the conductivities. Evaporation, vesicle rupture and impurities during preparation introduce variability in bulk conductivities, in particular, the conductivity of the small volume of solution in which the vesicles are grown. As a result, λ_{in} may change significantly. In order to determine the error in the interior conductivity, vesicles were diluted into a range of exterior solutions close to $R \sim 1$. The samples are then subjected to an AC electric field in the intermediate frequency range, 300kHz, where the vesicle deformation should be oblate for $R < 1$ and prolate for $R > 1$. The $R = 1$ ($\lambda_{ex} = \lambda_{in}$) delineates the samples with prolate and oblate shapes. In this way, knowing λ_{ex} , the post preparation λ_{in} can be estimated. This method was used for each individual batch of vesicles. We found that the interior bulk conductivities could change by as much as 20-30% compared to the original solution in which they were grown. This explains the scatter in the data seen in Figure 4.19.

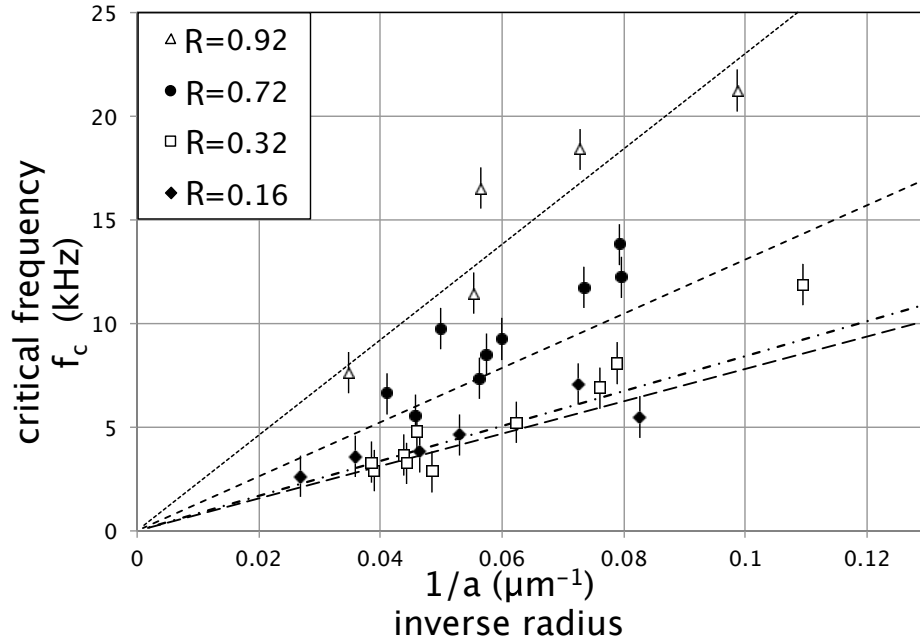


Figure 4.19: Transition frequency for PS2 polymersomes with interior conductivity $\lambda_{in} = 18 \mu S/cm$ in external solutions of different conductivity. All data are fit with $C_m = 0.22 \mu F/cm^2$ using Eq.[4.76].

4.4.3 Effect of field strength

The capacitance of BLMs (with solvent) increases quadratically with the applied voltage [18, 4, 174, 221], which has been attributed to membrane thinning associated with solvent exclusion. Enhanced fluctuations are also speculated to contribute to this effect [109]. For a spherical vesicle, the membrane electrostriction depends on the polar angle; it is maximal at the poles where the potential is largest. Therefore, the capacitance dependence on field strength is expected to be more complex.

The measurements with lipid vesicles did not detect voltage dependence of the prolate-oblate transition frequency. It is hypothesized that the polymer membranes could be more compressible due to the longer polymer chain length. However, Figure 4.20 shows that although polymersome deformation increases with field strength, the frequency for the prolate-oblate transition does not change. This implies that the

membrane thickness and capacitance are voltage-independent (in the investigated range of field strengths) in agreement with the reported behavior of solvent-free BLMs. An estimated 7% maximum areal strains shows that the polymersome is

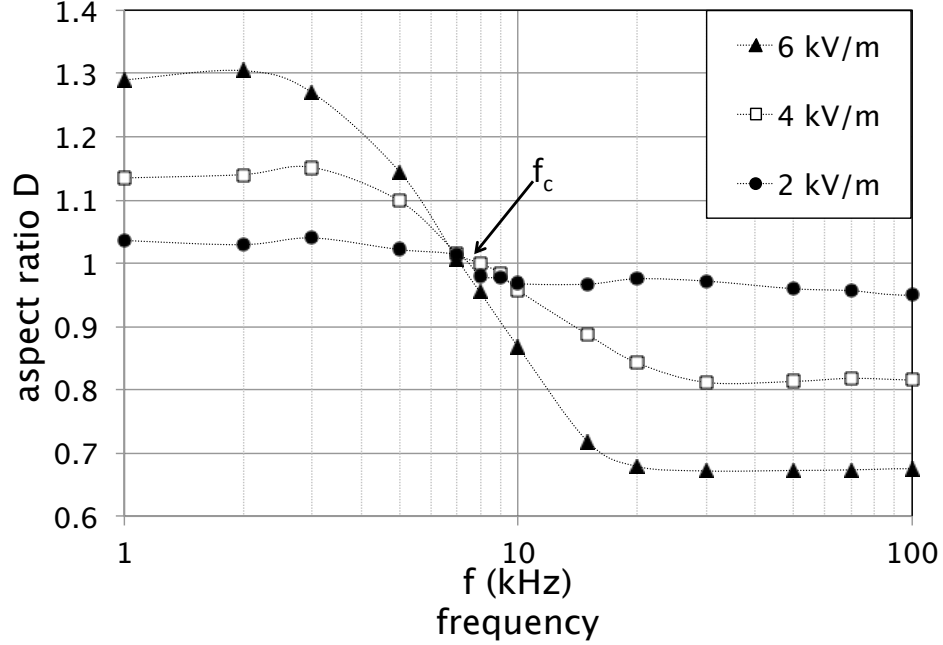


Figure 4.20: Shape elongation D variation with frequency at different field strengths $E_0 = 6, 4, 2 \text{ kV/m}$ for a $18 \text{ }\mu\text{m}$ polymersome (PS1) and conductivity conditions $\lambda_{\text{in}} = 20 \mu\text{S/cm}$ and $\lambda_{\text{ex}} = 40 \mu\text{S/cm}$. The transition between prolate and oblate ellipsoids occurs between 7-8 kHz for all field strengths, where $D=1$ corresponds to a spherical shape. There is no observable field strength dependence for the transition frequency (i.e. all transitions occur at the same approximate value). The greatest deformation observed, aspect ratio $D = 1.3$, corresponds to an areal strain $\Delta A/A = 7\%$.

indeed not in the stretching regime [48], and hence the membrane is not thinning due to stretching. Stronger fields are required in order to attain greater areal strain. However, in this case we discovered that polymersomes start to rotate.

Figure 4.20 shows the transition from prolate to oblate in more detail. Although vesicle deformation increases with field strength, the frequency for the prolate-oblate transition does not change. This implies that the membrane thickness and capacitance are voltage-independent in agreement with the reported behavior of solvent-free LBMs. The transition for polymer membranes also appears to be

voltage-independent. For this system, since the experiments require higher fields strengths compared to lipid vesicle, measurements are more difficult and the experimental error increases due to pearl-chaining and electrorotation.

The latter behavior, illustrated in Figure 4.21, is a novel, thus-far unreported feature of bilayer vesicles. In moderately strong fields ($E_0 \sim 5\text{kV/m}$), the oblatelly deformed polymersomes orient obliquely with respect to the field direction and the membrane rotates continuously. This dynamic is reminiscent of the rotation of fluid drops in a uniform electric field [168], which is the analog of the Quincke rotation of rigid dielectric spheres or shells [91, 203]. The tilted orientation makes the vesicle appear more prolate, which introduces error in determining the prolate-oblate transition. In stronger fields ($E_0 > 10\text{kV/m}$) the effect could be even more dramatic with the entire polymersome tumbling. The mechanism for rotation is currently unknown and possible directions for further study are outlined in the discussion section.

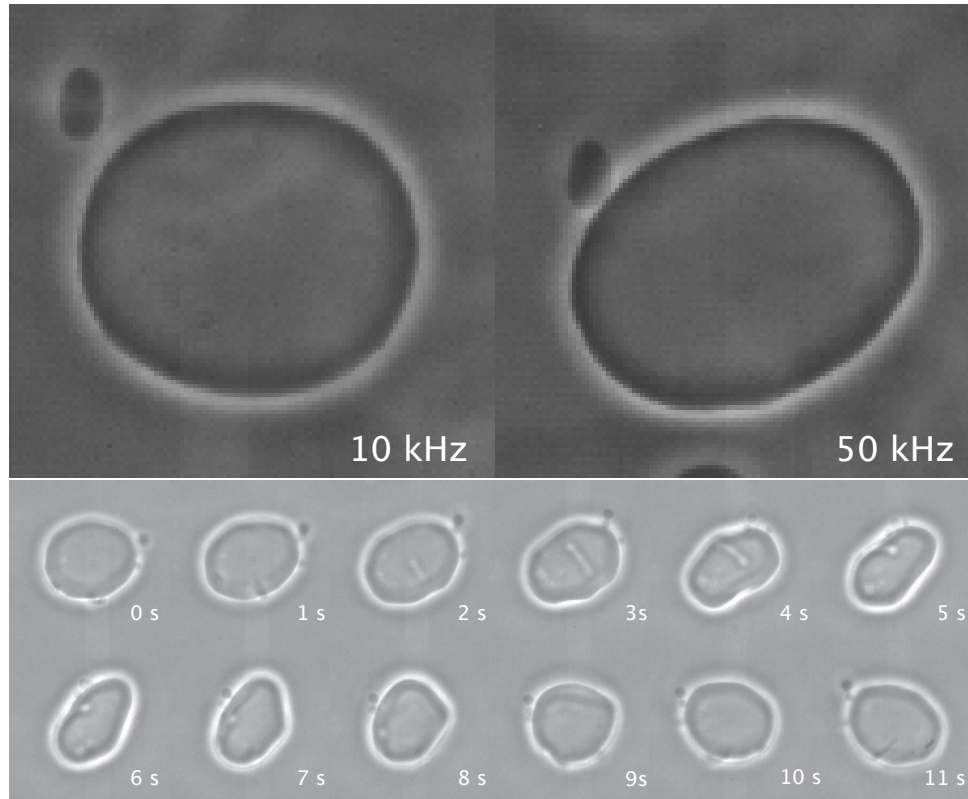


Figure 4.21: (A) $E_0=5$ kV/m : upon increase of frequency (from 10 to 50 kHz) a polymersome evolves towards a steady tilted orientation with respect to the applied field radius (B) $E_0=20$ kV/m frequency 100 kHz: Tumbling polymersome, $a=15\mu\text{m}$.

4.4.4 Time dependent deformation

As discussed in Section 4.2.9, the time dependent deformation is sensitive to membrane viscosity while the steady state shape is independent of viscous properties. The viscosity of lipid membranes can vary with membrane composition [51]. The viscosity of polymer membranes can also vary significantly with the length of the polymer chains. Using the theory outlined in Section 4.2.9, the influence of system parameters on time dependent deformation is investigated.

First, a single vesicle is exposed to an AC field of different strengths, each for 30-60 seconds. The field is then turned off and the vesicle is allowed to relax back to a sphere. The experiment for a PS2 vesicle is shown in Figure 4.22. The vesicle has no salt in either the interior or exterior fluids. The conductivity of the suspending glucose, $\lambda_{\text{in}} \sim 3\mu\text{S}/\text{cm}$ is slightly lower than the enclosed sucrose solution, $\lambda_{\text{ex}} \sim 5\mu\text{S}/\text{cm}$. This gives a conductivity ratio of $R = 1.7$ and prolate deformation at all frequencies. The vesicle is exposed to a 5 kV/m at 100 kHz field for about 30 seconds. After the field is turned off, the relaxation in shape back to a sphere is recorded. The experiment is immediately repeated at 10kV/m at 100 kHz. A plot of the entire shape evolution with time normalized by t_{hd} is shown as well as a plot of the relaxation with time normalized by t_{σ} .

The shape evolution is fitted with Eq.[4.62] using the same parameters given in Figure 4.22 for different field strengths. The value for membrane capacitance for PS2 is taken from Table 4.2. If the vesicle has no defects and does not porate, variables such as initial tension, radius, and solution conductivity will not change. The fit shown in Figure 4.22 shows good agreement between theory and experiment. Due to the difference in field strength, the maximum deformation differs when the field is on.

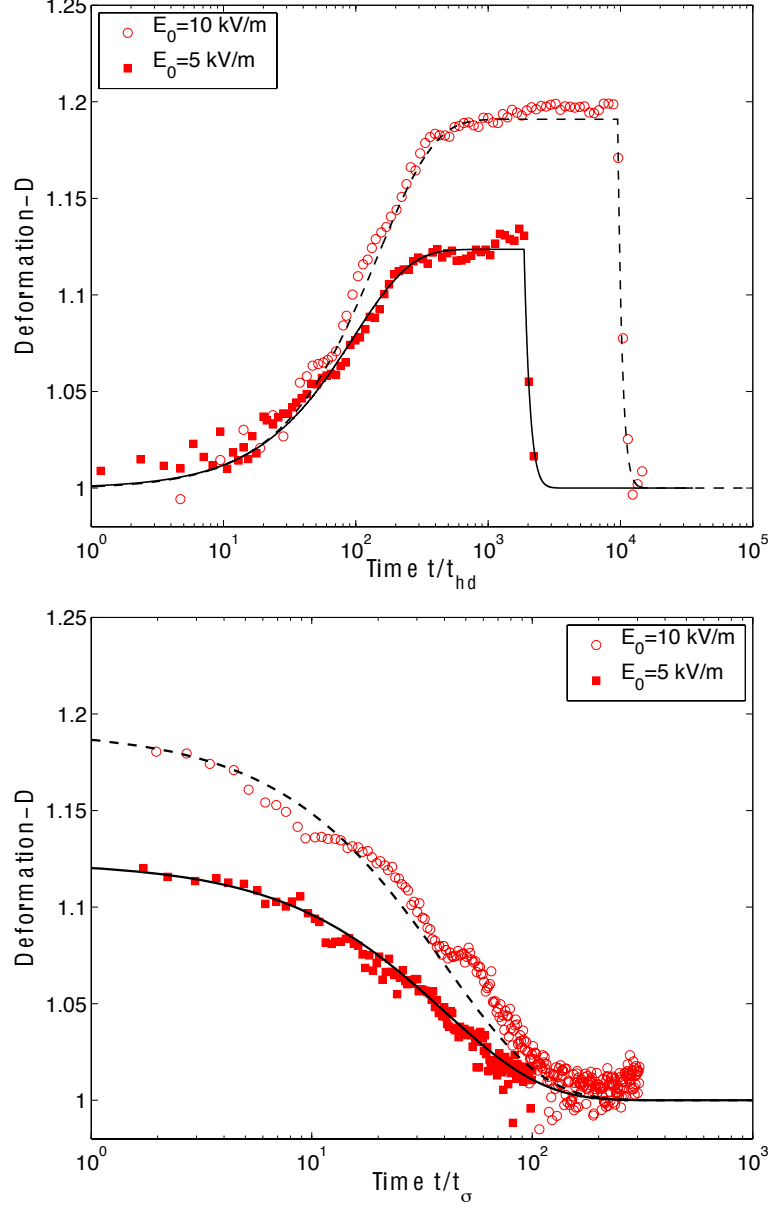


Figure 4.22: Time dependent deformation of a polymer (PS2) vesicle at different field strengths. A 100 kHz electric field is turned on at $t = 0$ s and turned off at approximately $t = 50$ s. The entire experiment is shown with time normalized by t_{hd} (top) and the relaxation is shown normalized by t_{σ} (bottom). The experiment is repeated on the same vesicle for electric field strengths $E_0 = 5$ kV/m (solid line), $E_0 = 10$ kV/m (dashed line). The vesicle size is measured to $a = 20.3 \mu\text{m}$, $\lambda_{in} = 5 \mu\text{S/cm}$, $\lambda_{ex} = 3 \mu\text{S/cm}$ ($R = 1.6$). Parameters for the fit are $\sigma_{eq} = 3 \times 10^{-7} \text{N/m}$, $C_m = 0.22 \mu\text{F/cm}^2$, $\kappa = 12 k_B T$, $\mu_{mm} = 120 \times 10^{-8} \text{Ns/m}$.

Despite the different maximum deformation, the relaxation occurs on approximately the same timescale. This can be explained by considering Eq.[4.70] and Eq.[4.62]. When there is no applied forcing, the excess area is stored in fluctuations, $\sigma_h = \sigma_{eq}$,

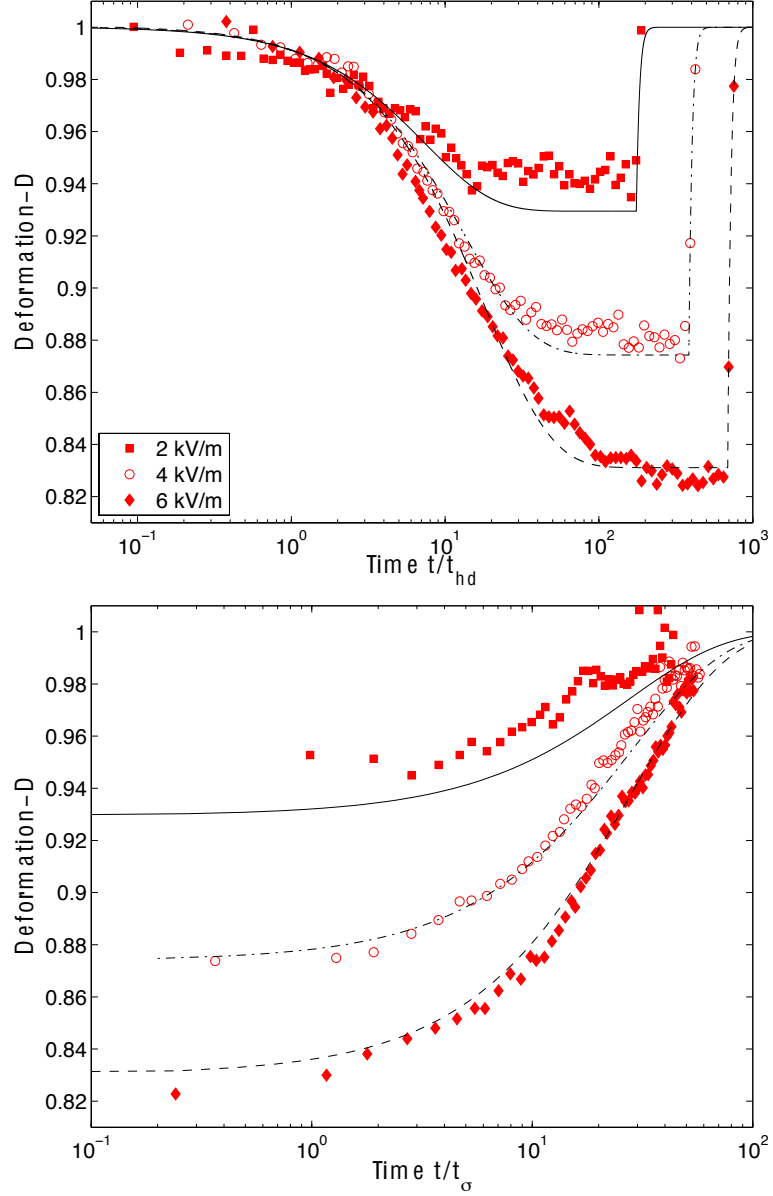


Figure 4.23: Time dependent deformation of a polymer (PS2) vesicle at different field strengths. A 100 kHz electric field is turned on at $t = 0s$ and turned off at approximately $t = 50s$. The entire experiment is shown with time normalized by t_{hd} (top) and the relaxation is shown normalized by t_{σ} (bottom). The experiment is repeated on the same vesicle for electric field strengths $E_0 = 4kV/m$ (solid line), $E_0 = 6kV/m$ (dash-dot line), $E_0 = 8kV/m$ (dashed line). The vesicle size is measured to $a = 27\mu m$, and the conductivities are $\lambda_{in} = 7\mu S/cm$, $\lambda_{ex} = 28\mu S/cm$ ($R = 0.25$). Parameters for the fit are $\sigma_{eq} = 4 \times 10^{-7} N/m$, $C_m = 0.22\mu F/cm^2$, $\kappa = 4k_B T$, $\mu_{mm} = 100 \times 10^{-8} Ns/m$.

and the evolution of vesicle is governed by the relaxation term in Eq.[4.62]. The accuracy of the fit for the relaxation shows that the isotropic tension term, defined by Eq.[4.70], is accurate and initial tension drives relaxation.

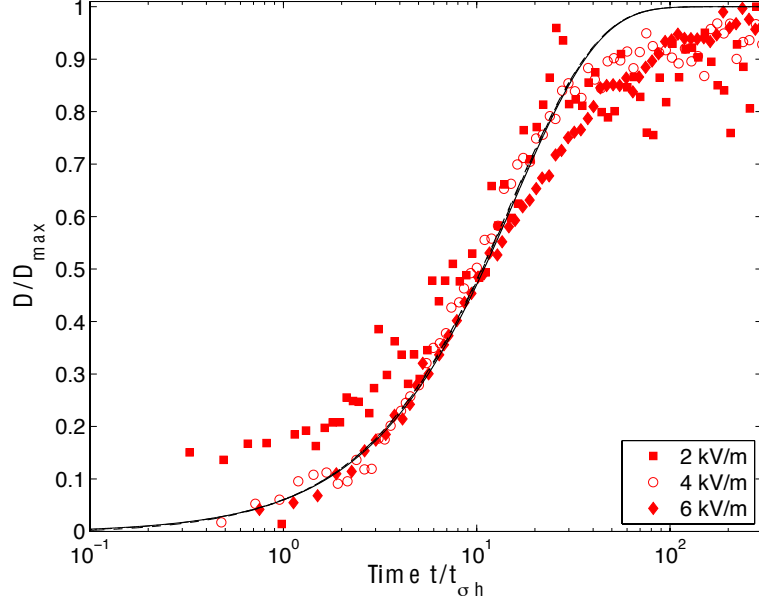


Figure 4.24: The time dependent deformation of a polymer (PS2) from Figure 4.23. Time is normalized by $t_{\sigma h} = a\mu(1 + \Lambda)/\sigma_h$. The magnitude of deformation is normalized by $D_{\max}$.

The deformation of a different PS2 vesicle with $R < 1$ is shown in Figure 4.23. The exterior conductivity is increased to $\lambda_{\text{ex}} = 28\mu\text{S}/\text{cm}$ for these experiments. The deformation of the vesicle is observed at three different field strengths, $E_0 = 4, 6, 8\text{ kV/m}$. The shape evolution is fit with the same parameters for each field strength, but the parameters differ slightly from Figure 4.22 because a different vesicle is used. The vesicle behaves as predicted by the theory, deformation increases with field strength and the rate of deformation scales with t_{hd} , while the relaxation scales with t_{σ} . When σ_h is used as the tension in t_{σ} and the deformation normalized by the maximum deformation, $D_{\max}$, the data collapse onto one curve, see Figure 4.24.

To demonstrate the effect of membrane tension on vesicle dynamics, two vesicles with different equilibrium tensions are exposed to a $E_0 = 10\text{ kV/m}$ 100 kHz field for 50-100 seconds, see Figure 4.25. The same experiment is repeated for a POPC vesicle with $R < 1$ and at a weaker field strength, see Figure 4.26. The equilibrium

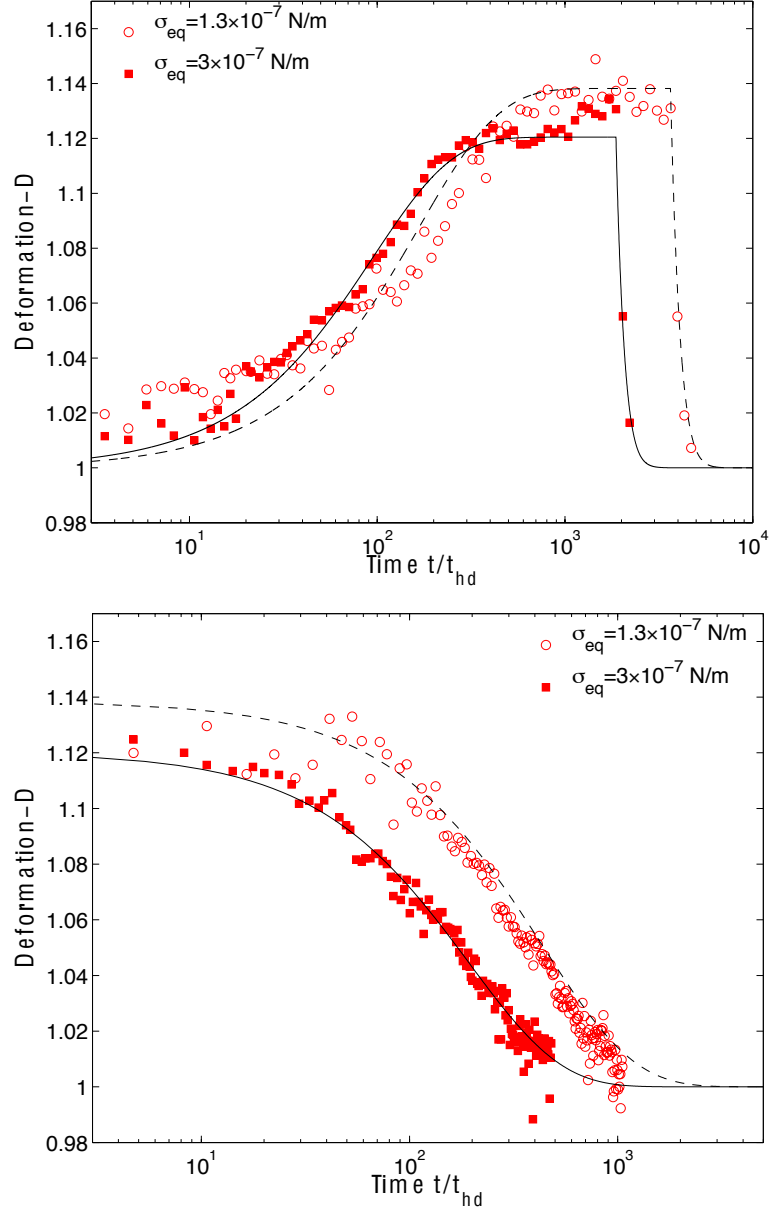


Figure 4.25: Time dependent deformation of a polymer (PS2) vesicle with different initial tensions. A 100 kHz 10 kV/m electric field is turned on at $t = 0s$ and turned off at approximately $t = 50s$. The entire experiment is shown with time normalized by t_{hd} . In this case the field strengths are equal and t_{hd} is identical for both experiments. The vesicles are fit with different initial tension $\sigma_{eq} = 4 \times 10^{-7} \text{N/m}$ (solid line), $\sigma_{eq} = 1.3 \times 10^{-7} \text{N/m}$ (dash-dot line). The tense vesicle size is measured to $a = 20.3 \mu\text{m}$ and the less tense is $a = 12.8 \mu\text{m}$. The conductivity for both vesicles are $\lambda_{in} = 5 \mu\text{S/cm}$, $\lambda_{ex} = 3 \mu\text{S/cm}$ ($R = 1.6$). Parameters for both vesicles are fit with $C_m = 0.22 \mu\text{F/cm}^2$, $\kappa = 13 k_B T$, $\mu_{mm} = 120 \times 10^{-8} \text{Ns/m}$.

tension does not significantly alter the rate of deformation as the vesicle transitions to a steady state. The maximum deformation is larger for the vesicle with lower equilibrium tension; the vesicle stores more excess area and can deform further. The

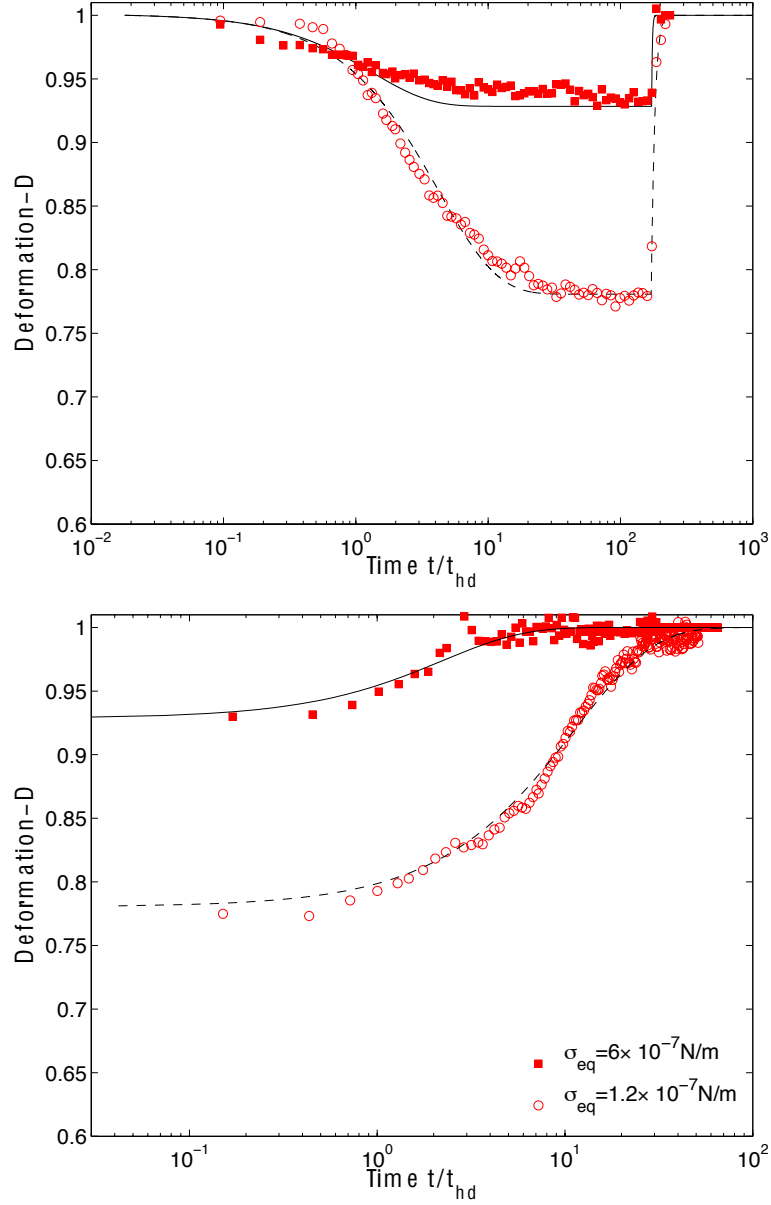


Figure 4.26: Time dependent deformation of a POPC vesicle with different initial tensions. A 100 kHz $4kV/m$ electric field is turned on at $t = 0s$ and turned off at approximately $t = 30s$. The entire experiment is shown with time normalized by t_{hd} (left) and the relaxation is shown normalized by t_{σ} (right). The vesicles are fit with different initial tension $\sigma_{eq} = 6 \times 10^{-7} N/m$ (solid line), $\sigma_{eq} = 1.2 \times 10^{-7} N/m$ (dash-dot line). The tense vesicle size is measured to $a = 20.3\mu m$ and the less tense is $a = 12.8\mu m$. The conductivity for both vesicles are $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 28\mu S/cm$ ($R = 0.18$). Parameters for both vesicles are fit with $C_m = 0.71\mu F/cm^2$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$. The bending rigidity is set at $\kappa = 20k_B T$ for the tense vesicle, and $\kappa = 3.5k_B T$ for the low tension vesicle.

difference in tension is most noticeable in the relaxation dynamics. Even a vesicle with a slightly lower tension will take longer to relax back to a sphere. In these

experiments, the relaxation is scaled by the t_{hd} timescale, which is identical for both vesicles because the field strength is the same. This scaling allows the difference in relaxation times to be apparent. If the relaxation is rescaled by the surface tension timescale for each vesicle, the data collapse and show similar relaxation behavior.

The fits in Figure 4.22 and 4.23 give an estimate for membrane viscosity. The value for membrane viscosity for PS2 membranes is relatively high, $\mu_{\text{mm}} = 100 - 120 \times 10^{-8} \text{Ns/m}$, in comparison to lipid vesicles, where $\mu_{\text{mm}} \sim 0.1 - 10 \times 10^{-8} \text{Ns/m}$, i.e. up to 1000 times more viscous than lipid membranes. The value for polymer membrane viscosity also agrees with previous measurements of $\mu_{\text{mm}} \sim 10^{-6} \text{Ns/m}$, which were made by relating the velocity of a sedimenting particle within the interface to membrane viscosity [48].

The deformation for three types of membranes, DOPC and POPC lipid membranes and PS2 polymer, are compared in Figure 4.27. The vesicles are exposed to the same field strength and allowed to reach equilibrium. Each experiment is scaled by the same EHD timescale, which is equal for these experiments because the field strength is identical. The polymer vesicle deforms at a slower rate than the lipid vesicles due to its higher membrane viscosity. The initial tension and size of the vesicles are similar, which results in a similar equilibrium shape. The effect of membrane viscosity is also observable by a slower relaxation. The accuracy of the fit is improved by considering both the evolution to a steady state shape and relaxation back to a sphere.

The values used for lipid membrane viscosity are within the range of values determined in previous experiments [51]. DOPC is representative of the membrane viscosity for lipid systems, which is found to be $\mu_{\text{mm}} = 1 \times 10^{-8} \text{Ns/m}$. POPC is a more viscous lipid membrane and the viscosity is found to be $\mu_{\text{mm}} = 10 \times 10^{-8} \text{Ns/m}$.

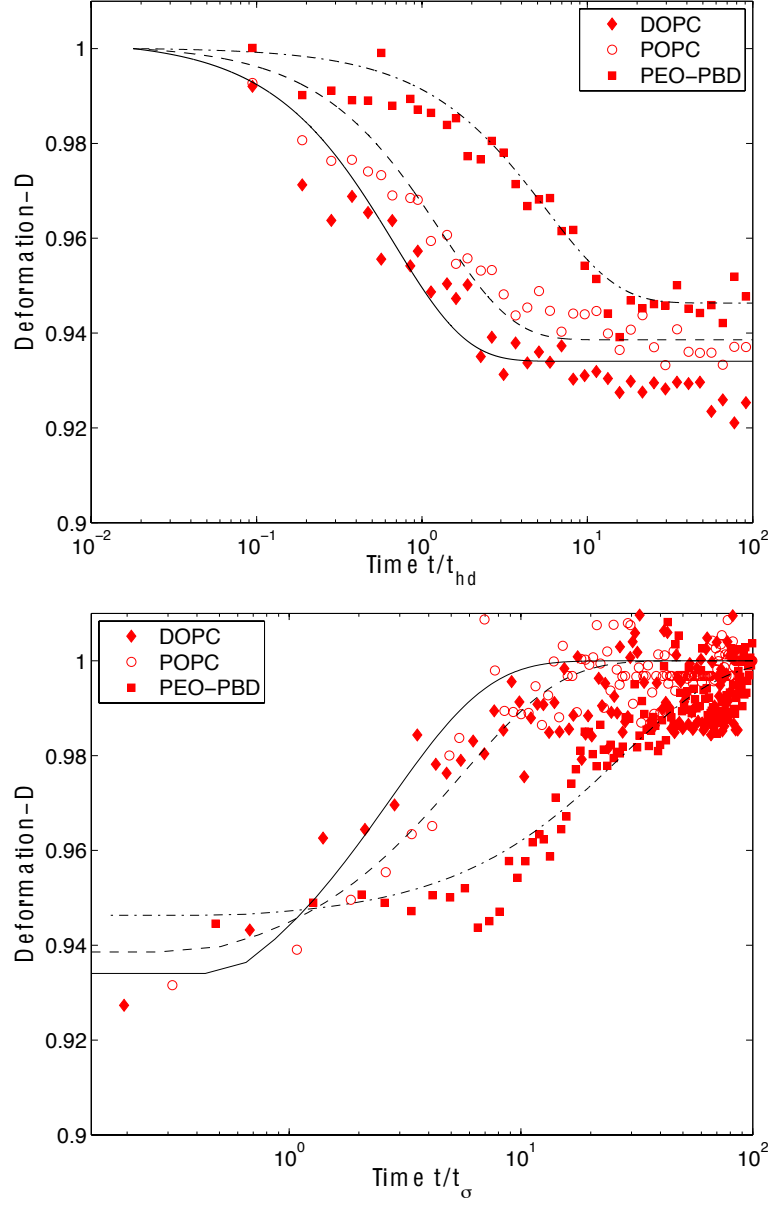


Figure 4.27: Time dependent deformation of DOPC, POPC, and PS2 vesicles. A 100 kHz $4kV/m$ electric field is turned on at $t = 0s$ and turned off at approximately $t = 30s$. The entire experiment is shown with time normalized by t_{hd} (left) and the relaxation is shown normalized by t_{σ} (right). The vesicles are fit with different membrane viscosities, the PS2 vesicle is fit with $\mu_{mm} = 100 \times 10^{-8}Ns/m$ (solid line), POPC with $\mu_{mm} = 10 \times 10^{-8}Ns/m$ (dashed line), and DOPC with $\mu_{mm} = 1 \times 10^{-8}Ns/m$ (dash-dot line). The PS2 is measured to $a = 27.0\mu m$, POPC is $a = 21.2\mu m$, and DOPC is $a = 19\mu m$. The conductivity for both vesicles are $\lambda_{in} = 7\mu S/cm$, $\lambda_{ex} = 28\mu S/cm$ ($R = 0.25$). Parameters for PS2 are fit with $C_m = 0.22\mu F/cm^2$, $\sigma_{eq} = 8.5 \times 10^{-7}N/m$, $\kappa = 40k_B T$. Parameters for POPC are fit with $C_m = 0.71\mu F/cm^2$, $\sigma_{eq} = 6.5 \times 10^{-7}N/m$, $\kappa = 24k_B T$. Parameters for DOPC are fit with $C_m = 0.71\mu F/cm^2$, $\sigma_{eq} = 5.5 \times 10^{-7}N/m$, $\kappa = 20k_B T$.

This method may not provide highly accurate values for low viscosity membranes, where viscous dissipation from the membrane is small. Relatively large differences in membrane viscosity, such as that seen between DOPC and POPC membranes is observable, but small changes in membrane viscosity may not result in a significant change in shape evolution. The method is best suited for measuring membranes with high viscosity, i.e. $\Lambda_s > \Lambda$, and the dissipation is primarily into the membrane. The current method also has the advantage of being non-invasive in that it does not require physical contact with the vesicle or insertion of probes into the membrane.

Low values of bending rigidity are used for fits in Figures 4.22-4.26 where the vesicles are either large deformations or low tension. As discussed in Section 5.9, these low values for bending rigidity are likely due to a surplus of excess area that is stored in a systematic deformation while resting on the cover glass.

Higher tension vesicles, shown in Figure 4.27, have more accurate values for bending rigidity, $\kappa \sim 20 - 40k_B T$. The higher tension vesicles were used for the comparison of membrane viscosity in order to reduce any errors arising from fits with low bending rigidities. Although the values for membrane viscosity are found to be identical for data from Figures 4.22-4.26. This is because the bending rigidity alters the amount of available excess area, which affects the maximum deformation but not the rate of deformation, see Figure 4.9. This suggests that electrodeformation is better suited for determining dynamic membrane properties such as membrane viscosity than bending rigidity.

4.5 Conclusions

This chapter demonstrates how AC electric fields can be used to determine the membrane properties of vesicles. Frequency-dependent vesicle deformation can serve as a facile method to determine the specific capacitance of bilayer membranes. The time dependent shape evolution is employed to estimate membrane viscosity.

The linear dependence of the transition frequency on inverse vesicle radius can be fit using Eq.[4.76] with the membrane capacitance as an adjustable parameter. Bilayers made of lipids have higher capacitance, $C_{\text{mm}} = 0.71\mu\text{F}/m^2$ for POPC, than block-copolymers, e.g., $C_{\text{mm}} = 0.26\mu\text{F}/m^2$ for PBd₂₁-b-PEO₁₄, which correlates with polymer's thicker membrane. The capacitance shows a power law dependence on polymer molecular weight. The membrane capacitance was found to increase with bulk salt concentration due to the thinning of the ion double layer adjacent to the membrane surfaces. The electric field strength does not affect the capacitance, indicating that membranes do not experience significant thinning due to electrostriction. This method can be applied to vesicles prepared from lipid extracts of the plasma membrane of cells, e.g., human red blood cells, to investigate the influence of naturally present transmembrane proteins. Knowledge about the electrical properties of model membranes with more complex composition as well as those of biological membranes is essential for correctly evaluating the mechanisms and conditions of electro-poration and -manipulation applied in technologies such as gene transfer and drug delivery.

The response of vesicles to an applied electric field can be used to determine values of membrane viscosity. The importance of membrane viscosity for polymer vesicles in electric fields indicates membrane viscosity is also likely important in flow. The

potential application for polymer vesicles in drug delivery applications, where the vesicles may be subjected to many applied stresses during processing and treatment, motivates the measurement of parameters affecting their dynamic response under an applied stress.

4.6 Future Directions

Measurement of polymer membrane viscosity at different temperatures.

