Size Exclusion Chromatography of Poly(2-ethyl-2-oxazoline) Homopolymers and Poly(ethylene oxide)-*b*-Poly(2-ethyl-2-oxazoline) Copolymers

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Abstract

Size exclusion chromatography is the method of choice for characterizing molecular weights and molecular weight distributions of polymers. An important advancement in SEC is multidetection SEC which includes multi-angle laser light scattering, viscometry, refractive index and UV spectroscopy to analyze block and graft copolymers as well as polymers with oligomeric molecular weights. Oligomeric molecular weights present special challenges since the light scattering and viscosity detectors are more sensitive to higher molecular weights and both detectors have low molecular weight threshold values.

The molecular weights and distributions of poly(2-ethyl-2-oxazoline) oligomers and block copolymers as well as poly(2-ethyl-2-oxazoline) were investigated by SEC using multiple detectors. Both a universal calibration method and light scattering were used to determine molecular weights and molecular weight distributions. The solvent was N-methylpyrrolidone that contained 0.05M LiBr used to minimize interactions among the polymers and solvent. SEC was used to establish that the diblock copolymers had heterogeneous compositional distributions. The low molecular weights of the diblock and homopolymer made it necessary to use the universal calibration method with combined refractive index and viscometry detectors to determine absolute molecular weights.

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I dedicate this work to my children, Rachel and Nicholas Hamm, may you always dream big and reach for the stars.

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CHAPTER 1: Introduction

Size exclusion chromatography is the most commonly used method for characterizing molecular weight distributions of polymers. It is a reliable, fast and relatively inexpensive method for determining accurate molecular weights and molecular weight distributions of polymers. Molecular weight analysis is crucial for understanding properties of the materials and developing a chromatographic method for each type of polymer is important to obtain accurate results. SEC coupled to a multi-detector system allows one to gain valuable information about the polymers that extends beyond molecular weight and molecular weight distribution. This is particularly important for analyzing block and graft copolymers where one component is often utilized as a macroinitiator for polymerizing the other component. Multi-detector systems often include multi-angle laser light scattering (MALS), viscometry, UV, and differential refractive index detectors (dRI). With these systems, information such as size and shape in solution and viscometric parameters can be obtained. Chapter 2 discusses the literature relevant to size exclusion chromatography.

Chapter 3 discusses a process for validating a SEC system and the deviation encountered for polystyrene standards. This process is necessary to ensure that the instruments are in proper working order so that data is not compromised. Chapter three also includes a discussion of differences in SEC results obtained by light scattering relative to using a “universal calibration”. A series of poly(2-ethyl-2-oxazoline) (PEOX) hompolymers were investigated in this study.

The fourth chapter describes the synthesis and characterization of poly(2-ethyl-2-oxazoline) (PEOX) hompolymers. These homopolymers were synthesized via cationic

living polymerization and characterized via size exclusion chromatography. This chapter chronicles the development of a suitable method for analyzing these PEOX homopolymers. Along with the molecular weight distribution results, the chromatography also provided significant insight into the effects of various synthetic methods.

Chapter 5 describes the synthesis and characterization of poly(ethylene oxide)-*b*-poly(2-ethyl-2-oxazoline) (PEO-*b*-PEOX) diblock copolymers. These copolymers were synthesized using cationic living polymerization with tosylate counterions. This chapter uses the method defined in chapter three for the chromatographic analysis of these complex diblock copolymers. Again SEC was used to investigate effects of the synthesis techniques. The importance of a multi-detector SEC system is highlighted in this chapter as additional peaks in the viscometric chromatograms showed evidence of residual macroinitiator and PEOX homopolymer in the desired copolymer final products.

CHAPTER 2: Literature Review

2.1 Overview

This literature review will introduce size exclusion chromatography (SEC) and its multiple uses. The main focus will be on SEC as it is used to analyze synthetic and natural homo- and copolymers as well as block copolymers. The first section will introduce the topic of SEC and will include advantages and limitations of the technique. The second section will focus on SEC of various polymer types and different analytical methods. Lastly it will cover new advances in SEC such as hyphenated SEC methods.

2.2 History of SEC

SEC as it is known today is a widely used method for analyzing polymeric materials. SEC separates polymers based on hydrodynamic size.[1] The polymer types that can be analyzed span a wide range and include biopolymers, proteins, and synthetic polymers as well as subsets of each category. SEC has grown immensely in capabilities over the last five decades and certainly since the introduction of chromatographic techniques over 100 years ago. Along with expanded capabilities, the instruments and technology have been greatly advanced as has our knowledge and understanding of the separation methods.

Many scientists have contributed to the development and rapid growth of size exclusion chromatography. The Russian scientist Tswett is credited with first developing the concept and practice of chromatography over a century ago. In his first paper titled "On a New Category of Adsorption Phenomena and Their Application to Biochemical Analysis" he separated chlorophyll pigments on a column comprised of calcium

carbonate. He called this technique “the chromatographic method" and the results from a column with the bands of separation a chromatogram. [2] He further noted that the assumption should be made that the separation mechanism was not restricted to colored compounds only. This development, even though it was in the area of adsorption chromatography, led the way for research on physical separations of chemical compounds.

Many more scientists advanced the field, but much of the research focused on separations in hydrophilic gels (sometimes called gel filtration chromatography). Gel filtration chromatography was generally used to describe the separation of water-soluble molecules using columns comprised of water-swollen gels. In 1959 Porath and Flodin described a technique for separating dextran and glucose fractions through a column packed with dextran gel.[3] However, “gel filtration chromatography” gave way to gel permeation chromatography that was broader in scope. This is what is now known as SEC. Gel filtration chromatography was utilized to show that separation times of molecules could be reduced to a few hours compared to the previous time requirements of possibly weeks.[4] “Gel Permeation Chromatography” is a term that was coined by J. C. Moore in 1964 when he explored the use of organic solvent-swollen styrene-divinylbenzene crosslinked beads to separate polystyrene polymers. He demonstrated the separation of large polystyrene molecules by gel permeation chromatography. The method left many areas for improvement. For example, the columns that were used were 0.305 inches in diameter and 12 feet long.[5]

2.3 Equipment

Equipment for SEC today includes many components that are commercially available from various suppliers. A typical instrument system includes a reservoir for the mobile phase (solvent), a degassing apparatus, an isocratic pump, an injector, columns and detectors.[6] For data collection and processing a computer and software are also required. Many equipment parameters are important to ensure analytical accuracy. These parameters include precise flow rate, temperature control, precise injector volumes, fast detection and a variety of detectors.[7] Typical SEC equipment is shown in **Figure 2.1**.

