STRUCTURE OF NEARLY IDEAL AND MULTI-COMPONENT POLYMERIC BIOMATERIALS

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STRUCTURE OF NEARLY IDEAL AND MULTI-COMPONENT POLYMERIC BIOMATERIALS

A Dissertation Presented
by
ERIKA M. SAFFER

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2014

Department of Chemical Engineering
STRUCTURE OF NEARLY IDEAL AND MULTI-COMPONENT POLYMERIC BIOMATERIALS

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by
ERIKA M. SAFFER

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ABSTRACT

STRUCTURE OF NEARLY IDEAL AND MULTI-COMPONENT POLYMERIC BIOMATERIALS

MAY 2014

ERIKA M. SAFFER, B.S., UNIVERSITY OF LOUISIANA AT LAFAYETTE
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Hydrogels have long been considered ideal candidates for biomaterial and tissue engineering applications due to their many desirable properties, such as high water content and tunable gelation conditions. Although these materials have undergone extensive research and development, some mechanical and physical properties are still difficult to achieve. The reason for this is often related to the structure of the hydrogel network. Understanding how network structures are influenced by changes in formulation parameters (i.e. polymer molecular weight, initial polymer concentration, ratio of hydrophilic to hydrophobic polymer), and correlating these results to known mechanical and physical properties would yield well characterized systems that are more easily tuned for specific applications. The work presented in this thesis focuses on the characterization of the micro- to nano-scale network structures of three distinct hydrogel systems: tetra-functional poly(ethylene glycol) (PEG)-based hydrogels, tetra-functional PEG/polydimethylsiloxane (PDMS)-based hydrogels, and commercial contact lenses.

The tetra-functional PEG and PEG/PDMS hydrogel systems were synthesized with a novel cross-linking technique that was developed by the Tew Group in the Polymer Science and Engineering department at the University of Massachusetts
Amherst. This technique was designed to reduce the formation of network defects. The resulting hydrogels are optically clear, and display highly resilient mechanical properties which suggest relatively defect free (or ideal) network structures. In collaboration with the Tew group, we performed a series of small-angle neutron scattering (SANS) studies on these systems. The results from the tetra-functional PEG hydrogels confirmed the presence of nearly ideal network structures. Additionally, those from the tetra-functional PEG/PDMS hydrogels revealed the presence of a two-phase network structure with a local, lamellar-like order. For both systems, the resulting structures were found to be dependent upon polymer molecular weight, initial polymer concentration, and the ratio of hydrophilic to hydrophobic polymer. These results confirm the effectiveness of the novel cross-linking technique used to synthesize the PEG and PEG/PDMS tetra-functional hydrogels. Their unique and predictable network structures provide an excellent starting point for the development of these systems for specific applications, such as tendon tissue engineering scaffolds.
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CHAPTER 1

MOTIVATION AND BACKGROUND INFORMATION

1.1 Introduction

Polymer networks remain critically important materials from both a fundamental and technological viewpoint. Networked materials are utilized in a wide variety of applications, including industrially important adhesives, high temperature epoxides, soft hydrogels found in biomaterials and consumer products, and biological materials, both naturally-occurring (e.g., tissues) and synthetic. While significant progress has been made in understanding the basic structure-property relationships of networks, much remains to be learned about how micro- to nano-scale network structures influence macroscopic mechanical and physical properties. This lack of understanding can result in difficulties when developing networked materials for applications that require difficult to achieve properties, such as highly elastic mechanical properties or uniform degradation profiles. The first step towards overcoming these challenges is to understand how changes in formulation parameters affect the micro- to nano-scale network structures of a given system. These structural changes can then be related to known mechanical and physical properties, resulting in a well characterized system that is more easily tuned for specific applications.

Hydrogels are water-swollen polymer networks. Their high water content, porous network structure, and the ability to manipulate gelation conditions are just a few of the qualities make these materials ideal candidates for biomaterial and tissue engineering applications. Many hydrogel systems have been developed from both natural polymers, such as alginate and collagen, and synthetic polymers, such as
poly(ethylene glycol). Hydrogel networks are formed through either physical or chemical cross-links. Physical cross-links are not permanent and can be dependent on environmental factors such as pH or temperature. Some examples of physical cross-links include ionic bonds and micelle-micelle interactions. Chemical cross-links create permanent networks junctions. Typically, these types of networks are formed from a functionalized macromer that can either cross-link with itself or with a cross-linker that has defined functionality.

By definition, an ideal hydrogel network is one in which each cross-link (or junction) connects the same number of polymer chains, the distance between these cross-links is uniform throughout the network, and each polymer chain participates equally in the network. If all hydrogel networks were ideal in structure, their mechanical and physical properties would be simple to predict and tune for specific applications. In practice, however, both physically and chemically cross-linked hydrogel networks contain defects such as elastically ineffective dangling ends or looping chains, entanglements, or fluctuations in polymer concentration in the network. Defects like these typically form unpredictably and can result in difficulty predicting physical properties, such as degradation rate or drug release profiles, and detract from the elastic properties and resilience of the network. Examples of ideal and real network structures are shown in Figure 1.1.
Many applications do not require ideal hydrogel networks. For example, alginate hydrogels are physically cross-linked and have very heterogeneous network structures, yet they are commonly used in applications such as cell encapsulation\textsuperscript{24, 25} and wound dressings\textsuperscript{26, 27}. However, a more ideal network structure would be desired for applications that required specific, tunable properties. For example, it is important for tissue engineering scaffolds to mimic the mechanical properties of the desired tissue to promote cell growth and development\textsuperscript{11, 28}. Tissues such as tendons or ligaments would require a scaffold with highly elastic, and resilient mechanical properties\textsuperscript{29, 30}. For this application, a hydrogel with a more ideal network would be desirable as this would result in very elastic mechanical properties. Polymer networks with minimal defects are also of interest for applications in energy storage. For example, poly(ethylene glycol) (PEG)-based networks are currently being investigated for energy storage application due to their ability to conduct lithium ions through chain relaxation\textsuperscript{31, 32}. The optimal network for this application should allow for maximum ion transport while maintaining robust mechanical
properties. This requires a network with minimal defects.\textsuperscript{33, 34} Whether a more ideal or heterogeneous network is desired, it is imperative to characterize the network structure of the system and understand how this changes with formulation parameters to determine how to best tune the system for a desired application.

A technique that has been utilized extensively to investigate the structure of hydrogel networks is small-angle neutron scattering (SANS).\textsuperscript{19, 35-38} A typical SANS instrument can probe structures between 1 nm and 200 nm in size. For most hydrogel systems, this range would probe the structure of a single polymer chain to that of several cross-links. SANS is a bulk measurement technique, and the scattering arises from the neutrons' interactions with the nuclei in the sample. Unlike small-angle x-ray scattering SAXS, where the scattering event is directly dependent on the atomic mass of the atoms in the sample (i.e. heavy elements scatter more due to their large electron shell), the neutron-nucleus interaction that occurs in SANS does not very systematically with atomic mass. For example, hydrogen scatters incoherently in SANS (i.e. scattering from hydrogel atoms cannot be detected), however its isotope, deuterium, scatters very well. This phenomenon makes SANS the preferred method for the study of biological and soft matter systems for two reasons: these systems predominately contain carbon, hydrogen, and nitrogen which do not scatter as well in SAXS, and SANS allows for labeling of certain components of a system by simply varying the hydrogen to deuterium ratio instead of labeling the region with a heavy metal and risking the disruption of the native structure.

In this work, we investigate the effect of formulation changes on the network structures of three hydrogel systems and relate these results to their mechanical and
physical properties. SANS is the predominate technique used in these studies, however, for one system we also employ stochastic optical reconstruction microscopy (STORM), to investigate the micro-scale structure of one system.

The first hydrogel system is the tetra-functional poly(ethylene glycol) hydrogel system. Poly(ethylene glycol), or PEG, is a hydrophilic, bio-inert polymer that has already been successfully utilized commercially in a variety of applications, including drug delivery vehicles\textsuperscript{39} and total hip replacements.\textsuperscript{40} PEG can be incorporated into both physically and chemically cross-linked hydrogel networks, however, those discussed in this work are chemically cross-linked hydrogels. Specifically, the systems discussed here are formed through a novel cross-linking technique designed to minimize the formation of network defects that was developed by the group of Greg Tew in the Polymer Science and Engineering Department at UMass Amherst. This cross-linking technique utilizes an end-functionalized PEG macromer that reacts with a cross-linker with a set functionality of four. If successful, this technique would yield networks with very uniform, well-defined structures. The resulting hydrogels are optically clear and display unique mechanical properties that suggest a more ideal network structure.\textsuperscript{41} This work discusses the relative homogeneity of the network structures of these systems compared to those obtained through more common cross-linking techniques, as well as how changes in PEG molecular weight and concentration affects the network structure.

The second hydrogel system contains both hydrophilic PEG chains and hydrophobic polydimethylsiloxane (PDMS) chains, and is formed through the same unique cross-linking technique discussed above. PDMS is a hydrophobic, biocompatible polymer\textsuperscript{42} that has been utilized in a wide array of commercial products, including
contact lenses and . The addition of PDMS to the PEG network allows for control over the degree of network swelling, and therefore, the resulting hydrogel modulus. These hydrogels are also optically clear, and display the same unique mechanical properties seen in the pure PEG hydrogel systems, suggesting that their networks are also more ideal. This work discusses how the network structures of these systems are affected by changes in the ratio of PEG to PDMS chains, and their molecular weights, during cross-linking.

1.2 Overview

In this work, the characterization of three distinct hydrogel systems will be discussed. In Chapter 2, the nano-scale structure of a nearly-ideal, poly(ethylene glycol) (PEG)-based hydrogel system is characterized with SANS. Chapter 3 details the structural characterization of a highly resilient PEG/Polydimethylsiloxane (PEG/PDMS) hydrogel system with SANS. In Chapter 4, any major conclusions will be summarized and possible directions for future work will be discussed.
1.3 References


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2.1 Introduction

The need for more homogeneous polymer networks has lead to the development of cross-linking techniques that allow for greater control over the resulting network microstructure. One of the most basic chemical cross-linking techniques is the photopolymerization of end-functionalized, or telechelic, polymers. While this technique allows for some control over the cross-link density of the network,\(^1\) it does not define cross-link functionality and commonly results in the formation of cross-linked clusters in the network (i.e. high functionality cross-links).\(^2,3\) A more recent approach utilizes click chemistry to control cross-linking in networks.\(^4,5\) Click reactions are highly efficient, have high functional group tolerance, and are highly active in water making them ideal for use as a hydrogel cross-linking strategy.\(^5,6\) Hydrogels formed through click chemistry have demonstrated high elastic moduli, suggesting that this cross-linking strategy can reduce the formation of defects in the network.\(^4,7\) Greater control over the cross-link functionality was obtained through the development of multifunctional cross-linkers designed to react with a specific number of telechelic polymer chains. Small angle neutron scattering (SANS) studies have revealed that defects are still present in these networks upon swelling.\(^8^{\text{–}14}\) A recent approach by Sakai and coworkers\(^{15}\) utilized 4-arm star-shaped polymers to reduce network defects and form highly elastic, remarkably

homogeneous hydrogels. They achieved this through the use of tetra-arm PEG macromers that cross-link through activated-ester chemistry. The resulting gels, referred to as Tetra-PEG gels, were found to have a remarkably homogeneous network structure through small-angle neutron scattering (SANS) and static-light scattering (SLS) studies.

Tew and coworkers recently developed a novel cross-linking technique utilizing thiol-norbornene chemistry designed to reduce the formation of network defects, or inhomogeneities, in PEG-based hydrogels (Figure 2.1). Also referred to as a “click reaction”, thiol-ene reactions are simple, highly efficient, produce no side products, and rapidly achieve high yield. Thiol-ene chemistry has been used to form several different types of materials, including nearly ideal, uniform polymer networks. The synthesis technique developed by the Tew group utilizes thiol-norbornene chemistry with a tetra-functional thiol cross-linker to produce PEG-based networks with well-defined cross-link functionalities and a narrow distribution of the molecular weight between cross-links. The resulting hydrogels are optically clear and have displayed high toughness and resilience. Resilience is a measure of a material’s ability to deform reversibly (elastically) without loss of energy. A recent publication demonstrates that tetra-functional PEG hydrogels with an equilibrium water content greater than 95% have a resilience $\geq 97\%$ at strains of up to 300%. As network defects typically contribute to viscous losses in mechanical behavior, the high resilience values suggest that these materials may have a relatively low level of defects.
Here, we have employed small-angle neutron scattering (SANS) to investigate the network microstructure and relative homogeneity of these tetra-functional PEG networks synthesized via thiol-norbornene chemistry. Four series of gels were created by varying the initial polymer concentration of 35,000 g/mol PEG, 12,000 g/mol PEG, 8,000 g/mol PEG and 4,000 g/mol PEG. These systems will be referred to as 35K, 12K, 8K and 4K tetra-functional PEG hydrogels, respectively. Analysis of the SANS data revealed that resulting network structure was dependent on the length of the PEG macromer. We find that the network structure in D$_2$O transitions from a homogeneous network to a unique, two-phase network as the length of the PEG macromer is decreased. This effect decreased significantly for gels swollen with deuterated $N,N$-dimethylformamide (d-DMF), suggesting that clustering of hydrophobic chain ends and crosslinker occurs in the lower molecular weight gels; however, it did not disappear completely in d-DMF,
indicating that there may also be more network defects at lower molecular weights that become “locked in” to the network structure during cross-linking in d-DMF. We have validated this through fitting of empirical models to the data sets. Additionally, the model fits revealed that the mesh size of the networks were tunable within each molecular weight series, varying inversely with initial polymer concentration as expected. A study of small molecule diffusion through the networks was conducted to further quantify the mesh size of these systems. The experimentally determined diffusion coefficient for a small molecule (riboflavin) through the networks was compared to that predicted by an empirical model that utilized the mesh size determined from the SANS experiments. Experimental and theoretical diffusion coefficients were similar, further validating the results of the SANS model fitting to these systems.

2.2 Materials and methods

2.2.1 Materials

Poly(ethylene glycol) (PEG) (Mn = 35 kDa, 12 kDa, 8 kDa, 4 kDa), exo-5-norbornenecarboxylic acid, triphenylphosphine, diisopropyl azodicarboxylate (DIAD), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP), 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropionophenone (PI), and N,N-dimethylformamide (DMF) and deuterated N,N-dimethylformamide (d-DMF) were purchased from Alfa Aesar, Sigma Aldrich, Acros Organics, or Fisher and used without further purification. Riboflavin was purchased from Thermo Fisher Scientific. Riboflavin stock solutions were made with water purified using a Thermo Scientific Barnstead NANOPure® Infinity System (nanopure water) purified to 18 mΩ-cm.
2.2.2 GPC characterization

Gel permeation chromatography (GPC) was conducted with a Polymer Laboratories PC-GPC50 with two 5 μm mixed-D columns, a 5 μm guard column, and a RI detector (HP1047A), with polystyrene standards and THF as the eluent at a flow rate of 1.0 mL/min. The polydispersity indexes (PDIs) of the resulting 35K, 12K, 8K and 4K PEG macromers were found to be 1.07, 1.07, 1.04, and 1.06 respectively.

2.2.3 Hydrogel preparation

The norbornene end-functionalized PEG (n-PEG-n) precursors were prepared by the Mitsunobu reaction according to the procedure described previously. The desired amount of n-PEG-n (0.077 g, 0.10 g, 0.14 g, 0.25 g, 0.33 g, or 0.50 g) was dissolved in DMF (1 mL) to form a clear solution. The tetra-functional cross-linker, PETMP, and the PI (0.5 wt% with respect to the polymer) were added to form the precursor solution. The molar ratio of the polymer (n-PEG-n) to the cross-linker (PETMP) was 2 to 1, so the molar ratio of norbornene to thiol groups was 1 to 1. After thorough mixing, the precursor solution was transferred to the desired mold (a customized Teflon or syringe mold) and exposed to ultraviolet light with a wavelength of 365 nm for 45 min. The cross-linked gel was removed from the mold and washed with excess DMF, which was replaced three times, to remove unreacted materials. The gel then was immersed in deionized water, which was replaced daily until equilibrium swelling was reached.

