Polymer Characteristics of Polyelectrolyte Polypeptides

Jorge Monreal
University of South Florida, jmonreal@mail.usf.edu

Follow this and additional works at: http://scholarcommons.usf.edu/etd
Part of the Physics Commons

Scholar Commons Citation

This Thesis is brought to you for free and open access by the Graduate School at Scholar Commons. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Scholar Commons. For more information, please contact scholarcommons@usf.edu.
Polymer Characteristics of Polyelectrolyte Polypeptides

by

Jorge Monreal

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy Department of Physics College of Arts and Sciences University of South Florida

Major Professor: Donald T. Haynie, Ph.D. Kirpal Bisht, Ph.D. Jianjun Pan, Ph.D.

Date of Approval:
June 29, 2016

Keywords: stress, strain, dielectric relaxation, polyelectrolyte, neo-Hookean, polypeptide

Copyright © 2016, Jorge Monreal
DEDICATION

This dissertation is dedicated to my Father and Mother. When my sister and I were young, they made a conscious decision to suffer the hardships and sometimes indignations of poor immigrants to ensure that both of us had a better life in the US. I can never repay or thank them enough for that. My dad, a Native American from the hills of San Luis, Potosi, Mexico, who didn’t have the benefits of even an elementary education, instilled in my sister and me the importance of an education. My mother, who received education up to sixth grade, did the same. They both put up with my incessant questions of how things worked. And they both tried to explain as best they could. Though we moved to several cities numerous times, the one constant was their interest in the new things we had learned in school.

Papá y mamá les dedico esta disertación a ustedes.
ACKNOWLEDGMENTS

As I have previously done in my master’s thesis from MIT, I would first and foremost like to thank God without whose grace I would not have completed this journey. It has been a long time in the making. Through various trials and tribulations God has guided me and has given me the desire to continue down this path. As happened with the apostle Paul, my plans were not God’s plans and ultimately God wins. To God be the glory.

Secondly, my spirituality would surely have suffered had it not been for the love and support exuded by brothers and sisters from both the Valrico and East Bradenton churches of Christ. It is amazing to see in retrospect how God’s love works.

This section would be incomplete without mention of my wife. There cannot be a limit on the thanks she deserves, along with gratitudes, for putting up with my ideosyncracies and my many days where I desired to toil alone rather than spend time with her. Truly I am blessed to have her by my side.

I would like to thank my family, especially my sister Raquel, whose unflinching support for my journey has always given me hope and a sense of admiration. In particular, I would like to thank my two grandsons, Julien and Jacob, who were born during the time this research took place. If you read this at some point in time let it be a testament that if I can do this, you too, or any one else, can do it if you set your mind to it. My love to both of you.

My thanks also go out to my advisor from whom I learned a lot during our long chats. And, finally, my gratitude goes out to all the friends and colleagues whom I’ve met at USF during my time here.
TABLE OF CONTENTS

LIST OF TABLES ................................................................. v

LIST OF FIGURES ............................................................... vi

ABSTRACT ................................................................. xiii

1. INTRODUCTION AND BACKGROUND ................................. 1
   1.1. Polypeptides ......................................................... 1
   1.2. Polyelectrolyte Charge Screening ............................... 4
   1.3. Polymer Relaxation Dynamics ................................... 9
   1.4. Mechanical Properties .......................................... 15
   1.5. Theoretical Models of Elasticity ............................... 19
      1.5.1. Microscopic Deformation: Freely Jointed Chain Model (FJC) ... 21
      1.5.2. Microscopic Deformation: Worm-like Chain Model (WLC) ........ 23
      1.5.3. Mesoscopic Treatment of Elastomer Deformation ............. 25
      1.5.4. Phenomenological Treatment of Elastomer Deformation ....... 29
   1.6. Dielectric Relaxation of Polymers ............................ 35

I. POLYELECTROLYTE STUDIES ........................................... 40

2. NONLINEAR ELASTICITY AND VISCOELASTICITY .................... 41
   2.1. Introduction ....................................................... 41
2.2. Background ................................................................. 42
  2.2.1. Materials and Methods ............................................. 45
  2.2.2. Polymers ............................................................. 45
  2.2.3. Mold ................................................................. 45
  2.2.4. Experimental Design ............................................... 46
  2.2.5. Materials Fabrication ............................................. 46
  2.2.6. Humidity Control ................................................. 47
  2.2.7. Materials Testing ................................................ 47
  2.2.8. Fitting and Parameter Determination ......................... 49
2.3. Results and Discussion ............................................. 51
  2.3.1. Force Versus Displacement ..................................... 51
  2.3.2. Nonlinear Elasticity Analysis .................................. 54
  2.3.3. Nonlinear Viscoelasticity Analysis ......................... 60
2.4. Conclusion ............................................................ 63
2.5. Supplementary Material .......................................... 65

3. DIELECTRIC RELAXATION OF POLYELECTROLYTE POLYPEPTIDE MELTS 86
  3.1. Introduction .......................................................... 86
  3.2. Material and Methods ............................................. 87
    3.2.1. Polypeptides .................................................. 88
    3.2.2. Capacitance Fixture ........................................... 88
  3.3. Dielectric Parameters and Capacitance Equations .............. 89
    3.3.1. Dielectric Parameters ....................................... 89
    3.3.2. Capacitance Equations ...................................... 91
  3.4. Results ............................................................. 93
  3.5. Discussion .......................................................... 95
  3.6. Conclusion .......................................................... 96
4. CONCLUSIONS FROM ELASTICITY AND RELAXATION STUDIES . . . . . . . . . . 101
   4.1. Elasticity Measurements .................................................. 101
      4.1.1. Future Work .......................................................... 105
   4.2. Relaxation Studies ......................................................... 105
      4.2.1. Future Work .......................................................... 107
5. CHARACTERIZATION OF CROSSLINKED RADA16 .............................. 111
   5.1. Introduction ................................................................. 111
   5.2. Experimental ............................................................... 112
      5.2.1. Materials ............................................................... 112
      5.2.2. Material Processing ................................................ 113
      5.2.3. Equipment ............................................................. 113
   5.3. Results and Discussion ................................................... 114
      5.3.1. SEM and TEM studies ............................................... 114
      5.3.2. NTA Measurements .................................................. 116
      5.3.3. FTIR Analysis ........................................................ 117
      5.3.4. Additional Analysis ................................................ 121
   5.4. Conclusion .................................................................. 122

II. ELECTROMAGNETIC HYDRODYNAMIC FLOW STUDY .......................... 126
6. GENERAL NS-LIKE P AND M-E EQUATIONS ..................................... 127
   6.1. Introduction ................................................................ 127
      6.1.1. System of Navier-Stokes Equations ............................. 127
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.</td>
<td>Electromagnetic “Flow” Differential Equations</td>
<td>130</td>
</tr>
<tr>
<td>6.2.1.</td>
<td>General Momentum and Mass-Energy Relations</td>
<td>130</td>
</tr>
<tr>
<td>6.2.2.</td>
<td>Euler-like equation</td>
<td>133</td>
</tr>
<tr>
<td>6.3.</td>
<td>Conclusion</td>
<td>135</td>
</tr>
</tbody>
</table>

REFERENCES | 136 |

APPENDIX A: PUBLICATIONS AND PRESENTATIONS | 146 |
| 1. | Refereed Publications | 146 |
| 2. | Manuscripts In Progress | 146 |
| 3. | Poster Presentations | 147 |

APPENDIX B: COPYRIGHT PERMISSION | 148 |

APPENDIX C: PHENOMENOLOGICAL STUDIES | 150 |

APPENDIX D: RADA16 FTIR | 157 |
# LIST OF TABLES

2.1. *Experimental factors of this study.* ................................. 45

2.2. *Youngs moduli values obtained by fitting a neo-Hookean model, \( \sigma = \mu(\lambda - 1/\lambda^2) \), to experimental data in Figures 2.17-2.19. First column lists experimental conditions; second lists shear modulus from global fits. \( E \) is the Young’s modulus. The fourth and fifth columns show samples at each condition with associated shear moduli from individual fits. The last column lists the standard deviation from individual fits (see Appendix C for plots of individual fits). .......................... 58

2.3. *Fitting parameters obtained by fitting the Mooney-Rivlin model, \( \sigma = (\mu_1 + \mu_2/\lambda)(\lambda - 1/\lambda^2) \), to experimental data in Figures 2.17-2.19. First column lists experimental conditions; second lists shear moduli from global fits. The third and fourth columns show samples at each condition with associated fitting parameters from individual fits (see Appendix C for plots of individual fits). .......................... 59

2.4. *Relaxation times for three representative samples at 33% RH and at high, medium or low strain.* ................................................. 62

3.1. *Conductivity of aqueous solutions.* ........................................ 94

4.1. *Reactants used to generate data for Figure 4.1. Both samples contain equal amount of reactant mass.* ............................................. 101

4.2. *Polymer volume fraction and mesh size for PLEY(4:1). Polymer volume fraction for PLE.* ...................................................... 104
LIST OF FIGURES

1.1. Peptide condensation mechanism .................................................. 1
1.2. NCA synthesis process [2] ............................................................ 3
1.3. Polyelectrolyte structures of a.) PLE and b.) PLEY (4:1). N signifies the degree of polymerization. ......................................................... 4
1.5. Charge screening on a.) Polyanion b.) Polycation. [3] ................. 6
1.6. Concentration regimes. First box indicates dilute concentration where \( c_m < c_m^* \). Middle box indicates overlap concentration \( c_m = c_m^* \). Third box is the semidilute concentration where \( c_m > c_m^* \) [4]. ......................... 10
1.7. Tube model with topological constraints in an uncrosslinked system. a.) Polymer chain in a fixed network; b.) Primitive chain within a tube formed by topological constraints [5]. ................................................. 11
1.8. Reptation dynamics within a tube.a.) Original tube; b.)-c.) Motion of the primitive chain as it moves to exit the tube; d.) Remaining tube at any time, \( t \), which eventually vanishes as the primitive chain exists the tube [5]. ........ 12
1.9. Typical tensile test stress-strain curve for linear polymer [6]. ........ 16
1.10. Youngs Modulus as a function of temperature for polymers [6]. .... 16
1.11. Effect on Youngs modulus due to increased cross-linking [6]. ........ 17
1.12. \( \overline{DP} \) effect on tensile strength [6]. ........................................ 18
1.13. Conformational changes in polymers: (a)Single molecule - top figure unstretched and with high entropy, lower figure stretched and with low entropy; (b) Crosslinked polymer- top figure unstretched, lower figure stretched [7]. 20
1.14. Coordinate system for a compressive test ................................. 20
1.15. Representation of polymer chain with \( N \) freely rotating chains of size \( l \) and end-to-end distance vector \( \mathbf{R} \). ........................................ 22
1.16. Worm-like chain model construction: \( S \) is the arc length, \( t_i \) are tangent vectors and \( \mathbf{R} \) is the end-to-end vector.

1.17. Chain deformation in three dimensions.

1.18. \( \chi \) maps every point, \( \mathbf{X} \in \mathcal{R}_u \) to every point, \( \mathbf{x} \in \mathcal{R}_d \). Points in \( \mathcal{R}_u \) are traced in Lagrangian spatial form and points in \( \mathcal{R}_d \) follow a Eulerian description.

1.19. Plot of \(-\sigma_{eng}/\mu\) versus \( \lambda \) in the compressive regime. Nonlinearity is evident.

1.20. Typical plots of permittivity (\( \varepsilon' \)) and dielectric loss (\( \varepsilon'' \)) versus log of frequency \( \omega \). Peak of dielectric loss occurs at the relaxation frequency, \( \omega_r \), which gives relaxation time through \( \tau_r = 1/\omega_r \).

1.21. Schematic representation of two different dipole polarization components of polymer chains: a.) dipole components parallel to the chain; b.) dipole components perpendicular to the chain.

2.1. Side chains of the amino acids of the present study. The side chain of an amino acid residue is attached to an alpha carbon atom in the polymer backbone. A peptide group is formed between backbone atoms of successive amino acid monomers.

2.2. Measurement apparatus. Cylindrical samples were compressed uniaxially along the vertical axis by adjusting the height of the lab jack. Displacement was quantified with a displacement gauge.

2.3. Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 30% w/v nominal PLEY concentration.

2.4. Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 40% w/v nominal PLEY concentration.

2.5. Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 50% w/v nominal PLEY concentration.

2.6. Force-displacement loading curves of samples at condition 310233 at 33% relative humidity with 30% nominal PLEY concentration.

2.7. Force-displacement loading curves of samples at condition 415433 at 33% relative humidity with 40% nominal PLEY concentration.

2.8. Force-displacement loading curves of samples at condition 520333 at 33% relative humidity with 50% nominal PLEY concentration.
2.9. Force-displacement plot with error bars for all conditions presented in Figures 2.3 - 2.8. Number after underscore represents relative humidity. For example, relative humidity for 3153_75 is 75% relative humidity. Error in force was calculated as \( F = C(w \pm \Delta \varepsilon_w) \), where \( w \) is weight and \( \Delta \varepsilon_w \) is standard deviation in weight measurement among the replicated samples. \( C \) is a constant to obtain force in Newtons.

2.10. Loading/unloading curves were obtained for samples in conditions 420275 and 410385. Specific samples are listed in legend of plot. Circles represent loading; stars, unloading. Unloading data for other conditions could not obtained due material fracture or other factors which prohibited measurement. (a) Condition 420275 at 75% RH. All three samples show pattern of nonlinear elastic deformation. (b) Condition 410385 at 85% RH. Samples show pattern of nonlinear elastic deformation.

2.11. Decision path followed to characterize mechanical properties of crosslinked material in this study.

2.12. Plots of Stress-Strain and Secant Modulus-Strain curves. Strain-Strain curve exhibits signs of strain hardening at high strain. Secant modulus follows a typical pattern of non-linear elastic biological material. Initial decrease in secant modulus, then increased secant modulus with increased strain [8, 9, 10]. (a) Stress versus curve for condition 515285; (b) Secant moduli versus strain curve for condition 515285.

2.13. Stress versus strain plot with error bars for the six conditions at 75% and 85% RH. Stress was calculated as \( \sigma = F/A \), where \( F \) is the applied force and \( A \) is the sample’s cross sectional area. Values of force were \( F = x_F \pm \Delta \varepsilon_F \), where \( \Delta \varepsilon_F \) is variance in force. Values of area were \( A = x_A \pm \Delta \varepsilon_A \), where the error in area, \( \Delta \varepsilon_A \), was calculated as \( \Delta \varepsilon_A = A \sqrt{2(\Delta \varepsilon_r/r)^2} \). Average diameter of samples was as given in subsection 2.2.5: 3.5 \( \pm \) 0.4 mm, so that \( r = 3.5/2 \) mm and \( \Delta \varepsilon_r = 0.4 \) mm. The stress with error was then calculated as \( \sigma = F/A \pm \Delta \varepsilon_\sigma \), where \( \Delta \varepsilon_\sigma = \sigma \sqrt{(\Delta \varepsilon_F/F)^2 + (\Delta \varepsilon_A/A)^2} \).

2.14. Secant modulus versus strain plot with error bars for the six conditions at 75% and 85% RH. Secant modulus was calculated as mentioned in the results and discussion section of Chapter 2. We have assumed that \( \Delta \varepsilon_\sigma >> \Delta \varepsilon_\varepsilon \). Therefore the secant modulus with error bars was calculated as \( E_s = \frac{1}{\varepsilon} (\sigma \pm \Delta \varepsilon_\sigma) \).

2.15. Change in secant modulus. (a) Ratio of change in secant modulus at each strain to maximum change in secant modulus versus strain for (a) 75% RH (Samples: 315375-1; 420275-1; 510475-1) and (b) 85% RH (Samples: 320485-1; 410385-2; 515285-2).
2.16. Nonlinear elastic behavior within each of the three regions in stress-strain response of crosslinked synthetic polypeptide co-poly-(L-glutamic acid$_4$, L-tyrosine$_1$). The data are for condition 515285 using sample 515285-3 to show behavioral characteristics within each of the three regions.

2.17. Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 315375 (b) Stress-stretch curves for condition 320485.

2.18. Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 420275. (b) Stress-stretch curves for condition 410385. Note*: Figure 2.18(b) excludes sample 410385-1 as data set is an outlier as seen in Tables 2.2 and 2.3.

2.19. Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 510475. (b) Stress-stretch curves for condition 515285.

2.20. Relaxation time data for samples 310233-1; 415433-2 and 520333-1 at 33% relative humidity and at high, medium and low strains. High strain corresponds to approximately 10% deformation, medium strain to 8% deformation and low strain to 4% deformation. Colored circles represent data; solid line represent the fit. Only relaxation times were important for this study. (a) Relaxation time data for sample 310233-1. (b) Relaxation time data for sample 415433-2. (c) Relaxation time data for sample 520333-1.

2.21. Isochronous stress-strain plots of samples in three different factors at 33% RH. The three lines represent 10 s, 100 s and 150 s in each case. (a) Sample 310233-1, (b) Sample 415433-2, (c) Sample 520333-1. Nonlinearity provides evidence of nonlinear viscoelasticity.

2.22. Ratio of sample volume/original sample volume as a function of time for sample at RH33% and RH85%. The equilibration time was approximately 72 h. The final volume was greater at RH85% than RH33%.
2.23. *Loading and unloading curves of apparatus with no sample. Circles represent loading; dashes represent unloading.* .......................................................... 84

2.24. *Plot of t-ratios for all four conditions studied.* ............................................. 85

3.1. a.) *Picture of test fixture as viewed from the top. Lexan board is transparent. Consequently, the copper ground strip on the backside of the fixture can be seen.* b.) *Schematic of equivalent circuit as measured by the impedance analyzer. Inductive ($X_L$) and capacitive ($X_C$) reactances are not separated by the analyzer. Measured reactance, $X$, is a combination of both so that $X = X_L + X_C$.* .................................................. 88

3.2. *Sample plots of 10, 15, 20% w/v PLE: a.) Plots of $X_{meas}$ bottom and $R_{meas}$ top for three PLE concentrations. Short circuit $R$ and $X$ are shown for reference; b.) Plots of conductance (top) and susceptance (bottom); c.) Ratio of $-C_{sc}/C_o$, on left axis, decreases from above 300 at low frequencies to about 1 at high frequencies due to electrode polarization at low frequencies. $C_{sc}/C_o$, on right axis, remains constant at about 17 then steadily increases to 22 at high frequencies due to capacitor fringe effects. Resonance peak at around 500 MHz can easily be seen.* .......................................................... 97

3.3. *Plot of permittivity (open circles, left axis) and dielectric loss (stars, right axis) as functions of frequency for a.) Deionized Water and b.) 15% w/v PLE. Points at resonance were omitted for ease of visibility.* ........................................... 98

3.4. *Plots of a.) permittivity versus frequency in the range of 50MHz $\leq \nu \leq$ 800MHz; b.) dielectric loss versus frequency in the same range as permittivity; c.) Cole-Cole and d.) conductivity versus frequency in the range of 1MHz $\leq \nu \leq$ 51MHz. Conductivity (d) is calculated with (3.8) for 1M NaCl; 10, 15 and 20% w/v PLE; 30, 40 and 50% w/v PLEY.* ........................................... 99

3.5. *Plots of relaxation time, $\tau$, versus peptide concentration in % (w/v) for two separate measurements each of PLE and PLEY (shown as open and closed circles; and open and closed squares, respectively). Relaxation times were obtained from peaks of dielectric loss in Figure 3.4b. Circles represent PLE. Squares show PLEY.* ........................................... 100

4.1. *Plots of force versus displacement. Data plotted with closed stars is hydrated PLE; data in open stars is dehydrated PLE; data plotted in open diamonds is PLEY(4:1).* ........................................... 102
4.2. Stress-Strain plots for samples containing 30% and 50% PLEY (4:1). At both 75% and 85% relative humidities samples with 30% PLEY (4:1) are stiffer than samples with 50% PLEY (4:1). (a) Stress-strain curves for conditions at 75% relative humidity. (b) Stress-strain curves for conditions at 85% relative humidity. Note: Data represented by black star is condition 410385 without the outlier data set from sample 410385-1 as seen in Table 2.2.

4.3. Plot of average mesh size $\xi$ as $\xi/a$ plotted versus monomer volume fraction, $\Phi$.

4.4. Relaxation time of PLEY(4:1) viscoelastic material versus concentration taken from Table 2.4 at three different strains.

4.5. Figure 3.5 modified to show power law scaling of PLEY(4:1) and PLE.


5.2. a.) SEM picture of 2% w/v RADA16 reacted with 20% EDC at 10 kX magnification. Monodisperse particles seen throughout sample. In addition to typical organic elements, EDS measurements showed significant traces of chlorine. ; b.) Same sample viewed under TEM at 28.7 kX magnification. Bar scale is 2000 nm. ; c.) TEM close-up view of a $\approx$70 nm nanocrystal at 824 kX magnification. Orthorhombic appearance of crystal is visible ; d.) Spherules were also present in sample. TEM view of spherules at 10.9 kX. Crosslinked RADA16 nanofibers in process of agglomeration are visible in the middle of picture and lower left corner. Bar scale is 10000 nm.

5.3. a.) TEM view of “plate-like” crystals present in 20% EDC dissolved in deionized water at 78.7 kX magnification.; b.) NTA measurement of 20% EDC only, DF=1000, in deionized water measured at 21 °C. ; c.) TEM view of solution made with 2% RADA16+20% EDC at 78.7 kX magnification. Visual inspection shows post-reaction crystals have different morphology than EDC crystals. Additionally, spherules appear in the mix. ; d.) NTA measurement of 2% w/v RADA16+20% EDC, DF=1000, in deionized water at 25 °C.; e.) TEM view of spherules from different location than figure 5.2d at 28.7 kX magnification. ; c.) Spherule size distribution statistics of e.) as measured with the TEM measuring tool.

5.4. FTIR of RADA16-NH$_3$ hydrogel at 7.8% w/v concentration. Peak at 1621 cm$^{-1}$ falls within the range of $\beta$-sheet values given in Barth, 2007 after taking into account the 4 cm$^{-1}$ equipment resolution.

5.5. FTIR signature of RADA16-NH$_3$ lyophilized powder. Peak at 1619 cm$^{-1}$ falls within the range of $\beta$-sheet values given by Chirgadze and Neuskaya (Biopolymers 1976 Apr;15(4):637-48) which gives a range of 1615 to 1637 cm$^{-1}$.
5.6. a.) FTIR spectra of 2% RADA16. The significant peak at 1621 cm$^{-1}$ is attributed to stable β-sheets. b.) FTIR spectra of 20% EDC. N=C=N bonds produce two distinctive peaks at 2130 and 1702 cm$^{-1}$, respectively. These disappear after a crosslinking reaction. c.) Overlaid FTIR spectra of unreacted 20% EDC (magenta) and RADA16+EDC (black) after reaction, diluted in deionized water.

5.7. Plots of dielectric relaxation of unreacted RADA16 (red) and product of RADA16 with EDC (green).

5.8. SEM pictures of electrospun blended PAA/RADA16 at several concentrations. Electrostatic modification of surface tension manifests in changes of fiber morphology.
ABSTRACT

Polypeptides are polymerized chains of amino acids linked covalently through peptide bonds. Polyelectrolyte polypeptides are polypeptides with electrolyte repeating groups. Several amino acids contain ionizable side chains which result in charge distributions when dissolved in aqueous solutions. This dissertation is motivated by a desire to gain knowledge of polyelectrolyte polypeptides as recent advances in chemical synthesis of polypeptides have made possible the fabrication of designed polypeptides that do not naturally occur in nature. Potential applications of newly designed polypeptides span the range from medical to clothing and energy even to robotics.

In this dissertation we compare the characteristic behavior of two polypeptide polyanions: Poly-(L-Glutamic Acid) [PLE] and Poly-(L-Glutamic Acid₄, Tyrosine₁) [PLEY(4:1)]. Comparative characteristic behaviors of each is conducted through relaxation phenomena in the context of mechanical elasticity measurements of hydrogels and dielectric relaxation of aqueous solutions in a radio frequency range of 1 MHz to 1000 MHz. Hydrogels are fabricated by crosslinking each polyanion with Poly-(L-Lysine) [PLK], a polycation, via the crosslinker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Elasticity and viscoelasticity measurements are conducted in a fixture designed by our lab. Dielectric relaxation behavior is studied on aqueous solution of both PLEY and PLE using a capacitive fixture, also designed in our lab. RF signals provided by an impedance analyzer are converted to permittivity and dielectric loss measurements. Peaks in dielectric loss provide evidence of relaxation mechanisms. A comparison of experimental results to theoretical expectations reveal both expected and some surprising behavior. Relaxation times for crosslinked hydro-
gels scale according to theoretical expectations according to so-called reptation dynamics. However, relaxation times of aqueous solutions did not scale as entangled polyelectrolytes. First, both PLEY and PLE scaled as neutral polymers rather than polyelectrolytes. This was expected because of the high concentrations studied. However, due to the high concentrations, it was expected that polypeptides were entangled in solutions. Data compared to theory did not support this expectation.

We, additionally, conducted a self-crosslinking experiment of a polyampholyte: RADA16. RADA16 is known to self-assemble into nano-fibers formed by $\beta$-sheet stacking. The self-crosslinking was also mediated by EDC. Results of crosslinking showed formation of polypeptide spherules as well as nano-crystals nominally orthorhombic in shape. It was not possible to ascertain composition of the nano-crystals due to both the limited amount of raw material available and the capabilities of measurement equipment as of this writing. It is hypothesized that nano-crystals are composed of some type of urea by-product from the crosslinking reaction. The spherules, on the other hand, seem to be described by the theory of hydrophobic polyelectrolytes.