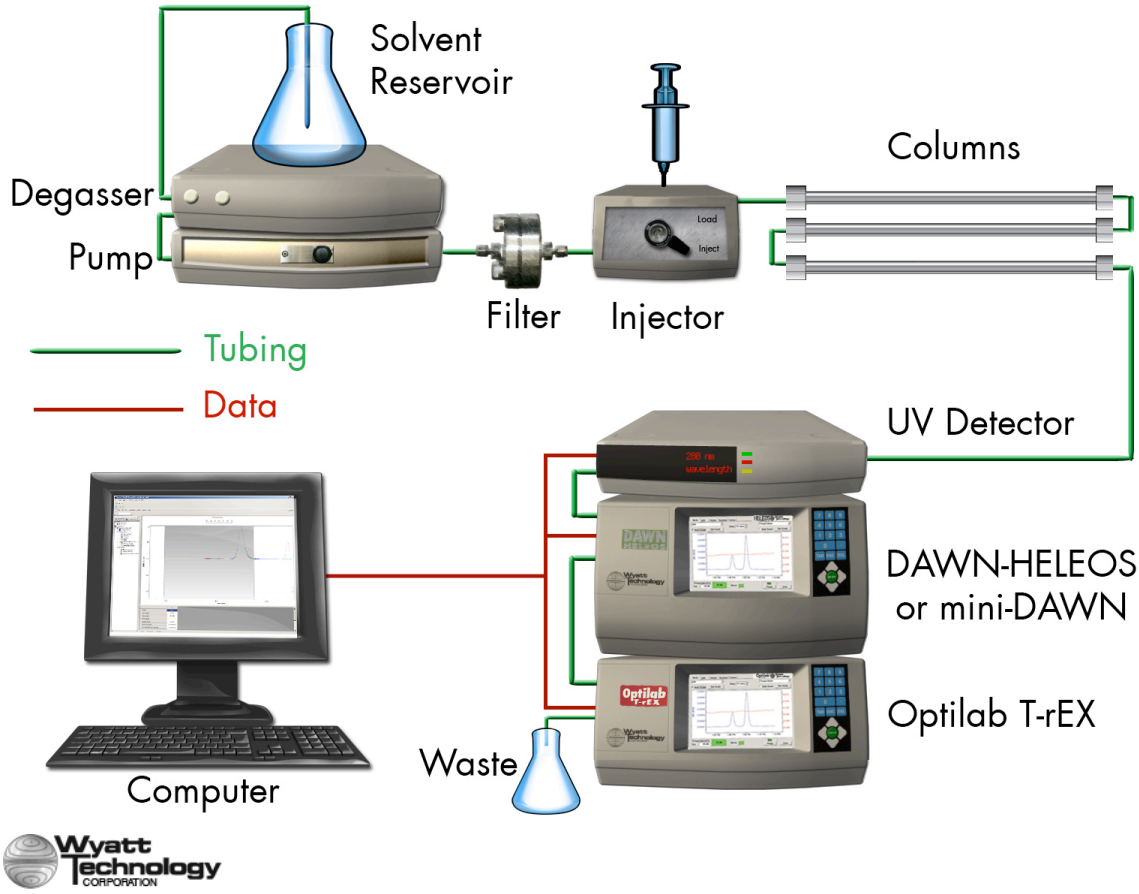


Figure 2.1: Typical SEC equipment with UV, light scattering and refractive index detectors (Figure courtesy of Wyatt Technology)

2.4 Separation of Molecules by SEC

Size exclusion chromatography separates molecules based on hydrodynamic size. The hydrodynamic size is determined by the solution properties of the polymer. The most probable conformation is the random coil.[6] In dilute solution polymer-polymer interactions are limited and polymer-solvent interactions dominate.[6] The coil rotates freely provided there are numerous single bonds, whereas multiple double bonds or aromatic structures contribute to rigidity in the conformation. Chain flexibility and polymer-solvent interactions contribute to determining hydrodynamic size, and thus mediate how the polymer transports through the column. Smaller polymers diffuse in and out of the pores in the column packing, while the larger molecules can only diffuse into the larger pores and thus elute at a lower elution volume. A schematic of this is shown in **Figure 2.2**. The curve produced from this order of elution is really a size distribution curve.[7]

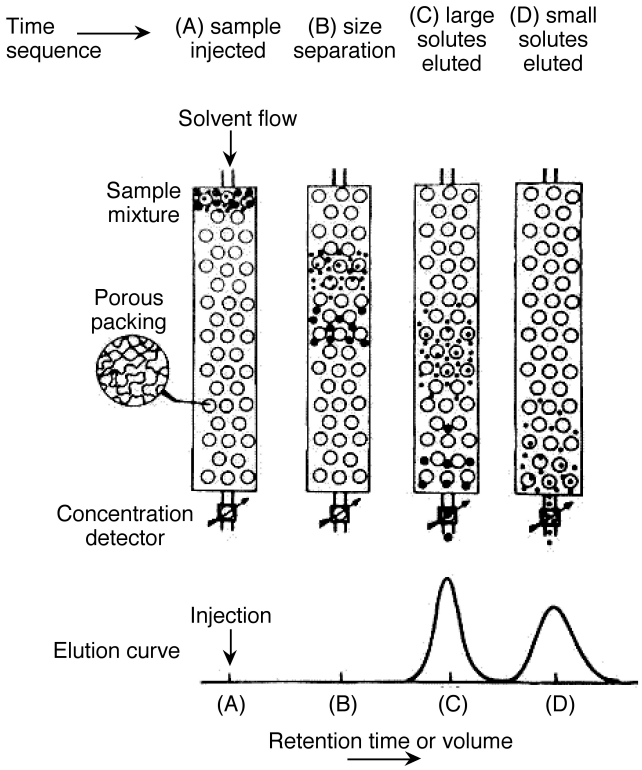


Figure 2.2: Schematic showing size separation of molecules[7]
(Figure reprinted with permission from John Wiley)

2.4.1 Molecular Weight Distribution and Molar Mass Averages

The size distribution and molecular weight distribution (MWD) or polydispersity index (PDI), are important properties of polymers. Molar mass averages are calculated from SEC chromatograms by using at least two detectors where one is a concentration detector. There are three main molar mass averages that are used in SEC. They are defined by the following equations[8]

$$\overline{M_n} = \frac{\sum_i N_i M_i}{N_i}$$

$$\overline{M_w} = \frac{\sum_i N_i M_i^2}{N_i M_i} \qquad (2.1)$$

$$\overline{M_z} = \frac{\sum_i N_i M_i^3}{N_i M_i^2}$$

where N_i is the number of molecules at each slice, and M_i is the molar mass at each eluting slice. In SEC, it is assumed that each eluting slice contains monodisperse molecules[8].

Number average molecular weight, M_n, is defined as the sum of the mass in grams over the distribution divided by the sum of the number of chains present over the distribution. Measuring M_n by SEC is challenging. For example, careful molecular weight calibration is required using a series of narrow distribution polymers if using column or universal calibration techniques. In the case of column calibration the measurement is relative to the structure of the polymers that are utilized as the standards.[9] If SEC is used in combination with multi-angle laser light scattering, weight average molecular weight, M_w, is directly calculated from the scattering intensities by first principles and is an absolute value, whereas M_n is calculated from M_w. M_n can be overestimated when measured by SEC-MALS at low molecular weight ranges since the very low molecular weights may not exhibit sufficient signal to be considered efficiently.[9]

The polydispersity (PDI) or measure of how broadly distributed the molar mass fractions of a polymer are, is defined as M_w/M_n. The closer the polymer is to being

monodisperse the closer the value is to 1.0. Polydispersity also plays a role in many polymer properties such as melt viscosity. Oftentimes polydispersity is influenced by the synthetic process. Linear step growth polymers have ideal PDI's of 2. Polymers prepared in conventional free radical polymerizations have ideal values of 1.5 (for termination by combination) or 2.0 (for termination by disproportionation), whereas controlled or living chain growth polymerizations have polydispersities nearer to 1.0.

2.5 Detectors

2.5.1 Multi-Angle Light Scattering Detector

Light scattering, whether static or dynamic, is applicable to a wide range of molar masses.[6] Light scattering is a result of light interacting with matter. In the case of polymer solutions or dispersions light is scattered by the molecules in solution. Information about the molecules is gained from the excess scattering, which is the difference between the scattered light due to the solvent relative to the scattered light from the macromolecular solution. The theory of light scattering has been explained in detail in many reviews and books and will only be briefly mentioned here. One such review is given by P. J. Wyatt.[8] Light scattering is based on two principles. First, the amount of scattered light is proportional to the concentration and molar mass of the sample as shown in **Equation 2.2** where $I(\theta)$ is the intensity of scattered light, M is the molar mass, c is the concentration of the sample and *dn/dc* is the differential refractive index.

$$I(\theta)_{scattered} \alpha \; Mc\left(\frac{dn}{dc}\right)^2 \qquad (2.2)$$

Secondly, the angular variation of scattered light is related to the size of the molecule. The two principles are combined in **Equation 2.3** where $I(\theta)$ is the intensity of scattered light, $R(\theta)$ is the excess Rayleigh ratio, K* is the physical constant for vertically polarized light defined by **Equation 2.4**, M is the molar mass, c is the concentration, $P(\theta)$ is the function that describes the large particle size effect or the ratio that occurs from the actual scattering and the scattering that occurs from small particles, and A_2 is the second virial coefficient.

$$I_{scattered}(\theta) \ \alpha \ R(\theta) \ = \ K^* McP(\theta)\left[1 - 2A_2 McP(\theta)\right] \quad (2.3)$$

Equation 2.4 defines the Rayleigh-Gans-Debye limit. The excess Rayleigh ratio is measured by the multi-angle laser light scattering instrument. This is shown in **Equation 2.4**

$$R(\theta) \ = \ K^* McP(\theta)\left[1 - 2A_2 McP(\theta)\right] \quad (2.4)$$

where $R(\theta)$ (the excess Rayleigh ratio) is the ratio of scattered and incident light intensity corrected for size of the scattering volume and distance from the scattering volume. K* is defined by **Equation 2.5**. M is molar mass in g/mole and c is concentration in g/mL. $P(\theta)$ is the scattering function and relates the scattering intensity to the mean square radius of the polymer. A_2 is the second virial coefficient that defines the interaction between the polymer and solvent In a good solvent A_2 goes to zero, so the second term of the equation has little affect in SEC. K* is defined by **Equation 2.5**

$$K^* = \frac{4\pi^2 n_0^2}{N_A \lambda_0^4}\left(\frac{dn}{dc}\right)^2 \quad (2.5)$$

where n_0 is the solvent refractive index, N_A is Avogadro's number, λ_0 is the vacuum wavelength of incident light, and dn/dc is the specific refractive index increment of the solution containing the dissolved polymer.