The membrane viscosity measurement method could be used to observe the change in membrane viscosity of polymer vesicles with temperature. The measurements in this chapter were performed at 20 C. In context of drug delivery applications, the measurement of membrane viscosity at physiological temperatures would be more applicable. A temperature control setup for the electrodeformation chamber can be used to adjust temperature and the time dependent deformation experiments repeated.

Rotation of polymersomes

The mechanism for the rotation of polymer vesicles at intermediate frequencies is an open question. The rotation occurs for deflated vesicles with conductivity ratio less than one ($R < 1$). The effect of Quincke rotation on spherical shells has been shown to decrease the critical field for rotation [202]. However, this theory predicts that thinner shells will result in a lower critical field. This would suggest that thinner lipid membranes would rotate before polymer vesicles. An estimate for the critical field based on the spherical shell model estimates a critical field on the order of $E_0 \sim 1$ kV/cm for aqueous systems, much higher than the field strengths where

rotation is observed.

Another possibility is that rotation is caused by an instability related to membrane mechanics. A perturbation to the membrane may result in symmetry breaking caused by the compressive force from the electric field. The perturbation may relax slowly enough to rotate the polymer vesicle. A dimple in the membrane can be seen in Figure 4.21b that relaxes as the vesicle rotates. The instability may also occur in extensional flow and in different types of membranes. A sphingomyelin:cholesterol vesicle, which has the thickness of lipid membranes but has a higher membrane viscosity, was also observed to rotate but at a higher field strength. Further experimental and theoretical investigation would be needed to test this hypothesis.

Instabilities produced by stretching

Experiments investigating the behavior of vesicles in elongational flow have shown a stretching transition to a dumbbell shape for very deflated vesicles [93]. Low frequency electric fields, and intermediated frequencies for vesicles with $R > 1$, produce electric stresses similar to elongation flow. Preliminary experiments on very deflated POPC vesicles in 100 Hz AC fields demonstrates a similar transition, see Figure 4.28.

The vesicle separates into two daughter vesicles connected by a lipid tether. The instability was observed to be asymmetric, with one daughter vesicle noticeably larger than the other. After the instability, the two vesicles begin to equalize and the tether becomes smaller as area is pulled into the vesicle. It was observed that in some vesicles, the two daughter vesicles rejoined and the instability repeated. Other vesicles stayed separate as two equal sized daughter vesicles. Although the instability

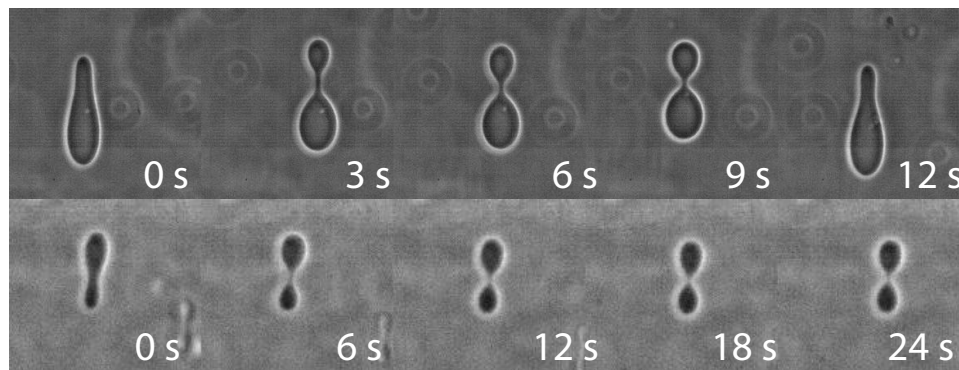


Figure 4.28: Transition to a two-dumbbell shape for a deflated vesicle in a 100 Hz 20 kV/m electric field. The vesicles transitions between a connected vesicle and a dumbbell shaped vesicle (top). Other vesicles remain in the two-dumbbell shape with two equally sized daughter vesicles (bottom).

that forms the two vesicles may have an asymmetric character, the two vesicles may need to vary their size to equilibrate the Laplace pressure between the two vesicles. A more detailed study of the behavior of this instability for different values of excess area and applied stress would give more insight into when this instability can occur.

Measurement of capacitance and conductance on real cells or vesicles with transmembrane proteins

The presence of transmembrane proteins may lead to a current across the membrane, which will affect the transition frequency in an AC field. The rate of transmembrane current will vary with the type of membrane protein and concentration. Including transmembrane proteins in vesicles would replicate the behavior of real cells in electric fields and would provide better control over the type and concentration of membrane proteins compared to real cells.

CHAPTER FIVE

Vesicle dynamics in DC electric pulses

5.1 Introduction

Living cells depend on electrochemical potentials across membranes for many crucial cell functions. Changes in the transmembrane electric field are necessary for such processes as signal propagation in neurons, directed cell migration, and ciliary motility in cell swimming [36, 15, 123, 124, 118, 24]. Electric fields are also used in engineering applications such as the electroporation of cells, allowing for the transport of molecules across a cell membrane, which is relevant for applications including drug delivery and gene therapy [140, 43].

During electroporation, a pore is formed in a cell membrane in response to the stress created by a pulsed DC electric field [161]. An electric pulse with appropriate strength and duration will create a stable pore in the membrane, allowing material to cross the membrane. Ideally, the pore will reseal after the pulse ends and the cell will remain intact. Giant unilamellar vesicles offer a convenient model system to

study electroporation because of their similarity to real cell membranes and large size (10-100 μm). The GUV membranes used in this chapter are assembled from lipids or amphiphilic block copolymers. Key observations about the membrane dynamics in a pulsed DC electric field can be made using phase contrast microscopy and recorded with a high speed camera [163, 161, 162].

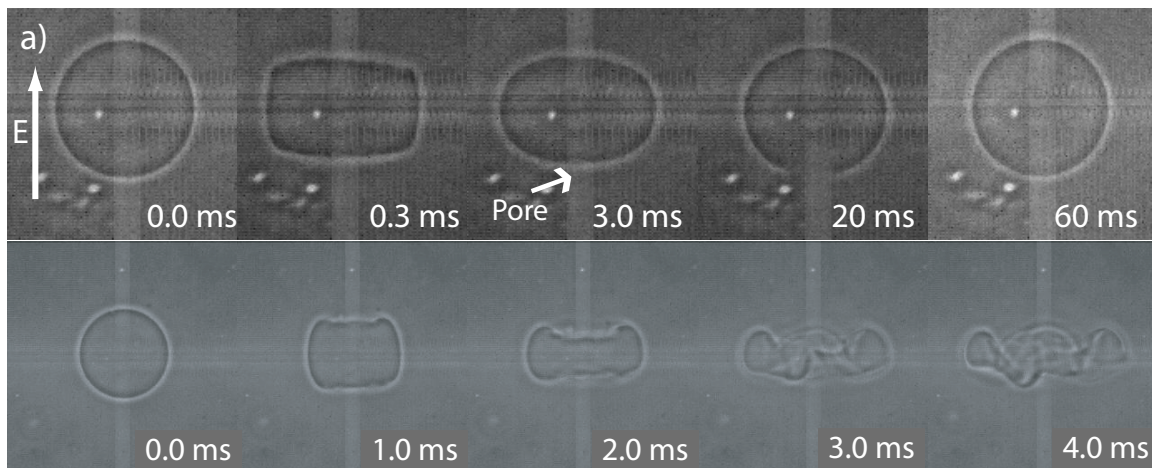


Figure 5.1: Examples of vesicle behavior: poration in a short strong DC pulse and collapse in a long weaker pulse. a) DOPC vesicle subjected to a $E_0 = 2kV/cm$, $300 \mu s$ pulse. A pore persists after the pulse and is visible due to the loss of contrast at the interface. The pore is fully resealed after approximately $60 ms$. b) DOPC vesicle subjected to a $E_0 = 1kV/cm$, $50 ms$ pulse. Although weaker, the longer pulse leads to collapse.

Previous experiments on vesicle dynamics in DC fields, conducted in Risk and Dimova (2005) and Riske and Dimova (2006), were limited to short pulses ($t_{pulse} < 300 \mu s$) [161, 162]. A question arising from this earlier work is: how do vesicles respond to long DC pulses ($t_{pulse} \sim 1 - 10 ms$)? To investigate this question, a set of experiments was conducted to observe vesicle behavior while subjected to pulses of various strengths and durations. The results showed that the response changed significantly between short and long pulses, see Figure 5.1. Specifically, short pulses produce transient pores that persist after the pulse has ended, but eventually reseal. Longer pulses result in membrane collapse during the pulse. The guiding hypothesis is that the difference in observed behavior can be attributed to the capacitive nature of the bilayer membrane.

This chapter focuses on the dynamics of vesicles in weak fields where poration does not occur in order to understand fundamental aspects of the transient response to electric fields. The electromechanical limits of bilayer membranes are discussed to establish an electric field strength limit below which the membrane remains intact. The theoretical model developed in Chapter 4 is adapted to describe the dynamics under applied DC pulses. New features are presented relating to the charging and discharging of the membrane capacitor. The complex dynamics seen in strong fields is left for further investigation in Chapter 6.

5.2 Physical system

As in Chapter 4, we consider an insulating charge-free lipid bilayer membrane with dielectric constant, ϵ_{mm} , and thickness, d . The bilayer is a spherical shell (vesicle) which separates two bulk fluids, each with their own conductivities, λ , viscosities, μ , and dielectric constants, ϵ . To characterize the mismatch in the fluid physical properties, the following ratios are introduced,

$$R = \frac{\sigma_{\text{in}}}{\sigma_{\text{ex}}}, \quad S = \frac{\epsilon_{\text{in}}}{\epsilon_{\text{ex}}}, \quad \Lambda = \frac{\mu_{\text{in}}}{\mu_{\text{ex}}}, \quad \Lambda_s = \frac{\mu_{\text{mm}}}{a\mu_{\text{ex}}}. \quad (5.1)$$

When an electric field is applied across an interface, charge is conducted from the bulk at a rate given by the characteristic charging timescale of the fluid, $t_{c,i} = \epsilon_i/\lambda_i$. Free charges will accumulate on both sides of the membrane and the membrane will act as a capacitor [207]. For a spherical vesicle in aqueous media, the membrane will

charge with the characteristic timescale for a spherical shell capacitor [69, 178, 97],

$$t_{\text{mm}} = \frac{aC_m}{\lambda_{\text{in}}} \left(1 + \frac{R}{2}\right), \quad (5.2)$$

where $C_m = \epsilon_{\text{mm}}/d$ is the capacitance of the membrane and a is the vesicle radius. The finite charging time of a vesicle is controlled by t_{mm} , and as a result controls the electric stress acting on the vesicle during charging. For systems with solution conductivity $\lambda \sim 0.1\mu\text{S}/\text{cm}$ and $a \sim 10\mu\text{m}$ the charging timescale is $t_{\text{mm}} \sim 1\text{ms}$.

The transmembrane potential is regulated by the capacitor charging timescale. The expression for the transmembrane potential in an electric field, $\mathbf{E}^\infty = E_0\hat{\mathbf{z}}$, is given by [177],

$$V_m = \frac{3}{2} (1 - \exp(-t/t_{\text{mm}})) a E_0 \cos(\theta). \quad (5.3)$$

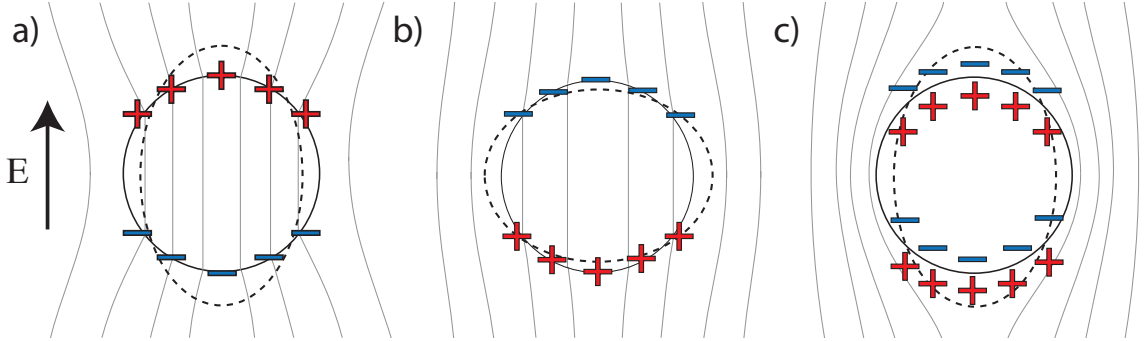


Figure 5.2: The steady electric field around a vesicle immersed in an electrolyte solution, following the imposition of a background uniform DC field (a) $t < t_{\text{mm}}$ $R > 1$ (b) $t < t_{\text{mm}}$ $R < 1$ (c) $t > t_{\text{mm}}$. Over a charging time t_{mm} Eq.[5.2] dipolar charge distribution forms in response to currents from the bulk.

Due to differences in the solution conductivities, ions accumulate at different rates at the membrane's physical surfaces. This gives rise to an imbalance in the charge densities on the inner and outer surface, which in the zero-thickness limit appears as induced surface charge, similar to simple fluid-fluid interfaces [125, 172]. The effective charge on the interface is dominated by the fluid with the faster charging timescale. If $R > 1$ ($t \ll t_{\text{mm}}$), the conduction in the vesicle interior is faster than

the suspending liquid. In this case, shown in Figure 5.2a, charges at the poles are attracted by the electrodes, pulling the vesicle into a prolate shape. In the opposite case, $R < 1$ ($t \ll t_{\text{mm}}$) shown in Figure 5.2b, there is excess of exterior ions at the interface and the polarization is reversed. In this induced-charge configuration, the vesicle becomes an oblate ellipsoid.

A very important distinction from drops is that for $t \gg t_{\text{mm}}$ the membrane capacitor is fully charged and the effective net charge vanishes. In this limit, the vesicle responds as an ideally-polarizable particle with vanishing electric field in the vesicle interior, see Figure 5.2c.

It can be seen from Figure 5.2 that vesicles with $R < 1$ will deform into an oblate before t_{mm} and then transition into prolate when $t > t_{\text{mm}}$. The main goal of this chapter is to observe this transition from oblate to prolate deformation. In general, this shows that the dynamics of the vesicle are dependent on membrane charging in a DC field. In an experiment, membranes can easily porate, leading to a breakdown of the membrane capacitor. An analysis of the relevant timescales is provided to demonstrate the electromechanical limits of the membrane and when the transition from oblate to prolate can be observed.

5.2.1 Timescales and Nondimensionalization

The electric stress acting to deform the vesicles must overcome viscous dissipation in the surrounding fluid. The timescale for deformation is given by the electrohydrodynamic (EHD) timescale,

$$t_{\text{hd}} = \frac{\mu_{\text{ex}}(1 + \Lambda)}{\epsilon_{\text{ex}} E_0^2}. \quad (5.4)$$

An important comparison of timescales is between t_{hd} with t_{mm} . This ratio compares the rate of deformation to the characteristic time of membrane charging,

$$\delta_m = t_{\text{hd}}/t_{\text{mm}}. \quad (5.5)$$

The EHD timescale is easily varied with field strength, with a range of $t_{\text{hd}} \sim 0.1 - 10 \text{ms}$ for 100 kV/m and 10 kV/m respectively. A weak field condition can be defined when $t_{\text{mm}} < t_{\text{hd}}$ ($\delta_m > 1$). In this limit, deformation occurs after the membrane is fully charged and only prolate deformation will be observed. In strong fields, $t_{\text{mm}} > t_{\text{hd}}$, deformation is observable while the vesicle is charging and depends on the conductivity ratio, R .

The relaxation timescale for the bilayer membrane can be given in terms of the bending rigidity,

$$t_\kappa = \frac{\mu_{\text{ex}}(1 + \Lambda)a^3}{\kappa}. \quad (5.6)$$

This timescale is useful because it is based on a material property of the membrane, while tension can vary as the vesicle deforms.

A comparison between the EHD timescale and the restoring timescale produces another important dimensionless parameter, given as a capillary number,

$$Ca_\kappa = \frac{a^3 \epsilon_{\text{ex}} E_0^2}{\kappa}. \quad (5.7)$$

The timescale over which a vesicle relaxes from a deformed shape is given by the tension relaxation timescale. The initial tension, σ_{eq} , of the membrane is used for relaxation timescale,

$$t_\sigma = \frac{\mu_{\text{ex}}(1 + \Lambda)a}{\sigma_{\text{eq}}}, \quad (5.8)$$

which describes the relaxation of the vesicle shape.

As discussed in Chapter 4, strong fields will produce critical transmembrane voltages leading to membrane poration [161]. The mechanism for poration is discussed in more detail here because poration is more prevalent in DC pulses. The transfer of compressive stress arising from the membrane capacitor to tension, σ_{el} , can be understood by considering the differential work done on a capacitor,

$$dW = \sigma_{\text{el}} h dA + \frac{1}{2} \epsilon_{\text{mm}} \left(\frac{V_m}{d} \right)^2 A dh, \quad (5.9)$$

where dA is a differential area and dh is the change in thickness of the capacitor. The differential work is given by $K_a \alpha dA$, where α is the increase in true area. If the membrane is modeled as incompressible, $A dh = -h dA$ and $\alpha = 0$. This reduces the equation to,

$$0 = \sigma_{\text{el}} - \frac{1}{2} \epsilon_{\text{mm}} \frac{V_m^2}{d}. \quad (5.10)$$

Rewriting the equation in terms of membrane capacitance gives the Lippmann equation [116],

$$\sigma_{\text{el}} = \frac{1}{2} C_m V_m^2. \quad (5.11)$$

This equation assumes the compressive stress on the capacitor acts over the entire thickness of the membrane. In the case of lipid membranes it is commonly assumed that the capacitor acts over the thickness of the inner dielectric layer, d_c , and that the entire membrane acts as an near incompressible material of thickness, d . In this case, the tension is related to the transmembrane voltage by [138],

$$\sigma_{\text{el}} = \epsilon_{\text{mm}} \left(\frac{d}{2d_c^2} \right) V_m^2. \quad (5.12)$$

While Eq.[5.12] has been used to determine the critical voltage for lipid membranes

[138, 161], measurements for polymer vesicles have used Eq.[5.11] [9]. The structure of the bilayer membrane and how charges interact with the interface will affect the relative values of d and d_c [135].

A membrane will form pores when the membrane tension exceeds a critical value, denoted as the lysis tension σ_{lys} . The lysis tension has been measured to be $\sigma_{\text{lys}} \sim 5 \times 10^{-3} \text{ N/m}$ for lipid vesicles and $\sigma_{\text{lys}} \sim 20 - 30 \times 10^{-3} \text{ N/m}$ for polymer vesicles [138, 161, 9]. This corresponds to a critical transmembrane voltage, V_c , of about $1V$ for lipid membranes and $V_c \sim 4 - 8V$ for polymer membranes [161].

A critical electric field strength can be estimated using Eq.[5.3],

$$E_c = \frac{2V_c}{3a}. \quad (5.13)$$

Strong fields, $E_0 > E_c$, will lead to poration for a fully charged membrane. For a typical vesicle size, $a = 20\mu\text{m}$, a lipid membrane will porate above field strengths of approximately $E_0 = 30kV/m$.

Previous work has not considered the finite time required to reach the critical transmembrane potential. This time is determined from the expression for transmembrane potential in an electric field, Eq.[5.3]. Setting $V_m = V_c$ and $\theta = 0$, Eq.[5.3] is solved for t , which gives the time when the critical transmembrane voltage is reached at the pole of the vesicle,

$$t_{\text{crit}} = -t_{\text{mm}} \ln \left[1 - \frac{2V_c}{3aE_0} \right]. \quad (5.14)$$

Note that this timescale is undefined for fields below the critical field strength, $E_0 < E_c$.

The typical membrane charging timescales is $t_{\text{mm}} \sim 1\text{ms}$ and is independent of field strength. Both t_{hd} and t_{crit} depend on field strength. In a weak field below the critical field for poration ($E_0 = 10\text{kV}/m$), a vesicle deforms on the timescale $t_{\text{hd}} \sim 10\text{ms}$. Therefore, a vesicle will deform only after the membrane has charged, which implies that only prolate deformation will be visible even if $R < 1$. If the field strength is increased to $E_0 = 100\text{kV}/m$, the EHD timescale will reduce to $t_{\text{hd}} \sim 0.1\text{ms}$ ($\delta_m \sim 0.1$). This indicates that deformation will occur while the membrane is charging and depends on conductivity ratio. Comparing this field strength to Eq.[5.13], it is clear that this exceeds the critical limit set by E_c . According to Eq.[5.14] the membrane will create pores at approximately $t_{\text{crit}} \sim 0.5\text{ms}$. This analysis shows that a single pulse will either be too weak to observe the oblate-prolate transition or will lead to poration.

The proposed solution for this problem is to use a two-step pulse to observe a transition from oblate to prolate. First, a strong field must be applied with duration $t_{\text{pulse}} \sim t_{\text{hd}} < t_{\text{mm}}$ in order to observe oblate deformation. To avoid membrane poration, the duration of the first pulse must be shorter than the critical timescale, $t_{\text{pulse}} < t_{\text{crit}}$. A step decrease in the field strength, below the critical field strength, will produce a transition to a prolate shape on the timescale $t_{\text{pulse2}} \sim t_{\text{hd}} > t_{\text{mm}}$. The second field strength must be below the critical field strength $E_0 < E_c$ to avoid poration. An example of the oblate-prolate transition using this method is shown in Figure 5.3.

5.2.2 Governing equations

The governing equations, hydrodynamic, and membrane stresses are identical to Chapter 4. The electric stresses differ slightly in DC fields compared to AC fields.

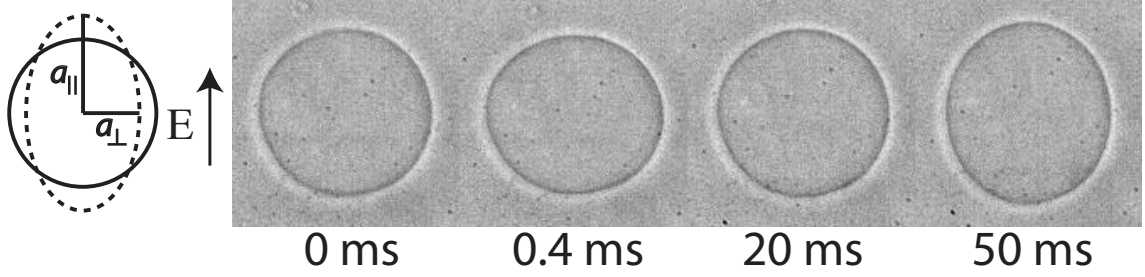


Figure 5.3: Transition from oblate to prolate for DOPC vesicle. A lipid membrane with capacitance $C_m = 0.71 \mu F/cm^2$, initial radius $a=24.5 \mu m$, $\lambda_{in}=3 \mu S/cm$, $\lambda_{ex}=10 \mu S/cm$. The charging time is $t_{mm}=0.95$ ms. A two pulse field is applied, pulse 1 is $E_0=400$ kV/m with duration $20 \mu s$ pulse followed by $E_0=20$ kV/m strength with duration 50 ms.

The governing equations are reiterated, but only the electric stresses are developed in detail. As before, the modeling approach uses the leaky dielectric assumptions; the bulk fluid is charge free and the electromechanical coupling occurs only at the interface.

Hydrodynamic field

Hydrodynamics inside and outside the vesicle obey the Stokes equation [139, 107],

$$\nabla \cdot \mathbf{T}^{hd} = 0, \nabla \cdot \mathbf{u} = 0, \quad (5.15)$$

where the hydrodynamic stress is computed by,

$$\mathbf{T}^{hd} = -p\mathbf{I} + \mu [\nabla \mathbf{u} + (\nabla \mathbf{u})^\dagger]. \quad (5.16)$$

If it is assumed that there is no bilayer slip or membrane permeability, the hydrodynamic boundary conditions are identical to that of a drop. The flow is

$$\mathbf{u}_{in} = \mathbf{u}_{ex} = \mathbf{u}_s, \quad (5.17)$$

where $\mathbf{u}_s$ is the flow at the interface. Membrane permeability can be neglected

if the vesicle is osmotically stable and the membrane is pore free. Although, the electric field can create pores in the membrane, the experiments with quantitative comparisons are limited to electric fields that do not induce poration, i.e. $E_0 < E_c$ or $t_{pulse} < t_{crit}$.

Electric field

The electric field is determined by the gradient of the electric potential,

$$\mathbf{E} = -\nabla\Phi. \quad (5.18)$$

The absence of bulk free charges determines that the electric field is solenoidal ($\nabla \cdot \mathbf{E} = 0$), thus the electric potential satisfies the Laplace equation,

$$\nabla^2\Phi = 0. \quad (5.19)$$

Far from the vesicle, the electric field is uniform and constant, $\mathbf{E}^\infty = E_0\hat{\mathbf{z}}$. The electric stresses are determined from the Maxwell stress tensor,

$$\mathbf{T}^{el} = \epsilon(\mathbf{E}\mathbf{E} - \mathbf{E}^2\mathbf{I}/2), \quad (5.20)$$

where $\mathbf{I}$ is the identity tensor. The electric potential is solved subject to the following boundary conditions at the interface.

The insulating bilayer membrane causes a jump in the potential field across the membrane,

$$\Phi_{in} - \Phi_{ex} = V_m. \quad (5.21)$$

The charging of the interface is determined by the conservation of current across the

membrane capacitor,

$$\mathbf{n} \cdot (\lambda_{\text{ex}} \mathbf{E}_{\text{ex}}) = \mathbf{n} \cdot (\lambda_{\text{in}} \mathbf{E}_{\text{in}}) = C_m \frac{dV_m}{dt}. \quad (5.22)$$

The transmembrane potential is determined as part of the solution for the potential. The effective net charge on the interface can be determined by the jump in the displacement field,

$$\mathbf{n} \cdot (\epsilon_{\text{ex}} \mathbf{E}_{\text{ex}} - \epsilon_{\text{in}} \mathbf{E}_{\text{in}}) = Q. \quad (5.23)$$

In the limit of zero-thickness membrane, Q is an effective charge on the membrane resulting from an imbalance in charge density between the inner and outer surface of the membrane. A mismatch in the rate of supply of charges gives rise to the net charge while charging; when the capacitor is fully charged, $Q = 0$.

Electric stresses

The electric stress is determined from the Maxwell stress tensor, Eq.[5.20], which first requires the electric field to be solved around the vesicle. The electric field is determined from the potential field around a spherical shell,

$$\Phi_{\text{ex}} = -E_0 \left[r + B_{\text{ex}} \frac{a^3}{r^2} \right] \cos \theta, \quad (5.24)$$

$$\Phi_{\text{in}} = -E_0 B_{\text{in}} r \cos \theta. \quad (5.25)$$

The jump in the potential across the membrane is written in terms of a time dependent variable and position dependence determined from Eq.[5.24] and Eq.[5.25],

$$V_m = \bar{V}(t) a E_0 \cos(\theta), \quad (5.26)$$

where $\bar{V}(t)$ is determined from the initial conditions.

The two boundary conditions for the potential jump across the membrane, Eq.[5.21], and the current conservation, Eq.[5.22], are used to solve for B_{in} and B_{ex} . An initial condition of zero transmembrane potential, $V_m = 0$, is assumed. The coefficients for the electric field are expressed in terms of the time dependent variable from Eq.[5.26],

$$B_{\text{in}} = \frac{3 - 2\bar{V}(t)}{R + 2}, \quad B_{\text{ex}} = \frac{(-R + 1) - R\bar{V}(t)}{R + 2}, \quad (5.27)$$

where,

$$\bar{V} = \frac{3}{2} (1 - \exp(-t/t_{\text{mm}})). \quad (5.28)$$

The electric stresses are computed from the Maxwell stress tensor, Eq.[5.20], and decomposed into normal and tangential components,

$$\mathbf{T}^{\text{el}} = \bar{p}^{\text{el}} [1 + 3 \cos(2\theta)] \hat{\mathbf{n}} + \bar{\tau}_s \sin(2\theta) \hat{\boldsymbol{\theta}}, \quad (5.29)$$

with magnitudes determined by,

$$\bar{p}^{\text{el}} = \frac{1}{2} [(E_r^{\text{ex}})^2 - (E_\theta^{\text{ex}})^2 - S((E_r^{\text{in}})^2 - (E_\theta^{\text{in}})^2)], \quad (5.30)$$

$$\bar{\tau}_s = E_\theta^{\text{ex}} Q + E_r^{\text{in}} (E_\theta^{\text{ex}} - E_\theta^{\text{in}}). \quad (5.31)$$

Substituting in the electric field in terms of B_{ex} and B_{in} yields,

$$\bar{p}^{\text{el}} = \frac{1}{12} [2 - 2B_{\text{ex}} + 5B_{\text{ex}}^2 - 2SB_{\text{in}}^2], \quad (5.32)$$

$$\bar{\tau}_s = \frac{1}{2} [-1 + B_{\text{ex}} + 2B_{\text{ex}}^2 + SB_{\text{in}}^2]. \quad (5.33)$$

These expressions are almost identical to the stress coefficients for an AC field, although both $\bar{p}^{\text{el}}$ and $\bar{\tau}_s$ are greater by a factor of 2 and all coefficients are real.

These stresses must be converted into vector spherical harmonics in order to match the other terms in the stress balance, Eq.[4.29]. The angular dependence corresponds to the $j = 2, m = 0$ vector spherical harmonics. The directions normal and tangential to the interface are given by $q = 2$ and $q = 0$ respectively. The vector spherical harmonics with the appropriate angle dependence are,

$$\mathbf{y}_{202} = \frac{1}{8} \sqrt{\frac{5}{\pi}} [1 + 3 \cos(2\theta)] \hat{\mathbf{r}}, \quad \mathbf{y}_{200} = -\frac{1}{4} \sqrt{\frac{15}{2\pi}} \sin(2\theta) \hat{\boldsymbol{\theta}}. \quad (5.34)$$

The electric stresses are rewritten in vector spherical harmonics which include the inverse of the coefficients from Eq.[5.34],

$$\tau_{202}^{\text{el}} = 8 \sqrt{\frac{\pi}{5}} \bar{p}^{\text{el}} \mathbf{y}_{202}, \quad \tau_{200}^{\text{el}} = -4 \sqrt{\frac{2\pi}{15}} \bar{\tau}_s \mathbf{y}_{200}. \quad (5.35)$$

Evolution equation

The driving force for vesicle deformation and hydrodynamic flow is the electric field. The characteristic electric stress, $\epsilon_{\text{ex}} E_0^2$, is also characteristic of the viscous stress, including surface viscous stresses. Membrane bending and tension are scaled by κ/a^3 . The stress balance is nondimensionalized using the capacitor charging time t_{mm} , vesicle radius a , and the characteristic magnitude of the bulk electric stress $\epsilon_{\text{ex}} E_0^2$.

The tangential component of the dimensionless stress balance, Eq.[4.29], is solved

first, this determines the non-isotropic part of the membrane tension,

$$\tilde{\sigma}_{20} = Ca_\kappa \left[\frac{-\tau_{200}^{\text{el}}}{\sqrt{6}} + c_{202} \frac{4 + (\Lambda + 8\Lambda_s)}{6} \right]. \quad (5.36)$$

Note that $\tilde{\sigma}_{20}$ is normalized by the bending rigidity and radius, $\tilde{\sigma}_{20} = \sigma_{20}a^2/\kappa$.

Using the expression for $\tilde{\sigma}_{20}$ in the normal stress balance, the normal surface velocity coefficient can be solved for,

$$c_{202} = C_{20} - Ca_\kappa^{-1} \frac{24(6 + \tilde{\sigma}_h)}{32 + 23\Lambda + 16\Lambda_s} f_{20}, \quad (5.37)$$

where $\tilde{\sigma}_h = \sigma_h a^2/\kappa$ is the bending rigidity normalized by the isotropic tension, which results from the choice of membrane tension in the capillary number. The forcing term C_{20} is determined from the electric stresses,

$$C_{20} = \frac{\sqrt{6} [2\tau_{200}^{\text{el}} + \sqrt{6}\tau_{202}^{\text{el}}]}{32 + 23\Lambda + 16\Lambda_s}, \text{ or } C_{20} = 16\sqrt{\frac{\pi}{5}} \frac{(3\bar{p}^{\text{el}} - \bar{\tau}_s)}{32 + 23\Lambda + 16\Lambda_s}. \quad (5.38)$$

The forcing term C_{20} can be rewritten in terms of the coefficients B_{in} and B_{ex} ,

$$C_{20} = 4\sqrt{\frac{\pi}{5}} \frac{(B_{\text{ex}} - 2)^2 - 4SB_{\text{in}}^2}{32 + 23\Lambda + 16\Lambda_s}, \quad (5.39)$$

and the complete expression is,

$$C_{20} = 9\sqrt{\frac{\pi}{5}} \exp\left[\frac{-2t}{t_{\text{mm}}}\right] \left(\frac{(R + \exp\left[\frac{t}{t_{\text{mm}}}\right](2 + R))^2 - 16S}{(32 + 23\Lambda + 16\Lambda_s)(2 + R)^2} \right). \quad (5.40)$$

The stress balance Eq.[2.14] yields a net flow normal to the interface, which

determines the shape evolution of the vesicle,

$$\frac{\partial f_{20}}{\partial t} = c_{202}. \quad (5.41)$$

The evolution equation written in terms of f_{20} becomes,

$$\delta_m \frac{\partial f_{20}}{\partial t} = C_{20} - \frac{\kappa}{a^3 \epsilon_{\text{ex}} E_0^2} \frac{24(6 + \tilde{\sigma}_h)}{32 + 23\Lambda + 16\Lambda_s}. \quad (5.42)$$

Note that time is normalized by t_{mm} in DC fields, where time was normalized by t_{hd} in the previous chapter. This was because the previous chapter addressed transient deformation with intermediate AC frequencies where the membrane never fully charges. In the AC case the timescale for deformation is controlled only by t_{hd} . In contrast, the evolution of vesicle shape in a DC field is related to how quickly the membrane capacitor charges, thus $\delta_m = t_{\text{hd}}/t_{\text{mm}}$ appears in Eq.[5.42].

As discussed in Chapter 4, the restoring force is dominated by membrane tension and the contributions from bending are negligible. This yields a simpler form for the relaxation term in the evolution equation,

$$\delta_m \frac{\partial f_{20}}{\partial t} = C_{20} - \frac{\sigma_h}{a \epsilon_{\text{ex}} E_0^2} \frac{24}{32 + 23\Lambda + 16\Lambda_s} f_{20}. \quad (5.43)$$

As can be seen in Eq.[5.43], the tension is the remaining variable to be determined. As the vesicle deforms, the excess area stored in fluctuations is transferred to systematic deformation [181]. The area stored in the deformed vesicle shape is determined by setting Eq.[5.43] to zero and solving for f_{20} . The area constraint, $\Delta = \Delta_s + \Delta_f$, is solved numerically at each time step to determine σ_h , see Section 4.2.7.

Two-step pulse

The equation developed above assumes an initial transmembrane voltage of zero. If the electric field strength is changed at a later time, as will be done in a two-step pulse, Eq.[5.42] will no longer be valid. The potential must be solved with the new electric field strength and a nonzero value for the initial transmembrane potential. It can be assumed that the field at infinity changes instantaneously because the bulk charging time is much faster than the membrane capacitor timescale, $t_c \ll t_{mm}$. When the applied electric field is changed, the values for the parameters δ_m and Ca will instantly change. Note that the transmembrane potential must be continuous when the field strength is changed.

The external field strength is changed from an initial field strength of E_0^1 to E_0^2 at a time $t = t_1$. The transmembrane potential is solved with a nonzero initial condition determined from the previous field strength,

$$\bar{V}_2(t) = \frac{3}{2} \left[1 + \exp \left[\frac{-t + t_1}{t_{mm}} \right] \left(-1 + \bar{V}_1(t_1) \frac{E_0^1}{E_0^2} \right) \right], \quad t > t_1, \quad (5.44)$$

where $\bar{V}_1(t_1)$ is the dimensionless transmembrane potential at $t = t_1$ determined from Eq.[5.28]. This expression can be applied whenever the field strength is varied using the previous values for field strength and transmembrane potential.

At times after the field has been changed, $t > t_1$, the transmembrane potential $\bar{V}_2(t)$ is substituted into the equation for C_{20} . The resulting equation gives a more

complicated expression for the forcing term,

$$C_{20} = \sqrt{\frac{\pi}{5}} \exp[-2t/t_{\text{mm}}] \left[\frac{(9e^{\lfloor \frac{2t}{t_{\text{mm}}} \rfloor} (2+R)^2 + e^{\lfloor \frac{2t_1}{t_{\text{mm}}} \rfloor} (R^2 - 16S)(3 - 2\bar{V}_1(t_1))^2 - 6e^{\lfloor \frac{t+t_1}{t_{\text{mm}}} \rfloor} R(R+2)(-3 + 2\bar{V}_1(t_1)))}{(32 + 23\Lambda + 16\Lambda_s)(2+R)^2} \right]. \quad (5.45)$$

Note that in the case of a strong initial field, the membrane may not be fully charged given the initial field strength. Although after the field is decreased, the transmembrane potential may exceed the equilibrium transmembrane potential for the weaker field strength. In this case, the membrane capacitor will discharge to adjust to the weaker field.

5.2.3 Discharging

A special case arises when the second field is set equal to zero, $E_0 = 0$, at a time $t = t_{\text{end}}$. This differs from the previous section because the form of the potential field changes in the absence of an externally applied field. The field can be assumed to relax at the bulk relaxation timescale, t_c , which can be considered instantaneous in comparison to the membrane capacitance timescale, t_{mm} . The discharging of the membrane will lead to a net force on the vesicle over a duration determined by the timescale, t_{mm} . The lack of an external field will also change the electric stresses and result in a different expression for the forcing term, C_{20} . The equation is normalized by the electric field strength before the field is turned off.

The potential field is recomputed with the boundary condition of $\Phi_{\text{ex}} = 0$ at

$r \rightarrow \infty$,

$$\Phi_{\text{ex}} = -E_0 B_{\text{ex}} \frac{a^3}{r^2} \cos \theta, \quad (5.46)$$

$$\Phi_{\text{in}} = -E_0 B_{\text{in}} r \cos \theta. \quad (5.47)$$

The boundary conditions for conservation of current, Eq.[5.22], and transmembrane potential, Eq.[5.21], remains the same. The dimensionless transmembrane potential at the time when field is turned off is used as an initial condition and is denoted, V_{end} . For a fully charged membrane, $V_{\text{end}} = 3/2$

The transmembrane voltage must be continuous when the field is turned off. The expression for transmembrane voltage changes to

$$\bar{V} = V_{\text{end}} \exp(-(t - t_{\text{end}})/t_{\text{mm}}), \text{ for } t > t_{\text{end}}. \quad (5.48)$$

The solution for the potential field yields the coefficients,

$$B_{\text{in}} = -\frac{2\bar{V}(t)}{2+R}, B_{\text{ex}} = \frac{R\bar{V}(t)}{2+R}. \quad (5.49)$$

The electric stress must be recomputed using the electric field determined from the potential Eq.[5.46] and Eq.[5.47]. The normal and tangential components remain the same as Eq.[5.29], but the change in potential field changes the coefficients, $\bar{p}^{\text{el}}$ and $\bar{\tau}_s$. The magnitude of the normal and tangential electric stresses computed from the Maxwell stress tensor becomes,

$$\bar{p}^{\text{el}} = \frac{1}{12}[5B_{\text{ex}}^2 - 2SB_{\text{in}}^2], \quad (5.50)$$

$$\bar{\tau}_s = \frac{1}{2}[2B_{\text{ex}}^2 + SB_{\text{in}}^2]. \quad (5.51)$$

Including the new expressions for $\bar{p}^{\text{el}}$ and $\bar{\tau}_s$ into the forcing term yields,

$$C_{20} = \frac{4V_{\text{end}}^2 \sqrt{\frac{\pi}{5}} \exp[-2t/t_{\text{mm}}](R^2 - 16S)}{(32 + 23\Lambda + 16\Lambda_s)(2 + R)^2}. \quad (5.52)$$

This equation is substituted into the evolution equation, Eq.[5.42] for $t > t_{\text{end}}$.

Without the external electric field, the charges on the surface of the vesicle will relax on the capacitive membrane timescale. If there is a mismatch between the interior and exterior conductivities, a net charge on the membrane will arise similar to the initial membrane charging. As a result, there is a transient stress that deforms the vesicle.

An interesting case arises for $R^2 < 16S$. Assuming the vesicle is at a steady state prolate shape and the membrane capacitor is fully charged. If $R^2 < 16S$, the forcing term will be negative and the vesicle will be pushed towards an oblate shape due to the field created by the discharging membrane. This can be understood physically by considering the charges on the exterior of the vesicle, which relax rapidly into the highly conductive exterior, leaving an excess of charges on the interior of the vesicle membrane. The interior charges on the opposite poles attract each other until they fully relax.

In the opposite case, $R^2 > 16S$, the forcing term will be positive and the vesicle will be forced into prolate deformation. Although for a fully charged membrane at steady state, the vesicle is already a prolate. The discharging will only prolong the prolate deformation before relaxing back to a spherical shape.