Additional research conducted with regards to electromagnetic hydrodynamic flows during the time frame of this dissertation is also included. The research uses hydrodynamic conservation equations as a starting point to derive one electromagnetic flow momentum equation analogous to the Cauchy momentum equation of hydrodynamics. It also introduces a mass-energy conservation equation for electromagnetic flow that has no hydrodynamic analogue.

We begin this dissertation by introducing in Chapter 1 some of the theoretical background necessary to understand results from experiments. Chapter 2 introduces experimental results from elasticity and viscoelasticity measurements and Chapter 3 explains the dielectric relaxation experiment. We then follow with Chapter 4 which presents conclusions from mechanical and dielectric relaxation experiments in a concise format. Results from the self-crosslinking of RADA16 are presented in Chapter 5. Finally, the additional research on
electromagnetic flow is presented in Chapter 6.
1.1. Polypeptides

Polypeptides are molecular chains of amino acids linked through covalent peptide bonds. Those covalent bonds are formed through a condensation mechanism. As shown in Figure 1.1, as two amino acids undergo condensation to form a peptide bond, the carboxyl group (C-terminus) on one of the amino acids loses an OH group. The amino group (N-terminus) from the other amino acid loses a hydrogen. The covalent peptide bond thus forms between the carbon at the C-terminus and the nitrogen at the N-terminus. Water (H$_2$O) is a result of this reaction.

Figure 1.1.: *Condensation mechanism of two amino acids.* (https://en.wikipedia.org/wiki/Peptide_bond; accessed 2/10/2016)
These biopolymers share many of the same physical characteristics as typical polymers and can be mesoscopically described with many of the same models appropriate for polymers. However, there are some differences. Unlike synthetically derived polymers, polypeptide polymers assemble hierarchically into stable ordered conformations categorized into secondary, tertiary and quaternary structures [12]. The type of structure into which a specific polypeptide self-assembles depends on amino acid side chain constituents. Secondary structures could include \( \alpha \)-helices, \( \beta \)-sheets and various types of coil turns. Tertiary structures, likewise, have a multitude of conformationally stable structures such as \( \beta \)-barrels made of \( \beta \)-strand-helix-\( \beta \)-strand units [12]. Finally, good examples of quaternary structures are collagen fibrils found in muscle tissue, tendons and ligaments.

Such a multitude of conformational arrangements available to polypeptides, but lacking in synthetic polymers, confer an advantage to polypeptides over polymers with regards to the range of mechanical properties, specifically. Consequently, many synthetically derived polypeptide materials can find applications as diverse as artificial tissues, biodegradable elastomers and elastomeric composites, biosensors, and even clothing.

Advances in chemical synthesis of polypeptides have made it possible to prepare several new designs of polypeptide sequences not available in nature. The oldest of these synthesis reactions has been the N-carboxyanhydride (NCA) polymerization process, see Figure 1.2. The basics of this process is that a protected amino acid group is reacted with solid triphosgene. The resulting N-carbamoylchlorides is then cyclized. Precipitated crude NCA is removed by filtration under Argon atmosphere, having been crystallized from a mixture of THF and hexane. Precipitate is then vacuum dried. The polymers are isolated, dissolved in trifluoroacetic acid (TFA), and protecting groups removed by reaction with HBr in acetic acid. After dissolution, triethylamine (TEA) is added to initiate polymerization. Reaction is quenched through precipitation in diethyl-ether. The precipitate is then filtered to obtain the desired polypeptide.
In this study we have used polyelectrolyte polypeptides which are in essence polypeptides with electrolyte repeating groups. Due to the ionic nature of these peptides, they are soluble in water which is in stark contrast to most neutral polymers. Aqueous solutions of polyelectrolyte polypeptides are electrically conductive due to a heavy presence of mobile ions. In dilute and semi-dilute solutions molecular chains adopt rod-like tube conformations due to charge repulsion.

Figures 1.3a and 1.3b show the polyelectrolyte structures of Poly-(L-Glutamic Acid) [PLE] and Poly-L-(Glutamic Acid4, Tyrosine1) [PLEY(4:1)], respectively. While the glutamic acid is a weak polyacid in solution, the tyrosine amino acid remains neutral but is a polar molecule.

The other two polypeptides we use in this study are Poly-L-Lysine (PLK) (Figure 1.4a) and RADA16 (Figure 1.4b). In this case, PLK turns out to be a weak base. RADA16 is an amphiphilic molecule with two distinctive sides: one side hydrophobic due to the alanine amino acid side group, the other hydrophilic due to both arginine and aspartic acid [13]. RADA16 studied here is acetylated with an amine N-terminus Ac-[RADA]$_4$-NH$_2$. The
arginine (R) and aspartic acid (D) amino acid residues are positively and negatively charged, respectively. Both PLE and PLEY are polyanions. PLK is a polycation. RADA16 is known as polyampholyte as it contains charged groups of both positive and negative signs.

1.2. Polyelectrolyte Charge Screening

As mentioned in the previous section, polyelectrolyte polypeptides are chains of peptides with ionizable repeating units. These peptides dissolve much more readily in aqueous media than typical neutral polymers. This is due to the amino acid side chain tendency to ionize in water. Ions remain in the chain while counterions surround them in solution. Mobility of charged particles through some induced potential leads to the characteristic conductivity of polyelectrolyte polypeptides. While neutral polymer chains adopt a variety of conformations primarily limited by excluded volume forces, conformations of charged polypeptides adopt more extended chains due to charge repulsion. As a consequence of the charges present, polyelectrolyte solutions have solution properties unlike that of neutral polymers. These are as follows:
Figure 1.4: Polyelectrolyte structures of a.) PLK and b.) RADA16. N signifies the degree of polymerization. In the case of RADA16, N=4.

- Extended chain conformations result in a regular distribution of chains within the solvent with equilibrium distances dependent on solution concentration [4].

- At low concentrations, the viscosity, $\eta$, is proportional to the square root of polypeptide concentration, $\eta \propto c^{1/2}$. Whereas neutral polymers at a same given viscosity scale with viscosity proportional to concentration [14].

- While neutral polymer solutions have no peak in the scattering function, homogeneous polyelectrolytes solutions have a well-pronounced peak.

- Counterions in solution are main contributors to osmotic pressure in polyelectrolyte polypeptides in solution, at low concentrations.

Additionally, since ionic Coulomb forces act over longer distances than weaker van der Waals forces it is possible that polyelectrolytes of opposite charges precipitate out of solution rather than crosslink.

Now, counter-ions do not diffuse away to be distributed homogeneously within the solvent. Rather, strong attractive forces between the cation (anion) and counterion prevent diffusive motility. Part of the counterions are kept within the neighborhood of the polyion as condensates so the effective charge density is reduced. Only at distances typically larger
than a monomer length do charges appear to have mobility. That length is known as the Bjerrum length, $\xi_B$, given by

$$\xi_B = \frac{e^2}{4\pi \varepsilon \varepsilon_0 k_b T},$$  \hspace{1cm} (1.1)

where $e$ is the elementary charge, $\varepsilon$ is the dielectric constant of the material, $\varepsilon_0$ is the vacuum permittivity, $k_b$ is Boltzmann’s constant and $T$ is absolute temperature. At the distance $\xi_B$, electrostatic force interaction between the charge on a polyelectrolyte chain along its contour and a nearby counterion is equal to thermal energy forces. For distances between neighboring charges $> \xi_B$, all counterions diffuse away. For distances $< \xi_B$, a fraction of the charges stays in the neighborhood of the polypeptide chain surface. In fact, due to this balance it charges appear primarily at distances $\xi_B$. The above holds $\forall(\xi_B < l_p)$, where $l_p$ is the persistence length. In the case of a monovalent isolated positive charge in a neutral solvent, the electrostatic potential of the charge is

$$V(r) = \frac{e}{4\pi \varepsilon \varepsilon_0 r}. \hspace{1cm} (1.2)$$

However, if one envisions charge screening on polyions as in Figure 1.5, where cations are surrounded by anions and anions are surrounded by cations, the presence of oppositely charged counterions screens the potential such that the spatial dependence on screening

Figure 1.5.: Charge screening on a.) Polyanion b.) Polycation. [3]
becomes
\[ V(r) = \frac{e}{4\pi\varepsilon_0 r} \exp \left( -\frac{r}{\xi_D} \right), \tag{1.3} \]
also known as a Yukawa potential. Here, \( \xi_D \) is the Debye length from the Debye-Hückel theory and is given by
\[ \xi_D = \left( \frac{\varepsilon_0 k_b T}{I_{io}} \right)^{1/2}, \tag{1.4} \]
where \( I_{io} \) is the ionic strength
\[ I_{io} = \sum_i \tilde{c}_i z_i^2 e^2. \tag{1.5} \]
Equation (1.5) contains \( \tilde{c}_i \), the charge number density, with charges \( \pm z_i e \). Physically, equation (1.3) shows that in the immediate vicinity of an ion, \( r \ll \xi_D \), the Coulomb attraction is quite strong. Conversely, for distances of increasing, \( r \), the Coulomb potential is screened by the \( r/\xi_D \) term and finally vanishes for \( r \geq \xi_D \). The Debye length effectively describes the size of a charge cloud surrounding an ion. For increased ionic strengths, screening sets in at decreased distances from the ion. This same mechanism appears in polyelectrolyte polypeptides. Repulsive electrostatic forces between polyion charges are screened by mobile counterion charges. The size of the shielding cloud around each charged amino acid residue is given by \( \xi_D \) as well as the ionic strength, \( I_{io} \).

Interestingly, charge screening of a highly diluted polyelectrolyte polypeptide solution increases with increasing polypeptide concentration. To explain, increasing the concentration also increases counterion concentration, which in turn increases the ionic strength. As a result, \( \xi_D \) decreases, as can be seen from equation (1.4). A decreased Debye length results in larger charge screening. In fact, screening can be so strong that polyelectrolyte polypeptides
lose their unique properties mentioned above and start behaving as neutral polymer chains [4]. This happens when the Debye length drops below the Bjerrum length, $\xi_D < \xi_B$.

Now, it has been found that the Debye length can also be described as the following [4]:

$$\xi_D = \left( \frac{l_{io}d^2}{\xi_B} \right)^{1/2}, \quad (1.6)$$

where

$$l_{io}d^2 \simeq \frac{1}{c_m \theta_{io}}. \quad (1.7)$$

The denominator in equation (1.7) describes both the polymer chain ions as well as the counterions in solution and, thus, determines the ionic strength as well.

Recent work has shown that there exists an electrostatic persistence length which is proportional to the square of the Debye screening length [15]. However, Dobrynin, et al. [14] state that computer simulations, as well as experiments, have shown that the distribution of ions surrounding charged polyelectrolyte polymers is perturbed more than expected from a Debye-Hückel theory. That signifies that there is a stronger charge screening of the electrostatic repulsion, thus, a shorter electrostatic persistence length than theory would indicate. Therefore, the electrostatic persistence length is directly proportional to $\xi_D$ rather than to $\xi_D^2$. This means that it is highly likely that the cross-over concentration from a polyelectrolyte polypeptide stiff rod to a "neutral-like" flexible chain can occur at very low polyionic concentrations. As this study was primarily conducted with high concentrations close to solubility limits, polymers were treated as neutral polypeptides.
1.3. Polymer Relaxation Dynamics

We begin this section by studying three dilution regimes of neutral polymer solutions which exhibit different polymer behaviors: dilute, semidilute, concentrated. The concentrated solution can also be referred to as a polymer melt. Polymer melt refers to a polymer liquid above its glass transition temperature. For polymer solutions, there exists a concentration at which polymer chains begin to interact and start to interpenetrate. That concentration is known as the overlap limit and is given by [4]

\[ c^*_m \simeq \frac{N}{R_F^3}, \]  

(1.8)

where \( N \) is the degree of polymerization and \( R_F \) is the Flory radius, which is a measure of the volume radius that encloses an expanded chain. The Flory radius is \( R_F = \langle R^2 \rangle^{1/2} \). The polymer volume fraction at the overlap concentration is

\[ \phi^* = v_m c^*_m, \]  

(1.9)

and for a neutral polymer in good solvent we have that typically \( \phi^* < N^{-4/5} \). This means that for a typical polymer with \( N \approx 10^4 \) polymer volume fraction should be less than \( 10^{-4} \) to be in the dilute regime, which is a very low concentration indeed. However, even at low polymer volume fractions in the range of \( 10^{-3} \) to \( 10^{-1} \) there already exists some chain overlap and interpenetration. The regime in which the polymer solution is dilute yet contains some polymer overlap is called the semidilute concentration. Figure 1.6 shows the three different concentration regimes. First box on the left indicates dilute concentration where \( c_m < c^*_m \). Middle box indicates overlap concentration \( c_m = c^*_m \). Third box on the right is the semidilute concentration where \( c_m > c^*_m \). The concentrated regime falls far above the semidilute at \( c_m > c_e \), where \( c_e \) is the concentration at onset of entanglement.

Now in the dilute regime one can model polymer dynamics as Brownian motion. If one
Figure 1.6: Concentration regimes. First box indicates dilute concentration where $c_m < c_m^*$. Middle box indicates overlap concentration $c_m = c_m^*$. Third box is the semidilute concentration where $c_m > c_m^*$ [4].

represents the polymer as a set of beads connected by springs the Rouse model is obtained. The beads are representative of a chain’s center of mass, while the springs represent entropic forces similar to Hooke’s law exhibited by polymers upon extension. If we let $R_n$ be the positions of all the beads, this system can be modeled by the linear equation below [5]:

$$ \zeta \frac{dR_n}{dt} = -k(2R_n - R_{n+1} - R_{n-1}) + f_n, $$

(1.10)

where $\zeta$ is the friction coefficient of each bead and

$$ k = \frac{3k_bT}{b^2}. $$

(1.11)

Here, $b^2$ is the mean squared end-to-end distance of each Rouse sequence of beads and $f_n$ represents Gaussian distributed random forces. As we are primarily interested in studying relaxation time, we give the relaxation time obtained as a solution to equation 1.10. The Rouse relaxation time is

$$ \tau_R = \frac{\zeta N^2 b^2}{3\pi^2 k_b T}. $$

(1.12)

We, thus see that $\tau_R \propto N^2 \propto M^2$. The relaxation time is proportional to the square of the degree of polymerization, $N$, as well as molecular weight, $M$. 
However, the Rouse model does not take into account hydrodynamic drag force of each polymer bead due to solvent as would be the case in dilute solutions. That is each bead, as it moves in the solvent, creates a flow field in the same direction as the random force that caused the motion. With this modification Doi and Edwards [5] give relaxation time as:

\[
\tau_z = \frac{\eta_s (\sqrt{N}b)^3}{\sqrt{3\pi k_b T}},
\]

where \( \eta_s \) is viscosity of the solvent. This shows that \( \tau_z \propto N^{3/2} \propto M^{3/2} \) instead of that given by 1.12.

Neither of these models appropriately describes relaxation times upon onset of entanglement. The most well-known model to describe entanglement is the reptation model. Figure

Figure 1.7.: Tube model with topological constraints in an uncrosslinked system. a.) Polymer chain in a fixed network; b.) Primitive chain within a tube formed by topological constraints [5].
1.7 presents a schematic for the tube model envisioned in reptation dynamics. A polymer melt can be viewed as a network of interlocking polymer chains called entanglements as envisioned in the rightmost box of Figure 1.6. Those entanglements cause topological constraints on individual polymer chains that limit their movement. This also limits the response of the polymer to mechanical deformations. Such topological constraints on a polymer chain are represented by the dots in Figure 1.7a, while the chain itself is represented by the “wiggly” solid line. The set of topological constraints can be seen to encompass a tube within which the length of the polymer is constrained. While the polymer can have any configuration of defects, or “wiggles”, within the tube, over long enough time scales those defects average out to a primitive chain as seen in figure 1.7b. The dynamics of the primitive chain are necessarily then along the tube as shown schematically in Figures 1.8a-1.8d. For any applied stress, the primitive chain starts within its tube, but then moves either right or left. At some time,

Figure 1.8: Reptation dynamics within a tube. a.) Original tube; b.)-c.) Motion of the primitive chain as it moves to exit the tube; d.) Remaining tube at any time, t, which eventually vanishes as the primitive chain exists the tube [5].
$\tau_d$, the chain eventually exits the tube. Relaxation time for a reptation mechanism, then, is given by

$$\tau_d = \frac{\zeta N^3 b^4}{\pi^2 k_b T a^2}, \quad (1.14)$$

where $a$ is the step length of the primitive chain and all other variables are as defined previously.

Up until now nothing has been said about the solvents in which the polymers are dissolved. There are three types of solvents: poor solvents, theta solvents and good solvents. In poor solvents, individual monomers in the polymer chain try to minimize contact with solvent molecules. Consequently, polymer chains collapse into a spherical configuration. Theta solvents allow for a balance of interaction forces between polymer chain and solvent. Thus, the polymer settles into an an ideal, non-perturbed chain configuration. In a good solvent, chain monomers try to maximize interactions with solvent. Therefore, the chain becomes expanded. What makes a poor, theta or good solvent is related to the Gibbs free energy of mixing between polymer and solvent as described through a Flory-Huggins treatment. The Gibbs free energy is given by

$$\Delta G_{\text{mix}} = N k_b T \left[ \frac{\phi_p}{DP} \ln \phi_p + \phi_s \ln \phi_s + \chi \phi_p \phi_s \right], \quad (1.15)$$

where $N$ is number of chains, $DP$ is the polymer’s degree of polarization, and $\phi_p$ and $\phi_s$ are volume fractions of polymer and solvent, respectively. Here, $\chi$ is the interaction energy, due to enthalpic contributions, between polymer and solvent. Interaction energy, $\chi$, is what determines the type of solvent. It is also dependent on temperature and concentration.

As mentioned above, the polymer finds itself in different configurations depending on the solvent. Now, polymers are known to be fractal objects. That is, dimensionality of the polymer varies with scale due to a self-similarity, which for polymers there is a limit. Theory
due to Flory established a Flory scaling exponent given by

$$\nu = \frac{1}{d_f},$$  \hspace{1cm} (1.16)

where \(d_f\) is a fractal dimension. For an ideal chain with random walk, \(d_f = 2\), therefore, \(\nu = 1/2\). Thus, a theta solvent has \(\nu = 1/2\). Poor and good solvents have \(\nu = 1/3\) and \(\nu = 3/5\), respectively. It has been found that a more accurate number for \(\nu\) of a good solvent is \(\nu = 0.588\).

With this information, scaling laws of relaxation times with respect to polymer concentrations can be introduced. In his 2012 talk at the Boulder School for Condensed Matter and Materials Physics at Yale University, Rubinstein presented the following [3] for neutral polymers:

Unentangled (\(\nu = 0.588\)) :

$$\tau \sim N^2 c^{(2-3\nu)/(3\nu-1)} \rightarrow \tau_{gs} \sim N^2 c^{0.31}$$  \hspace{1cm} (1.17)

(\(\nu = 1/2\)) :

$$\tau \sim N^2 c^{(2-3\nu)/(3\nu-1)} \rightarrow \tau_{\Theta s} \sim N^2 c$$  \hspace{1cm} (1.18)

Entangled (reptation, \(\nu = 0.588\)) :

$$\tau_d \sim N^3 c^{3(1-\nu)/(3\nu-1)} \rightarrow \tau_{dgs} \sim N^3 c^{1.6}$$  \hspace{1cm} (1.19)

Equation 1.17 gives relaxation time, \(\tau_{gs}\), for unentangled neutral polymers in a good solvent. Equation 1.18 gives relaxation time, \(\tau_{\Theta s}\), for unentangled neutral polymers in a \(\Theta\)-solvent. Equation 1.19 gives a reptation dynamics relaxation time, \(\tau_{dgs}\), for entangled neutral polymers in a good solvent. These differ significantly from relaxation times as functions of concentration for polyelectrolytes which are given as [3, 16, 17, 18] :

**Unentangled Polyelectrolyte Polymers** :

$$\tau_{pe} \sim N^2 c^{-1/2}$$  \hspace{1cm} (1.20)

**Entangled Polyelectrolyte Polymers** :

$$\tau_{dpe} \sim N^3 c^0.$$  \hspace{1cm} (1.21)

It will be seen that the samples in this study, though polyelectrolyte polypeptides, behaved as
given by equations 1.17, 1.18 and 1.19. That was due to the high concentrations involved.

1.4. Mechanical Properties

The physical properties of a polymeric substance will be a function of the underlying chemical structure of the polymers and temperature. Further, polymer properties such as average degree of polymerization (DP), solubility and viscosity are important parameters for development of scaled-up manufacturing processes. Also important are mechanical properties such polymers exhibit in macroscopic materials. A useful measure of the mechanical properties of an elastic polymeric material is Youngs modulus, or stress/strain. In the case of tensile measurements a force is applied to a standard test specimen shape under defined conditions, and the force is increased at a constant rate until the material fractures. Typical tensile test Stress-Strain curves for polymeric samples follow a pattern as in Figure 1.9.

The linear region of the stress/strain curve obeys Hookes law. It is in this region that Youngs modulus can be obtained according to the simple relation, \[ \sigma = E \varepsilon, \]

where \( \sigma = F/A_o \), \( F \) is the applied force along a single axis, \( A_o \) is the original cross-sectional area of the sample, \( E \) is Youngs modulus and \( \varepsilon = \Delta L/L_o \), where \( L_o \) is the original sample length. Other variables are per Fig 1.9. Figure 1.10 shows how Youngs modulus for a polymer varies as a function of temperature at a fixed loading time [6]. Below the glass transition temperature, \( T_g \), a polymer exhibits brittle-like characteristics, while above it behaves as a viscoelastic fluid. Therefore, if a polymer has a \( T_g \) below room temperature it will have soft, rubbery, of even fluid-like characteristics. Conversely, for a polymer with a \( T_g \) above room temperature, say 100 °C, it will be brittle and solid-like at room temperature.

Plasticizers and cross-linkers also influence how polymer molecules associate. Transient intermolecular interactions and crystallinity may increase rigidity. The directionality and relative orientation of molecules may translate into anisotropic bulk properties. Figure 1.11 below, shows the effects of cross-linking on polyisoprene. Youngs modulus increases as cross-
linking increases. The average degree of polymerization ($\overline{DP}$) also affects tensile strength (force/area required to fracture material) of a polymer. Figure 1.12 below shows that higher tensile strengths result from longer polymer chains. This could be due to an increased
propensity for chain entanglement and crystallization [6].

Polymer solids display qualitatively different properties from conventional crystalline solids and from low molecular mass liquids. Crystalline solids usually behave as elastic bodies in the low stress limit. Liquids usually display viscous forces only. In contrast, polymeric substances are both elastic and viscous in the solid state and in the liquid. These materials are also anelastic; reversibility is time-dependent. The weighting of the elastic, anelastic and viscous components depends on polymer structure and thermal energy, so that, above the glass transition temperature, $T_g$, a polymer without crosslinks is melt-like, or inelastic and weak, and a polymer with crosslinks is rubbery.

The greater structural complexity of polypeptides relative to synthetic organic polymers results in differences in mechanical properties of materials [2]. As a result of applied stress, polypeptide conformations in $\beta$ sheets, random coils and alpha helices could result in categorically different elastic responses for any given stress. In contrast, applying a mechanical
force to a polymer material will make individual polymer molecules reorient. A process that will continue until the global free energy minimum of the material is reached. Locally, the free energy minimum would necessarily be larger than thermal energy. While proteins consist of just 20 amino acids, X-ray diffraction and NMR studies have revealed polypeptides can fold into a plethora of geometrical shapes. As mentioned previously, it is likely that polypeptides will display a larger range of mechanical properties under stress than will typical synthetic polymers. That said, polypeptide materials will not necessarily be stronger than synthetic polymer materials. It is known, however, that certain proteins such as collagen and spider silk, are stronger than most polymeric materials. This suggests that it may be possible to realize peptide materials that have physical properties comparable if not superior to those of nylon or Kevlar. The ability of polypeptides to form more complex structures than is possible for typical synthetic organic polymers could be relevant to strain hardening. Additionally, irreversible network reorganization under stress seems more likely for polypeptides than polymers. The reversibility of polypeptide chain reorientation following deformation is likely to depend on hydration, because the polymer backbone and many amino acid side chains are hydrophilic. In such cases, water acts as a plasticizer.
1.5. Theoretical Models of Elasticity

Polymers can be represented by “spaghetti-like” long chains of monomeric units that arrange themselves into conformational states which maximize entropy. Mechanical properties of polymeric material are driven by entropy- a measure of the state of disorder in a system- with \( E \propto \frac{1}{S} \), where \( S \) represents entropy. Polymers that adopt a large number of molecular conformations have a large multiplicity of microstates. Multiplicity of microstates (\( \omega \)) is directly related to entropy through, \( S = k_b \ln(\omega) \), where \( k_b \) is Boltzmann’s constant and \( S \) is entropy. For two materials: if \( \omega_1 > \omega_2 \Rightarrow S_1 > S_2 \), and we have \( E_2 > E_1 \). Higher entropy results in a “softer” polymer with lower \( E \). Conversely, lower entropy results in a “stiffer” material. Stretching a metal will increase its potential energy by increasing the distance between atoms, and heating a metal at constant stress will increase the amplitude of fluctuations about the average inter-atomic distance at equilibrium. In an elastomer, by contrast, stretching will decrease chain disorder and thus entropy. The retractive force will therefore be due largely to the tendency of molecules to increase entropy, which is a maximum at equilibrium, that is, in the unstressed material. A temperature increase will increase polymer chain disorder, making the average chain conformation more as in the unstretched state. Adding heat to a polymer under a constant applied force will therefore decrease its length. Stiffness will increase, because a greater applied force will be needed to achieve the same extension. Figure 1.13a shows the conformational change in polymers and figure 1.13b shows stretching of crosslinked polymers. Without crosslinks or entanglements polymer molecules would slide past each other with no uncoiling. Crosslinking provides a means of microscopically spreading a load among entangled molecules upon placement of a macroscopic force on the material. During uniaxial compression of a homogeneous isotropic material composed of crosslinked polymers one axis experiences compressive deformation and axes perpendicular to axis of compression undergo tensile stretching. The opposite occurs for a uniaxial tensile stretch along the stretch axis. In either compression or tensile
Figure 1.13.: Conformational changes in polymers: (a) Single molecule - top figure unstretched and with high entropy, lower figure stretched and with low entropy; (b) Crosslinked polymer - top figure unstretched, lower figure stretched [7].

deformation, polymer chains stretch with increasing stresses, thereby subjecting the polymer chain networks to increased entropic forces as available conformational states of polymers are continually reduced.