2.2.4 Small-angle neutron scattering

Samples for small-angle neutron scattering (SANS) were prepared as described above, except the immersion and equilibration steps were performed in D₂O. SANS measurements were conducted on the 30 m small angle neutron scattering instrument on
the NG-7 beamline at the National Institute of Standards and Technology (NIST) Center for Neutron Research, Gaithersburg, MD. Spectra were obtained at room temperature in quartz sample cells with a path length of 2 mm. Gels were synthesized prior to placement in the sample cells according to the procedure listed above. Great care was taken to produce gels with a thickness of 2 mm and diameter of 0.75” in order to fill the cell. Once the sample was in place, excess D$_2$O was added to the cell to maintain equilibrium swollen conditions and prevent any solvent evaporation during scattering. Spectra were collected for 105 minutes per sample. The sample to detector distance varied from 1.0 to 13 m, resulting in $q$-range for these experiments of $0.003 \text{ Å}^{-1} < q < 0.5 \text{ Å}^{-1}$. Data reduction and normalization were performed using standard techniques. Model fitting to the first sample run for the 12K tetra-functional hydrogel at an initial polymer concentration of 0.14 g/mL gave non-physical results, so the values reported here are from a second, rehydrated sample.

### 2.2.5 Small molecule diffusion

Diffusion measurements were performed using a side-by-side two-chamber diffusion cell system (LabECX, Santa Clarita, CA) and measurements were taken in a method utilized by our laboratory in diffusion experiments on another system that was adapted from previously published work (Figure 2.2). Gels synthesized as disks with a thickness between 1 and 2 mm and a diameter of 40 mm. Prior to experimentation, gels were then cut to a diameter of 35 mm, allowing them to fit into a custom cartridge (schematic in Figure 2.2). The donor chamber was filled with 60 mL of riboflavin solution maintained at 75 mg/L while the receiver chamber was initially filled with 60 mL of nanopure water. The first sample was taken after the system reached pseudo-
steady state, which was approximately 3 hours after the initial start time. 700 μL samples were taken from the receiver chamber every 30 minutes for 270 minutes. To maintain a constant volume on the receiving side, 700 μL of nanopure water was added after each sample was taken. Diffusion experiments were run at 25°C. The concentration of riboflavin in the receiver side at each time point was determined by measuring the absorbance at 450 nm using a μQuant spectrophotometer (BioTek Instruments, Richmond, VA) and compared to a riboflavin standard curve. All samples were run in triplicate (n ≥ 3).

Figure 2.2: Schematic of diffusion cell experimental set-up and sample holder.
Effective diffusivity was determined from the change in riboflavin concentration in the receiver cell after pseudo-steady state was reached. For pseudo-steady diffusion through a membrane,\textsuperscript{36}

\[ V_R \frac{d c_R}{d t} = A_M j_M + Q_B \] \hspace{1cm} (2.1)

\[ j_M = \frac{D_{\text{eff}}}{L} (c_D - c_R) \] \hspace{1cm} (2.2)

Where \( V_R \) is the volume of the receiver chamber (cm\(^3\)), \( c_R \) is the concentration of riboflavin in the receiver chamber (µg/cm\(^3\)), \( A_M \) is the effective area of diffusion (cm\(^2\)), \( j_M \) is the flux through the membrane per unit area (µg/cm\(^2\)/s), \( t \) is time (s), and \( Q_B \) is the experimental baseline input of riboflavin into the receiver chamber (µg/s). In Equation 2.2, \( D_{\text{eff}} \) is the effective diffusion coefficient (cm\(^2\)/s), \( L \) is the diffusion path length (cm; gel thickness), and \( c_D \) is the concentration of riboflavin in the donor chamber (µg/ cm\(^3\)), which was kept constant at 75 µg/ cm\(^3\). \( Q_B \) was set to zero for these experiments as riboflavin is unable to penetrate the polystyrene sample holder.\textsuperscript{25} Combining Equations 2.1 and 2.2 and rearranging yields:

\[ \frac{d c_R}{(c_D - c_R)} = D' dt \] \hspace{1cm} (2.3)

\[ D' = \beta' D_{\text{eff}} \] \hspace{1cm} (2.4)

\[ \beta' = \frac{A_M}{L V_R} \] \hspace{1cm} (2.5)

Solving Equation 2.3 using the initial condition: at \( t = 0 \), \( c_R = c_{R0} \), yields Equation 2.6:
\[
\ln \left( \frac{c_D - c_{R,0}}{c_D - c_R} \right) = D' t \quad (2.6)
\]

\(D'\) was determined through a plot of \(\ln \left( \frac{c_D - c_{R,0}}{c_D - c_R} \right)\) vs. time. \(D_{\text{eff}}\) is the effective diffusivity, or permeability, for the hydrogel. It is the product of diffusivity, \(D\), and the partition coefficient \(k\) (mL riboflavin in hydrogel/mL in water), as shown by Equation 2.7.

\[
D_{\text{eff}} = Dk \quad (2.7)
\]

From this relationship, it can be seen that an increase in \(k\) (i.e. a solute’s preference for the hydrogel over aqueous solution), results in a decrease in the diffusion coefficient for a given solute.

To measure \(k\), a hydrogel with a known volume was placed in a 1.5 mL microcentrifuge tube with 1 mL of Riboflavin stock solution \((C_{\text{blk},i} = 75 \text{ mg/L})\). The sample was kept at 25°C for 24 hrs, during which time equilibrium was achieved between the gel and bulk solution. The initial and final concentration of riboflavin in the bulk solution was measured as described above, and equation 2.8 was used to calculate \(k\).\textsuperscript{25,26}

\[
k = \frac{C_{\text{gel}}}{C_{\text{bulk}}} = \frac{V_{\text{blk}}(C_{\text{blk},i} - C_{\text{blk},f})}{V_{\text{gel}}C_{\text{blk},f}} \quad (2.8)
\]

Where \(C_{\text{blk},i}\) and \(C_{\text{blk},f}\) are the initial and final concentrations of the bulk solution, \(V_{\text{blk}}\) is the volume of the bulk solution, \(V_{\text{gel}}\) is the volume of the gel, and \(C_{\text{gel}}\) and \(C_{\text{bulk}}\) are the final concentrations of riboflavin in the gel and the bulk solution, respectively. Partition coefficients were determined for 4K hydrogels with initial polymer
concentrations of 0.25 g/mL and 0.50 g/mL, as well as for the 12K hydrogel with an initial polymer concentration of 0.10 g/mL.

Reported diffusion coefficients ($D$ or $D_{\text{eff}}$), represent an average of three samples. Reported $k$ values for the 4K 0.25 g/mL hydrogel and the 12K 0.10 g/mL hydrogel represent an average of the results from 4 hydrogel sections taken from the same hydrogel. The reported $k$ value for the 4K 0.50 g/mL hydrogel represents an average of eight hydrogel sections taken from 2 different hydrogels (four sections from each gel).

2.3 Results and discussion

2.3.1 Qualitative analysis

SANS experiments were carried out on tetra-functional PEG hydrogels formed from norbornene-functionalized 4K, 8K, 12K and 35K PEG. The initial polymer concentration was varied from 0.077 g/mL to 0.50 g/mL to form a series of hydrogels at each molecular weight. Due to its high molecular weight, cross-linking reactions done with 35K PEG had consistently lower yields than the other three PEG chain lengths. This is most likely due to the lower concentration of functional groups, especially at very low concentrations for this system. Therefore, the lowest initial polymer concentration used for that series was 0.10 g/mL. An additional hydrogel was formed at an initial polymer concentration of 0.33 g/mL for the 35K series as this is the critical overlap concentration ($C^*$) for this molecular weight of PEG. Figure 2.3 contains spectra from hydrogels with varying PEG length at initial polymer concentrations of 0.10 g/mL, 0.25 g/mL and 0.50 g/mL.
Figure 2.3: Scattering spectra from tetra-functional PEG hydrogels formed with 4K, 8K, 12K, and 35K MW PEG with varying initial polymer concentrations between 0.077 g/mL and 0.50 g/mL. Spectra have been shifted for clarity.
These SANS experiments probed network structures between 180 nm and 2 nm. As the size of polymer chains used to form these networks range from 4.9 nm to 14.5 nm, the size range probed by SANS would provide structural information on the conformation of single polymer chains as well as multiple cross-linking sites (i.e. nano-scale network structures). Network defects that occur on length scales larger than 180 nm would not be captured in this SANS experiment, however, an upturn in the spectra at low $q$ would indicate their presence. These spectra plotted in Figure 2.3 qualitatively demonstrate that a change in network structure occurs with changes in the length of the PEG macromer. While all spectra contain a broad shoulder and upturn at low $q$, the shoulder becomes more pronounced as the length of the PEG is decreased. Structural differences are most striking at the highest initial polymer concentration, 0.50 g/mL, where a distinct peak forms in the spectra of the 4K hydrogel. The other three gels exhibit a broad shoulder that shifts towards lower $q$ as the molecular weight of PEG is increased.

The presence of a peak is commonly found in scattering spectra from networks with high junction functionality.$^{2, 3, 28}$ These types of networks have a higher density of polymer near the junctions, resulting in the junction points serving as scattering centers. Due to the cross-linking chemistry used to form the tetra-functional PEG networks, we would expect these systems to have a uniform junction functionality of 4 and would therefore not expect to see a peak in the scattering spectra. For the 35K and 12K gels, the absence of a maximum in the spectra confirm this low junction functionality. The presence of a broad shoulder indicates that the mesh size is fairly uniform. Similar behavior has been observed in SANS studies of polydimethylsiloxane (PDMS) and polystyrene (PS) networks with low cross-link functionalities.$^{9, 29}$ Spectra obtained from
these studies lack a correlation peak as the cross-link junctions are small in comparison to the rest of the network, preventing them from acting as distinct scattering centers.\textsuperscript{2,9,29}

While the SANS results for the 35K and 12K systems indicate a relatively homogeneous structure for the majority of the $q$ range probed, the upturn at low $q$ suggests the presence of structural heterogeneities on larger length scales. The shape of the scattering curves from the 35K and 12K tetra-functional PEG systems are notably similar to those obtained for PEG solutions.\textsuperscript{30} Scattering spectra from PEG solutions have also shown an upturn at low $q$ which has been attributed to clustering of the chains in solution. Several groups have compared scattering from a semi-dilute polymer solution to that of the cross-linked gel in order to investigate network homogeneity.\textsuperscript{9,17,18,31} For many of these systems, the scattering from the unswollen (or as-prepared) gel was similar to scattering from the semi-dilute precursor solution. However, at higher degrees of swelling, scattering from the gel began to deviate from that of the semi-dilute solution. In these instances, the high $q$ scattering from both systems remained similar, while at low $q$, the gel exhibited scattering at a higher intensity than the solution scattering.\textsuperscript{9,31} The excess in scattering was attributed to concentration fluctuations in the network that result in regions of inhomogeneity due to the formation of “hard-to-swell” zones.\textsuperscript{9,32}

We have qualitatively compared the scattering from the 35K and 12K tetra-functional PEG hydrogels to scattering from linear PEO chains in solution ($M_w = 100K$ at 90$^\circ$C, 10 wt\%) reported by Hammouda and coworkers.\textsuperscript{30} For both systems, the upturn at low $q$ is slight but still present. Matsunaga and coworkers\textsuperscript{18} observed a similar upturn in SANS from as-prepared Tetra-PEG hydrogels, which were also compared to scattering from PEG in solution (102K PEO at 10$^\circ$C reported by Hammouda and coworkers\textsuperscript{33}).\textsuperscript{18}
The 5K Tetra-PEG gel exhibits scattering that was most similar that of the linear PEG in solution at low $q$, displaying a slope greater than -2. Scattering from 10K, 20K, and 40K Tetra-PEG as-prepared hydrogels exhibit similar features to that of the linear PEG solution, however, they exhibit a lower slope at low $q$ (slope $\approx$ -2).\textsuperscript{18}

The spectra obtained for the 8K and 4K series are noticeably different than those obtained for the higher MW series. Hydrogels formed at initial polymer concentrations of 0.077 g/mL and 0.10 g/mL have two broad shoulders in their spectra, one at low $q$ and one at high $q$. As the initial polymer concentration increases from 0.10 g/mL to 0.14 g/mL, the high $q$ shoulder becomes more pronounced, decreasing in broadness and increasing in intensity. At the highest initial polymer concentration (0.50 g/mL), the high $q$ shoulder becomes more pronounced for the 8K series, and for the 4K tetra-functional hydrogel the shoulder becomes a peak. Similar spectra were obtained from SANS studies of randomly cross-linked PEG hydrogels.\textsuperscript{2, 3} The presence of a peak in the scattering spectra for these systems indicates the presence of scattering centers, and is a common feature in scattering from highly branched gels and end-cross-linked gels with high end group functionality.\textsuperscript{28} For these systems, a peak indicates clusters or highly cross-linked regions within the network. A study by Lin-Gibson and coworkers\textsuperscript{3} recently investigated the structure of randomly cross-linked 1K, 2K, 4K, and 8K PEG-dimethacrylate hydrogels through SANS. They concluded that it was reasonable to assume a network structure of cross-linked clusters in a solution like matrix. The absence of a defined peak occurred at low polymer concentrations when the polymer was too diffuse to form uniform clusters, resulting in large defects in the network. Waters and co-workers\textsuperscript{2} investigated the scattering from randomly cross-linked 3.4K, 4.6K, and 8K PEG-
diacrylate and PEG-diacrylamide hydrogels. They also observed a defined peak in the scattering spectra of these systems, and related it to the average spacing between dense cross-linked junction regions in the network.

For the tetra-functional PEG systems discussed in this paper, it is unlikely that the correlation peak seen in the spectra is due to highly cross-linked junctions as is the case in the randomly cross-linked PEG networks discussed above. The cross-linking technique used to form the tetra-functional PEG systems should result in junctions of low functionality regardless of the length of PEG macromer used. Additionally, both the 4K and 8K systems demonstrate highly resilient mechanical properties similar to the 12K and 35K systems, suggesting that their network structures are similar.  

Therefore, we believe that these structural differences between different molecular weight tetra-functional PEG hydrogels are mainly due to the formation of domains rich in the hydrophobic components of the network (norbornene end-groups and tetra-thiol cross-linker) upon swelling in water. It is important to note here that all hydrogels are synthesized in DMF, and that both the hydrophilic PEG with the norbornene end-groups and the hydrophobic cross-linker are readily soluble in DMF. The cross-linked gels are then washed with DMF to remove any unreacted material that is not connected to the network.

As the length of the PEG macromer is decreased, the ratio of hydrophobic material (norborne end-groups and tetra-thiol cross-linker) to hydrophilic material (PEG chains) in the network increases. At the same polymer concentration, there will be a greater amount of low molecular weight chains present in solution which will result in a higher cross-link density (i.e. more hydrophobic norbornene end-groups and tetra-thiol
cross-linker are contained in the gel). The reduction in PEG length also results in less hydrophilic units between the hydrophobic norbornene end-groups which could result in clustering due to end effects when the network is swollen with water. As discussed further below, there is also evidence of a higher incidence of defects in lower molecular weight samples, which also contributes to the development of a shoulder and low \( q \) scattering in the spectra. However, the structural differences observed as molecular weight decreases appears to be mainly due to a microphase separation of chain ends and cross-linker.