Figure 5.4 shows the magnitude of C_{20} for charging (left) and discharging from a fully charged state (right). When the electric field is turned off, the potential field

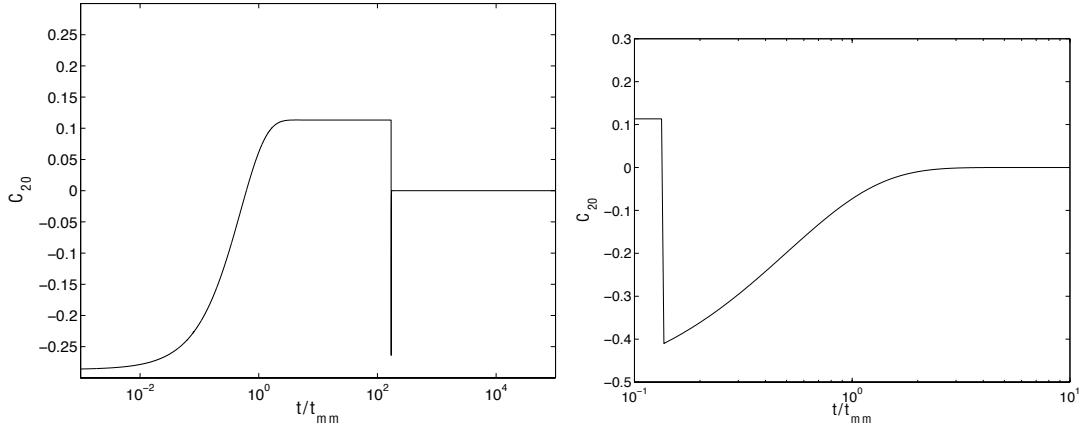


Figure 5.4: (left) The electric forcing term, $C_{20}(t)$, for an initial uncharged vesicle with a field turned on at $t = 0$. (right) The electric forcing term, $C_{20}(t)$, for an initially fully charged vesicle after the field is turned off. The relevant parameters are $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 50\mu\text{S}/\text{cm}$ ($R = 0.1$), $a = 20\mu\text{m}$, $\mu_{\text{mm}} = 1 \times 10^{-8}\text{Ns}/\text{m}$.

changes instantaneously due to the assumption that charges in the bulk relax much faster than the capacitor charging time. This results in the jump in the value for C_{20} seen in Figure 5.4 (right).

5.2.4 Model results

The evolution of drop shape depends on many physical parameters. In the experiment some parameters cannot be adjusted in isolation, particularly the membrane properties. For instance, the polymer membranes are thicker and therefore have a lower capacitance than lipid membranes, but the thicker membrane also increases bending rigidity and membrane viscosity. Adjusting individual parameters in the model provides a way of understanding the effect of each parameter on vesicle dynamics.

The effect of field strength is illustrated in Figure 5.5. The field strength of the first pulse is varied in Figure 5.5(left) and the field strength of the second pulse is

varied in Figure 5.5(right). As expected, stronger fields lead to greater deformation. If the first pulse is changed, the membrane does not charge faster, the transition to prolate occurs over the same timescale, t_{mm} , which is independent of field strength. If the second pulse is increased in strength, the vesicle will deform more and reach a steady state more quickly. Interestingly, immediately after the field is turned off a vesicle in the stronger field can retract to a less deformed shape compared with a vesicle in a weaker field. This is due to the greater amount of charge on the membrane which creates a stronger field while discharging. The magnitude of retraction also depends on other factors, such as the capacitive charging time and conductivity ratio.

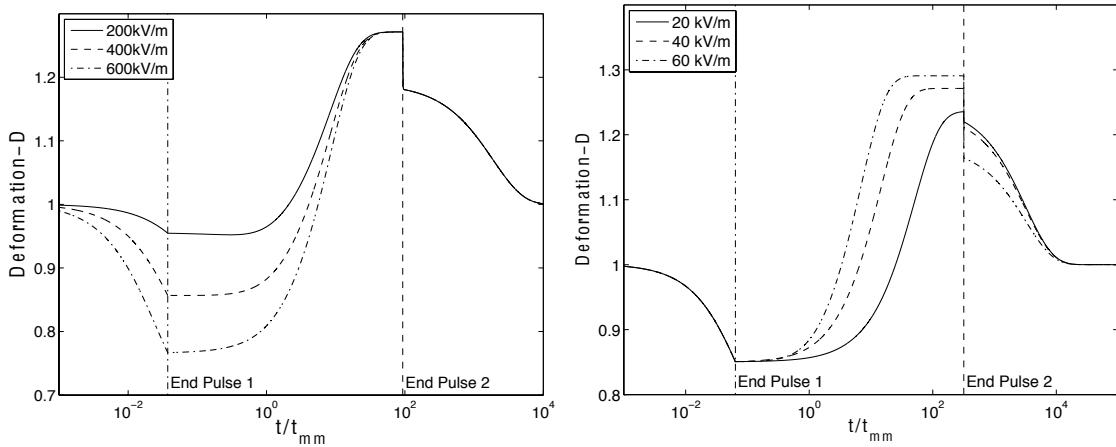


Figure 5.5: Effect of field strength by changing magnitude of the first step (left) and second step (right). The duration of the first pulse is $20 \mu s$ and the second is $50 ms$. The other vesicle parameters are constant. $\lambda_{in} = 3 \mu S/cm$, $\lambda_{ex} = 10 \mu S/cm$, $C_m = 0.7 \mu F/cm^2$, $a = 20 \mu S/cm$, $\kappa = 20 k_B T$, $\mu_{mm} = 10^{-8} N s/m$.

The effect of membrane tension is shown in Figure 5.6. The tension has little influence on the dynamics during the first pulse because the forcing term overwhelms the relaxation term. When the vesicle transitions to prolate, the degree of maximum deformation is closely related to initial tension. Higher initial tension leads to less deformation, as a result of having less excess area that can be transferred into systematic deformation. The relaxation of vesicles with different initial tensions are shown in Figure 5.6(right). Although the vesicles start at a different maximum

deformation, the magnitude of retraction due to membrane discharging is similar. In most cases, the capacitance timescale, $t_{\text{mm}} \sim 0.1 - 1\text{ms}$, is faster than the tension relaxation timescale, $t_\sigma \sim 10 - 100\text{ms}$. Only in cases of very tense vesicles will a vesicle relax at the same timescale as discharging. The effect of tension is most apparent in the time needed for relaxation back to a sphere. A fairly tense vesicle will retract on the order of 1 second ($10^3 \times t_{\text{mm}}$) while a less tense vesicle will take over 10 seconds ($10^4 \times t_{\text{mm}}$) to relax.

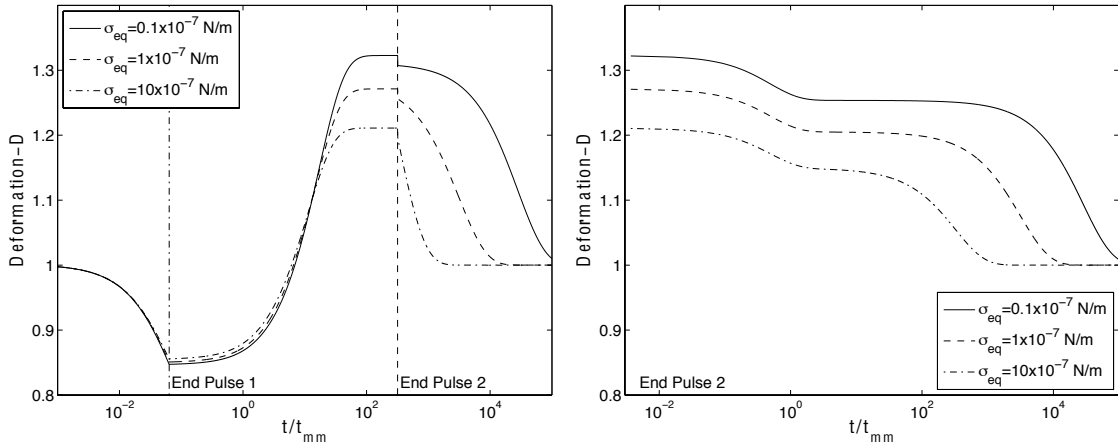


Figure 5.6: Effect of membrane tension on vesicle evolution (left) and relaxation (right). The membrane tension is varied from $\sigma_{\text{eq}} = 0.1, 1, 10 \times 10^{-7} \text{ Nsm}$. The first pulse step is 400kV/m for $20\mu\text{s}$ and then 40kV/m for 50ms . The other vesicle parameters are constant. $\lambda_{\text{in}} = 3\mu\text{S/cm}$, $\lambda_{\text{ex}} = 10\mu\text{S/cm}$, $C_m = 0.7\mu\text{F/cm}^2$, $a = 20\mu\text{S/cm}$, $\kappa = 20k_B T$, $\mu_{\text{mm}} = 10^{-8} \text{ Nsm}$.

The effect of bending rigidity is shown in Figure 5.7. The effect of bending rigidity is similar to that of membrane tension in that bending rigidity regulates the amount of excess area stored in fluctuations. A membrane with higher bending rigidity will store less area in fluctuations and lead to less deformation. Bending has little effect during the transition from oblate to prolate, but significantly alters the maximum deformation. The main difference between bending and initial tension is that bending does not influence the relaxation back to a sphere. This is because once all applied stresses have vanished, the excess area returns to membrane fluctuations and the relaxation is driven by initial tension.

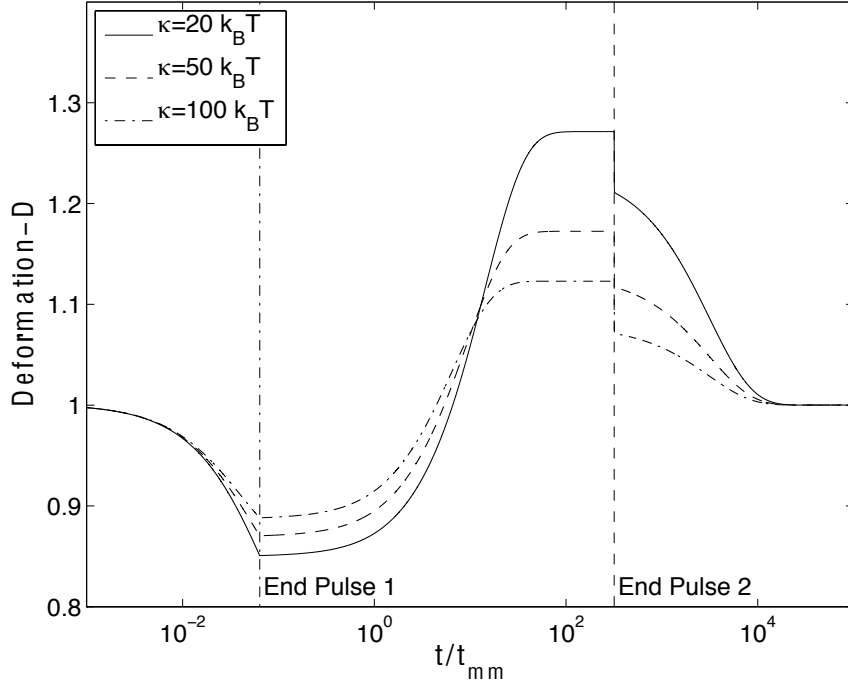


Figure 5.7: Effect of bending rigidity on shape evolution. Larger bending rigidity limits resisting transfer of excess area from fluctuations to systematic deformation. Bending rigidity is varied from $\kappa = 20, 50, 100k_B T$. The first pulse step is $400kV/m$ for $20\mu s$ and then $40kV/m$ for $50ms$. The other vesicle parameters are constant. $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$, $C_m = 0.7\mu F/cm^2$, $a = 20\mu S/cm$, $\sigma_{eq} = 10^{-7}N/m$, $\mu_{mm} = 10^{-8}Ns/m$.

The effect of membrane viscosity is shown in Figure 5.8. An increase in membrane viscosity slows the dynamics of the vesicle at all stages. There is significantly less deformation during the strong pulse and the vesicle takes longer to reach the equilibrium shape. Although as was discussed in Chapter 4, membrane viscosity has no influence on the maximum deformation. The retraction due to discharging is inhibited by greater membrane viscosity as well.

The effect of membrane viscosity can be understood by considering the viscous term: $32 + 23\Lambda + 16\Lambda_s$. When membrane viscosity is $\mu_{mm} \sim 10^{-8}Ns/m$ the dimensionless term, Λ_s , is much less than $\Lambda = 1$. In this limit, the membrane viscosity does not influence the viscous resistance. If membrane viscosity is increased to $\mu_{mm} \sim 50 \times 10^{-8}Ns/m$, Λ_s becomes much greater than one. In this case, membrane viscosity becomes the dominant source of viscous dissipation.

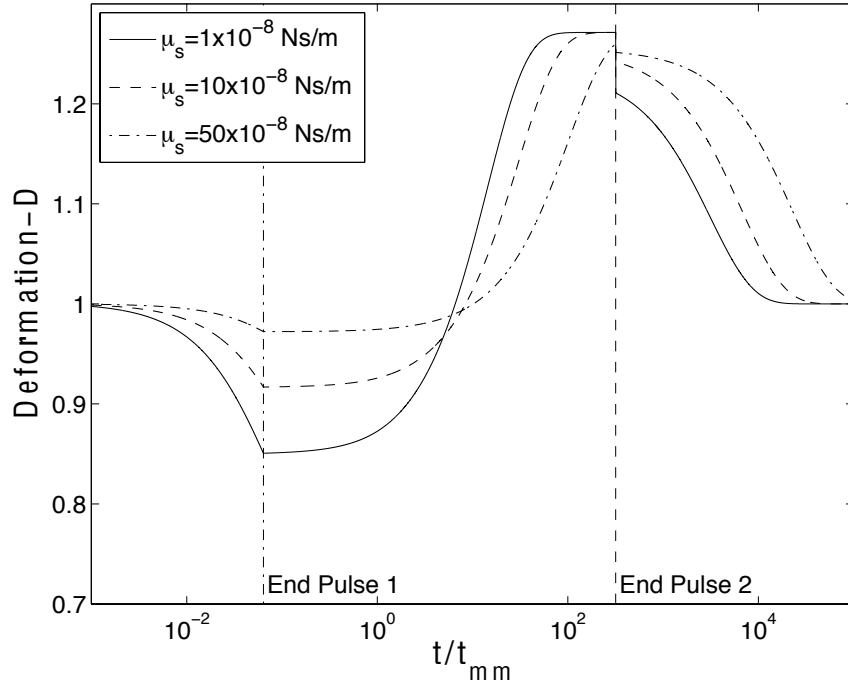


Figure 5.8: Membrane viscosity slows the rate of deformation, but does not change the equilibrium shape. Membrane viscosity is varied from $\mu_{\text{mm}} = 1, 10, 50 \times 10^{-8} \text{Ns/m}$. The first pulse step is 400kV/m for $20 \mu\text{s}$ and then 40kV/m for 50ms . The other vesicle parameters are constant: $\lambda_{\text{in}} = 5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $C_m = 0.7 \mu\text{F/cm}^2$, $a = 20 \mu\text{S/cm}$, $\sigma_{\text{eq}} = 10^{-7} \text{N/m}$.

The effect of membrane capacitance is shown in Figure 5.9. A decrease in membrane capacitance leads to faster membrane charging. This leads to a quicker transition from oblate to prolate and less time for the vesicle to deform into an oblate. If the capacitance is small, the membrane can be close to fully charged at the end of the first pulse; the membrane will have to discharge slightly to adapt to the weaker field. This can be seen in the solid line in Figure 5.9. The membrane charges rapidly during the strong pulse, when the field is decreased, the vesicle continues to deform into an oblate because of the stress created from discharging. In comparison, vesicles with high capacitance values quickly turn to prolate deformation because the transmembrane potential matches the fully charged state at the weaker field.

The maximum deformation does not vary with membrane capacitance. After the field is turned off, vesicles with higher capacitance have a longer timescale for

discharging because the membrane holds more charge. This creates a more persistent field due to the discharging membrane and a greater retraction.

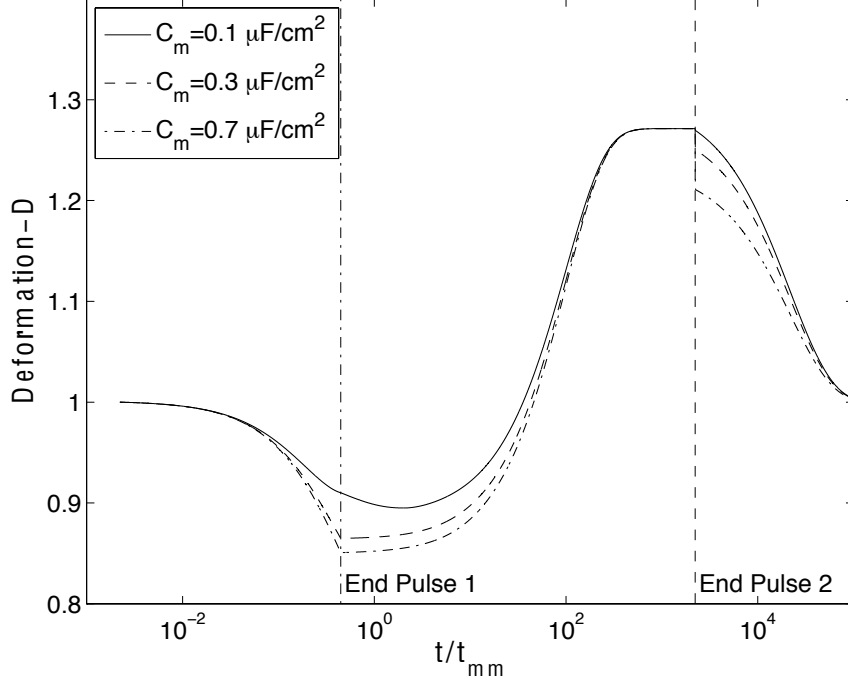


Figure 5.9: Effect of membrane capacitance. A greater capacitance leads to slower charging. This causes greater deformation into oblate and a greater effect of membrane discharging. Membrane capacitance is varied from $C_m = 0.1, 0.3, 0.7 \mu F/cm^2$. The other vesicle parameters are constant: $\lambda_{in} = 5 \mu S/cm$, $\lambda_{ex} = 10 \mu S/cm$, $a = 20 \mu S/cm$, $\sigma_{eq} = 10^{-7} N/m$, $\mu_{mm} = 1 \times 10^{-8} Ns/m$.

The effect of adjusting the interior and exterior electrical conductivity is shown in Figure 5.10. The effect of exterior conductivity is most pronounced during the first pulse. A higher exterior conductivity produces a lower conductivity ratio, this increases the electric stress while the membrane is charging. After the membrane capacitor has fully charged, the effect of conductivity ratio vanishes and the deformation is similar. For $R < 1$, the effect of exterior conductivity on discharging is smaller because the exterior conductivity has less influence on the charging timescale compared with the interior conductivity.

For vesicles with $R < 1$, the interior conductivity has more influence on vesicle

dynamics because it can significantly change both the capacitance timescale and conductivity ratio. In the capacitance timescale the influence of interior conductivity outweighs that of exterior conductivity when $R < 1$. As a result, a low internal conductivity gives a similar effect as high capacitance. Although in contrast to membrane capacitance, adjusting interior conductivity also adjusts the conductivity ratio. If the internal conductivity is increased, which is analogous to low capacitance, the membrane will charge too quickly for deformation into oblate to occur. As a result, experiments are performed on vesicles with low internal conductivity to give a longer capacitor charging timescale.

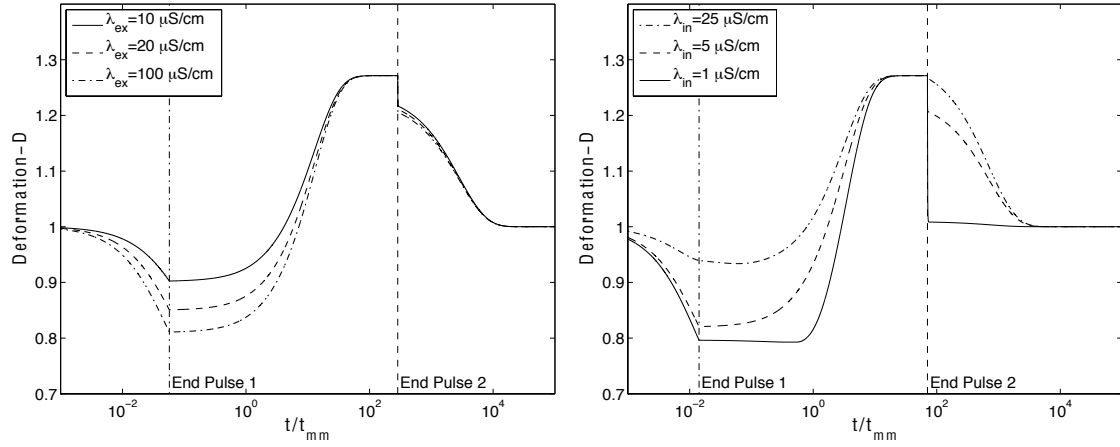


Figure 5.10: Shape evolution for different solution conductivities. An increase in exterior conductivity mostly changes conductivity ratio, R , leading to slightly faster charging. The internal conductivity affects both conductivity ratio and capacitor charging time. A lower internal conductivity leads to slower charging and greater deformation. (left) The external conductivity is varied $\lambda_{\text{ex}} = 10, 20, 100 \mu\text{S}/\text{cm}$ and is constant at $\lambda_{\text{in}} = 5 \mu\text{S}/\text{cm}$. (right) The internal conductivity is varied $\lambda_{\text{in}} = 1, 5, 25 \mu\text{S}/\text{cm}$ and is constant at $\lambda_{\text{in}} = 50 \mu\text{S}/\text{cm}$. The other vesicle parameters are constant: $C_m = 0.7 \mu\text{F}/\text{cm}^2$, $a = 20 \mu\text{S}/\text{cm}$, $\sigma_{\text{eq}} = 10^{-7} \text{N}/\text{m}$, $\mu_{\text{mm}} = 1 \times 10^{-8} \text{Ns}/\text{m}$.

5.3 Experimental methods

Vesicles are prepared using the electroformation method in a 100mM sucrose solution without the addition of salt. The conductivity of the interior sucrose solution is measured to be in the range of $3 - 5\mu S/cm$. The experiments are conducted by diluting the vesicles into glucose solution. The conductivity of the exterior solution is varied by adjusting the concentration of NaCl from 0-0.1mM. About 1 mL of the diluted vesicle solution is filled into the electrodeformation chamber. Vesicles are observed using a phase contrast microscopy and recorded using a high speed camera.

Preparation of giant vesicles

Giant vesicles are prepared from lipids (DOPC) and polyethyleneoxide-b-polybutadiene (PBd_n-b-PEO_m), diblock copolymers using the electroformation method [6]. The copolymers were obtained from Polymer Source Inc. (Montreal, Canada) and the lipids were purchased from Avanti.

The same procedure as Chapter 4 is followed. Briefly, a small volume (10 μ l) of the lipid solution is spread on the conductive surfaces of two glass plates coated with indium tin oxide (ITO). Organic solvent is removed using a vacuum, then the chamber is assembled across a 2 mm Teflon spacer, conductive sides facing inwards. The chamber is gently filled with 20 mM sucrose solution, then connected to an alternating current (1.8V-4.0V, 10 Hz frequency) for 1-2h at room temperature. The vesicle solution is removed from the electroswellling chamber and diluted into an isotonic glucose and salt solution. The exterior fluid conductivity is adjusted by

adding NaCl solution (0.01mM to 1 mM concentrations).

Electrodeformation set-up

The electrodeformation experiments were conducted in a chamber (Eppendorf, Germany), which consists of a Teflon frame confined from above and below by two glass slides. A pair of parallel cylindrical electrodes with a radius of $92\ \mu\text{m}$ and a separation of $0.5\ \text{mm}$ are fixed to the lower glass.

The field is applied using a function generator (Agilent 2300) connected to high voltage amplifier (Matsusada AMT-1BG0). The function generator is programmed to apply either a single or two-step pulse using the arbitrary function command. The strength and duration of the pulses are varied by adjusting the signal strength from the function generator. The high voltage amplifier increases voltage amplitude from the function generator by 100 times. The pulse is initiated from the function generator, which triggers the high speed camera (Photron SA1.1) with a TTL signal.

A 2-step DC pulse electric field is used in order to observe behavior at times before and after t_{mm} . An example of a two-pulse signal is shown in Figure 5.11. A strong pulse, $E_0 > E_c$, is used for early times ($t < t_{\text{mm}}$). The pulse must have sufficient strength and duration to deform the vesicle, which requires that the pulse time is longer than the EHD timescale $t_{\text{pulse}} > t_{\text{hd}}$. Because this initial field strength is stronger than the critical field for poration, the field strength is subsequently decreased to a value below E_c . The strength and duration of the first pulse is limited to produce a field that does not result in a transmembrane potential above the critical value V_c .

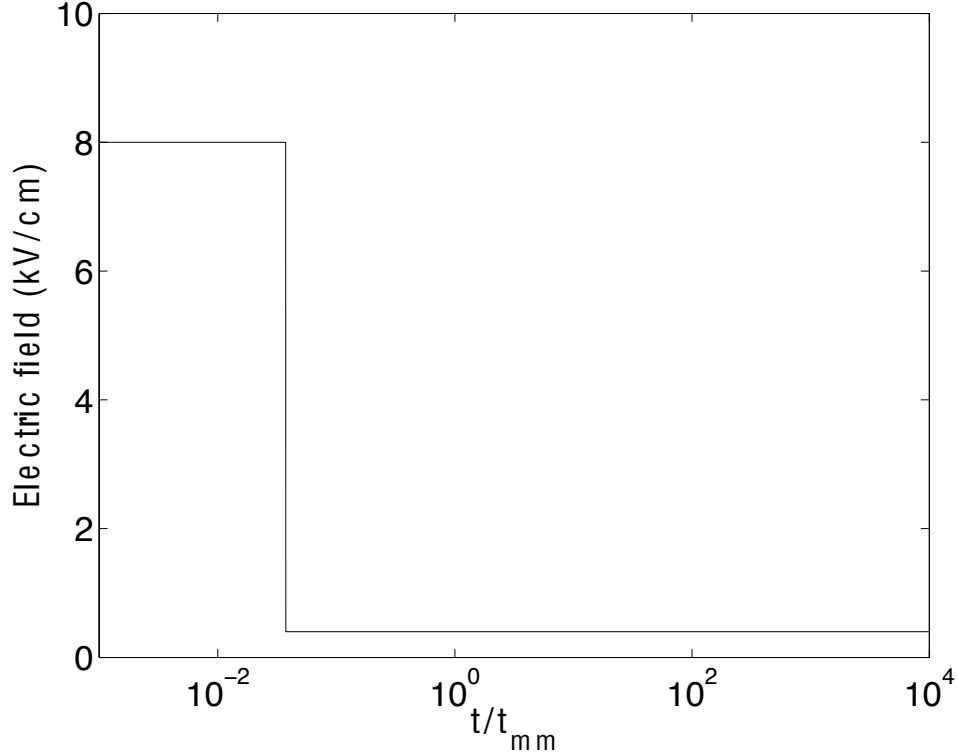


Figure 5.11: Example of the voltage profile 2-step DC pulse.

Optical microscopy and imaging

Due to the differences in density and refractive index between the sucrose and glucose solutions, the vesicles are stabilized by gravity close to the bottom of the chamber and have good contrast when observed with phase contrast microscopy. An inverted microscope Axio Observer.A1 (Zeiss, Germany) equipped with 20× or 40× Ph2 objectives are used to image the vesicle under phase contrast mode. Images are recorded with the camera at 30,000 frames per second.

Before an electric pulse is applied, the tension and bending rigidity of a vesicle is measured by observing membrane fluctuations. The camera is set to 60 frames per second (fps) and shutter rate of 1/5,000 seconds. 6,000-8,000 frames are recorded and then analyzed using the routine developed by [68]. If the vesicle survives the electric

pulse, the measurement is repeated on the same vesicle. These measurements are to confirm that the equilibrium tension does not change significantly over the course of the experiment. This procedure also gives a comparison for bending rigidity and tension values used to fit the shape evolution.

Deformation parameter

The evolution of the vesicle shape is recorded using the high speed camera and analyzed using in-house image analysis software [68]. Using contour detection, two semi axes $a_{\parallel}$ and $a_{\perp}$ are determined using the Fourier series expansion, where $a_{\parallel}$ is parallel and $a_{\perp}$ is normal to the field direction.

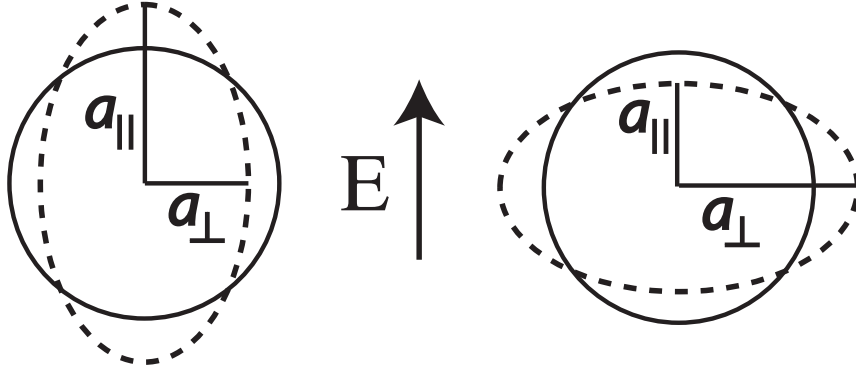


Figure 5.12: Definition of axes where $a_{\parallel}$ and $a_{\perp}$ are the axes parallel and perpendicular to the symmetry axis respectively. Deformation is shown for prolate (left) and oblate (right). The deformation parameter is defined as $D = a_{\parallel}/a_{\perp}$.

A deformation parameter is defined as the axis parallel to the field divided by the axis perpendicular to the field, see Figure 5.17. Theoretically, the deformation parameter is computed from the amplitude of the spherical harmonic, f_{20} , by,

$$D_{theory} = \frac{1 + f_{20}1/2\sqrt{5/\pi}}{1 - f_{20}1/4\sqrt{5/\pi}}, \quad D_{exp} = \frac{a_{\parallel}}{a_{\perp}}. \quad (5.53)$$

5.4 Results: experiment

In this section, the results of experimental observations of single and two-step pulses are given for different field strength. The oblate-prolate transition is demonstrated using a two-step pulse. The effect of membrane viscosity, membrane capacitance, and initial tension on the shape evolution are explored. All experiments are compared to theory developed in Section 5.2.

5.4.1 Weak fields: prolate deformation

First, theory and experiment are compared for a single long pulse ($t_{pulse} > t_{mm}$) where the field is below the critical field for poration, $E_0 < E_c$. In a weak field, the oblate to prolate transition is not observable because t_{hd} is longer than t_{mm} . An example of a vesicle in a weak field is given in Figure 5.13. The experiment is fit with the values given in the caption of Figure 5.13.

The evolution of the vesicle shape in DC fields provides a method for determining membrane viscosity similar to AC fields in Chapter 4. In contrast to POPC membranes used in Chapter 4, which had membrane viscosity values $\mu_{mm} \sim 10 \times 10^{-8} \text{Ns/m}$, DOPC membranes have a lower value of membrane viscosity $\mu_{mm} \sim 0.1 - 1 \times 10^{-8} \text{Ns/m}$. Using the values for DOPC, the membrane viscosity ratio is small, $\Lambda_s \sim 0.05$, compared with the bulk viscosity ratio $\Lambda = 1$. This means that the contribution to viscous resistance from DOPC membranes is small compared to bulk viscosity. This implies that the ability to determine values of low viscosity membranes, such as DOPC, is limited with this method. Nevertheless, as can be seen in Figure 5.13, the evolution of DOPC vesicle shape fits fairly well with low

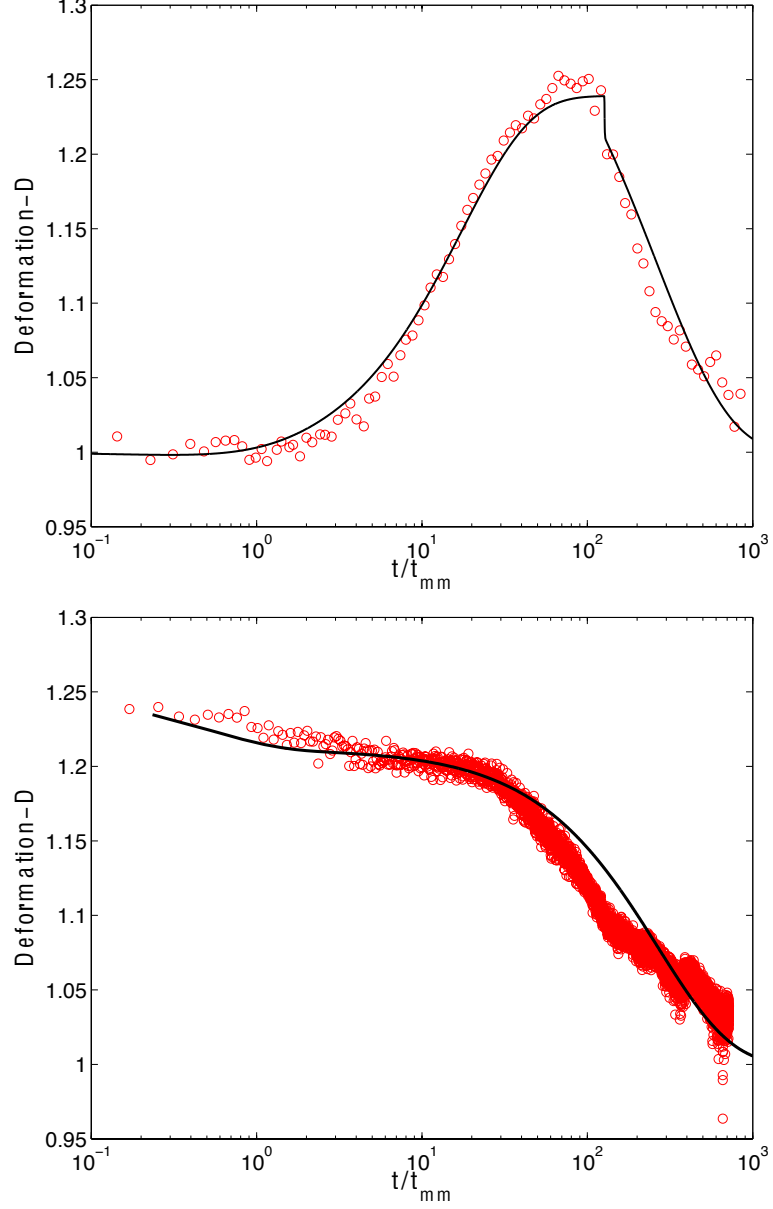


Figure 5.13: DOPC vesicle behavior in a single DC pulse. The electric field is turned on at $t = 0$ for a duration of $50ms$. The field strength is $E_0 = 20kV/m$. The vesicle is fit with $\sigma_{eq} = 5 \times 10^{-7} N/m$, membrane viscosity $\mu_{mm} = 0.1 \times 10^{-8} Ns/m$, or $\Lambda_s \sim 0.04$, $C_m = 0.71\mu F/cm^2$, $a = 24\mu m$, $\kappa = 13k_bT$, $\lambda_{in} = 5.5\mu S/cm$, $\lambda_{ex} = 10\mu S/cm$.

values of membrane viscosity.

The retraction due to membrane discharging can be seen in the rapid decrease of elongation in Figure 5.13(top), although the logarithmic scale obscures this deformation. The retraction is more clearly seen in Figure 5.13(bottom), which

is a plot of the deformation data after the field is turned off, i.e. $t \gtrsim 10^2 t_{\text{mm}}$. The data is replotted to more clearly separate the retraction due to discharging and the relaxation back to a sphere driven by membrane tension. The theory captures both the magnitude of retraction and the timescale, which occurs a time comparable to t_{mm} . The relaxation back to a sphere driven by membrane tension is fairly well captured by the theory.

Experimental error arises in resolving the shape of the vesicle, particularly as it relaxes back to a sphere. When the field is applied, the vesicle will lift off the glass slightly. This has been reported in previous experiments using vesicles in electric fields [190]. When the field is turned off, the vesicle will settle back to the glass surface. The small change in position causes the vesicle to move out of focus. The focal plane can be set slightly above the vesicle before the pulse is applied to reduce this error. The movement is greater in strong fields, where gradients in the electric field are more significant. Despite these slight measurement errors, the timescale over which the vesicle relaxes back to a sphere is still captured. As discussed in Section 5.2.4, the duration for relaxation is the important quantity for determining membrane tension.

The shape evolution is compared for the same vesicle at different field strengths in Figure 5.14. The experiment is repeated at the new field strength immediately after the vesicle relaxes back to a sphere. The experiment is fit with identical experimental parameters except for field strength. The fit correctly estimates the faster response as field strength is increased, but slightly overestimates the deformation observed at the weakest field strength. The presence of the retraction after the field is turned off is small, but is noticeable in the higher field strength. The theory captures the magnitude of this retraction in high fields, as well as it being negligible in weaker fields. After the retraction due to membrane discharging, the vesicle relaxes back

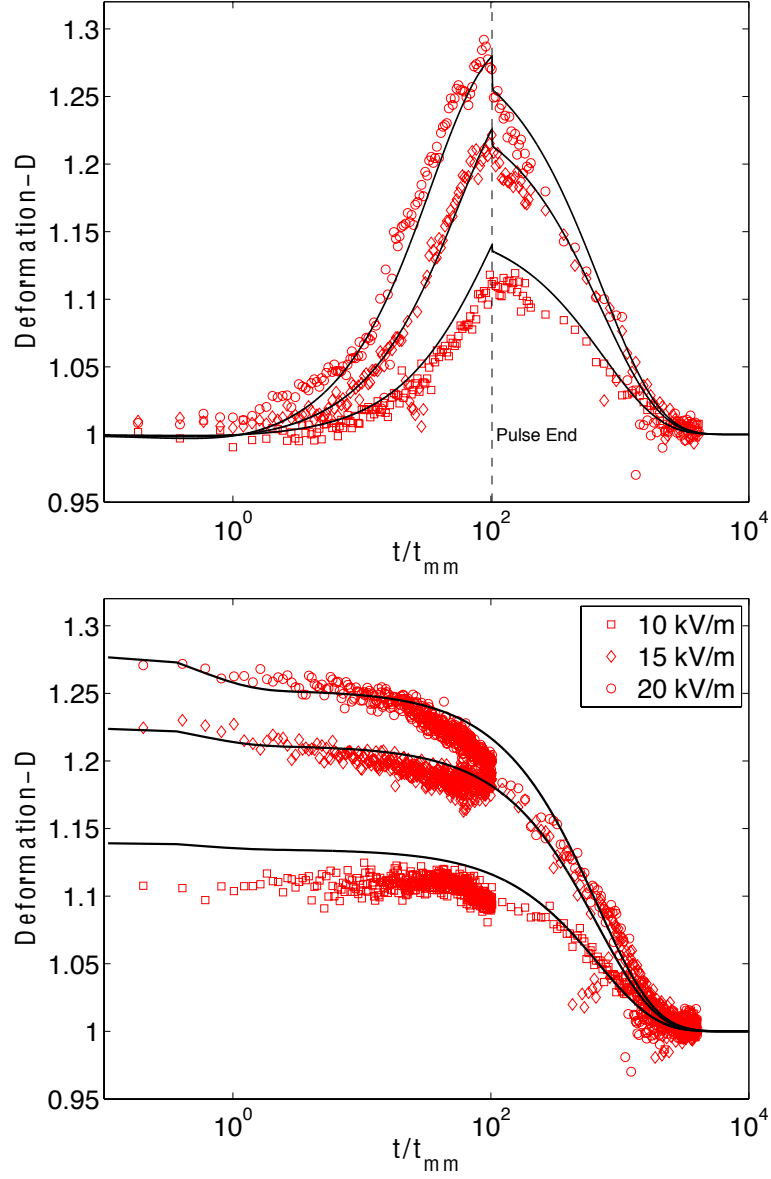


Figure 5.14: Prolate deformation for a single vesicle subjected to 10,15, and 20 kV/m 50 μ s pulse. The vesicle parameters are $a = 18\mu\text{m}$, $C_m=0.71 \mu\text{F/cm}$, $\lambda_{\text{in}}=3 \mu\text{S/cm}$, $\lambda_{\text{ex}}=10 \mu\text{S/cm}$, $\mu_{\text{mm}}=0.3 \times 10^{-8} \text{Ns/m}$, $\sigma_{\text{eq}}=1.7 \times 10^{-7} \text{N/m}$, $\kappa=14 k_b T$.

to a sphere over approximately the same timescale, t_σ , which is independent of field strength.

5.4.2 Strong fields: oblate deformation and collapse

If the field is increased, the EHD timescale becomes faster than the timescale for capacitive charging, $t_{\text{hd}} < t_{\text{mm}}$. In this limit, the vesicle can deform before the membrane fully charges. If conductivity ratio is less than unity, $R < 1$, oblate deformation will be observed for times shorter than the capacitance timescale, $t < t_{\text{mm}}$. Once the membrane charges, the electric stress changes to pull the vesicle into a prolate. As discussed in Section 5.2.1, the theory is only valid for $V_m < V_c$, where effects of membrane poration can be neglected. The membrane can reach the critical transmembrane potential at t_{crit} .

Once a pore forms, the transmembrane potential can decrease due to conduction through the membrane [97, 80]. Figure 5.15 shows a vesicle in a single strong DC pulse ($E_0 > E_c$). The vesicle initially deforms into an oblate ellipsoidal shape as predicted but does not transition to prolate. Instead, the oblate deformation continues and eventually leads to collapse. Another feature apparent in Figure 5.15 is the formation of sharp edges in the membrane and a cylindrical deformation at 1 ms. The edges only appear in strong fields where poration can occur. The effects of poration on vesicle dynamics is more closely examined in Chapter 6.