Figure 1.14.: Coordinate system for a compressive test. Compressive forces are exacted along the $\hat{z}$ direction resulting in polymer stretches along the $\hat{y}$ and $\hat{x}$ directions.
1.5.1. Microscopic Deformation: Freely Jointed Chain Model (FJC)

Assuming the polymer network of crosslinked polypeptides deforms affinely (end-to-end distances of each molecular segment increase or decrease by the same ratio) as proposed by [19, 20, 21], the stress $\sigma_{\alpha\beta}$ will be proportional to the change in free energy density due to an infinitesimal change in strain, $\varepsilon_{\alpha\beta}$ such that

$$
\sigma_{\alpha\beta} = \frac{\partial A}{\partial \varepsilon_{\alpha\beta}} \bigg|_{\varepsilon=0} .
$$

(1.22)

In this and other treatments of polymer elasticity we will be primarily concerned with material deformations at constant volume and temperature. Therefore, a convenient thermodynamic energy is the Helmholz free energy, $A = U - TS$, where internal energy, $U$, is assumed to be negligible for an ideal elastomer and, thus, set to zero. The two variables $\sigma$ and $\varepsilon$ are defined as before, thus representing force and extension, respectively. For one individual chain free to move about randomly, the best guess approximation is a Gaussian distribution of conformational space sampling which is also the multiplicity of states for the chain. The distribution of end-to-end vector, $R$, per figure 1.15 [5, 22] is thus

$$
p(R) = \left( \frac{3}{2\pi Nl^2} \right)^{3/2} \exp \left( -\frac{3R^2}{2Nl^2} \right) .
$$

(1.23)

Here $R$ is the magnitude of the end-to-end vector, $N$ is the number of Kuhn length segments, and $l$ is the length of the Kuhn segment, or Kuhn length. It immediately follows that entropy for the chain is $S = k_b \ln (p(R))$. The force required to perform a one-dimensional stretch along the x-direction is $F = d\mathcal{E}/dx$, where $\mathcal{E}$ is the energy. With $A = -TS$ as the energy under consideration, we have $F = \frac{dA}{dx} = -\frac{d}{dx} [k_b T \ln (p(x))] = -k_b T \frac{d}{dx} \left( -\frac{3x^2}{2Nl^2} \right)$ so that we finally have,

$$
F = \frac{3k_b T}{Nl^2} x .
$$

(1.24)
If we let, $K = 3k_b T/Nl^2$ we readily see that equation 1.24 represents Hooke’s law for a polymer, $F = Kx$. The difference between metals and polymers is that a polymer’s spring constant, $K$, increases with temperature due to the entropic nature of conformational states. Because polymer stiffness is driven by entropy, we expect that stretching polymer chains results in increased polymer stiffness. As the chain is stretched, the number of available conformational states decreases, therefore, the multiplicity of states, as well as entropy, decreases. Consequently, the relationship, $E \propto \frac{1}{S}$, leads one to understand that polymer stiffness increases with increased stretch. In subsequent chapters we will see that this phenomena gives rise to marked nonlinearity in elastomeric materials. Young’s modulus increases as strain increases. It is not likely that the chain will continue to have a Gaussian probability distribution as it is continually stretched close to its contour length by an external force, however.

The FJC model assumes independent chain segments which are unrestricted in their orientation. Therefore, the total energy is separable so that $\mathcal{E}(\hat{l}_i) = -FR \cdot \hat{x} = -F \sum_i \hat{l}_i \cdot \hat{x} = -FL \sum_i \cos \theta_i$. Strick, et al. [22] offered a good review where the partition function is
\[ Z = \sum_{l_i} e^{-E_i/k_bT} = \sum_{l_i} \prod_{i=1}^{N} e^{F_l \cos \theta_i/k_bT} = \left[ \int d\Omega e^{F_l \cos \theta_i/k_bT} \right]^N, \] so that

\[ Z = \left[ \frac{2\pi k_b T}{F_l} \sinh \frac{F_l}{k_b T} \right]^N. \quad (1.25) \]

The free energy of the chain is, \( E_{fjc} = -k_b T \ln Z \), from which one can compute an average extension, \( \langle x \rangle \), using that \( \langle x \rangle = -\frac{\partial E_{fjc}}{\partial F} \). Upon differentiating we obtain,

\[ \langle x \rangle = NL \left[ \coth \left( \frac{F_l}{k_b T} \right) - \frac{k_b T}{F_l} \right]. \quad (1.26) \]

At low forces (\( F \ll k_b T/l \)) Hooke’s law, equation (1.24), is recovered. At high forces the model diverges, however.

1.5.2. Microscopic Deformation: Worm-like Chain Model (WLC)

Compared to the FJC model, a better representation of stiff polymer chains is the worm-like chain model. The WLC model is a continuous flexible rod where the Khun length of the FJC model goes to zero, \( \ell \to 0 \). Figure 1.16 is a schematic of the structure used in the WLC.

![Figure 1.16: Worm-like chain model construction: S is the arc length, t are tangent vectors and R is the end-to-end vector.](image)

In general, the radius of curvature of a bent rod will be a function of arc length, \( s \), which
will extend along the rod from one end to the other, and there will be both enthalpic and entropic contributions to free energy. The Kratky-Porod model accounts for both possibilities [23].

Now,

\[ E_{\text{bend}} = \frac{K_{\text{eff}}}{2} \int_0^{l_0} \left( \frac{dt}{ds} \right)^2 ds, \]  

(1.27)

where \( ds \) is an increment of arc; \( dt/ds \), a derivative of the tangent vector, measures the curvature of the rod; \( K_{\text{eff}} = \xi k_b T \) is the bending modulus of the chain and the integration over \( 0 < s < l_0 \) represents the entire length of the rod with \( l_0 \) being the contour length of the polymer chain. The persistence length, \( \xi = \ell/2 \) (half the Khun length), is a characteristic distance along the chain over which tangent vector correlations die off and roughly measures the length over which a rod is rigid. For example, if \( s << \xi \); the rod looks stiff. If each accessible polymer conformation is weighted by its elastic energy, as in classical statistical mechanics, the partition function \( Z_{\text{bend}} = \int D\mathbf{t} \exp \left[ -E_{\text{bend}}/k_b T \right] \) so that,

\[ Z_{\text{bend}} = \int D\mathbf{t} \exp \left[ \frac{2}{\xi} \int_0^{l_0} \left( \frac{dt}{ds} \right)^2 ds \right], \]  

(1.28)

where \( \mathbf{t}(s) \) is the tangent vector of the curve, the integration is over \( \mathbf{t}(s) \) for all possible chain configurations and \( D\mathbf{t}(s) \) is a unit tangent vector increment. Applying tensile force \( F(x) \) to the ends of a chain will increase its energy by \( E_{\text{tensile}} = -Fx = -F \int t_x ds \). The average extension thus becomes

\[ \langle x \rangle = \frac{\partial \ln Z(f)}{\partial F} = \int D\mathbf{t} \times p(x) \]

\[ = \left( \int D\mathbf{t} \times x \exp \left[ -\left( E_{\text{bend}} + E_{\text{tensile}} \right)/k_b T \right] \right) / Z(f) \]

\[ = \left( \int D\mathbf{t} \times x \exp \left[ -\left( \frac{\xi}{2} \right) \int \left( \frac{dt}{ds} \right)^2 ds + F \int t_x ds \right] \right) / Z(f), \]  

(1.29)
where $p(x)$ is the probability of a conformation with extension $x$, the partition function $Z(f)$ accounts for the applied force $F = f k_b T$, the reduced force $f$ has units of inverse length, and $x$ is the extensive thermodynamic conjugate of the intensive variable $f$.

Marko and Siggia [24] proposed the interpolation formula,

$$F = \frac{k_b T}{\xi} \left( \frac{1}{4(1 - x/l_0)^2} + \frac{x}{l_0} - \frac{1}{4} \right),$$  

(1.30)

which diverges as $x/l_0 \to 1$ and thus more accurately models chain behavior than the freely-jointed chain in the high-force limit. The worm-like chain model can be discretized by putting a fixed harmonic bending potential between successive units [25]. An enthalpic contribution to elasticity could also be used in applications of the worm-like chain model where a chain’s restoring force is a strong component of stretching dynamics and cannot be neglected. In this case,

$$F = \frac{k_b T}{\xi} \left( \frac{1}{4(1 - x/l_0 + F/K_0)^2} + \frac{x}{l_0} - \frac{1}{4} - \frac{F}{K_0} \right),$$  

(1.31)

where $K_0$ is the elastic modulus [26].

1.5.3. Mesoscopic Treatment of Elastomer Deformation

Equation (1.24) presented the restoring force of a polymer chain. We can represent the one-dimensional stretch along the x-direction by the principal stretch, $\lambda_x$, where $\lambda = l/L_0$ with $l$ the deformed length and $L_0$ the original length. Additionally, the denominator represents the mean squared end-to-end distance, $\langle R^2 \rangle = N l^2$. So one can re-write equation (1.24) as

$$F = 3k_b T \frac{\lambda_x}{\langle R^2 \rangle}.$$  

(1.32)

25
This form makes it intuitively obvious how extension force varies with the ratio of stretch exerted relative to the mean squared end-to-end distance and temperature. For example, extension force decreases with increasing mean squared end-to-end distance. Conversely, it increases with increasing stretch and/or temperature.

Equation (1.32) relates to the extension of one polymer chain. To understand what happens in three-dimensional affine deformations where \( N \) chains are present we can follow a similar line of reasoning as was used to derive the freely jointed chain model. Figure 1.17 presents an affine stretch in three dimensions of a single polymer chain. The assumption is that one end of the polymer chain remains at the origin and is affinely stretched from vector \( \vec{r}_1(x_1, y_1, z_1) \) to \( \vec{r}_2(x_2, y_2, z_2) \). Stretches in the \( x, y, z \) directions are denoted by the principal stretches \( \lambda_x, \lambda_y, \lambda_z \), respectively. Consequently, the moving end of the polymer reaches the second point by moving \( x_2 = \lambda_x x_1; y_2 = \lambda_y y_1; z_2 = \lambda_z z_1 \). Making use of equation (1.23), we find that the probability that the end of the chain not at the origin is at \( (x_1, y_1, z_1) \) will be

![Figure 1.17: Chain deformation in three dimensions.](image)
\[ p_1(r_1) = \left( \frac{\beta}{\sqrt{\pi}} \right)^3 \exp \left( -\beta^2 r_1^2 \right), \quad (1.33) \]

where \( \beta = \sqrt{\frac{3}{2N} / l} \).

Equation (1.33) can be rewritten as \( p_1(r_1) = \left( \frac{\beta}{\sqrt{\pi}} \right)^3 \exp \left[ -\beta^2 (x_1^2 + y_1^2 + z_1^2) \right] \).

It is, then, obvious that upon a stretch deformation, the probability that the chain end that was at \( r_1 \) is now at \( r_2 \) will be

\[ p_2(r_2) = \left( \frac{\beta}{\sqrt{\pi}} \right)^3 \exp \left[ -\beta^2 (\lambda_x^2 x_1^2 + \lambda_y^2 y_1^2 + \lambda_z^2 z_1^2) \right]. \quad (1.34) \]

The relative change in probabilities between the undeformed and deformed state is

\[ \ln \frac{p_2}{p_1} = -\beta^2 \{ (\lambda_x^2 - 1)x_1^2 + (\lambda_y^2 - 1)y_1^2 + (\lambda_z^2 - 1)z_1^2 \}. \quad (1.35) \]

Let the initial position of the segment end at \( x_1, y_1, z_1 \) be such that \( x_1^2 = y_1^2 = z_1^2 = \langle R^2 \rangle / 3 \), where \( \langle R^2 \rangle = Nl^2 \) as defined before. Upon substitution of these relations into equation (1.35), we obtain that

\[ \ln \frac{p_2}{p_1} = -\frac{1}{2} (\lambda_x^2 + \lambda_y^2 + \lambda_z^2 - 3). \quad (1.36) \]

Therefore, the change in entropy of the chain upon deformation from state 1 to state 2 is

\[ \Delta S = k_b \ln \frac{p_2}{p_1} = -\frac{k_b}{2} (\lambda_x^2 + \lambda_y^2 + \lambda_z^2 - 3). \quad (1.37) \]

For an assembly of \( N \) chains per unit volume we then have that the total entropy per unit
volume is

\[ \Delta S = - \frac{Nk_B}{2} (\lambda_x^2 + \lambda_y^2 + \lambda_z^2 - 3). \]  

(1.38)

An interesting feature of this relation is that the length scale at the microscopic chain segment level is no longer required. The appropriate length scale is now at the aggregate chain level which translates to macroscopically measured quantities. One consequence is that internal energy changes can be deemed negligible. Therefore, the work, or energy, per unit volume required to change the entropy from state 1 to state 2 is \( W = -TdS \), for infinitesimal changes in entropy or \( W = -T\Delta S \) for macroscopic changes. Therefore, the strain energy density contained in an ideal elastomer stretched by \( \lambda_i \) such that \( i \in \{1, 2, 3\} \), is

\[ W = \frac{Nk_B T}{2} (\lambda_x^2 + \lambda_y^2 + \lambda_z^2 - 3). \]  

(1.39)

Now we consider an elastomeric polymer undergoing a uniaxial tensile stretch along the \( \hat{x} \)-direction with \( \lambda_x \) as in Figure 1.14. There will be accompanying contractions along the \( \hat{y} \) and \( \hat{z} \) directions with \( \lambda_y \) and \( \lambda_z \), respectively. Elastomer deformation is typically isochoric (i.e. constant volume). Therefore, the change in volume of a cubical sample expressed as a function of principal stretches is zero, such that \( \Delta V = 0 = (x_1 \lambda_x)(y_1 \lambda_y)(z_1 \lambda_z) - x_1 y_1 z_1 = x_1 y_1 z_1 (\lambda_x \lambda_y \lambda_z - 1) \) This means that \( \lambda_x \lambda_y \lambda_z = 1 \) for the isochoric deformation. Contractions in the \( y \) and \( z \) directions are thus

\[ \lambda_y = \lambda_z = \frac{1}{\sqrt[3]{\lambda_x}} = \frac{1}{\sqrt[3]{\lambda}}, \]  

(1.40)

where \( \lambda = \lambda_x \). Substituting the relationships in (1.40) into (1.39) we can obtain the force needed to induce deformation by taking the derivative of the strain energy density, equation...
(1.39), with respect to the stretch, \( F = \frac{\partial W}{\partial \lambda} \). Carrying out the differentiation we obtain that

\[
F = N k_b T \left( \lambda - \frac{1}{\lambda^2} \right). \tag{1.41}
\]

The force is actually exerted over the cross-sectional area, \( A_0 \), of a sample as shown in Figure 1.6. Therefore, dividing (1.41) by the cross-sectional area and noting that \( \sigma = F/A_o \) is the engineering stress, we obtain a stress-stretch relationship for the isochoric uniaxial deformation of an elastomer as

\[
\sigma = N k_b T \left( \lambda - \frac{1}{\lambda^2} \right). \tag{1.42}
\]

Arruda and Boyce [28] introduce \( G = N k_b T \) as the rubbery modulus. As defined above, for uniaxial deformations \( \lambda > 1 \) represent extensions and \( 0 < \lambda < 1 \) signify compressive deformations. The state of \( \lambda = 1 \) is the undeformed state and is also at the origin of a stress-stretch plot. Young’s Modulus is defined for the region of small deformations, close to \( \lambda = 1 \). One can obtain Young’s Modulus at low strains, by taking the derivative of stress with respect to stretch at \( \lambda = 1 \). This gives that

\[
E = \left. \frac{d\sigma}{d\lambda} \right|_{\lambda=1} = 3 N k_b T. \tag{1.43}
\]

For a perfect incompressible material, \( E = 3 \mu \), where \( \mu \) is the shear modulus. Evidently \( \mu = N k_b T \) in the region of small deformations where Hooke’s law is applicable.

1.5.4. Phenomenological Treatment of Elastomer Deformation

**Theoretical Development**

Rather than continuing to build on a microscopic model based on statistical thermodynamics, one can refer to phenomenological models utilizing concepts from continuum mechanics. For
Figure 1.18.: \( \chi \) maps every point, \( X \in \mathcal{R}_u \) to every point, \( x \in \mathcal{R}_d \). Points in \( \mathcal{R}_u \) are traced in Lagrangian spatial form and points in \( \mathcal{R}_d \) follow a Eulerian description.

A body undergoing rigid deformation from a reference undeformed state, \( \mathcal{R}_u \), to a deformed state, \( \mathcal{R}_d \), \( \exists \{\chi: \mathcal{R}_u \mapsto \mathcal{R}_d\} \). \( \chi \) is function that offers one-to-one mapping, is at least twice differentiable and is non-singular [29, 30, 27]. Therefore,

\[
x = \chi(X), \quad \forall \{X \in \mathcal{R}_u\},
\]

(1.44)

where \( x \) is a position vector of point \( X \) in \( \mathcal{R}_d \). Since \( \chi \) is non-singular we can write it’s inverse to obtain the reverse mapping

\[
X = \chi^{-1}(x), \quad \forall \{x \in \mathcal{R}_d\}.
\]

(1.45)

It must be noted that positions of points, \( X \in \mathcal{R}_u \), are described by a Lagrangian spatial reference and positions of points, \( x \in \mathcal{R}_d \), are described by a Eulerian spatial reference (ie. laboratory reference frame). Additionally, for simplicity of explanation we are calling the reference body undeformed, though, generally, the reference body can be in any state of deformation. The mapping of interest is from the reference body to any subsequent state of deformation.

Now, the deformation gradient tensor, \( \mathbf{F} \), is the function that represents \( \chi \) in the general description given above. The deformation gradient tensor at a generic particle \( X \) is defined
For $F$ to be non-singular, the Jacobian determinant must not vanish, $J = detF \neq 0$. In addition, for orientation preserving deformation considered here, $j = detF > 0$, making $F$ positive determinant.

Since $F$ completely characterizes rigid deformation, where the distance between all pairs of particles is preserved under deformation, it contains a portion which is rigid rotation and a portion which is strain (or distortion). It can, thus, be written in two forms

$$F = RU = VR,$$ (1.47)

where $R$ is a proper orthogonal tensor that represent the rotational part of $F$; $U$ represents the strain part and is called the right stretch tensor; and $V$ is called the left stretch tensor. Now, $U$ allows for computation of geometric quantities in the deformed configuration, $R_d$, in terms of pre-images from the undeformed configuration, $R_u$, therefore, it is a Lagrangian stretch tensor. Conversely, $V$ allows for computation of geometric quantities in the undeformed configuration, $R_u$, in terms of images in the deformed configuration, $R_d$, therefore, it is a Eulerian stretch tensor.

From equation (1.47), we find that $U = (F^T F)^{1/2}$ and $V = (FF^T)^{1/2}$. However, due to mathematical complexity it is not desirable to calculate stretch tensors through these relationships. Much better is to calculate the square of the stretch tensors, such that

$$C = F^T F = U^2, \quad B = FF^T = V^2,$$ (1.48)

where $C$ and $B$ are the right and left Cauchy-Green deformation tensors, respectively. Eigen-
values for $\mathbf{C}$ and $\mathbf{B}$ are $\lambda_i^2$, where $\lambda_i; \ i \in \{1, 2, 3\}$, are the principal stretches. Scalar-valued functions of both $\mathbf{C}$ and $\mathbf{B}$, also called their principal scalar invariants, are

\begin{align*}
I_1(\mathbf{C}, \mathbf{B}) &= \text{tr}(\mathbf{C}, \mathbf{B}); \\
I_2(\mathbf{C}, \mathbf{B}) &= \frac{1}{2} \left[ \text{tr}(\mathbf{C}, \mathbf{B})^2 - (\text{tr}(\mathbf{C}, \mathbf{B}))^2 \right]; \\
I_3(\mathbf{C}, \mathbf{B}) &= \det(\mathbf{C}, \mathbf{B}),
\end{align*}

where we use $(\mathbf{C}, \mathbf{B})$ to state that the invariants for both tensors coincide. In terms of the principal stretches, the scalar invariants can be written as

\begin{align*}
I_1(\mathbf{C}, \mathbf{B}) &= \lambda_1^2 + \lambda_2^2 + \lambda_3^2; \\
I_2(\mathbf{C}, \mathbf{B}) &= \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_3^2 \lambda_1^2; \\
I_3(\mathbf{C}, \mathbf{B}) &= \lambda_1^2 \lambda_2^2 \lambda_3^2.
\end{align*}

As we saw in the previous section, the free energy density depends only on the local strain. Since it is a scalar quantity, it must also be invariant under SO(3) rotations. Therefore, it is expected that the free energy density has a functional dependence in the form of $W(I_1, I_2, I_3)$. It is apparent from relations of the principal invariants that $I_1$ describes uniaxial elongations or contractions, $I_2$ describes three dimensional interactions among elongations and contractions, and $I_3$ generally relates to relative volume changes. For incompressible materials, which typically undergo isochoric deformations so that volume changes are negligible, $\Delta V = 0$. This leads to $I_3(\mathbf{B}) = \lambda_1^2 \lambda_2^2 \lambda_3^2 = 1$ for a Euclidian laboratory reference frame, in which we are typically interested. Due to $I_3$, the requirement for material incompressibility is then

\begin{equation}
\prod_{i=1}^{3} \lambda_i = 1 \text{ where } \lambda_i, \ i \in \{1, 2, 3\}.
\end{equation}
**Experimental Application**

PLEY materials in our study displayed neo-Hookean non-linear elasticity. The stress-strain relationship is linear under small deformations. Then, above some value of strain, the relationship becomes non-linear and thereafter exhibits strain hardening.

Fu and Ogden [29] give the Cauchy stresses for unconstrained, incompressible, homogeneous and isotropic materials as

$$\sigma_i = \lambda_i \frac{\partial W}{\partial \lambda_i} - p, \ i \in \{1, 2, 3\}.$$  \hspace{1cm} (1.52)

The neo-Hookean strain-energy density function characterizing the material is

$$W = \frac{\mu}{2} (I_1 - 3).$$  \hspace{1cm} (1.53)

Application of the two equations above lead to the constitutive relation

$$\boldsymbol{\sigma} = \mu \mathbf{B} - p \mathbf{I},$$  \hspace{1cm} (1.54)

where $\boldsymbol{\sigma}$ is the Cauchy stress tensor, $\mu$ is the shear modulus of the material, $\mathbf{B}$ is the left Cauchy-Green deformation tensor defined in (1.48), $p$ is a hydrostatic pressure determined by boundary conditions and $\mathbf{I}$ is the identity matrix.