**2.3.2 Effect of solvent on hydrogel structure**

In order to investigate these structural differences further, we conducted a SANS experiment on tetra-functional PEG networks swollen in deuterated DMF (d-DMF). Results were obtained for the following hydrogels: 35K at 0.33 g/mL, 12K at 0.50 g/mL, 8K at 0.10 g/mL, and 4K at 0.10 g/mL (Figure 2.4). The intensity of background scattering was determined with the high \( q \) scaling approximation (Equation 2.9), where \( n \) is a high-\( q \) Porod exponent and \( B \) is incoherent background.

\[
I(q) = \frac{A}{q^n} + B
\]  

(2.9)

A linear plot of \( q^n I(q) \) vs \( q^n \) yields a linear plot that has a slope, \( B \), and intercept \( A \). The value for background determined from these plots was subtracted from the original scattering spectra. Spectra for hydrogels swollen in D\(_2\)O have been shifted up so both spectra can be seen clearly as they would otherwise overlap.
Figure 2.4: Spectra from hydrogels swollen in D$_2$O and d-DMF with background scattering subtracted.

The effect of solvent on network structure is most clearly seen for the 4K, 8K, and 12K hydrogels. Swelling these systems in d-DMF appears to result in a more homogeneous network structure for all systems, which is indicated by the reduced presence of high $q$ features that are prominent in spectra from their D$_2$O-swollen counterparts. Additionally, the slope of the upturn at low $q$ that is present for the 12K system is greatly reduced when swollen in d-DMF. These results support the theory that the networks undergo phase separation when swollen in D$_2$O. The only system that remains relatively unaffected by the change in solvent is the 35K system, which would have the highest ratio of hydrophilic to hydrophobic components due to the high MW of
the PEG macromer used. Therefore, this system would be less likely to undergo a large degree of phase separation in D₂O even at the highest initial polymer concentration as the hydrophobic content of the network is much less than the hydrophilic content. However, both D₂O and d-DMF swollen networks display an upturn at low \( q \), suggesting that the large scale inhomogeneities in these systems form independently of solvent and could possibly be the result of chain entanglements that become locked into place after cross-linking.

It is interesting to note that, while spectra from all d-DMF gels show a more homogenous structure than is obtained in D₂O, the 8K and 4K samples in d-DMF still exhibit a small upturn at low \( q \) and development of a small shoulder. These indicate that there is some presence of clusters or inhomogeneity in the polymer segment density for these lower molecular weights, even in d-DMF. Thus, the inhomogeneity in network structure that begins to appear for shorter length PEG cannot completely be attributed to phase separation. We believe there may be some structural defects in the network, such as loops or dangling ends, which become locked into place as the gels are cross-linked in d-DMF, and that these are more significant for the lower molecular weight samples. Results from swelling studies confirm that there is some deviation from ideal network behavior for lower molecular weight hydrogels in d-DMF.²⁷

### 2.3.3 Kratky plot analysis

The differences in gel structure with changes in PEG chain length and solvent can be seen more clearly by replotting the data in a Kratky plot. A Kratky plot is used to highlight scattering at high \( q \), and has been used to investigate the structure of hydrogel networks.³⁵⁻³⁹ The shape of the Kratky plot indicates the conformation of the scattering
unit. For a rod at high \( q \), \( I(q) \approx 1/q \). Therefore, a Kratky plot of scattering from a rod-like object would become linear at high \( q \) as \( q^2 I(q) = A + Bq \). Scattering from Gaussian chains at high \( q \) approximates to \( I(q) \approx 1/q^2 \), while scattering from a three-dimensional object at high \( q \) approximates to \( I(q) \approx 1/q^4 \). Therefore, Kratky plots of scattering from Gaussian chains would increase monotonically with \( q \) and would reach a plateau at high \( q \), while that for a three dimensional object should have a peak and would then decrease as \( 1/q^2 \) at high \( q \).

A large peak at low \( q \) is commonly observed in the Kratky plots of polymer gels and indicates the presence of frozen inhomogeneities in the gel network.\(^{38}\) Shinohara and coworkers\(^{36}\) and Karino and coworkers\(^{38}\) recently used the Kratky plot to provide further evidence that their cross-linking technique, which forms cross-links that can move position by sliding along polymer chains in the network, could be used to reduce inhomogeneities in the networks of their hydrogels. Kratky plots for all four tetra-functional hydrogels with varying initial polymer concentrations are shown in Figure 2.5.
Figure 2.5: Kratky plots for 35K, 12K, 8K and 4K tetra-functional hydrogels in D_2O. Background has been subtracted through the same method discussed in a previous section.

The Kratky plots for all four of the 35K and 12K hydrogels do not show a maximum and instead resemble scattering from a polymer solution, indicating the absence of large inhomogeneities. Alternatively, the Kratky plots for the 4K hydrogels yield a defined peak around \( q = 0.08 \) Å\(^{-1}\) for all initial polymer concentrations, indicating that these networks contain frozen inhomogeneities. While the location of this peak does not change, it becomes narrower and increases in intensity as the initial polymer concentration is increased. The transition between the phase-separated 4K system and the more homogeneous 12K and 35K systems is clearly represented by the Kratky plots for the 8K system. While there is no defined peak, the slope at low \( q \) is more similar to that
of the 4K system and the transition from linear scattering to a plateau is much sharper than seen in the 12K and 35K Kratky plots.

Kratky plots of scattering from networks swollen in d-DMF vs D$_2$O provide further insight into solvent-based structural changes (Figure 2.6)

![Figure 2.6: Kratky plots for 35K, 12K, 8K and 4K tetra-functional hydrogels in D$_2$O and d-DMF. Background has been subtracted through the same method discussed in a previous section.](image)

For all systems, the Kratky plots indicate that the polymer chains in the network become more swollen in d-DMF. The most drastic change occurs for the 4K system, and is seen in the elimination of the peak when the network is swollen in d-DMF.
2.3.4 Model fitting results for 35K and 12K tetra-functional hydrogels

More insight into the nano-scale structure of these networks can be obtained by fitting the SANS data with an empirical model. The scattering spectra for the 35K and 12K PEG tetra-functional hydrogel series were successfully fit with the correlation length model through a nonlinear, least squares fit (Figure 2.7).²³

Figure 2.7: Scattering spectra for 35K and 12K tetra-functional hydrogel series. Symbols indicate scattering data, while solid lines indicate the fit of the correlation length model to the data is indicated by the solid line. Spectra have been shifted for clarity.

This model was developed by Hammouda and coworkers³⁰ and has been used to analyze the scattering spectra of polymer solutions as well as scattering from other hydrogel systems.¹⁸,⁴⁰⁻⁴² Scattering intensity is modeled by Equation 2.10:

\[
I(q) = A \frac{n}{q^n} + \frac{C}{1 + (q \xi_L)_m^n} + B k g
\]  
(2.10)

where \(I(q)\) is the scattering intensity, \(q\) is the scattering vector, and \(Bkg\) is scattering from background. Parameters \(n\), \(m\), and \(\xi_L\) are the Porod exponent, the Lorentzian exponent, and the Lorentzian screening length, respectively. The Porod exponent characterizes the fractal structure of the gel, while the Lorenztian exponent characterizes the
polymer/solvent interactions and therefore describes the thermodynamics of the system. The Lorenzian screening length, $\xi_L$, is the correlation length for polymer chains$^{30}$ and in the case of a gel network gives an indication of the gel mesh size. Results of the fit of this model to the 35K and 12K SANS spectra are shown in Table 2.1.

### Table 2.1: Results from correlation length model fit to 35K and 12K PEG hydrogels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>35K PEG</th>
<th>12K PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$ (g/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>2.5 ± 0.08</td>
<td>2.1 ± 0.05</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>$13.0 ± 0.8$</td>
<td>$7.3 ± 0.2$</td>
</tr>
<tr>
<td>$m$</td>
<td>1.7 ± 0.01</td>
<td>1.7 ± 0.01</td>
</tr>
<tr>
<td>$Bkg$ (1/cm)</td>
<td>0.07 ± 8e-5</td>
<td>0.08 ± 1e-4</td>
</tr>
</tbody>
</table>

The reduced $\chi^2$ values for all the fits were equal to or less than 2.6, indicating a good agreement between the model and the data. All of the gels are mass fractal, indicated by a Porod exponent, $n$, of 2 or greater, with the exception of the 35K and 12K tetra-functional gel with an initial polymer concentration of 0.33 g/mL and 0.143 g/mL respectively. A Porod exponent, $n$, of 2 or greater suggests that the 35K and 12K tetra-functional PEG hydrogels are a one-phase system.$^{18}$ The correlation length model was also used to fit master curves of SANS from as-prepared Tetra-PEG hydrogel systems by Matsunaga et al.$^{18}$ Similar to our results, they obtained a Porod exponent of 2, which they argued suggested homogeneity of their hydrogel network structure.$^{18}$
The Lorentzian exponent, $m$, for all of 35K and 12K tetra-functional PEG hydrogels was less than or equal to 2, indicating that the polymer chains in the system are behaving as though in a good solvent. The 12K hydrogel with an initial polymer concentration of 0.143 g/mL is again an outlier, with a Lorentzian exponent slightly greater than 2 indicating a state between theta and bad solvent. The outlying values obtained for both the 12K and 35K systems at a single concentration are difficult to understand. Qualitatively, the spectra from these gels do not appear significantly different than the rest of the gels in the series. Also, mechanical and swelling data support the fact that these systems are very reproducible. However, these fitting results could indicate slight variations between samples on a nano-scale level that would not affect the macroscopic properties of these systems.

The Lorentzian screening length, or gel mesh size, decreased with increasing initial polymer concentration as expected, and with values of $3.7 \, \text{nm} \leq \xi_L \leq 13.0 \, \text{nm}$ and $2.1 \, \text{nm} \leq \xi_L \leq 6.3 \, \text{nm}$ for 35K and 12K gels. We can compare this mesh size to the end-to-end distance of 35K and 12K PEG chains. Assuming a random walk Gaussian chain confirmation, the end-to-end distance can be calculated as:

$$r_0 = bN^{v_1},$$  \hspace{1cm} (2.11)

where $r_0$ is the end-to-end distance, $b$ is the Kuhn length for PEG (0.76 nm), $N$ is the number of Kuhn segments, and $v_1$ is the scaling exponent (equal to 0.5 for an ideal Gaussian chain modeled as a random walk). Using Equation 2.11, we estimate $r_0 = 14.5 \, \text{nm}$ and $r_0 = 8.5 \, \text{nm}$ for a 35K and 12K PEG chain in an ideal Gaussian chain conformation. The correlation length found through the model fit is similar to the estimated $r_0$ at low concentrations and decreases with increasing polymer concentration.
as we would expect. We speculate that the chains are in an environment similar to a semi-dilute solution. Classic work by de Gennes\textsuperscript{45} supports the idea that the correlation length, $\xi_L$, in homogeneous gels should be equal to or less than size of the average mesh, and should not differ significantly from the correlation length of a polymer solution at the same concentration.\textsuperscript{31} A schematic of the 35K and 12K tetra-functional PEG network structure is shown in Figure 2.8.

![Figure 2.8: Representation of 12K tetra-functional PEG hydrogel network, net-like mesh structure with minimal inhomogeneities. $\xi_L$ indicates the Lorentzian screening length.](image)

2.3.5 Model fitting results for 8K and 4K tetra-functional hydrogels

Scattering spectra from both the 8K and 4K tetra-functional hydrogels contain an additional broad shoulder that becomes more well-defined at higher concentrations, and therefore the correlation length model could not be used to fit the entire scattering spectra. We hypothesize that at low concentrations the presence of two shoulders indicates two correlation lengths; the first corresponding to the formation of relatively hydrophobic domains of cross-linker and chain ends, and the second corresponding to the mesh size of the hydrophilic gel matrix. As the polymer concentration is increased, the second shoulder becomes more pronounced. In the case of the 4K system, this shoulder
transitions into a well-defined peak, indicating the presence of a sharp boundary between domains that corresponds to a specific $d$-spacing.

In an effort to elicit more information about these structural changes, we chose to fit the shoulder that appears at low $q$ for all spectra with the correlation length model (Figure 2.9). The background (calculated from Equation 2.9), was held constant during the fitting process for consistency. In spectra that contain a well-defined peak, the position of the peak maxima was determined and related to a $d$-spacing according to the relation $d = 2\pi/q$. The values obtained from the model fit and $d$-spacing analysis can be seen in Table 2.2.

![Figure 2.9: 8K and 4K tetra-functional PEG hydrogels at varying initial polymer concentrations. Symbols indicate scattering data, while solid lines indicate the fit of the correlation length model to the data. The dashed lines indicate the portion of the model that was not fit to the data. Spectra have been shifted for clarity.](image)
Table 2.2: Results of the fit of the correlation length model to 8K and 4K PEG tetra-functional hydrogels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>8K PEG</th>
<th>4K PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.077</td>
<td>0.10</td>
</tr>
<tr>
<td>$C_0$ (g/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.12</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>3.4 ± 0.09</td>
<td>3.0 ± 0.08</td>
</tr>
<tr>
<td>$m$</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>$Bkg$ (1/cm)*</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Peak Position (Å⁻¹)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d-spacing (nm)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

As expected, the Lorentzian screening length, $\xi_L$, decreased with increasing initial polymer concentration and ranged from $2.6 \text{ nm} \leq \xi_L \leq 4.0 \text{ nm}$ for the 8K series and $1.4 \text{ nm} \leq \xi_L \leq 3.2 \text{ nm}$ for the 4K series. These values were also consistently smaller than the end-to-end distance of 8K and 4K PEG chains assuming a random walk Gaussian chain confirmation ($4K \text{PEG} = 4.9 \text{ nm}$, $8K \text{PEG} = 7.0 \text{ nm}$). The Lorentzian exponents indicate good solvent conditions for the 8K system and theta to poor solvent conditions for the 4K system. This further supports our hypothesis that the features seen at low $q$ describe the structure of the swollen gel network of these systems.

The $d$-spacing found for both systems decreased as the initial polymer concentration increased, ranging from 12.3 to 11.4 nm in the 8K system and 9.2 to 8.5 nm in the 4K system. This supports our theory that these structural changes are due to phase separation. The increase in polymer concentration corresponds to an increase in hydrophobic content (e.g. norbornene end-groups and cross-linker). Therefore, it is
expected that these hydrophobic domains would grow in size and the distance between them would decrease as the concentration of polymer in the system is increased. The \( d \)-spacings are smallest in the 4K systems, likely due to the higher ratio of hydrophobic to hydrophilic content in these networks. This is also supported by the well-defined peak present in these spectra, which suggests a sharp transition between hydrophobic domains and hydrophilic gel matrix. A depiction of the resulting network structures of these systems is shown in Figure 2.10. Fitting with additional models was done in an effort to fully describe the structure of these systems. Those results are shown in Appendix A.

![Figure 2.10](image.png)

Figure 2.10: Representation of the 4K and 8K tetra-functional hydrogel network in D\(_2\)O. The two-phase, net-like mesh structure that occurs in the water swollen network contains phase-separated regions (indicated by the circles) separated by a characteristic length scale, \( d \). The mesh size in these networks is denoted by \( \xi_L \).

### 2.3.6 Model fitting for hydrogels in deuterated DMF

These spectra were all fit with the correlation length model, which yields a mesh size and information about chain conformation. Results of these fits are shown in Figure 2.11 and Table 2.3. There was a good agreement between the model fit and the data for all d-DMF networks, which was indicated by reduced \( \chi^2 \) values of 2.2 or less. However,
the model fit to the 4K system does not capture the shoulder in the low \( q \) region even though the reduced \( \chi^2 \) value of 2.0 indicates a good agreement.

Figure 2.11: Correlation length model fits to tetra-functional PEG networks swollen with \( \text{d-DMF} \). Open symbols represent the scattering spectra, while the model fit is indicated by the solid line. Spectra have been shifted for clarity.