Figure 3 shows a representative image of a multi-angle laser light scattering cell and photodiodes incorporated into a multi-angle laser light scattering instrument.

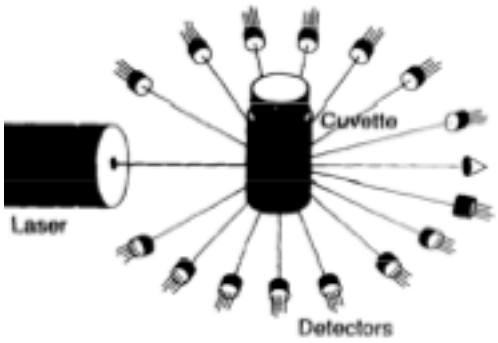


Figure 2.3: Multi-angle light scattering schematic showing multiple detectors around the sample flow cell or cuvette[8] (Figure courtesy of Wyatt Technology)

2.5.2 Refractive Index Detector

The refractive index (RI) detector is usually one of the main components of an SEC system. The refractive index is a measure of how light bends as it passes through one medium to another. The RI detector can serve as the concentration detector for either a SEC-MALS experiment or SEC-viscosity experiment. In conjunction with either of these additional detectors determination of molar mass averages are possible. An essential part of determining the molar mass averages with either set of detector types is knowledge of the dn/dc or specific refractive index increment. The dn/dc is dependent upon the chemical composition of the polymer, the temperature, and the wavelength of the incident radiation of the instrument.[7] The dn/dc is a measure of how the refractive

index of the solution changes with concentration. With a refractive index detector this can be measured in two ways. One method is to inject a series of polymer samples with varying concentration into the detector using a syringe pump or injection box. The data is plotted on a dRI (differential refractive index) versus concentration graph. The slope of the line is equal to the dn/dc of the analyte in that solvent. The second method is to measure the dn/dc by running a chromatogram with a known concentration of polymer in solution, assuming 100% recovery of the polymer, then calculating the dn/dc from the peak area.

In SEC, it is usually assumed that the dn/dc is consistent across the molecular weight distribution of a homopolymer.[7] However, due to chemical heterogeneity it can vary across the distribution of random, block, and alternating copolymers. In this case the dn/dc can generally be expressed as a weight fraction of each component, but this does not lead to accurate SEC molecular weights if it is not constant across the distribution.[7]

2.5.3 Viscosity Detector

Specific viscosity can be measured with a SEC system comprised of a concentration detector and viscosity detector. Specific viscosity is defined by **Equation 2.6** shown below.

$$\eta_{sp} = \eta/\eta_0 - 1 \quad (2.6)$$

This is measured by the viscometer detector from an equation relating the differential pressure across a capillary bridge. The differential viscometer equipment is shown in **Figure 4**.

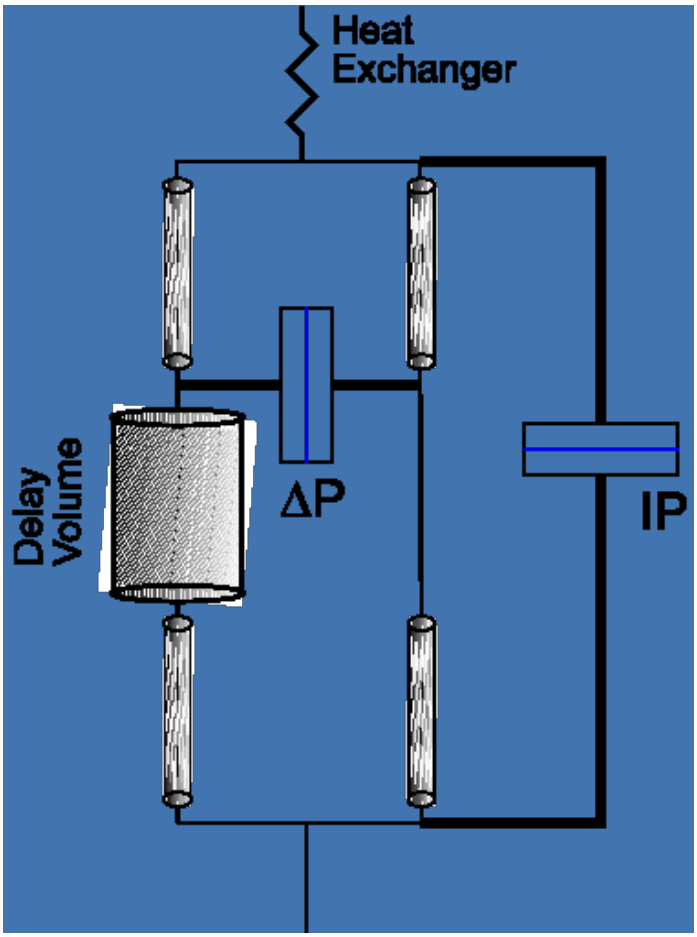


Figure 2.4: Schematic of the inside of a differential viscosity detector (Figure courtesy of Wyatt Technology)

In differential viscometry the polymer solution and pure solvent are split upon injection into the viscometer. The polymer solution flows through one side while the pure solvent flows through the other side creating a pressure difference.[7] The specific viscosity is then defined by **Equation 2.7**.

$$\eta_{sp} = 4\Delta P/(1P - 2\Delta P) \quad (2.7)$$

Where η_{sp} is the specific viscosity, ΔP is the imbalance pressure across the bridge, and IP is the pressure from the top to the bottom of the bridge.

Intrinsic viscosity can then also be determined through **Equation 2.8**

$$[\eta] = \lim_{c \to 0} \eta_{sp} / c \qquad (2.8)$$

In **Equation 2.8** [η] is the intrinsic viscosity, η_{sp} is the specific viscosity and c is the concentration. Specific viscosity is defined by **Equation 2.9**. Where η is the viscosity of the sample and η_0 is the viscosity of the pure solvent.

$$\eta_{sp} = \eta / \eta_0 - 1 \qquad (2.9)$$

Other important parameters can also be determined with the viscosity detector. The Mark-Houwink parameters can be determined along with the hydrodynamic radius. The Mark-Houwink parameters relate intrinsic viscosity to molar mass through **Equation 2.10**. Where [η] is intrinsic viscosity, M is molar mass and K and a are constants.

$$[\eta] = KM^a \qquad (2.10)$$

These constants are for a given polymer, solvent, temperature system and are available in the literature for many such systems. Using information from the viscosity detector these constants can be determined by plotting intrinsic viscosity versus molar mass of a series of the particular materials with a known molecular weight on a double logarithmic scale. The intercept then equals K and the slope the a value. This "a" value provides insight relative to the conformation of the polymer where low values represent a random coil structure and a value around 2 represents rigid rods. When molar mass averages cannot be determined through use of SEC-MALS due to insufficient sensitivity, the viscosity detector along with a concentration detector can be used to create a universal calibration curve. This calibration can also yield absolute molar mass averages.

2.6 Calibration Methods

SEC-MALS is an absolute method that does not require calibration. However, there are times when SEC-MALS may not be applicable. This can result from low molar mass or low dn/dc. When this is the case a calibration must be used to determine molar mass. Two common calibration methods are a relative calibration (commonly referred to as column calibration) and a universal calibration.

2.6.1 Column Calibration or Relative Calibration

Column calibration is a relative method where a series of narrow molecular weight standards are used. Most commonly these standards are narrow distribution polystyrene prepared by living anionic polymerization, but others are also available such as poly(ethylene oxide) and poly(methyl methacrylate). These narrow molecular weight standards are analyzed via SEC and the peak apexes are assigned known molar mass values provided by the manufacturer.[7] A calibration curve is then constructed with log M_p vs retention volume. This curve is used to find the molar mass of the unknown sample by relating elution volume to log M_p on the calibration curve. The samples have to be analyzed on the same columns, with the same flow rate, solvent and temperature.[6] This method however has limitations. The calibration only yields absolute molecular weights when the chemical structure of the analyte is the same as the standards. This is due to the fact that different polymers interact with the solvent differently and do not have the same molecular weight-hydrodynamic size relationship.