To describe the stress-strain experiment presented in Chapter 2, we refer to Figure 1.14 and consider a cube undergoing uniaxial strains in the $\hat{x}$-direction such that boundary conditions are $\sigma_{11} \neq 0$, and $\sigma_{22} = \sigma_{33} = 0$. Accounting for material incompressibility so that $\lambda_1 =
\( \lambda, \lambda_2 = \lambda_3 = \lambda^{-1/2} \), we have

\[
\sigma = \begin{bmatrix}
\sigma_{11} & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0
\end{bmatrix}, \quad \mathbf{F} = \begin{bmatrix}
\lambda & 0 & 0 \\
0 & \frac{1}{\sqrt{\lambda}} & 0 \\
0 & 0 & \frac{1}{\sqrt{\lambda}}
\end{bmatrix}.
\]  \hspace{1cm} (1.55)

Application of equation (1.54) gives the following:

\[
\begin{bmatrix}
\sigma_{11} & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0
\end{bmatrix} = \begin{bmatrix}
\mu \lambda^2 - p & 0 & 0 \\
0 & \mu(1/\lambda) - p & 0 \\
0 & 0 & \mu(1/\lambda) - p
\end{bmatrix}.
\]  \hspace{1cm} (1.56)

This gives that the hydrostatic pressure, \( p = \mu/\lambda \). Finally, substituting the relation for \( p \) into (1.56), we obtain the true stress of the deformed body given by the stress-stretch relation

\[
\sigma_{11} = \mu \left( \lambda^2 - \frac{1}{\lambda} \right) .
\]  \hspace{1cm} (1.57)

Components of the first Piola-Kirchhoff stress tensor give the engineering normal stress on the reference (i.e.: “undeformed”) body through the equation \( \mathbf{S} = \sigma \mathbf{F}^{-T} \). Application of this relation leads to \( S_{11} = (\sigma_{11})(\lambda^2_2) = (\sigma_{11})(1/\lambda) \), which finally gives

\[
\sigma_{\text{eng}} = \mu \left( \lambda - \frac{1}{\lambda^2} \right) ,
\]  \hspace{1cm} (1.58)

where the engineering normal stress, \( \sigma_{\text{eng}} = S_{11} \). Below is a plot of \(-\sigma_{\text{eng}}/\mu\) versus \( \lambda \) for \( 0 < \lambda < 1 \) which lie in the compressive deformation regime. This is the expected behavior of neo-Hookean material under compressive deformation tests.
1.6. Dielectric Relaxation of Polymers

When a dielectric substance is placed within a capacitive system, say a parallel-plate capacitor, application of an electric field induces anisotropy of charge such that a net polarization appears. The electric field within such a capacitor causes a slight shift in the charge “cloud” distribution, normally in equilibrium, such that negative charges appears opposite the direction of positive charges. That shift in charge distribution within the dielectric medium produces a net electric dipole moment per volume termed a dielectric polarization, \( P \). Due to charge polarization an electric field arises within the dielectric medium such that it opposes the applied electric field. In the case of a fluid medium, it is also possible that the molecules re-orient so their axes are aligned with the imposed electric field. A measure of how difficult it is for an electric field to be generated within a polarized dielectric material is known as permittivity, \( \varepsilon \). A material with low \( \varepsilon \) more easily sets up an internal electric field within the medium. Permittivity is typically expressed relative to permittivity of vacuum so that \( \varepsilon = \varepsilon_r \varepsilon_o \), where \( \varepsilon_r \) is the material’s relative permittivity and \( \varepsilon_o \) is permittivity of vacuum.
vacuum. The relative permittivity can be directly related to polarization via the electric
field, \( E \), between the parallel plates of the capacitor by:

\[
P = \varepsilon_o (\varepsilon_r - 1) E.
\]  \hspace{1cm} (1.59)

If an alternating electric field of the form \( E = E_o e^{-i\omega t} \) is applied to the dielectric, an alternating polarization will be produced such that

\[
P = \varepsilon_o (\varepsilon_r - 1) E_o e^{-i\omega t},
\]  \hspace{1cm} (1.60)

where \( \omega \) is the frequency, \( i = \sqrt{-1} \) and \( t \) is time. In material where molecules re-orient with applied electric field, the dipolar orientational polarizability at a given electric field is [4]

\[
P_{or} \simeq c_m \frac{|p|^2 |E|}{3k_B T},
\]  \hspace{1cm} (1.61)

where \( c_m \) is the density of re-orienting units and \( p \) is their dipole moment. Now, if the equilibrium orientational polarization at any given electric field is \( P_{or} \), according to Debye theory the polarization some time \( t \) after application of the field is

\[
P(t) = P_{or} [1 - \exp(-t/\tau_r)],
\]  \hspace{1cm} (1.62)

where \( \tau_r \) is the relaxation time [31]. From equations (1.61) and (1.62) we see that at a certain electric field and temperature, there will be an equilibrium polarization determined by a balance between field and thermal forces. However, some finite time is required for polar molecules to rotate into a new equilibrium distribution after field perturbation. That finite time is given by the relaxation time \( \tau_r \), which is given by \( \tau_r = 1/\omega_r \), where \( \omega_r \) is the relaxation frequency.

For \( \omega \tau_r \ll 1 \) dipoles will be able to keep up with the changing electric field. Therefore, the
dielectric constant will have no imaginary component. Conversely, for \( \omega \tau_r \gg 1 \), orientational polarization will not occur since molecules cannot keep up with changing electric field. At intermediate frequencies, however, the dielectric constant will be complex. Letting \( \varepsilon(\omega) \) be the frequency-dependent dielectric constant and \( \varepsilon(0) \) the DC contribution, we have

\[
\varepsilon(\omega) = \varepsilon' + i\varepsilon'' = \frac{\varepsilon(0)}{1 - i\omega\tau_r}. \tag{1.63}
\]

This relaxation mechanism gives the real component to the dielectric constant (permittivity) as

\[
\varepsilon' = \varepsilon(0) \left( \frac{1}{1 + \omega^2 \tau_r^2} \right), \tag{1.64}
\]

and that of the imaginary component (dielectric loss) as

\[
\varepsilon'' = \varepsilon(0) \left( \frac{\omega\tau_r}{1 + \omega^2 \tau_r^2} \right). \tag{1.65}
\]

As shown in figure 1.20, the value of permittivity starts at the DC value at low frequencies, but steadily decreases with increasing frequency. Dielectric loss is zero at very low and very high frequencies, but goes through a maximum at \( \omega_r \). At this peak, electrical energy is dissipated as heat.

Now, polymer melts exhibit similar dielectric behavior as stated above. However, in the case of polymer melts there are typically multiple relaxation mechanisms. The dipole moment per unit volume in the case of amorphous polymer melts is given by

\[
P = \frac{1}{V} \sum_{all\ chains} \sum_{chain} \sum_{monomer} \mu_i. \tag{1.66}
\]

As can be seen from equation (1.66) information on permittivity and dielectric loss can become very complicated as polymers are randomly distributed throughout the medium.
In general, one sees relaxation processes at higher frequencies due to segmental motion, which is related to a polymer’s glass transition temperature. Molecular motion or rotations characterized by entanglement spacing or end-to-end vector motion are evident at lower frequencies. These are related to viscoelastic properties of the melt. Now, if the
polymer melt is primarily composed of type A molecules peaks in the dielectric loss curves will typically signal overall rotation of the molecule. If the melt is primarily type B molecules, the peak will give information about micro-Brownian motion, which is a form of segmental motion. In most cases, melts are a combination of type A and B molecules.
Part I.

POLYELECTROLYTE STUDIES
CHAPTER TWO:

NONLINEARITY IN A CROSSLINKED POLYELECTRIC POLYPEPTIDE:
A PHENOMENOLOGICAL STUDY

2.1. Introduction

The following is a phenomenological study of co-poly-(L-glutamic acid$_4$, L-tyrosine$_1$) [PLEY (4:1)] crosslinked with poly-L-lysine (PLK) via 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (EDC). The overarching goal of the study was to show that under conditions of high relative humidity the crosslinked material exhibited evidence of nonlinear elasticity, as opposed to, say, linear elasticity. Conversely, at low relative humidities the material exhibited viscoelastic properties. Upon further study it was determined that viscoelasticity was nonlinear for this crosslinked material.

The following types of plots were used in the evaluation of nonlinear elasticity for material with conditions at 75% and 85% relative humidities: Stress-Strain; Secant Modulus-Strain; Change in Secant Modulus-Strain; and Stress-Stretch, from which a shear modulus was obtained through fits of a Neo-Hookean model. Viscoelasticity of material in conditions of 33% relative humidity was evaluated through relaxation times at several strains. Isochronous stress-strain plots showed presence of nonlinear viscoelasticity in crosslinked material at low relative humidities.
Abstract

Youngs modulus (E) of soft solids composed of crosslinked synthetic polypeptides has been determined under different conditions. Co-poly-(L-glutamic acid4, L-tyrosine1) [PLEY (4:1)] was crosslinked with poly-L-lysine (PLK) and 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (EDC). Elasticity was assessed by subjecting samples to a compressive strain. Crosslinked material at high relative humidity, RH 75-85%, exhibited Non-linear elastic. Stress-strain response was approximately linear at low strain but nonlinear above a threshold strain. Analysis of the secant modulus revealed apparent softening of samples at low strain and hardening at high strain, as in biological soft tissues. Fitting stress-strain data with a neo-Hookean model yielded $40 \leq E \leq 300$ kPa at high RH. Viscoelasticity was nonlinear at low RH. The average viscosity-driven relaxation time was 13 min at high strain and 6 min at low strain. Analysis of the derivative of the secant modulus for non-linear elastic materials revealed a transient response up to a strain of $\varepsilon \approx 0.18-0.20$. Above this range, oscillations tended to zero. Non-linear viscoelastic materials showed lower-amplitude oscillations than samples at high RH up to $\varepsilon \approx 0.06$ and strong damping thereafter. The data suggest that it will be possible to engineer mechanical properties of polypeptide materials.

2.2. Background

Protein- and polypeptide-based biomaterials are of increasing interest in medicine, biotechnology and biodegradable materials [12, 32]. Advantages of structural proteins for such materials include intrinsic biocompatibility, the ability to self-assemble into complex higher-order structures, for example, collagen fibers, and a remarkable range of mechanical properties, for example, high-performance elasticity and toughness [12, 32]. Some protein elastomers can withstand over 100 elongation without rupture and return to the original length on removal of stress [33, 34]. The rubber-likeness displayed by some proteins will depend on physical properties of individual chains.
Most structural proteins have repetitive amino acid sequences [35, 36]. Different sequence motifs are found in different structural proteins, which display different mechanical properties and biological functions [35, 36]. Specifically how amino acid sequence translates into protein elasticity is, however, largely unclear. The essential features of rubber-like elasticity are, by contrast, clear enough: long chains enable deformation, at least some independence in chain behavior is required, and crosslinks limit deformation [37]. One of the most extensively studied elastic proteins is the connective tissue protein elastin. The wild-type protein features numerous valine-proline-glycine-valine-glycine repeats, and lysine residues enable enzyme-catalyzed crosslinking of chains, turning soluble individual chains into an insoluble fibrous aggregate [38, 39, 40].

There are three main models of elastic elasticity: random chain network, a solvent-related mechanism and extension-dependent damping of internal chain dynamics. The random chain network model was developed by Flory [41, 42]. Hoeve and Flory [43] reported a low value the ratio of the internal energy to the total elastic force for bovine elastin and concluded that the material was a network of random chains. The authors affirmed the viewpoint was affirmed over a decade later [44]. In the model of Weis-Fogh and Anderson [45], by contrast, extending an elastin fiber will increase the exposure of hydrophobic side chains to solvent, lowering the entropy of water as it forms a cage at the fiber-solvent interface [46, 47]. The backbone of a polymer, however, not the solvent, must bear the tensile load. As to chain dynamics, the \( \beta \) spiral conformation of an elastin chain will permit oscillations of the \( \phi \) and \( \psi \) angles of amino acid residues other than proline. Chain stretching could dampen the amplitude and influence the frequency of the oscillations [48, 49]. This view appears to gain support from dielectric relaxation and acoustic absorption experiments and computational studies summarized in [50]. The amplitude of the oscillations will, however, depend mainly on thermal energy; a compressive or tensile force is not expected to have marked impact on the oscillations, provided that the chain is not stretched too much. The elastic properties
of other proteins are assumed to depend on different mechanisms, though of course basic principles will be generally valid.

Crosslinked polymer networks display non-linear elasticity under some conditions. Various theoretical and descriptive models have been proposed. Rubinstein and Panyukov [51], for example, have developed a molecular model of non-linear elasticity for entangled polymer networks. Storm et al. [52] have proposed a molecular model of the non-linear elasticity of actin, collagen, fibrin, vimentin and neurofilaments. More recently, Carrillo et al. [53] used a combination of theoretical analysis and molecular dynamics simulations to develop a model of networked deformations, which the authors then used to describe non-linear mechanical properties of polymers and biological gel networks. Synthetic polymer networks deform reversibly at an applied stress in the $10^4 - 10^7$ Pa range. Networks of the proteins actin and collagen, by contrast, deform at stresses as low as $10^{-1} - 10^2$ Pa. The elastic response of a polymer network will normally contain both entropic and enthalpic components, the balance depending on the polymers involved and how they interact.

Here we assayed for the response of crosslinked synthetic polypeptides to an applied compressive stress. The polymer chosen for the experiments was a random co-polymer of L-glutamic acid (E) and L-tyrosine (Y) in a four-to-one molar ratio [PLEY (4:1)]. PLEY molecules were crosslinked with PLK, a polycationic homopolymer, and EDC, a diimide reagent. We then compared the mechanical properties of the materials with biological tissues, polymer networks, and biological gels. To the authors’ knowledge, there are no available studies on cross-linked PLEY(4:1) in the field. However, synthetically designed polypeptides are becoming increasingly important in diverse areas such as biodegradable devices, medical implants and mechanical dampers. This study initiates mechanical property studies of materials that have not previously been investigated. The data suggest that it is possible to engineer mechanical properties of polypeptide materials.
2.2.1. Materials and Methods

2.2.2. Polymers

PLEY (4:1) (MW 20-50 kDa) and PLK (MW 15-30 kDa) were from Sigma-Aldrich (USA). EDC was obtained from TCI America (USA). The chemical structures of the side chains are shown in Figure 2.1. The nominal pKas are 4.1 (E), 10.5 (Y) and 10.5 (K).

![Chemical structures of amino acids](image)

Figure 2.1.: Side chains of the amino acids of the present study. The side chain of an amino acid residue is attached to an alpha carbon atom in the polymer backbone. A peptide group is formed between backbone atoms of successive amino acid monomers.

2.2.3. Mold

Samples were formed in a custom-made aluminum mold. Fabricated in the USF Department of Physics machine shop, the base and lid of the mold were cut from a 0.5”-thick plate, cylinders were cut into a 0.125”-thick plate, and all flat surfaces were milled to a roughness of 2.5-25 µm. The mold was designed to produce 1 to 1, length to diameter cylinders, each having a volume of ≈ 50 mm³. All samples had a nominal diameter and height of 4 mm.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLEY (4:1) concentration (%) w/v</td>
<td>-1</td>
</tr>
<tr>
<td>PLK concentration (%) w/v</td>
<td>10</td>
</tr>
<tr>
<td>EDC concentration (%) w/v</td>
<td>20 (1.0 M)</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 2.1.: Experimental factors of this study.
2.2.4. Experimental Design

Table 2.1 summarizes the details of sample composition. Three experimental factors were the concentrations of PLEY (4:1), PLK and EDC, and each had three possible values. RH, a fourth factor, also had three possible values. A full-factorial experiment would have involved $3^4 = 81$ trials, exceeding the scope of the project, so the number of trials was reduced to $3^3 = 27$. Table C1 in Appendix C, generated with JMP statistical software (SAS, USA), shows a randomized trial for the selected factors. PLK at low concentration was predicted to be unlikely to precipitate PLEY (4:1) by way of interpolyelectrolyte complex formation, the lysine side chains of PLK binding to glutamic acid side chains of PLEY by Coulomb interactions. The three-dimensional polymer network thus formed is more likely to precipitate at a high concentration of PLK, as the odds of saturating available sites on PLEY will be higher. Table C1 in Appendix C also presents the composition of each of the 27 samples of this study. Sample 1, for instance, was prepared by mixing 40 $\mu$L of 30% (w/v) PLEY with 40 $\mu$L of 40% (w/v) EDC and 40 $\mu$L of 20% (w/v) PLK. The sample was then equilibrated in a chamber at 85% relative humidity prior to analysis. The sample compositions of this study were 3 replicates of 9 different conditions, not 27 different conditions. Not all samples survived processing. This is evident in conditions with only two replicates as presented in raw data graphs Figures 2.3-2.8. Materials were fabricated down the list according to Table C1 so as to not introduce bias.

2.2.5. Materials Fabrication

Equal volumes (40 $\mu$L) of PLEY solution, EDC solution and PLK solution were mixed in a 1.5-mL tube using a 250 $\mu$L micro-pipette. Volumes delivered were expected to be within 2 $\mu$L of nominal, or 5% variation. EDC was mixed first with PLEY to prevent immediate crosslinking. EDC is reactive towards carboxylic acid, but the anhydride is unstable; a peptide bond is formed when EDC reacts with a carboxylic acid group and an amino group.
PLK was then added to the PLEY-EDC mixture. Nominal concentrations at 30%, 40% and 50% PLEY were made thus. Unfortunately, there are no chromophores available to determine reactant concentrations more accurately with UV instruments. The final reaction mixtures were then transferred to the mold with a 1-mL syringe and allowed to set up over a 24-h period. Samples were removed from the mold and placed in a humidity chamber. Each sample was allowed to come to equilibrium over a period of 3 days prior to mechanical analysis. The samples had a diameter of $(3.5 \pm 0.4)$ mm and a height of $(3.6 \pm 0.3)$ mm. Individual height and diameter measurements were considered accurate to within 0.5 mm.

2.2.6. Humidity Control

Desired RH values were obtained with different saturated aqueous solutions of salt: KCl for 85%, NaCl for 75% and MgCl₂ for 33% [54]. Saturated salt solutions were prepared by adding deionized water to a minimum of 5 g of salt previously deposited in a 15-mm tall petri dish. Saturated salt solutions were deposited in separate petri dishes, sealed with parafilm and allowed to come to equilibrium at 22 °C over several days. This method has been shown to provide relative humidities with 2% of nominal values, if prepared correctly. Samples were removed from the mold and placed in the designated relative humidity chamber. Each sample was then allowed to come to equilibrium at 22 °C over a period of 3 days prior to mechanical analysis. Figure 2.22 in supporting information provides experimental evidence to support the chosen equilibrium period.

2.2.7. Materials Testing

**Force-Displacement (F-D) measurements**

Uniaxial compression tests of crosslinked samples were performed as illustrated in Figure 2.2. A digital scale (Centech, USA) with a maximum loading capacity of 1000 g (9.8 N) and a resolution of 0.1 g was placed on top of a lab jack, giving continuous displacement
in the vertical dimension. An analog displacement gauge (Pittsburgh Dial Indicator, USA, 0.001 in (0.025 mm) resolution) was used to quantify compression. The sample was placed between the scale and a fixed arm. This system enabled measurement of spring constants in the 10-1100 N/m range. Displacement was the independent variable; compressive force was the dependent variable. A loading curve was obtained for each sample, an unloading curve for selected samples. All data were acquired at 22 °C. Figure 2.23 in supporting information shows the loading and unloading curve of the apparatus itself over the force range of 0.5-6 N.

Figure 2.2: Measurement apparatus. Cylindrical samples were compressed uniaxially along the vertical axis by adjusting the height of the lab jack. Displacement was quantified with a displacement gauge.

**Relaxation time measurements**

Conditions at 33% relative humidity were analyzed through relaxation times. To obtain relaxation times, a sample was placed on a lab scale utilizing the apparatus shown in Figure 2.2. A predetermined strain was applied to the sample by raising the lab jack by a displacement coinciding with the desired strain. At the point where the desired strain was reached, as measured by the displacement gauge, the sample was allowed to relax down to its equilibrium value over time. Values of stress were determined by the values of weight measured on
the balance. To capture relaxation as a function of time, a video of the relaxation process was started at the point of maximum stress for the given strain. Relaxation stress values at each time were extracted from analysis of the video.

2.2.8. Fitting and Parameter Determination

Young’s modulus

There are several phenomenological and mechanistic models of varying levels of complexity that can be used to describe hyperelastic materials. For biological materials and cross-linked polymers, specifically, a neo-Hookean model is widely used. There are some disadvantages to the use of a neo-Hookean model. In general, its predictive nature is less accurate than models such as the Mooney-Rivlin, Ogden and Arruda-Boyce models, as examples. Additionally, the neo-Hookean model does not predict accurately at large strains. However, given that our samples showed significant variability it was decided that the neo-Hookean model is appropriate to serve our purpose of elasticity comparisons among different conditions. The simplicity of the model allowed for a standard method to be applied to each condition and, thereby, determine if patterns arise due to changing experimental conditions of concentration and relative humidity. This study did not seek to determine exact values of shear moduli at each given condition. Rather, our aim was to understand changes in elasticity due to changes in condition on a condition-to-condition comparative basis. We, nonetheless, compared stiffness trends obtained by the neo-Hookean model with that of a two-parameter Mooney-Rivlin model.

All fitting parameters were obtained with Matlab® implementing a trust region nonlinear least squares algorithm.

Engineering normal stress is calculated as $\sigma = \frac{F}{A_o}$, where $F$ is the applied force along a single axis and $A_o$ is the original cross-sectional area of the sample. Engineering strain is defined as $\varepsilon = \frac{l - L_o}{L_o}$, where $l = L_o - \delta$ is the length of the sample after a compressive
displacement $\delta$ and $L_o$ is the original sample length. Principal stretches are defined by engineering strain, $\varepsilon = \lambda - 1$ where $\lambda = \frac{l}{L_o}$. For an incompressible, homogeneous and isotropic material, true stress $\sigma_{true} = \mu \left( \lambda^2 - \frac{1}{\lambda} \right)$, where $\mu = \frac{E}{2(1+\nu)}$ is the shear modulus, $\nu$ is Poisson's ratio, and $E$ is Young's modulus. The engineering normal stress will be

$$\sigma_{eng} = \mu \left( \lambda - \frac{1}{\lambda^2} \right) \quad (2.1)$$

on inserting $\sigma_{eng} = \frac{\sigma_{true}}{1+\varepsilon}$. If $\nu=0.5$, as is the case for incompressible isotropic materials undergoing isochoric deformation, then $E = 3\mu$. The neo-Hookean stress model for engineering stress can be fit to stress-strain data to determine $\mu$ and thus $E$.

The Mooney-Rivlin model used here contains two parameters, though up to nine parameters can be used. These material constants, as they are called, are $C_1$ and $C_2$ and here expressed as $\mu_1 = 2C_1$ and $\mu_2 = 2C_2$ to keep notation comparable to that used in the neo-Hookean model. The Mooney-Rivlin model is in essence an extension to the neo-Hookean model. The engineering normal stress will be

$$\sigma_{eng} = \left( \mu_1 + \frac{\mu_2}{\lambda} \right) \left( \lambda - \frac{1}{\lambda^2} \right) \quad (2.2)$$

Linearization of equation 2.2 gives the following form

$$\sigma_{eng}^* = \mu_1 + \mu_2 \beta, \quad (2.3)$$

where $\sigma_{eng}^* = \sigma_{eng}/(\lambda - 1/\lambda^2)$ and $\beta = 1/\lambda$. On a plot of $\sigma_{eng}^*$ versus $\beta$ one finds that $\mu_1$ is the intercept and $\mu_2$ is the slope. In general, due to our compressive tests we will find negative values of $\mu_2$. 50
Relaxation time
The time dependence of stress at a given strain was modeled as follows. For two elements of a Maxwell material in parallel, each consisting of a spring and a dashpot in series, the differential equation for the stress-strain response is

\[ \sigma + p_1 \dot{\sigma} + p_2 \ddot{\sigma} = q_1 \dot{\varepsilon} + q_2 \ddot{\varepsilon}, \]  

where \( p_1 = \tau_1 + \tau_2; \) \( p_2 = \tau_1 \tau_2; \) \( q_1 = \eta_1 + \eta_2; \) \( q_2 = \eta_1 \tau_2 + \tau_1 \eta_2. \) Here \( \tau_i = \eta_i / E_i, i \in \{1,2\} \) is relaxation time and \( \eta_i \) is viscosity. The relaxation times were determined by fitting

\[ \sigma(t) = \varepsilon_o \left( E_1 e^{-\frac{t}{\tau_1}} + E_2 e^{-\frac{t}{\tau_2}} \right) \]

(2.5)

to individual experimental data sets.

2.3. Results and Discussion

2.3.1. Force Versus Displacement

A force-displacement curve was obtained for each sample. Figures 2.3-2.5 shows loading curves for samples in each of the conditions at relative humidities of 75% and 85%. See Table C1 in Appendix C for specific compositions and summary of samples within each condition. Labels for each condition contain information about PLEY, PLK and EDC concentration as well as relative humidity. For example, 315375 represents 30% (w/v) PLEY, 15% (w/v) PLK, 30% (w/v) EDC and 75% RH. Not all samples survived processing. This is evident in conditions with only two replicates. We, additionally, obtained force-displacement curves for samples at relative humidities of 33%. These are shown in Figures 2.6-2.8. Figure 2.9 presents force-displacement data for all conditions with each condition averaged over replicates. To obtain the error bars in the force-displacement plot the standard deviation in
Figure 2.3: Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 30% w/v nominal PLEY concentration.

Weight among samples for each condition was obtained at each displacement value setting. Error in force was calculated as $F = C(w \pm \Delta \varepsilon_w)$, where $w$ is weight and $\Delta \varepsilon_w$ is standard deviation in weight measurement among the replicated samples. $C$ is a constant to obtain force in Newtons comprised of $g = 9.8 \text{ m/s}^2$ and a conversion factor to change from grams to kilograms.

Non-linearity was apparent at higher strains for high RH samples as seen in Figs. 2.3-2.5. Additionally, on samples for which unloading curves could be obtained comparison of
loading and unloading curves provided initial evidence of a non-linear elasticity in the present materials. Figure 2.10 shows force-displacement loading and unloading curves for samples at 75% and 85% RH. Circles represent loading, stars unloading. Loading was clearly non-linear, and unloading closely followed loading. This behavior presented initial indications that the material might have non-linear elasticity. Such behavior alone might not necessarily indicate non-linear elasticity, however. Given the apparent non-linear elasticity exhibited in the force-displacement plots by samples at high RH, we proceeded to further analyze such material through application of phenomenological models in section 2.3.2.

Samples at 33% RH were significantly stiffer and more brittle than those at high RH. In both low and high RH, linear regions typically appeared at low strains. Arbitrarily fitting a line to the first six data values of the F-D plots Figures 2.3-2.8 gives a rough estimate of the material’s spring constant as obtained from the slope of the fitted line. On average, spring constants for materials at each relative humidity were: 290 N/m for 75% RH; 240 N/m for 85% RH and 1340 N/m for 33% RH. Force versus displacement measurements revealed a difference in time-dependent behavior between crosslinked polymers at high RH versus those at low RH. High RH samples immediately reached an equilibrium value in reaction force at a given strain. Those at low RH relaxed over a certain period of time prior to settling on an equilibrium force. For samples at 33% RH at strains up to about 0.04 the resulting force relaxed very quickly to equilibrium, typically less than six seconds. But at strains beginning at around 0.06 and larger the material took progressively longer to relax to equilibrium. To obtain force versus displacement curves, a force reading was obtained after about 10 seconds of relaxation time at each strain. High RH samples showed no evidence of time dependence relaxation within the same 10 second time period. As mentioned above, for each sample at high RH equilibrium force was reached immediately at each imposed strain, within the range of strains in these experiments. Therefore, one can say that the minimum time limit over which samples at high RH exhibited non-linear elasticity was 10 seconds. That is to
say that within a 10 second time period, stress at each given strain did not relax down to a
different value for samples at high RH.