A comparison of the model fit results for networks swollen in \( \text{D}_2\text{O} \) and \( \text{d-DMF} \) is shown in Table 2.3. The effect of solvent quality on network structure can most clearly be seen in the change in mesh size, \( \xi_L \). As expected, all systems demonstrated an increase in mesh size in \( \text{d-DMF} \) when compared to \( \text{D}_2\text{O} \). The Lorentzian exponent for gels swollen in \( \text{d-DMF} \) is less than for gels swollen in \( \text{D}_2\text{O} \), indicating that \( \text{d-DMF} \) is a better solvent for the network.
Table 2.3: Results of correlation length model fits to tetra-functional PEG networks swollen in D$_2$O and d-DMF.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D$_2$O</th>
<th>d-DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1.4 ± 0.03</td>
<td>1.7 ± 0.04</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>2.9 ± 0.1</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>m</td>
<td>1.8 ± 0.02</td>
<td>1.5 ± 0.01</td>
</tr>
<tr>
<td>Bkg (1/cm)</td>
<td>0.09 ± 2e-4</td>
<td>0.32 ± 4e-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D$_2$O</th>
<th>d-DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2.3 ± 0.02</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>2.1 ± 0.01</td>
<td>4.0 ± 0.06</td>
</tr>
<tr>
<td>m</td>
<td>2.0 ± 0.005</td>
<td>1.6 ± 0.01</td>
</tr>
<tr>
<td>Bkg (1/cm)</td>
<td>0.11 ± 2e-4</td>
<td>0.27 ± 3e-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D$_2$O</th>
<th>d-DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2.5 ± 0.13</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>3.4 ± 0.09</td>
<td>4.7 ± 0.05</td>
</tr>
<tr>
<td>m</td>
<td>1.9 ± 0.12</td>
<td>1.6 ± 0.01</td>
</tr>
<tr>
<td>Bkg (1/cm)</td>
<td>0.08</td>
<td>0.27 ± 2e-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D$_2$O</th>
<th>d-DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2.6 ± 0.12</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>2.6 ± 0.03</td>
<td>3.7 ± 0.03</td>
</tr>
<tr>
<td>m</td>
<td>2.0 ± 0.05</td>
<td>1.7 ± 0.01</td>
</tr>
<tr>
<td>Bkg (1/cm)</td>
<td>0.07</td>
<td>0.28 ± 2e-4</td>
</tr>
</tbody>
</table>

However, the resulting d-DMF mesh sizes are still less than the predicted length of the same length PEG polymer in a random walk configuration (Table 2.4) even though they are thermodynamically behaving as though in a good solvent (indicated by a Lorentzian exponent, $m$, less than 2). This, in conjunction with the persistence of the upturn at low $q$ for all spectra, indicates that there are still some inhomogeneities present in these networks.
Table 2.4: Comparison of mesh size (Lorentzian screening length) for tetra-functional PEG hydrogels in D$_2$O and d-DMF, and comparison to calculated length of PEG macromer assuming a random walk confirmation.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Lorentzian Screening Length (nm)</th>
<th>Random Walk Length (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4K</td>
<td>8K</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>2.6</td>
<td>3.4</td>
</tr>
<tr>
<td>d-DMF</td>
<td>3.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Random Walk Length (nm)</td>
<td>4.9</td>
<td>7.0</td>
</tr>
</tbody>
</table>

2.3.7 Small molecule diffusion

The theoretical mesh sizes for these tetra-functional hydrogels have been calculated by the Tew group using a variety of approaches, including scaling approximations and model fitting of stress-strain ($\sigma$-$\lambda$) curves. The mesh sizes predicted by fitting a three-chain model to the $\sigma$-$\lambda$ curves of the hydrogels in tension yielded the smallest mesh sizes (17 to 20 nm for the 12K system and 7 to 10 nm for the 4K system). However, they were still significantly larger than those found through SANS model fitting (2.1 to 6.3 nm for the 12K system and 1.4 to 3.2 nm for the 4K system). The reason for this difference is unclear.

A small molecule diffusion study was performed in an effort to validate the mesh sizes predicted through the SANS model fits, as well as demonstrate the ability to tune the mesh size by varying polymer concentration and polymer molecular weight. 12K and 4K tetra-functional hydrogels at three different initial polymer concentrations (0.10 g/mL, 0.25 g/mL, and 0.50 g/mL), were investigated in this study. Riboflavin is a small, water soluble molecule with a hydrodynamic radius of 0.58 nm and should therefore be able to pass through the networks for all gels. Effective diffusivities were determined for all
Partition coefficients were determined for the 12K system at an initial polymer concentration of 0.10 g/mL, and 4K systems at initial polymer concentrations of 0.25 g/mL and 0.50 g/mL. These partition coefficients were used to calculate a corrected diffusivity using Equation 2.7. These results are listed in Table 2.5 and are shown in Figure 2.12.

![Figure 2.12: Effective diffusivity ($D_{\text{eff}}$) and corrected diffusivity ($D_c$) values for the diffusion of riboflavin through 12K and 4K tetra-functional hydrogels.](image)

In order to validate the mesh sizes determined from the SANS model fitting, these experimentally determined diffusivities will be used to calculate a mesh size through the use of a diffusion model. Several models have been developed over the years to describe the mechanism for small molecule diffusion through hydrogel networks, and are based on one of three basic theories of diffusion: hydrodynamic theory, obstruction theory, and free volume theory. A review by Amsden provides an excellent summary of the work done in these fields. For the purposes of our work, a model based on the free volume
theory was chosen to calculate the theoretical diffusion coefficient for the tetra-functional PEG systems. Models based on the free volume theory stem from a theory originally put forward by Cohen and Turnbull\textsuperscript{47} which states that in a pure liquid, a solute diffuses by jumping between voids formed by the redistribution of liquid molecules. For a given temperature, the rate at which this occurs is dependent upon the probability of the formation of a void, or free volume, large enough to accommodate a solute of a given size.\textsuperscript{47}

In a hydrogel network, this process is complicated by the presence of the polymer chains as the distance between them must be large enough to allow the solute to pass through to allow for diffusion. Therefore, diffusion of a solute through a gel network is dependent upon the product of the probabilities of a solute molecule finding the proper free volume in the liquid and a large enough opening between polymer chains.\textsuperscript{48-52} The free volume diffusion model used in this work was developed by Lustig and Peppas,\textsuperscript{49} (Equation 2.12), and has been used to predict solute diffusion many hydrogel systems\textsuperscript{46} including and randomly cross-linked polyethylene glycol hydrogels.\textsuperscript{53}

\[
\frac{D_g}{D_0} = \left(1 - \frac{r_s}{\xi_D}\right) \exp\left(-Y \left(\frac{v_2}{1-v_2}\right)\right)
\]  

(2.12)

where $D_g$ is the diffusivity of the small molecule in the gel, $D_0$ is the diffusivity of the small molecule in water, $r_s$ is the hydrodynamic radius of the solute (for riboflavin, $r_s = 0.58$ nm), $\xi_D$ is the average mesh size (or correlation length) of the polymer network, $Y$ is the ratio of critical volume required for translational movement of the solute molecule to the average free volume per molecule of liquid (assumed to be unity),\textsuperscript{49} and $v_2$ is the
volume fraction of polymer in the swollen hydrogel. This model takes into that a solute will only pass through the mesh if it has an effective radius that is smaller than the average mesh size of the network, or correlation length, $\xi_D$, through the use of the sieving factor, $\left(1 - \frac{r_s}{\xi_D}\right)$. It was chosen over other possible models due to its simplicity and use of the scaling correlation length between cross-links, $\xi_D$, which can be directly compared to the correlation lengths determined from model fitting of the SANS spectra ($\xi_L$) from these systems (described in the previous section). The value of $D_0$ was calculated with the Stokes Einstein equation (Equation 2.13) and was found to be $4.19 \times 10^{-10}$ m$^2$/s.

$$D_0 = \frac{k_BT}{6\pi \eta r_s} \quad (2.13)$$

where $k_B$ is Boltzmann’s constant, $T$ is the temperature, $\eta$ is the viscosity of the solvent, and $r_s$ is the hydrodynamic radius of the solute. For riboflavin at 25°C, $D_0$ is $4.19 \times 10^{-10}$ m$^2$/s. The equilibrium polymer volume fractions ($v_2$) for the 12K and 4K tetra-functional hydrogel systems were determined in a previous study$^{27}$ and are shown in Table 2.5. These values, along with either the experimentally determined effective diffusivity ($D_{\text{eff}}$) or the corrected diffusivity ($D_c$) for each system were substituted into Equation 2.12 to determine $\xi_D$. The values for experimentally determined effective diffusivities ($D_{\text{eff}}$), experimentally determined partition coefficients ($k$), corrected diffusivities ($D_c$), the mesh sizes determined from the diffusion model ($\xi_D$), and the mesh sizes determined from SANS ($\xi_L$) are listed in Table 2.5.
Table 2.5: Equilibrium polymer volume fraction $v_2$, effective diffusivity ($D_{\text{eff}}$), partition coefficient ($k$), corrected diffusivity ($D_c$), mesh size predicted by diffusion model ($\xi_D$), and the mesh size predicted from SANS model fits ($\xi_L$) for 12K and 4K tetra-functional hydrogels. IPC stands for initial polymer concentration.

<table>
<thead>
<tr>
<th>12K IPC</th>
<th>$v_2$</th>
<th>$D_{\text{eff}}$ ($m^2/s$) $\times 10^{10}$</th>
<th>$k$</th>
<th>$D_c$ ($m^2/s$) $\times 10^{10}$</th>
<th>$\xi_D$ (nm)</th>
<th>$\xi_L$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10 g/mL</td>
<td>0.024 ± 0.001</td>
<td>4.00 ± 0.20</td>
<td>1.01 ± 0.07</td>
<td>3.76 ± 0.33</td>
<td>6.9 ± 0.61</td>
<td>5.8 ± 0.10</td>
</tr>
<tr>
<td>0.25 g/mL</td>
<td>0.045 ± 0.003</td>
<td>3.47 ± 0.19</td>
<td>N/A</td>
<td>N/A</td>
<td>4.4 ± 0.26</td>
<td>2.9 ± 0.02</td>
</tr>
<tr>
<td>0.50 g/mL</td>
<td>0.074 ± 0.002</td>
<td>2.22 ± 0.35</td>
<td>N/A</td>
<td>N/A</td>
<td>1.5 ± 0.23</td>
<td>2.1 ± 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4K IPC</th>
<th>$v_2$</th>
<th>$D_{\text{eff}}$ ($m^2/s$) $\times 10^{10}$</th>
<th>$k$</th>
<th>$D_c$ ($m^2/s$) $\times 10^{10}$</th>
<th>$\xi_D$ (nm)</th>
<th>$\xi_L$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10 g/mL</td>
<td>0.057 ± 0.001</td>
<td>2.84 ± 0.33</td>
<td>N/A</td>
<td>N/A</td>
<td>2.1 ± 0.24</td>
<td>2.6 ± 0.03</td>
</tr>
<tr>
<td>0.25 g/mL</td>
<td>0.086 ± 0.002</td>
<td>3.06 ± 0.55</td>
<td>1.57 ± 0.37</td>
<td>1.95 ± 0.57</td>
<td>1.2 ± 0.35</td>
<td>1.4 ± 0.34</td>
</tr>
<tr>
<td>0.50 g/mL</td>
<td>0.094 ± 0.003</td>
<td>3.18 ± 0.41</td>
<td>1.68 ± 0.11</td>
<td>1.89 ± 0.27</td>
<td>1.2 ± 0.17</td>
<td>1.4 ± 0.18</td>
</tr>
</tbody>
</table>

± One standard deviation

For both the 12K and 4K systems, the mesh sizes determined from the diffusion experiments ($\xi_D$) are very close to those determined from the model fitting of the SANS data ($\xi_L$), validating those results. It is still unclear why there is a large difference in the mesh sizes predicted from swelling data and those predicted from SANS model fitting and diffusion. However, the results of this study indicate that for more homogeneous hydrogel systems, SANS and diffusion studies are more accurate methods for determining mesh size.

The diffusion studies also provide insight into the structure of the 4K tetra-functional hydrogel systems. For hydrogels formed with initial polymer concentrations of 0.25 g/mL and 0.50 g/mL, the partition coefficient for riboflavin was found to be greater than one (1.57 and 1.68 respectively). These results suggest that for these systems, riboflavin is selectively partitioning into the hydrogel network over aqueous solution. As discussed in the previous section, the 4K tetra-functional hydrogels have more cross-linker content than the 12K tetra-functional hydrogels due to the short chain length of the
4K PEG macromer. The SANS results suggest that the cross-linker groups in the 4K tetra-functional systems undergo phase separation when the networks are swollen with water. While riboflavin is water soluble, it does have some functional groups that could cause it to selectively partition into more hydrophobic regions. The higher $k$, and therefore, $D_{\text{eff}}$, values for the 4K systems could therefore be explained by an interaction between riboflavin and the large clusters of hydrophobic cross-linker.

The $k$ value for the 12K system at an initial polymer concentration of 0.10 g/mL is essentially 1 ($k = 1.01$). SANS results for the 12K system indicate a homogeneous network structure. The absence of cross-linker clusters is explained by the large chain length of the PEG macromer and low concentration of cross-linker relative to the 4K system. Therefore, for the 12K systems it is reasonable to assume that $k$ is unity.

2.4 Conclusions

Tetra-functional chemically cross-linked 35K, 12K, 8K and 4K poly(ethylene glycol) (PEG) hydrogels with well-defined network structures were synthesized via photo-initiated thio-norbornene chemistry. A previous publication has discussed the highly resilient mechanical properties of these hydrogels, which suggests that they have more homogeneous network structures. Through qualitative analysis and model-fitting of SANS data, we have shown that the 35K and 12K tetra-functional PEG hydrogels have a remarkably homogeneous network structure with low junction functionality. SANS results from the 8K and 4K tetra-functional PEG hydrogels suggest a deviation from the homogeneous network structure seen in the 35K and 12K systems that we believe is primarily due to some segregation of the hydrophobic chain ends and cross-linker upon swelling the network in water. This effect becomes more significant as PEG chain length
decreases and is supported by spectra of gels swollen in d-DMF, which indicate a more homogeneous structure than the gels in D₂O. However, there are still some small indications of inhomogeneity for the 8K and 4K networks even in d-DMF, suggesting a higher level of defect formation during cross-linking for these systems. Diffusion of riboflavin through the 12K and 4K networks further demonstrated the effect of polymer molecular weight and concentration on mesh size. These results also validated the mesh sizes determined by SANS.
2.5 References


CHAPTER 3
HIGHLY RESILIENT POLY(ETHYLENE GLYCOL)/POLYDIMETHYLSILOXANE HYDROGELS

3.1 Introduction

Polyethylene glycol-based hydrogels are of great interest for many applications, including biomaterial and tissue engineering applications. The tetra-functional PEG-based hydrogels discussed in Chapter 2 are highly resilient and have nearly ideal network structures that can be tuned by altering the molecular weight or initial polymer concentration of PEG. These properties make them desirable for many biomaterial and tissue engineering applications. However, the use of a single, hydrophilic polymer to form these networks limits the degree to which their swelling and mechanical properties can be tuned. The introduction of a hydrophobic polymer into this system would allow for control over network swelling and therefore, the resulting mechanical properties. It would also enable additional the system to perform other functions, such as encapsulation and release of a hydrophobic active agent or transport of oxygen through the network.

The Tew group has developed tetra-functional hydrogels made with both PEG and polydimethylsiloxane (PDMS).