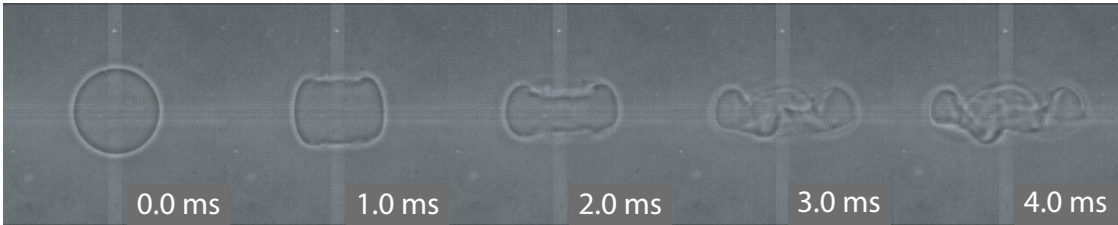


Figure 5.15: Example of vesicle collapse in a DC pulse. DOPC vesicle subjected to a 1 kV/cm , 50 ms pulse.

The goal of this chapter is to provide a quantitative description of vesicle behavior in DC fields, thus poration must be avoided. This is done by applying electric fields

that are either weak, such that $E_0 < E_c$ or short enough so that $t_{pulse} < t_{crit}$. The following section demonstrates how a two-step DC pulse can be used to observe the transition in vesicle from oblate to prolate.

5.4.3 Two-step pulse

Vesicle collapse limits the strength and duration for the first pulse and in general there is only a narrow window of pulse parameters which produce the oblate-prolate transition without poration. In this section, the first pulse is kept constant at $E_0 = 100$ kV/m, for a duration of 20 μ s. The field is then decreased to a field strength less than the critical field strength and turned off at $t=50$ ms. The results of this deformation is compared to the theory that includes the two-step pulse.

Examples of the oblate-prolate transition are shown in Figure 5.16. When $t/t_{mm} \sim 1$, both experiment and theory show the vesicle shape transition from an oblate to prolate shape. As with the weak field experiments, the rate of shape change is sensitive to membrane viscosity. The rapid retraction after the field is turned off is observable only at stronger field strengths. Once the membrane has fully discharged, the vesicle relaxes back to a sphere on a timescale governed by the initial membrane tension.

The behavior of a polymer vesicle (PS1) is compared to a DOPC lipid vesicle in Figure 5.16. The two vesicles were subjected to the same pulse strength and duration and were chosen to have similar size, solution conductivities, and initial tension. The parameters for the fits are given in the caption. Polymersomes have a thicker membrane than DOPC membranes, therefore they have a lower membrane capacitance and a higher membrane viscosity. The lower capacitance leads to faster

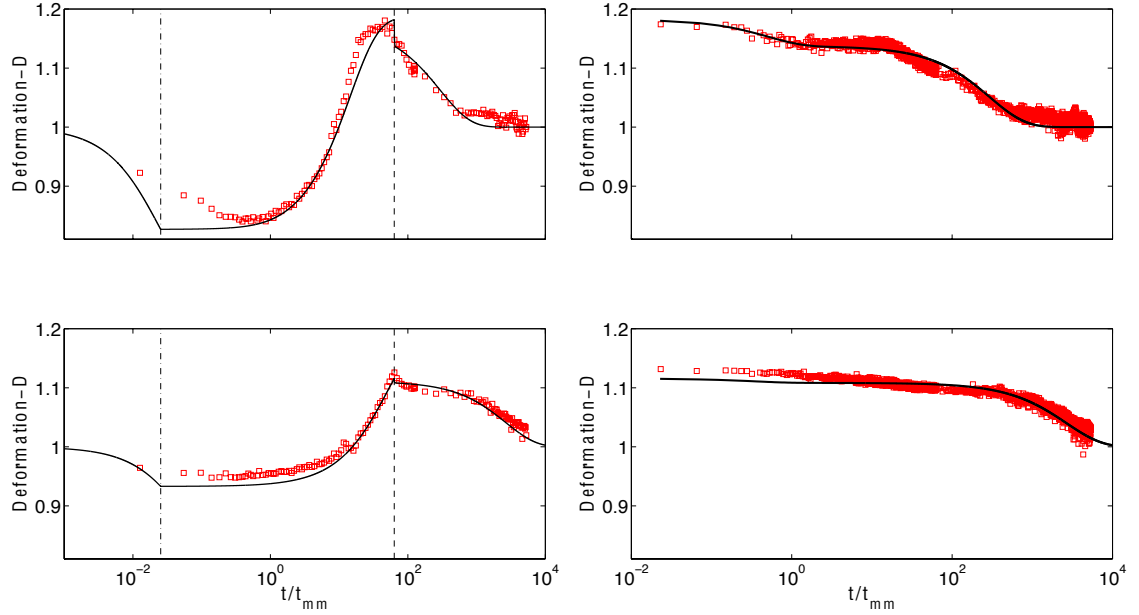


Figure 5.16: Oblate prolate deformation for vesicle. An initial field of strength 400 kV/m for 20 μ s is applied, the field strength is decreased to 20 kV/m for 50 ms. (top) The DOPC vesicle parameters are $a = 20.2\mu\text{m}$, $C_m=0.71 \mu\text{F}/\text{cm}$, $\lambda_{\text{in}}=2 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}}=10 \mu\text{S}/\text{cm}$, $\mu_{\text{mm}}=0.3 \times 10^{-8} \text{ N-s}/\text{m}$, $\sigma_{\text{eq}}=4 \times 10^{-7} \text{ N}/\text{m}$, $\kappa=23 k_B T$. (bottom) The polymer vesicle parameters are $a = 27.2\mu\text{m}$, $C_m=0.27 \mu\text{F}/\text{cm}$, $\lambda_{\text{in}}=2 \mu\text{S}/\text{cm}$, $\lambda_{\text{ex}}=10 \mu\text{S}/\text{cm}$, $\mu_{\text{mm}}=30 \times 10^{-8} \text{ N-s}/\text{m}$, $\sigma_{\text{eq}}=2.5 \times 10^{-7} \text{ N}/\text{m}$, $\kappa=40 k_B T$.

membrane charging and a shorter duration of negative forcing directed towards oblate deformation. The higher membrane viscosity also reduces the rate of shape change and therefore limits the magnitude of oblate deformation. The slower rate of deformation is observed in the oblate to prolate transition as well as a slower relaxation after the field is turned off.

In contrast, the lipid vesicle experiences a faster rate of deformation and a more pronounced oblate shape. This can be attributed to both higher membrane capacitance and lower membrane viscosity. The theory appears to fit well with the exception of the deformation immediately after the field strength is decreased. One possible explanation for this discrepancy is that the membrane has a rate-dependent membrane viscosity for high shear rates. This discrepancy may also be due to errors in matching the image with the starting time for the pulse.

The comparison between the two relaxation curves shows a more dramatic retraction due to discharging for the lipid membrane, see Figure 5.16(right). The higher membrane capacitance allows for more charge to be stored and the duration of the retraction, governed by t_{mm} , is longer. In addition, the lower membrane viscosity reduces the viscous resistance to shape change. A small retraction is possible in the polymer membranes, but is less pronounced unless stronger fields are applied.

The effect of initial membrane tension is compared for two DOPC vesicles in Figure 5.17. The field strengths and durations of the two-step pulse are identical for the two vesicles. The lower membrane tension has little influence on the magnitude of oblate deformation; the lower tension vesicle deforms only slightly more. The membrane tension in the relaxation term of Eq.[5.43] does not become large until the vesicle deforms significantly. The dynamics under small deformations occurs over very short timescale, given by the electrohydrodynamic timescale, and the surface tension timescale is irrelevant.

Both vesicles transition from oblate to prolate at approximately the same time. This is a result of a similar membrane charging timescales, which depends on similar values of radius, membrane capacitance, and solution conductivities. The low tension vesicle deforms slightly more than the high tension membrane, but the effect of membrane tension is more noticeable during the relaxation, see Figure 5.17(right). Both vesicles retract a similar amount due to membrane discharging. As predicted by the model, the membrane tension has little influence on this retraction. The retraction is most sensitive to the t_{mm} timescale and conductivity conditions, both of which are similar in the two vesicles. The effect of membrane tension is dramatically different in the relaxation back to a sphere. The high tension vesicle relaxes back to a spherical shape in about $10^2 \times t_{\text{mm}}$ while the low tension membrane takes almost $10^4 \times t_{\text{mm}}$ to fully relax to a sphere.

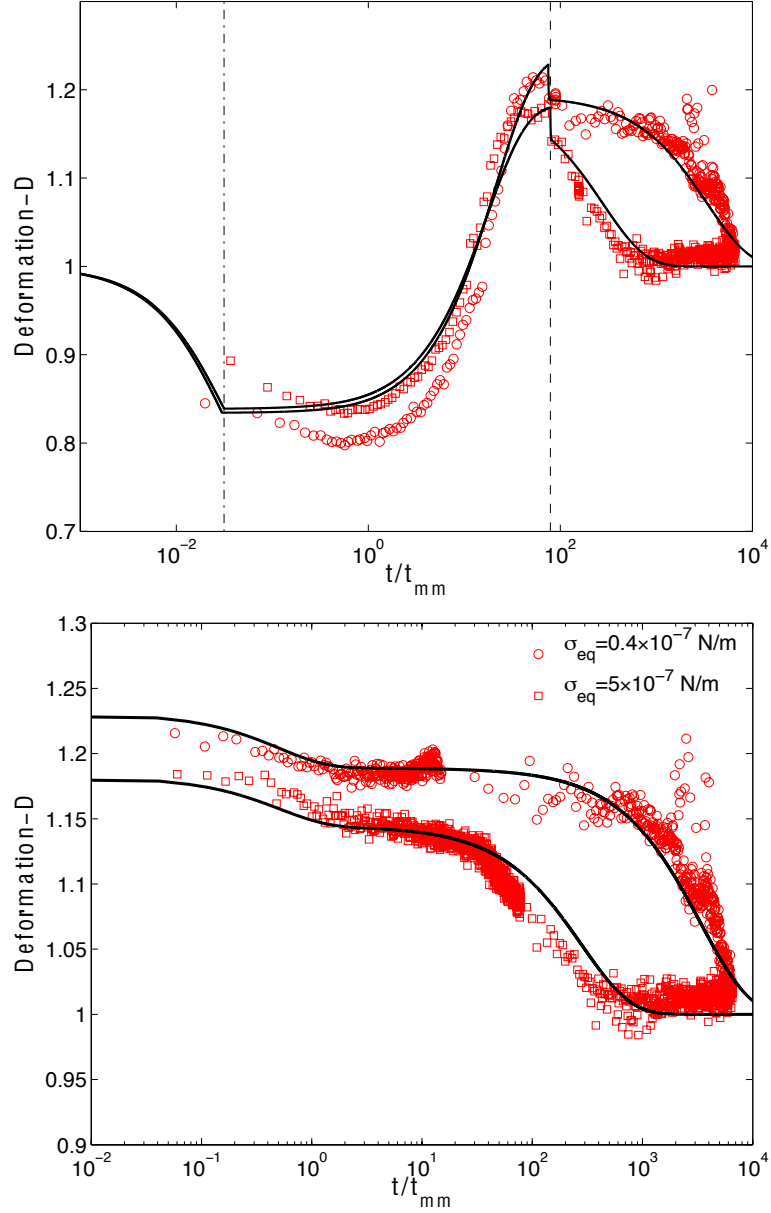


Figure 5.17: Oblate to prolate deformation for two different DOPC vesicle exposed to the same two-step pulse with different initial tensions. The shape deformation for vesicle with a higher initial tension is denoted by squares (□) and the vesicle with a lower initial tension is denoted by circles (○). An initial field of strength 400 kV/m for 20 μ s is applied, the field strength is decreased to 20 kV/m for 50 ms. The high tension DOPC vesicle parameters, radius $a = 19.9 \mu\text{m}$, $C_m = 0.71 \mu\text{F/cm}$, $\lambda_{\text{in}} = 2.5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 0.1 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 5 \times 10^{-7} \text{ N/m}$, $\kappa = 22 k_B T$. The low tension $\circ$ DOPC vesicle parameters, radius $a = 20.9 \mu\text{m}$, $C_m = 0.71 \mu\text{F/cm}$, $\lambda_{\text{in}} = 2.5 \mu\text{S/cm}$, $\lambda_{\text{ex}} = 10 \mu\text{S/cm}$, $\mu_{\text{mm}} = 0.1 \times 10^{-8} \text{ N-s/m}$, $\sigma_{\text{eq}} = 0.4 \times 10^{-7} \text{ N/m}$, $\kappa = 24 k_B T$.

Figure 5.18 compares the deformation of two polymer vesicles exposed to different field strengths for the second step of the pulse. The two vesicles also have different radii; the larger vesicle experience larger deformation. The polymer membranes also

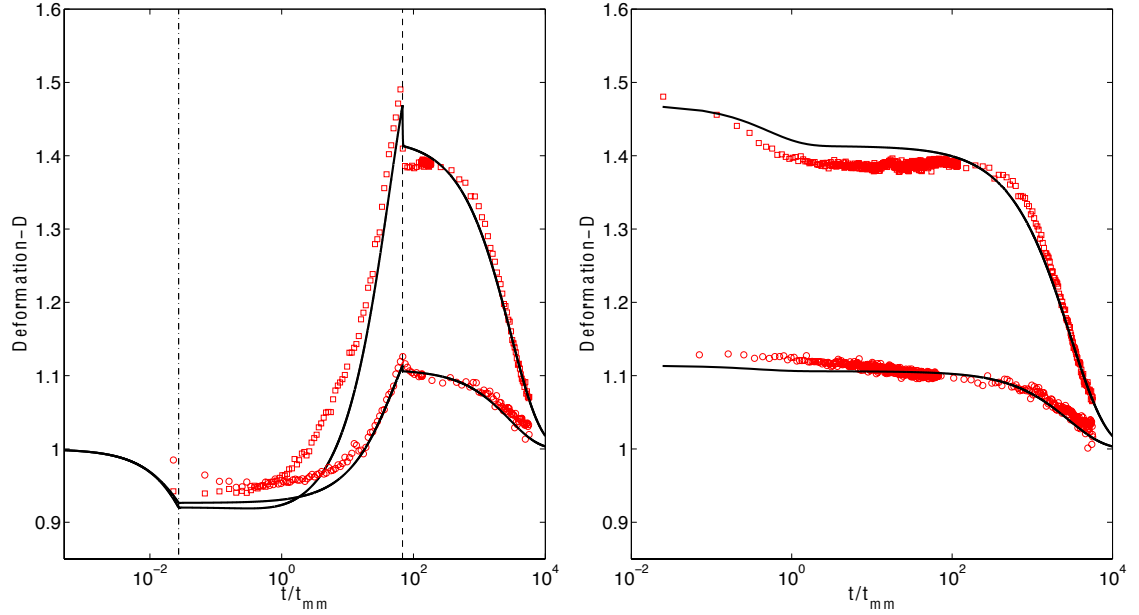


Figure 5.18: Oblate to prolate deformation for two polymer vesicles. An initial field of strength 400 kV/m for 20 μ s is applied, the field strength is decreased to 20 kV/m or 40 kV/m for 50 ms. The polymer vesicle (PS1) parameters are radius 20.9 μ m, $C_m=0.27$ μ F/cm, $\lambda_{in}=0.8$ μ S/cm, $\lambda_{ex}=10$ μ S/cm, $\mu_{mm}=20 \times 10^{-8}$ N-s/m. $\sigma_{eq}=1.5 \times 10^{-7}$ N/m, $\kappa=4 k_B T$. The polymer vesicle (PS1) parameters are radius 27.2 μ m, $C_m=0.27$ μ F/cm, $\lambda_{in}=1.5$ μ S/cm, $\lambda_{ex}=10$ μ S/cm, $\mu_{mm}=30 \times 10^{-8}$ N-s/m, $\sigma_{eq}=2.1 \times 10^{-7}$ N/m, $\kappa=30 k_B T$.

have a higher critical voltage for poration than lipid membranes. This allows the stronger field to be used for the experiments shown in Figure 5.18. The strong field leads to a pronounced retraction due to discharging, even though the membrane capacitance is less than half the value of lipid membrane capacitance. The model shows a similar magnitude of retraction, although the large deformations make a quantitative comparison difficult.

As with the AC field experiments, the fit for the larger vesicle uses a low value for bending rigidity. The low value of bending rigidity, $\kappa = 4k_B T$, is likely due to a surplus of excess area caused by deformation not accounted for by Eq.[4.66]. If large deformations are avoided by using weaker fields and higher tension vesicles, the theoretical model shows good quantitative agreement with accurate values for bending rigidity.

5.4.4 Phase diagram

A phase diagram is constructed to demonstrate the effect of pulse strength and duration on vesicle response. The phase diagram shows the behavior in a two-step pulse. The first pulse is varied in strength and duration while the second pulse is the same for all experiments. The second pulse is below the critical field ($E_0 < E_c$) with a duration much longer than t_{mm} . The purpose of these experiments is to compare vesicle behavior with two timescales, t_{hd} and t_{crit} (Eq.[5.4] and Eq.[5.14]), which predict the rate of deformation and the critical limit for poration respectively.

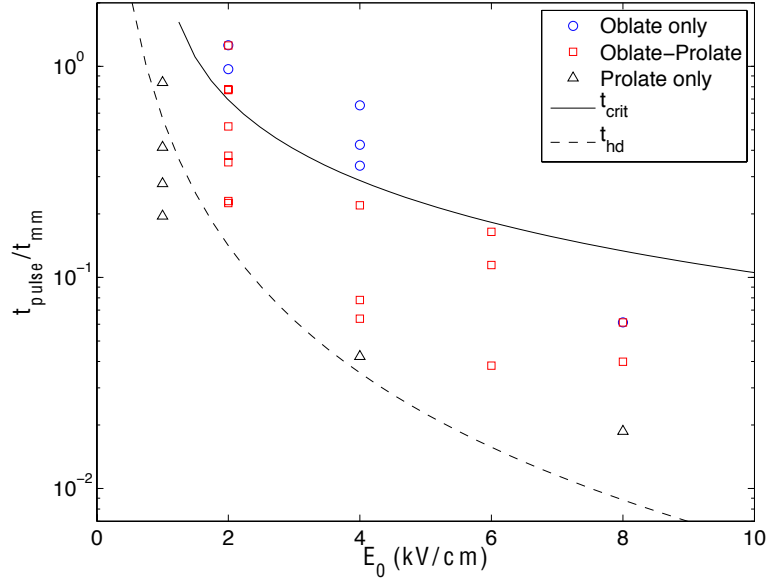


Figure 5.19: Vesicle behavior as a function of the duration and field strength of the first pulse, the duration of the first pulse is normalized by the capacitor charging time t_{mm} . The second pulse is set at $E_0 = 20kV/m$ for $50\mu s$. Three outcomes are observed, only prolate deformation, the transition from oblate-to prolate, and only oblate deformation. The oblate only deformation occurs when the vesicle has porated the membrane capacitor is short circuited. The comparison with the timescale t_{crit} is shown with $E_c = 1kV/cm$. The comparison with t_{hd} is shown normalized with $t_{mm} = 0.5ms$.

The results in Figure 5.19 show the crossover from prolate-only to oblate-prolate corresponds with the t_{hd} timescale. When the vesicle experiences a field with sufficient strength and duration to overcome viscous forces at short times, the oblate-prolate transition will be visible.

The t_{crit} timescale is compared with the crossover between oblate-prolate and oblate-only behavior. If the entire membrane became perfectly conducting when pores formed, there should only be oblate deformation because the membrane capacitor is short-circuited. The observation of oblate only deformation indicates that the pores are sufficiently large to short-circuit the membrane. Poration also occurs during some of the oblate-prolate results, which indicates that the presence of some pores may not result in enough conduction to alter the vesicle dynamics. In this case, the capacitance of the membrane is intact and the charged part of the membrane pulls the vesicle into a prolate shape. A critical field strength of $E_c = 1\text{kV/cm}$, which corresponds to a $V_c = 3V$ for a 20 micron vesicle, is used to plot t_{crit} in Figure 5.19. Note that t_{crit} is determined from the transmembrane potential at the pole of the vesicle where the critical transmembrane potential is reached first. A stronger field is required to produce transmembrane conduction over enough membrane area such that the vesicle will not return to prolate.

5.5 Conclusions

This chapter provides a quantitative comparison between theory and experiment for the deformation of vesicles in DC fields. The capacitive charging of the membrane is shown to regulate the shape evolution. A theoretical model is modified from previous work to consider changes in the applied electric field strength [208, 177]. In addition, a description of vesicle dynamics after the field is turned off is developed and shown to agree with experiments. The shape evolution of vesicles in DC fields is also shown to be sensitive to membrane properties, particularly membrane viscosity.

The behavior of vesicles made from two types of membranes are compared in DC

pulses, DOPC lipid membranes and polymer (PS1) membranes. The values used to fit DOPC membranes are within the range $\mu_{\text{mm}} = 0.1-0.3 \times 10^{-8}$ Ns/m. These values agree with previous measurements of lipid membrane viscosity [46]. The low value for DOPC membrane viscosity makes the contribution to viscous resistance negligible compared to the bulk viscosity. As a result, small changes in lipid membrane viscosity will not dramatically change vesicle dynamics. In contrast, the polymer membrane (PS1) vesicles have a much higher membrane viscosity, $\mu_{\text{mm}} = 30 \times 10^{-8}$ Ns/m. This results in a dominant contribution to viscous resistance and a much slower response to the applied electric field compared with DOPC membranes. Like AC fields, DC pulses can also be used to determining membrane viscosity, although a single weak pulse is better suited as a measurement method compared to the two-step pulse where there are rapid changes in vesicle shape.

The theory can be used to accurately describe the shape deformation in a two-step pulse, which is used to produce an oblate-prolate deformation. A strong initial pulse is needed to produce significant deformation before the membrane capacitor charges, producing either oblate or prolate deformation depending on the conductivity ratio. The step down in field strength is required to avoid membrane poration, which can lead to conduction across the membrane and a short-circuiting of the membrane capacitor. Without poration, the weaker field pulls the vesicle, now with a fully charged membrane, into a prolate shape.

A new shape retraction due to the capacitive discharging of the membrane is observed in experiments for vesicles with conductivity ratio $R < 1$ and described by theory. The retraction is sensitive to the amount of charge stored by the membrane capacitor. The amount of charge is greater if either membrane capacitance is higher or the applied field is stronger. As with charging, the duration for the discharging force is regulated by the membrane capacitor timescale, t_{mm} . If this timescale is

longer, the force during discharging will act over a longer period of time and lead to greater retraction.

The behavior of vesicles in DC fields is well described by an electrohydrodynamic model with a capacitive interface. The assumption of a non-conducting membrane capacitor works well in weak fields, but the application of a strong field produces membrane pores which can lead to conduction. This motivates the question: how do membrane pores affect vesicle dynamics? The next chapter investigates this question using the approach introduced here.

5.6 Future Directions

Effect of vesicle dynamics from ion channels

Ion channels are transmembrane proteins which are responsible for many cell processes, such as signaling and the regulation of cell functions [2]. For instance, nerve cells open and close ion channels in order to transmit electric signals along the cell [74]. GUVs with active proteins offer a simpler model than real cells to study the activity of ion channels [119, 120, 60]. The effect of transmembrane current, which can arise from the presence of ion channels, has been studied theoretically [229, 230]. The effect of ion channels on vesicle dynamics may give insight into the response of different types of ion channels in the presence of electric fields. The dynamic response of the vesicle could be compared to the same model developed in this chapter, but with the addition of a transmembrane current [208].

Wrinkling of polymer membrane

The wrinkling of polymer vesicles was observed in strong DC pulses. The wrinkling is similar to the wrinkling behavior seen in DPPC gel phase vesicles [99]. DPPC vesicles are known to have shear elastic behavior that can induce wrinkling. The polymer vesicles have recently been shown to have some shear elastic behavior [100]. The viscoelastic behavior of polymer vesicles may also influence the polymer vesicle behavior in flow. Investigating behaviors such as membrane wrinkling may give insight into the rheology of polymer membranes and dynamics of polymer vesicles.

CHAPTER SIX

Cylindrical deformation and vesicle collapse in DC electric pulses

6.1 Introduction

The transport of molecules across a cell membrane is required for developing medical technologies such as gene transfection, where a series of electric pulses produce transient pores in the cell membrane, allowing foreign DNA to be delivered into the cell. The charging of the membrane capacitor produces a compressive stress on the membrane which leads to the formation of pores. It has also been shown that a conducting membrane becomes unstable in an electric field [183], although remains poorly understood [56, 156].

During electroporation, a pore is formed in a cell membrane through the stress created by a pulsed DC electric field [161]. Ideally, the pore will reseal after the field is turned off and the cell will remain intact. An example of a pore forming and

resealing after a pulsed electric field is shown in Figure 5.1(top). The dynamic process of formation and resealing for an individual pore has been studied theoretically and in experiments [95, 155]. The models have been shown to accurately describe the dynamics of a single pore, which is created by a uniform tension, due to osmotic pressure and micropipette aspiration [170].

Nonuniform tension and multiple pores have not been analyzed and hence the process of pore formation is not well understood [155]. The poration of vesicles in electric fields has been investigated for short DC pulses [161], and the process of pore resealing has been studied after an applied pulse [155]. A vesicle in short strong DC pulses was observed to adopt a cylindrical shape deformation [162]. The cylindrical shapes were observed when salt was included in the suspending media, although a conclusive explanation of the cylindrical deformation behavior was not determined.

An explanation for the collapsing and cylindrical deformations motivate the study of vesicles in strong DC pulses. Membrane poration is proposed as the underlying cause for both behaviors. GUV assembled from lipids or amphiphilic block copolymers are used to study the effect of poration on vesicle dynamics. Observations of membrane dynamics in a pulsed DC electric field are made using phase contrast microscopy and recorded with a high speed camera [163, 161, 162]. The behavior of vesicles with conductivity ratio $R < 1$ are investigated in strong fields, i.e. field strengths that produce transmembrane potentials above V_c , Eq.[5.13]. Field strengths similar to those used in previous studies are applied, although pulse durations are extended beyond the $300\mu s$ pulses previously used [163, 161, 162]. The evolution of the vesicle is studied for different values of field strength and membrane composition.

Two dynamical features of poration are investigated in detail. Strong DC pulses

are shown to produce vesicle collapse as seen in Figure 6.2. This behavior is related to membrane poration and breakdown of the membrane structure under a large electric stress. Additionally, membrane poration is shown to produce visible corners in the membrane and result in the cylindrical deformation previously observed [162]. The corner location, which measures the degree of squaring, correlates with the critical transmembrane potential.

6.2 Vesicle collapse: observations

The same experimental procedure described in Chapter 5 is followed. A vesicle is subjected to a single DC pulse and the deformation is recorded with a high speed camera at 30,000 frames per second. In contrast to Chapter 5, the field strength is increased to produce transmembrane voltages that exceed the critical transmembrane potential, V_c . For conductivity ratios greater than one ($R > 1$), the vesicles deform into prolate and pores form at the poles, as described in [161, 162]. Vesicles with conductivity ratios less than one ($R < 1$) are observed to collapse rather than simply opening pores.

Collapsing behavior is seen for both lipid and polymer membrane systems. Lipid vesicles, which have a low poration threshold, collapse in lower field strengths. Lipid vesicles also collapse faster due to their lower membrane viscosity. In contrast, polymer vesicles collapse more slowly due to their higher membrane viscosity and at a higher field strength. The character of the collapse also changes with field strength. Examples of the different collapsing behavior are shown in Figures 6.1, 6.2, and 6.3.

Figure 6.1 shows vesicle collapse in a relatively weak field as the two regions

around the poles of the vesicle come towards each other. At 0.4ms, the region near the pole appears to have flattened, while the rest of the vesicle appears undisturbed. A sharp edge is observed at the end of this flat region. The vesicle continues to deform further until the two sides of the vesicle interact. After the vesicle has fully collapsed, the vesicle can either be completely destroyed or it can reform. When the vesicle is still intact, it can take about 1-10s to completely reform, see Figure 6.1. After the vesicle reforms, the phase contrast across the membrane is reduced as a result of mixing between interior and exterior fluids. This process indicates that the membrane pores do not grow large enough to break up the membrane, but the membrane has become porous, allowing the exchange of fluid and molecules across the membrane.

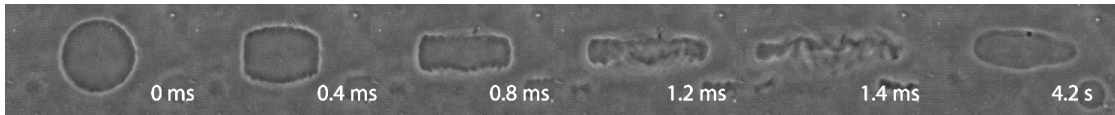


Figure 6.1: POPC vesicle in a $E_0 = 2kV/cm$ 2ms DC pulse. The vesicle fully collapses but is able to reform into a vesicle. The phase contrast decreases after it reforms.

At an increased field strength, seen in Figure 6.2, the character of collapse changes. An edge appears near the equator and the region appears to buckle. The region near the equator is expelled away from the vesicle center as the top and bottom hemispheres of the vesicle compress towards each other. The timescale for collapse is much shorter in the strong field, the entire process occurs in less than $200\mu s$ compared to 1-4ms in the weak fields. The vesicle can also reform after the strong field is turned off, as long as the pulse is turned off immediately after collapse. If the field is left on for a long time after collapse, the vesicle was not observed to reform.

Vesicle collapse is also observed for polymer membranes, although at stronger field strengths than for lipid membranes. The initial deformation for polymer vesicles

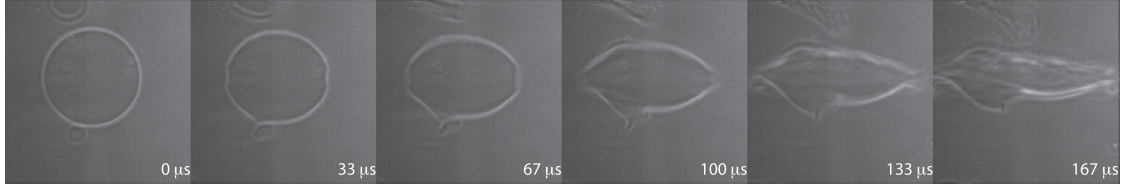


Figure 6.2: Examples of lipid vesicle collapse in a DC pulse. The vesicle radius is $a = 18.4\mu m$ and the applied DC electric pulse is $E_0 = 8kV/cm$ and duration $150\mu s$.

is similar; the pole regions of the vesicle flatten producing a cylindrical deformation. Although in the polymer case, the vesicle reaches a highly deformed shape and remains in a similar shape before eventually collapsing. This period of pausing can be seen from about $300 - 600\mu s$.

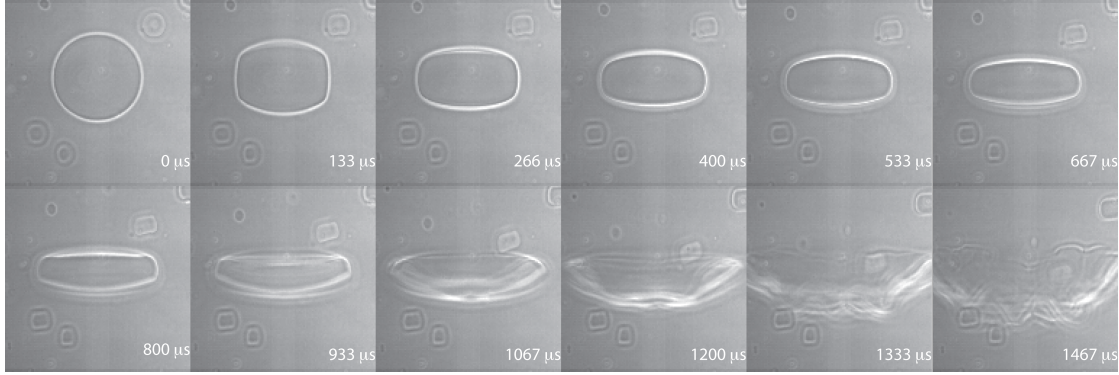


Figure 6.3: Polymer vesicle pausing between $266\mu s$ and $667\mu s$ (PS1 radius $18\mu m$) in $E_0 = 4kV/cm$ $5ms$ DC pulse.

The higher molecular weight systems, PS2 and PS3, require very strong fields to produce collapsing, $E_0 > 6kV/cm$. Just below this field strength, the vesicle can deform and remain in oblate deformation. This indicates that the membrane has porated, otherwise the fully charged state would result in prolate deformation, but the fact that the vesicles do not collapse suggests that even though the membrane has porated, the membrane structure is strong enough to balance the electric stress.

The higher molecular weight systems, PS2, PS3, and PS4, can also rotate before collapsing. A sequence of rotation for a PS3 vesicle is shown in Figure 6.4. The vesicle deforms, then rotates 3-4 times before eventually collapsing. This behavior appears

to be similar to the rotation of polymer vesicles seen at intermediate values in AC fields. The vesicles also translate in strong fields, which is likely due to gradients in the electric field. As a result, the vesicle may leave the field of view before collapsing.

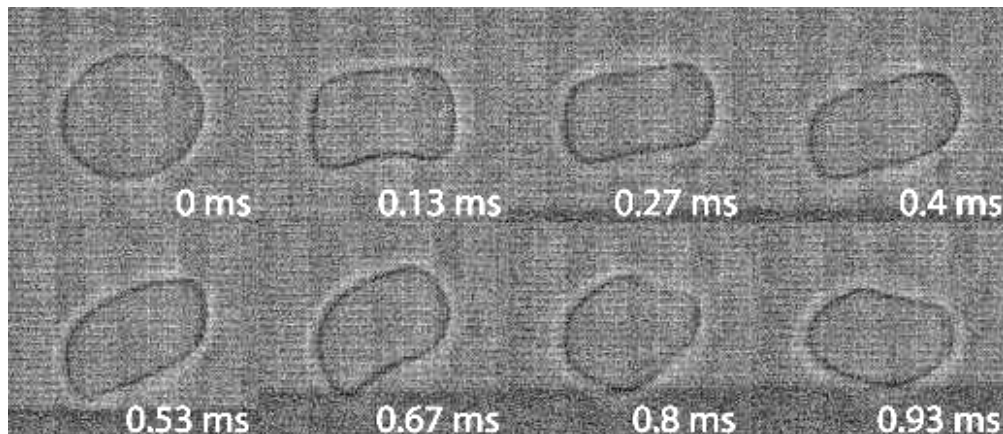


Figure 6.4: Rotation of a PS3 vesicle rotating in a 6 kV/cm DC field.

6.2.1 Rate of deformation

The influence of membrane poration can be investigated by recording vesicle deformation before the vesicle collapses. Plots of deformation for POPC and PS1 systems are shown in Figure 6.5. Time is normalized by t_{hd} . The collapsing of the different experiments indicates that the capacitive charging of the membrane does not significantly influence the collapsing dynamics. The last point plotted is the last image where the interface can be reliably detected.

The pausing behavior of polymer vesicles can be seen as a plateau in Figure 6.5(bottom). The length of time over which the pausing occurs varies, but usually begins after $t > 50t_{\text{hd}}$ and can stay in a similar shape until about $100 - 200t_{\text{hd}}$. The rate of deformation is also slower than lipid vesicles. Lipid vesicles collapse within $20t_{\text{hd}}$ while polymer vesicles take $\sim 50t_{\text{hd}}$ to reach a paused state and then $\sim 200t_{\text{hd}}$ to fully collapse.

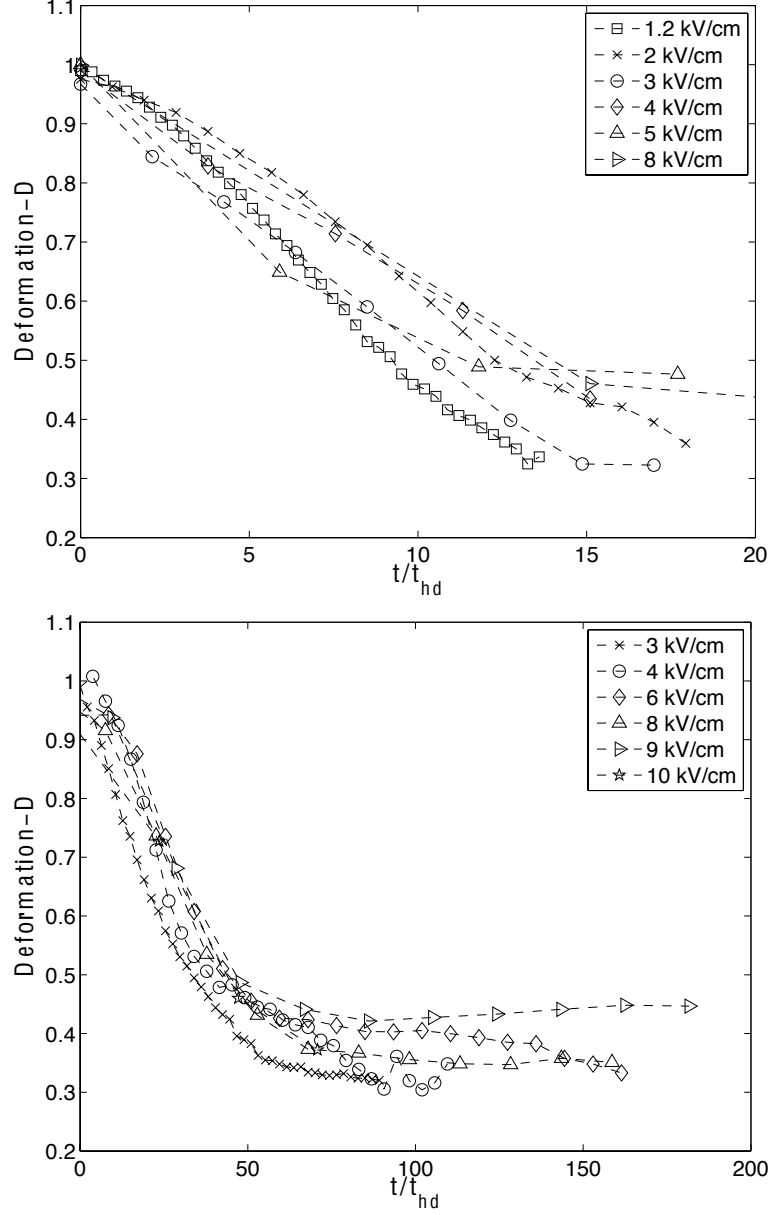


Figure 6.5: Deformation as a function for t/t_{hd} for POPC and polymer (PS1) vesicles. The rate of deformation scales as E_0^2

The rate of deformation for lipid and polymer systems are compared in Figure 6.6. The initial slope before pausing is taken for the polymer vesicles. The rate of collapse is about 2-3 times faster for the lipid vesicles than PS1 vesicles. This difference in rate can be explained by considering the viscous term in C_{20} given by $32 + 23\Lambda + 16\Lambda_s$. Using the membrane viscosity values determined from Chapters 4 and 5, lipid membranes were found to have membrane viscosity values $\mu_{mm} \sim$

$0.1 - 10 \times 10^{-8} \text{Ns/m}$ and PS1 was found to have $\mu_{\text{mm}} \sim 30 \times 10^{-8} \text{Ns/m}$. This gives proportionally different values of $\Lambda_s \sim 0.1 - 5$ (lipid) and $\Lambda_s \sim 15$ (PS1). The magnitude of the viscous term for PS1 is about 2-3 times greater than POPC, which explains the different rates of deformation seen in experiments.

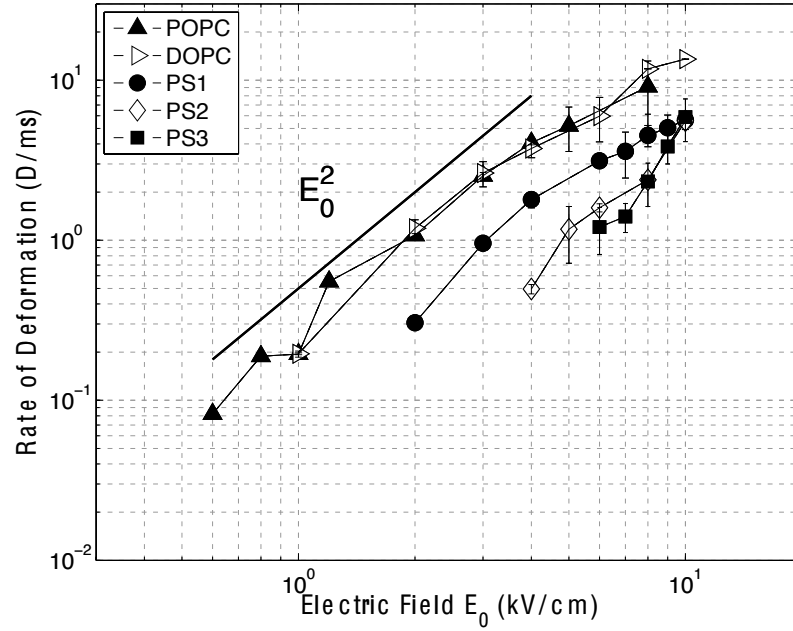


Figure 6.6: Deformation as a function for t/t_{hd} for lipid and polymer vesicles. The rate of deformation scales as E_0^2

Two main features of the vesicle behavior are investigated in detail in the following sections. First, the deformation parameter, defined by the ratio of the two main axes, is compared to a theoretical model based on the theory developed in Chapter 5, but is modified to include the effect of membrane poration. Second, membrane poration is proposed as an explanation for the squaring behavior seen in DC strong pulses. The location of the edge observed in experiments is compared to the angle corresponding to a transmembrane potential exceeding V_c .