Materials at the three low RH conditions clearly exhibited viscoelasticity. Consequently,
application of hyper-elastic models, such as the neo-Hookean or Mooney-Rivlin, were inap-
propriate to assess elasticity for two reasons. Firstly, the materials exhibited viscoelasticity
as opposed to elasticity. The models mentioned above are only applicable to elastic ma-
terials. Secondly, stress-stretch plots would show stress values obtained after a 10 second
time interval at higher stretches. That would lead to misleading parameter values obtained
from such models and not be indicative of actual material behavior. Therefore, our efforts
were focused on time-dependent viscoelastic analysis to study relaxation characteristics of
materials at low RH as presented in section 2.3.3.

Figure 2.11 presents the decision path followed to characterize mechanical properties of
crosslinked material in this study. Material at high RH was analyzed for non-linear elasticity
since it exhibited no stress relaxation within the range of strains applied and limiting time
period used in this study. Material at 33% RH was analyzed as viscoelastic material since
it did exhibit stress relaxation at each strain above approximately 0.06.

2.3.2. Nonlinear Elasticity Analysis

Many biomaterials display non-linear elasticity [55]. Glandular and fibrous breast tissue
under compression, for example, displays mostly linear stress-strain curves below 10% strain;
a modulus of 28-35 kPa for glandular tissue and about 96-116 kPa for fibrous tissue at 5%
strain, and a modulus of 48-66 kPa and 218-244 kPa at 20% strain [56]. Strain stiffening is
also displayed by artery walls [57], cornea [58], blood clots [59] and other biological tissues.

To confirm our crosslinked material behaved similar to biological materials, we utilized F-
D loading data for analysis of stress-strain curves and looked for evidence of strain hardening
in secant modulus versus strain plots. Stress-strain curves were plotted to assess possible
non-linear elasticity. Figure 2.12a shows a typical stress-strain curve. Here it is for condition 515285. All conditions are presented in Figure 2.13. To obtain error bars in the stress-strain plot, first stress was calculated as \( \sigma = \frac{F}{A} \), where \( F \) is the applied force and \( A \) is the sample’s cross sectional area. Values of force were \( F = x_F \pm \Delta \varepsilon_F \), where \( x_F \) is each individual value of force at each strain and \( \Delta \varepsilon_F \) is variance in force determined from prior calculations. Values of area were \( A = x_A \pm \Delta \varepsilon_A \), where the error in area, \( \Delta \varepsilon_A \), was calculated as \( \Delta \varepsilon_A = A \sqrt{\frac{2(\Delta \varepsilon_r)}{r^2}} \). Average diameter of samples was as given in subsection 2.2.5: 3.5 ± 0.4 mm, so that \( r = 1.75 \text{mm} \) and \( \Delta \varepsilon_r = 0.2 \text{mm} \). The stress with error was then calculated as \( \sigma = \frac{F}{A} \pm \Delta \varepsilon_\sigma \), where \( \Delta \varepsilon_\sigma = \sigma \sqrt{\left(\frac{\Delta \varepsilon_F}{F}\right)^2 + \left(\frac{\Delta \varepsilon_A}{A}\right)^2} \). It can be seen from the plots that the stress-strain relationship is linear for small deformations. Then, above a threshold value of strain, the relationship becomes non-linear and the material displays strain hardening. Secant moduli are often used in the analysis of non-linear stress-strain relationships to gain insight on the variation in elasticity as a function of strain [8, 9, 10]. The secant modulus is approximately the same as the tangent modulus within the linear regime of small deformations, but it deviates substantially from the tangent modulus in the non-linear region. In the present work, the secant modulus was evaluated at each data point \( i \) in the stress-strain curve as \( (E_s)_i = \sigma_i / \varepsilon_i \), where \( \sigma_i \) is engineering stress and \( \varepsilon_i \) is engineering strain.

Figure 2.12b shows the data of Figure 2.12a plotted as secant moduli. Typical curves showed an initial decrease in secant modulus at low strain (\(< 0.05\)), signaling strain softening. This is probably attributable to polypeptide chain movement and re-ordering upon compressive loading of the material. A further increase of load resulted in strain hardening. Analysis of variation in elasticity, through the secant modulus as a function of strain, provided evidence for strain softening at low strains otherwise not noticeable in the stress-strain plot. Additionally, the analysis pointed to an approximate strain value, different for each condition, where strain softening transitioned to strain hardening. This analysis further sub-
stantiated the non-linear elasticity of PLEY material. Figure 2.14 presents secant moduli for all six conditions. Secant modulus was calculated as mentioned in the results and discussion section of Chapter 2. We have assumed that \( \Delta \varepsilon_\sigma >> \Delta \varepsilon_\varepsilon \). Therefore the secant modulus with error bars was calculated as \( E_s = \frac{1}{\varepsilon}(\sigma \pm \Delta \varepsilon_\sigma) \), where \( \Delta \varepsilon_\sigma \) was calculated previously.

We were interested in calculating the change in secant modulus at each data point, or the approximate second derivative of the stress at each value of strain, to see if there any further information could be gleaned from stress-strain data. We calculated the change in secant modulus as a modified centered-difference second derivative of the stress with respect to strain at each point:

\[
\frac{dE_i}{d\varepsilon} \bigg|_{\varepsilon=\varepsilon_i} = \frac{d^2\sigma_i}{d\varepsilon^2} \bigg|_{\varepsilon=\varepsilon_i} \approx \frac{\sigma_{i+1} - 2\sigma_i + \sigma_{i-1}}{\varepsilon_i^2}. \tag{2.6}
\]

The change in secant modulus, a type of transient response at low to medium strain, was analogous to the second-order transient response to an impulse in an electrical circuit. As strain increased, the change in \( E_s \) settled to zero above a certain strain, the value depending on the sample. Figure 2.15 displays the ratio \( \frac{d^2\sigma_i}{d\varepsilon^2} \bigg|_{\varepsilon=\varepsilon_i} / \frac{d^2\sigma_i}{d\varepsilon^2} \bigg|_{\varepsilon=\varepsilon_{max}} \) at each value of the strain for samples at 75% and 85% RH. One sample from each condition was chosen at random and plotted in this new type of plot for the sole purpose of displaying our findings. The interesting behavior, however, calls for additional studies to be conducted using this type of plot. Transient responses are evident up to a strain of \( \varepsilon \approx 0.18-0.2 \). For \( \varepsilon > 0.2 \), \( \frac{d^2\sigma_i}{d\varepsilon^2} \bigg|_{\varepsilon=\varepsilon_i} \approx 0 \). The character of the response appears to support the hypothesis that polypeptide chain movement and re-ordering occur when \( \varepsilon \leq 0.18-0.20 \) in materials at 75% and 85% RH. Increased strain could increase polymer alignment and intermolecular interactions. Strain hardening then begins to take place when \( \varepsilon > 0.20 \) as polymer chains reach an internal entropic force of equal magnitude to that imparted to the material as the polymers elongate between crosslinks. During this stage, the secant modulus fluctuates little if at all, because changes in the elastic modulus between successive strain values are primarily driven by
enthalpy rather than chain re-orientation. Consequently, the stress-strain curve becomes non-linear. Fluctuations were greater in general for samples at 85% RH than at 75% RH. Figure 2.16 compares stress, secant modulus and \( \frac{d^2\sigma}{d\varepsilon^2} \) versus strain for condition 515285, sample 515285-3 (85% RH). The data suggest three distinct regions of response to strain. In the first, the secant modulus decreases with increasing strain. This appears to reflect the movement of polypeptide chains relative to each other in a chain re-ordering process. The second derivative displays a transient response in this region. In the second region, there appears to be evidence for strain hardening. The second derivative oscillates around zero with a small amplitude, indicating that the polypeptide chain is still reordering. In the third region, \( \frac{d^2\sigma}{d\varepsilon^2} \bigg|_{\varepsilon=\varepsilon_i} \approx 0 \). The secant modulus is increasing in response to increasing strain, indicating a continued but moderate rise in stress at each strain in the stress-strain curve. This region corresponds to strain hardening. Similar behavior is displayed by nonlinear elastic biological materials [9, 10]. Samples at high RH exhibited non-linear elastic deformation, whereas those at low RH showed non-linear viscoelastic deformation, as will be discussed below.

Figures 2.17-2.19 present stress-stretch data for all conditions at 75% and 85% relative humidities. The applied stress was a uniaxial compression, so the maximum stretch was 1 for \( \delta = 0 \). Data is represented by the colored open circles. A neo-Hookean model for stress, \( \sigma = \mu \left( \lambda - \frac{1}{\lambda^2} \right) \), was globally fit to the experimental data with \( \mu \) as the fitting parameter and represented by a black line. Youngs modulus was calculated as \( E = 3\mu \) under the assumption of perfect material incompressibility. Table 2.2 lists shear modulus, \( \mu \) from global fits in the second column and calculated Young’s Modulus, E, on the third column. The fourth column of table 2.2 lists the samples at each condition and the fifth column presents shear moduli for each of the samples (see Appendix C for plots of individual fits). The last column calculates the standard deviation of shear moduli among samples within each condition. The sole exception is sample 410385-1, whose data was not included in the global fits of condition 410385.
In the same figures we, additionally, present a global fit of data using a two-parameter Mooney-Rivlin model, \( \sigma = (\mu_1 + \frac{\mu_2}{\lambda}) (\lambda - \frac{1}{\lambda^2}) \) represented by the blue dotted line. Table 2.3 lists the fitting parameters from global fits in the second column. The third and fourth columns show samples at each condition with associated fitting parameters from individual fits (see Appendix C for plots of individual fits).

**Table 2.2:** Young's moduli values obtained by fitting a neo-Hookean model, \( \sigma = \mu(\lambda - 1/\lambda^2) \), to experimental data in Figures 2.17-2.19. First column lists experimental conditions; second lists shear modulus from global fits. E is the Young’s modulus. The fourth and fifth columns show samples at each condition with associated shear moduli from individual fits. The last column lists the standard deviation from individual fits (see Appendix C for plots of individual fits).

| Conditions | \(|\mu|\) (kPa) | E (kPa) | Sample | \(|\mu|\) (kPa) | Std Dev (kPa) |
|------------|----------------|---------|--------|----------------|---------------|
| 315375     | 82             | 246     | 1 2    | 102            | 24            |
| 320485     | 45             | 135     | 1 2    | 64             | 25            |
| 420275     | 43             | 129     | 1 2 3  | 43 33 57       |               |
| 410385     | 139            | 417     | 1 3    | 139            |               |
| 410385     | 41             | 123     | 2 3    | 70 13          |               |
| 510475     | 40             | 120     | 2 3    | 57 45          |               |
| 515285     | 12             | 36      | 2 3    | 15 9           | 4             |

The values shown in the conditions column of table 2.2 signify the concentrations of PLEY, PLK, EDC and RH. For example, 315375 represents 30% (w/v) PLEY, 15% (w/v) PLK, 30% (w/v) EDC and 75% RH. Data in table 2.2 shows larger stiffness occurs at PLEY concentrations of 30% compared with 50%. We attribute this to a higher degree of crosslinking for lower PLEY concentrations within the range of EDC concentrations used in this study. Higher concentrations of PLEY might not have resulted in additional crosslinking. Rather, unreacted aqueous PLEY, as well as PLK and EDC, probably remained embedded within
Table 2.3: Fitting parameters obtained by fitting the Mooney-Rivlin model, \( \sigma = (\mu_1 + \mu_2/\lambda)(\lambda - 1/\lambda^2) \), to experimental data in Figures 2.17-2.19. First column lists experimental conditions; second lists shear moduli from global fits. The third and fourth columns show samples at each condition with associated fitting parameters from individual fits (see Appendix C for plots of individual fits).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>([\mu_1, \mu_2]) (kPa)</th>
<th>Sample</th>
<th>([\mu_1, \mu_2]) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>315375</td>
<td>[240, -260]</td>
<td>1</td>
<td>[360, -370]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>[170, -190]</td>
</tr>
<tr>
<td>320485</td>
<td>[60, -90]</td>
<td>1</td>
<td>[100, -130]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>[50, -60]</td>
</tr>
<tr>
<td>420275</td>
<td>[220, -210]</td>
<td>2</td>
<td>[90, -100]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>[470, -420]</td>
</tr>
<tr>
<td>410385</td>
<td>[540, -590]</td>
<td>1</td>
<td>[540, -590]</td>
</tr>
<tr>
<td></td>
<td>[210, -220]</td>
<td>2</td>
<td>[460, -460]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>[16, -25]</td>
</tr>
<tr>
<td>510475</td>
<td>[40, -60]</td>
<td>1</td>
<td>[60, -60]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>[50, -80]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>[40, -60]</td>
</tr>
<tr>
<td>515285</td>
<td>[-1, -7]</td>
<td>2</td>
<td>[-6, -6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>[6, -11]</td>
</tr>
</tbody>
</table>

the crosslinked network leading to lower elastic moduli. t-ratios (see Figure 2.24 in supporting information) showed that RH made the most significant contribution to stiffness in the low strain region. The next most influential factors were concentration of PLEY and concentration of PLK. The experiments also revealed that changes in the EDC concentration had little to no effect on the spring constant within the 20-40% range. Polypeptides were covalently crosslinked by EDC but also non-covalently by ionic interactions between PLEY and PLK. A control experiment revealed that a mixture of PLEY and PLK formed a precipitate but the reactants did not crosslink due to the absence of EDC. This indicates that EDC crosslinked PLEY with PLK as expected. 1M EDC was possibly more than sufficient for crosslinking the concentrations used in present experiments so that increased concentrations did not affect crosslinking significantly. The higher values of shear modulus at \( \mu = 139 \) kPa for sample 410385-1 seems to be an outlier as it doesn’t seem to follow a pattern of decreasing
μ relative to conditions at 30% and 50% PLEY. Samples 410385-2 & 3, seem to adhere to the pattern, however. It might be possible that measurement system set-up was somehow slightly different for samples 410385-2 and 410385-3 than that of 410385-1.

Comparing results of |μ| from global fits of the neo-Hookean model to |μ2| from global fits of the Mooney-Rivlin model we see a similar trend. Conditions at 75% RH decrease for increasing PLEY concentration, with |μ2|=260 kPa for 30% PLEY and |μ2|=60 kPa at 50%. Again, this was not expected. The trend for values at 85% RH seem less obvious as the condition at 410385 seems to run counter to the trend at 75%. This needs to be investigated further. Regardless, stiffness at 50% RH is lower than stiffness values of either 30% or 40%.

Samples at high RH exhibited non-linear elastic deformation, whereas those at low RH showed non-linear viscoelastic deformation, as will be discussed below.

2.3.3. Nonlinear Viscoelasticity Analysis

Viscoelastic materials play an important role in applications requiring energy absorption. Examples include earthquake dampers and cushioning in seats and shoes. Such materials could benefit from engineering viscoelastic properties. Viscoelasticity is an important feature of biological materials. The time dependence of stress-strain relationships could potentially be engineered to meet the requirements of medical or non-biological applications of polypeptide materials. The present materials and others based on designed polypeptides have potential applications in cartilage replacement. We therefore determined certain viscoelastic properties of the present polypeptide materials.

Samples at 33% RH showed a viscoelastic response under compression. At each increment of displacement from equilibrium (strain), time was required for the stress measurement to come to equilibrium, the amount depending on the sample. In general, samples at 75% RH and 85% RH did not display such behavior, the sole exception being Sample 515285-2, which was compressed to a relatively large deformation. Due to the observed viscoelastic response,
relaxation times for samples at RH33% were therefore obtained.

There were three conditions for material at 33% RH of three samples each for a total of nine samples. Due to material brittleness some of the samples that survived processing did not survive F-D measurements. However, samples that survived processing and F-D measurements were used to study relaxation characteristics. It was hypothesized that samples available for time relaxation studies would not behave much different, in general, than other samples within each respective condition. This hypothesis can be disproved in future studies. Measurements on available samples were, thus, obtained to study characteristic relaxation behavior under low, medium and high strains.

To obtain relaxation times, we applied a predetermined strain at a low value, where it was assumed that Hooke’s law applied, at a medium value, and a high value, well above the probable elastic region. In general, the first eight data points of samples at 33% RH extended to displacements of \( \delta = 0.2 \) mm, and stress showed a linear response to strain. For \( \delta > 0.2 \) mm, stress-strain relations became nonlinear up to the maximum displacements measured in this study, \( \delta \approx 0.7 \) mm. The high strain was obtained by displacing samples by 0.41 mm, a compressive deformation of c. 10%. This enabled sample analysis at a point close to the upper end of the non-linear region. Medium strain was at a displacement of 0.30 mm, a c. 8% deformation. Low strain was obtained by displacing the sample by 0.15 mm, a c. 4% deformation.

Figure 2.20a shows relaxation data for Samples 310233-1; 415433-2 and 520333-1 at high, medium and low strain. Red symbols are for 10% deformation, green for 8% deformation, and blue for 4% deformation. Figure 2.20b shows the fit \( \sigma(t) = \varepsilon_o \left( E_1 e^{-t/\tau_1} + E_2 e^{-t/\tau_2} \right) \) to experimental low-strain data. Table 2.4 presents relaxation times of selected samples as obtained by fitting \( \sigma(t) = \varepsilon_o \left( E_1 e^{-t/\tau_1} + E_2 e^{-t/\tau_2} \right) \) to experimental data sets. Shorter values of \( \tau_1 \) can be attributed to the fast relaxation due to the spring components of the Maxwell system, larger values of \( \tau_2 \) are attributable to damping of the dashpot, or viscous,
Table 2.4: Relaxation times for three representative samples at 33% RH and at high, medium or low strain.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample</th>
<th>High Strain (ε = 0.10)</th>
<th>Medium Strain (ε = 0.08)</th>
<th>Low Strain (ε = 0.04)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>τ₁ (s)</td>
<td>τ₂ (s)</td>
<td>τ₁ (s)</td>
</tr>
<tr>
<td>310233</td>
<td>310233-1</td>
<td>7.6</td>
<td>470</td>
<td>8.9</td>
</tr>
<tr>
<td>415433</td>
<td>415433-2</td>
<td>13</td>
<td>770</td>
<td>6.9</td>
</tr>
<tr>
<td>520333</td>
<td>520333-1</td>
<td>43</td>
<td>1100</td>
<td>2.4</td>
</tr>
</tbody>
</table>

components. For example, Figure 2.20b shows a steep decrease in stress within the first 13 s of strain at ε = 0.04. This fast relaxation could be attributable to the effect of short-order polymer network re-alignment in response to the step increase in stress, typically modeled as a Heaviside step function. Further network re-alignment is slowed down by the viscous component of surrounding medium, which results in longer-term stress relaxation. Figure 2.20b shows such gradual decrease in stress for times greater than 13 s.

Figure 2.21 presents isochronous stress-strain plot [60]. A nonlinear isochronous stress-strain plot signifies nonlinear viscoelasticity. The samples were at 33% RH. The strain values were as in Table 2.4, and data were compared at three different time points: 10 s, 100 s and 150 s. Relaxation plots similar to Figure 2.20b were obtained for each value of the strain. For example, Figure 2.20b shows relaxation data for sample S8 at low strain, and at 10 s, σ = 18 kPa, at 100 s, σ = 11 kPa, and at 150 s, σ = 10 kPa. These data are plotted for ε = 0.04 in Figure 2.21b. The same process gave points for ε = 0.08 and ε = 0.10. The upper curve in each panel of Figure 2.21 is for 10 s, the middle curve, 100 s, and the lower curve, 150 s. It is clear that these 33% RH samples behaved as nonlinear viscoelastic materials. Materials at 33% RH showed obvious viscoelastic behavior throughout the range of applied strain.

Materials at 75% or 85% RH could potentially display viscoelasticity beyond a strain threshold. This possibility is supported by analysis of sample 515285-2. At 85% RH, the sample exhibited viscoelastic behavior at displacements beyond 0.86 mm, or 22% deformation.
on a sample height of 4 mm. At displacements above 0.86 mm viscoelastic behavior was found. Single-exponential relaxation times were found to be 1400 s and 650 s for high and low stress, respectively.

2.4. Conclusion

Post-processing of data revealed significant variability among replicate samples within each condition. Variability among samples and conditions stemmed from processing and measurement methods. First, reactant mixtures were made to nominal concentrations, each with approximately 2% uncertainty due to weighing of lyophilized polymer and addition of solvent water with micro-pipettes. Transfer of reactants to test tube proved to be a difficult task at high PLEY concentrations due its high viscosity. Mixture of reactants resulted in approximately 5% uncertainty a previously stated. Transfer of reactant mixture at nominally 120 µL to the mold with a syringe resulted in additional losses of about five to six microliters, or 5% uncertainty. Relative humidity chambers are expected to be within 2% of nominal. However, typically four to five samples were placed in their respective relative humidity chambers as called for in Table C1. Not all samples in each RH chamber were measured at once due to a strict adherence to conduct measurements down the list shown in Table C1. Therefore, some samples experienced cycles of changing RH as chambers were opened and closed to measure samples that were chronologically “next in line”. Due to this changing environment, it is estimated that samples experienced relative humidities within a 4% uncertainty. The sum total of these uncertainties is 20%. Additionally, care had to be taken during measurement to ensure the fixed arm was making proper contact with the sample. Improper contact between fixed arm and sample created immediately noticeable errors in readings. It was therefore critical that proper contact be made at each measurement run. Resolution of scale was within 0.1 g and the displacement gage was within 0.025 mm. It is possible that the measurement apparatus as a whole provided approximately 10% uncertainty. Plots
with error bars clearly show that higher variability occurred at high strains. Most samples within each condition seem to follow similar patterns at low displacements (strains), with the exception of condition 410385. This seems to indicate that a majority of sample variability arose from sample fabrication. The sum total of fabrication and measurement is about 30% uncertainty.

Using the coefficient of variation, \( CV = (\text{Std Dev})/(\text{mean} \ |\mu|) \), to estimate data value dispersion we find uncertainty in shear modulus, obtained from the neo-Hookean model, to be the following for each condition: \( CV_{315375} = 30\% \); \( CV_{320485} = 56\% \); \( CV_{420275} = 28\% \); \( CV_{410385} = 71\% \); \( CV_{510475} = 45\% \); \( CV_{515785} = 33\% \). Other than the two conditions where \( CV > 50\% \) the rest seem to be close to expectation.

Of particular importance is the uncertainty with regards to relative humidity. Due to the 4% uncertainty in RH it is difficult to distinguish and compare stiffness properties between conditions at 75% and 85%. However, within the 75% RH condition a trend of decreasing stiffness with increasing PLEY concentration is readily apparent. This trend is not so obvious at 85% RH since condition 410385 does not follow the same pattern. Excluding the first sample as an outlier, shows that condition 410385 is similar in stiffness as 320485. This could be either due to errors in processing, measurement, difference in PLK concentration or a combination of all three. It could also be possible that measurements are correct and represent actual material behavior. This should be investigated further in future studies.

Due to sample variability in this study, values for stiffness here are to be understood as for comparative purposes only to ascertain trends as they relate to changes in conditions.

As far as trends are concerned, we conclude that the mechanical properties exhibited by cross-linked PLEY are typical of biomaterials. Loading/unloading curves revealed that the present polypeptide materials displayed nonlinear viscoelasticity at low relative humidity (33%). This was in contrast to the display of nonlinear elasticity at high relative humidity (75-85%). At low strains, the material shows low elasticity as polypeptide chains move
relative to each other. As strain increases beyond a certain point, the material shows sign of strain hardening as the secant modulus increases. The material seems to behave close to a neo-Hookean model from solid mechanics. It is presumed that water inclusion within polypeptide cross-linked structures gives rise to this behavior. However, more testing needs to be conducted.

Further, we conclude that out of the four factors studied here, the most significant with regards to material stiffness was relative humidity. Higher relative humidities result in lower stiffness. Lower relative humidities result in higher stiffness. This is ascribed to the higher load bearing capability of cross-linked polypeptides primarily comprised of covalent bonds that are comparatively stronger than the hydrogen or van der Waals bonds manifested by water molecules at higher relative humidities.

Lastly, it was shown that cross-linked polypeptide material at low humidities exhibit relaxation times on the order of minutes. For those studied in this paper, at high deformation viscosity-driven relaxation times averaged 13 min. At low deformation, viscosity-driven relaxation times averaged 6 min.

We hope this work opens new areas of research into the engineering of designed polypeptides to obtain specifically desired relaxation times for biomedical applications or applications where biodegradability is desired.

2.5. Supplementary Material.

Fitting a single exponential decay model to volumetric change data for a cylindrical sample that was 4 mm high and 4 mm in diameter yielded time constants of 22 h at RH85 and 14 h at RH33 and amplitudes of 63% at RH85 and 74% at RH33 (Figure 2.22). By 72 h, then, the decay will have been 96% complete for RH85 and over 99% complete for RH33.

The loading and unloading curve of the apparatus itself over the force range of 0.5-6 N was measured (Figure 2.23). The fixed arm was directly on the scale. The curves were
essentially linear and coincident in this range. The corresponding displacement range was \(0.05 \text{ mm} \leq \delta \leq 0.5 \text{ mm}\). All samples of the present study were compressed less than 2N. \(t\)-ratios were computed for the various sample conditions studied here (Figure 2.24). The values were obtained with JMP statistical software.
Figure 2.4: Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 40% w/v nominal PLEY concentration.
Figure 2.5: Force-displacement loading curves of conditions at a.) 75% and b.) 85% relative humidities at 50% w/v nominal PLEY concentration.
Figure 2.6: Force-displacement loading curves of samples at condition 310233 at 33% relative humidity with 30% nominal PLEY concentration.