PDMS is a biocompatible polymer whose excellent gas permeability, high elasticity when lightly cross-linked, and prior use in biomaterial applications (i.e. contact lenses, tissue engineering scaffolds), make it an excellent candidate for incorporation into these systems. PEG/PDMS-based networks formed with different cross-linking techniques have been studied previously. Bailey and coworkers added star-shaped PDMS macromers to PEG-DA networks in an effort to exhibit some control over the
swelling properties of their PEG networks without altering the resulting mechanical properties. This type of control is important for developing a material for tissue engineering applications as it is important to understand how cells interact with the mechanical environment of a material. As the degree of swelling, or mesh size, alone can impact cell behavior, the ability to increase or decrease the modulus of a PEG-based hydrogel without changing the swelling is a desirable in this field. Erdodi and coworkers formed thin PEG/PDMS-based hydrogels with the goal of increased oxygen permeability for contact lens applications. Similar to the PDMS star-PEG-DA systems developed by Bailey and coworkers, Erdodi and coworkers found that these materials provided excellent control over the degree of swelling by simply altering the PEG/PDMS ratio. The resulting networks were found to have high oxygen permeability, tensile strength (0.71-1.0 MPa), modulus (0.67-1.10 MPa), and elasticity (elongation = 118-175%). As with the degree of swelling, these properties were also tunable by altering the PEG/PDMS ratio. The addition of PDMS to water swollen networks increases the oxygen permeability of these materials.

Utilizing the same cross-linking technique discussed in Chapter 2, the Tew group has synthesized tetra-functional PEG/PDMS hydrogels. These hydrogels are optically clear and were found to have tunable swelling properties and predictable mechanical properties that varied with the PEG/PDMS ratio (Table 3.1). They were also found to be highly resilient which indicates that the addition of PDMS likely does not introduce additional defects into the network.

Here, we have employed small-angle neutron scattering (SANS), to investigate the nanoscale structure of these systems. An initial study investigated the structure of as
series of hydrogels formed with 12K PEG and 4.5K PDMS at four molar ratios of PEG to PDMS (100/0, 70/30, 50/50, 30/70) and their corresponding dry polymer networks at three molar ratios of PEG to PDMS (70/30, 50/50, 30/70). The spectra from the dry polymer networks and swollen, PDMS-containing hydrogels contained a very pronounced peak in the spectra. This was expected as the hydrophobic PDMS chains would collapse upon themselves as the gel became swollen with water. Additionally, spectra from these systems contain several higher order peaks, suggesting a more ordered, lamellar-type structure. A second study investigated changes in network structure as the molecular weight of PEG and PDMS used were varied. Two series of hydrogels were formed at three molar ratios of PEG to PDMS (70/30, 50/50, and 30/70) and two different combinations of polymer molecular weight (4K PEG/4K PDMS, 12K PEG/10K PDMS). These systems also display a pronounced peak and several higher order peaks in their SANS spectra, again suggesting a more ordered, lamellar-type structure is occurring in these systems. The position of the peak denotes the spacing between scattering structures in the system, and can be altered by varying the polymer molecular weight and/or PEG to PDMS ratio.

3.2 Materials and methods

3.2.1 Materials

5-norbornene-2-carboxylic acid (99% exo), diisopropyl azodicarboxylate (DIAD), triphenylphosphine, poly(ethylene glycol) (PEG) (Mn = 12.3 kDa, according to 1H NMR), hydroxyl terminated polydimethylsiloxane (PDMS) (Mn= 4.4 kDa, according to 1H NMR), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP), and 2-hydroxy-4’-
(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) were purchased from Sigma Aldrich, Acros Organics, Alfa Aesar, or Gelest and used without further purification.\textsuperscript{2,3}

3.2.2 Preparation of hydrogels

Precursor solutions consisted of norbornene end-functionalized PEG (nor-PEG-nor, Mn = 12 kDa or 4 kDa) or the desired ratio of norbornene end-functionalized PEG and norbornene end-functionalized PDMS (nor-PDMS-nor, Mn = 4.5 kDa or 10 kDa), pentaerythritol terakis(3-mercaptopropionate) (PETMP), and 2-hydroxy-4’-(2-hydroxyethoxy)-2-methyl-propiophenone as photoinitiator were dissolved in THF or chlorobenzene. The precursor solutions were put into the desired mold and then exposed to ultraviolet light with a wavelength of 365 nm for 1 hour. The cross-linked gel was removed from the mold and repeatedly washed with excess THF or chlorobenzene to remove unreacted materials. Finally, the gel was immersed in excess deionized water, which was replaced daily until equilibrium swelling was reached.\textsuperscript{2,3}

3.2.3 Ultra-small- and small-angle neutron scattering (USANS and SANS)

For this study, the equilibration and swelling steps were performed in D\textsubscript{2}O. Great care was taken to form hydrogels of the correct thickness (between 1 and 2 mm), and diameter (0.75 inches). The gels were placed in quartz sample cells with a path length of 1 mm or 2 mm. After loading each sample, excess D\textsubscript{2}O was added to the sample cell to maintain swelling equilibrium and fill any voids. USANS measurements were conducted on the perfect crystal diffractometer ultra-high resolution small-angle neutron scattering instrument (BT-5 beamline) at the National Institute for Standards and Technology (NIST) Center for Neutron Research in Gaithersburg, MD.\textsuperscript{9} Spectra were collected for (INSERT TIME) per sample at 25°C. SANS measurements were conducted on the 30m
small angle neutron scattering instrument (NG-7 beamline) at NIST in Gaithersburg, MD.\textsuperscript{10} Spectra were collected for one hour and forty-five minutes per sample at 25°C. The $q$-range covered in these experiments was $0.003 \text{ Å}^{-1} < q < 0.5 \text{ Å}^{-1}$. The sample to detector distance was 1.0 to 13 m, continuously variable. Data reduction and normalization were performed using standard techniques.\textsuperscript{11}

3.3 Results and discussion

The first PEG/PDMS network that will be discussed is that formed with 12K PEG and 4.5K PDMS. A series of hydrogels, and their corresponding dry polymer networks, was formed by varying the molar ratio of PEG to PDMS. The initial and final volume fractions of PEG, PDMS, and total polymer, as well as the equilibrium swelling ratio ($Q$) for these samples are listed in Table 3.1. It is important to note that the molecular weight of the PEG macromer used to form these networks is much larger than that of the PDMS macromer, and therefore, the molar ratios do not directly correspond to the ratio of the volume of hydrophilic to hydrophobic groups in the network. This can be more clearly seen by looking at the volume fraction of PEG and PDMS in the initial and hydrated state. For a molar ratio of 70/30 PEG to PDMS, polymer network itself is actually composed of 86% by volume PEG and 14% PDMS. The polymer network with the highest amount of PDMS, a molar ratio of 30/70 PEG to PDMS, contains 50% by volume PEG and 50% PDMS.
Table 3.1: Equilibrium swelling ratios ($Q$) and volume fraction of PEG, PDMS, and total polymer in the preparation and equilibrium swollen (hydrated) states from Cui et al. \(^3\)

<table>
<thead>
<tr>
<th>PEG/PDMS Ratio</th>
<th>Preparation State</th>
<th>Hydrated State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Phi_{PEG,0}$</td>
<td>$\Phi_{PDMS,0}$</td>
</tr>
<tr>
<td>100/0</td>
<td>0.084</td>
<td>0</td>
</tr>
<tr>
<td>70/30</td>
<td>0.069</td>
<td>0.011</td>
</tr>
<tr>
<td>50/50</td>
<td>0.059</td>
<td>0.023</td>
</tr>
<tr>
<td>30/70</td>
<td>0.043</td>
<td>0.041</td>
</tr>
</tbody>
</table>

3.3.1 SANS and USANS from 12K PEG/4.5K PDMS tetra-functional dry polymer networks and hydrogels

Small-angle and ultra-small angle neutron scattering studies (SANS and USANS), were performed on 12K PEG/4.5K PDMS tetra-functional dry polymer networks and hydrogels with an initial polymer concentration of 0.10 g/mL and varying molar ratios of PEG to PDMS. USANS investigates structures from 15 μm to 240 nm, while SANS investigates structures from 180 nm to 2 nm. This size range includes the structure and of single polymer chains to the macroscopic structure of the network, yielding a comprehensive picture of the network structure for these hydrogels. The SANS results that detail the nanoscale structure of the system will be discussed first. The results from the dry polymer networks are shown in Figure 3.1.
Figure 3.1: SANS spectra from 12K PEG/4.5K PDMS dry networks with PEG to PDMS ratios of 70/30, 50/50, and 30/70. Spectra have been shifted for clarity.

The presence of a well defined peak in the spectra from the dry network indicates the presence of two phases in these networks even though they are un-swollen. It is unlikely that this peak is due to contrast between the PEG and PDMS macromers as their scattering length densities are relatively close (6.41E-7 Å⁻² and 6.35E-8 Å⁻² respectively). This has been seen in SANS from other systems containing two types of polymers.\textsuperscript{12,13} Therefore, the peak in the spectra is most likely due to the formation of domains with different structures as the network dries. One structure, or phase, is PEG-rich (crystalline) and the other is PDMS-rich domains (amorphous). The presence of a strong upturn at low \( q \) for all spectra suggests that this phase separation could be occurring on a larger scale as well (spacings >200 nm). The position of the dominate peak can be related to the space between scattering domains, or d-spacing, in the sample through the relationship \( d = \frac{2\pi}{q} \).

Interestingly, for these systems the dominate peak is followed by one or more weak,
higher-order peaks. Higher-order peaks in SANS spectra can occur due to the form factor of the scattering object (i.e. spherical structures), or as a result of the overall structure having some degree of order, such as body-centered cubic (BCC) or face-centered cubic (FCC) crystal and lamellar structures. For the PEG/PDMS hydrogel systems discussed here, it is believed that the higher-order peaks are indicative of some local order occurring in the network due to the position of these peaks relative to the initial peak.

SANS spectra from a lamella-like ordered structure contains a dominant peak at $q^*$, followed by one or more higher order peaks occurring at numeric intervals of $q^*$, (i.e. $2q^*$, $3q^*$, $4q^*$, etc.). This scattering pattern has been observed for several systems, including amphiphilic conetwork films, surfactant based hydrogels, and human stratum corneum.

PEG/PDMS dry polymer networks at all ratios of PEG to PDMS contain a dominate peak at $q^*$, followed by a weak higher-order peak occurring at approximately $2q^*$ that indicates some order is occurring in the system. Spectra from the 30/70 dry polymer network contains higher order peaks at approximately $2q^*$ and $3q^*$, indicating a greater degree of order than the systems that only display one higher-order peak. While the positions of these peaks indicate a lamellar-like structure is present in this system, their broadness and low intensity indicates that this order is mostly local (i.e. weak long-range order). Table 3.2 lists the position of the dominant peak ($q^*$), the observed positions of the higher order peaks ($q_2$, $q_3$, etc.), the theoretical positions of higher order peaks assuming a lamella-like structure ($2q^*$, $3q^*$, etc.), and the d-spacing for each sample. The spectra in Figure 3.2 are plotted in a Lorentz corrected plot ($q^2I(q) vs q$) to highlight the peaks, and the positions of the higher order peaks are marked.
Table 3.2: Analysis of peak positions for 12K PEG/4.5K PDMS dry polymer networks with PEG/PDMS ratios of 70/30, 50/50, and 30/70. The initial peak in the spectra is denoted by $q^*$, and the observed position of the higher order peaks are listed as $q_2$ and $q_3$. The peak positions that would correspond to a lamellar structure were calculated from the initial peak position and are listed as $2q^*$ and $3q^*$.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>$q^*$ (Å$^{-1}$)</th>
<th>$q_2$ (Å$^{-1}$)</th>
<th>$q_3$ (Å$^{-1}$)</th>
<th>$2q^*$ (Å$^{-1}$)</th>
<th>$3q^*$ (Å$^{-1}$)</th>
<th>d-spacing (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/30</td>
<td>0.027</td>
<td>0.056</td>
<td>-</td>
<td>0.054</td>
<td>0.081</td>
<td>23.3</td>
</tr>
<tr>
<td>50/50</td>
<td>0.024</td>
<td>0.053</td>
<td>-</td>
<td>0.048</td>
<td>0.072</td>
<td>26.2</td>
</tr>
<tr>
<td>30/70</td>
<td>0.018</td>
<td>0.031</td>
<td>0.053</td>
<td>0.036</td>
<td>0.054</td>
<td>34.9</td>
</tr>
</tbody>
</table>

The space between domains in these systems appears to increase with increasing PDMS content, and in fact the largest d-spacing (34.9 nm) is found in the dry network with a PEG to PDMS ratio of 30/70. From this information, it is assumed that the d-spacing for these systems describes the distance between PEG-rich (crystalline) regions of the dry polymer network. This same dry network also displayed two higher-order peaks, indicating a greater degree of local order than seen for the other two ratios of PEG to PDMS. As the volume fraction ratio of PEG to PDMS in the polymer network is close to 50/50, this higher degree of order is not surprising.
Figure 3.2: Lorentz corrected ($q^2 I(q)$ vs $q$) SANS peak position analysis for 12K PEG/4.5K PDMS dry networks with PEG to PDMS ratios of 70/30, 50/50, and 30/70. Background has been subtracted, spectra have been shifted and only a portion of the curve has been plotted to highlight the position of the peaks.

The full-width at half-maximum, or broadness, of the dominant peak can be related to a correlation length for which a given d-spacing occurs (i.e. distance that the ordered structure is maintained). A system with long range order, such as a crystalline
material, would display a very sharp peak. Broader peaks indicate that order is occurring on a shorter range. The PEG/PDMS hydrogels have been fit with a Lorentzian peak model (Equation 3.1) to determine the exact peak position and width.

\[ I(q) = \frac{I_0}{1 + \left(\frac{(q - q_0)}{B}\right)^2} + Bkg \]  

(Equation 3.1)

where \( I_0 \) is the peak height, \( q_0 \) is the center of the peak, \( B \) is the half-width-half-maximum (HWHM), and \( Bkg \) is the incoherent background. This model would not fit the entirety of the SANS spectra for these materials, and was therefore only fit to the portion of the curve containing the peak as shown in Figure 3.3.

Figure 3.3: Fit of Lorentzian peak model to the 12K PEG/4.5K PDMS dry polymer networks. The solid lines indicate the portion of the spectra that was fit with the model, while the dashed lines represent the unfit portion of the model.
The results of the model fit were related to d-spacing and correlation length, and are shown in Table 3.3.

Table 3.3: Results of the Lorentzian peak model fit to SANS spectra from 12K PEG/4.5K PDMS dry polymer networks.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Peak Position (Å⁻¹)</th>
<th>HWHM (Å⁻¹)</th>
<th>Bkg (1/cm)</th>
<th>d-spacing (nm)</th>
<th>Correlation Length, ξ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/30</td>
<td>0.026 ± 2e⁻⁵</td>
<td>0.007 ± 4e⁻⁵</td>
<td>0.53</td>
<td>24.1</td>
<td>44.3</td>
</tr>
<tr>
<td>50/50</td>
<td>0.023 ± 1e⁻⁵</td>
<td>0.005 ± 2e⁻⁶</td>
<td>0.43</td>
<td>27.3</td>
<td>58.2</td>
</tr>
<tr>
<td>30/70</td>
<td>0.018 ± 5e⁻⁶</td>
<td>0.003 ± 6e⁻⁶</td>
<td>0.46</td>
<td>34.9</td>
<td>101</td>
</tr>
</tbody>
</table>

The fit results indicate that the correlation length for the lamellar-like structure increases with the amount of PDMS in the system. At a PEG/PDMS ratio of 30/70, the volumetric ratio of PEG to PDMS is close to 50/50 and the correlation length spans 103 nm. This range of order spans about five times the d-spacing for the system, meaning that local order is preserved for at least five lattice distances. The dominant peak broadens as the PDMS content of the system is decreased, and the correlation length decreases from two d-spacings for the 50/50 molar ratio of PEG to PDMS to less than one d-spacing for a ratio of 70/30 PEG to PDMS. This indicates that the relative volume fractions of PEG to PDMS in the network have more influence on the resulting structure than the mole fractions of PEG to PDMS chains. A schematic of the dry polymer network is shown in Figure 3.4.
Figure 3.4: Schematic of dry polymer network structure, where $\xi$ represents the correlation length of the lamellar-like structure and $d$ represents the distance between PEG and PDMS-rich domains.