6.2.2 Comparison with simple interfaces

The theoretical model considered is identical to Chapter 5 except that a purely conducting rather than a capacitive membrane is considered. For a conducting membrane, the vesicle will act similarly to a fluid drop, which lacks interfacial capacitance. In the case of a drop, deformation will be oblate for $R < 1$ and reach an equilibrium oblate shape when balanced by surface tension. In the case of the vesicle, because the membrane has porated, the integrity of the membrane is likely compromised. Once a pore has formed, the membrane area can no longer be assumed as constant and the expression for tension Eq.[4.70] can no longer be considered valid. The vesicle eventually collapses as the membrane is free to stretch as the pores expand.

Once a pore nucleates, membrane tension acts to pull the pore open while membrane viscosity resists the in plane motion. The evolution of pore radius can be described by the timescale [95],

$$t_{\text{pore}} \sim \mu_{\text{mm}}/\sigma. \quad (6.1)$$

The typical values of membrane tension that induce poration are of the magnitude $\sigma \sim 10^{-2}\text{N/m}$ [9]. The values for membrane viscosity can vary significantly from $\mu_{\text{mm}} \sim 10^{-9}\text{Ns/m}$ for lipids to $\mu_{\text{mm}} \sim 10^{-6}\text{Ns/m}$ for polymers. This implies that the timescale for pore opening can vary from $t_{\text{pore}} \sim .1 - 100\mu\text{s}$. Thus, the pore can open quite quickly for lipid membranes once the critical field is reached, but might require longer time for more viscous membranes. Note that this timescale is based off the dynamics of an individual pore. If multiple pores nucleate in a region of the membrane, the pores may release the tension quickly and remain small. In addition,

conduction through the membrane will reduce the transmembrane potential in the membrane regions surrounding the pore and therefore reduce the compressive stress across the membrane capacitor. Therefore, the timescale t_{pore} is likely only valid for a short time when the membrane tension is still high.

The observations in Section 6.2 suggest that pores form when the transmembrane potential exceeds the critical value, V_c , but do not expand to the point where they are visible in phase contrast. The deformation, which occurs on $t_{\text{hd}} \sim 10^{-5}\text{s}$, is likely slower than the time for pores to open and allows conduction. Although the deformation may be faster than the time needed to grow beyond the nanometer scale. Following this argument, a membrane composed of many pores will rapidly lead to conduction across the membrane. In the limit of a perfectly conducting membrane, i.e. no electric resistance across the membrane, the transmembrane potential becomes zero, $V_m = 0$.

To investigate the influence of membrane pores theoretically, the entire membrane is considered conducting. Two hypothesis for membrane tension are tested. First, the membrane is assumed to obey the constant area constraint and remain intact and incompressible. Second, the membrane tension, and the entire relaxation term, is neglected. These two cases are plotted in Figure 6.7. A theoretical model can only be considered a qualitative comparison due to the large deformations seen in experiment.

In the limit of a conducting interface, the boundary conditions change to a continuous potential field across the interface,

$$\Phi_{\text{in}} - \Phi_{\text{ex}} = 0. \quad (6.2)$$

Electric current is conserved across the interface,

$$\mathbf{n} \cdot (\lambda_{\text{ex}} \mathbf{E}_{\text{ex}}) - \mathbf{n} \cdot (\lambda_{\text{in}} \mathbf{E}_{\text{in}}) = 0. \quad (6.3)$$

As before, it is assumed that charges accumulate at the interface instantaneously. This assumption is valid if $t_{\text{mw}} < t_{\text{hd}}$. Even in strong fields with $E_0 \sim 1 - 5 \text{ kV/cm}$, the EHD timescale, $t_{\text{hd}} \sim 10^{-6} \text{ s}$, is still slower than the Maxwell-Wagner polarization time, $t_{\text{mw}} \sim 10^{-7} \text{ s}$.

In the limit of strong fields, the transmembrane potential will reach the critical value before the membrane is fully charged. This is seen by comparing the timescales t_{crit} and t_{mm} . For a field $E_0 \sim 1 - 5 \text{ kV/cm}$, the timescale for critical field is $t_{\text{crit}} < 0.1 t_{\text{mm}}$. This shows that even before the membrane porates, the displacement current through the membrane is significant due to membrane charging. This demonstrates that the vesicle behaves as a drop before and after pores form in the membrane.

These assumptions lead to the boundary conditions, Eq.[6.2] and Eq.[6.3], which are analogous to the boundary conditions for a drop with instantaneous charging. The electric field is solved with these boundary conditions, which corresponds to the same solution given in the Chapter 5 with $V_m = 0$. An alternative description is to consider an interface with infinite capacitance, which implies that the membrane will never fully charge. By setting $C_m \rightarrow \infty$, the forcing term C_{20} is easily modified to reflect a simple fluid-fluid interface.

This analysis only changes the forcing term in the evolution equation. The

expression simplifies to,

$$C_{20} = 36\sqrt{\frac{\pi}{5}} \left(\frac{(1+R)^2 - 4S}{(32 + 23\Lambda + 16\Lambda_s)(2+R)^2} \right). \quad (6.4)$$

This replaces the forcing term in the evolution equation derived in Chapter 5. The equation is rewritten as,

$$\delta_m \frac{\partial f_{20}}{\partial t} = C_{20} - Ca_\kappa^{-1} M_2 f_{20}, \quad (6.5)$$

where,

$$Ca_\kappa = \frac{a^3 \epsilon_{ex} E_0^2}{\kappa}, \text{ and } M_2 = \frac{24(6 + \tilde{\sigma}_h)}{32 + 23\Lambda + 16\Lambda_s}. \quad (6.6)$$

Note that $\tilde{\sigma}_h = \sigma_h a^2 / \kappa$ is also a constant if C_{20} is constant with time. σ_h is determined by Eq.[4.70], which assumes that the membrane area is conserved.

Without any dependence on time in C_{20} , an explicit equation for f_{20} can be determined,

$$f_{20} = \frac{C_{20} Ca_\kappa}{M_2} \left(1 - \exp \left[-\frac{M_2}{Ca_\kappa \delta_m} t \right] \right). \quad (6.7)$$

If the effect of membrane tension is neglected due to the effect of poration, the solution for f_{20} simplifies further,

$$f_{20} = \frac{C_{20}}{\delta_m} t. \quad (6.8)$$

In both expressions for f_{20} , the rate of deformation will increase with field strength and decrease with higher membrane viscosity, see Eq.[6.4]. The expression that includes membrane tension, Eq.[6.7], will saturate at a value determined by membrane tension. The expression without tension, Eq.[6.8], will continue to deform indefinitely. Because this is a small deformation theory, this can only be considered

valid within a limited range of deformation.

Three cases are considered in Figure 6.7 and compared to experiments for lipid and polymer vesicles. The first uses the capacitance model derived in Chapter 5. The second keeps the assumption of constant membrane area and uses the forcing term for a porated membrane, Eq.[6.7]. The third neglects any restoring force of membrane tension, this also is the limit of $Ca \sim \infty$ where electric stresses greatly outweigh restoring stresses from the membrane. The results in Figure 6.7 show that the experiment most closely follows the model without membrane tension. This indicates that the electric stress greatly exceeds the restoring stress from membrane tension. The resistance to deformation results only from viscous dissipation, which increases with higher membrane viscosity. This results in a slower response for polymer vesicles in comparison with lipid vesicles.

The pausing exhibited by polymer vesicles may indicate a period where the membrane is stretching and the membrane provides enough resistance through tension to prevent immediate collapse. Although eventually the electric stress destabilizes the membrane and causes vesicle collapse. The large deformations and membrane stretching prevent a quantitative comparison, but deformation of the polymer vesicles shows similarity to the conducting membrane with tension shown in Figure 6.7.

The deformation before collapse is shown at three different field strengths for lipid and polymer vesicles in Figure 6.8. The deformation is fit with Eq.[6.8] using identical parameters for the lipid and polymer experiments, only vesicle size and electric field strength are varied. The deformation for lipid vesicles before collapse demonstrates the trend of faster collapse at higher field strengths. The largest deviation from theory is seen at low field strengths where the experiment shows

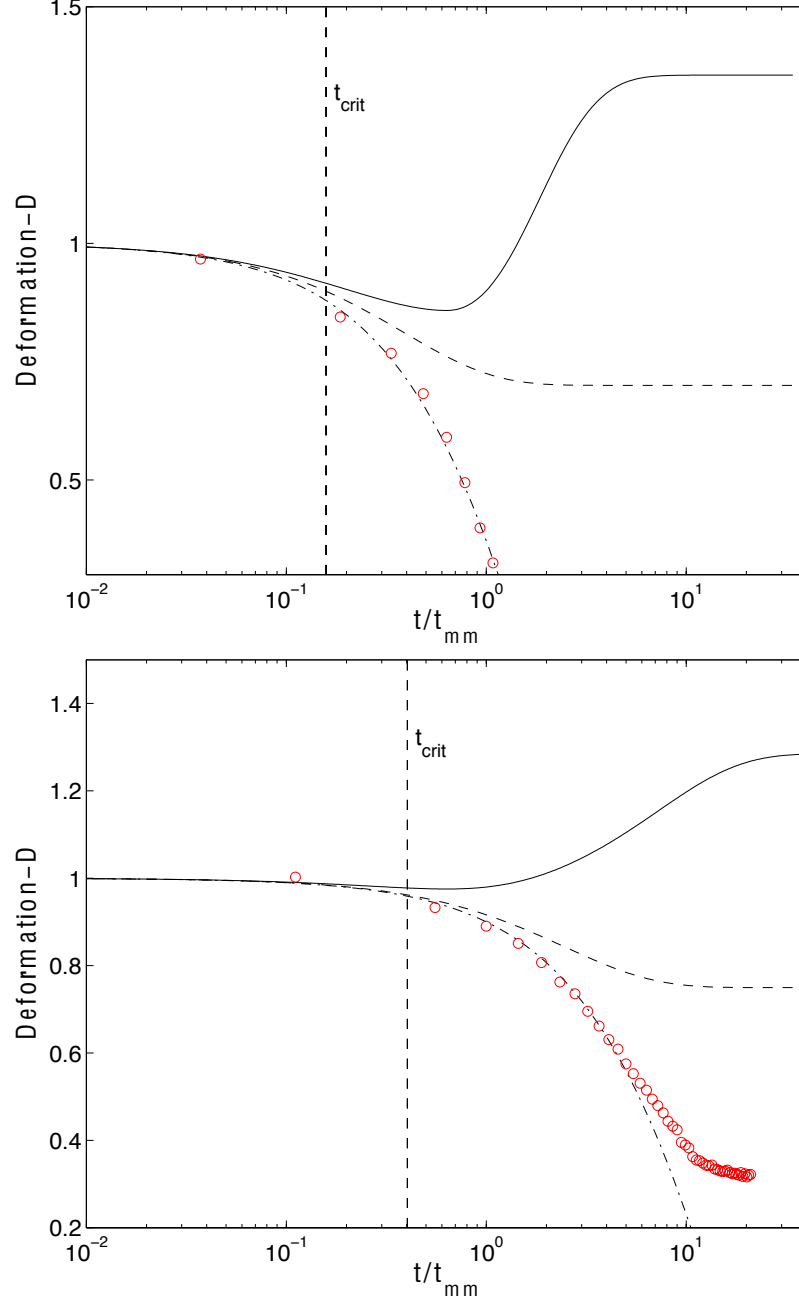


Figure 6.7: Collapse of a POPC vesicle (top) and Polymer-PS1 vesicle (bottom) in a single DC pulse. The symbols are experimental data. The solid line corresponds to the model with capacitance, the dashed line is the model of a highly conducting membrane, the dash-dot line is the model with a conducting membrane and no membrane tension. The electric field is turned on at $t = 0$ for a duration of $10ms$. The strength of the field for both experiments is $3kV/cm$. The POPC vesicle is fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $C_m = 0.71 \mu F/cm^2$, $a = 15.2 \mu m$, $\kappa = 20k_bT$, $\sigma_{in} = 5.0 \mu S/cm$, $\sigma_{ex} = 68 \mu S/cm$. The PS1 vesicle is fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$, $\mu_{mm} = 30 \times 10^{-8} Ns/m$, $C_m = 0.27 \mu F/cm^2$, $a = 13.4 \mu m$, $\kappa = 30k_bT$, $\sigma_{in} = 5.0 \mu S/cm$, $\sigma_{ex} = 68 \mu S/cm$.

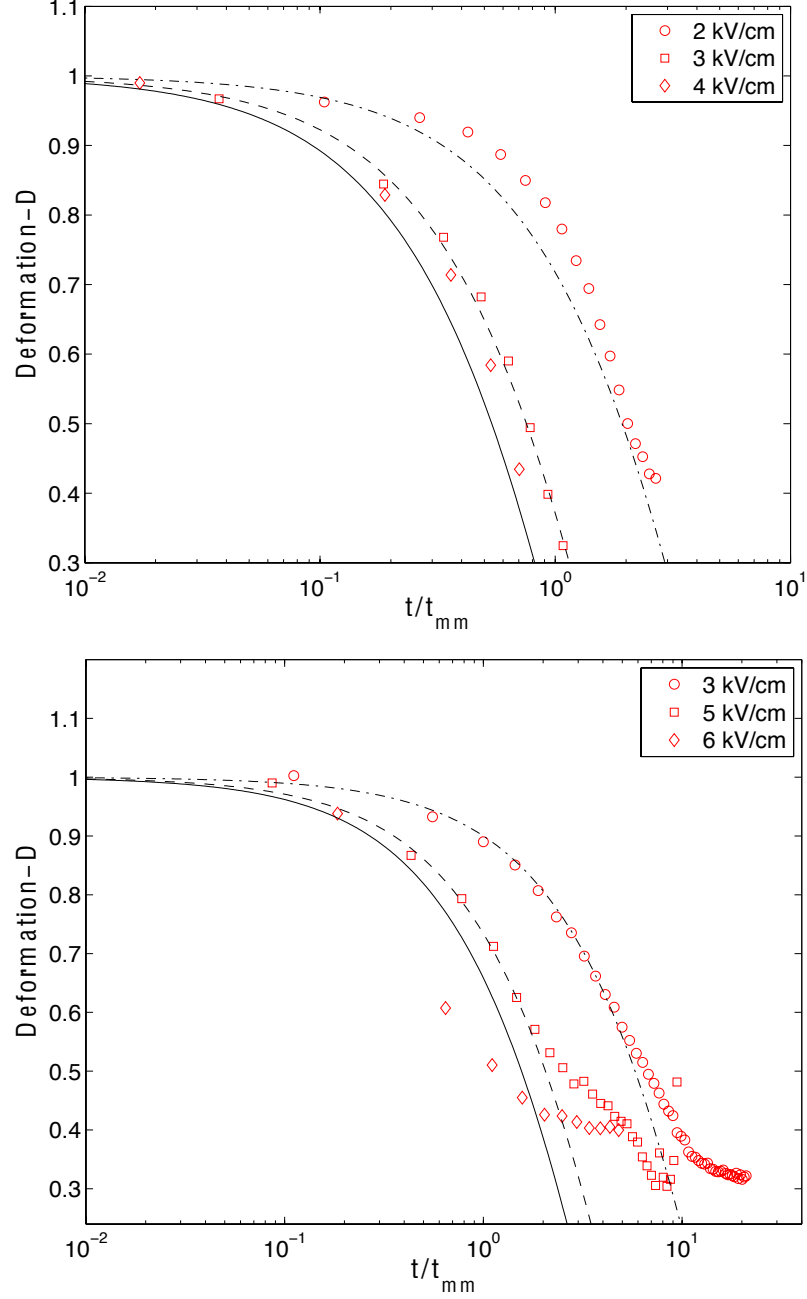


Figure 6.8: Collapse of a POPC (top) and polymer (PS1) (bottom) vesicles at different field strengths. The lines show the results from the evolution equation Eq.[6.8] for different field strengths. (left) The solid line corresponds 2 kV/cm, the dashed line is 3 kV/cm, the dash-dot line is 4 kV/cm. The vesicle is fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$ membrane viscosity $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $a = 14.1, 15.2, 13.2 \mu m$. (right) The solid line corresponds 3 kV/cm, the dashed line is 5 kV/cm, the dash-dot line is 6 kV/cm. The PS1 vesicles are fit with $\sigma_{eq} = 1 \times 10^{-7} N/m$, $\mu_{mm} = 10 \times 10^{-8} Ns/m$, $a = 13.7, 17.2, 12.9 \mu m$. The conductivities for both experiments are $\sigma_{in} = 5.0 \mu S/cm$, $\sigma_{ex} = 68 \mu S/cm$

slower deformation than predicted. This may be a result of the membrane capacitor needing a longer time to reach the critical transmembrane potential in weaker fields. Until the membrane porates, the capacitor is still intact and the collapse is delayed. The deformation of polymer vesicles before collapse shows fairly good agreement until pausing, see Figure 6.8(bottom). The largest deviation is seen at the highest field strength, where deformation is very fast and errors can result from the rapid deformation.

The agreement with theory supports the assumption that the collapse is a result of membrane poration. The experiments also suggest that the membrane pores remain small while the vesicle deforms. This is likely due to the reduction in tension due the disturbance in the field created by the pore. Although enough tension probably remains to prevent resealing. Contribution to membrane tension from the systematic deformation of the vesicle, σ_h , may also prevent the pore from resealing.

6.3 Cylindrical deformation from membrane poration

As demonstrated in the previous section, membrane poration can significantly change vesicle dynamics. Cylindrical deformation is apparent in the vesicle shape before collapse in both lipid and polymer experiments, see Figure 6.2. The model described in the previous section assumes that the entire membrane is conducting and the transmembrane potential is zero over the entire vesicle. Although this assumption appears to capture the ellipsoidal deformation and collapse of the vesicle, it is unlikely that poration occurs at all locations on the vesicle. Rather, poration is likely to occur only in regions of the vesicle where the transmembrane potential exceeds V_c .

As seen in Eq.[5.26], the transmembrane potential, V_m , for a spherical capacitor varies with position by $\cos(\theta)$. As a result, for all field strengths where $V_m(\theta = 0) > V_c$, there will be a region around the poles where $V_m > V_c$ and a region around the equator where $V_m < V_c$.

In the regions where the membrane has porated, the membrane capacitance has been short circuited by a membrane conductance. A completely short-circuited interface acts similarly to a fluid-fluid interface. In this case, the ratio of interior to exterior charging timescales determines the net charging of the interface. For $R < 1$, the forces are directed inward toward the center of vesicle while for $R > 1$, the forces are reversed and are directed outward. Regions that have not porated remain as a capacitor with zero conductance. In the case of vesicles with conductivity ratio $R < 1$, poration causes the poles to be forced inwards, while the non-porated region is forced outward once the membrane capacitor is charged. This effect is shown graphically in Figure 6.9.

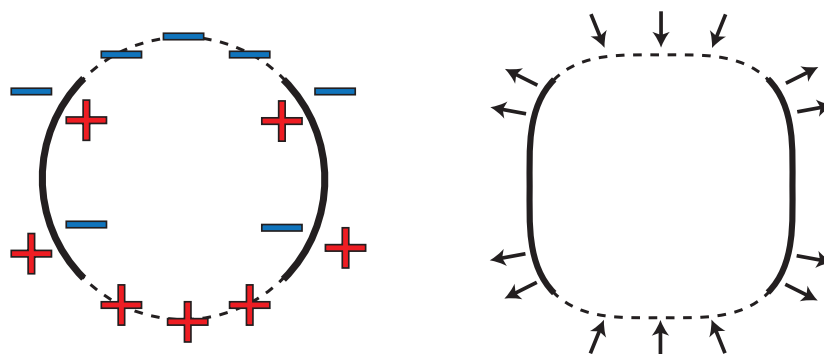


Figure 6.9: (left)Charges on a vesicle with porated regions at poles. (right) Deformation and direction of forces for $R < 1$ with porated regions near poles.

The sensitivity of the capacitor charging timescale to the solution conductivities may explain why cylindrical deformation is only seen in higher conductivity solutions

[162]. If conductivities are high, the capacitor charges quickly and the difference in electric stresses between the porated and non-porated regions occurs faster than the vesicle can deform, $t_{\text{mm}} < t_{\text{hd}}$. If the vesicle is in low conductivity solutions, the vesicle interface charges slowly and it takes longer to reach the critical transmembrane potential. The vesicle may deform quickly and as a result of the slow charging, the transmembrane potential does not reach a critical value.

In order to quantify the effect, it is possible to determine the angular position where $V_m \sim V_c$. The transmembrane potential is set equal to V_c ,

$$V_c = \frac{3}{2} (1 - \exp(-t/t_{\text{mm}})) a E_0 \cos(\theta). \quad (6.9)$$

The location on the vesicle where the membrane has porated is found by solving for θ . This is denoted by a critical angle,

$$\theta_c = \arccos \left(\frac{2V_c}{3(1 - \exp(-t/t_{\text{mm}})) a E^\infty} \right). \quad (6.10)$$

This expression is compared with experiments at different field strengths by measuring the angular location of the edge in time. The results can be fit for different values of V_c .

Note that this expression assumes the vesicle is a sphere and the field does not change due to the porated regions. The rapid deformation may also invalidate the assumption of zero Reynolds number. During collapse, the membrane is moving at an approximate velocity of $U \sim 0.01 - 0.1 \text{ m/s}$. For a vesicle of radius $a \sim 10^{-5} \text{ m}$ in water ($\rho = 1000 \text{ kg/m}^3$, $\mu = 10^{-3} \text{ Pa}\cdot\text{s}$), Reynolds number is of the order $Re \sim 0.1 - 1$. The effect of inertia may alter the hydrodynamic response of the vesicles. Although deformation, membrane poration, and high velocities will likely affect the position

of θ_c , Eq.[6.10] provides a useful estimate for comparison with the edges seen in experiments.

6.3.1 Experiment

The critical angle is measured at the edge observed in the experiment as shown in Figure 6.10 using image processing software (imageJ). A central axis is drawn through the vesicle collinear with the field direction. A second line is then drawn from the central point of the axis (center of the vesicle) to the midpoint of the corner. The other corners are measured in a similar manner to establish an error estimate of ± 5 degrees. This error also reflects the limited resolution of the edge and slight asymmetry in the vesicle shape.

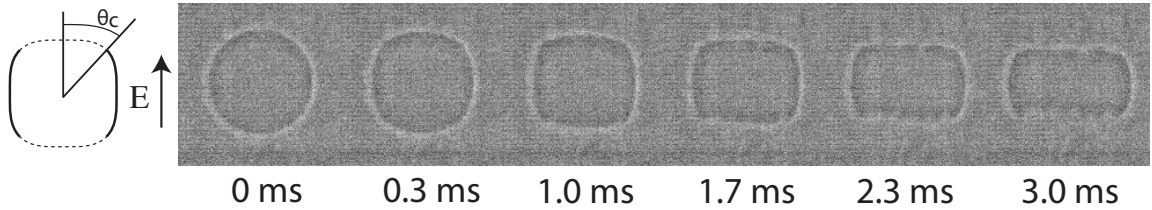


Figure 6.10: Sequence of deformation for in a $E_0 = 1kV/cm$ DC pulse. The angle of the edge is measured from the field axis.

Measurements are taken on both lipid (DOPC) and polymer membranes. Polymers of different molecular weights are utilized to observe the effect of membrane thickness on corner formation. Adjusting the membrane thickness changes both the membrane capacitance as well as the poration threshold [9]. The location of the critical angle is measured in time for different pulse strengths. The duration of the pulses is much longer than the capacitive charging timescale t_{mm} and long enough for corners to form. A pulse duration of 10 ms is used for most experiments. The observations are made using a phase contrast microscope, Zeiss Axio Observer.A1 (Zeiss, Germany) with a 40x Ph2 objective. The images are recorded with a

high speed camera (Photron SA1.1) at 30,000 frames per second. The media conductivities are measured during preparation and the vesicle radius is determined from the undeformed shape. The observations are compared to Eq.[6.10] using a single value for the critical membrane voltage, V_c , for each membrane type.

The position of the edge is shown in Figure 6.11 at different field strengths using DOPC vesicles. The data for the highest field strengths end because the vesicle either collapses or moves out of view of the camera. This is usually a result of gradients in the electric field, which can be significant in large fields [190].

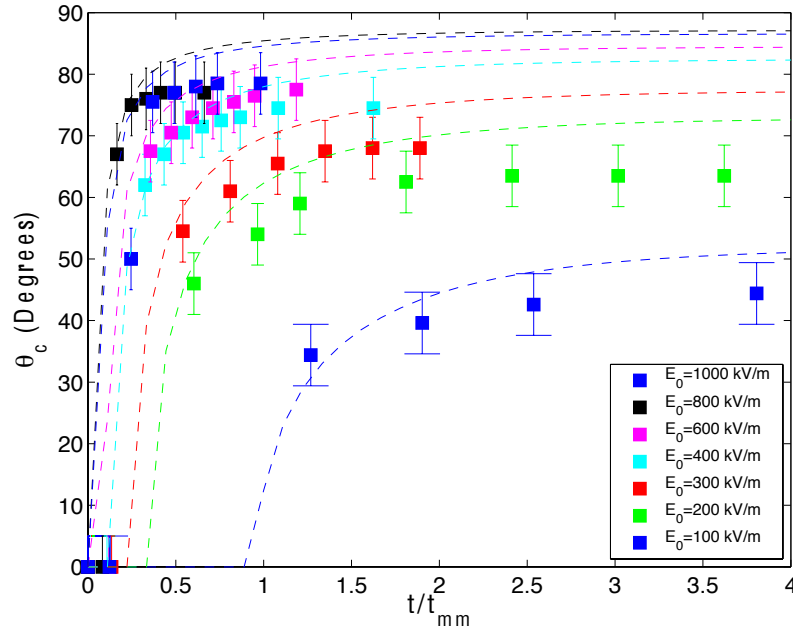


Figure 6.11: The location of θ_c compared t/t_{mm} for DOPC vesicles. The data is fit for different field strength values, but with the same parameters: $V_c = 2.0V$, $\lambda_{in} = 5\mu S/cm$, $\lambda_{ex} = 70\mu S/cm$.

Comparison between experiment and theory shows agreement for a critical transmembrane voltage of, $V_c = 2.0V$. Although the value of V_c is about twice the value of found in previous measurements [161, 9], the agreement with Eq.[6.10] supports the relation between membrane poration and the formation of edges.

Figures 6.12-6.15 show the measurements of θ_c for different polymer membranes.

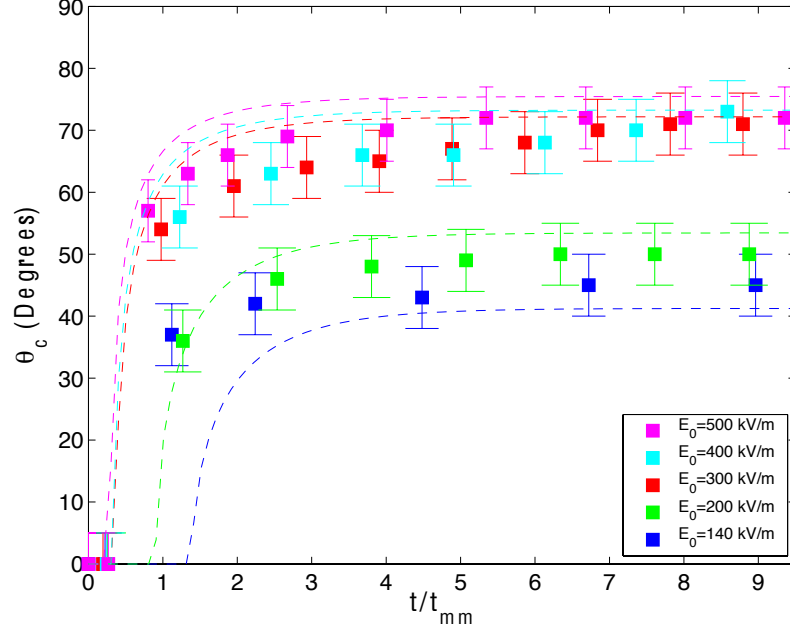


Figure 6.12: Critical angle measurements for polymer systems PS1. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=4.2$. The systems all have conductivities $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 70\mu\text{S}/\text{cm}$.

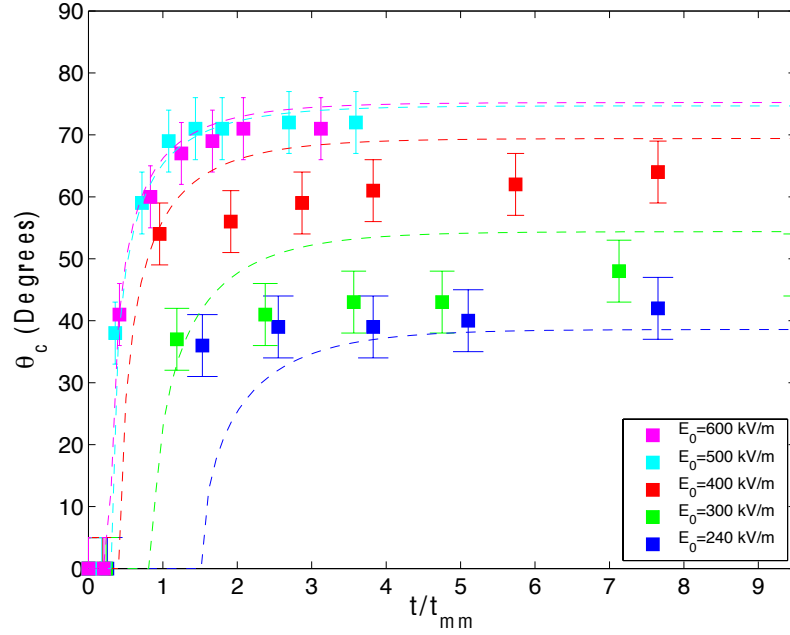


Figure 6.13: Critical angle measurements for polymer systems PS2. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=6.5$. The systems all have conductivities $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 70\mu\text{S}/\text{cm}$.

θ_c increases with field strength in all experiments. The critical transmembrane voltage increases with polymer molecular weight. With the exception of the highest

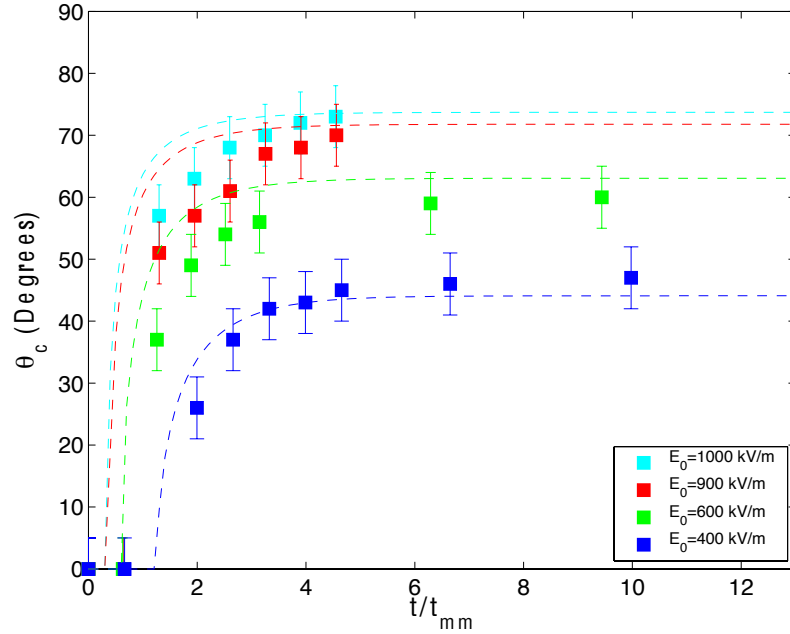


Figure 6.14: Critical angle measurements for polymer systems PS3. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=7$. The systems all have conductivities $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 70\mu\text{S}/\text{cm}$.

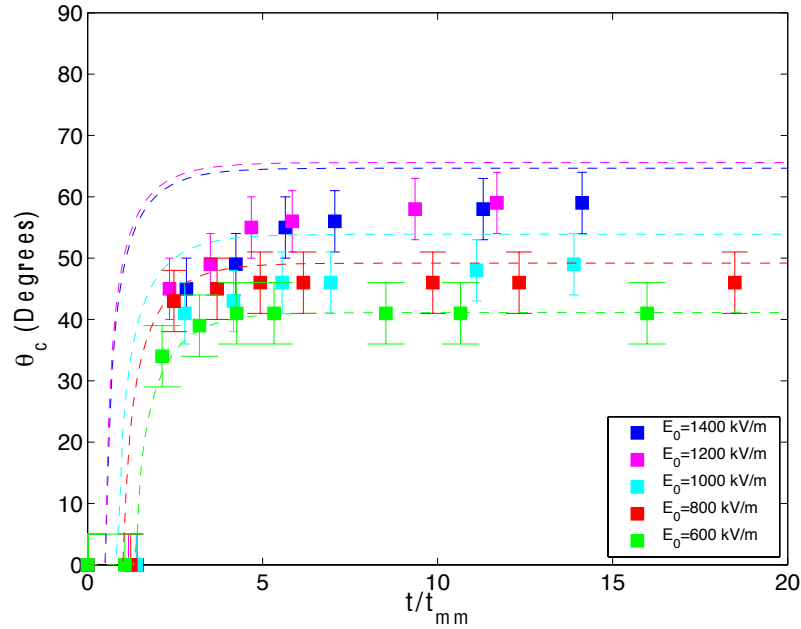


Figure 6.15: Critical angle measurements for polymer systems PS4. The critical angle progression in time, scaled by the capacitor charging time t/t_{mm} . The data is fit with one value of V_c for each polymer, $V_c=16$. The systems all have conductivities $\lambda_{\text{in}} = 5\mu\text{S}/\text{cm}$, $\lambda_{\text{ex}} = 70\mu\text{S}/\text{cm}$.

molecular weight polymer, the range of critical membrane potentials is within the same range of critical transmembrane potentials, $V_c \sim 4 - 8\text{V}$, seen in previous

experiments for polymer vesicles [9].

The process of pore formation in polymer membranes may be more complicated as a result of membrane compressibility. Although membrane compression was not observed to influence the transition frequency in AC fields, the fields used in Chapter 4 were much weaker. If the membrane compresses, the change in thickness will alter the membrane capacitance. The compression of the membrane will also absorb stress rather than transfer the compression into tension. The weaker agreement with Eq.[6.10] seen in high molecular weight polymers may be a result of membrane compression.

The angular position of the edges gives a rough estimate for the critical transmembrane voltage. With knowledge of the membrane capacitance and critical transmembrane voltage, the lysis tension can be estimated using the Lippman equation [116],

$$\sigma_{\text{lys}} = \frac{1}{2}C_m V_c^2, \quad (6.11)$$

which assumes that the other contribution to membrane tension, such as the isotropic tension σ_h , is smaller than the compressive stress on the membrane capacitor.

Using the values for membrane capacitance determined in Chapter 4 and the fitted values for V_c , the lysis tension is estimated with Eq.[6.11]. The value of C_m for POPC is used as an estimate for DOPC membranes.

Previous measurements on the electromechanical limits of polymer membranes have used micropipette aspiration to control tension and then apply an electric field to produce membrane breakdown [9]. From the applied tension and transmembrane voltage, they were able to determine values for critical transmembrane voltage and lysis tension. Their approach was to apply a 60 μs DC pulse and then observe when

Name	C_m ($\mu\text{F}/\text{cm}^2$)	V_c (V)	σ_{lys} (N/m)
DOPC	0.710	2.0	0.017
PS1	0.270	4.2	0.024
PS2	0.227	6.5	0.048
PS3	0.172	7.0	0.042
PS4	0.066	16.0	0.084

Table 6.1: Values for membrane capacitance, critical transmembrane voltage, and lysis tension estimated from the Eq.[6.11].

poration had occurred after the field is turned off, similar to the pore formation shown in Figure 5.1. The pore cannot be observed during the pulse because a pore will only nucleate during the pulse and then grow to an observable size after the field has turned off. The values for critical voltage and lysis tension given in Table 6.1 are slightly higher than those found using this method [9]. For instance, previous measurements of lipid membranes gives $V_c \sim 1.1V$ compared to $V_c = 2.0V$ in Table 6.1. Despite the many complexities arising from vesicles in strong DC fields, the similar range of values for V_c and σ_{lys} indicates that the formation of edges can be attributed to membrane poration.

6.4 Conclusions

Membrane poration can dramatically change the dynamics of vesicles. Pores lead to conduction across the membrane and change the electric stresses at the interface. The breakdown of the membrane capacitor produces stresses that can lead to vesicle collapse. The ellipsoidal deformation can be approximated by a conducting membrane without membrane tension. Without tension, the forces opposing deformation are limited to viscous resistance, which delays the rate of deformation and can be significantly increased in the case of highly viscous membranes. The

accuracy of the fit is surprising given the complexity seen in experiment.

The formation of corners exemplifies this complexity. The variation in transmembrane potential, a consequence of the spherical geometry, leads to porated regions near the vesicle poles and an intact region near the equator. The abrupt change in electric stress resulting from the jump in poration explains the presence of sharp edges seen in experiments. Although the critical transmembrane potential appears to be higher than previously measured, this may be a result of a number of complications including deformation, disturbances to the electric field, and the effect of inertia. The process of pore formation and vesicle deformation resulting from an applied electrical potential is a topic which requires further research.

6.5 Future Directions

Development of nano pores

Theoretical models for pore dynamics have focused primarily on individual pores. The dynamics of pore formation under an electric stress remains a difficult problem due to the gradients in stress and electrical conduction through the membrane. In the case of a single pore, membrane tension pulls the pore open to a maximum size. If there are many pores present, the tension may be quickly relieved by the opening of many small pores. The lifetime and evolution of pores are likely to differ from the case of an individual pore.

Improved imaging techniques may yield better information about pore evolution. For instance, the pores may be visible from a view above the vesicle, in the direction of the electric field. The transmission of material through small pores could also be

visualized using fluorescent molecules.

Poration control with AC fields

Macro-pores are usually formed in electric fields using short and strong DC pulses [155]. The timescale for resealing of a macro-pore has been shown to be dependent on the membrane edge tension [95, 155]. It may be possible to adjust this timescale by application of a weak AC electric field while the pore is resealing. The weak electric field could create a slight tension on the membrane and delay pore closure. The size of the pore could be tracked using methods similar to those previously developed [155]. A longer pore closure time may lead to an enhance transmission of material across the membrane. The efficacy of this method could be tested by observing fluorescent molecules that only fluoresce after mixing with other reactants inside the vesicle [165]. If the two reactants are separated in the interior and exterior media, fluorescence would only occur when material had crossed the membrane.

CHAPTER SEVEN

Membrane fluctuations in AC electric fields

7.1 Introduction

Lipid membranes are soft and easily bent by thermal noise. Equilibrium shape fluctuations of vesicle membranes are stable and can be observed using light microscopy [145, 68]. Analysis of the fluctuations can be used to determine membrane properties, specifically bending rigidity and membrane tension.

In the presence of electric fields, the fluctuations of the membrane will be modified by the presence of charge. It is predicted that charge on the membrane surface will increase bending rigidity and lower tension [230, 5]. The effective membrane property values are described by a sum of the inherent values (in the absence of electric field) and an electric contribution,

$$\kappa_{eff} = \kappa + \kappa^{\text{el}}, \quad \sigma_{eff} = \sigma + \sigma^{\text{el}}. \quad (7.1)$$

In the case of a lipid membrane, which is nearly incompressible, the compressive stress from the transmembrane potential is transferred into tension. The repulsion between charges adds resistance to change in membrane curvature. This resistance leads to a higher effective bending rigidity of the interface. The presence of surface charge gives rise to a diffuse layer of ions in the bulk surrounding the membrane. The double layers affect both tension and bending rigidity of the membrane [230]. The influence of the double layer on membrane tension comes from a compressive effect similar to the internal field. The influence on bending is due to the finite thickness of the double layers.

The effect of electric fields on membrane properties has been studied theoretically, but experimental observations are limited [230]. An experiment that isolates the influence of electric fields on membrane fluctuations is difficult because the electric stresses usually lead to vesicle deformation. As discussed in Chapter 4, a systematic deformation of the vesicle will alter membrane fluctuations. Previous experimental work has studied the influence of electric fields on suspended planar membranes using neutron scattering [108]. This study confirmed the increase in bending rigidity and decreasing membrane tension in low conducting solutions. The effect of an electric field on a vesicle membrane, which more closely represents a real cell, has not been studied.

As discussed in Chapter 4, the transition from prolate to oblate in AC fields occurs at a critical frequency, f_c , for systems with $R < 1$. At the transition frequency, the sum of forces on the vesicle cancel and the vesicle remains spherical. This frequency is unique because the vesicle has a nonzero transmembrane voltage as well as an effective net charge on the membrane surface. Because the vesicle is a sphere, the excess membrane area remains in fluctuations, which can be observed optically. These fluctuations can be recorded and analyzed using techniques

previously developed [145, 127]. The presence of the electric field results in a non-equilibrium system [208]. Accordingly, the temporal correlations of membrane fluctuations are measured.