Figure 2.7: Force-displacement loading curves of samples at condition 415433 at 33% relative humidity with 40% nominal PLEY concentration.
Figure 2.8: Force-displacement loading curves of samples at condition 520333 at 33% relative humidity with 50% nominal PLEY concentration.
Figure 2.9: Force-displacement plot with error bars for all conditions presented in Figures 2.3 - 2.8. Number after underscore represents relative humidity. For example, relative humidity for 3153_75 is 75% relative humidity. Error in force was calculated as $F = C(w \pm \Delta \varepsilon_w)$, where $w$ is weight and $\Delta \varepsilon_w$ is standard deviation in weight measurement among the replicated samples. $C$ is a constant to obtain force in Newtons.
Figure 2.10.: Loading/unloading curves were obtained for samples in conditions 420275 and 410385. Specific samples are listed in legend of plot. Circles represent loading; stars, unloading. Unloading data for other conditions could not obtained due material fracture or other factors which prohibited measurement. (a) Condition 420275 at 75% RH. All three samples show pattern of nonlinear elastic deformation. (b) Condition 410385 at 85% RH. Samples show pattern of nonlinear elastic deformation.
Figure 2.11: Decision path followed to characterize mechanical properties of crosslinked material in this study.
Figure 2.12: Plots of Stress-Strain and Secant Modulus-Strain curves. Strain-Strain curve exhibits signs of strain hardening at high strain. Secant modulus follows a typical pattern of non-linear elastic biological material. Initial decrease in secant modulus, then increased secant modulus with increased strain [8, 9, 10]. (a) Stress versus curve for condition 515285; (b) Secant moduli versus strain curve for condition 515285.
Figure 2.13: Stress versus strain plot with error bars for the six conditions at 75% and 85% RH. Stress was calculated as $\sigma = F/A$, where $F$ is the applied force and $A$ is the sample’s cross sectional area. Values of force were $F = x_F \pm \Delta \varepsilon_F$, where $\Delta \varepsilon_F$ is variance in force. Values of area were $A = x_A \pm \Delta \varepsilon_A$, where the error in area, $\Delta \varepsilon_A$, was calculated as $\Delta \varepsilon_A = A \sqrt{2(\Delta \varepsilon_r/r)^2}$. Average diameter of samples was as given in subsection 2.2.5: 3.5 ± 0.4 mm, so that $r = 3.5/2$ mm and $\Delta \varepsilon_r = 0.4$ mm. The stress with error was then calculated as $\sigma = F/A \pm \Delta \varepsilon_{\sigma}$, where $\Delta \varepsilon_{\sigma} = \sigma \sqrt{(\Delta \varepsilon_F/F)^2 + (\Delta \varepsilon_A/A)^2}$.
Figure 2.14: Secant modulus versus strain plot with error bars for the six conditions at 75% and 85% RH. Secant modulus was calculated as mentioned in the results and discussion section of Chapter 2. We have assumed that $\Delta \varepsilon_\alpha >> \Delta \varepsilon_\epsilon$. Therefore the secant modulus with error bars was calculated as $E_s = \frac{1}{\dot{\varepsilon}}(\sigma \pm \Delta \varepsilon_\sigma)$. 
Figure 2.15: Change in secant modulus. (a) Ratio of change in secant modulus at each strain to maximum change in secant modulus versus strain for (a) 75% RH (Samples: 315375-1; 420275-1; 510475-1) and (b) 85% RH (Samples: 320485-1; 410385-2; 515285-2).
Figure 2.16: Nonlinear elastic behavior within each of the three regions in stress-strain response of crosslinked synthetic polypeptide co-poly-(L-glutamic acid, L-tyrosine). The data are for condition 515285 using sample 515285-3 to show behavioral characteristics within each of the three regions.
Figure 2.17: Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 315375 (b) Stress-stretch curves for condition 320485.
Figure 2.18.: Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 420275. (b) Stress-stretch curves for condition 410385*. Note *: Figure 2.18(b) excludes sample 410385-1 as data set is an outlier as seen in Tables 2.2 and 2.3.
Figure 2.19: Determination of stiffness parameters with global fitting in Matlab®. Circles represent data values; Solid black line is a global fit with a neo-Hookean model. Blue dotted line is a global fit with a Mooney-Rivlin model. Global and individual values of $|\mu|$ are listed in Table 2.2 for the neo-Hookean model and Table 2.3 for the Mooney-Rivlin model. (a) Stress-stretch curve for condition 510475. (b) Stress-stretch curves for condition 515285.
Figure 2.20: Relaxation time data for samples 310233-1; 415433-2 and 520333-1 at 33% relative humidity and at high, medium and low strains. High strain corresponds to approximately 10% deformation, medium strain to 8% deformation and low strain to 4% deformation. Colored circles represent data; solid line represent the fit. Only relaxation times were important for this study. (a) Relaxation time data for sample 310233-1. (b) Relaxation time data for sample 415433-2. (c) Relaxation time data for sample 520333-1.
Figure 2.21.: Isochronous stress-strain plots of samples in three different factors at 33% RH. The three lines represent 10 s, 100 s and 150 s in each case. (a) Sample 310233-1, (b) Sample 415433-2, (c) Sample 520333-1. Nonlinearity provides evidence of nonlinear viscoelasticity.
Figure 2.22: Ratio of sample volume/original sample volume as a function of time for sample at RH33\% and RH85\%. The equilibration time was approximately 72 h. The final volume was greater at RH85\% than RH33\%.

Figure 2.23: Loading and unloading curves of apparatus with no sample. Circles represent loading; dashes represent unloading.
Figure 2.24: Plot of t-ratios for all four conditions studied.
CHAPTER THREE :

DIELECTRIC RELAXATION OF POLYELECTROLYTE POLYPEPTIDE MELTS

3.1. Introduction

Dielectric relaxation has been used extensively to probe the molecular structure of biological liquids and solids through several methods which include open-ended coaxial lines, parallel plate capacitors and wave guides [61, 62, 63]. A popular tool employed to study dielectric properties is the open-ended coaxial line probe that can be used in field testing for a variety of materials including agricultural products [64]. For the most part, however, milliliter-size sample volumes are required for conducting typical measurements. That presents a problem when studying polypeptides that are either high-cost or can be synthesized only in limited quantities. Hagl et.al [65] studied the minimum volume required to yield accurate dielectric measurements of breast tissues through comparison of two open-ended coaxial probes. The 3.58 mm diameter probe required a minimum of 3.0mm thickness. The 2.2 mm probe required at least 1.5 mm of tissue thickness. Assuming a cylindrical sensing volume, the 3.58 mm probe requires 30 µL of tissue. The 2.2 mm probe needs 6 µL. Here we present a parallel-plate capacitor fixture that can measure dielectric loss of polyelectrolyte solutions for volumes in the 13-20 µL range. The ease with which the fixture can be used to obtain preliminary data yields savings in time and cost.

Poly(L-glutamic acid)|PLE] has been one of the most studied amino acids as it presents an analogue to the investigation of proteins. Of particular interest has been the investigation of
its conformational dynamics as a function of pH which tend from helix at low pH to random coil at pH greater than 6-7 [66]. In contrast, Poly-L-(glutamic acid$_4$, Tyrosine$_1$) [PLEY (4:1)] has not been as extensively studied. Both polypeptides are weak polyacids. Dielectric relaxation is a consequence of molecular polarizability. PLE contains a dipole moment parallel to the chain contour which is responsible for polymer displacement as well as a perpendicular component responsible for micro-Brownian side-chain dynamics [67]. PLEY has similar dipole moments as PLE with the addition that tyrosine gives it an additional polar molecule perpendicular to the backbone. Using time domain reflectometry with a coaxial line method of measurement, Mashimo et al observed dielectric relaxation in the frequency region between 10 - 500 MHz. Authors attributed this relaxation to electric dipole fluctuations due to micro-Brownian motion of polymer side chains [66, 67]. Relaxation time due to the parallel component reveals correlation time for reorientation of the end-to-end distance vector of the longest-lived relaxation mode and strength of dielectric relaxation directly reveals a polymer chain’s mean square end-to-end distance [68]. Dielectric studies are important because they relate molecular relaxation mechanism at the microscopic level to material properties at the macroscopic level. Here we present a quick and cost effective measurement mechanism that provides information of a polyelectrolyte’s dielectric loss using µL-size droplets of solution. Such a measurement device has not been characterized previously to the authors’ knowledge. Additionally, we conduct dielectric loss measurements on PLEY (4:1) which has not been characterized in the field previously.

3.2. Material and Methods

We used an Agilent 4191A RF Impedance Analyzer set up to obtain Resistance (R) and Reactance (X) measurements in series equivalent mode. Measurements were recorded in two separate parts: $1\text{MHz} \leq \nu \leq 51\text{MHz}$ and $50\text{MHz} \leq \nu \leq 800\text{MHz}$. The impedance analyzer samples 50 frequency points. Therefore, low frequency measurements gather data at a step
frequency of 1 MHz. High frequency data was recorded at 20 MHz steps. Each frequency sweep was run five times and results at each frequency averaged.

3.2.1. Polypeptides

Poly(L-glutamic acid) [PLE] of molecular weight 64 kDa, Poly-L-(glutamic acid$_4$, Tyrosine$_1$) [PLEY (4:1)] of molecular weight 20-50 kDa were used as obtained from Sigma-Aldrich (USA). Aqueous solutions were prepared with deionized water of about 1 $\mu$S/cm. Nominal pH for PLE aqueous solutions were in the range of 7.5-7.6 placing it in the random coil regime. PLEY had a pH of $\approx$ 6.2. The solubility limit for PLE is about 20% w/v. PLEY is soluble up to about 50% w/v. We studied highly concentrated samples of each polypeptide.

3.2.2. Capacitance Fixture

Figure 3.1.: a.) Picture of test fixture as viewed from the top. Lexan board is transparent. Consequently, the copper ground strip on the backside of the fixture can be seen. b.) Schematic of equivalent circuit as measured by the impedance analyzer. Inductive ($X_L$) and capacitive ($X_C$) reactances are not separated by the analyzer. Measured reactance, $X$, is a combination of both so that $X = X_L + X_C$.

Overall length of our fixture was 7.7 cm and similar in construction as [69], with the exception of the transmission lines (see Fig. 3.1). We utilized two lengths of 30 gauge
uninsulated copper wire soldered to SMA connectors. The ends of the wires not on SMA connectors were soldered to a circular copper plate: D=3.18 mm, thickness=0.75 mm. SMA connectors with copper plates were fixed to opposite sides of a Lexan board with a groove in the middle to facilitate placement of copper plate. A channel along the middle of the Lexan board allowed the wires to be placed flat against the board to minimize any kinks that might form. The SMA connector on the other end of the test port is fitted with a 50 Ω termination end-cap. The gap between the round parallel plates was ℓ=1.64 mm. With these dimensions one could easily calculate the theoretical open-air capacitance, \( C_o \). Assuming \( \varepsilon_o=8.8542 \times 10^{-12} \) F/m and using \( C_o = \varepsilon_o A/\ell \) we obtained a \( C_o=0.04 \) pF. This value was significantly lower than the average \( C_{oc}=7 \) pF obtained from the impedance analyzer over the spanned frequency range indicating severe electrode fringe effects. We used \( C_{oc} \) values as obtained from the impedance analyzer as a sort of “cell constant” [18] to better represent sample conditions. As will be shown later, our test set up produced a resonance peak at around 500 MHz.

It must be noted that the fixture only required a maximum of approximately 26 µL of sample to obtain measurements. Liquid surface tension maintained appropriate electrode coverage and additionally formed a liquid cylinder between the electrodes. Due to the relative speed of measurements evaporation was negligible. All data was taken at a constant ambient temperature of 22 °C.

3.3. Dielectric Parameters and Capacitance Equations

3.3.1. Dielectric Parameters

Complex permittivity of a lossy medium is well-known. As a function of angular frequency it is \( \varepsilon^* = \varepsilon' + i\omega\varepsilon'' \) [70, 4, 71, 62], where \( \varepsilon' \) is the real part of the permittivity that describes a material’s capability of storing energy, \( i = \sqrt{-1}, \omega = 2\pi\nu \), where \( \nu \) is the measured
frequency and $\varepsilon''$ is the dielectric loss that quantifies energy dissipation within the medium. Further, the dielectric loss can have dipolar and ionic components [71]. It can be written as $\varepsilon''_{dipolar} = \varepsilon'' - \sigma \omega \varepsilon_0$, where $\sigma$ is the ionic mobility sometimes termed the dc conductivity, $\sigma_{dc}$. At low frequencies ionic conductivity can be experimentally determined through a plot of $\sigma_{dc} = \varepsilon_0 \omega \varepsilon''$, in units of $\Omega^{-1} m^{-1}$, versus $\omega$ using a simple extrapolation. However, the samples in this study are highly concentrated polyelectrolytes that will tend to have an increased screening effect at higher concentrations [4]. Therefore, in this study ionic mobility was neglected and only the dipolar component analyzed.

It is clear to see in our fixture (Fig. 3.1a), that there is a combination of inductance and capacitance in the shorted circuit when there is either a shorting conductor or a sample between the capacitor plates. That combination of inductance and capacitance gives rise to reactance measurements captured by the analyzer equipment (Fig. 3.1b). Inductance arising from an open circuit in our test fixture is negligible. As will be seen, fringing effects at the sample-capacitor plate junctions increase with increasing frequencies. We take the simple approach of accounting for these through the use of the open circuit capacitance, $C_{oc}$, as measured with the impedance analyzer as well as with dissipation factors.

It is possible to convert impedance analyzer measurements from a series to a parallel equivalent circuit [72, 73, 70] through the use of the tangent loss, $t_\ell = \tan \delta$. It must be noted that the analyzer used in this study was hardwired to obtain measurements as a series equivalent circuit when measuring $R$ and $X$.

The relation for permittivity used in this study is given below.

$$\varepsilon' = \xi \left( \frac{C_{ms}}{C_{oc}} \right) \left( \frac{1}{1 + t_\ell^2} \right) - C_f,$$

(3.1)

where $\xi$ is a calibration constant found by measuring deionized water with the fixture. Here, $\xi=4$. $C_{ms}$ is the measured sample capacitance and $C_{oc}$ is the measured open circuit capacitance. The term after the capacitance ratio is a dissipation factor for obtaining $\varepsilon'$.
when capacitance is measured as a series equivalent circuit given by [70]. The second term is purely a fringe effect term given by

\[
C_f = \left( \frac{C_{sc}}{C_o} \right) \left( \frac{C_{oc}}{C_{os}} \right).
\]  

(3.2)

The equation above contains the ratio of short circuit capacitance, \( C_{sc} \), caused by fringe effects, to theoretical open air capacitance, \( C_o \). The ratio is multiplied by the average value of \( C_{oc}/C_{os} \), where \( C_{os} \) is calculated by the difference in susceptance between an open and short circuit. \( C_{oc} \) was previously defined.

The relation for dielectric loss used here is

\[
\varepsilon'' = \zeta \left( \frac{C_{ms}}{C_{oc}} \right) \left( \frac{t_t}{1 + i t_t^2} \right).
\]

(3.3)

Similar to permittivity, \( \zeta \) is a calibration constant found by measuring deionized water with the fixture and is here \( \zeta = 1/2 \). The term after the capacitance ratio is the dissipation factor for obtaining \( \varepsilon'' \) when capacitance is measured as a series equivalent circuit given by [70].

The first terms in both equations are the well-known ratios that give permittivity and dielectric loss[70, 4, 71], respectively.

3.3.2. Capacitance Equations

The impedance analyzer was set to only measure resistance (R) and reactance (X) components of impedance. Since the 4191 Agilent impedance analyzer is hardwired to give R and X measurements as series equivalent circuits [72, 74], we converted those readings to series
equivalent conductance, susceptance and capacitance as follows:

\[ G = \frac{R}{R^2 + X^2} \]  
\[ B = \frac{-X}{R^2 + X^2} \]  
\[ C = \frac{B}{\omega} \]

Tangent loss was calculated according to

\[ t_\ell = \tan \delta = \frac{G}{B}. \]

After calibration at the measurement port with a zero ohm cap, a zero conductance cap and a 50 Ω cap, we characterized the fixture. We first took R and X measurements with an open circuit, where there was no sample between the capacitor plates, and thus obtained \( Z_{oc} \). The circuit was then shorted by placing a thick piece of copper between the parallel plates, thus, measuring \( Z_{sc} \). Utilizing both measures we arrived at the test fixture characteristic impedance, \( Z_o \), according to \( Z_o = \sqrt{Z_{oc}Z_{sc}} \). A 50 Ω end cap was used during characterization and sample measurements.

With this method, and using (3.4)-(3.6), we calculated \( B_{xx}, G_{xx}, \) and \( C_{xx} \), where \( xx= \) short circuit (sc), open circuit (oc) and measured sample (ms). Tangent loss was calculated by \( t_\ell = G_{ms}/B_{ms}. \) Since \( X_{oc} \gg R_{oc} \) for an open circuit, we found \( C_{oc} \) was the same calculated as either a series or parallel equivalent circuit. The \( C_{os} \) denominator appearing in (3.2) was calculated as \( C_{os} = (B_{oc} - B_{sc})/\omega. \)

Figures 3.2a,b present data for three concentrations of PLE: 10, 15 and 20% w/v. Figure 3.2a presents sample data as obtained from the impedance analyzer: R (top of graph) and X (bottom of graph). It also shows R and X data for a shorted circuit as a reference. Figure 3.2b shows calculated conductance (top) and susceptance (bottom) for those same PLE concentrations. Conductance decreases at higher frequencies. Susceptance initially decreases
at lower frequencies but sharply increases at higher frequencies. The fixture resonance peak at around 500 MHz can easily be seen in these two plots.

Figure 3.2c shows data characteristic to the fixture. Ratio of $-C_{sc}/C_o$, on left axis, decreases from above 300 at low frequencies to about 1 at high frequencies due to electrode polarization at low frequencies. $C_{oc}/C_o$, on right axis, remains constant at about 17 then steadily increases to 22 at high frequencies due to capacitor fringe effects. These effects are taken into account in the permittivity calculation.

3.4. Results

R and X data obtained from the impedance analyzer was processed with a Matlab© program to obtain plots of permittivity and dielectric loss using equations (3.1) and (3.3), respectively. Figure 3.3a shows the relationship between permittivity (open circles, left axis) and dielectric loss (stars, right axis) for deionized water. DI water was used to calibrate fixture and obtain values for $\xi$ and $\zeta$. Electrode polarization is evidenced at lower frequencies. Permittivities greater than 82 occur at frequencies below 55 MHz. As evidenced by the dielectric loss two peaks are present: a larger one at 235 MHz with $\varepsilon'' = 2.6$ and a smaller one at 460 MHz with $\varepsilon'' = 0.5$. Dielectric loss peaks coincide with permittivity decreases. It is known that free pure water has a maximum dielectric loss at 2.45 GHz. However, the polar nature of water molecules permits rotational motion within microwave frequencies. Indeed the dielectric loss peaks appear within the range of studies by Komarov et. al. [75] where it was found that DI water had $\varepsilon'' = 0.03$ at 27 MHz and $\varepsilon'' = 3.6$ at 915 MHz.

Values for $\xi$ and $\zeta$ were applied to equations (3.1) and (3.3) to generate subsequent plots. Figure 3.3b shows permittivity and dielectric loss for a representative sample of 15% w/v PLE. Points at resonance were omitted for ease of visibility. Dielectric loss increases as permittivity decreases then shows a peak at the relaxation frequency, thereafter decreasing towards a plateau along with permittivity. This is the typical relationship between permit-
tivity and dielectric loss.

Figure 3.4 (a)-(c) shows plots of permittivity and dielectric loss versus frequency in the range of 50MHz ≤ ν ≤ 800MHz and Cole-Cole plots.

As a double check, we measured conductivity of 1M NaCl with our fixture and compared it against the theoretical value given by Stogryn’s equation [1] at 22 °C of σ = 8.075 Ω⁻¹m⁻¹. We calculated conductivity as

\[
σ = 3 \left( \frac{ɛ_0 G_{ms}}{C_0} \right),
\]

(3.8)

Figure 3.4d presents conductivity as a function of frequency calculated with (3.8) for 1M NaCl, three concentrations of PLE and three concentrations of PLEY. The value for conductivity of each material was obtained through a linear extrapolation of the plot of σ versus ω at frequencies in the range of 1MHz ≤ ν ≤ 51MHz. Conductivity of 1M NaCl as obtained with (3.8) was σ=8.1. This is in 0.3% error with Stogryn’s equation at 22 °C. Table 3.1 presents conductivities as obtained through this procedure. The high conductivities of PLE and PLEY are not surprising given the high polyelectrolyte concentrations.

Table 3.1.: Conductivity of aqueous solutions. Conductivity of 1M NaCl at 22 °C was at 0.3% error compared with Stogryn [1].

<table>
<thead>
<tr>
<th>Solution</th>
<th>σ(Ω⁻¹m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M NaCl</td>
<td>8.1</td>
</tr>
<tr>
<td>10 % w/v PLE</td>
<td>3.6</td>
</tr>
<tr>
<td>15 % w/v PLE</td>
<td>3.8</td>
</tr>
<tr>
<td>20 % w/v PLE</td>
<td>3.8</td>
</tr>
<tr>
<td>30 % w/v PLEY</td>
<td>4.3</td>
</tr>
<tr>
<td>40 % w/v PLEY</td>
<td>4.2</td>
</tr>
<tr>
<td>50 % w/v PLEY</td>
<td>5.7</td>
</tr>
</tbody>
</table>
3.5. Discussion

We briefly discuss here some information gleaned from measurements of PLE and PLEY. Peaks in the dielectric loss curves are related to relaxation time, which is a molecular probe of dielectric material. Relaxation time is inverse of relaxation frequency according to $\tau_c = 1/\omega_c = 1/2\pi \nu_c$. Therefore, lower relaxation times occur at higher relaxation frequencies. Conversely, higher relaxation times occur at lower frequencies.

There are three prevailing mechanisms that affect relaxation time of a polymer: 1.) For micro-Brownian motion of the polymer side chain, it increases with increasing concentration\cite{76, 68}; 2) In general, it increases with increasing molecular weight of polymer\cite{77}; and 3.) It increases for increasingly prevailing rigid polymer chains\cite{76, 16, 67, 66}. Figure 5b shows the first trend where relaxation frequency for both PLE and PLEY decreases with increasing concentration, such that relaxation time increases as concentration is increased. Molecular weight of PLE was 64 kDa as measured by viscometry. It was 20-50 kDa for PLEY. Therefore, one would expect relaxation times for PLE to be larger, on average, than PLEY. However, Figure 5b shows that, in general, PLEY peaks of the dielectric loss are red-shifted relative to peaks of PLE. The structural difference between PLE and PLEY is that PLEY contains a tyrosine side chain, which would convey slightly more rigidity to the PLEY polypeptide chain relative to PLE, due to its imposition of limits in conformational states. Evidently, PLEY molecular chain rigidity effects prevail over molecular weight effects so that despite PLEY being of lower molecular weight than PLE, it shows higher relaxation times due to higher chain rigidity stemming from the tyrosine side chain. Figure 3.5 plots relaxation time as a function of concentration for two separate measurements each of PLE and PLEY. There was a lapse of one week between measurements. The general trend in both cases, is that relaxation time increases for increasing concentrations.
3.6. Conclusion

We have shown that it is possible to measure permittivity and dielectric loss of concentrated polyionic peptides in aqueous solutions at high frequencies utilizing a parallel-plate fixture with resistance and reactance measurements. It serves as a tool that can obtain preliminary data quickly and cost-effectively. The fixture also makes it possible to conduct various types of additional dielectric studies such as laser induced relaxation as well as a study of magnetic effects on relaxation.
Figure 3.2.: Sample plots of 10, 15, 20% w/v PLE: a.) Plots of $X_{\text{meas}}$ bottom and $R_{\text{meas}}$ top for three PLE concentrations. Short circuit $R$ and $X$ are shown for reference; b.) Plots of conductance (top) and susceptance (bottom); c.) Ratio of $-C_{sc}/C_o$, on left axis, decreases from above 300 at low frequencies to about 1 at high frequencies due to electrode polarization at low frequencies. $C_{sc}/C_o$, on right axis, remains constant at about 17 then steadily increases to 22 at high frequencies due to capacitor fringe effects. Resonance peak at around 500 MHz can easily be seen.
Figure 3.3: Plot of permittivity (open circles, left axis) and dielectric loss (stars, right axis) as functions of frequency for a.) Deionized Water and b.) 15% w/v PLE. Points at resonance were omitted for ease of visibility.
Figure 3.4: Plots of a.) permittivity versus frequency in the range of $50\text{MHz} \leq \nu \leq 800\text{MHz}$; b.) dielectric loss versus frequency in the same range as permittivity; c.) Cole-Cole and d.) conductivity versus frequency in the range of $1\text{MHz} \leq \nu \leq 51\text{MHz}$. Conductivity (d) is calculated with (3.8) for $1\text{M NaCl}; 10, 15$ and $20\% \text{ w/v PLE}$; $30, 40$ and $50\% \text{ w/v PLEY}$. 
Figure 3.5.: Plots of relaxation time, $\tau$, versus peptide concentration in % (w/v) for two separate measurements each of PLE and PLEY (shown as open and closed circles; and open and closed squares, respectively). Relaxation times were obtained from peaks of dielectric loss in Figure 3.4b. Circles represent PLE. Squares show PLEY.
CHAPTER FOUR :

CONCLUSIONS FROM ELASTICITY AND RELAXATION STUDIES

4.1. Elasticity Measurements

From data obtained in elasticity measurements, it was immediately clear that hydration contributes significantly to non-linear elasticity. One additional experiment was conducted to see if this was indeed the case. Figure 4.1 shows reactants fabricated according to table 4.1. PLE (closed stars) shows clear signs of non-linearity previously explained. In general, elasticity was lower than PLEY(4:1) (in open diamonds). Data in open stars shows force versus displacement for dehydrated PLE. Obviously, dehydrated PLE has both a higher stiffness and lack of non-linearity. It is presumed that in the hydrated crosslinked network, hydrogen bonds among the chain network are primary agents for bearing the force load. Hydrogen bonds are not as stiff as covalent bonds present in the polymer network. Therefore, non-linearity is evidenced. On the other hand, less hydration results in loading forces being primarily supported by the crosslinked network. Load on the crosslinked network leads to two viscoelastic relaxation mechanisms. There is a short time relaxation attributed to network realignment in response to the step increase in stress. Additionally, there is a longer

<table>
<thead>
<tr>
<th>Polypeptide Melt</th>
<th>EDC (40 %w/v)</th>
<th>PLK (20 %w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 (\mu\text{L}) of 50% (w/v) PLEY(4:1)</td>
<td>40 (\mu\text{L})</td>
<td>40 (\mu\text{L})</td>
</tr>
<tr>
<td>100 (\mu\text{L}) of 20% (w/v) PLE</td>
<td>40 (\mu\text{L})</td>
<td>40 (\mu\text{L})</td>
</tr>
</tbody>
</table>
relaxation time. Here polymer motion is slowed by viscous components of the surrounding medium as the polymer network moves through a reptation mechanism. A second note of explanation is owed on the reason that stiffness at a concentration of 30% PLEY(4:1) is higher then at 50% as seen in table 2.2 and clearly shown in Figure 4.2a for material at 75% RH. While it is difficult to distinguish stiffness at 30% from 40% PLEY and 40% from 50% PLEY, stiffness at 30% PLEY is different from 50% PLEY at strains > 0.15. Less obvious is the difference in stiffness from plots in Figure 4.2b. With the outlier data set from sample 410385-1 it appears condition at 40% PLEY is stiffer than both 30% and 50% PLEY. However, after extracting that data set and re-plotting the condition-represented by black
stars- 30% and 40% appear indistinguishable. As mentioned previously, one possible reason for this could have been the uncertainty in relative humidity conditions plus measurement uncertainty. Regardless, both 30% and 40% PLEY are higher in stiffness than 50% PLEY, particularly 30% is stiffer than 50% at strains > 0.15. This characteristic behavior was not originally expected.