The ordered structure seen in the dry polymer networks is also represented in the SANS spectra from the swollen polymer networks (Figure 3.5). All of the hydrogels formed from 12K PEG and 4.5K PDMS are optically clear, suggesting that no macro-phase separation is occurring. However, spectra from these systems still contain a pronounced peak indicating that some nano-scale phase separation is occurring in these networks. This is expected, as the PDMS chains should collapse upon swelling with water. The peaks seen in the spectra for the PEG/PDMS hydrogels are much broader than those peaks seen in the dry polymer system, indicating that the order seen in these systems is occurring much shorter length scales. For the swollen networks, this peak can be related to the spacing between regions of the gel that are highly swollen (PEG-rich) and regions that are more collapsed (PDMS-rich). Higher-order peaks are still present in hydrogels with PEG to PDMS ratios of 70/30 and 50/50, indicating that they might
maintain some of the local, lamellar-like structure seen in the dry samples (Figure 3.5). The positions of these peaks, as well as their corresponding d-spacing, are listed in Table 3.4.

![Graph showing SANS full spectra and peak position analysis](image)

Figure 3.5: SANS full spectra and peak position analysis for 12K PEG/4.5K PDMS hydrogels with PEG to PDMS ratios of 70/30, 50/50, and 30/70. Incoherent background has not been subtracted from the plot of the full SANS spectra, however, spectra have been shifted for clarity. For the plot of the peak position analysis, incoherent background has been subtracted, spectra have been shifted and only a portion of the curve has been plotted to highlight the position of the peaks.

Table 3.4: Analysis of peak positions for 12K PEG/4.5K PDMS hydogels with PEG/PDMS ratios of 70/30, 50/50, and 30/70. The initial peak in the spectra is denoted by $q^*$, and the observed position of the higher order peaks are listed as $q_2$ and $q_3$. The peak positions that would correspond to a lamellar structure were calculated from the initial peak position and are listed as $2q^*$ and $3q^*$.

<table>
<thead>
<tr>
<th>12K/4.5K PEG/PDMS Swollen Hydrogels</th>
<th>$q^*$ (Å$^{-1}$)</th>
<th>$q_2$ (Å$^{-1}$)</th>
<th>$q_3$ (Å$^{-1}$)</th>
<th>$2q^*$ (Å$^{-1}$)</th>
<th>$3q^*$ (Å$^{-1}$)</th>
<th>d-spacing (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>$q_1$</td>
<td>$q_2$</td>
<td>$q_3$</td>
<td>$2q_1$</td>
<td>$3q_1$</td>
<td></td>
</tr>
<tr>
<td>70/30</td>
<td>0.017</td>
<td>0.045</td>
<td>-</td>
<td>0.051</td>
<td>-</td>
<td>37.0</td>
</tr>
<tr>
<td>50/50</td>
<td>0.022</td>
<td>0.047</td>
<td>-</td>
<td>0.044</td>
<td>-</td>
<td>28.6</td>
</tr>
<tr>
<td>30/70</td>
<td>0.016</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39.3</td>
</tr>
</tbody>
</table>
Hydrogels formed at PEG to PDMS ratios of 70/30 and 30/70 have the highest d-spacings at 37 nm and 39 nm respectively, while the 50/50 hydrogel has the smallest d-spacing. This is attributed to the majority of the network structure transitioning from being predominately swollen with dispersed collapsed regions to predominately collapsed with disperse swollen regions. When analyzing the SANS spectra from the hydrogel with a PEG to PDMS ratio of 70/30, it is reasonable to assume that the d-spacing is related to the distance between PDMS-rich domains as they would act as scattering centers in the PEG-rich network. As more PDMS is added to the network, the space between these PDMS-rich regions decreases as seen in the spectra from the 50/50 hydrogel. Upon further addition of PDMS to the system, however, the d-spacing increases. If this spacing were still characterizing the distance between PDMS-rich domains, this result would not make sense as with the addition of more PDMS the distance between these domains should decrease again. Therefore, what is occurring is a transition from a network that is swollen and rich in PEG macromer to one that is more collapsed and equivalent in the volume of PEG and PDMS. The d-spacing now characterizes the distance between swollen, PEG-rich regions in a predominately collapsed, PDMS-rich network. A depiction of the network transition with increasing PDMS content is shown in Figure 3.6.
Figure 3.6: Representation of structural changes in PEG to PDMS network structure with increasing PDMS content.

To probe the network structures on a larger scale, ultra-small angle neutron scattering (USANS), was performed on the 12K PEG/4.5K PDMS hydrogel series. USANS probes larger length scales (15 μm to 240 nm), and would therefore provide information on any macro-phase separation that is occurring in these systems. Combined USANS and SANS spectra are shown in Figure 3.7.
Figure 3.7: Desmeared USANS and smeared SANS spectra for 12K PEG/4.5K PDMS hydrogels with PEG to PDMS ratios of 70/30, 50/50, and 30/70. USANS spectra have been shifted to match with SANS intensity at low \(q\), and the combinations of USANS and SANS spectra have been shifted for clarity.

An analysis of the slope of the SANS and USANS spectra over at least one decade of \(q\) values can yield information about the fractal structure of the network. Mass fractal scattering occurs when the spectra decays as \(I(q) \sim q^{-D_m}\) where \(D_m\) is the mass fractal exponent and ranges from 1 to 3.\(^{18,19}\) Surface fractal scattering occurs when \(I(q) \sim q^{-(6-D_s)}\) where \(D_s\) is the surface fractal exponent and ranges from 2 to 3.\(^{19}\) The USANS data from the 70/30 and 50/50 hydrogels indicate a mass fractal structure is occurring at long length scales from these systems. Therefore at large length scales, the network structures for these gels appear to be one phase (i.e. no micro-phase separation).\(^{20}\) At smaller length scales (high \(q\)), both systems decay with \(q^{-3}\) or \(q^{-4}\) indicating sharp boundaries between phase separated regions (i.e. surface fractal scattering). The USANS and SANS spectra for the 30/70 hydrogel both decay with \(q^{-4}\),
indicating the presence of sharp boundaries between phases on the micro-and nano-scale. Therefore, as the amount of PDMS in these systems is increased, we see a transition from a mass fractal network structures to a surface fractal network with sharp boundaries between domains of PEG within a PDMS network. It is important to note that the hydrogels formed from all three ratios of PEG to PDMS are optically clear, indicating that this phase separation does not occur on a macro-scale.

The USANS data was fit with the correlation length model discussed in Chapter 2, yielding information about the correlation length for these systems (Figure 3.8) and the results are listed in Table 3.5. Similar to the trend seen in the d-spacings for the SANS data, the largest correlation lengths occurred in the 70/30 and 30/70 hydrogel systems, while the smallest occurred in the 50/50 hydrogel. This again supports the transition from a predominately swollen network to a more collapsed network upon the addition of PDMS.
Figure 3.8: Fit of correlation length model (solid line) to smeared USANS data (open symbols) from 12K PEG/4.5K PDMS hydrogels. Spectra have been shifted for clarity.

Table 3.5: Fitting results of the correlation length model to the smeared USANS spectra from 12K PEG/4.5K PDMS hydrogels.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Porod Exponent</th>
<th>Lorentzian Screening Length (nm)</th>
<th>Lorentzian Exponent</th>
<th>Bkg (1/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/30</td>
<td>6.69 ± 0.20</td>
<td>770 ± 20</td>
<td>3.85 ± 0.10</td>
<td>150 ± 10</td>
</tr>
<tr>
<td>50/50</td>
<td>5.46 ± 0.06</td>
<td>670 ± 20</td>
<td>3.57 ± 0.05</td>
<td>240 ± 20</td>
</tr>
<tr>
<td>30/70</td>
<td>5.55 ± 0.20</td>
<td>1500 ± 170</td>
<td>3.86 ± 0.10</td>
<td>1500 ± 10</td>
</tr>
</tbody>
</table>

3.3.2 SANS from 12K PEG/10K PDMS and 4K PEG/4.5K PDMS tetra-functional hydrogels

To investigate the effect of macromer molecular weight on the resulting network structure, a second SANS study was performed on PEG/PDMS systems with the same molar ratios of PEG to PDMS (70/30, 50/50, and 30/70) while varying the molecular
weights of PEG and PDMS used. One system was formed with 12K PEG and 10K PDMS while the other was formed with 4K PEG and 4.5K PDMS, which shall be referred to as the high $M_w$ and low $M_w$ systems from here on. The initial polymer concentration for the high $M_w$ system was slightly less (0.07 g/mL), than that of the low $M_w$ and 12K PEG/4.5K PDMS system (0.10 g/mL), due to solubility issues of the higher $M_w$ PDMS. For both of these systems, the molecular weights of the PEG and PDMS chains are similar, so the mole ratio of chains is similar to the volume fraction of PEG and PDMS in the polymer network. SANS spectra obtained from these systems are shown in Figure 3.9. It is important to note that for these systems, some degree of macro-phase separation was visually observed. For the lower $M_w$ PEG/PDMS system, gels formed at ratios of 50/50 and 30/70 were opaque, had some observable surface roughness, and curled in on themselves when in aqueous solution. This behavior was seen for all PEG/PDMS ratios formed with the higher $M_w$ PEG/PDMS.
Similar to the SANS results from the 12K PEG/4.5K PDMS hydrogels, SANS from the 12K/10K and 4K/4.5K PEG PDMS hydrogels contain a dominant broad peak followed by one or more higher-order peaks at regular intervals of $q^*$ (i.e. 2$q^*$, 3$q^*$, etc.). The positions of these higher-order peaks, as well as the calculated values of their positions for a lamellar-like structure ($2q^*$, $3q^*$, etc.), are listed in Table 3.6 and displayed in Figure 3.10. These peaks are most prominent in the high $M_w$ system, where up to four higher order peaks are observed in the sample with a 50/50 ratio of PEG to PDMS. This sample would have approximately equal volumes of PEG to PDMS, so this higher degree of local order is not surprising. This order is lost upon the transition to a network with predominately hydrophobic chains, as is seen in the appearance of only one higher-order peak for this system.

For the low $M_w$ system, three higher-order peaks are observed for the PEG to PDMS ratio of 70/30 and only two are seen for the other ratios. This system also does not
follow the same trend in d-spacing as the other two systems, displaying a gradually increasing d-spacing with PDMS content. This can be explained by the use of the 4K PEG as the hydrophilic macromer. SANS from PEG hydrogels formed with this same cross-linking technique revealed that hydrogels formed with 4K PEG displayed some nano-scale phase separation due to the shortness of the PEG chain and increased hydrophobic content (norbornene and cross-linker groups) of that network compared to networks formed with larger PEG chains at the same initial polymer concentration. Therefore, for the PEG/PDMS system, it is reasonable to believe that a ratio of 70/30 PEG to PDMS for this system could already have a more collapsed network structure due to the added phase separation of the hydrophobic norbornene and cross-linking groups. The d-spacing would therefore characterize the distance between swollen regions dispersed in a predominately collapsed network.

Table 3.6: Analysis of peak positions for 12K PEG/10K PDMS and 4K PEG/4.5K PDMS hydogels with PEG/PDMS ratios of 70/30, 50/50, and 30/70. The initial peak in the spectra is denoted by $q^*$, and the observed position of the higher order peaks are listed as $q_2$, $q_3$, and $q_4$. The peak positions that would correspond to a lamellar structure were calculated from the initial peak position and are listed as $2q^*$, $3q^*$, etc.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>$q^*$ (Å$^{-1}$)</th>
<th>$q_2$ (Å$^{-1}$)</th>
<th>$q_3$ (Å$^{-1}$)</th>
<th>$q_4$ (Å$^{-1}$)</th>
<th>$2q^*$ (Å$^{-1}$)</th>
<th>$3q^*$ (Å$^{-1}$)</th>
<th>$4q^*$ (Å$^{-1}$)</th>
<th>$5q^*$ (Å$^{-1}$)</th>
<th>d-spacing (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/30</td>
<td>0.012</td>
<td>0.024</td>
<td>0.062</td>
<td>-</td>
<td>0.024</td>
<td>0.036</td>
<td>0.048</td>
<td>0.060</td>
<td>52.4</td>
</tr>
<tr>
<td>50/50</td>
<td>0.014</td>
<td>0.025</td>
<td>0.047</td>
<td>0.075</td>
<td>0.028</td>
<td>0.042</td>
<td>0.056</td>
<td>0.070</td>
<td>44.9</td>
</tr>
<tr>
<td>30/70</td>
<td>0.011</td>
<td>0.025</td>
<td>-</td>
<td>-</td>
<td>0.022</td>
<td>0.033</td>
<td>0.044</td>
<td>0.055</td>
<td>57.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio</th>
<th>$q^*$ (Å$^{-1}$)</th>
<th>$q_2$ (Å$^{-1}$)</th>
<th>$q_3$ (Å$^{-1}$)</th>
<th>$q_4$ (Å$^{-1}$)</th>
<th>$2q^*$ (Å$^{-1}$)</th>
<th>$3q^*$ (Å$^{-1}$)</th>
<th>$4q^*$ (Å$^{-1}$)</th>
<th>$5q^*$ (Å$^{-1}$)</th>
<th>d-spacing (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/30</td>
<td>0.028</td>
<td>0.052</td>
<td>0.096</td>
<td>-</td>
<td>0.056</td>
<td>0.084</td>
<td>0.112</td>
<td>-</td>
<td>22.4</td>
</tr>
<tr>
<td>50/50</td>
<td>0.027</td>
<td>0.064</td>
<td>-</td>
<td>-</td>
<td>0.054</td>
<td>0.081</td>
<td>0.108</td>
<td>-</td>
<td>23.2</td>
</tr>
<tr>
<td>30/70</td>
<td>0.017</td>
<td>0.039</td>
<td>-</td>
<td>-</td>
<td>0.034</td>
<td>0.051</td>
<td>0.068</td>
<td>-</td>
<td>36.9</td>
</tr>
</tbody>
</table>
Figure 3.10: SANS peak position analysis for 12K PEG/10K PDMS and 4K PEG/4.5K PDMS hydrogels with PEG to PDMS ratios of 70/30, 50/50, and 30/70. Background has been subtracted, spectra have been shifted and only a portion of the curve has been plotted to highlight the position of the peaks.

The d-spacings found for the high M_w system display a similar trend to that of the 12K PEG/4.5K PDMS in that two extreme ratios (70/30 and 30/70 PEG to PDMS) have the highest d-spacings while the middle ratio has the lowest. This suggests that a similar transition from a more swollen network structure (PEG-rich) with dispersed collapsed regions (PDMS-rich) to a predominately collapsed structure with dispersed swollen regions.

3.4 Conclusions

The Tew group at UMass Amherst has developed a novel cross-linking technique designed to reduce the formation of inhomogeneities in hydrogel networks during cross-linking. This cross-linking technique has been applied to form tetra-functional PEG/PDMS hydrogels with varying molar ratios of PEG to PDMS. Three systems of
these hydrogels were formed with varying molecular weights of PEG and PDMS: 12K PEG/4.5K PDMS, 12K PEG/10K PDMS, and 4K PEG/4.5K PDMS, and dry networks were formed from 12K PEG/4.5K PDMS. SANS results from the dry polymer networks and hydrogels formed with 12K PEG/4.5K PDMS indicate the presence of a short range, lamellar-like order in these systems. In the dry polymer network, this structure is most likely formed by regions of PEG-rich domains (crystalline) and PDMS-rich domains (amorphous). For the hydrogel networks, this lamellar-like order is also the result of PEG-rich and PDMS-rich domains, however the PEG-rich domains are swollen while the PDMS-rich domains are more collapsed. As the PDMS content in these networks is increased, the structure transitions from a predominately swollen structure with collapsed, PDMS-rich domains to a predominately collapsed structure with regions of swollen, PEG-rich domains. USANS results indicate that this transition is also seen on larger length scales then probed with SANS.