The classic model for membrane fluctuations is first introduced. The experimental methods for measuring and analyzing membrane fluctuations are given. The effect on bending rigidity and tension are shown at different field strengths.

7.2 Membrane fluctuations

The classic description of the relaxation of membrane fluctuations, driven by thermal noise, depends on the ratio of membrane stresses to viscous dissipation into the bulk fluids. A more complete model developed by Seifert and Langer (1994) included dissipation within the membrane. This model is summarized in Appendix C. The classical model for a membrane, first described by Brochard and Lennon (1975), is summarized in this section [180].

The location of the membrane is described by height function, $h(x, y, t)$, which describes the height relative to a mean position. The fluid velocity field around the membrane is described by the Stokes equation, which includes a point force term $\mathbf{K}(\mathbf{r})$,

$$\nabla p - \mu \nabla^2 \mathbf{u} = \mathbf{K}(\mathbf{r}), \quad (7.2)$$

where $\mathbf{r}$ is the location of the point force. The fluid is also assumed to be incompressible,

$$\nabla \cdot \mathbf{u} = 0. \quad (7.3)$$

These equations can be solved through a Fourier transformation,

$$\mathbf{u}_{\mathbf{q}} = \int \exp(-i\mathbf{q} \cdot \mathbf{r}) \mathbf{v}(\mathbf{r}) b d^3\mathbf{r}, \quad (7.4)$$

$$\mathbf{K}_{\mathbf{q}} = \int \exp(-i\mathbf{q} \cdot \mathbf{r}) \mathbf{K}(\mathbf{r}) d^3\mathbf{r}, \quad (7.5)$$

and can be decomposed into components,

$$\mathbf{u}_{\mathbf{q}}(z) = u_{\mathbf{qz}}(z)\hat{\mathbf{z}} + u_{\mathbf{qq}}(z)\hat{\mathbf{q}} + u_{\mathbf{qk}}(z)\hat{\mathbf{k}}, \quad (7.6)$$

$$\mathbf{K}_{\mathbf{q}}(z) = K_{\mathbf{qz}}(z)\hat{\mathbf{z}} + K_{\mathbf{qq}}(z)\hat{\mathbf{q}} + K_{\mathbf{qk}}(z)\hat{\mathbf{k}}, \quad (7.7)$$

where $\hat{\mathbf{q}}$ is the unit vector parallel to the wave vector, $\mathbf{q}$, $\hat{\mathbf{k}}$ is the in-plane vector, which is perpendicular to $\hat{\mathbf{q}}$ and $\hat{\mathbf{z}}$. The expression for the velocity and point force components are inserted into Eq.[7.4] and Eq.[7.5]. An analysis of the coupling between components shows that the normal displacements do not couple with displacements in the transverse direction, $\hat{\mathbf{k}}$. A force density is assumed in the directions $\hat{\mathbf{z}}$ and $\hat{\mathbf{q}}$,

$$\mathbf{K}_{\mathbf{qz}}(z) = \delta(z) [K_{\mathbf{qz}}\hat{\mathbf{z}} + K_{\mathbf{qq}}\hat{\mathbf{q}}]. \quad (7.8)$$

When this force density is inserted into Eq.[7.4] and Eq.[7.5], the forces acting in the $\hat{\mathbf{z}}$ direction decouple from the in-plane components and as a result, the relation between force and velocity becomes,

$$\mathbf{u}_{\mathbf{qz}}(z=0) = \frac{1}{4\mu q} K_{\mathbf{qz}}. \quad (7.9)$$

Analogous expression can be written for $\mathbf{q}$ in place of $\mathbf{z}$. The force from the membrane is found by the derivative of the membrane energy,

$$F_0 = \int \left[\frac{\kappa}{2} (2H - C_0)^2 + \sigma \right] dA, \quad (7.10)$$

$$K_{\mathbf{qz}} = -\frac{\partial F_0}{\partial h_q} = -(\kappa q^4 + \sigma q^2)h_{\mathbf{q}}. \quad (7.11)$$

It is assumed that the membrane position moves with the normal component of the velocity field.

$$\frac{\partial h_{\mathbf{q}}}{\partial t} = \mathbf{u}_{\mathbf{qz}}(z=0) \quad (7.12)$$

Combining Eq.[7.12] with Eq.[7.11] and Eq.[7.9] yields an equation of motion that describes the motion of the interface

$$\frac{\partial h_{\mathbf{q}}}{\partial t} = -\frac{\kappa q^3 + \sigma q}{4\mu} h_{\mathbf{q}}. \quad (7.13)$$

The solution of this equation of motion is given by,

$$h_{\mathbf{q}}(t) = h_{\mathbf{q}}(0) \exp \left[\frac{-4\mu}{\kappa q^3 + \sigma q} t \right]. \quad (7.14)$$

This model assumes that viscous dissipation is limited to the bulk fluid. The dissipation rate is given by the term in the exponential,

$$s_1(q) = \frac{-4\mu}{(\kappa q^3 + \sigma q)}, \quad (7.15)$$

where q is wavenumber. The tension affects the relaxation rates for low wave numbers while bending rigidity primarily affects higher modes.

The final step of the analysis is to multiply Eq.[7.14] by $h_{-\mathbf{q}}(t + \Delta t)$ and then average over the thermal fluctuations to obtain the autocorrelation function [180],

$$C(q, \Delta t) = \langle h_{\mathbf{q}}(t) h_{-\mathbf{q}}(t + \Delta t) \rangle = \frac{k_B T}{\sigma q^2 + \kappa q^4} \exp(s_1(q) \Delta t). \quad (7.16)$$

This equation will be used to analyze the membrane fluctuations measured in experiment. However, a more useful form of the equation is found by normalizing

with $\langle h_{\mathbf{q}}(t)h_{-\mathbf{q}}(t) \rangle$ where $\Delta t = 0$. This normalized expression is denoted by $C_{\text{norm}}(\Delta t)$,

$$C_{\text{norm}}(q, \Delta t) = \exp \left(\frac{-4\mu}{\kappa q^3 + \sigma q} \Delta t \right). \quad (7.17)$$

This model assumes that dissipation is dominated by bulk viscosity. Seifert and Langer (1994) accounted for viscous dissipation in the membrane and showed that the mechanism for viscous dissipation, either membrane or bulk viscosity, varies with wavenumber and system parameters [182]. The details of their model are given in Appendix C. The purpose of developing the Seifert-Langer model is to verify that the fluctuations observed in experiments dissipate energy only into the bulk fluid and not by other mechanisms. Once this is assumed, the simplified Milner and Safran (1987) approach can reliably be used [129].

The Seifert-Langer model provides wavenumber cutoffs that predict the transitions between different dissipation mechanisms. For low wave numbers, $q < q_1$, viscous dissipation into the bulk is dominant.

$$q_1 = \frac{2\mu K_a}{b(\kappa + 2K_a d_c^2)}, \quad (7.18)$$

where K_a is the area expansion modulus, b is the intermonolayer slip, and d is the thickness of the hydrophobic core. For a lipid membrane, $K_a \sim 0.3\text{N/m}$, $b \sim 10^8\text{Ns/m}^3$, and $d_c \sim 2.9\text{nm}$. This results in a cutoff wavenumber around $q_1 \sim 20\mu\text{m}^{-1}$. This means that fluctuations of wavelength greater than about 50nm will relax at a timescale related to dissipation into the bulk. This length scale is below the resolution of the microscope, approximately $0.2\mu\text{m}$. As a result, only the relaxation timescale related to bulk dissipation will be observable. The Seifert-Langer model also predicts dissipation due to bilayer slip for $q < q_1$. The slip results from

differences in lipid density across the bilayer. This rate of dissipation is given by $s_2 = \frac{K_a}{2b} q^2$. A scaling analysis based on typical values for K_a , b , and q gives a value for dissipation rates much faster than the bulk dissipation. More details are given in Appendix C.

The dynamical correlation function has also been derived for a spherical geometry [173, 129]. The expression for $s_1(q)$ differs in the case of a sphere, and is denoted by ζ_1 ,

$$\zeta_1(n) = \left[\frac{n(n+1)}{\mu a^3 (2n+1)(2n^2+2n-1)} \right] (n+2)(n-1)[\kappa(n+1)n + \sigma a^2], \quad (7.19)$$

where n is the mode number and is related to the wavenumber by $q \sim n/a$. This expression is better suited for vesicles due to their spherical shape. Note that for large wave numbers, Eq.[7.19] reduces to the expression for planar geometry, Eq.[7.15]. The normalized correlation function is given by,

$$C_{\text{norm}}(n, \Delta t) = \exp(-\zeta_1(n)\Delta t). \quad (7.20)$$

For $q < q_1$, the dynamic height-height correlation function is given in terms of the height above the mean position of the membrane, $h_q(t)$ [181, 228]. Through observations of membrane fluctuation amplitudes at equilibrium, the bending rigidity and tension can be determined using Eq.[7.16]. More recently, the normalized temporal correlation function, Eq.[7.17], was used to measure bending rigidity and tension [228].

With the addition of an electric field, it cannot be assumed that the membrane is in thermal equilibrium [208]. The advantage of using the temporal correlations is

it captures dynamic character of the membrane. The specific objective is to observe how the electric field modifies $\zeta_1(q)$, which can be used to determine renormalized values for κ and σ . The frequency dependent deformation of a vesicle, discussed in detail in Chapter 4, provides an opportunity to measure membrane fluctuations on a spherical vesicle in an electric field. A valid measurement of $\zeta_1(n)$ is possible by observing fluctuations at the critical frequency, f_c (Eq.[4.76]), where the vesicle is a sphere and Eq.[7.16] is applicable.

7.3 Experimental procedure

DOPC vesicles are prepared according to the same procedure described in Chapter 4. The vesicles are grown in 200 mM aqueous sucrose solutions and then diluted into osmotically matched glucose solution. The vesicles are observed using a phase contrast microscope Axio Observer.A1 with 100x 1.2 NA oil immersion objective. A Photron high speed camera records membrane fluctuations at 125 frames per second (fps) and has a shutter speed of 1/5000 seconds [68]. The use of the faster shutter reduces the influence of integration time errors which can create a blurred image during recording [145]. 6000-8000 frames are recorded for each experiment, approximately one minute of recording time. The membrane contour is found using a sub-pixel resolution contour recognition software developed by [68].

An alternative method for determining the membrane contour was developed in MATLAB based on the approach taken in [127]. This method takes the gradient of pixel intensity along radial slices of the image. Then the positive gradient data, corresponding to transition from low to high pixel intensity at the interface, is fit using a Lorentzian function to determine the location of highest gradient. The fitted

point is used as the initial guess for the fit on the adjacent slice. Both methods return similar results and the software developed in [68] is used for purposes of speed.

The contour data for each frame is given in polar coordinates $r(\theta, t)$, where the centroid is determined from the contour data [145]. The radial contour information is converted into a height function, $h(\theta, t)$, defined as the deviation from a mean position. The mean radius for each frame is averaged over the entire contour,

$$h(\theta, t) = r(\theta, t) - \langle r(t) \rangle_\theta, \quad (7.21)$$

where $\langle \rangle_\theta$ denotes average over θ . The mean radius is equivalent to the initial radius of the vesicles $\langle r \rangle_\theta = a$. The height function is transformed into Fourier space,

$$h_n(t) = \frac{a}{2\pi} \int_0^{2\pi} h(\theta, t) \exp(-in\theta) d\theta, \quad (7.22)$$

where n is the mode number, which is related to wavenumber by $n = aq$. The height function can be written in terms of either the mode number or wavenumber, but is kept in terms of mode number for comparison with Eq.[7.20],

The height-height temporal correlation function is found by [145],

$$C(n, \Delta t) = \langle |h(n, t)| |h(n, t + \Delta t)| \rangle_t - \langle |h(n, t)| \rangle_t^2, \quad (7.23)$$

where $\langle \rangle_t$ denote average over time. The temporal correlation function is normalized by $C(n, \Delta t = 0)$ to isolate the effect of the relaxation rates. The normalized function is then compared to Eq.[7.20] for each mode number,

$$C_{\text{norm}}(n, \Delta t) = \frac{C(n, \Delta t)}{C(n, \Delta t = 0)}. \quad (7.24)$$

The relaxation rates are determined as a function of mode number. This data can be compared to the theoretical values for $\zeta_1(n)$ and then fit for values of bending and tension. A least squares method is used for both fitting procedures.

Although the measured relaxation rates are compared to a model for fluctuations on a sphere, the lowest modes are influenced by the presence of the cover glass. The glass dampens fluctuations and possibly give errant results. A lower concentration of sugar solutions could be used, but the resolution of the contour suffers due to the loss of phase contrast. A lower mode cutoff is used for the fitting procedure. The modes which are not influenced by the glass are estimated to be $n > 6 - 8$, which roughly corresponds to a full wavelength over a $\pi/4$ angular slice of the vesicle surface.

The highest modes used for fitting is determined by the resolution of the microscope. The resolution can be estimated by,

$$\text{Resolution} = \frac{\lambda_l}{2\text{NA}} \quad (7.25)$$

where λ_l is the wavelength of light, and NA is the numerical aperture of the objective (a 1.2 NA is used for the experiment). Assuming a mid-spectrum wavelength of light at 550 nm, the resolution can be estimated to be about 230 nm. The resolution of the camera also affects the ability to detect the membrane contour. The pixel resolution of the camera is approximately the same length, $\sim 0.18\mu\text{m}/\text{pixel}$. A common measure to resolve a fluctuation wavelength is approximately 4-5 pixels [145]. This gives a large wavenumber cutoff $q \sim 1\mu\text{m}^{-1}$, which corresponds to an upper limit on mode number of around $n = 20$ for a $20\mu\text{m}$ vesicle. Larger vesicles will have a larger number of modes that can be analyzed accurately. The range of mode numbers used for most vesicles is within the range of $n \sim 6 - 18$, because many vesicles are slightly less than $20\mu\text{m}$.

As discussed in Chapter 4, the membrane capacitance leads to a frequency dependent shape in AC fields. For a vesicle with conductivity ratio less than one, a transition from prolate to oblate occurs at a critical frequency. At this transition point, the vesicle is spherical due to a net canceling of the electric stresses. A set of images recording membrane fluctuations is first taken in the absence of electric fields. An AC field is then applied at the transition frequency, which is determined by adjusting the frequency until the vesicle remains spherical. The field strength is increased in increments of 1 kV/m from $E_0 = 1 - 5$ kV/m. A full set of 6000-8000 frames are recorded at each field strength.

Measurements at field strengths higher than 5 kV/cm are difficult due to interactions between vesicles. The vesicles begin to attract each other in strong fields resulting from interactions between the induced dipoles on the vesicles. The vesicles will align in pearl chain formations and disrupt membrane fluctuations. Measurements where vesicles come in contact with surrounding vesicles are discarded.

Vesicles have also been observed to lift off the cover glass when the field is applied [190]. In order to compare vesicles with and without electric field, gravity should be negligible in both cases. The criterion for determining the effect of gravity compared to vesicle tension can be determined by the relation [79, 145]

$$\Delta\rho ga^4/\kappa \lesssim \frac{\sigma a^2}{\kappa}. \quad (7.26)$$

Solving this inequality for tension yields a value for tension where gravitational forces will affect the fluctuations,

$$\sigma \lesssim \Delta\rho ga^2. \quad (7.27)$$

Using 200 mM sugar solution the density difference is approximately $\Delta\rho \sim 12$ kg/m³

(Sucrose = 1009 kg/m³ and Glucose = 1021kg/m³). For typical values of radius ($a = 20\mu m$), the effect of gravity will not be significant for tensions $\sigma > 5 \times 10^{-8} \text{N/m}$. The effect of gravity will become more significant for larger vesicles. Thus, very large ($a > 20\mu m$) and very deflated vesicles ($\sigma \lesssim \times 10^{-8} \text{N/m}$) are avoided.

7.4 Results

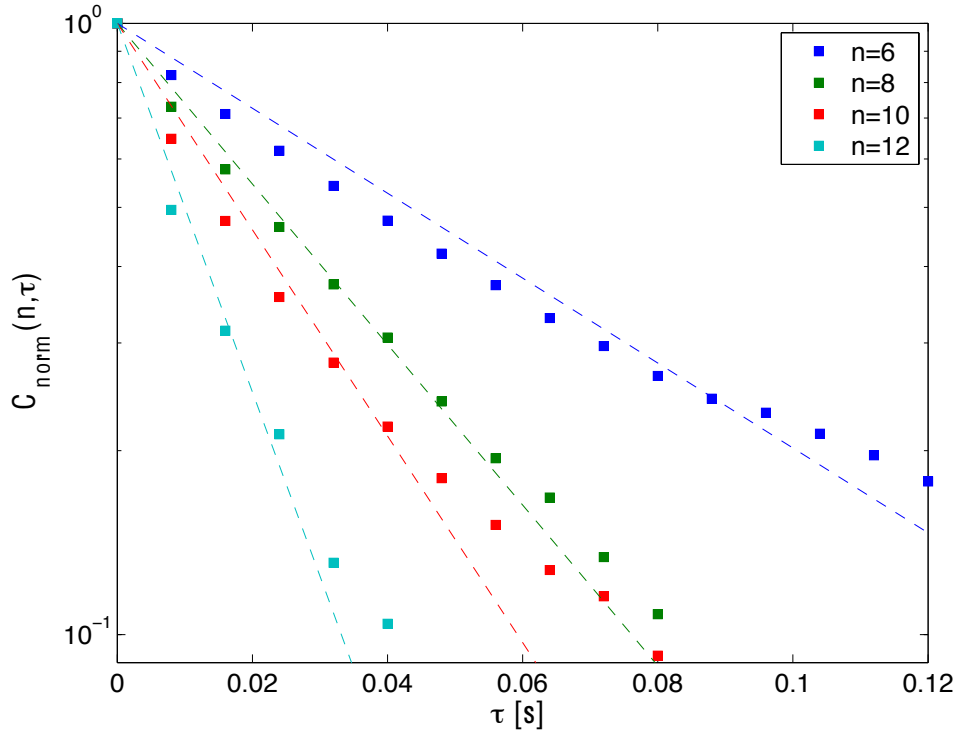


Figure 7.1: Normalized correlation function for a DOPC vesicle. Average radius of the vesicle is $a=14.3 \mu m$. The symbols represent experimental data calculated using Eq.[7.24] and the lines are the fits of the data for each mode number using Eq.[7.20].

The procedure outlined in the previous section is used to measure the bending rigidity and tension for DOPC vesicles with and without electric fields. The correlation function determined from experiments using Eq.[7.24] is compared to the exponential decay predicted by Eq.[7.20]. A comparison between theory and experiment is shown in Figure 7.1 with and without an applied electric field. The

results show fairly good agreement with the exponential decay predicted by Eq.[7.20]. As expected from Eq.[7.19], the relaxation rate at higher wavenumbers is faster. The recording rate limits the number of points that can be used to fit the higher mode numbers. An acquisition rate greater than 125 fps could be used, but this would reduce the total amount of observation time due to the limit on frames that can be recorded.

The fit for each mode using Eq.[7.20] is then plotted as a function of mode number. An example is given in Figure 7.2. The errors associated with the exponential fits are determined from a 90% confidence interval. The higher modes have a larger error due to the fewer number of points available to fit. The fit appears appropriate for the range of modes considered ($n = 8 - 18$). The lack of agreement for the lowest modes is likely due to the influence of the glass. The highest modes still appear to follow the fitted expression, but the error begins to become large due to the lack of temporal and spatial resolution.

The influence of the electric field on $\zeta_1(n)$ is demonstrated in Figure 7.2. The presence of electric field indicating an increase of the relaxation rate and a lower tension. The slope from the lowest to highest modes is steeper in the presence of electric field, which indicate a higher effective bending rigidity.

The fitting procedure shown in Figure 7.2 for the $E_0 = 0\text{kV/cm}$ is repeated for the range of applied electric field strengths (1-5 kV/m). Figure 7.3 shows the bending rigidity and tension determined from these fits for a set of experiments on one vesicle at different field strengths. The change in bending rigidity and tension is plotted for each field strength in Figure 7.3. The bending rigidity without electric field is found to be about $\kappa = 25 k_B T$, which is within the range of previous measurements for DOPC, $\kappa \sim 20 - 25 k_B T$ [68]. This confirms that the procedure can accurately

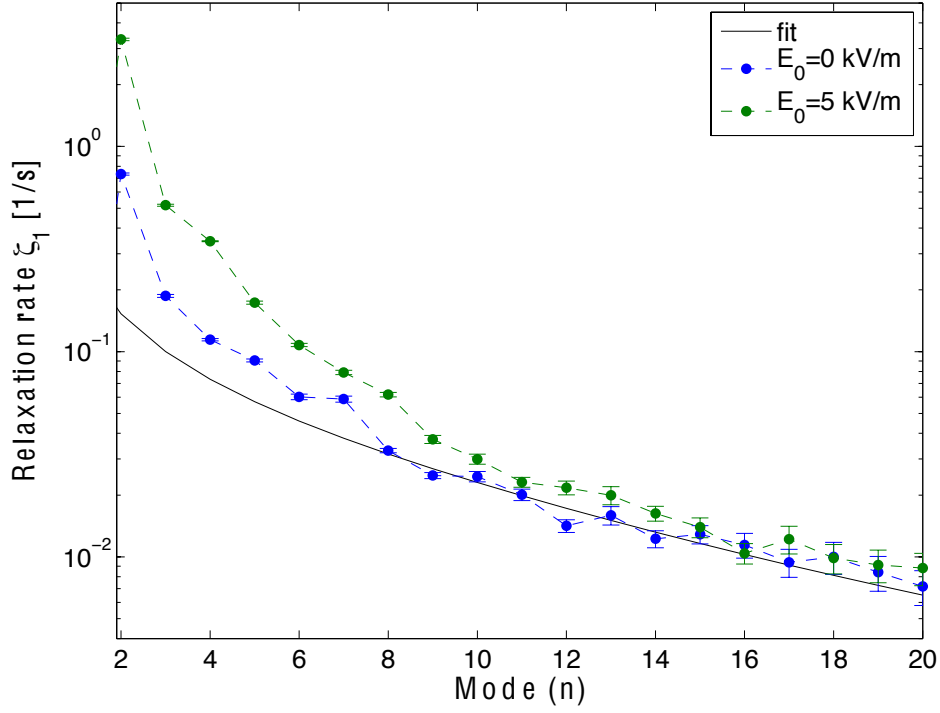


Figure 7.2: Relaxation rates as a function of modes n . Theoretical curves show the effect of higher bending rigidity (dashed) and lower tension (dotted). The relaxation rates for a $14.3 \mu\text{m}$ DOPC vesicle with and without electric field are compared.

determine bending rigidity values.

The bending rigidity is observed to increase with field strength while the effective tension decreases with electric field strength. The bending rigidity value at the highest electric field strength (5 kV/m) is roughly twice the original bending rigidity. The highest field strength decreases the tension to less than half the original value.

The largest contribution to the scatter seen in Figure 7.3 is likely a result of the movement of vesicles due to nonuniformities in the electric field. This can make the vesicle move out of focus, which leads to a decrease in contour resolution.

The effect of the electric field on membrane properties is likely to scale with electric stress, which depends on electric field strength by $\mathbf{T}^{\text{el}} \sim E_0^2$. The curves plotted in Figure 7.3 are effective membrane property values assuming the form

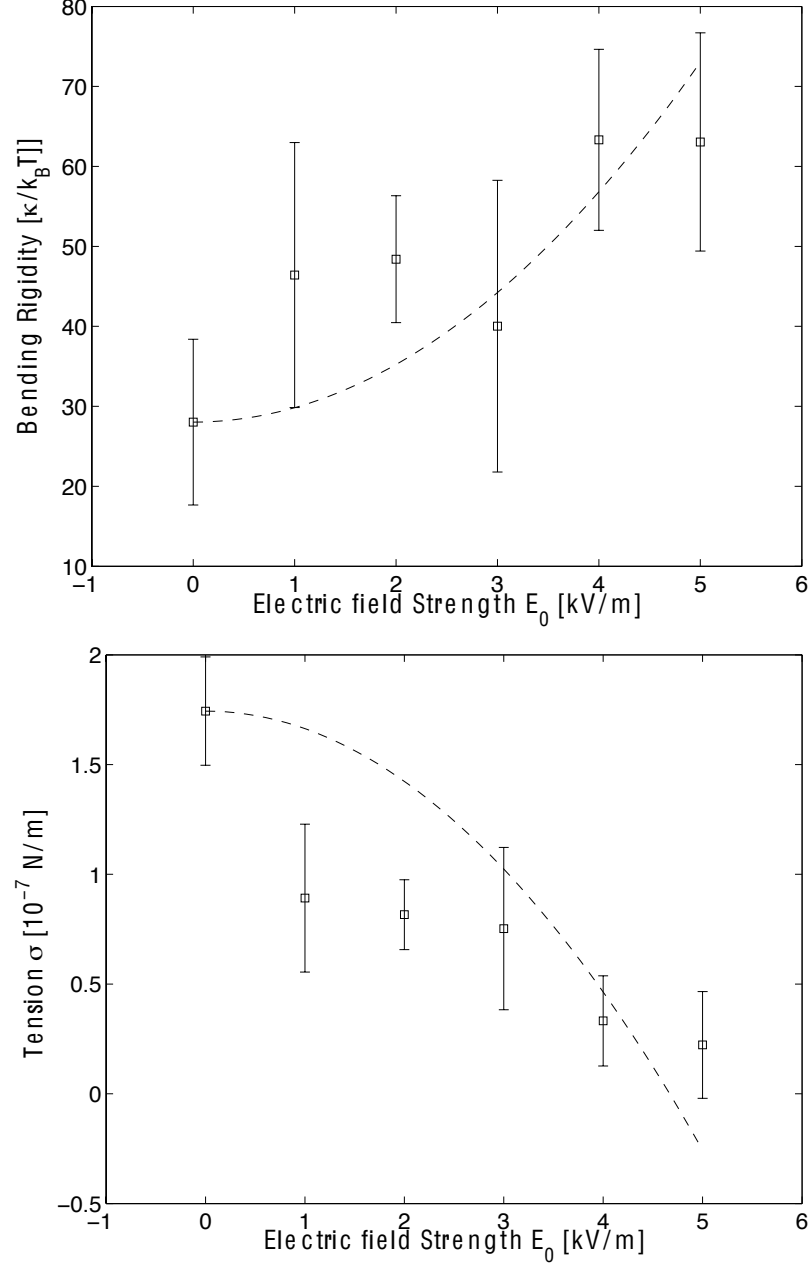


Figure 7.3: Bending rigidity (top) and membrane tension (bottom) as a function of field strength for one vesicle. The effective bending rigidity given by Eq.[7.1] is plotted for $\kappa^{\text{el}} = 1.8[k_B T/(kV/m)^2]E_0^2$, where E_0 is the electric field in kV/m. The effective tension is plotted for $\sigma^{\text{el}} = -0.08 \times 10^{-7}[N/m/(kV/m)^2]E_0^2$, where E_0 is the electric field in kV/m.

given in Eq.[7.1] and that $\kappa^{\text{el}} \sim E_0^2$ and $\sigma^{\text{el}} \sim -E_0^2$. The coefficients given in Figure 7.3 are estimates based on the magnitude of change seen in the results. Although the change in membrane properties do not agree closely with the E_0^2 scaling, the general trends of increasing bending rigidity and decreasing tension are observable.

7.5 Conclusions

The frequency dependent shape in an AC electric field allows for the observation of membrane fluctuations in electric fields. The observations confirm trends predicted in theoretical models that show an increase in effective bending rigidity and a decrease in effective tension. A quantitative comparison with theory is difficult due to the use of AC fields and the spherical geometry of the vesicles. Theoretical models have mainly addressed planar membranes in DC fields [230].

The spherical shape causes a variation in transmembrane potential with angular position. This variation likely results in a gradient in effective membrane properties. Because the membrane fluctuations are observed around the entire vesicle, there is a spatial averaging of the fluctuations. Limiting the fluctuation analysis to the regions near the poles may result in observing a more dramatic effect from the electric field.

The use of an AC field at the shape transition frequency, f_c , allows the vesicle to remain spherical. At the transition frequency, the interface has a nonzero transmembrane potential as well as an effective net charge resulting from the mismatch in charging timescales of the surrounding fluids. Both transmembrane potential and effective net charge may explain the change in membrane properties. The effect of net charge may be similar to that of membranes with fixed charges, which have been shown to increase bending rigidity and lower tension[224, 110, 62, 153, 41]. Both effects are likely to decrease with salt concentration, which motivates further investigation using this method.

7.6 Future directions

Influence of salt

Measurements were only taken for vesicles with no salt in the interior solution and low salt concentrations in the exterior. This was done in the hope of seeing the largest influence on bending rigidity as predicted by theory. Comparing measurements at different salt concentrations would demonstrate the influence of double layers and surface charge. The amount of net surface charge, Q , related to conductivity ratio by [208],

$$Q \sim \frac{R - S}{R + 2}. \quad (7.28)$$

Low conductivity ratios, $R \ll 1$, will provide a maximum value of $Q \sim 1/2$, while $R \sim 1$ will give smaller values for Q . Adjusting the conductivity ratio might isolate the effect of net surface charge.

Polymer Membranes

The fluctuations of polymer membranes shows behavior indicative of two relaxation rates, see Figure 7.4. This is likely due to dissipation within the membrane, although further analysis is needed to identify the mechanism of dissipation. The Seifert-Langer theory predicts dissipation within the membrane arising from intermonolayer slip, although there may be other explanations for the two exponential behavior such as the effect of membrane viscosity. Additionally, the polymer membranes may have a nonzero shear elasticity. Future work requires comparisons with other theoretical models and additional observations for different types of polymer membranes.

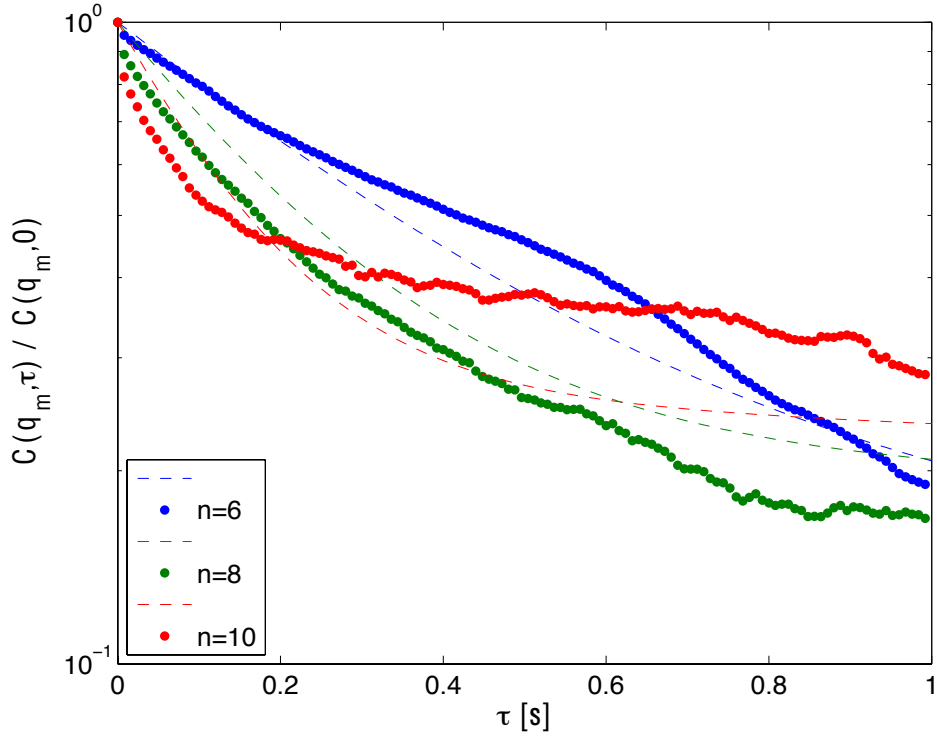


Figure 7.4: Normalized correlation function for PEO-Pbd (0.6-b-1.2) membrane compared to normalized Eq.[C.17]. The parameter for the fit are $\kappa = 40k_bT$, $\sigma = 0.3 \times 10^{-7}N/m$, $d = 2.5 \times 10^{-9}m$, $K_a = 0.01N/m$, $b = 50 \times 10^8Ns/m^3$, $\mu_{mm} = 1 \times 10^{-6}Ns/m$

CHAPTER EIGHT

Conclusion

The goal of this thesis was to describe complex behavior arising from interfacial charging in electric fields. The electromechanical coupling at the interface drives deformation in both drops and vesicles. In drops, the coupling between electric charge, drop shape, and surface flow lead to nonlinear behavior. In vesicles, the capacitive charging of the interface is shown to produce a frequency dependent shape in AC fields that can be exploited to measure membrane capacitance. In DC fields, the capacitive charging of the interface leads to an oblate-prolate shape transition. The electric stress can also induce membrane pores, which causes conduction through the interface leading to cylindrical deformations and vesicle collapse. The interfacial charge also leads to changes to membrane fluctuations which is observed in experiments.

A silicone oil drop immersed in castor oil is used to study the behavior of a simple fluid-fluid interface in electric fields. The drop rotates due to an electric torque created by the induced dipole interacting with the applied field. The interfacial electric stresses also induce drop deformation and fluid flow. After fluid rotation is induced, the drop adopts a steady tilted shape and constant fluid flow. The main contribution of Chapter 3 is to show that the interaction between the

surface charge, drop shape, and fluid flow lead to nonlinear drop behavior, including shape oscillations and chaotic tumbling. The character of the nonlinear behavior is dependent on viscosity ratio. Low viscosity drops have easily deformable interfaces leading to shape oscillations while high viscosity drops deform slowly and tumble. The large deformations experienced during the unsteady shape behavior also lead to drop breakup. A theoretical model is developed that includes the effect of ellipsoidal shape and surface flow tangential to the interface. The model includes charge convection arising from the surface flow, which produces behavior similar to that seen in experiments and not captured by previous models. A number of future directions arise from the present research for both theory and experiment. Improvements to the theoretical model include the possibility for time-dependent shape deformations and a more complete description of the fluid flow field. The study of surfactant-laden interfaces, interactions between rotors, and new fluid systems in electric fields are proposed as future directions for experiments.

While complexity arises in simple fluid-fluid interfaces from charge convection, the capacitive charging due to the finite thickness of a bilayer membrane introduces complexity for vesicles. The bilayer also produces membrane stresses that leads to bending resistance and tension within the membrane, which alters the restoring forces in comparison with drops. The fluid nature of the membranes produces viscous dissipation within the membrane. Membrane capacitance leads to a frequency dependent shape, where electric stresses change at a frequency comparable to the membrane charging timescale. The primary focus in Chapter 4 is to demonstrate a novel method for measuring membrane capacitance using a frequency dependent shape transition in AC electric fields. Experimental result of vesicle shape change in AC fields is compared to theory. The time-dependent shape evolution of a vesicle after a field is turned on or off is shown to be sensitive to membrane viscosity.

The potential application of these methods for determining membrane capacitance and membrane viscosity may include measuring polymer membranes at different temperatures and the capacitance of real cells.

With knowledge of membrane capacitance for different membrane systems, the behavior of vesicles in DC pulses is investigated in Chapter 5. In DC fields, the capacitive charging of the membrane leads to time-dependent stresses. Theoretical models have predicted a transition from oblate to prolate shape as the membrane capacitor charges. The fragility of the membrane leads to a timescale for membrane poration not previously considered. Comparing this timescale to the capacitive timescale and the characteristic deformation (EHD) timescale inspires the development of a two-step pulse experiment capable of observing the oblate to prolate transition without producing membrane pores. Similar to AC fields, the shape evolution in DC pulses can be used to estimate membrane viscosity.

The cylindrical deformation and collapse of vesicles in strong DC fields is shown to be related to membrane poration in Chapter 6. The deformation agrees with a theoretical model for a conducting interface, indicating that electrical conduction through pores short circuits the membrane capacitor. The electric stresses also overcome the mechanical resistance of the membrane, leading to collapse. The rate of deformation before collapse is inhibited by viscous resistance and is slower for high viscosity membranes. Gradients in electric stress along the vesicle produce poration only in the cap regions of the vesicle. The appearance of edges correlates with the critical angle where the electric stress exceeds the mechanical limits of the membrane. An estimate for the lysis tension can be made based on this angle for lipid and polymer membranes. The influence of pores on vesicle dynamics in DC pulses gives rise to many questions about the dynamics of pore formation under an electric stress. A more complete understanding of pore formation may lead to more

efficient methods of electroporation.

A method for investigating the effect of electric fields on membrane fluctuations, based on the frequency dependent shape, is given in Chapter 6. Observations of membrane fluctuations in the presence of electric fields have been limited to planar membranes. Fluctuation analysis on spherical vesicles has been limited to vesicles at equilibrium because electric fields deform vesicles and pull out membrane fluctuations. Measurements of membrane fluctuations are taken at the oblate to prolate transition frequency in AC fields where the vesicle is spherical. The measurements show an increase in bending rigidity and decrease in tension. These observations motivate a physical description of the effect of AC electric fields on membrane fluctuations. Further experiments may also give insight into the effect of transmembrane voltage and induced surface charge on membrane fluctuations.

In all the systems investigated in this thesis, the electromechanical coupling at the interface drives the dynamics. The applied stresses are regulated by interfacial charging, which produce a variety of behaviors that can be exploited to advance technologies ranging from electrorheology to the manipulation of cells.

APPENDIX A

Vesicle deformation in an external field: general equations

A.1 Hydrodynamic stresses

The velocity field outside the vesicle is,

$$\mathbf{u}_{jm0}^- = \frac{1}{2}r^{-j}(2-j+jr^{-2})\mathbf{y}_{jm0} + \frac{1}{2}r^{-j}[j(j+1)]^{1/2}(1-r^{-2})\mathbf{y}_{jm2}, \quad (\text{A.1})$$

$$\mathbf{u}_{jm2}^- = \frac{1}{2}r^{-j}(2-j)\left(\frac{j}{j+1}\right)^{1/2}(1-r^{-2})\mathbf{y}_{jm0} + \frac{1}{2}r^{-j}(j+(2-j)r^{-2})\mathbf{y}_{jm2}. \quad (\text{A.2})$$

The velocity field inside the vesicle is,

$$\mathbf{u}_{jm0}^+ = \frac{1}{2}r^{j-1}(-(j+1)+(j+3)r^2)\mathbf{y}_{jm0} + \frac{1}{2}r^{j-1}[j(j+1)]^{1/2}(1-r^2)\mathbf{y}_{jm2}, \quad (\text{A.3})$$

$$\mathbf{u}_{jm2}^+ = \frac{1}{2}r^{j-1}(3+j)\left(\frac{j+1}{j}\right)^{1/2}(1-r^2)\mathbf{y}_{jm0} + \frac{1}{2}r^{j-1}(j+3-(j+1)r^2)\mathbf{y}_{jm2}. \quad (\text{A.4})$$

These are associated with the stresses,

$$\tau_{jm0}^{\text{hd,in}} = (2j+1)c_{jm0} - 3\left(\frac{j+1}{j}\right)^{1/2}c_{jm2}, \quad (\text{A.5})$$

$$\tau_{jm0}^{\text{hd,ex}} = -(2j+1)c_{jm0} + 3\left(\frac{j}{j+1}\right)^{1/2}c_{jm2}, \quad (\text{A.6})$$

$$\tau_{jm0}^{\text{hd,in}} = 3\left(\frac{j}{j+1}\right)^{1/2}c_{jm0} - \frac{4+3j+2j^2}{j+1}c_{jm2}, \quad (\text{A.7})$$

$$\tau_{jm0}^{\text{hd,ex}} = -3\left(\frac{j}{j+1}\right)^{1/2}c_{jm0} - \frac{3+j+2j^2}{j}c_{jm2}. \quad (\text{A.8})$$

$$(\text{A.9})$$

A.2 Membrane stresses

The bending stresses are given by,

$$\tau_{jm2}^{\kappa} = j(j+1)(j-1)(j+2)f_{jm}, \quad \tau_{jm0}^{\kappa} = 0. \quad (\text{A.10})$$

The stresses due to membrane tension are given by,

$$\tau_{jm2}^{\sigma} = 2\sigma_{jm} + \sigma_h(j-1)(j+2)f_{jm}, \quad \tau_{jm0}^{\sigma} = -\sqrt{j(j+1)}\sigma_{jm}. \quad (\text{A.11})$$

The surface viscous stresses have only in-plane contributions,

$$\tau_{jm0}^{\mu} = -2\sqrt{j(j+1)}K_sc_{202} + j(j+1)K_sc_{200} + (-2+j+j^2)\mu_sc_{200}, \quad (\text{A.12})$$

$$\tau_{jm2}^{\mu} = K_s(-4c_{202} + 2\sqrt{j(j+1)}c_{200}), \quad (\text{A.13})$$

where K_s is a dilational viscosity, which does not come into effect for an incompressible membrane. The surface elastic stresses are,

$$\tau_{jm0}^\eta = \bar{K}_A \sqrt{j(j+1)} (-d_{jm2} + \sqrt{j(j+1)} d_{jm0}) + \bar{\eta} (j-1)(j+2) d_{jm0}, \quad (\text{A.14})$$

$$\tau_{jm2}^\eta = \bar{K}_A (2d_{jm2} - \sqrt{j(j+1)} d_{jm0}), \quad (\text{A.15})$$

where $\bar{K}_A$ is a dimensionless compression modulus $\bar{K}_A = K_A/(a\epsilon E_0^2)$, and $\bar{\eta}$ is the shear elastic modulus $\bar{\eta} = \eta/(a\epsilon E_0^2)$. These terms are zero for the vesicle model.