One reason is that $E_{50\%}$ has a higher degree of chain overlap than $E_{30\%}$. As a result, both EDC and PLK reactants have a tougher time diffusing into the entangled network to effect crosslinking effectively. This seems a likely explanation given that the mesh size of a temporary polymer network decreases with increasing concentration as $\xi = a\Phi^{-3/4}$ shown in Figure 4.3. Here, $\xi$ is the mesh size, $a$ is the persistence length, and $\Phi$ is the monomer volume fraction. Now, if we approximate the monomer volume fraction with the polymer volume fraction, we have that

$$\phi^* \sim N^{-4/5},$$

(4.1)

where equation (4.1) is scaling for the critical polymer overlap volume fraction in an ideal solvent. In the cases of PLEY(4:1) and PLE, calculations show that $N \approx 200$ for either of the polymers used in the experiments conducted here. Therefore, the critical polymer fraction for both PLEY(4:1) and PLE is $\phi^* \sim (200)^{-4/5} = 0.014$. As stated previously, average molecular weights for PLEY(4:1) and PLE are 35 kDa and 30 kDa, respectively. Estimates for polymer density are PLEY=1.54 g/cm$^3$ and PLE=1.52 g/cm$^3$. Additionally, PLEY(4:1) has an approximate persistence length of 2.0 nm. Using this information the actual polymer volume fraction for both PLEY and PLE can be calculated with the use of

$$\phi = \frac{c}{\rho},$$

(4.2)

where $c$ is the polymer concentration and $\rho$ is its density. Table 4.2 shows estimated polymer
volume fractions for both PLEY and PLE. It also shows the mesh size for PLEY. One result

Table 4.2: Polymer volume fraction and mesh size for PLEY(4:1). Polymer volume fraction for PLE.

<table>
<thead>
<tr>
<th>PLEY(4:1): $\phi$</th>
<th>PLEY(4:1): $\xi$ nm</th>
<th>PLE: $\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_{30%} = 0.20$</td>
<td>$\xi_{30%} = 6.8$</td>
<td>$\phi_{10%} = 0.07$</td>
</tr>
<tr>
<td>$\phi_{40%} = 0.26$</td>
<td>$\xi_{40%} = 5.5$</td>
<td>$\phi_{15%} = 0.10$</td>
</tr>
<tr>
<td>$\phi_{50%} = 0.33$</td>
<td>$\xi_{50%} = 4.6$</td>
<td>$\phi_{20%} = 0.13$</td>
</tr>
</tbody>
</table>

that is immediately apparent is the comparative size of the overlap volume fraction for both PLEY and PLE relative to the critical overlap volume fraction. Since the dilute regime falls in the range of $\phi < \phi^*$, neither of the polypeptide solutions are dilute. However, they both are in the semidilute range, $\phi > \phi^*$. In fact, $\phi_{PLEY(4:1)}$ is 14 to 23 times larger than $\phi^*$. However, $\phi_{PLE}$ is only 5 to 9 times larger than $\phi^*$. While both neutral in aqueous solutions, as will be seen in the next sections, their concentrations relative to the critical concentration differentiate their state with regards to chain behavior in either a $\Theta$ or good solvent. PLEY(4:1) seems to form ideal chains at the chosen concentrations and PLE forms extended chains analogous to chains in good solvent. One additional bit of information is that, as expected, mesh size for PLEY(4:1) decreases from 6.8 nm to 4.6 nm giving credence to the notion that EDC and PLK have to diffuse through decreasing mesh sizes.

A second reason the Young’s modulus, $E_{30\%} > E_{50\%}$, has to do with entropy. As was mentioned in the introductory chapter, a higher entropy results in a softer polymer with a lower Young’s modulus, $E \propto \frac{1}{S}$. In the case of the crosslinked network, on a per molecule basis $S_{50\%} < S_{30\%}$ since topological constraints limit motion of the primitive chain. However, on a total volume basis, $S_{50\%} > S_{30\%}$ particularly as seen through the entropy of mixing

$$\Delta S_{mix} = -k_b N \left[ \frac{\phi_p}{DP} \ln \phi_p + \phi_s \ln \phi_s \right]. \quad (4.3)$$

For the polymer, the degree of polymerization greatly reduces the volume fraction contribution relative to the solvent volume fraction. As polymer concentration increases, the polymer
volume fraction increases, but the solvent volume fraction decreases by almost \((DP)\phi_p\) the amount. That change increases \(\Delta S_{\text{mix}}\) so that overall entropy increases. Thus, the entropy of a higher concentration is greater than the entropy at a lower concentration, \(S_{50\%} > S_{30\%}\). As such, \(E_{30\%} > E_{50\%}\). Therefore, there are two possible explanations for this finding.

4.1.1. Future Work

Due to significant sample variability, it is recommended that for each sample two to three repeated measurements are made in immediate succession to more accurately assess measurement error. Additionally, the large variations indicate unexplained issues in either the sample preparation stage or the elasticity measurement stage. Such large fluctuations also indicate that a larger set of samples are needed to make the experimental studies statistically significant. Different sample geometries, such as cross-section area and length, should also be used to avoid possible systematic experimental error.

4.2. Relaxation Studies

Step strain-induced relaxation studies of PLEY(4:1) viscoelastic material showed that relaxation at high strain scales as an entangled neutral polymer in good solvent according to the reptation model in equation 1.19. Figure 4.4 shows that the high strain curve (in orange filled circles) scales close to \(\tau \sim c^{1.6}\). At high strain is where one would expect that the crosslinked polymer network “feels” the full load of the force which causes reptation motion of the network.

Studies from dielectric relaxation yielded similar results regarding PLEY(4:1). Figure 4.5 shows that PLEY(4:1) scale as \(\tau \sim c^{0.9-1.1}\), which is close to the scaling law predicted for unentangled neutral polymers in a \(\Theta\)-solvent. Deviations appear due to experimental variations. Nonetheless, PLEY(4:1) evidently behaves as an ideal chain with \(\nu = 1/2\). One
would have expected a high degree of entanglement closer to that described by the reptation model due to the high concentration. Surprisingly, that was not the case. Yet, it is known that a highly concentrated neutral polymer melt does behave as an ideal chain polymer. So in this sense, the scaling behavior seems to be explained. A surprising find was the scaling trend for PLE. As shown in figure 4.5, PLE scaled as $\tau \sim c^{0.3}$. This scaling changes with concentration analogously to the unentangled neutral polymer in good solvent of equation 1.17! The possibility that PLE solutions could have been in the dilute regime was discounted by the rough estimates calculated in table 4.2. In good solution a polymer exhibits chain swelling due to excluded volume effects. Given that PLE is primarily a polyanion, there would be charge repulsion if chains intermingle too heavily. It is likely that a combination of excluded volume effects in combination with charge repulsion leads to the unentangled nature of PLE. If it is the case that PLEY is not entangled, the mechanics behind such inability to entangle in aqueous solutions are a bit more difficult to explain. The presence of tyrosine, which is a polar amino acid, in the polymer chain could be a source of maintaining a balance of forces between monomer-monomer and monomer-solvent interactions so that PLEY(4:1) scales as an unentangled ideal chain. In a separate experiment, an attempt was made to electrospin a blend of PLEY(4:1) with RADA16. Electrospun fiber fabrication proved to be a very difficult task with PLEY(4:1). For electrospinning to work efficiently, polymers must be already entangled in solution. Evidently one reason PLEY(4:1) could not spin might have been due to a lack of chain entanglement. However, it seems more likely that the polypeptide chains are so densely packed that the polymer behaves as ideal. The most interesting result is that neither PLEY nor PLE scaled as polyelectrolytes, where unentangled polyelectrolytes would scale in relaxation time as $\tau \sim c^{-0.5}$ and entangled polypeptide solutions would scale as $\tau \sim c^0$. Rather, both scaled as neutral polymers.
4.2.1. Future Work

In the dielectric studies here, only one fixture of fixed geometry is used for measurements. Although PLEY concentrations were measured twice with a span of one week between measurements, PLE concentrations should have additional measurements. Additionally, experimental data from the use of different fixtures, such as of different distance between the two electrodes, would also be beneficial. This would exclude possible experimental errors and draw more definitive experimental conclusions on the dielectric properties of samples. With regards to viscoelastic relaxation, it would also be useful to conduct relaxation studies at different concentrations multiple time to be able to understand the underlying statistical nature of the material.
Figure 4.2. Stress-Strain plots for samples containing 30% and 50% PLEY (4:1). At both 75% and 85% relative humidities samples with 30% PLEY (4:1) are stiffer than samples with 50% PLEY (4:1). (a) Stress-strain curves for conditions at 75% relative humidity. (b) Stress-strain curves for conditions at 85% relative humidity. Note: Data represented by black star is condition 410385 without the outlier data set from sample 410385-1 as seen in Table 2.2.
Figure 4.3.: Plot of average mesh size $\xi$ as $\xi/a$ plotted versus monomer volume fraction, $\Phi$.

Figure 4.4.: Relaxation time of PLEY(4:1) viscoelastic material versus concentration taken from table 2.4 at three different strains.
Figure 4.5: Figure 3.5 modified to show power law scaling of PLEY(4:1) and PLE.
5.1. Introduction

The ability of RADA16 to self-assemble into nanofibers has been studied extensively for use as cell culture scaffolding and drug delivery [78, 79, 80, 81, 82]. It is known that RADA16 conforms into $\beta$-sheets and self-assembles into nano-fibers with widths in the range of 3-10nm in diameter [83, 84, 85, 13] forming hydrogels when dissolved in water. Self-assembly produces two distinctive sides: one side hydrophobic due to alanine, the other hydrophilic due to arginine and aspartic acid [13]. At least one study has crosslinked a peptide made of a combination of RADA16-Bone morphogenic protein with poly(lactic-co-glycolic acid) via EDC for bone regeneration [86]. Here we study self-crosslinking of the RADA16 peptide via EDC, which could lead to an entirely new range of possible designed peptides with a myriad of functional characteristics.

Figure 5.1.: Acetylated RADA16 with N-terminus [11].

RADA16 studied here is acetylated with an amine N-terminus Ac-[RADA]$_4$-NH$_2$. The arginine (R) and aspartic acid (D) amino acid residues are positively and negatively charged,
respectively. There are a total of 17 peptide bonds: 15 between RADA amino groups; one at the acetyl end; one at the primary amine. It contains 17 C=O and 16 N-H bonds in the backbone. A total of nine sp\(^3\) hybridized C bonds stemming from the acetyl end and alanine (A) amino acid subgroups are also present in RADA16. Side chains in aspartic acid provide a total of four carboxyl groups on the hydrophilic side available for crosslinking by a carbodiimide reaction mechanism. The N-terminus primary amine is available for crosslinking. In addition, there are four amines in the arginine guanidinium group that could possibly take part of a crosslinking reaction. EDC is a zero-length crosslinker which reacts with carboxyl groups to form amine reactive intermediates. These react with amino groups to form peptide bonds. An N-substituted urea forms when the intermediate fails to react with the amine [87]. N-acylurea could also form as a side reaction during crosslinking. However, the reaction is limited to carboxyls in hydrophobic regions of a protein or polypeptide. Given that alanine, which forms the hydrophobic region of RADA16 and only contains -CH\(_3\), the side reaction was not expected to occur here.

5.2. Experimental

5.2.1. Materials

RADA16 was obtained from 3D Matrix as a lyophilized powder that was prepared by exchanging TFA for HCl [11] so that the arginine had a chlorine counterion and the aspartic acid was protonated. It was reconstituted in deionized water at a nominal 2.0% (w/v) to give a solution with pH \(\approx\) 2-3.

EDC was obtained from TCI America (USA) as a hydrochloride with a MW=191.70 g/mol and of 98.0% purity. It was dissolved in deionized water to obtain a nominal 20 % (w/v) solution with pH 7.68 as measured with a Sensorex polymer electrode.
5.2.2. Material Processing

Procedure for reacting RADA16 with EDC was the following. To 100µL of 2% (w/v) RADA16 gel we added 50µL of 20% (w/v) EDC. The mixture was shaken vigorously for approximately 5 minutes on a Vortex Genie mixer at setting 7 then placed in a lab bench Fisher-Scientific centrifuge for two minutes. To improve mixing, we let the mixture sit overnight for approximately 24 hrs. The resulting aqueous solution had a pH=3.53. All reactions were carried out at 22 °C.

In preparation for viewing the sample under SEM, 250 µL of 70% (w/v) ethanol was added to reactant mixture to both dissolve any unreacted polymer and aid in evaporation of the solution. Approximately 100 µL of the solution was placed on a coverglass that was cleaned by immersion in ethanol and sonicated for 10 minutes. The product solution on the coverglass was then evaporated for about 6 minutes on top of a hotplate set at 90 °C. To view under SEM, an approximately 10 nm layer of Au-Pd was deposited on top of the dried RADA16/EDC film with a Denton sputtering system. Preparation of samples for viewing under TEM required nominal dilution factors of DF=1000. Samples were vacuum dried at 45 °C and negatively dyed.

Nanoparticle Tracking Analysis equipment required volumes in the range of 0.8-1 mL. We used dilution factors of DF=1000 in deionized water to study the distribution of particle sizes in our sample.

FTIR studies were conducted at room temperature, 22 °C, using the same dilution factor.

5.2.3. Equipment

A JEOL JSM-63900LV SEM equipped with an energy-dispersive X-ray spectroscopy (EDS) detector from Oxford Instruments was used to obtain SEM pictures and material composition data. RADA16 FTIR spectra were obtained at 4 cm-1 resolution on a Jasco FT/IR 4100 with a multi-reflection Attenuated Total Reflectance (ATR) accessory equipped with a ZnSe
crystal. FTIR spectra for EDC and RADA16+EDC product were measured on a Bruker Vertex 70 spectrometer with a single pass ATR accessory. Nanoparticle Tracking Analysis (NTA) was performed on a Malvern Instruments Nanosight LM10 with capability of tracking particles in the size range of 10 - 2000 nm. TEM data was obtained in collaboration with the Microscopy Core Facility.

5.3. Results and Discussion

5.3.1. SEM and TEM studies

Figure 5.2a shows an SEM picture of the resulting product from a reaction between RADA16 hydrogel and EDC prepared as detailed in the Experimental section, both previously dissolved in deionized water. Nanoparticles of approximately 70-80 nm are readily visible and randomly dispersed throughout the film surface. To rule out contamination from NaCl or other types of salts, we measured elemental X-ray dispersion with the EDS detector on a 1 µm x 1 µm field of view at four different sample locations. In addition to elements typical of organic compounds such as carbon, oxygen, nitrogen and hydrogen, EDS measurements showed significant traces of chlorine. No other elements were found. We attribute the presence of chlorine to counterions in the RADA16 arginine amino acid residues as well as the hydrochloride from EDC. Higher magnifications of nanoparticles resulted in pictures that were very fuzzy due to surface charge build-up. TEM pictures provided better details. Figure 5.2b shows the sample viewed under TEM at 28.7 kX magnification and exhibits a similar nanoparticle monodispersity as seen under SEM. Under TEM, it is readily apparent that nanoparticles appear to be crystalline in nature and randomly located. Figure 5.2c shows a close-up picture taken with TEM at 824 kX magnification of one of these nanocrystals. This particle appears to have either an orthorhombic or tetragonal crystal structure. Studies of additional TEM pictures, led us to believe there is a preponderance of orthorhombic structures.
with regards to the nanocrystals. Mixed in with the nanocrystals, and somewhat hidden in Figures 5.2b and 5.3c, are larger sized spherules. Figure 5.2d presents these spherules, which are bigger in size and in general tend to be $> 0.5 \, \mu m$. Interestingly, one could also observe the presence of crosslinked RADA16 nanofibers in process of agglomeration in figure 5.2d at the middle and lower left corner of the picture.
5.3.2. NTA Measurements

Figure 5.3.: a.) TEM view of “plate-like” crystals present in 20% EDC dissolved in deionized water at 78.7 kX magnification.; b.) NTA measurement of 20% EDC only, DF=1000, in deionized water measured at 21 °C.; c.) TEM view of solution made with 2% RADA16+20% EDC at 78.7 kX magnification. Visual inspection shows post-reaction crystals have different morphology than EDC crystals. Additionally, spherules appear in the mix.; d.) NTA measurement of 2% w/v RADA16+20% EDC, DF=1000, in deionized water at 25 °C.; e.) TEM view of spherules from different location than figure 5.2d at 28.7 kX magnification.; c.) Spherule size distribution statistics of e.) as measured with the TEM measuring tool.
To ensure the nanocrystals were not due to unreacted EDC, we measured particle distribution of the reactant using NTA on a sample at DF=1000 in deionized water. We additionally viewed the same dilution sample under TEM. Figure 5.3a, TEM picture at 78.7 kX magnification, shows that indeed there are “plate-like” square particles or flakes within the EDC solution. NTA showed particles to be typically in the range of 46 - 300 nm. Less probable were particles of sizes ranging between 500-700 nm. Figure 5.3b presents data for one set of measurements at 21 °C. A visual comparison of figure 5.3a with figure 5.3c, also taken with TEM at 78.7 kX magnification and showing reaction product nanocrystals, reveals crystal morphologies are different. Whereas crystals in EDC are “plate-like” flakes at various stages of dissolution, product nanocrystals are solid, well-formed orthorhombic-like structures. NTA quantified the size distribution of the mix of nanocrystals and spherules in the product solution. Figure 5.3d presents data obtained for one set of measurements from a sample of product solution diluted in deionized water at DF=1000 and measured at 25 °C. It shows particles present in the 100-600 nm range within which the majority appear to be nanocrystals. Larger sizes, > 900 nm, most likely stem from spherules. Indeed, in a representative area covered primarily with spherules, 5.3e, a manual count of N=13 spherules yielded an average size D= 987 nm with standard error = 59 nm. The 95% confidence interval in this region is [859, 1115] nm. Therefore, we attribute the size distribution peaking at 902 nm in Fig. 5.3d to spherules. Such distribution of sizes did not appear in NTA measurements of EDC.

5.3.3. FTIR Analysis

From SEM and TEM pictures, as well as NTA, it was not immediately clear that there was a crosslinking reaction taking place between RADA16 and EDC. To gather further evidence that the spherules and nanocrystals were not just a result of desegregated RADA16 hydrogel and unreacted EDC, respectively, FTIR measurements were conducted. FTIR measurements
Figure 5.4: FTIR of RADA16-NH$_3$ hydrogel at 7.8% w/v concentration. Peak at 1621 cm$^{-1}$ falls within the range of $\beta$-sheet values given in Barth, 2007 after taking into account the 4 cm$^{-1}$ equipment resolution.

were obtained for RADA and EDC alone as well as RADA+EDC after reaction. Figure 5.6a is an FTIR plot of RADA16 hydrogel prior to reaction with EDC. It clearly shows a distinctive $\beta$-sheet peak at 1621 cm$^{-1}$ [88]. Appendix D shows typical position of beta sheet peaks in FTIR from Barth, 2007 and specifically for RADA16 from Arosio et. al., 2012. The broad peak at 2116 cm$^{-1}$ covers the range of alkyne (C≡C) and nitrile (C≡N) stretches, which are not thought to be present in RADA16. Therefore, this broad peak is unknown as of this writing. Figure 5.6b shows FTIR data for EDC prior to reaction with EDC. Of particular importance are the peaks at 2130 and 1702 cm$^{-1}$ as these distinctive
peaks for EDC disappear after the crosslinking reaction with RADA16. The peak at 2130 cm\(^{-1}\) is attributed to the N=C=N bonds of EDC \cite{89}. We attribute the peak at 1702 cm\(^{-1}\) to stretching of the cumulated C=N bonds since C is an sp hybridized carbon. It is expected that these bonds would no longer be present after reaction of the primary amine with the unstable intermediate o-acylisourea. That is in fact what we found. Figure 5.6c shows overlaid plots of EDC reactant (magenta) with the RADA16+EDC product (black). Peaks at 2130 and 1702 cm\(^{-1}\) are conspicuously absent, confirming that a crosslinking reaction
indeed took place. The two most prominent peaks of the RADA16+EDC product (black curve) appear at 1619 and 1571 cm$^{-1}$. Both peaks could be attributed to different modes of N-H bending vibrations of primary amine groups such as those present in urea. Given the relative signal strength of the peaks, it is also possible that they are produced by bending vibrations of amide groups. It could also be the case that the peak at 1619 cm$^{-1}$ signifies some presence of RADA16 due to incomplete crosslinking reaction possibly due to an inefficient reaction mechanism or insufficient EDC. In general, however, the absence of EDC confirms crosslinking has taken place.

Crosslinking confirmed, we would expect the peaks to be produced by the presence of both amide bonds from crosslinked peptides and amine bonds from the urea by-product. The peak at 1571 cm$^{-1}$ could also be produced by a nitro group (-NO$_2$) asymmetric stretch, though we believe this type of bond is less likely to occur in the current crosslinking environment. The next highest peaks of the RADA16+EDC product curve appear at 1481 and 1281 cm$^{-1}$. Bending and rocking deformations of alkane groups (CH$_2$; CH$_3$) are attributed to 1481 cm$^{-1}$. It seems likely that the C-N stretch of an amine group causes the peak at 1281 cm$^{-1}$. The low energy peaks at 878, 849 and 813 cm$^{-1}$ which appear on the product curve, but not on the EDC curve could be due to C-Cl bond stretching. However, that needs to be studied in more detail and we will not mention them further. On the higher energy side of the product spectrum we attribute the peak at 2982 cm$^{-1}$ to sp$^3$ hybridized C-H bonds signifying the presence of acetyl groups. It seems the 2754 cm$^{-1}$ peak on the RADA16/EDC product curve could be produced by the O-H stretch of regenerated carboxylic acids that did not react with a primary amine. It is not likely that the peak is produced by the C-H stretch of aldehydes.

FTIR data, thus, lends support to the existence of a proposed crosslinking reaction of RADA16 activated by EDC. It is likely that crosslinking proceeds through EDC activation of the carboxyl groups present in the aspartic acid amino acid residues. The unstable, amine-reactive O-acylisourea intermediate that results from activation of the arboxyl groups then
reacts with available primary amines. Primary amines available for reaction either come from the N-terminus or the guanidinium group of the arginine subgroup. While the guanidinium cation is highly stable in an aqueous solution, reactions stemming from a combination of both the N-terminus and possibly guanidinium groups cannot be ruled out.

5.3.4. Additional Analysis

It should be pointed out that Powder XRD analysis of the product yielded inconclusive results. It was not possible to obtain significant readings with the amount of raw material at hand. Currently available TEM is not equipped with XRD capability to further analyze nanocrystalline structures.

An additional measurement was performed to characterize dielectric relaxation differences between the unreacted RADA16 and the product of RADA16 with EDC. Figure 5.7 shows a comparison of the dielectric relaxation curve between unreacted RADA16 (red) and the product of RADA16 with EDC (green). It can easily be seen that peak relaxation responses occur in the range of 100-200 MHz where one would expect normal mode relaxation processes to dominate. Normal mode processes are related to translational molecular motions. Peaks at higher frequencies around 850-900 MHz are attributed to bound water relaxation processes. Calculations of relaxation times as $\tau_r = 1/\omega_c$ give that $\tau_r = 1.6$ ns and $\tau_r = 0.8$ ns for RADA16 and product of RADA16/EDC, respectively. Qualitatively, this means that RADA16 molecules are longer, thus take a longer time to relax, than those molecules resulting from RADA16/EDC product, which would be shorter.