2.6.2 Universal Calibration

Due to the limitations of the column calibration technique there was a need for a better calibration method that would apply to most polymers. In 1967 Benoit proposed a

universal calibration method.[10] The universal calibration is based on the fact that SEC separates by hydrodynamic volume, and therefore every species eluting at the same elution volume are the same in size.[6] The Einstein viscosity relationship in **Equation 2.11**

$$[\eta]M = K(V/M) \qquad (2.11)$$

shows that $[\eta]$, the limiting viscosity index, is related to V (the hydrodynamic volume of the particles), M (their molecular weight) and a constant K. This equation shows that $[\eta]M$ is a direct measure of the hydrodynamic volume of the particles.[10] Benoit suggested that log $[\eta]M$ be used instead of log M as in the column calibration method. He was able to show empirically that a series of different polymers ranging from narrowly-distributed linear polystyrenes and polydienes to graft copolymers of polystyrene and poly(methyl methacrylate) fell on the same calibration curve and could thus be utilized to calculate absolute molecular weights.

Universal calibration requires a viscosity detector and a concentration (usually refractive index) detector. A series of known narrow molecular weight polymer standards are analyzed that cover the range of expected molecular weights of the unknown samples. A plot of $\log[\eta]M$ versus elution volume is used to create a universal calibration curve. As with any method there are still limitations. It has been reported that deviations in absolute molecular weights using the universal calibration can occur with highly polar polymers, ionic polymers, or polymers measured in theta solvents and it has also been shown to fail with low molar mass oligomers because the viscosities are too low to obtain sufficient signal to noise ratios with the viscosity detector.[6] Chance *et al.* reported the failure of the universal calibration for oligomeric polystyrene (MW less than 1,000

g/mole) due to the negative value of the intrinsic viscosity in this low molecular weight range.[11] The calibration can also fail in this range because polymer characteristics that are constant at high molecular weight range such as dn/dc and density may not be constant at low molecular weight for many polymers due to endgroup effects.

2.7 Multi-Detector SEC

Every polymer has a story, a story that tells how it has been synthesized, how it interacts with other components in a solution or column, its size, and its composition. SEC tells the part of the story of the polymer's size and at times the effects of synthetic conditions. Multiple detectors such as MALS, viscosity, RI, and UV are the authors of this story.

2.7.1 Multi-Detector SEC for Analysis of Star Polymers and Micelles

Three, four and six arm star poly(methyl methacrylate) polymers were synthesized by group transfer polymerization.[12] The authors wanted to investigate if "core first" synthesis had occurred. In order to show the size of the polymers and molecular weights, SEC with MALS, RI, and viscosity was used. They showed that star polymers with the expected molecular weights were created and cleavage of the arms showed that the core remained intact. Using the viscosity data they confirmed from the shrinking factors that the synthesis had been successful by the core first method. The shrinking factor was defined as a ratio of the intrinsic viscosity of a branched polymer to the intrinsic viscosity of a linear polymer with the same molecular weight. For a branched polymer the shrinking factor is less than 1. In this paper the authors showed that the shrinking factor was less than 1 so they concluded that they had branched molecules. The molecular weights were measured by universal calibration and light

scattering. They found that the molecular weights measured by light scattering were closer to the theoretical values. This is due to the fact that branched molecules are often higher in molecular weight but have a smaller hydrodynamic volume causing them to elute later than linear polymers of the same molecular weight.

In contrast Cho et al. used an arm first method of synthesis to create star polymers of a poly(ethylene glycol) base with a cationic degradable core created by the addition of 2-(dimethylamino)ethyl methacrylate with a bis(2-methacryloyloxyethyl) disulfide crosslinker for siRNA delivery. These polymers were analyzed using SEC-MALS for M_w measurements and by a universal calibration for M_n and PDI calculations using Polymer Standard Services polystyrene-DVB columns in THF at 35° C.[13] By universal calibration the M_n values were around 40,000 g/mol and by light scattering the M_w values were around 90,000 g/mol. They showed that the M_w measured by MALS was higher than the apparent molecular weight measured by universal calibration. The authors attributed this to the compact nature of the structures thus impacting the hydrodynamic volume.

Stereoisomer-induced changes in micelle stability and/or degradation properties of poly(lactic acid)-*block*-poly(ethylene glycol) copolymers were studied with the aid of SEC-MALS-RI.[14] The M_n and M_w were measured by SEC MALS using polystyrene DVB columns in THF at 50° C and the values were found to be comparable to the M_n values calculated by NMR. These polymers were around 4,500 to 6,000 g/mole. Although they used SEC MALS to measure the molecular weight the issue of *dn/dc* was not addressed in this paper. The authors do not mention the *dn/dc* value they used or how it was obtained. This important value would have a direct effect on the molecular weight

measurement. SEC analysis of the degraded copolymers identified short chain oligomers and correlated changes in M_n. With this data along with thermal analysis they showed that isomer composition was the major factor in determining degradation properties under certain conditions.

2.7.2 Multi-Detector SEC for Analysis of Solution Properties

SEC can be used to investigate solution properties of different polymers. These solution properties include the effect of short and long-range intrachain interactions along the backbone on persistence length, solvation, dilute solution conformation, specific refractive index increment and instrinsic viscosity.[15] Intrachain interactions can affect *dn/dc* by causing a change in chemical composition in that area of the polymer chain therefore changing the overall *dn/dc*. Using SEC-MALS-viscosity-RI Ahmad and Striegel investigated properties of polystyrene, poly(vinyl chloride), and poly(vinylbenzyl chloride). Molecular weight was determined in an online mode as well as by Zimm plots in an offline mode. A Zimm plot is a double extrapolation plot of Kc/R_θ versus $\sin 2\theta + K'c$. Where K is an optical constant, c is concentration and R_θ is the Rayleigh ratio. A Zimm plot is obtained by making measurements of Kc/R_θ at various concentrations as a function of scattering angle. From the Zimm plot they were able to determine the second virial coefficient (A_2). For accurate molecular weight measurements *dn/dc* was also determined offline. Plots of the radius of gyration (R_g) and instrinsic viscosity versus molar mass were constructed. R_g is defined as the average radius of the distribution of mass about the center of gravity of the molecule. It was found that intrachain repulsion between monomers and the influence of A_2 affected chain stiffness making it less stiff and solvation was also affected, ultimately enhancing solubility. In an additional paper by

Ahmad he demonstrated the relationship between sequence length heterogeneity and dilute solution conformations of copolymers. He employed SEC-MALS-UV-VISC-RI to determine molar mass and changes across the molar mass distribution of block, random, alternating, and gradient polymers of styrene and methyl methacrylate and homopolymers of each. Plots of viscometric radius ($R\eta$), radius of gyration (R_g) and $R\eta/R_g$ were plotted versus molar mass to investigate these characteristics.[16] The viscometric radius is the radius of a hard sphere that alters the viscosity of the solvent by the same amount as does the polymer.[16] The $R\eta/R_g$ change with molar mass can be used as a metric of sequence length heterogeneity as shown by this paper. This ratio changes depending on polymer architecture and conformation in dilute solution. With increasing polymer compactness the ratio is increased and decreases with more extension of the polymer. Therefore by studying this ratio the authors were able to determine how the sequence length heterogeneity influenced the compactness of the polymer chain or if intrachain repulsion occurred causing the polymer to extend.

Another group investigated the chain stiffness of alternating copolymers containing stilbene using SEC-MALS-VISC.[17] Using a viscosity detector, persistence length can be estimated by the relationship between viscosity, molar mass, and radius of gyration. Without a viscosity detector, persistence length can be determined by graphing $(M/<Rg>)^{1/2}$ versus 1/M where M is molar mass and R_g the radius of gyration. The persistence length can then be obtained from the y intercept of the line. The addition of a viscosity detector can aid in determining persistence length for polymers of low molar mass where R_g is too small to be measured by light scattering. The intrinsic viscosity value is used to construct a Bohdanecky plot where $(M^2/[\eta])^{1/3}$ versus $M^{1/2}$ is plotted and

the persistence length is given by the y intercept. In addition, the viscometer allows measurements of viscosity at lower molecular weights where light scattering is relatively insensitive. The persistence length is a measurement of the chain whose bond direction "persists" in the same direction as the first bond. This length can be a good indication of chain stiffness. The stiffer the chain the higher the value.