Isotropic Pressure

From the calculation of electric stress, an isotropic pressure term remains. The term is given by

$$p_{iso} = \frac{1}{6} (-1 - 8B_{\text{ex}} + 2B_{\text{ex}}^2 + B_{\text{in}}^2) \quad (\text{A.16})$$

which has been multiplied by the Y_{00} coefficient $1/\sqrt{4\pi}$. This term is absorbed by the hydrostatic pressure and can be neglected.

A.3 Results

Only membrane bending, tension, and surface viscous stresses are considered. Surface elastic stresses are ignored in this model. The tangential stress balance is solved first, this determines the non-isotropic part of the membrane tension,

$$\sigma_{jm} = Ca_\kappa \left[\frac{-\tau_{jm0}^{el}}{\sqrt{j(j+1)}} + c_{jm2} \frac{2+j+(j-1)(\Lambda+2(j+2)\Lambda_s)}{j(j+1)} \right]. \quad (\text{A.17})$$

Using the expression for σ_{jm} in the normal stress balance, the normal surface velocity coefficient can be solved for,

$$c_{jm2} = C_{jm} + Ca^{-1}(\Gamma_1 + \sigma_h \Gamma_2) f_{jm} \quad (\text{A.18})$$

and

$$C_{jm} = \frac{\sqrt{j(j+1)}}{d(\Lambda, j)} \left[2\tau_{jm0}^{\text{el}} + \sqrt{j(j+1)}\tau_{jm2}^{\text{el}} \right], \quad (\text{A.19})$$

$$\Gamma_1 = -(j+2)(j-1)[j(j+1)]^2 d(\Lambda, j)^{-1}, \quad (\text{A.20})$$

$$\Gamma_2 = -(j+2)(j-1)[j(j+1)] d(\Lambda, j)^{-1}, \quad (\text{A.21})$$

and

$$d(\eta, j) = (4 + 3j^2 + 2j^3) + (-5 + 3j^2 + 2j^3)\Lambda + 4(-2 + j + j^2)\Lambda_s. \quad (\text{A.22})$$

APPENDIX B

Higher order vesicle deformation

This appendix outlines an approach for calculating the excitation of higher order shape modes resulting from disturbances in the electric field caused by ellipsoidal deformation.

B.1 Physical model

The vesicle interface is described by an expansion in scalar spherical harmonics,

$$r_s = a(1 + f(\theta, \phi)), \quad (\text{B.1})$$

where,

$$f(\theta, \phi) = \sum_{j=2}^{\infty} \sum_{m=-j}^j f_{jm} Y_{jm}. \quad (\text{B.2})$$

The original electrostatic problem is solved for around a sphere. The potential can be expanded,

$$\Phi = \Phi_0(r, \theta) + \epsilon \Phi_1(r, \theta) + O(\epsilon^2), \quad (\text{B.3})$$

where ϵ is a small parameter. Each potential must satisfy Laplace's equation,

$$\nabla^2 \Phi = \nabla^2 \Phi_0(r, \theta) + \epsilon \nabla^2 \Phi_1(r, \theta) + O(\epsilon^2) = 0, \quad (\text{B.4})$$

$$\nabla^2 \Phi_0(r, \theta) = 0, \quad (\text{B.5})$$

$$\nabla^2 \Phi_1(r, \theta) = 0. \quad (\text{B.6})$$

The solution to Φ_0 satisfies the boundary conditions

$$\Phi_0^{\text{ex}} = -E_0 \left[r + B_{\text{ex}} \frac{a^3}{r^2} \right] \sum_{n=-1}^1 e_{1n}^\infty Y_1 n, \quad \Phi_0^{\text{in}} = -E_0 B_{\text{in}} r \sum_{n=-1}^1 e_{1n}^\infty Y_1 n, \quad (\text{B.7})$$

where,

$$B_{\text{ex}} = \frac{(1 - R) + R\bar{V}(t)}{R + 2}, \quad B_{\text{in}} = \frac{3 - 2\bar{V}(t)}{R + 2}, \quad (\text{B.8})$$

and,

$$\bar{V}(t) = \frac{3}{2} \left[1 - \exp \left(-\frac{t}{t_m} \right) \right]. \quad (\text{B.9})$$

The solution to Φ_1 will satisfy zero potential at $r = \infty$ because this boundary condition was satisfied by Φ_0 .

$$\Phi_1(r = \infty, \theta) = 0 \quad (\text{B.10})$$

and the boundary condition on the interface remains,

$$\mathbf{n} \cdot (\lambda_{\text{ex}} \mathbf{E}_{\text{ex}}) = \mathbf{n} \cdot (\lambda_{\text{in}} \mathbf{E}_{\text{in}}) = C_m \frac{dV_m}{dt} \quad (\text{B.11})$$

where $V_m = \Phi^{\text{ex}}(r = 1, \theta) - \Phi^{\text{in}}(r = 1, \theta)$. The boundary condition at the interface

also includes an $O(\epsilon)$ contribution from Φ_0 by expanding around $r = 1$,

$$\Phi_0(r, \theta) = \Phi_0(r = 1, \theta) + \epsilon f_{20} \frac{\partial \Phi_0(r = 1, \theta)}{\partial r}. \quad (\text{B.12})$$

The boundary condition becomes,

$$C_m \frac{\partial V_m}{\partial t} = \mathbf{n} \cdot (-\lambda_{\text{ex}} \nabla \Phi_1^{\text{ex}} - f_{20} E_0 \cos(\theta) (1 - 2B_{\text{ex}})), \quad (\text{B.13})$$

$$C_m \frac{\partial V_m}{\partial t} = \mathbf{n} \cdot (-\lambda_{\text{in}} \nabla \Phi_1^{\text{in}} - f_{20} E_0 \cos(\theta) B_{\text{in}}). \quad (\text{B.14})$$

Because Φ_1 must satisfy Laplace's equation, the general solution for Φ_1 remains,

$$\Phi_1 = \sum_{n=0}^{\infty} (A_n r^n + B_n r^{-1-n}) B_n(\cos(\theta)). \quad (\text{B.15})$$

We can anticipate a solution up to order $n = 3$, the boundary conditions at $r = \infty$ and $r = 0$ reduce the problem to,

$$\Phi_1^{\text{ex}} = A_1 r B_1(\cos(\theta)) + A_2 r^2 B_2(\cos(\theta)) + A_3 r^3 B_3(\cos(\theta)), \quad (\text{B.16})$$

$$\Phi_1^{\text{in}} = B_1/r^2 B_1(\cos(\theta)) + B_2/r^3 B_2(\cos(\theta)) + B_3/r^3 B_3(\cos(\theta)). \quad (\text{B.17})$$

Applying the first two terms in Eq.[B.11] provide an algebraic relationship between A_n and B_n terms.

$$B_1 = -\frac{1}{10} \left(E_0 f_{20} \sqrt{\frac{5}{\pi}} ((2B_{\text{ex}} - 1) + B_{\text{in}} R) + 5R A_1 \right) \quad (\text{B.18})$$

$$B_2 = -\frac{2R A_2}{3} \quad (\text{B.19})$$

$$B_3 = -\frac{3}{40} \left(E_0 f_{20} \sqrt{\frac{5}{\pi}} ((2B_{\text{ex}} - 1) + B_{\text{in}} R) + 10 R A_3 \right) \quad (\text{B.20})$$

Now balancing the other two terms in Eq.[B.11], a set of differential equations for A_n are found by equating terms with the same angle dependence. They are solved using the initial condition $V_m(t=0) = 0$ and $f_{20}(t=0) = 0$.

$$A_1 = -\frac{E_0 f_{20} B_{\text{in}}}{\sqrt{5\pi}}, \quad A_2 = 0, \quad A_3 = -\frac{E_0 f_{20} B_{\text{in}}}{2a^2 \sqrt{5\pi}} \quad (\text{B.21})$$

$$B_1 = -\frac{E_0 a^3 f_{20} (1 - 2B_{\text{ex}})}{2\sqrt{5\pi}}, \quad B_2 = 0, \quad B_3 = -\frac{3E_0 a^5 f_{20} (1 - 2B_{\text{ex}})}{8\sqrt{5\pi}} \quad (\text{B.22})$$

B.2 Stresses

The stress on the vesicle due to Φ_1 can be calculated using the Maxwell stress tensor,

$$\mathbf{T}^{\text{el}} = \epsilon_{\text{ex}} \left[(\hat{r} \cdot \mathbf{E}_{\text{ex}}) \mathbf{E}_{\text{ex}} - \frac{1}{2} \mathbf{E}_{\text{ex}} \cdot \mathbf{E}_{\text{ex}} \hat{r} \right] - \epsilon_{\text{in}} \left[(\hat{r} \cdot \mathbf{E}_{\text{in}}) \mathbf{E}_{\text{in}} - \frac{1}{2} \mathbf{E}_{\text{in}} \cdot \mathbf{E}_{\text{in}} \hat{r} \right]. \quad (\text{B.23})$$

From this calculation, which involves recoupling of vector spherical harmonics, there are terms proportional to $j = 0$, $j = 2$, $j = 4$, and $j = 6$, $m = 0$ for all terms. The $j = 4$ terms will be important for describing square deformation, the lower terms are higher order terms to the isotropic pressure and ellipsoidal deformation,

$$p_{\text{el}} = E_0^2 f_{20}^2 \frac{-1576 B_{\text{in}}^2 + 1157 (1 - 2B_{\text{ex}})^2 S}{6160 \sqrt{\pi}}, \quad (\text{B.24})$$

$$\tau_s = -E_0^2 f_{20}^2 \frac{212B_{\text{in}}^2 + 137(1 - 2B_{\text{ex}})^2 S}{308\sqrt{5\pi}}. \quad (\text{B.25})$$

When including B_{in} and B_{ex} ,

$$p_{\text{el}} = \frac{9 \exp(-2t/t_{\text{mm}}) E_0^2 f_{20}^2 (-1576 + 1157R^2 S)}{6160\sqrt{\pi}(R+2)^2}, \quad (\text{B.26})$$

$$\tau_s = -\frac{9 \exp(-2t/t_{\text{mm}}) E_0^2 f_{20}^2 (212 + 137R^2 S)}{308\sqrt{5\pi}(R+2)^2}. \quad (\text{B.27})$$

The stresses are converted to spherical harmonics,

$$\mathbf{T}_{\text{el}} = \frac{2\sqrt{\pi}}{3} p_{\text{el}} \mathbf{Y}_{202}(\theta, \phi) - \frac{32\sqrt{\pi/5}}{3} \tau_s \mathbf{Y}_{200}(\theta, \phi). \quad (\text{B.28})$$

The forcing term for C_{40} is solved using,

$$C_{jm} = -\frac{\sqrt{j(j+1)}}{d(\Lambda, j)} \left[2\tau_{jm0}^{\text{el}} + \sqrt{j(j+1)} \tau_{jm2}^{\text{el}} \right], \quad (\text{B.29})$$

which can now be included in the evolution equation for f_{40} ,

$$C_{40} = -\frac{4(5\tau_{jm2}^{\text{el}} - \sqrt{5}\tau_{jm0}^{\text{el}})}{9(20 + 19\eta + 8\eta_s)}. \quad (\text{B.30})$$

The overall evolution equation is given by

$$\frac{\partial f_{40}}{\partial t} = C_{40} - C a_{\kappa}^{-1} \frac{40(20 + \tilde{\sigma}_h) f_{40}}{20 + 19\Lambda + 8\Lambda_s}. \quad (\text{B.31})$$

APPENDIX C

Membrane fluctuations

C.1 Seifert-Langer theory

The following section summarizes the model for fluctuations of a planar membrane developed by Seifert and Langer (1993).

The lipid membrane immersed in a fluid that obeys the Stokes equation and is considered incompressible

$$\nabla p - \eta \nabla^2 \mathbf{v} = \mathbf{K}(\mathbf{r}) , \quad \nabla \cdot \mathbf{v} = 0, \quad (\text{C.1})$$

where $\mathbf{K}(\mathbf{r})$ is a point force.

The equations can be solved by first taking the Fourier transform of both variables, $\mathbf{v}$ and $\mathbf{K}$,

$$\int \exp(-i\mathbf{k} \cdot \mathbf{r}) \mathbf{v}(\mathbf{r}) d\mathbf{x}, \quad (\text{C.2})$$

$$\int \exp(-i\mathbf{k} \cdot \mathbf{r}) \mathbf{K}(\mathbf{r}) d\mathbf{x}. \quad (\text{C.3})$$

The solution after taking the variable transforms is,

$$v = \frac{1}{\eta k^2} \left(\delta_{ij} - \frac{k_i k_j}{k^2} \right) \mathbf{K}_{kj} \quad (\text{C.4})$$

The force density for small deformations can be approximated by,

$$\mathbf{K}_{qz}(z) = \delta(z) [\mathbf{K}_{qz} \hat{\mathbf{z}} + \mathbf{K}_{qq} \hat{\mathbf{q}}], \quad (\text{C.5})$$

where $\hat{\mathbf{q}}$ is the unit vector parallel to the wave vector, $\mathbf{q}$, $\hat{\mathbf{k}}$ is the in-plane vector, which is perpendicular to $\hat{\mathbf{q}}$ and $\hat{\mathbf{z}}$. This gives the relation between velocity and force density for displacement z ,

$$v_{qz} = \frac{1}{4\eta q} \mathbf{K}_{qz} \quad (\text{C.6})$$

and the same for q in place of z .

A description of the normal bending force must be derived. This is done by taking the derivative of a membrane energy F , which is given by

$$F = \frac{1}{2} (h_q, \rho_q, \rho_q) \begin{pmatrix} \tilde{\kappa} q^4 + \sigma q^2 & -2kdq^2 & 0 \\ -2kdq^2 & 2k & 0 \\ 0 & 0 & 2k \end{pmatrix} \begin{pmatrix} h_q \\ \rho_q \\ \rho_q \end{pmatrix}, \quad (\text{C.7})$$

where $\tilde{\kappa} = \kappa + 2d^2k$.

If one first considers the height of the bilayer, the force is given by,

$$\mathbf{K}_{qz} = -\frac{\partial F}{\partial h_q} = -[\tilde{\kappa} q^4 h_q - 2kdq^2 \rho_q]. \quad (\text{C.8})$$

Including this equation in Eq.[C.6] and writing $v_q = \partial h_q / \partial t$, the equation of motion

becomes,

$$\frac{\partial h_q}{\partial t} = -\Gamma(q)[\tilde{\kappa}q^4 h_q - 2kdq^2 \rho_q] \quad (\text{C.9})$$

. This equation includes the local density difference ρ , and therefore another equation must be developed to determine ρ . This is accomplished by considering the in-plane force balance for each monolayer,

$$-\tilde{\nabla}\sigma^\pm + -T_{xz}^\pm \mp b(\tilde{\mathbf{v}}^+ - \tilde{\mathbf{v}}^-) + \mu\tilde{\nabla}\tilde{\mathbf{v}}^\pm = 0 \quad (\text{C.10})$$

At small values, the local density difference is related to the membrane velocity by

$$\frac{\partial \rho^\pm}{\partial t} = -\tilde{\nabla} \cdot \tilde{\mathbf{v}}^\pm \quad (\text{C.11})$$

A no-slip boundary between the membrane and the surrounding fluid is assumed. An equation of motion for the upper and lower densities is found by inserting the continuity equation into Eq.[C.10]. The difference between the upper and lower layers is found by subtracting the two equations. A Fourier transform gives the equation in the same form as Eq.[C.6], which includes information of mean position and lipid density,

$$\frac{\partial}{\partial t} \begin{pmatrix} h_q \\ \rho_q \end{pmatrix} = -\Gamma(q)E(q) \begin{pmatrix} h_q \\ \rho_q \end{pmatrix} \quad (\text{C.12})$$

where $\Gamma(q)$ is the matrix of kinetic coefficients,

$$\Gamma(q) = \begin{pmatrix} 1/4\eta q & 0 \\ 0 & \frac{q^2}{2(2b+2\eta q+\mu q^2)} \end{pmatrix} \quad (\text{C.13})$$

and $E(q)$ is defined by Seifert as the 'energy matrix', because it gives the total energy

of the bilayer membrane when it is integrated over all modes, q , $E(q)$ is given by,

$$E_q = \begin{pmatrix} \tilde{\kappa}q^4 + \sigma q^2 & -2kdq^2 \\ -2kdq^2 & 2k \end{pmatrix} \quad (\text{C.14})$$

The eigenvalues of the matrix $\Gamma(q)E(q)$ give two timescales, γ_1 and γ_2 . γ_1 has three different characteristic wavelengths, each with a different q dependence. When an unbounded, zero tension, membrane is considered, two cutoff wavelengths are given by $q_1 = 2\eta k/b\tilde{\kappa}$ and $q_2 = \sqrt{2b/\mu}$.

$$\gamma_1 = \begin{cases} \frac{\tilde{\kappa}}{4\eta}q^3 & q \ll q_1 \\ \frac{k}{2b}\frac{\tilde{\kappa}}{\tilde{\kappa}}q^2 & q_1 \ll q \ll q_2 \\ \frac{k}{\mu}\frac{\tilde{\kappa}}{\tilde{\kappa}} & q_2 \ll q \end{cases}, \gamma_2 = \begin{cases} \frac{k}{2b}q^2 & q \ll q_1 \\ \frac{\tilde{\kappa}}{4\eta}q^3 & q_1 \ll q \end{cases}. \quad (\text{C.15})$$

The solution to Eq.[C.12] for the height and density function is given by exponential functions,

$$\begin{pmatrix} h_q(t) \\ \rho_q(t) \end{pmatrix} = \exp(-\Gamma(q)E(q)t) \begin{pmatrix} h_q(0) \\ \rho_q(0) \end{pmatrix}. \quad (\text{C.16})$$

The quantity of interest, which is measurable with experiments, is the height-height correlations. The general expression is,

$$\langle h_q(t)h_{-q}(0) \rangle = \frac{k_b T}{\tilde{\kappa}q^4 + \sigma q^2} (A_1(q) \exp(-\gamma_1(q)t) + A_2(q) \exp(-\gamma_2(q)t)), \quad (\text{C.17})$$

where,

$$A_1(q) = \frac{\gamma_2(q) - (\kappa q^3 + \sigma q)/4\eta}{\gamma_2(q) - \gamma_1(q)}, \quad A_2(q) = 1 - A_1(q). \quad (\text{C.18})$$

C.2 Values of material properties

The material properties of various bilayer membranes have been studied and can be used to plot diagrams of γ_1 and γ_2 versus q . The material properties for representative systems are given in the table below. The parameters include elastic stretching modulus K_a , hydrophobic core thickness d , bending rigidity, κ , intermonolayer friction, b , membrane viscosity μ_s .

Membrane Type	κ 10^{-20} J	K_a (N/m)	$b(Ns/m^3)(10^8)$	$\mu_s(Ns/m)$	Source
PEO-Pbd	16.9	.1-.470	1-2	1-4x10 ⁻⁶	2), 5),
SM:Chol (60:40)	30-55.6	2-3	~12	~10x10 ⁻⁶	1), 3),4)
POPC	15.5	.2	~2	300x10 ⁻⁶	5)
DOPC	10	.3	.1-1	.005x10 ⁻⁶	3), 5)

Table C.1: Material values for various membrane types. Citations: 1) [68], 2) [48], 3) [160], 4) [57], 5) [136]

These values can be used to estimate the order of magnitudes for the cutoff wavelengths and relaxation rates.

Membrane Type	q_1 $1/\mu m$	q_2 $1/\mu m$
PEO-Pbd	0.6	7
SM:Chol (60:40)	0.3	15
POPC	2.13	1.15
DOPC	23.5	89.4

Table C.2: Cutoff wave vectors for different membranes.

Membrane Type	$\gamma_1 (q \ll q_1)1/s$	$\gamma_1 (q_1 \ll q \ll q_2)1/s$	$\gamma_1 (q_2 \ll q)1/s$
PEO-Pbd	$4 \times 10^{-17} q^3$	$2.5 \times 10^{-11} q^2$	1254
SM:Chol (60:40)	$7.5 \times 10^{-17} q^3$	$2.3 \times 10^{-11} q^2$	5535
POPC	$3.875 \times 10^{-17} q^3$	$8.25 \times 10^{-11} q^2$	110
DOPC	$.25 \times 10^{-17} q^3$	$5.8 \times 10^{-10} q^2$	4.7×10^6

Table C.3: Wave vector dependence for γ_1 for various membrane types

Membrane Type	$\gamma_2 (q \ll q_1)1/s$	$\gamma_2 (q_1 \ll q)1/s$
PEO-Pbd	$5x10^{-10} q^2$	$8.4 \times 10^{-16} q^3$
SM:Chol (60:40)	$8.3x10^{-11} q^2$	$2.7 \times 10^{-16} q^3$
POPC	$5x10^{-10} q^2$	$2.3 \times 10^{-16} q^3$
DOPC	$7.5x10^{-9} q^2$	$3.2x 10^{-16} q^3$

Table C.4: Wave vector dependence for γ_2 for various membrane types

C.3 Relaxation rates: dependence on membrane properties

The following are estimates of the two membrane relaxation rates γ_1, γ_2 vs. wavevector q using the material values given in Table 1. The red line indicates q_1 , the blue line indicates q_2 . The shaded box is an estimate for the regime that can be observed using fluctuation analysis, i.e. optical microscopy, for a vesicle with radius, $R_{ves} \sim 20 \mu m$. This gives $q = n/R_{ves}$ where $n \sim 2-10$. A higher value for n is attainable for larger radius vesicles, although the accessible wavenumbers in the experiment are limited by resolution.

Increasing intermonolayer slip shifts both cutoff wave vectors, see Figure C.1. The first crossover wave vector is shifted lower because $q_1 \sim 1/b$ while the second crossover wave vector shifts higher, $q_2 \sim \sqrt{b}$. For $q < q_1$ the second relaxation rate γ_2 is decreased as $\gamma_2 \sim 1/b$ while the first relaxation rate is unaffected. In the range $q_1 < q < q_2$, the first relaxation rate γ_1 is also decreased as $\gamma_1 \sim 1/b$.

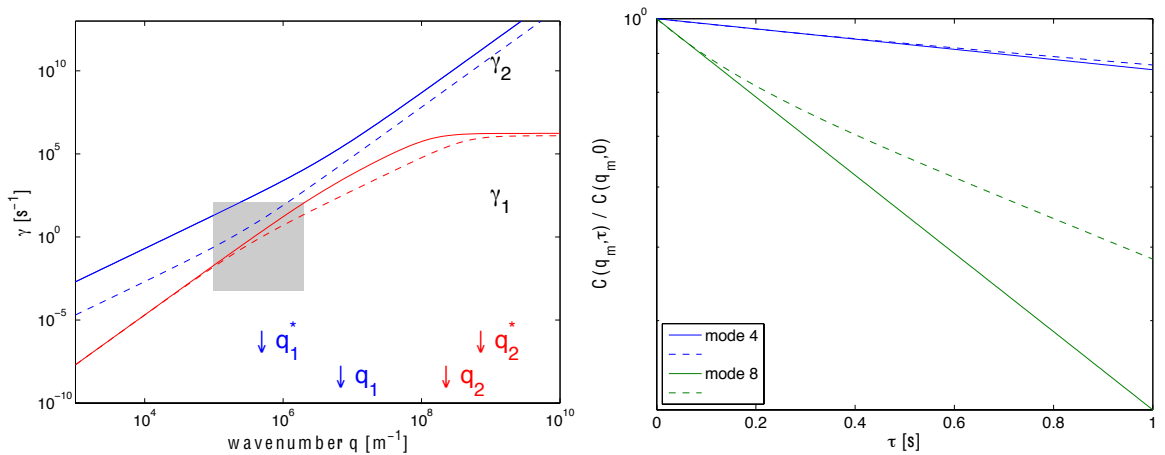


Figure C.1: The effect of intermonolayer slip, b , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8 Ns/m^3$. The dashed line shows relaxation rates for identical parameters except for $b = 10 \times 10^8 Ns/m^3$.

An increase in membrane thickness, d , shifts the first cutoff wave vectors q_1 , see Figure C.2. The first crossover wave vector is shifted through a change in $\tilde{\kappa}$ because $q_1 \sim 1/\tilde{\kappa} \sim 1/d^2$. The second crossover wave vector is unaffected. For $q < q_1$, both timescales are unchanged. In the range $q_1 < q$ the first relaxation rate γ_1 is also decreased as $\gamma_1 \sim 1/\tilde{\kappa} \sim 1/d^2$.

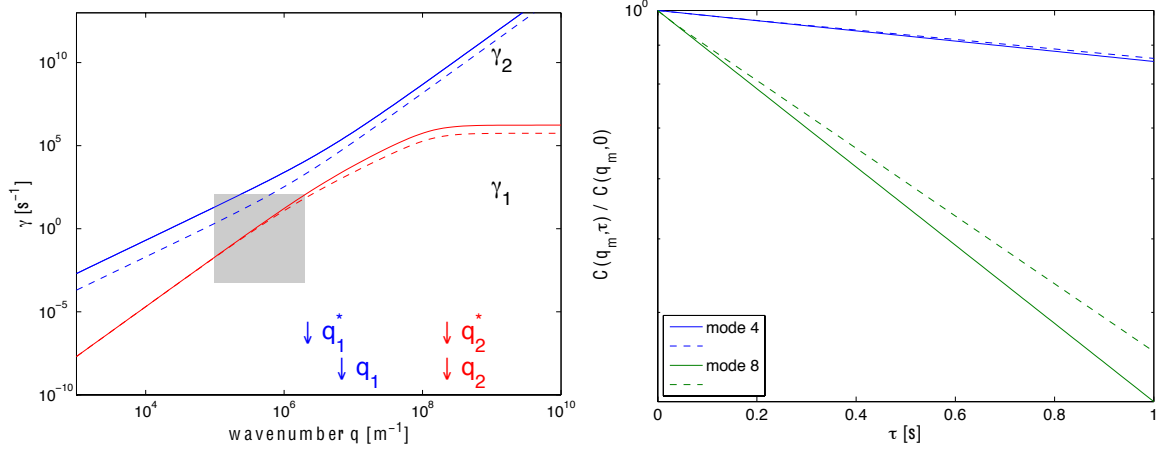


Figure C.2: The effect of membrane thickness, d , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9} \text{m}$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9} \text{Ns/m}$, $b = 1 \times 10^8 \text{Ns/m}^3$. The dashed line shows relaxation rates for identical parameters except for $d = 2.5 \times 10^{-9} \text{m}$.

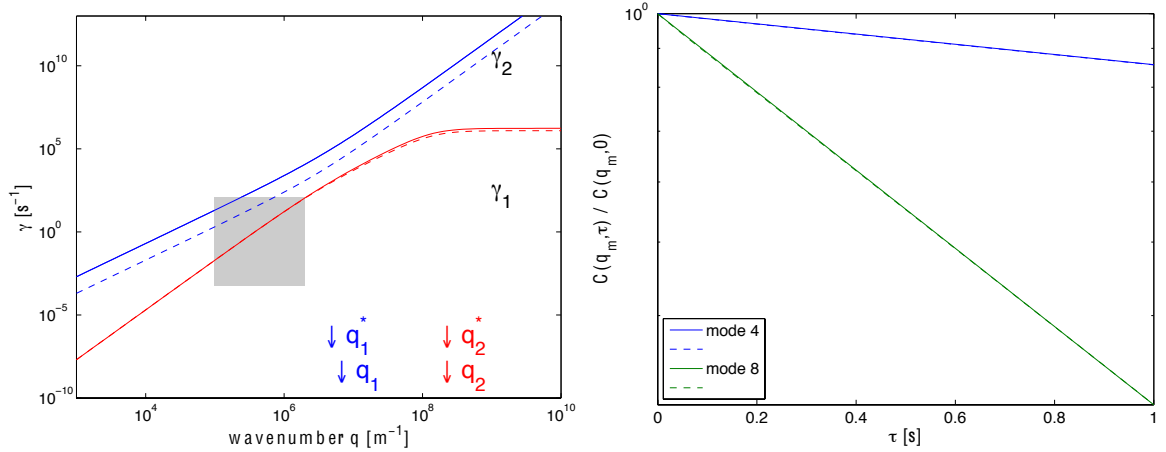


Figure C.3: The effect of membrane stretching modulus, K_a , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid line shows data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9} \text{m}$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9} \text{Ns/m}$, $b = 1 \times 10^8 \text{Ns/m}^3$. The dashed line shows relaxation rates for identical parameters except for $K_a = 0.04N/m$.

An increase in membrane stretching modulus, k , shifts the first cutoff wave

vectors q_1 , see Figure C.3. The first crossover wave vector is shifted as $q_1 \sim k$. The second crossover wave vector is unaffected. For $q < q_1$, only the second timescale γ_2 is increased linearly, $\gamma_2 \sim k$. In the range $q_1 > q$ the first relaxation rate γ_1 is not significantly affected if $d^2k > \kappa$, in this limit $\gamma_1 \sim k/\tilde{\kappa} \sim k/k$.

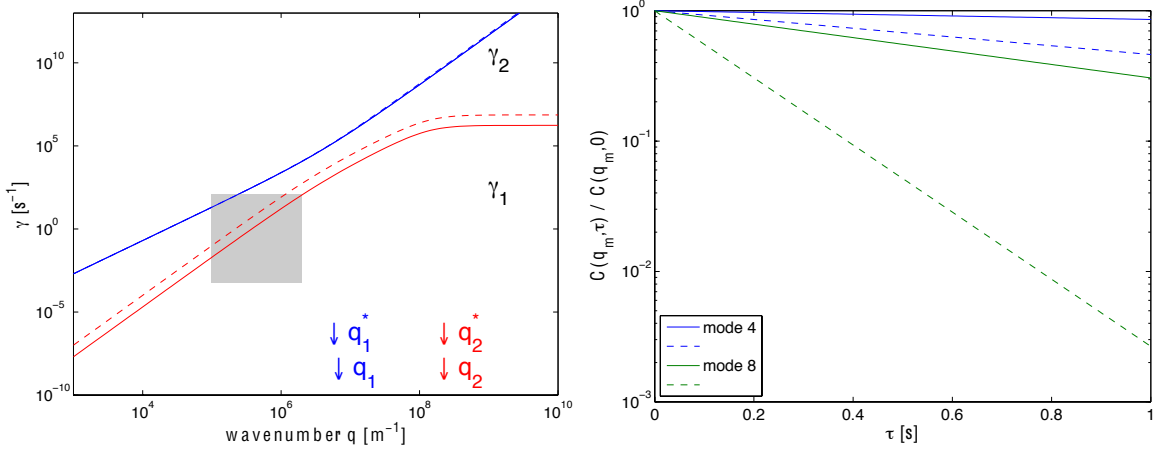


Figure C.4: The effect of membrane bending rigidity, κ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$.

An increase in membrane stretching modulus, k , shifts the first cutoff wave vectors q_1 , see Figure C.4. The first crossover wave vector is shifted as $q_1 \sim k$. The second crossover wave vector is unaffected. For $q < q_1$, only the second timescale γ_2 is increased linearly, $\gamma_2 \sim k$. In the range $q_1 > q$ the first relaxation rate γ_1 is not significantly affected if $d^2k > \kappa$, in this limit $\gamma_1 \sim k/\tilde{\kappa} \sim k/k$. Also in this limit, $\gamma_2 \sim k$ through $\tilde{\kappa}$. If $d^2k < \kappa$ then $\gamma_1 \sim k$.

An increase in membrane viscosity primarily affects the highest wave vectors, see Figure C.5.

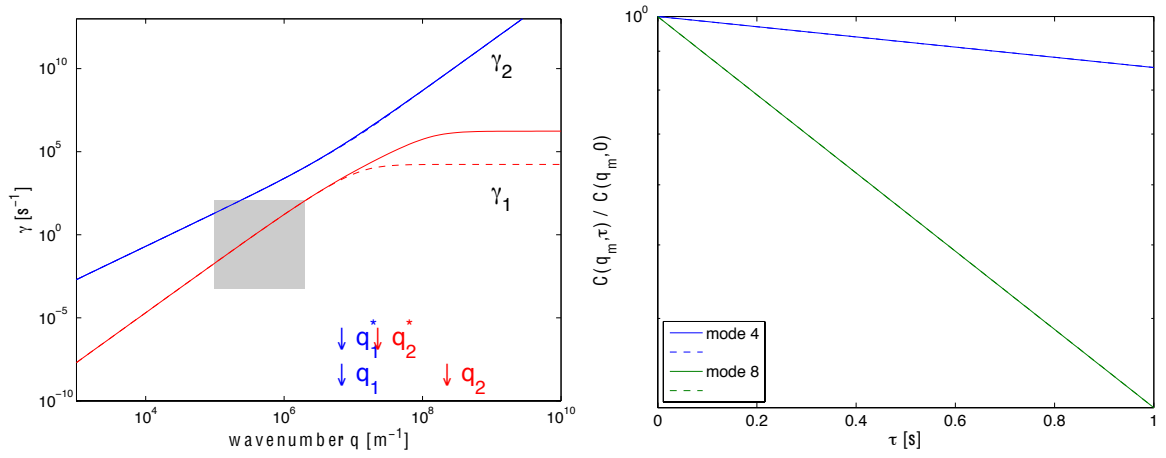


Figure C.5: The effect of membrane viscosity, μ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\mu_s = 1 \times 10^{-6}$.

The effect of tension, which primarily affects low wave vectors, is shown in Figure C.6.

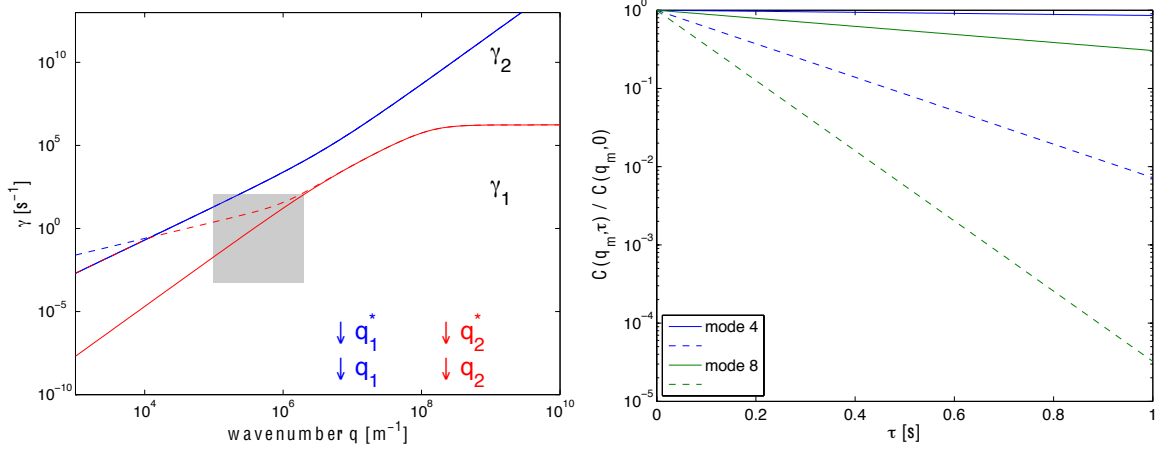


Figure C.6: The effect of membrane tension, σ , on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0\text{N/m}$, $d = 1.5 \times 10^{-9}\text{m}$, $K_a = 0.4\text{N/m}$, $\mu_s = 10 \times 10^{-9}\text{Ns/m}$, $b = 1 \times 10^8\text{Ns/m}^3$. The dashed lines shows relaxation rates for identical parameters except for $\sigma = 1 \times 10^{-7}\text{N/m}$.

The combined effect using the properties for polymer and lipid membranes with and without tension is shown in Figures C.7 and C.8.

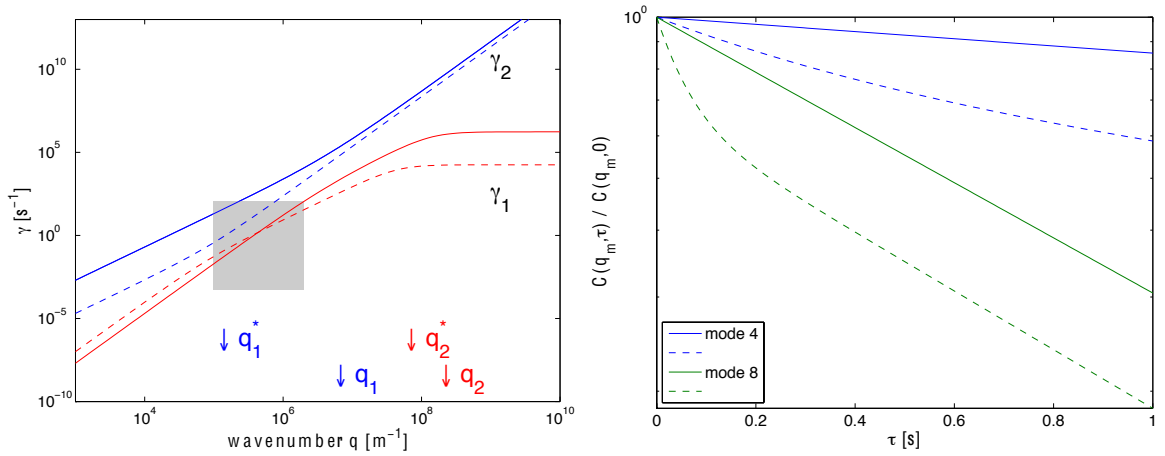


Figure C.7: The effect of all parameters excluding tension, on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data with $\kappa = 20k_bT$, $\sigma = 0\text{N/m}$, $d = 1.5 \times 10^{-9}\text{m}$, $K_a = 0.4\text{N/m}$, $\mu_s = 10 \times 10^{-9}\text{Ns/m}$, $b = 1 \times 10^8\text{Ns/m}^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$, $\sigma = 0$, $d = 2.5 \times 10^{-9}\text{m}$, $K_a = 0.04\text{N/m}$, $\mu_s = 1 \times 10^{-6}$, $b = 10 \times 10^8\text{Ns/m}^3$.

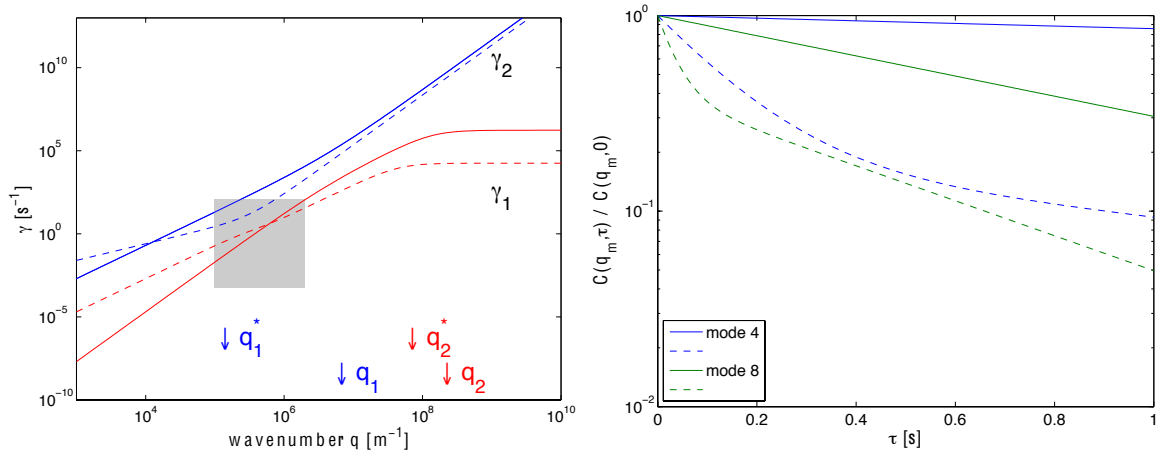


Figure C.8: The effect of all parameters, including tension, on the relaxation times γ_1 and γ_2 for a tensionless membrane. The solid lines show data for with $\kappa = 20k_bT$, $\sigma = 0N/m$, $d = 1.5 \times 10^{-9}m$, $K_a = 0.4N/m$, $\mu_s = 10 \times 10^{-9}Ns/m$, $b = 1 \times 10^8Ns/m^3$. The dashed lines shows relaxation rates for identical parameters except for $\kappa = 100k_bT$, $\sigma = 1 \times 10^{-7}N/m$, $d = 2.5 \times 10^{-9}m$, $K_a = 0.04N/m$, $\mu_s = 1 \times 10^{-6}$, $b = 10 \times 10^8Ns/m^3$.