The networks formed with 12K PEG/10K PDMS and 4K PEG/4.5K PDMS yielded SANS spectra with similar features to the 12K PEG/4.5K PDMS SANS spectra. Specifically, the short range, lamellar-like order appears to also be present in these systems. Unlike the 12K PEG/10K PDMS and 12K PEG/4.5K PDMS networks, the 4K PEG/4.5K PDMS networks appear to be predominately collapsed for all three ratios of PEG to PDMS. This is most likely due to the short PEG chains in this system and consequently higher concentration of hydrophobic cross-linker.
3.5 References


CHAPTER 4

CONCLUSIONS AND FUTURE WORK

4.1 Concluding remarks

This thesis explored the nano- to micro-scale network structures of three polymeric biomaterial systems: tetra-functional poly(ethylene glycol) (PEG)-based hydrogels, tetra-functional PEG and polydimethylsiloxane (PDMS)-based hydrogels, and a commercial contact lens system. The overall goal of this work was to develop an understanding of how formulation parameters, such as initial polymer concentration and macromer molecular weight, influence the resulting network structures. This information would allow for a better understanding of how to tune the structures of these systems to yield desired mechanical and physical properties.

Chapter 2 discussed the nano-scale network structures of highly-resilient PEG-based hydrogels that were developed by our collaborators, the Tew group in the Polymer Science and Engineering Department at the University of Massachusetts Amherst. The novel cross-linking technique used to form these systems was developed to minimize network defects, and the resulting highly resilient nature of these systems suggest a more ideal network structure. Small-angle neutron scattering (SANS) studies revealed that networks synthesized with higher M_w PEG (32K and 12K) had more ideal network structures, while those formed with lower M_w PEG (8K and 4K) had a unique, two-phase network structure. This two-phase structure was attributed to the increased hydrophobic content in these networks. Model fitting was done to elicit the mesh size of these systems, and a small molecule diffusion study was done on the 12K and 4K systems to confirm these results.
Chapter 3 discussed the network structure of hydrogels containing both hydrophilic (PEG) and hydrophobic (PDMS) macromers, and the work is again in collaboration with the Tew group. These networks were synthesized with the same cross-linking technique used to form the PEG systems discussed in Chapter 2, and also display highly resilient mechanical properties, suggesting that the addition of PDMS does not affect the homogeneous network structure of these systems. Ultra-small- and small-angle neutron scattering (USANS and SANS) studies were done on a series of hydrogels formed with the same initial polymer concentration (0.10 g/mL), with varying PEG and PDMS molecular weights (12K PEG/4.5K PDMS, 12K PEG/10K PDMS, and 4K PEG/4.5K PDMS), and molar ratios of PEG to PDMS (70/30, 50/50, and 30/70). The structure of a series of dry polymer networks (12K PEG/4.5K PDMS at PEG to PDMS ratios of 70/30, 50/50, and 30/70), were also investigated with SANS. These studies revealed the presence of a weakly-ordered network structure was present in both the dry polymer networks and hydrogel systems. The positions of the peaks in the SANS spectra for these systems suggest a lamellar like structure with equal spacing between regions that PEG-rich and those that are PDMS-rich. The distance between these regions (or d-spacing), and the correlation length of this order (i.e. distance along which this ordered structure can be seen), can be altered by varying the molar ratio of PEG to PDMS as well as the molecular weight of PEG and PDMS macromers.
4.2 Future directions

4.2.1 Tetra-functional PEG-based hydrogel system

While the average distance between phase-separated regions in the 8K and 4K systems has been determined with SANS, little is known about the structure of the collapsed regions and relative prevalence in the network. This could be better characterized with cryo-transmission electron microscopy (cryo-TEM). Cryo-TEM has been used to visualize the structures of several aqueous systems, including peptide-based hydrogels\(^2\) and poly(vinyl alcohol) hydrogels.\(^3\) While this technique would allow for further characterization of phase-separated regions, there could be some difficulty in preparing the samples as they must be very thin (100 nm or less). Access to this instrumentation can also be an issue, as it is currently not available at the University of Massachusetts Amherst. Therefore, to do this experiment, one would have to apply for and obtain time on a cryo-TEM instrument at a national lab.

The Tew group has also successfully synthesized a similar series of hydrogels using a tri-functional cross-linker.\(^4\) The swelling studies done on these systems suggest a different network structure than networks formed with the tetra-functional cross-linker, potentially containing more network defects.\(^4\) A SANS study on these systems would reveal more information about the changes in the network structures that occur with changes in cross-linker functionality.

4.2.2 Tetra-functional PEG/PDMS-based hydrogel system

While the highly resilient mechanical properties of these systems suggests that they have a more homogeneous network structure,\(^5\) \(^6\) this has yet to be confirmed. Therefore, a second SANS study should be performed on a series of tetra-functional
PEG/PDMS networks swollen in a good solvent for both PEG and PDMS, such as deuterated tetrahydrofuran (d-THF). It would also be interesting to perform a SANS study on networks swollen with a solvent that is poor for PEG but good for PDMS, such as diethyl ether. This would complement the SANS study on water swollen systems as a reversal in the d-spacing trend would further validate our analysis of the SANS spectra from water-swollen networks.

These systems would also benefit from a cryo-TEM study, as the size and shape of the phase-separated PDMS regions was not able to be determined through SANS. Cryo-TEM would allow for confirmation of the lamellar-like structure seen in the SANS spectra, give some insight into the prevalence and thickness of those regions, and would confirm the transition from a swollen network with dispersed collapsed regions to a collapsed network with dispersed swollen regions.

A study of oxygen diffusion through these systems would provide insight into the interconnectivity of the collapsed, PDMS-rich regions. The chain flexibility and bulkiness of the siloxane groups in the PDMS chain allow for enhanced oxygen diffusion through PDMS materials. If the PDMS regions in the PEG/PDMS hydrogels are interconnected, we would see greater oxygen diffusivity than if those regions are segregated from one another.
4.3 References


APPENDIX A

COMBINED MODEL FIT TO SANS FROM 8K AND 4K TETRA-FUNCTIONAL HYDROGELS

A.1 Fit of correlation length and Teubner-Strey models to 8K and 4K SANS results

Scattering spectra from both the 8K and 4K tetra-functional hydrogels contain well defined shoulders and peaks at high concentrations, and therefore could not be fit with the correlation length model alone. Instead, a linear combination of the correlation length model and the Teubner-Strey model\(^1\)\(^-\)\(^2\) were used to fit the data. The scattering spectra and the model fits for the 8K and 4K series are shown in Figure A.1.

![Figure A.1](image_url)

Figure A.1: 8K and 4K tetra-functional PEG hydrogels at varying initial polymer concentrations. Symbols indicate scattering data, while solid lines indicate the fit of the
summed correlation length model and Teubner-Strey model to the data. Spectra have been shifted for clarity.

The Teubner-Strey model was originally developed for the characterization of microemulsions, but has been used by Iannuzzi and coworkers to analyze scattering from porous hydrogels. The model is given by:

\[ I(q) = \frac{1}{a_2 + c_1 q^2 + c_2 q^4} + Bkg \]  

Once a fit has been obtained, the parameters \( a_2 \), \( c_1 \), and \( c_2 \) can be used to determine the correlation length, \( \xi \), and the characteristic length of the concentration fluctuations, \( d \), as follows:

\[ \xi = \left[ \left( \frac{1}{2} \right) \left( \frac{a_2}{c_2} \right)^{1/2} + \left( \frac{1}{4} \right) \left( \frac{c_1}{c_2} \right) \right]^{-1/2} \]  

\[ d = 2\pi \left[ \left( \frac{1}{2} \right) \left( \frac{a_2}{c_2} \right)^{1/2} - \left( \frac{1}{4} \right) \left( \frac{c_1}{c_2} \right) \right]^{-1/2} \]

A linear combination of this model with the correlation length model was used to fit the 8K and 4K scattering spectra (Figure A1). This combination is shown by Equation 4:

\[ I(q) = A_1 \left( \frac{A}{q^n} + \frac{c}{1 + (q\xi)^m} \right) + A_2 \left( \frac{1}{a_2 + c_1 q^2 + c_2 q^4} \right) + Bkg \]

where \( A_1 \) and \( A_2 \) are constants that multiply each individual model, and \( Bkg \) is the background scattering. This model fit both the 8K and 4K data remarkably well, resulting in reduced \( \chi^2 \) values of 1.8 or less for all the spectra. The model fit indicates the presence of a two-phase network structure, one phase consisting gel network similar to that found
in the 35K and 12K systems, while the other phase consists of the hydrophobic components which undergoes phase separation during the solvent exchange from D$_2$O to d-DMF. The fit results for all hydrogels in the 8K and 4K series are listed in Table 2.

Table A.1: Results of the fit of the summed Teubner-Strey and correlation length models to 8K and 4K PEG tetra-functional hydrogels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>8K PEG</th>
<th>4K PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$ (g/mL)</td>
<td>0.077</td>
<td>0.10</td>
</tr>
<tr>
<td>$n$</td>
<td>2.7 ± 0.07</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>$\xi_L$ (nm)</td>
<td>4.3 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>$m$</td>
<td>1.9 ± 0.01</td>
<td>1.9 ± 0.01</td>
</tr>
<tr>
<td>$a_I$</td>
<td>70 ± 16</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>$c_1 \times 10^4$</td>
<td>-4.3 ± 1.2</td>
<td>-2.4 ± 0.5</td>
</tr>
<tr>
<td>$c_2 \times 10^6$</td>
<td>9.8 ± 2.3</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>Bkg (1/cm)</td>
<td>0.09 ± 1e-4</td>
<td>0.09 ± 2e-4</td>
</tr>
<tr>
<td>$\xi$ (nm)</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>$d$ (nm)</td>
<td>12.7</td>
<td>12.6</td>
</tr>
</tbody>
</table>

According to Teubner and coworkers$^2$, when fitting the Teubner-Strey model to a two-phase system, the results should yield $a_2 > 0$, $c_2 > 0$ and $c_1 < 0$. All of the fit results obtained for the 8K and 4K systems follow these criteria, further validating the use of this model to fit our data. The two Teubner-Strey model parameters, $\xi$ and $d$, characterize the network on a larger scale; $d$ represents the distance between the non-network domains which have a characteristic length of $\xi$ (Figure A.2). Iannuzzi and coworkers$^3$ utilized...
these parameters in a similar way to characterization their porous hydrogels, where $\zeta$ denoted the distance between pores (or size of the network domains) and $d$ represented the distance between network domains, or pore diameter. The correlation length parameters are representative of the polymer mesh phase in the hydrogel, with the $\zeta_L$ parameter indicating the mesh size. The network structure of the 8K and 4K tetra-functional PEG hydrogels as well as a depiction of the fit parameters are shown in Figure A.2.

![Figure A.2: Representation of the 4K and 8K tetra-functional hydrogel network in D_2O.](image)

The two-phase, net-like mesh structure that occurs in the water swollen network contains phase-separated regions with a correlation length of $\zeta$ separated by a characteristic length scale, $d$. The mesh size in these networks is denoted by $\zeta_L$.

Results from the correlation length model component of the fit indicate that the polymer chains are behaving as though in a theta or poor solvent, with all Lorentzian exponents nearly or greater than 2. As expected, the Lorentzian screening length, $\zeta_L$, decreased with increasing initial polymer concentration and ranged from $1.3 \text{ nm} \leq \zeta_L \leq 4.3 \text{ nm}$ for the 8K series and $1.1 \text{ nm} \leq \zeta_L \leq 3.1 \text{ nm}$ for the 4K series. These values were also consistently smaller than the correlation length obtained for the 35K and 12K series,
as well as the end-to-end distance of 8K and 4K PEG chains assuming a random walk Gaussian chain confirmation (4K PEG = 4.9 nm, 8K PEG = 7.0 nm).

The Teubner-Strey component of the fit yielded values for $a_2$, $c_1$, and $c_2$, which were used in equations (2) and (3) to calculate $\zeta$ and $d$ for the systems. It was found that $\zeta$ ranged from $4.5\,\text{nm} \leq \zeta \leq 6.5\,\text{nm}$ for the 8K system and $3.6\,\text{nm} \leq \zeta \leq 5.5\,\text{nm}$ for the 4K system, while $d$ ranged $12.7\,\text{nm} \leq d \leq 11.0\,\text{nm}$ and $8.1\,\text{nm} \leq d \leq 9.1\,\text{nm}$ respectively.
A.2 References


APPENDIX B

SANS FROM ALGINATE/HYALURONIC ACID FILMS

B.1. Introduction and background information

In recent years, the production of scaffolds for tissue repair, drug delivery, and tissue regeneration has been of great interest in both the industrial and academic communities. Alginate\textsuperscript{1, 2} and hyaluronan\textsuperscript{3, 4} are two polydissacharides that are widely researched for their wound healing properties, and are active components in many commercially available tissue support devices. However, these systems typically exhibit poor mechanical properties and degrade too quickly for most applications. In an effort to address these issues, the Schmidt group in the Biomedical Engineering department at the University of Texas at Austin has developed a novel synthesis technique to produce films made with alginate and glycidyl methacrylated hyaluronic acid (GMHA) with robust handling properties and tunable degradation.

The films are fabricated via solvent evaporation with two subsequent cross-linking steps. Recent evidence suggests that compositional changes induce phase separation due to immiscibility during these cross-linking steps. This phase separation results in drastically different degradation and suggests that at some composition of alginate greater than 50% w/v, percolation of the GMHA occurs. Results of swelling and tensile tests confirm that an increase in porosity occurs with increasing GMHA concentration in the cast films.

In collaboration with the Schmidt group, we conducted a small-angle neutron scattering study (SANS) to investigate how the nanoscale structure of these hydrogel
films is affected by variations in the alginate/GMHA ratio as well as the cross-linking of the GMHA. To our knowledge, no research has detailed the phase separation that occurs in alginate/hyaluronan polymer films with varying composition. Any information on the nanoscale structure of these systems would be highly impactful as these materials have already generated great interest from both the academic and industrial communities.

B.2. Materials and methods

B.2.2. Materials

Alginate/GMHA films were received from the Schmidt group and were synthesized according to a proprietary procedure. Deuterated water was purchased from Fisher Scientific and was used without further purification.

B.2.3. Small-angle neutron scattering (SANS)

All samples were run at Oak Ridge National Laboratory (ORNL) Spallation Neutron Source (SNS) on the extended Q-range small-angle neutron scattering diffractometer (EQ-SANS) instrument. Spectra were obtained at 25°C in quartz sample cells with a path length of 0.5 mm. The films were pressed against the front quartz window and the excess space was filled with deuterated water. Deuterated water was used to quantify the solvent scattering. The use of two sample-to-detector distances (5 m and 1.3 m) with two different wavelength bands (starting at 10 Å and 5 Å, respectively), resulted in a $q$-range of approximately $0.0040 < q < 0.62$ Å$^{-1}$. An 8 mm sample aperture was used to collimate the incidence beam. Data reduction was conducted following standard procedures implemented in MantidPlot. This software was also used to merge the data from the two wavelength bands into a single profile.
B.3. Results and discussion

SANS was conducted on a series of four films cast with varying ratios of alginate to GMHA: 100/0, 50/50, 60/40, 67/33. The ratio of alginate to GMHA in the film affects the ability of GMHA to percolate into the alginate film. At a ratio of 50/50, the concentration of GMHA is above the percolation threshold and therefore, the final film only contains alginate. The film at a ratio of 60/40, however, GMHA can percolate into the alginate film. Based on mechanical and physical properties of this film, the Schmidt group has hypothesized that it contains clusters of GMHA. The 67/33 film is also below the percolation threshold but was additionally template with urea. Its structure should therefore contain dense fibers of polymer. The 100% alginate film (100/0) should have the smallest pore size of all the films.