2.7.3 Multi-Detector SEC for Analysis of Chemical Heterogeneity

For block and gradient copolymers the *dn/dc* sometimes changes across the molar mass distribution. Because of this change it is important to know how the *dn/dc* changes across the distribution so that accurate molar mass can be determined by SEC-MALS. For random copolymers this is often just a simple calculation of the weight percentage of each polymer times the *dn/dc* for that polymer. However, in block and graft copolymers the calculations are not as simple. In a paper by Ahmad and Striegel they present a method to obtain the absolute corrected molar mass averages of a gradient copolymer of styrene and methyl methacrylate where the styrene percentage decreases from 30% to 19% with increasing molar mass.[18] In this method the multi-detector system includes MALS, viscosity, refractive index, and ultraviolet absorption spectroscopy (UV). Since this polymer has styrene as one of its components they can rely on the absorption of styrene at 260 nm to determine chemical composition. The percent styrene is then used to determine the *dn/dc* at each elution slice. The percent styrene was calculated based on **Equation 2.12**.[18]

$$(\%St)_i = \frac{Z_i\left(\frac{dn}{dc}\right)_{PMMA}}{F\left(\frac{dn}{dc}\right)_{PS} - Z_i\left[\left(\frac{dn}{dc}\right)_{PS} - \left(\frac{dn}{dc}\right)_{PMMA}\right]} \times 100\%$$

$$\text{where } F = \frac{S_{UV,PS}}{S_{DRI,PS}} = \text{constant and } Z_i = \frac{S_{UV,i}}{S_{DRI,i}} \qquad (2.12)$$

In this equation F is the ratio of the signal of the UV detector to that of the RI detector for a polystyrene homopolymer and Z_i is the ratio of the UV signal to the RI signal for the gradient copolymer at each slice *i*. The *dn/dc* has to be corrected across the distribution so that there is no bias in the molar mass averages determined by light scattering. **Equation 2.13** was used to correct for this bias.

$$M_{corrected,i} = M_{uncorrected,i} \times \frac{\left(\frac{dn}{dc}\right)_{uncorrected}}{\left(\frac{dn}{dc}\right)_{corrected,i}} \qquad (2.13)$$

where $(dn/dc)_{uncorrected}$ is the specific refractive index increment of the copolymer solution, $M_{uncorrected}$ is the uncorrected molar mass obtained via traditional SEC-MALS calculations, and $(dn/dc)_{corrected,i}$ is the corrected *dn/dc* at each slice. These equations are accurate for copolymers that have only one component that absorbs in a specific UV region so that the chemical heterogeneity over the distribution can be assessed via UV detection. The corrected *dn/dc* is calculated using **Equation 2.14**.

$$\left(\frac{dn}{dc}\right)_{corrected,i} = \left(\frac{dn}{dc}\right)_{PS}(\%St)_i + \left(\frac{dn}{dc}\right)_{PMMA}(\%MMA)_i \qquad (2.14)$$

Using a similar method Schlaad and Kilz calculated accurate molecular weights of diblock copolymers of polystyrene-block-poly(methyl methacrylate), polystyrene-block-polybutadiene and polystyrene-block-poly(L-lysine).[19]

Using a quintuple-detector system comprised of MALS, dynamic light scattering, UV, viscosity, and refractive index, Striegel characterized copolymers for absolute molecular weight, chemical heterogeneity, radius of gyration, and polymer architecture.[20] Four PL Aquagel-OH columns were used in series with an aqueous mobile phase containing 0.5 M acetic acid and 0.5 M NaCl. Once again the chemical heterogeneity had to be accounted for since copolymers of polyacrylamide and *N,N*-dimethylacrylamide were used. This was done in a similar manner as mentioned before by correcting for differences in dn/dc at each elution slice. This article shows the strength of a multi-detector system and the information that can be gained where polymers with overlapping UV signals were analyzed.

2.7.4 Multi-Detector SEC for Analysis of charged polymers

Aqueous SEC was used to determine the molar mass averages of zwitterionic polybetaines.[21] Charged polymers can be difficult to analyze via SEC due to enthalpic interactions with the stationary phase and due to polyelectrolyte effects. These polyelectrolyte effects can be due to charges on the chain which cause inter and intra-chain interactions leading to extension in solution or even insolubility. Because of this, oftentimes mobile phases with high salt concentrations are used to screen these interactions. For the analysis of these zwitterionic polymers an aqueous mobile phase containing 0.1 M NaBr was used. The authors still found that the molar mass averages calculated by light scattering were significantly larger than the theoretical values or those calculated by NMR. They attributed this to the aggregated nature of these polymers in solution.

Many times charged polymers play a large role in biological agents and systems. Because of the charged nature of these polymers they are often analyzed in mobile phases with high salt concentrations that do not mimic an *in vivo* environment. In a paper by Gao *et al.* a commercial polymer (Polymer JR) which was a cationic hydroxyethyl cellulose derivative was analyzed using MALS in a batch experiment.[22] The batch experiment involved injecting several solutions at different concentrations directly into the MALS detector. This bypassed the columns and avoided potential column interactions. With this method only the average weight average molar mass is obtained due to lack of separation. Using this method the authors were able to investigate the properties in a more *in vivo* like environment as the samples were dissolved in a phosphate buffer. This could not be conducted using columns as enthalpic interactions would dominate and fractionation would not occur solely on the basis of hydrodynamic volume.

2.7.5 Multi-Detector SEC for Analysis of Branched Polymers

Branched molecules exhibit another potential complication in SEC analysis. This complication arises from the branches interacting with the pores and trapping or delaying the molecule from eluting. Multi-detector SEC can be particularly useful in determining properties of branched molecules. Although useful, determination of the molecular weights of branched polymers are often highly variable.[23]

One example is in a paper by Milic who investigated solution properties of hyperbranched polyols using multi-detector SEC.[24] SEC and batch mode analyses were performed to follow the process of hyperbranching during polymerization of hydroxylated fatty acid methyl esters from soybean oil into polyols. The batch mode analyses were conducted by injecting samples of varying concentration directly into the

light scattering detector or by placing samples in cuvettes into the light scattering detector. A Zimm plot is obtained from which M_w, R_g and A_2 can be determined. The authors found that, as expected, the sizes of the molecules were more compact than their linear counterparts. Plots of the radius of gyration versus molar mass determined the size of the molecules. It was also noticed that some high molar mass molecules eluted late, as indicated by an upturn in the graph. This co-elution was attributed to possible branches interacting with column pores or molecules being trapped by the pores due to their compact size.

Another way to analyze branched polymers is to use SEC to investigate the branching factors that describe the architectures of the polymers compared to their linear counterparts of the same molecular weight. This was done in an article by Puskas where branched polyisobutylene and polystyrene were analyzed using multi-detector SEC.[25] The authors were able to show that using molecular weight data along with radii of gyration and hydrodynamic radii, these branching factors could be calculated using the equations shown below.

$$g = \left(R^2_{gz,br} / R^2_{gz,lin} \right)_{Mw}$$

$$h = \left(\langle R_h \rangle_{z,br} / \langle R_h \rangle_{z,lin} \right)_{Mw} \qquad 2.15$$

$$\rho = \left(R_g^{2\ 1/2} / \langle R_h \rangle_z \right)$$

This analysis required the use of UV, MALS, viscosity and refractive index detectors.

Crosslinked acrylic latexes cannot be analyzed via SEC due to a portion of the sample that is insoluble. However, the sol fraction still contains information on branching architecture that can be important to the overall properties. To investigate the branching

properties of the sol fraction, SEC with triple detection was employed.[26] Using both MALS and universal calibration the molar masses were calculated. It was found that the local dispersities described by the ratio of M_w to M_n at each elution slice were significant and the authors were able to infer information about long chain branching in the material. To calculate the local dispersity, they used the ratio of M_n obtained from universal calibration and the M_w obtained from SEC MALS. This also showed that co-elution had likely occurred because the local dispersity was close to one at low elution volumes but increased as elution volume increased. Due to contraction of the polymer chains in branched polymers, a branched polymer of the same hydrodynamic volume as a linear polymer will have higher molecular weight. This can lead to co-elution because at each elution volume you are likely to have a dispersion of chains of different molar masses and branching degrees.

2.8 Hyphenated SEC Methods

Polymers are complex structures that have distributions in molar mass, chemical composition, functionality, and molecular architecture.[27] Due to this complexity it is often necessary to couple SEC to other techniques to acquire a full picture and understanding of the polymer and its properties. These methods have been extensively reviewed by Pasch[27] and will only be briefly mentioned here. One such technique involves coupling SEC to spectroscopic detectors such as NMR and FT-IR. This allows for analysis of chemical composition as well as molar mass. In one such article by Hiller *et al.* SEC-NMR was used as a molar mass detector as well as to determine the monomer units of the copolymer.[28] By injecting 100 μL of a 2.7 mg/mL sample solution the signal to noise ratio was sufficient for analysis. It was found that above 3 mg/mL the molar

mass calculation was compromised. This is another way to investigate chemical heterogeneity across the molar mass distribution of copolymers.