The combination of FTIR and DR measurements ascertain that indeed there is a type of self-crosslinking mechanism occurring within RADA16 upon addition of EDC. That leaves the puzzle of what is the composition of resulting crystals and spherules. A TEM with EDS and XRD capability will definitely pinpoint the crystallographic and composition of nanocrystals. However, those capabilities were not available as of this writing. Regard-
ing spherules, Figure 5.2b seems to show “beaded necklaces” of the type described for a hydrophobic polyelectrolyte in Dobrynin et al. [90]. The paper described a clumping of globules connected by strings as effective charge on a chain decreases, which occurs as polymer concentration increases. The clumped globules would then exist in a form similar to a liquid droplet. This sort of mechanism would seem likely since self-crosslinking of RADA16 would result in the predominant existence of positively charged arginine groups along with hydrophobic alanine groups, thus a hydrophobic polyelectrolyte. This type of beading is reminiscent of what was found during electrospinning of blends composed of Polyacrylic acid (PAA) and RADA16 at various PAA and RADA16 concentrations. As Figure 5.8 shows electrostatic charges from RADA16 seem to modify surface tension which is manifested as changes to fiber morphology. Particularly telling are SEM pictures in the 15% w/v PAA concentration row. The top row are fibers deposited on a glass substrate. The bottom row shows samples deposited on an aluminum substrate, which is a conductor. Whereas in the case of fiber deposition on a glass substrate, fibers with beads appear, in the case of fiber deposition on a conductor beads tend to adopt a “flatter” more interconnected structure suggesting charge dissipation. The interconnected branches seem to be a result of charged hydrodynamic flow.

5.4. Conclusion

We have provided evidence that crosslinking in RADA16 is activated by EDC. It is likely that crosslinking proceeds through EDC activation of the carboxyl groups present in the aspartic acid amino acid residues reacting with primary amines either from the N-terminus and/or the guanidinium group of the arginine subgroup. The reaction produces nanocrystals and micron-sized spherules. Further studies are required to understand the mechanisms leading to crosslinking as well as formation of nanocrystals and spherules.
Figure 5.6: a.) FTIR spectra of 2% RADA16. The significant peak at 1621 cm$^{-1}$ is attributed to stable $\beta$-sheets. b.) FTIR spectra of 20% EDC. N=C=N bonds produce two distinctive peaks at 2130 and 1702 cm$^{-1}$, respectively. These disappear after a crosslinking reaction. c.) Overlaid FTIR spectra of unreacted 20% EDC (magenta) and RADA16+EDC (black) after reaction, diluted in deionized water.
Figure 5.7: Plots of dielectric relaxation of unreacted RADA16 (red) and product of RADA16 with EDC (green).
Figure 5.8: SEM pictures of electrospun blended PAA/RADA16 at several concentrations. Electrostatic modification of surface tension manifests in changes of fiber morphology.
Part II.

ELECTROMAGNETIC HYDRODYNAMIC FLOW STUDY
CHAPTER SIX :
GENERAL NAVIER-STOKES-LIKE MOMENTUM AND MASS-ENERGY EQUATIONS

6.1. Introduction

Generally, the concept of Navier-Stokes equations is applied in the context of conservation laws. Specifically, conservation of mass and momentum. In this section, we take the notion of hydrodynamic conservation laws, add energy conservation, and apply the system to non-relativistic electromagnetic field flow.

6.1.1. System of Navier-Stokes Equations

Several groups have applied the Navier-Stokes (NS) equations to Electromagnetic (EM) fields through analogies of EM field flows to hydrodynamic fluid flow. Most recently, Boriskina and Reinhard made a hydrodynamic analogy utilizing Euler’s approximation to the Navier-Stokes equation in order to describe their concept of Vortex Nanogear Transmissions (VNT), which arise from complex electromagnetic interactions in plasmonic nanostructures [91].

In 1998, H. Marmanis published a paper that described hydrodynamic turbulence and made direct analogies between components of the NS equation and Maxwell’s equations of electromagnetism[92]. Kambe formulated equations of compressible fluids using analogous Maxwell’s relation and the Euler approximation to the NS equation[93]. Lastly, in a recently published paper John B. Pendry, et. al. developed a general hydrodynamic model approach to plasmonics [94].
In the cases of Kambe and Boriskina, et.al, the groups built their models through analogous Euler-like equations along with relevant mass continuity analogues, respectively shown below.

\[
\frac{Dv}{Dt} = -\nabla p, \quad (6.1)
\]
\[
\frac{Dr}{Dt} + \rho \nabla \cdot \mathbf{v} = 0 \quad (6.2)
\]

where \( \mathbf{v} \) is the velocity vector, \( \nabla = \frac{\partial}{\partial x_i} \hat{e}_i \) is the del operator, \( p \) is pressure, \( \rho \) is fluid density, and \( \frac{D}{Dt} = \partial/\partial t + \mathbf{v} \cdot \nabla \) is a material derivative operator. Marminis and others [95, 96] utilized the Navier-Stokes equation (6.3) to build their EM analogues:

\[
\rho \left( \frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla)\mathbf{v} \right) = -\nabla p + \mu \nabla^2 \mathbf{v} + \mathbf{f}. \quad (6.3)
\]

The terms on the left side of the equation represent the fluid’s inertia per volume. The \( \frac{\partial \mathbf{v}}{\partial t} \) term represent an unsteady state acceleration, while \( \mathbf{v} \cdot \nabla \mathbf{v} \) is a non-linear advection term. On the right hand side, the sum of the pressure gradient, \( \nabla p \), and the viscosity, \( \mu \nabla^2 \mathbf{v} \), represent the divergence of a stress tensor. Finally, \( \mathbf{f} \) represents the sum of all other body forces acting on the system. Equation (6.3) is the momentum equation that describes fluid flow, while equation (6.1) is its approximation under zero body forces and inviscid flow, neglecting heat conduction, also termed the Euler approximation.

As others have done, we, likewise, begin with an analogy of hydrodynamic conservation equations mapped to corresponding electromagnetic conservation equations, assuming non-relativistic flow in an isotropic medium, to finally derive a new system of Navier-Stokes-like equations that model electromagnetic flow. This new set of equations could potentially be useful in gaining a different perspective and better understanding of electromagnetic mass, energy, momentum behavior.
6.1.2. General Momentum, Mass, Energy Conservation Hydrodynamic Equations

Equation (6.3) is not in its most general form to describe fluid momentum. A more general equation is the Cauchy Momentum equation into which one substitutes in an appropriate stress tensor and constitutive relations relative to the problem at hand. Such substitution then leads to the NS equation. Making use of the material derivative operator, the Cauchy Momentum Equation is:

\[ \rho \frac{Dv}{Dt} = \nabla \cdot \sigma + f. \] (6.4)

where \( \nabla \cdot \sigma \) is the divergence of a stress tensor, which can be further broken down into the sum of a pressure tensor, \( -\nabla p \), and a deviatoric tensor, \( \nabla \cdot \tau \). So that, \( \nabla \cdot \sigma = -\nabla p + \nabla \cdot \tau \).

Here we have opted to represent tensors as boldface lower-case Greek letters.\(^1\)

Given the above, the question then becomes: What is necessary to generally define a hydrodynamic model obeying Navier-Stokes-type equations. The answer comes in the form of conservation of momentum, mass and energy. In terms of the material derivative operator these three are:

**Momentum**: \[ \rho \frac{Dv}{Dt} - \nabla \cdot \sigma - f = 0 \] (6.5)

**Mass**: \[ \frac{D\rho}{Dt} + \rho \nabla \cdot v = 0 \] (6.6)

**Energy**: \[ \frac{DS}{Dt} - \frac{Q}{T} = 0 \] (6.7)

where \( Q \) and \( T \) are the heat transfer rate and temperature, respectively. The above equations

\(^1\)In component form, the stress tensor can be represented as \( \sigma_{ij} = \tau_{ij} + \pi \delta_{ij} \), where \( \tau_{ij} \) is the stress deviator tensor that distorts a volume component, while \( \pi \delta_{ij} \) is the volumetric stress tensor that tends to change the volume of a stressed body due to pressure exertion. Thus, to derive the Navier-Stokes equation from the Cauchy momentum equation a stress tensor of the form: \( \sigma_{ij} = -p \delta_{ij} + 2\mu \epsilon_{ij} \) is used, with \( \mu \epsilon_{ij} \) representing the viscosity component and \( p \) the pressure.
(6.5-6.7) plus relative constitutive equations lead to hydrodynamic models for non-relativistic flows within continuum space dynamics.

6.2. Electromagnetic “Flow” Differential Equations

6.2.1. General Momentum and Mass-Energy Relations

Comparing analogues of hydrodynamic conservation equations to electromagnetic conservation equations leads to some useful electromagnetic flow relations. Starting with a comparison to the Cauchy momentum equation, we must first look for a term analogous to the hydrodynamic stress tensor, $\sigma_{ij}$. While there is still some controversy over correct electromagnetic momentum relations (Abraham vs Minkowski), thus, also controversy over the appropriate form of stress tensor model, a seeming consensus appears to be for using a Minkowski form [97, 98]. In general, the constitutive relations are $D = \varepsilon_o E + P$ and $B = \mu_o (H + M)$, where $E$ and $B$ are the electric and magnetic fields, respectively; $P$ and $M$ are the polarization and magnetization fields, respectively; $D$ and $H$ are the displacement and magnetic “H” fields, respectively; and $c = 1/\sqrt{\varepsilon_o \mu_o}$ is the speed of light in vacuum. We have also used $\varepsilon_o$ as permittivity and $\mu_o$ as permeability both of free space. The Minkowski stress tensor is then of the form

$$\tau = [ED + HB - \frac{1}{2} I (E \cdot D + H \cdot B)]$$  \hspace{1cm} (6.8)$$

where, $I$ is the identity matrix. The third term of equation (6.8) contains the energy density defined as, $u = \frac{1}{2} (E \cdot D + H \cdot B)$.

To derive desired electromagnetic momentum and mass-energy relations without unnecessary mathematical complications, we choose to work in a non-magnetic, negligibly polarizable, isotropic medium. With the Minkowski stress tensor, EM conservation of linear momen-
tum, derived from forces on a charged particle of arbitrary volume traveling through an EM field, is [99, 100]:

\[ \nabla \cdot \tau = \frac{\partial g}{\partial t} + f \]  

(6.9)

Here \( g \) is defined as EM field momentum density (\( g \equiv S/c^2 \)) and \( S = E \times H \) is the Poynting vector. On the left-hand side of (6.9), \( \nabla \cdot \tau \) represents the total momentum flowing through the surface of an arbitrary volume per unit time, while on the right-hand side \( \partial g/\partial t \) represents the rate of change of field momentum density within such volume and \( f \) is the rate of change of mechanical momentum within the volume [100] imposed by the Lorenz force. Conservation of electromagnetic momentum, equation (6.9), is analogous to the hydrodynamic conservation of momentum, equation (6.5), in the following way

\[ \frac{Dg}{Dt} - (v_{em} \cdot \nabla g) - \nabla \cdot \tau + f = 0 \]  

(6.10)

where the second term on the left side of (6.10) must be included to balance the material derivative term to obtain equation (6.9). We, thus, introduce a new vector term which represents a time-independent momentum density convective acceleration

\[ A_g = v_{em} \cdot \nabla g \]  

(6.11)

where \( v_{em} \) represents the velocity field of the EM field in space-time and is analogous to the hydrodynamic velocity field convective acceleration, \( v \cdot \nabla v \). Now, \( A_g \) is a form of force exerted due to flow of momentum density interacting with the velocity field. In other words, the gradient of the momentum density vector points in the direction of the velocity field vector. As such, we group the two forces that appear in (6.10) into one total force so that \( F = A_g - f \), the first being a time independent convective force, the second being a time
dependent rate of change in mechanical momentum. As shown in equation (6.15) below, this completes the analogy with the hydrodynamic Cauchy momentum equation (6.5).

Unfortunately, there is no conservation of mass equation for an EM field. But, there is a conservation of energy equation for electromagnetic fields and it is given by the following relation [100]:

$$\frac{\partial u}{\partial t} + \nabla \cdot \mathbf{S} + \mathbf{j} \cdot \mathbf{E} = 0$$  \hspace{1cm} (6.12)

where $u = \varepsilon_0 \frac{1}{2} (E^2 + c^2 B^2)$ and $\mathbf{j}$ is charge current, $\mathbf{j} = \rho \mathbf{v}$ with $\rho = qn$ as charge density and $\mathbf{v}$ as charge velocity.

Utilizing Einstein’s non-relativistic mass-energy relationship, $E = mc^2$, we establish a relation between conservation of electromagnetic energy and conservation of mass from hydrodynamic flow by taking equation (6.6), after expanding the material derivative operator, multiplying it by $c^2$ and setting it equal to equation (6.12) to get the following relation

$$\frac{\partial u}{\partial t} + \nabla \cdot \mathbf{S} + \mathbf{j} \cdot \mathbf{E} = \left\{ \frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) \right\} \times c^2$$ \hspace{1cm} (6.13)

dividing by $c^2$ and re-arranging terms we get

$$\frac{\partial \rho_{em}}{\partial t} + \nabla \cdot \mathbf{g} + \frac{1}{c^2} \mathbf{j} \cdot \mathbf{E} - \nabla \cdot (\rho_{ns} \mathbf{v}_{em}) = 0$$ \hspace{1cm} (6.14)

where $\rho_{em} = \rho_{ed} - \rho_{ns}$ and $\rho_{ed} = u/c^2$, while $\rho_{ns}$ is a material density of the medium and $\mathbf{v}_{em}$ is as before. Equation (6.14) has no analogue to hydrodynamic equations.

In summary, we have the following two EM field conservation relations here derived. These form a system of Navier-Stokes-like equations whereby additional insight could be gained into
electromagnetic flows.

\[ \text{Momentum} : \quad \frac{Dg}{Dt} - \nabla \cdot \tau - \mathcal{F} = 0 \quad (6.15) \]

\[ \text{Mass-Energy} : \quad \frac{\partial \rho_{em}}{\partial t} + \nabla \cdot g + \frac{1}{c^2} \mathbf{j} \cdot \mathbf{E} - \nabla \cdot (\rho_{ns} \mathbf{v}_{em}) = 0 \quad (6.16) \]

Equation (6.15) is analogous to the Cauchy momentum equation (6.5). It is a vector equation that describes the time rate of change of the EM field momentum density, under assumptions made here. The Mass-Energy equation is a scalar equation that describes the time rate of change of a so-called EM density given by the difference in energy density, \( u \), per \( c^2 \) and a second density obtained from the medium of the EM field. It has no analogue to hydrodynamics. The electromagnetic momentum and mass-energy equations are coupled through the momentum density vector, \( \mathbf{g} \), and the velocity vector, \( \mathbf{v}_{em} \), in a similar fashion to hydrodynamic conservation equations.

6.2.2. Euler-like equation

Interestingly, we obtain a Euler-like approximation for equation (6.15) upon moving \( \nabla \cdot \tau \) to the right-hand side. First, let us represent the divergence of Maxwell stress tensor in component notation. Since it is a second rank tensor we will have the following,

\[
\nabla \cdot \tau = \frac{\partial \tau_{ij}}{\partial x_j} e_i = \varepsilon_o \left\{ \frac{E_i}{\partial x_j} + c^2 B_j \frac{\partial B_i}{\partial x_j} - \delta_{ij} \frac{\partial (u/\varepsilon_o)}{\partial x_j} \right\} e_i \quad (6.17)
\]
where the $\mathbf{e}_i$ are basis vectors. Assuming a non-conducting, vacuum medium so that $\mathbf{f} = 0$, equation (6.15) in component notation becomes

\[
\left( \frac{\partial g_i}{\partial t} + v_j \frac{\partial g_i}{\partial x_j} \right) - v_j \frac{\partial g_i}{\partial x_j} \mathbf{e}_i
= \varepsilon_0 \left\{ E_j \frac{\partial E_i}{\partial x_j} + c^2 B_j \frac{\partial B_i}{\partial x_j} \mathbf{e}_i - \frac{\partial (u/\varepsilon_0)}{\partial x_j} \right\}.
\]

(6.18)

or in vector notation

\[
\frac{\partial \mathbf{g}}{\partial t} = \varepsilon_0 \left\{ (\mathbf{E} \cdot \nabla) \mathbf{E} + c^2 (\mathbf{B} \cdot \nabla) \mathbf{B} - \nabla (u/\varepsilon_0) \right\}.
\]

(6.19)

Since the electromagnetic wave is propagating in a vacuum, from Maxwell’s equations $\nabla \cdot \mathbf{E} = \nabla \cdot \mathbf{B} = 0$. After rearranging, equation (6.19) becomes

\[
\frac{\partial \mathbf{g}}{\partial t} = -\nabla u.
\]

(6.20)

Now, the energy density term, $u$, on the right hand side of the equation is measured in energy per unit volume, which is also a measure of pressure as a force per unit area. Through simple dimensional analysis one can ascertain that

\[
\text{Energy per Volume} = \frac{\mathbf{F} \cdot d}{\mathbf{A} \cdot d} = \frac{\mathbf{F}}{\mathbf{A}} = P
\]

where $\mathbf{F}$ is a force, $\mathbf{A}$ is unit area and $d$ is distance. As a consequence, the energy density term can be thought of as a pressure component so that we can let $p_{em} = u$, as a representation of pressure. With this substitution, the analogy with Euler approximation is evident. Under non-divergent $\mathbf{v}$ such that $\nabla \cdot \mathbf{v} = 0$, Navier-Stokes equation (6.1) becomes $\frac{\partial \mathbf{v}}{\partial t} = -\frac{\nabla p}{\rho}$, which is analogous to (6.21) below.

\[
\frac{\partial \mathbf{g}}{\partial t} = -\nabla p_{em}.
\]

(6.21)
We have distinguished electromagnetic pressure from NS pressure through the subscript, em.

6.3. Conclusion

We have shown that by starting with a general form of a hydrodynamic momentum conservation equation, Cauchy momentum, we can analogously derive a general form of electromagnetic momentum conservation. As aid to construction, we can likewise use the hydrodynamic conservation of mass equation to derive an electromagnetic relation between mass-energy applicable to electromagnetic “fluid” flow. While, by no means, can these generalized EM Navier-Stokes-like equations be applied to any electromagnetic flow at hand, they could be useful if applied carefully to a system. One must first decide on appropriate flow assumptions regarding steady-state, vorticity, and constitutive relations appropriate to the medium within which EM flow occurs. In future work, we will apply these EM conservation equations to describe several well-known problems in electromagnetism.
REFERENCES


APPENDIX A:
PUBLICATIONS AND PRESENTATIONS

1. Refereed Publications


2. Manuscripts In Progress


3. Poster Presentations

Order Completed

Thank you very much for your order.

This is a License Agreement between Jorge Monreal ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number 3654370272271
License date Jun 22, 2015
Licensed content publisher Elsevier
Licensed content publication Annals of Physics
Licensed content title General Navier-Stokes-like momentum and mass-energy equations
Licensed content author Jorge Monreal
Licensed content date March 2015
Licensed content volume 354
Licensed content issue number n/a
Number of pages 5
Type of Use reuse in a thesis/dissertation
Portion full article
Format both print and electronic
Are you the author of this Elsevier article? Yes
Will you be translating? No
Title of your thesis/dissertation Nonlinear Mechanical, Optical and Electrical Properties of Polypeptides
Expected completion date May 2016
Estimated size (number of pages) 400
Elsevier VAT number GB 494 6272 12
Permissions price 0.00 USD
VAT/Local Sales Tax 0.00 USD / 0.00 GBP
Total 0.00 USD
APPENDIX C:
PHENOMENOLOGICAL STUDIES

Table C1: Randomized sample fabrication obtained from a design of experiment table. There are three replicates of each condition.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>PLEY</th>
<th>PLK</th>
<th>EDC</th>
<th>RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>320485-1</td>
<td>30%</td>
<td>20%</td>
<td>40%</td>
<td>85%</td>
</tr>
<tr>
<td>420275-1</td>
<td>40%</td>
<td>20%</td>
<td>20%</td>
<td>75%</td>
</tr>
<tr>
<td>515285-1</td>
<td>50%</td>
<td>15%</td>
<td>20%</td>
<td>85%</td>
</tr>
<tr>
<td>520333-1</td>
<td>50%</td>
<td>20%</td>
<td>30%</td>
<td>33%</td>
</tr>
<tr>
<td>420275-2</td>
<td>40%</td>
<td>20%</td>
<td>20%</td>
<td>75%</td>
</tr>
<tr>
<td>520333-2</td>
<td>50%</td>
<td>20%</td>
<td>30%</td>
<td>33%</td>
</tr>
<tr>
<td>415433-1</td>
<td>40%</td>
<td>15%</td>
<td>40%</td>
<td>33%</td>
</tr>
<tr>
<td>415433-2</td>
<td>40%</td>
<td>15%</td>
<td>40%</td>
<td>33%</td>
</tr>
<tr>
<td>510475-1</td>
<td>50%</td>
<td>10%</td>
<td>40%</td>
<td>75%</td>
</tr>
<tr>
<td>410385-1</td>
<td>40%</td>
<td>10%</td>
<td>30%</td>
<td>85%</td>
</tr>
<tr>
<td>410385-2</td>
<td>40%</td>
<td>10%</td>
<td>30%</td>
<td>85%</td>
</tr>
<tr>
<td>310233-1</td>
<td>30%</td>
<td>10%</td>
<td>20%</td>
<td>33%</td>
</tr>
<tr>
<td>510475-2</td>
<td>50%</td>
<td>10%</td>
<td>40%</td>
<td>75%</td>
</tr>
<tr>
<td>415433-3</td>
<td>40%</td>
<td>15%</td>
<td>40%</td>
<td>33%</td>
</tr>
<tr>
<td>320485-2</td>
<td>30%</td>
<td>20%</td>
<td>40%</td>
<td>85%</td>
</tr>
<tr>
<td>315375-1</td>
<td>30%</td>
<td>15%</td>
<td>30%</td>
<td>75%</td>
</tr>
<tr>
<td>420275-3</td>
<td>40%</td>
<td>20%</td>
<td>20%</td>
<td>75%</td>
</tr>
<tr>
<td>510475-3</td>
<td>50%</td>
<td>10%</td>
<td>40%</td>
<td>75%</td>
</tr>
<tr>
<td>515285-2</td>
<td>50%</td>
<td>15%</td>
<td>20%</td>
<td>85%</td>
</tr>
<tr>
<td>310233-2</td>
<td>30%</td>
<td>10%</td>
<td>20%</td>
<td>33%</td>
</tr>
<tr>
<td>315375-2</td>
<td>30%</td>
<td>15%</td>
<td>30%</td>
<td>75%</td>
</tr>
<tr>
<td>315375-3</td>
<td>30%</td>
<td>15%</td>
<td>30%</td>
<td>75%</td>
</tr>
<tr>
<td>310233-3</td>
<td>30%</td>
<td>10%</td>
<td>20%</td>
<td>33%</td>
</tr>
<tr>
<td>515285-3</td>
<td>50%</td>
<td>15%</td>
<td>20%</td>
<td>85%</td>
</tr>
<tr>
<td>520333-3</td>
<td>50%</td>
<td>20%</td>
<td>30%</td>
<td>33%</td>
</tr>
<tr>
<td>320485-3</td>
<td>30%</td>
<td>20%</td>
<td>40%</td>
<td>85%</td>
</tr>
<tr>
<td>410385-3</td>
<td>40%</td>
<td>10%</td>
<td>30%</td>
<td>85%</td>
</tr>
</tbody>
</table>
Individual fits with neo-Hookean model of conditions at a.) 75% and b.) 85% relative humidities at 30% w/v nominal PLEY concentration.
Individual fits with neo-Hookean model of conditions at a.) 75% and b.) 85% relative humidities at 40% w/v nominal PLEY concentration.
Individual fits with neo-Hookean model of conditions at a.) 75% and b.) 85% relative humidities at 50% w/v nominal PLEY concentration.
Individual fits with Mooney-Rivlin model of conditions at a.) 75% and b.) 85% relative humidities at 30% w/v nominal PLEY concentration.
Individual fits with Mooney-Rivlin model of conditions at a.) 75% and b.) 85% relative humidities at 40% w/v nominal PLEY concentration.
Individual fits with Mooney-Rivlin model of conditions at a.) 75% and b.) 85% relative humidities at 50% w/v nominal PLEY concentration.
APPENDIX D:
RADA16 FTIR

1. Table from Barth, 2007 shows range of $\beta$-sheet values in the range of 1623-1641 cm$^{-1}$ in H$_2$O. Graph of RADA16-I, which has a different terminus than that used in this study, comes from Arosio et al., 2012. Beta sheet is clearly shown at 1627 cm$^{-1}$ for both in H$_2$O and original powder.
### Assignment of amide I band positions to secondary structure

<table>
<thead>
<tr>
<th>Secondary structure</th>
<th>Band position in $^1$H$_2$O/cm$^{-1}$</th>
<th>Band position in $^2$H$_2$O/cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Extremes</td>
</tr>
<tr>
<td>α-helix</td>
<td>1654</td>
<td>1648–1657</td>
</tr>
<tr>
<td>β-sheet</td>
<td>1633, 1634</td>
<td>1623–1641, 1674–1695</td>
</tr>
<tr>
<td>Turns</td>
<td>1672</td>
<td>1662–1686</td>
</tr>
<tr>
<td>Disordered</td>
<td>1654</td>
<td>1642–1657</td>
</tr>
</tbody>
</table>

---

RADA16-I in water at pH 4.5 (+) and in original powder (−). Max at 1627 cm$^{-1}$.  

*Barth, A., Biochimica et Biophysica Acta 1767 (2007) 1073–1101*