The SANS spectra from these systems were fit with the two correlation length model given by Equation B.1.

\[
I(q) = \frac{A}{1 + (q\xi_1)^n} + \frac{C}{1 + (q\xi_2)^m} + B \tag{B.1}
\]

Where \(A\) and \(C\) are Lorentzian scales, \(\xi_1\) and \(\xi_2\) are correlation lengths, and \(n\) and \(m\) are Lorentzian exponents. Results of the model fit to the data are shown in Table B.1.

Table B.1: Two correlation length model fit to the SANS spectra from the Alginate/HA films.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>67/33</th>
<th>50/50</th>
<th>60/40</th>
<th>100/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>4.9 ± 1.9</td>
<td>3.3 ± 0.4</td>
<td>2.0 ± 0.7</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>(\xi_1) (nm)</td>
<td>29.0 ± 6.2</td>
<td>20.4 ± 1.1</td>
<td>23.8 ± 3.7</td>
<td>18.5 ± 1.8</td>
</tr>
<tr>
<td>(n)</td>
<td>3.2 ± 0.2</td>
<td>4.0 ± 0.1</td>
<td>3.4 ± 0.2</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>(C)</td>
<td>0.03 ± 0.002</td>
<td>0.02 ± 0.001</td>
<td>0.03 ± 0.002</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td>(\xi_2) (nm)</td>
<td>1.9 ± 0.05</td>
<td>1.8 ± 0.05</td>
<td>1.9 ± 0.05</td>
<td>2.2 ± 0.08</td>
</tr>
<tr>
<td>(m)</td>
<td>5.3 ± 0.4</td>
<td>6.4 ± 0.6</td>
<td>6.0 ± 0.5</td>
<td>28.3 ± 29.3</td>
</tr>
<tr>
<td>(Bkg (1/cm))</td>
<td>0.05 ± 7.0e-5</td>
<td>0.05 ± 7.1e-5</td>
<td>0.04 ± 6.9e-5</td>
<td>0.12 ± 9.5e-5</td>
</tr>
</tbody>
</table>
Figure B.1: SANS from alginate/hyaluronic acid films with varying ratios of alginate to hyaluronic acid. The open symbols indicate the SANS spectra, while the solid lines indicate the fit of the two correlation length model to the data.

The model fit well to all of the data with the exception of the 100% alginate film, which had a very large error for the second Lorentzian exponent ($m$). This is most likely due to the absence of the second shoulder in the scattering spectra. Spectra from systems that were cast with both alginate and GMHA contain a second shoulder around $q = 0.04$. This shoulder corresponds to the second correlation length in the model fit, and is around 2 nm for all systems cast with both alginate and GMHA. Very little difference is seen in the fit results for these systems, and the spectra are very similar. The differences in structure would most likely be seen at lower $q$, and therefore USANS or VSANS would
be best able to look at the structures. However, the introduction of GMHA induces the presence of the second shoulder as it is not seen in the pure alginate film. This suggests that some interaction between the GMHA and alginate results in the development this small scale structural change. The correlation length could indicate the presence of a very small mesh size or very small clusters. In discussions with the Schmidt group, it is not clear what could be causing the development of the second shoulder. Perhaps more information could be gained from USANS or VSANS studies.
B.4 References


C.1. Introduction and background information

Drug delivery research strives to solve or improve problems that arise when introducing a drug into the body. Targeted delivery, tunable release kinetics, enhanced therapeutic efficacy, and increased half-life of the drug being delivered are just a few benefits that new micelle and gel formulations could potentially offer.\textsuperscript{1,2} Polymeric micelle systems composed of amphiphilic polymers, such as poly(sebacic anhydride)/poly(ethylene glycol) (PSA-PEG),\textsuperscript{3} poly(capolactone)/poly(ethylene glycol) (PCL-PEG),\textsuperscript{4} and poly(lactic acid)/poly(ethylene glycol) both diblock and triblock forms, (PLA-PEG) and (PLA-PEG-PLA),\textsuperscript{5,6} are a few of these systems that have drawn much attention from the scientific community. When present in an aqueous environment, these polymeric systems self assemble into a micellar structure with the hydrophobic segments forming the inner core and the hydrophilic segments forming the outer shell (or corona).\textsuperscript{2} At increasing polymer concentrations, these micelles begin to interact with one another, allowing them to form physical hydrogels.\textsuperscript{7}

The hydrophobic blocks used in these polymeric systems typically consists of biodegradable and biocompatible polymers. In nature, long-chain unsaturated ω-hydroxyl carboxylic acids are used in the synthesis of important plant polyesters.\textsuperscript{8} Monomers such as these could become the building blocks for synthetic, bio-degradable polymer systems, however, their disadvantages lie in the complex chemistry and high costs required to produce them.\textsuperscript{9}
The Gross research group at the Polytechnic Institute of NYU has successfully synthesized a series of biobased ω-carboxy fatty acid monomers, including 1,18-cis-9-octadecenedioic acid (or oleic diacid). Their technique exploited *C. tropicalis* ATCC20962, a microorganism capable of converting n-alkanes and fatty acids to their corresponding α,ω-diacids through an ω-oxidation pathway.\(^9\)\(^{-12}\) Copolymers containing these monomer groups have not been synthesized before, so it was not previously known what properties these polymers will have once in solution.

The aim of this project was to produce and characterize amphiphilic block copolymers in which the hydrophobic block is poly(oleic diacid). As the first step towards this, alternating copolymers containing the oleic diacid monomer and various hydrophilic monomers have been synthesized by the Gross group for characterization. The work presented here details the characterization that has been done on these systems utilizing dynamic light scattering (DLS), fluorescence microscopy, and rheology. The chemical structure of the oleic diacid monomer and the structures of the four hydrophilic monomers it has been polymerized with are shown in Figure C.1.
Figure C.1: The chemical structure of the biologically derived oleic diacid monomer, as well as the structures of the diol monomers it has been polymerized with (as reported by Yixin Yang, at the Polytechnic Institute at NYU).

These monomers were used to synthesize five unique amphiphilic copolymers, which are listed in Table C.1 along with their molecular weights and polydispersity index.

Table C.1: The name, number average molecular weight, and polydispersity index for each of the five amphiphilic copolymers (as reported by Yixin Yang, at the Polytechnic Institute at NYU).

<table>
<thead>
<tr>
<th>Polymer Name</th>
<th>Molecular Weight (Mₙ)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(oleic diacid-co-sorbitol)</td>
<td>4100</td>
<td>3.2</td>
</tr>
<tr>
<td>Poly(oleic diacid-co-glycerol)</td>
<td>21,750</td>
<td>4.6</td>
</tr>
<tr>
<td>Poly(oleic diacid-co-di-glycerol)</td>
<td>11,080</td>
<td>3.1</td>
</tr>
<tr>
<td>Poly(oleic diacid-co-di-poly(ethylene glycol))</td>
<td>16,540</td>
<td>3.7</td>
</tr>
<tr>
<td>Poly(oleic diacid-co-sorbitol-co-glycerol)</td>
<td>13,710</td>
<td>3.9</td>
</tr>
</tbody>
</table>

DLS was selected as the primary method used to determine the CMC of these systems. The advantage of this method is that it allows the experimenter to determine the CMC, particle (micelle) size and polydispersity of a sample simultaneously.
C.2 Materials and methods

C.2.1 Materials

Poly(oleic diacid) (POD) copolymers were synthesized by the Gross group according to a method described in previous publications,\textsuperscript{9-12} and were characterized without further purification. Polymer solutions were made with water purified using a Thermo Scientific Barnstead NANOPure\textsuperscript{®} Infinity System (nanopure water) purified to 18 mΩ-cm. Rhodamine (R110) was purchased from Molecular Probes and was used without further purification. Polymer solutions were prepared by adding the desired amount of polymer to nanopure water, stirring this solution overnight, and allowing it to sit at room temperature for a minimum of 24 hrs prior to experimentation.

C.2.2 Particle size analysis

Dynamic light scattering (DLS) studies were performed on two different instruments. For the polymers in solution at pH of 7, a 200 mW Innova Ar-ion laser (\(\lambda = 488\) nm) with a Brookhaven Instruments BI-9000AT correlator was used. Data was collected for one or two minutes at an incident angle of 90° and a temperature of 25°C. Sample times. For the polymers in solutions of pH 8, 9, 10, and 11, a Brookhaven Instrument Corporation Zeta Plus Particle Size Analyzer (Holtzville, NY) was used. Data was collected for 30 minutes at 25°C using a wavelength of 660 nm and angle of 90°.

C.2.3. Fluorescence microscopy

For imaging experiments, a small amount of the hydrophobic dye was added to nanopure water and a drop of this solution was added to the prepared polymer solution. Images were obtained using an Olympus (Center Valley, PA) IX71 inverted
epifluorescent microscope. The hydrophobic dye was excited at a wavelength of 488 nm. Image analysis was done using ImageJ software.

C.2.4 Rheology

Rheological data was obtained on a TA-Instruments AR2000 stress-controlled rheometer with an environmental test chamber (ETC) to ensure that the desired temperature was maintained throughout the duration of the experiment. Disposable 25 mm aluminum parallel plates were used due to the adhesive nature of these polymers. Samples were loaded onto the bottom plate, and the top plate was lowered until a gap of 800 μm was reached. The sample was then sheared manually to ensure an even coating of the polymer on the geometry, and any excess polymer was removed. Stress sweeps, frequency sweeps, and viscosity measurements were performed on poly(oleic diacid-co-di-glycerol, -co-di-PEG, -co-glycerol, and -co-sorbitol-co-glycerol. Poly(oleic diacid-co-sorbitol) is solid at room temperature, so no rheological measurements were performed on this sample. Based on the results from the frequency sweep of POD-co-di-PEG, these tests were repeated at increasing temperatures ranging from 40°C to 60°C (increasing in increments of 5°C).

C.3 Results and discussion

The raw poly(oleic diacid) copolymers are yellow/green in color and have a slight odor. With the exception of poly(oleic diacid-co-sorbitol) which is a soft solid at room temperature, all of the copolymers are viscous liquids. They are impressively adherent, and are difficult to remove from surfaces (i.e. metal spatulas, lab counter, gloves, etc.). Solutions of all five polymers were prepared in nanopure water at various weight percents. Even at low weight percents, these solutions have a milky appearance. DLS
data was obtained for all five polymers at 0.004 wt%. The results reveal that while there appear to be some single chain polymers in solution in water at a pH of 7 and a temperature of 25˚C, the majority of the polymers are present in large aggregates between 56 and 2000 nm in size. An example of the average particle size distribution obtained for these solutions is shown in Figure C.2.

Figure C.2: Average particle sizes and intensity for 0.004 wt% poly(oleic diacid-co-di-PEG) in water. The intensity value for each size indicates the prevalence of that particle size in solution. For example, an intensity of 100 would mean that particles of that size are most prevalent in solution.
In an attempt to solubilize these system, different solvents were used including methanol, ethanol and tetrahydrofuran. Particle size was again measured with DLS, and revealed results similar to those in water. For poly(oleic dicacid-co-sorbitol-co-glycerol), an experiment was done to investigate the effect of solution pH on particle size. Solutions varied from pH 11 to pH 8. The results of this study revealed that pH had little to no affect on the particle size of these solutions as they still contained very large particles. An example of a typical result from this study can be seen in Figure C.3.

![Poly(oleic diacid-co-sorbitol-co-glycerol) pH11 vs. pH8](image)

Figure C.3: Average particle size and intensity for 0.05 wt% poly(oleic diacid-co-sorbitol-co-glycerol) in solutions at pH 11 (blue bars) and pH 8 (peach bars).
It is important to note again that the raw copolymer has both a color and a smell, which indicates that there may be some free monomer or cross-linking agent present. To investigate this further, the copolymer solutions were lyophilized. The recovered copolymers were solid and white in color, indicating that whatever impurity was present before had been removed. These purified copolymers were again placed into solution and DLS was used to investigate particle sizes. The results were similar to those seen from raw polymer solutions as the majority of the solution consisted of large particles.

To determine whether the large particles formed are clusters of micelles, crystalline polymer aggregates, or microgels, fluorescence experiments were carried out under microscopy. The hydrophobic fluorescent dye, Rhodamine 110, was introduced into the polymer solution. If there are clearly segregated hydrophobic domains, the dye would concentrate there and we would be able to observe this under microscopy. Crystalline domains would limit dye penetration. Images obtained for three of the polymers are shown in Figure C.4 a, b, and c.
Figure C.4 a, b, and c: Images under bright and dark field for a) poly(oleic diacid-co-di-PEG), b) poly(oleic diacid-co-sorbitol-co-glycerol), and c) poly(oleic diacid-co-sorbitol) in the presence of hydrophobic fluorescent dye, Rhodamine 110.

These images show that there are some differences between the aggregates formed by these two polymer systems. The images of poly(oleic diacid-co-sorbitol) reveal that the hydrophobic dye was either unable to penetrate the aggregate due to
crystalline domains, or that there was no clear segregation of the hydrophobic domains to attract the dye. The images of poly(oleic diacid-co-di-PEG) and poly(oleic diacid-co-sorbitol-co-glycerol) reveal that the dye was able to penetrate aggregates formed by these polymers. Therefore, it can be concluded that these aggregates are amorphous and contain clearly segregated hydrophobic regions that attracted the dye.

If these copolymers are to be synthesized and processed industrially, it is important to understand the mechanical and flow properties of the raw polymer. To do this, a rheological study was performed on all of the copolymers except poly(oleic diacid-co-sorbitol), which is a waxy solid at room temperature. The results from the viscosity study are shown in Figure C.5.

Figure C.5: Viscosity of poly(oleic diacid) copolymers at 37°C. POD stands for poly(oleic diacid).
The copolymer containing sorbitol monomers has the highest viscosity. This is unsurprising as the poly(oleic diacid-co-sorbitol) copolymer is a soft solid at room temperature, suggesting that the sorbitol groups have a high affinity for themselves. Also unsurprisingly, both polymers containing glycerol groups have similar viscosities. The copolymer containing the PEG groups has the lowest viscosity. Frequency sweeps were done for all copolymers (except poly(oleic diacid-co-sorbitol)). The results of these experiments were most interesting for poly(oleic diacid-co-di-PEG) as the $G'$ and $G''$ curves were close to reaching a cross-over point. To investigate this further, frequency sweeps were done at increasing temperatures to see if and when this cross-over occurs. The results are shown in Figure C.6.
Figure C.6: Frequency sweep at increasing temperatures for poly(oleic diacid-co-di-PEG)

For temperatures above 50°C, a cross-over point is seen at or near an angular frequency of 280 rad/s. There is also a slight decrease in viscosity decreases as temperature is increased, as see in Figure C.7.
Of greater concern for the processing of the raw copolymers is their ability to adhere to metallic surfaces and the cost that would be incurred by methods/solvents required to remove them from these surfaces. It would, therefore, be of interest to investigate post synthesis steps to purify these polymer systems before further characterization or processing.

C.4. Conclusions

A series of bio-derived, amphiphilic copolymers were synthesized by the Gross group at Brooklyn Polytechnic University. Through a novel synthetic technique, these copolymers were synthesized from hydrophobic poly(oleic diacid) copolymerized with various hydrophilic groups (sorbitol, glycerol, polyethylene glycol). The subsequent characterization of these systems by our lab revealed that when present in aqueous solution, the copolymers form large, insoluble clusters or microgels. Through fluorescence microscopy, it was found that some of these copolymers formed clusters
that contained amorphous hydrophobic domains, which could be useful for drug delivery applications. Rheological studies of the raw copolymer product revealed that these systems have relatively high viscosities, but that these viscosities can be reduced by increasing temperature. For future work, the biggest concern with these systems is their ability to adhere to surfaces. Post synthesis steps should be implemented to remove impurities before future characterization or processing.
C.5 References


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