Another hyphenated SEC method is 2D liquid chromatography. In 2D-LC, separation usually occurs by both molar mass and chemical composition. This method combines HPLC with SEC, thereby achieving not only size separation but also separation by chemical composition. In one such study liquid chromatography at critical conditions was coupled to SEC to render a detailed study of polystyrene-*block*-polyisoprene diblock copolymers.[29]

CHAPTER 3: SEC Analysis of Variance

3.1. Synopsis

This chapter discusses the error analysis established in our SEC laboratory. Method validation is an important part of any analytical laboratory. In order to establish a proper method and know that data is reasonable and as expected one must know the limits of the instrument being used and things that can affect the data such as sample preparation. Molecular weight was measured by both light scattering and universal calibration for several samples. The dn/dc was also measured. Proper and consistent handling of the raw data must be carried out systematically to know that the data is correct and statistically valid. These processes are demonstrated through use of an acceptable method for measuring both polystyrene standards and poly(2-ethyl-2-oxazoline) (PEOX) polymers.

The measurement of uncertainty tells us the range of values one should reasonably expect to obtain from a measurement. Therefore it tells us about the validity of the measurement. The uncertainty may be given by the simple calculation of a standard deviation or a multiple thereof with a given confidence interval. In this section the uncertainty was measured by multiplying the standard deviation by two and dividing it by the mean. This calculation then provided a measurement of uncertainty that is expressed in terms of a percentage of the mean value. Using this value it was determined that with a 95% confidence interval, data should fall within that range.

The polystyrene standard that was utilized has a reported molecular weight of 21,720 g/mole with a PDI of 1.02. This is measured each time a sample sequence is

analyzed. PEOX samples were measured via an acceptable method (study established in chapter 4 of this thesis) by light scattering and universal calibration to obtain molar mass averages.

3.2 Experimental

3.2.1 Materials

A 21,720 g/mole polystyrene standard was purchased from Agilent Technologies and used as received and stored in a desiccator between uses. PEOX samples were obtained from research polymers that were submitted to the laboratory for analysis. The PEOX samples were vacuum dried at 60 °C 2-3 hours before use and stored in a desiccator between runs. The SEC mobile phase, *N*-methylpyrrolidone (NMP), was purchased from Fisher Scientific, stirred over phosphorus pentoxide (P_2O_5), distilled under vacuum, and filtered through a 0.2 μm PTFE filter before use. After distillation but before filtration, 4.34 g of lithium bromide (LiBr) was added per liter of NMP to provide a 0.05 M solution. The LiBr was purchased from Sigma Aldrich and dried in a vacuum oven before being added to the NMP. Three Agilent PLgel 10 μm Mixed BLS polystyrene-divinylbenzene columns 300x7.5 mm connected in series were used as the stationary phase. An isocratic pump (Agilent 1260 infinitiy, Agilent Technologies, USA) with an online degasser (Agilent 1260), autosampler and column oven was used for mobile phase delivery and sample injection. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology Corporation, USA), operating at a wavelength of 658 nm; a viscometer detector (Viscostar, Wyatt Technology Corporation, USA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, USA) provided online results of the homopolymer SEC's. The

system was corrected for interdetector delay, band broadening, and the MALS signals were normalized using a 21,720 g/mole polystyrene standard obtained from Agilent Technologies with each set of samples. Data acquisition and analysis was completed using Astra 6 software from Wyatt Technology Corporation.

3.2.2 SEC Analysis of the Polystyrene Standard

A polystyrene standard with a M_p = 21,720 g/mole and PDI = 1.02 was analyzed by SEC twenty times. The polystyrene is of low enough molecular weight to be an isotropic scatterer. This avoids any angular dependence of the scattered light when passing through the multi-angle laser light scattering detector. An isotropic scatterer is used for validation of the multi-angle laser light scattering detector (HELEOS II). Alignment of the signals from each detector, band broadening and normalization must be performed for the standard in order to validate the HELEOS II detector. These parameters are then applied to the sample chromatograms following each run of the polystyrene standard. For a narrow PDI sample the peaks of each of the detectors should align. The software aligns the signals and accounts for the delay volume between the detectors. Band broadening is a result of the mixing that occurs during the flow path as the sample travels to each detector. This also must be accounted for in the software before analysis can be completed. The third step of the validation process is to apply a normalization routine. Normalization relates the measured voltages at each angle of the multi-angle laser light scatterer to that of the 90° detector which is always equal to 1. The normalization process provides a coefficient for each of the detector voltages around the flow cell. This becomes important as the light scattering detector measures the excess Rayleigh ratio that takes into account the detector signal voltages of the solution, solvent and laser.

The polystyrene standard was analyzed twenty times at a concentration of approximately 3 mg/mL in NMP + 0.05 M LiBr at a flow rate of 0.5 mL/min. This process consisted of several different samples analyzed multiple times. The data presented is the number average molecular weight (M_n), or M_p in the case of a monodisperse standard.

The *dn/dc* was also calculated via two methods. One method consisted of direct injection of a series of solutions ranging from 0.1 to 1 mg/mL into the refractive index detector. This is considered an offline method because the sample is not passed through the columns. The intensity of each peak versus concentration is then plotted on a graph creating a line where the slope represents the *dn/dc* of the sample. The second method uses the software to calculate the *dn/dc* by assuming 100% recovery of the sample.

3.2.3 SEC Analysis of Poly(2-ethyl-2-oxazoline)

Three poly(2-ethyl-2-oxazoline) homopolymers (Samples A-C) were analyzed to establish differences in the measurement of molar mass via light scattering and universal calibration. Each sample was prepared three separate times and was run and analyzed three separate times giving 9 analyses of each sample. These samples were analyzed via light scattering and universal calibration to obtain molar mass averages. The *dn/dc* was also calculated using the 100% recovery method.

3.3. Results and Discussion

3.3.1. Polystyrene Standard Analysis

Figure 3.1 shows a chromatogram of the polystyrene standard before being processed for peak alignment, band broadening and normalization. From the chromatogram one can observe that the curves of the three detectors do not overlay as they should. In **Figure 3.2** the raw data has been processed to account for interdetector

delay (peak alignment), band broadening and normalization. As observed in **Figure 3.2** this process overlays the peaks correctly. This processed data is then saved and applied to polymer samples for analysis. The saved configuration applies the delay volumes and normalization coefficients to the sample data prior to analysis.

Table 3.1 shows the results of the twenty sample analyses of the polystyrene standard. Since this is a narrow molecular weight standard, the M_p value is similar to M_n. The results confirm that the experimental means of M_n and M_p differ by less than 1%. Compared to the manufacturer's value of M_p = 21, 730 g/mol the experimental M_p value differs by less than 2%. The measurement of uncertainty for M_n, M_p, and PDI is around 9% indicating that measurements within 9% of the mean value cannot be distinguished as different and variation within the measurements is low. When running this standard if the measurement falls within 9% of the mean then it is accepted that the system is functioning properly and therefore no system errors should have an effect on the data.

The *dn/dc* was measured twice via direct injection into the Optilab T-rEX refractive index detector. This method produced an average *dn/dc* of 0.1378. The software method using the assumption of 100% recovery of seven 21K polystyrene standards produced a *dn/dc* of 0.1368 with a standard deviation of 0.0099. Therefore a value around 0.13 is used as the *dn/dc* for polystyrene in NMP + 0.05 M LiBr.

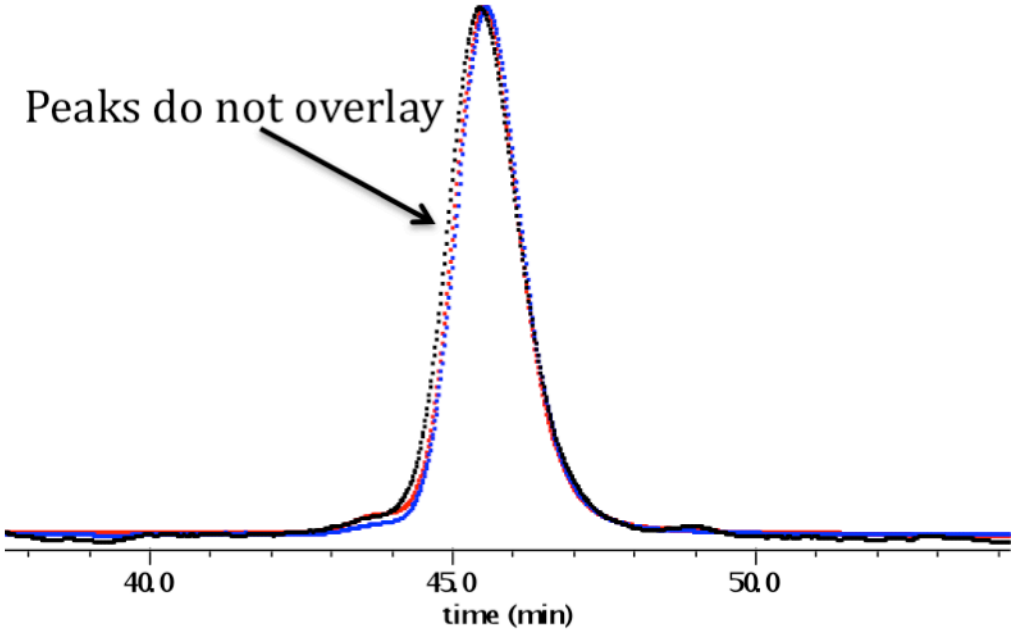


Figure 3.1 Chromatogram of a 21,720 g/mole polystyrene standard before correction for interdetector delay and peak broadening