Bibliography

- [1] http://www.nature.com/horizon/livingfrontier/background/figs/membrane_f2.html.
- [2] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. Molecular biology of the cell. Garland Publishing Inc., New York, 4th Edition, 2002.
- [3] Kathleen T Alligood, Tim D Sauer, and James A Yorke. Chaos: an introduction to dynamical systems. Springer, 1996.
- [4] O. Alvarez and R. Latorre. Voltage-dependent capacitance in lipid bilayers made from monolayers. Biophys. J., 21:1–17, 1978.
- [5] T. Ambjornsson, M. A. Lomholt, and P. L. Hansen. Applying a potential across a biomembrane: Electrostatic contribution to the bending rigidity and membrane instability. Phys. Rev. E, 75:051916, 2007.
- [6] M. I. Angelova and D. S. Dimitrov. Liposome electroformation. Faraday Discuss. Chem. Soc., 81:303– 311, 1986.
- [7] K. Antonova, V. Vitkova, and M. D. Mitov. Deformation of giant vesicles in ac electric fields-dependence of the prolate-to-oblate transition frequency on vesicle radius. Eur. Phys. Lett., 89:38004, 2010.
- [8] S. Aranda, K. A. Riske, R. Lipowsky, and R. Dimova. Morphological transitions of vesicles induced by ac electric fields. Biophys. J., 95:L19–L21, 2008.
- [9] Discher DE Aranda-Espinoza H, Hammer DA. Electromechanical limits of polymersomes. Phys. Rev. Lett., 87:208301, 2001.
- [10] H Aref and S Balachandar. Chaotic advection in a stokes flow. Physics of Fluids, 29(11):3515–3521, 1986.
- [11] A.V. Babakov, L. N. Ermishkin, and E. A Liberman. Influence of electric field on the capacity of phospholipid membranes. Nature, 210:953–955, 1966.
- [12] J. C. Bacri and D. Salin. Bistability of ferrofluid magnetic drops under magnetic field. J. Magn. Magn. Mater., 39:48–50, 1983.
- [13] Giuseppe Battaglia and Anthony J. Ryan. Bilayers and interdigitation in block copolymer vesicles. Journal of the American Chemical Society, 127(24):8757–8764, 2005.

- [14] M. Z. Bazant, K. Thornton, and A. Ajdari. Diffuse-charge dynamics in electrochemical systems. Phys. Rev. E, 70:021506, 2004.
- [15] B.P. Bean. The action potential in mammalian central neurons. Nature Reviews: Neuroscience, 8:451–465, 2007.
- [16] Nikolaos Benteitis and Sonja Krause. Droplet deformation in dc electric fields: the extended leaky dielectric model. Langmuir, 21(14):6194–6209, Jul 2005.
- [17] BJ Bentley and LG Leal. Experimental investigation of drop deformation and breakup in steady, two-dimensional linear flows. Journal of Fluid Mechanics, 167:241–83, 1986.
- [18] R. Benz and K. Janko. Voltage-induced capacitance relaxation of lipid bilayer membranes -effect of membrane composition. Biochimica et Biophysica Acta, 455:721–738, 1976.
- [19] J. Bławdziewicz, P. Vlahovska, and M. Loewenberg. Rheology of a dilute emulsion of surfactant-covered spherical drops. Physica A, 276:50–80, 2000.
- [20] H Block and JP Kelly. Electro-rheology. Journal of Physics D: Applied Physics, 21(12):1661, 2000.
- [21] Harry Block, JP Kelly, A Qin, and T Watson. Materials and mechanisms in electrorheology. Langmuir, 6(1):6–14, 1990.
- [22] M. Bloom, E. Evans, and O. G. Mouritsen. Physical properties of the fluid lipid bilayer component of cell membranes - a perspective. Quarterly reviews of biophysics, 24:293–397, 1991.
- [23] D. Boal. Mechanics of the Cell. Cambridge University Press, 2002.
- [24] N. M. Bonini, M. C. Gustin, and D. L. Nelson. Regulation of ciliary motility by membrane potential in paramecium: A role for cyclic amp. Cell Motility and the Cytoskeleton, 6:256272, 1986.
- [25] F Bordi, C Cametti, and S Sennato. Electrical properties of aqueous liposome suspensions. Advances in Planar Lipid Bilayers and Liposomes, 4:281–320, 2006.
- [26] Michelle R Bringer, Cory J Gerdt, Helen Song, Joshua D Tice, Rustem F Ismagilov, Michelle R Bringer, Cory J Gerdt, Helen Song, Joshua D Tice, and Rustem F Ismagilov. Microfluidic systems for chemical kinetics that rely on chaotic mixing in droplets. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 362(1818):1087–1104, 2004.
- [27] F. Brochard and J. F. Lennon. Frequency spectrum of the flicker phenomenon in erythrocytes. J. Phys.(France), 36:1035–1047, 1975.
- [28] F. Brochard-Wyart, P. G. de Gennes, and O. Sandre. Transient pores in stretched vesicles: role of leak-out. Physica A, 278:32–51, 2000.

- [29] W. Cai and T. C. Lubensky. Hydrodynamic and dynamic fluctuations of fluid membranes. Phys. Rev. E, 52:4251–4266, 1995.
- [30] P. B. Canham. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. J. Theor. Biol., 26:61–81, 1970.
- [31] A. Cebers. Chaos in polarization relaxation of a low-conducting suspension of anisotropic particles. J. Magn. Magn. Mat., 122:277–280, 1993.
- [32] A. Cebers, E. Lemaire, and L. Lobry. Electrohydrodynamic instabilities and orientation of dielectric ellipsoids in low-conducting fluids. Phys. Rev. E, 63:016301, 2000.
- [33] A. Cebers, E. Lemaire, and L. Lobry. Flow modification induced by quinke rotation in a capillary. Int. J. Modern Phys., 16:2603–2609, 2002.
- [34] Chuan-Hua Chen. Electrohydrodynamic stability. Electrokinetics and Electrohydrodynamics in Microsystems, pages 177–220, 2011.
- [35] Y. A. Chizmadzhev and V. F. Pastushenko. Electrical stability of biological and model membranes. Biologicheskie Membrany, 6:1013–1045, 1989.
- [36] K.S. Cole. Membranes, ions and impulses. University of California Press, 1968.
- [37] H. G. L. Coster. Dielectric and electrical properties of lipid bilayers in relation to their structure. In H.T. Tien and A. Ottova-Leitmannova, editors, Planar lipid bilayers (BLMs) and their applications, page 75108. Elsevier, Amsterdam, 2003.
- [38] H. G. L. Coster, T. C. Chilcott, and A. C. F. Coster. Impedance spectroscopy of interfaces, membranes and ultrastructures. Bioelectrochem. Bioenergetics, 40:79–98, 1996.
- [39] Anton Daitche and Tamás Tél. Dynamics of blinking vortices. Physical Review E, 79(1):016210, 2009.
- [40] Debasish Das and David Saintillan. Electrohydrodynamic interactions of spherical particles under quinke rotation. Bulletin of the American Physical Society, 57, 2012.
- [41] N Delorme and A Fery. Direct method to study membrane rigidity of small vesicles based on atomic force microscope force spectroscopy. Physical Review E, 74(3):030901, 2006.
- [42] J. D. Deng, K. H. Schoenbach, E. S. Buescher, P. S. Hair, P. M. Fox, and S. J. Beebe. The effects of intense submicrosecond electrical pulses on cells. Biophys. J., 84:2709–2714, 2003.
- [43] D.S. Dimitrov. Electroporation and electrofusion of membranes. In R. Lipowsky and E. Sackmann, editors, Structure and dynamics of membranes, pages 854–900. Elsevier, 1995.

- [44] R. . Dimova, B. Poligny, and C. Dietrich. Pretransitional effects in dmpe-vesicle membranes: optical dynamometry study. Biophys. J., 79:340–356, 2000.
- [45] R. Dimova, N. Bezlyepkina, M. D. Jord, R. L. Knorr, K. A. Riske, M. Staykova, P. M. Vlahovska, T. Yamamoto, P. Yang, and R. Lipowsky. Vesicles in electric fields: Some novel aspects of membrane behavior. Soft Matter, 5:3201 – 3212, 2009.
- [46] R Dimova, C Dietrich, A Hadjiisky, K Danov, and B Pouligny. Falling ball viscosimetry of giant vesicle membranes: finite-size effects. The European Physical Journal B-Condensed Matter and Complex Systems, 12(4):589–598, 1999.
- [47] R. Dimova, K. A. Riske, S. Aranda, N. Bezlyepkina, R. L. Knorr, and R. Lipowsky. Giant vesicles in electric fields. Soft matter, 3:817–827, 2007.
- [48] R. Dimova, U. Seifert, B. Poligny, S. Førster, and H.-G. Døbereiner. Hyperviscous diblock copolymer vesicles. Eur. Phys. J. D, 7:241–250, 2002.
- [49] D. E. Discher and F. Ahmed. Polymersomes. Annu. Rev. Biomed. Eng., 8:323–341, 2006.
- [50] H. Dobereiner. Properties of giant vesicles. Curr. Opinion Coll. Int. Sci., 5:256–263, 2000.
- [51] R. E, S. Aranda, N. Bezlyepkina, V. Nikolov, K. A. Riske, and R. Lipowsky. A practical guide to giant vesicles. probing the membrane nanoregime via optical microscopy. J. Phys. Cond. Matt., 18:S1151–S1176, 2006.
- [52] D. A. Edwards, H. Brenner, and D. T. Wasan. Interfacial Transport Processes and Rheology. Butterworth-Heinemann, Boston, 1991.
- [53] Marjan Enayati, Ming-Wei Chang, Felix Bragman, Mohan Edirisinghe, and Eleanor Stride. Electrohydrodynamic preparation of particles, capsules and bubbles for biomedical engineering applications. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 382(1):154–164, 2011.
- [54] Lebedev A.V. Engel, A. and K.I Morozov. http://www.nature.com/horizon/livingfrontier/background/figs/membrane_f2.html. Z. Naturforsch.
- [55] J. S. Eow, M. Ghadiri, A. O. Sharif, and T. J. Williams. Electrostatic enhancement of coalescence of water droplets in oil: a review of the current understanding. Chem. Eng. J., 84:173–192, 2001.
- [56] J.M. Escoffre, T. Portet, L. Wasungu, J. Teissie, D . Dean, and M.P. Rols. What is (still not) known of the mechanism by which electroporation mediates gene transfer and expression in cells and tissue. Molecular biotechnology, 41:286–295, 2009.
- [57] Gabriel Espinosa, Iván López-Montero, Francisco Monroy, and Dominique Langevin. Shear rheology of lipid monolayers and insights on membrane fluidity. Proceedings of the National Academy of Sciences, 108(15):6008–6013, 2011.

- [58] E. Evans and W. Rawicz. Entropy driven tension and bending elasticity in condensed-fluid membranes. Phys. Rev. Lett., 64:2094–2097, 1990.
- [59] E. Evans and R. Skalak. Mechanics and Thermodynamics of Biomembranes. CRC Press, Boca Raton, Florida, 1980.
- [60] MD El Alaoui Faris, D Lacoste, J Pécrcéaux, J-F Joanny, J Prost, and P Bassereau. Membrane tension lowering induced by protein activity. Physical review letters, 102(3):038102, 2009.
- [61] J. Q. Feng. A 2d electrohydrodynamic model for electrorotation of fluid drops. J. Coll. Int. Sci., 246:112–121, 2002.
- [62] A Fogden, DJ Mitchell, and BW Ninham. Undulations of charged membranes. Langmuir, 6(1):159–162, 1990.
- [63] Stephan Förster and Eckart Krämer. Synthesis of pbpeo and pipeo block copolymers with alkylolithium initiators and the phosphazene base t-bup4. Macromolecules, 32(8):2783–2785, 1999.
- [64] K. R. Foster and H. P. Schwan. Dielectric properties of tissues and biological materials: a critical review. Crit. Rev. Biomed. Eng., 17:25–104, 1989.
- [65] J.-B. Fournier, A. Ajdari, and L. Peliti. Effective-area elasticity and tension of micromanipulated membranes. Phys. Rev. Lett., 86:4970–4973, 2001.
- [66] L.J. Gentet, G.J. Stuart, and J.D. Clements. Direct measurement of specific membrane capacitance in neurons. Biophys. J., 79:314–320, 2000.
- [67] K. D. Gillis. Techniques for membrane capacitance measurements. In B. Sackmann and E. Neher, editors, Single Channel Recording, pages 155–198. Plenum, New York, 1995.
- [68] R. S. Gracia, N. Bezlyepkina, R. L. Knorr, R. L. Lipowsky, and R. Dimova. Effect of cholesterol on the rigidity of saturated and unsaturated membranes: fluctuation and electrodeformation analysis of giant vesicles. Soft Matter, page DOI:10.1039/B920629A, 2010.
- [69] C. Grosse and H. P. Schwan. Cellular membrane potentials induced by alternating fields. Biophys. J., 63:1632–1642, 1992.
- [70] J. W. Ha and S. M. Yang. Effects of surfactant on the deformation and stability of a drop in a viscous fluid in an electric field. J.Coll. Int. Sci., 175:369–385, 1995.
- [71] J. W. Ha and S. M. Yang. Electrohydrodynamics and electrorotation of a drop with fluid less conductive than that of the ambient fluid. Phys. Fluids, 12:764–772, 2000.
- [72] D. A. Hammer and D. E. Discher. Synthetic cells:self-assembling polymer membranes and bioadhesive colloids. Ann. Rev. Mater. Res., 31:387–404, 2001.
- [73] J. Hanna and P. M. Vlahovska. Surfactant-induced migration of a spherical drop in stokes flow. Phys. Fluids, 22:013102, 2010.

- [74] Leland H Hartwell, John J Hopfield, Stanislas Leibler, and Andrew W Murray. From molecular to modular cell biology. Nature, 402:C47–C52, 1999.
- [75] Hui He, Paul F. Salipante, and Petia M. Vlahovska. Electrorotation of a viscous droplet in a uniform direct current electric field. Physics of Fluids, 25(3):032106, 2013.
- [76] W. Helfrich. Elastic properties of lipid bilayers - theory and possible experiments. Z. Naturforsch., 28c:693–703, 1973.
- [77] W. Helfrich. Deformation of lipid bilayer spheres by electric fields. Z. Naturforsch., 29c:182–183, 1974.
- [78] W. Helfrich and R. M. Servuss. Undulations, steric interactions and cohesion of fluid membranes. Nuovo Cimento Soc. Ital. Fis., 3D:137–151, 1984.
- [79] Jonas Rosager Henriksen and John Hjorth Ipsen. Thermal undulations of quasi-spherical vesicles stabilized by gravity. The European Physical Journal E, 9(4):365–374, 2002.
- [80] Masahiro Hibino, Masaya Shigemori, Hiroyasu Itoh, Kuniaki Nagayama, and K Kinosita. Membrane conductance of an electroporated cell analyzed by submicrosecond imaging of transmembrane potential. Biophysical journal, 59(1):209–220, 1991.
- [81] Paul G Higgs and Jean-François Joanny. Enhanced membrane rigidity in charged lamellar phases. Journal de Physique, 51(20):2307–2320, 1990.
- [82] Jonathan R Howse, Richard AL Jones, Anthony J Ryan, Tim Gough, Reza Vafabakhsh, and Ramin Golestanian. Self-motile colloidal particles: from directed propulsion to random walk. Physical review letters, 99(4):048102, 2007.
- [83] Y. T. Hu, D. J. Pine, and L. G. Leal. Drop deformation, breakup, and coalescence with compatibilizer. Phys. Fluids, 12:484–489, 2000.
- [84] H. Hyuga, K. Kinosita Jr., and N. Wakabayashi. Transient and steady-state deformation of a vesicle with an insulating membrane in response to step-function or alternating electric fields. Jpn. J. Appl.Phys., 30:2649–2656, 1991.
- [85] Nancy E Jackson and Charles L Tucker III. A model for large deformation of an ellipsoidal droplet with interfacial tension. Journal of Rheology, 47:659, 2003.
- [86] ATSA Jaworek and AT Sobczyk. Electro spraying route to nanotechnology: An overview. Journal of Electrostatics, 66(3):197–219, 2008.
- [87] G.B. Jeffrey. The motion of ellipsoid particles immersed in a viscous fluid. Proc. R. Soc. Lond. A, 102:169–179, 1923.
- [88] J. T. Jenkins. The equations of mechanical equilibrium of a model membrane. SIAM J. Appl. Math., 32:755, 1977.

- [89] J. T. Jenkins. Static equilibrium configurations of a model red blood cell. J. Math. Biol., 4:149, 1977.
- [90] T. B. Jones. Quincke rotation of spheres. IEEE Trans. Industry Appl., 20:845–849, 1984.
- [91] T. B. Jones. Quincke rotation of spheres. IEEE Trans. Industry Appl., 20:845–849, 1984.
- [92] T. B. Jones. Electromechanics of particles. Cambridge University Press, New York, 1995.
- [93] Vasilij Kantsler, Enrico Segre, and Victor Steinberg. Critical dynamics of vesicle stretching transition in elongational flow. Physical review letters, 101(4):48101, 2008.
- [94] Vasilij Kantsler and Victor Steinberg. Transition to tumbling and two regimes of tumbling motion of a vesicle in shear flow. Physical review letters, 96(3):36001, 2006.
- [95] E. Karatekin, O. Sandre, H. Guitouni, N. Borghi, P.H. Puech, and F. Brochard-Wyart. Cascades of transient pores in giant vesicles: Line tension and transport. Biophys. J., 84:1734–1749, 2003.
- [96] S. R. Keller and R. Skalak. Motion of a tank -reading ellipsoidal particle in shear flow. J. Fluid Mech., 120:27–47, 1982.
- [97] K. Kinoshita Jr., I. Ashikawa, N. Saita, H. Yoshimura, H. Itoh, K. Nagayama, and A. Ikegami. Electroporation of cell membrane visualized under a pulsed laser fluorescence microscope. Biophys. J., 53:1015–1019, 1988.
- [98] Knut Brøndbo Kjerstad. Clay-Oil Droplet Suspensions in Electric Field. PhD thesis, Norwegian University of Science and Technology, 2012.
- [99] R. L. Knorr, M. Staykova, R. S. Gracia, and R. Dimova. Wrinkling and electroporation of giant vesicles in the gel phase. Soft Matter, page DOI:10.1039/b925929e, 2010.
- [100] Marta Kocun, Waltraut Mueller, Michael Maskos, Ingo Mey, Burkhard Geil, Claudia Steinem, and Andreas Janshoff. Viscoelasticity of pore-spanning polymer membranes derived from giant polymersomes. Soft Matter, 6(11):2508–2516, 2010.
- [101] P. Kramar, D. Miklavcic, M. Kotulska, and A. M. Lebar. Voltage- and current-clamp methods for determination of planar lipid bilayer properties. In A. Iglic, editor, Advances in Planar Lipid Bilayers and Liposomes, vol. 11, pages 29–66. Elsevier, 2010.
- [102] Martin Kraus, Wolfgang Wintz, Udo Seifert, and Reinhard Lipowsky. Fluid vesicles in shear flow. Physical review letters, 77(17):3685–3688, 1996.
- [103] S. Krause and P. Chandratreya. Electrorotation of deformable fluid droplets. J. Coll. Int. Sci., 206:10–18, 1998.

- [104] M. Kummrow and W. Helfrich. Deformation of giant lipid vesicles by electric fields. Phys. Rev. A, 44:8356–8360, 1991.
- [105] E. Lac and G. M. Homsy. Axisymmetric deformation and stability of a viscous drop in a steady electric field. J. Fluid. Mech, 590:239–264, 2007.
- [106] D. Laver. Electrical methods for determining surface charge density and electrolyte composition at the lipid bilayer -solution interface. In A. Iglic, editor, Advances in Planar Lipid Bilayers and Liposomes, vol. 9, pages 87–105. Elsevier, 2009.
- [107] L. G. Leal. Particle motions in a viscous fluid. Ann. Rev. Fluid Mech, 12:435–476, 1980.
- [108] Sigolène Lecuyer, Giovanna Fragneto, and Thierry Charitat. Effect of an electric field on a floating lipid bilayer: A neutron reflectivity study. The European Physical Journal E: Soft Matter and Biological Physics, 21(2):153–159, 2006.
- [109] S. L. Leikin. Thermal fluctuations and capacitance of bilayer lipid membranes in electric field. Biologicheskies Membrany, 2:820–831, 1985.
- [110] H. N. W. Lekkerkerker. The electric contribution to the curvature elastic modulus of charged fluid interfaces. Physica A, 159:319, 1989.
- [111] E. Lemaire and L. Lobry. Chaotic behavior in electro-rotation. Physica A, 314(1-4):663–671, November 2002.
- [112] Elisabeth Lemaire, Laurent Lobry, N Pannacci, and Francois Peters. Viscosity of an electro-rheological suspension with internal rotations. Journal of Rheology, 52:769, 2008.
- [113] G Liao, II Smalyukh, JR Kelly, OD Lavrentovich, and A Jákli. Electrorotation of colloidal particles in liquid crystals. Physical Review E, 72(3):031704, 2005.
- [114] E. M. Lifshitz, L. D. Landau, and L. P. Pitaevskii. Electrodynamics of Continuous Media. Butterworth-Heinemann, 1984.
- [115] R. Lipowsky and E. Sackmann. Structure and dynamics of membranes. Elsevier, Amsterdam, 1995.
- [116] Gabriel Lippmann. Beziehungen zwischen den capillaren und elektrischen erscheinungen. Annalen der Physik, 225(8):546–561, 1873.
- [117] Caterina LoPresti, Hannah Lomas, Marzia Massignani, Thomas Smart, and Giuseppe Battaglia*. Polymersomes: nature inspired nanometer sized compartments. J. Material Chemistry, 19:3576–3590, 2009.
- [118] H. Machemer and M. Eckert. Electrophysiological control of reversed ciliary beating in paramecium. J. General Physiology, 61:572–587, 1973.
- [119] J-B Manneville, P Bassereau, D Levy, and J Prost. Activity of transmembrane proteins induces magnification of shape fluctuations of lipid membranes. Physical review letters, 82(21):4356–4359, 1999.

- [120] J. B. Manneville, P. Bassereau, S. Ramaswamy, and J. Prost. Active membrane fluctuations studied by micropipet aspiration. Phys. Rev. E, 64:021908, 2001.
- [121] D. Marsh. Elastic curvature constants of lipid monolayers and bilayers. Chem. Phys. Lipids, 144:146159, 2006.
- [122] M. W. Matsen and F. S. Bates. Testing the strong-stretching assumption in a block copolymer microstructure. Macromolecules, 28(26):8884–8886, 1995.
- [123] C. D. McCaig, A. M. Rajnicek, B. Song, and M. Zhao. Controlling cell behavior electrically: Current views and future potential. Physiol. Rev., 85:943–978, 2005.
- [124] C. D. McCaig, B. Song, and A. M. Rajnicek. Electrical dimensions in cell science. J. Cell Sci., 122:4267–4276, 2009.
- [125] J. R. Melcher and G. I. Taylor. Electrohydrodynamics - a review of role of interfacial shear stress. Annu. Rev. Fluid Mech., 1:111–146, 1969.
- [126] P. Meleard, C. Gerbeaud, P. Bardusco, N. Jeandaine, M.D. Mitov, and L. Fernandez-Puente. Mechanical properties of model membranes studied from shape transformations of giant vesicles. Biochimie, 80:401–413, 1998.
- [127] Philippe Méléard, Tanja Pott, Hélène Bouvrais, and John Hjort Ipsen. Advantages of statistical analysis of giant vesicle flickering for bending elasticity measurements. The European Physical Journal E: Soft Matter and Biological Physics, 34(10):1–14, 2011.
- [128] L. Miao, M. A. Lomholt, and J. Kleis. Dynamics of shape fluctuations of quasi-spherical vesicles revisited. Eur. Phys. J. E., 9:143–160, 2002.
- [129] Milner and Safran. Dynamical fluctuations of droplet microemulsions and vesicles. Phys. Rev. A, 36:4371–4379, 1987.
- [130] Mario Minale. Models for the deformation of a single ellipsoidal drop: a review. Rheologica acta, 49(8):789–806, 2010.
- [131] C. Misbah. Vacillating breathing and tumbling of vesicles under shear flow. Phys. Rev. Lett., 96:028104, 2006.
- [132] M. D. Mitov, P. Meleard, M. Winterhalter, M. I. Angelova, and P. Bothorel. Electric-field-dependent thermal fluctuations of giant vesicles. Phys. Rev. E, 48:628–631, 1993.
- [133] J. D. Moroz and P. Nelson. Dynamically stabilized pores in bilayer membranes. Biophys. J., 72:2211–2216, 1997.
- [134] A. W. Musial. Nonlinear electrohydrodynamics of non-spherical particles. MS Thesis, Dartmouth College, 2011.
- [135] J. F. Nagle and S. Tristram-Nagle. Lipid bilayer structure. Curr. Opin. Struct. Biol., 10:474–480, 2000.

- [136] Jin Nam, Paul A Beales, and T Kyle Vanderlick. Giant phospholipid/block copolymer hybrid vesicles: mixing behavior and domain formation. Langmuir, 27(1):1–6, 2010.
- [137] M. Naumowicz and Z. A. Figaszewski. Chronopotentiometric technique as a method for electrical characterization of bilayer lipid membranes. J. Membrane Biol., 240:47–53, 2011.
- [138] D. Needham and R. M. Hochmuth. Electromechanical permeabilization of lipid vesicles. role of membrane tension and compressibility. Biophys. J., 55:1001–1009, 1989.
- [139] P. Nelson. Life in the slow lane: The low reynolds-number world. In Biological Physics: Energy, Information, Life, pages 158–194. Freeman, 2004.
- [140] E. Neumann, A. E. Sowers, and C. A. Jordan. Electroporation and electrofusion in cell biology. Plenum Press, New York, 1989.
- [141] Hiroshi Noguchi and Gerhard Gompper. Fluid vesicles with viscous membranes in shear flow. Physical review letters, 93(25):258102, 2004.
- [142] P. Olla. The behavior of closed inextensible membranes in linear and quadratic shear flows. Physica A, 278:87–106, 2000.
- [143] J. Ouellette. Smart fluids move into the marketplace. The industrial physicist, 9:<http://www.aip.org/tip/INPHFA/vol-9/iss-6/p14.html>, 2003.
- [144] Jang-Ung Park, Matt Hardy, Seong Jun Kang, Kira Barton, Kurt Adair, Deep kishore Mukhopadhyay, Chang Young Lee, Michael S Strano, Andrew G Alleyne, John G Georgiadis, et al. High-resolution electrohydrodynamic jet printing. Nature materials, 6(10):782–789, 2007.
- [145] J. Pecreaux, H.-G. Dobereiner, J. Prost, J.-F. Joanny, and P. Bassereau. Refined contour analysis of giant unilamellar vesicles. Eur. Phys. J. E., 13:277–290, 2004.
- [146] M Jacques Pecreaux. Mesure du spectre de fluctuations de vsicules gantes par analyse de contours ; application aux membranes passives et actives. PhD thesis, UNIVERSITE PARIS 6, 2004.
- [147] P. Peterlin. Frequency-dependent electrodeformation of giant phospholipid vesicles in ac electric field. J. Biol. Phys., 36:339–354, 2010.
- [148] P. Peterlin, S. Svetina, and B. Zeks. The prolate-to-oblate shape transition of phospholipid vesicles in response to frequency variation of an ac electric field can be explained by the dielectric anisotropy of a phospholipid bilayer. J. Phys. Cond. Phys., 19:136220, 2007.
- [149] F. Peters, L. Lobry, and E. Lemaire. Experimental observation of lorenz chaos in the quinke rotor dynamics. Chaos, 15:013102, 2005.
- [150] R. Pethig and D.B. Kell. The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology. Phys. Med. Biol., 32:933–970, 1987.

- [151] Horia I Petrache, Stephanie Tristram-Nagle, Klaus Gawrisch, Daniel Harries, V Adrian Parsegian, and John F Nagle. Structure and fluctuations of charged phosphatidylserine bilayers in the absence of salt. Biophysical journal, 86(3):1574–1586, 2004.
- [152] Robert Brooks Phillips, Jane Kondev, Julie Theriot, Nigel Orme, and Hernan Garcia. Physical biology of the cell. Garland Science New York, 2009.
- [153] P Pincus, J-F Joanny, and D Andelman. Electrostatic interactions, curvature elasticity, and steric repulsion in multimembrane systems. EPL (Europhysics Letters), 11(8):763, 1990.
- [154] T. Portet, F.C.I. Febrer, J.M. Escoffre, C. Favard, M.P. Rols, and D. S. Dean. Visualization of membrane loss during the shrinkage of giant vesicles under electropulsation. Biophys. J., 96:4109–4121, 2009.
- [155] Thomas Portet and Rumiana Dimova. A new method for measuring edge tensions and stability of lipid bilayers: effect of membrane composition. Biophysical journal, 99(10):3264–3273, 2010.
- [156] Thomas Portet, Chloé Mauroy, Vincent Démery, Thibault Houles, Jean-Michel Escoffre, David S Dean, and Marie-Pierre Rols. Destabilizing giant vesicles with electric fields: An overview of current applications. The Journal of Membrane Biology, 245(9):555–564, 2012.
- [157] Emil Prodan, Camelia Prodan, and John H Miller. The dielectric response of spherical live cells in suspension: an analytic solution. Biophysical journal, 95(9):4174–4182, 2008.
- [158] G. Quincke. Ueber rotation em im constanten electrischen felde. Ann. Phys. Chem., 59:417–86, 1896.
- [159] W. Rawicz, K.C. Olbrich, T. McIntosh, D. Needham, and E. Evans. Effect of chain length and unsaturation on elasticity of lipid bilayers. Biophys. J., 79:328–339, 2000.
- [160] W Rawicz, BA Smith, TJ McIntosh, SA Simon, and E Evans. Elasticity, strength, and water permeability of bilayers that contain raft microdomain-forming lipids. Biophysical journal, 94(12):4725, 2008.
- [161] K. A. Riske and R. Dimova. Electro-deformation and poration of giant vesicles viewed with high temporal resolution. Biophys. J., 88:1143–1155, 2005.
- [162] K. A. Riske and R. Dimova. Electric pulses induce cylindrical deformations on giant vesicles in salt solutions. Biophys. J., 91:1778–1786, 2006.
- [163] K. A. Riske, R. Lipowsky, and R. Dimova. High temporal resolution of electro-poration, fusion and deformation of giant vesicles. Biophys. J., 86:518A, 2004.
- [164] NJ Rivette and JC Baygents. A note on the electrostatic force and torque acting on an isolated body in an electric field. Chemical engineering science, 51(23):5205–5211, 1996.

- [165] Yosef Rosemberg and Rafi Korenstein. Electroporation of the photosynthetic membrane: A study by intrinsic and external optical probes. Biophysical journal, 58(4):823–832, 1990.
- [166] P. F. Salipante. Electrohydrodynamics of drops in strong fields. MS Thesis, Dartmouth College, 2009.
- [167] P. F. Salipante, R. Knorr, R. Dimova, and P. M. Vlahovska. Electrodeformation method for measuring the capacitance of bilayer membranes. Soft Matter, submitted, 2011.
- [168] P. F. Salipante and P. M. Vlahovska. Electrohydrodynamics of drops in strong uniform dc electric fields. Phys. Fluids, 22:112110, 2010.
- [169] P. S. Salipante. Electrohydrodynamics of drops in strong fields. MS Thesis, Dartmouth College, 2009.
- [170] O. Sandre, L. Moreaux, and F. Brochard-Wyart. Dynamics of transient pores in stretched vesicles. PNAS, 96:10591–10596, 1999.
- [171] H. Sato, N. Kaji, T. Mochizuki, and Y. H. Mori. Behavior of oblatelly deformed droplets in an immiscible dielectric liquid under a steady and uniform electric field. Phys. Fluids, 18:127101, 2006.
- [172] D. A. Saville. Electrohydrodynamics: The taylor-melcher leaky dielectric model. Annu. Rev.Fluid Mech., 29:27–64, 1997.
- [173] MB Schneider, JT Jenkins, and WW Webb. Thermal fluctuations of large quasi-spherical bimolecular phospholipid vesicles. Journal de Physique, 45(9):1457–1472, 1984.
- [174] P. Schoch, D.F. Sargent, and R. Schwyzer. Capacitance and conductance as tools for the measurement of asymmetric surface potentials and energy barriers of lipid bilayer membranes. J Membr Biol, 46:7189, 1979.
- [175] K.H. Schoenbach, S.J. Beebe, and E.S. Buescher. Intracellular effect of ultrashort electrical pulses. Bioelectromagnetics, 22:440–448, 2001.
- [176] J. Schwalbe, P. M. Vlahovska, and M. Miksis. Lipid membrane instability driven by capacitive charging. Physics of Fluids, 23:04170, 2011.
- [177] Jonathan T Schwalbe, Petia M Vlahovska, and Michael J Miksis. Vesicle electrohydrodynamics. Physical Review E, 83(4):046309, 2011.
- [178] H. P. Schwan. Dielectrophoresis and rotation of cells. In E. Neumann, A. E. Sowers, and C. A. Jordan, editors, Electroporation and electrofusion in cell biology, pages 3–21. Plenum Press, 1989.
- [179] U. Seifert. The concept of effective tension for fluctuating vesicles. Z. Phys. B, 97:299–309, 1995.
- [180] U. Seifert. Configurations of fluid membranes and vesicles. Advances in physics, 46:13–137, 1997.

- [181] U. Seifert. Fluid membranes in hydrodynamic flow fields: Formalism and an application to fluctuating quasispherical vesicles. Eur. Phys. J. B, 8:405–415, 1999.
- [182] U. Seifert and S.A. Langer. Viscous modes of fluid bilayer membranes. Europhys. Lett., 23:71–76, 1993.
- [183] Jacopo Seiwert, Michael J Miksis, and Petia M Vlahovska. Stability of biomimetic membranes in dc electric fields. Journal of Fluid Mechanics, 1(1):1–13, 2012.
- [184] Jamal Seyed-Yagoobi. Electrohydrodynamic pumping of dielectric liquids. Journal of electrostatics, 63(6):861–869, 2005.
- [185] J. D. Sherwood. Breakup of fluid droplets in electric and magnetic fields. J. Fluid Mech., 188:133–146, 1988.
- [186] K. C. Smith, J.C. Neu, and W. Krassowska. Model of creation and evolution of stable electropores for dna delivery. Biophys. J., 86:2813–2826, 2004.
- [187] Helen Song, Michelle R Bringer, Joshua D Tice, Cory J Gerdtz, and Rustem F Ismagilov. Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels. Applied Physics Letters, 83(22):4664–4666, 2003.
- [188] C. Sozou. Electrohydrodynamics of a liquid drop - time-dependent problem. Proc. Royal Soc. A, 331:263–272, 1972.
- [189] T. M. Squires and M. Z. Bazant. Induced-charge electro-osmosis and electrophoresis. J. Fluid Mech., 509:217–252, 2004.
- [190] M. Staykova, R. Lipowsky, and R. Dimova. Membrane flow patterns in multicomponent giant vesicles induced by alternating electric fields. Soft Matter, 4:2168–2171, 2008.
- [191] HA Stone, Ali Nadim, and Steven H Strogatz. Chaotic streamlines inside drops immersed in steady stokes flows. Journal of Fluid Mechanics, 232(1):629–646, 1991.
- [192] T. Sun and H. Morgan. Single-cell microfluidic impedance cytometry: a review. Microfluidics and nanofluidics, 8:423–443, 2010.
- [193] Rongjia Tao. Electrorheology for efficient energy production and conservation. Journal of Intelligent Material Systems and Structures, 22(15):1667–1671, 2011.
- [194] G. I. Taylor. Studies in electrohydrodynamics. i. circulation produced in a drop by an electric field. Proc. Royal Soc. A, 291:159–166, 1966.
- [195] J. Teissie, M. Golzio, and M. P. Rols. Mechanisms of cell membrane electroporabilization: A minireview of our present (lack of ?) knowledge. BBA, 1724:270–280, 2005.

- [196] J. Teissie and T. Y. Tsong. Electric field induced transient pores in phospholipid bilayer vesicles. Biochemistry, 20:1548–1554, 1981.
- [197] E. Tekle, R. D. Astumian, W. A. Friauf, and P. B. Chock. Asymmetric pore distribution and loss of membrane lipid in electroporated dopc vesicles. Biophys. J., 81:960–968, 2001.
- [198] H. T. Tien. Bilayer Lipid Membranes (BLM): Theory and Practice. Marcel Dekker, New York, 1974.
- [199] R. Tran-Son-Tay, SP. Suter, and PR. Rao. Determination of red blood cell membrane viscosity from rheoscopic observations of tank-treading motion. Biophysical journal, 46(1):65–72, 1984.
- [200] A. Tsebers. Chaotic solutions for the relaxation equations of electrical polarization. Magnetohydrodynamics, 27:251–258, 1991.
- [201] I. Turcu. Electric field induced rotation of spheres. J. Phys. A: Math. Gen., 20:3301–3307, 1987.
- [202] I. Turcu and C. M. Lucaciu. Dielectrophoresis -a spherical shell model. J. Phys. A, 22:985–993, 1989.
- [203] I. Turcu and C. M. Lucaciu. Electrorotation -a spherical shell model. J. Phys. A, 22:995–1003, 1989.
- [204] V. Vitkova, M. Mader, and T. Podgorski. Deformation of vesicles flowing through capillaries. Europhys. Lett., 68:398–404, 2004.
- [205] P. Vlahovska, J. Bławdziewicz, and M. Loewenberg. Nonlinear rheology of a dilute emulsion of surfactant-covered spherical drops in time-dependent flows. J. Fluid Mech., 463:1–24, 2002.
- [206] P. Vlahovska, J. Bławdziewicz, and M. Loewenberg. Small-deformation theory for a surfactant-covered drop in linear flows. J. Fluid Mech., 624:293–337, 2009.
- [207] P. M. Vlahovska. Non-equilibrium dynamics of lipid membranes: deformation and stability in electric fields. In A. Iglic, editor, Advances in Planar Lipid Bilayers and Liposomes, vol. 12, pages 103–146. Elsevier, 2010.
- [208] P. M. Vlahovska. Non-equilibrium dynamics of lipid membranes: deformation and stability in electric fields. In A. Iglic, editor, Advances in Planar Lipid Bilayers and Liposomes, vol. 12, pages 103–146. Elsevier, 2010.
- [209] P. M. Vlahovska. On the rheology of a dilute emulsion in a uniform electric field. J. Fluid Mech., 670:481–503, 2011.
- [210] P. M. Vlahovska, R. Gracia, A. Aranda, and R. Dimova. Electrohydrodynamic model of vesicle deformation in alternating electric fields. Biophys. J., page submitted, 2008.
- [211] P. M. Vlahovska, R. S. Gracia, S. Aranda-Espinoza, and R. Dimova. Electrohydrodynamic model of vesicle deformation in alternating electric fields. Biophys. J., 96:4789–4803, 2009.

- [212] P. M. Vlahovska and R.S. Gracia. Dynamics of a viscous vesicle in linear flows. Phys. Rev. E, 75:016313, 2007.
- [213] P. M. Vlahovska, T. Podgorski, and M. Misbah. Vesicles and red blood cells: from individual dynamics to rheology. Comptes Rendus Physique, 10:775789, 2009.
- [214] Petia M Vlahovska. On the rheology of a dilute emulsion in a uniform electric field. Journal of Fluid Mechanics, 670:481–503, 2011.
- [215] PM Vlahovska, YN Young, G Danker, and C Misbah. Dynamics of a non-spherical microcapsule with incompressible interface in shear flow. Journal of Fluid Mechanics, 678:221, 2011.
- [216] T Ward and GM Homsy. Electrohydrodynamically driven chaotic mixing in a translating drop. ii. experiments. Physics of Fluids, 15:2987, 2003.
- [217] Thomas Ward and GM Homsy. Electrohydrodynamically driven chaotic mixing in a translating drop. Physics of Fluids, 13:3521, 2001.
- [218] J. C. Weaver and Y. A. Chizmadzhev. Theory of electroporation: A review. Bioelectrochem.Bioenerg., 41:135–160, 1996.
- [219] Eric D Wetzel and Charles L Tucker. Droplet deformation in dispersions with unequal viscosities and zero interfacial tension. Journal of Fluid Mechanics, 426(1):199–228, 2001.
- [220] S. H. White. A study of lipid bilayer membrane stability using precise measurements of specific capacitance. Biophys. J., 10.
- [221] S. H. White and W. Chang. Voltage dependence of capacitance and area of black lipid membranes. Biophys. J., 36:449–453, 1981.
- [222] Stephen Wiggins, Julio M Ottino, Stephen Wiggins, and Julio M Ottino. Foundations of chaotic mixing. Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 362(1818):937–970, 2004.
- [223] M. Winterhalter and W. Helfrich. Effect of voltage on pores in membranes. Phys. Rev. A, 36:5874–5876, 1987.
- [224] M Winterhalter and Wolfgang Helfrich. Effect of surface charge on the curvature elasticity of membranes. The Journal of Physical Chemistry, 92(24):6865–6867, 1988.
- [225] T. Yamamoto, S. Aranda-Espinoza, R. Dimova, and R. Lipowsky. Stability of spherical vesicles in electric fields. Langmuir, 26:12390–12407, 2010.
- [226] D. V. Zhelev and D. Needham. Tension-stabilized pores in giant vesicles: determination of pore size and pore line tension. Biochim. Biophys. Acta, 1147:89–104, 1993.

- [227] Jing Zheng, Weixing Shen, David ZZ He, Kevin B Long, Laird D Madison, Peter Dallos, et al. Prestin is the motor protein of cochlear outer hair cells. Nature, 405(6783):149–155, 2000.
- [228] Hernan Zhou, Beatriz Burrola Gabilondo, Wolfgang Losert, and Willem van de Water. Stretching and relaxation of vesicles. Physical Review E, 83(1):011905, 2011.
- [229] F. Ziebert, M. Z. Bazant, and D. Lacoste. Effective zero-thickness model for a conductive membrane driven by an electric field. Phys. Rev. E, 81:031912, 2010.
- [230] Falko Ziebert and David Lacoste. A planar lipid bilayer in an electric field: Membrane instability, flow field, and electrical impedance. Advances in Planar Lipid Bilayers and Liposomes, 14:63, 2011.