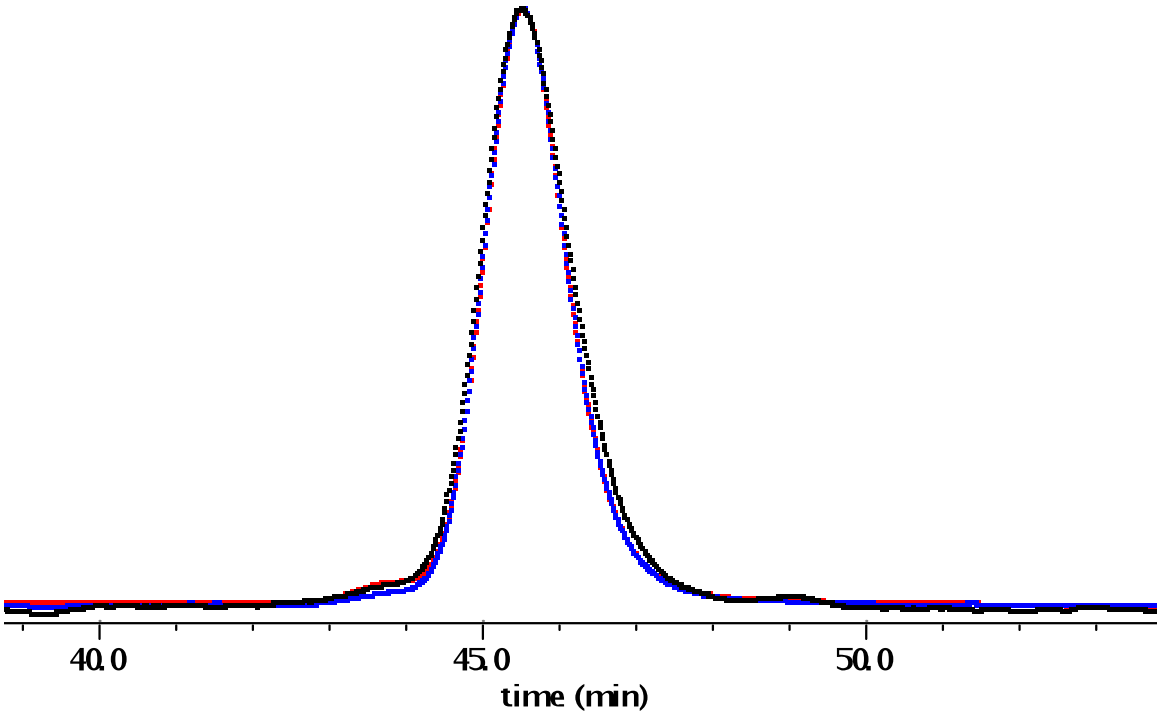


Figure 3.2 Chromatogram of a 21,720 g/mole polystyrene standard after correction for interdetector delay and peak broadening showing overlaying peaks

Table 3.1 Data from twenty polystyrene standard analyses

	Mn (kg/mol)	Mp (kg/mol)	Polydispersity (Mw/Mn)
Mean of 20 runs	**21.4**	**21.3**	**1.00**
Standard deviation	**0.944**	**1.17**	**0.004**
Measurement of Uncertainty (%)	**8.8**	**11.0**	**0.80**

3.3.2 Poly(2-ethyl-2-oxazoline) molar mass averages measured by light scattering and universal calibration

Light scattering and universal calibration analysis methods can lead to differences in molar mass average values of the same sample. Light scattering is a direct measure of weight average molar mass (M_w) by using the equations set forth for measuring mass via the excess Rayleigh ratio. This is detailed in **Equations 2.2 -2.5**. Universal calibration on the other hand is a calibration technique based on the empirical finding that at least low dispersity polymers with the same log(ηM) will elute at the same elution volume. A calibration curve is established where $\log[\eta]$M versus elution volume is plotted. Both of these methods represent absolute methods for calculating molar mass averages, so the values obtained with the two methods were compared herein. ANOVA was used to analyze differences in the values obtained by light scattering and with the universal calibration for three PEOX homopolymers. ANOVA is a statistical test to measure variances in sets of data. From the data in **Table 3.2** it is noted that for samples A-C the molar mass averages for M_n and M_w calculated via light scattering are lower than those calculated by universal calibration by around 1,000 g/mole. By comparing the standard deviations and uncertainties it is also noted that the variance in the universal calibration is

smaller as the standard deviation and uncertainty is less than that of the light scattering method. This trend is observed across all samples. The ANOVA analysis showed *p* values less than 0.05 indicating that for the molar mass averages calculated via light scattering and universal calibration that the groups are statistically different.

Table 3.2 Average, standard deviation, and uncertainty of nine sample runs from Samples A-C (molecular weights in kg/mol, LS=light scattering, UC=universal calibration)

		M_n via LS	M_w via LS	PDI via LS	M_n via UC	M_w via UC	PDI via UC
A	Mean	6.1	6.2	1	6.6	7.4	1.1
	Standard deviation	0.26	0.28	0.01	0.12	0.18	0.02
	Uncertainty	8.6%	9.1%	1.0%	3.4%	4.7%	3.2%
B	Mean	6	6.2	1	7	8	1.1
	standard deviation	0.37	0.44	0.01	0.17	0.18	0.01
	Uncertainty	12.1%	14.2%	2.7%	4.8%	4.5%	1.5%
C	Mean	6.5	6.6	1.01	7	7.8	1.13
	Standard deviation	0.36	0.4	0.01	0.24	0.27	0.01
	Uncertainty	11.0%	12.0%	1.7%	6.9%	6.8%	1.5%
	Average Uncertainty	10.6%	11.8%	1.8%	5.0%	5.4%	2.1%

CHAPTER 4: Size Exclusion Chromatography of Poly(2-ethyl-2-oxazoline) Homopolymers

4.1 Synopsis

Polyoxazolines have great potential for use as drug carriers and other biological agents. They possess many of the same properties as poly(ethylene glycol) in that they are immunogenic and have "stealth" like properties that limit proteolysis and rapid clearance of small molecule therapeutics. [30] Like poly(ethylene glycol, polyoxazolines are nonionic, stable and highly soluble in organic and aqueous solvents. [30] Poly(2-oxazolines) are of particular interest due to the chemically modifiable side chain. This side chain can be tailored to have a hydrophilic or hydrophobic group making possible variations that could be important in many applications. [31] Poly(2-oxazolines) are also of interest in the research realm as they have lower critical solution temperatures and they exhibit antimicrobial properties.[31]

This chapter discusses the polymerization of poly(2-ethyl-2-oxazoline) (PEOX) homopolymers via cationic polymerization. The focus of this chapter is on the development of a method for characterizing the molecular weight distribution of these polymers via size exclusion chromatography (SEC). SEC characterization of the homopolymers was useful for detecting differences in the homopolymers as a result of various synthetic conditions.

4.2. Experimental

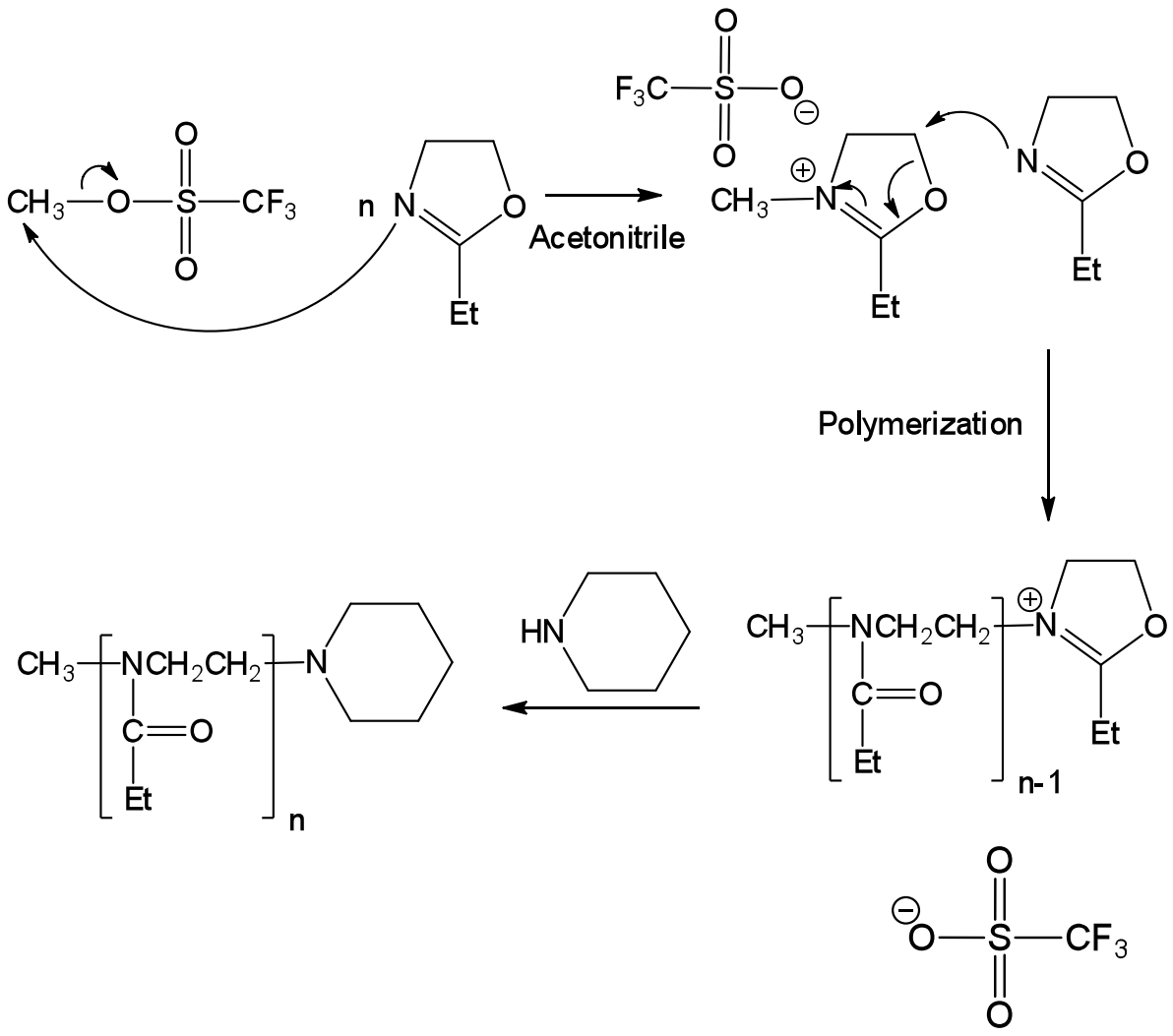
4.2.1. Materials

PEOX samples for analysis were vacuum dried at 60 °C for 2-3 hours before use and stored in a desiccator. The SEC mobile phase, *N*-methylpyrrolidone (NMP), was purchased from Fisher Scientific, stirred over phosphorus pentoxide (P_2O_5), distilled

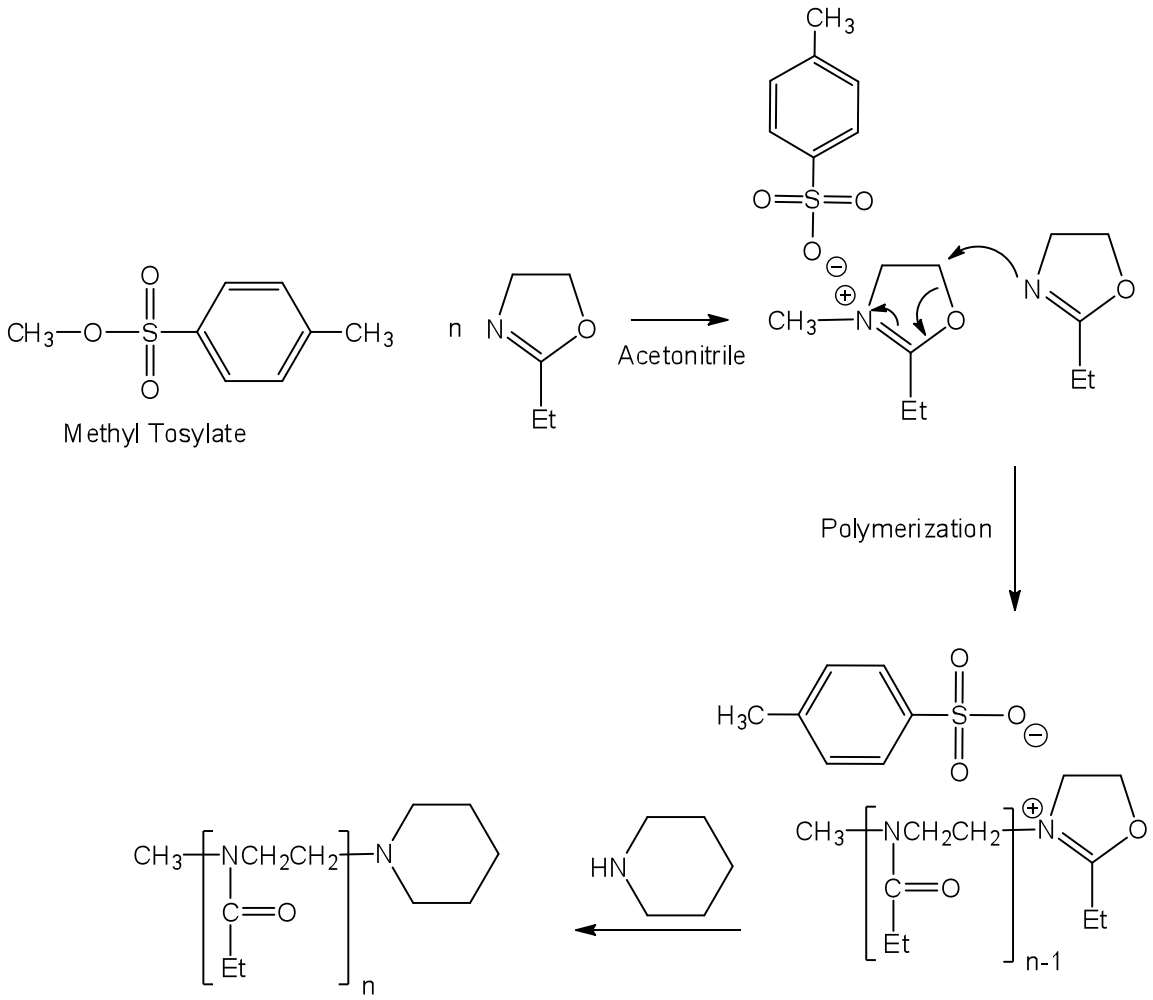
under vacuum, and filtered through a 0.2 μm PTFE filter before use. After distillation but before filtration, 4.34 g of lithium bromide (LiBr) was added per liter of NMP to provide a 0.05 M solution. The LiBr was purchased from Sigma Aldrich and used as received. In the method development phase, three different sets of columns were used to optimize separation of the homopolymers by hydrodynamic volume. A column set that consisted of three Viscotek PLgel polystyrene/divinylbenzene mixed bed columns connected in series was used first. The Viscotek LT5000L medium, LT4000L low and LT3000L ultra low were used in addition to a guard column with the same stationary phase. The Viscotek column stationary phase beads is are a polystyrene-divinylbenzene resin with particle sizes of 10, 7 and 6 μm respectively. A second column set consisted of three α-M Tosoh poly(HEMA) mixed bed columns connected in series with a guard column of the same stationary phase. The α-M stationary phase is a 13-μm particle size column. The third column set was made up of three Agilent PLgel 10-μm Mixed B polystyrene/divinylbenzene columns 300x7.5mm connected in series with a guard column of the same stationary phase. An isocratic pump (Agilent 1260 infinity, Agilent Technologies, USA) with an online degasser (Agilent 1260), autosampler and column oven was used for mobile phase delivery and sample injection. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology Corporation, USA), operating at a wavelength of 658 nm; a viscometer detector (Viscostar, Wyatt Technology Corporation, USA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, USA) provided online results of the homopolymer SECs. The system was corrected for interdetector delay, band

broadening, and the MALS signals were normalized using the 21,720 g/mol polystyrene standard obtained Agilent Technologies with each set of samples. Data acquisition and analysis was completed using Astra 6 software from Wyatt Technology Corporation, USA.

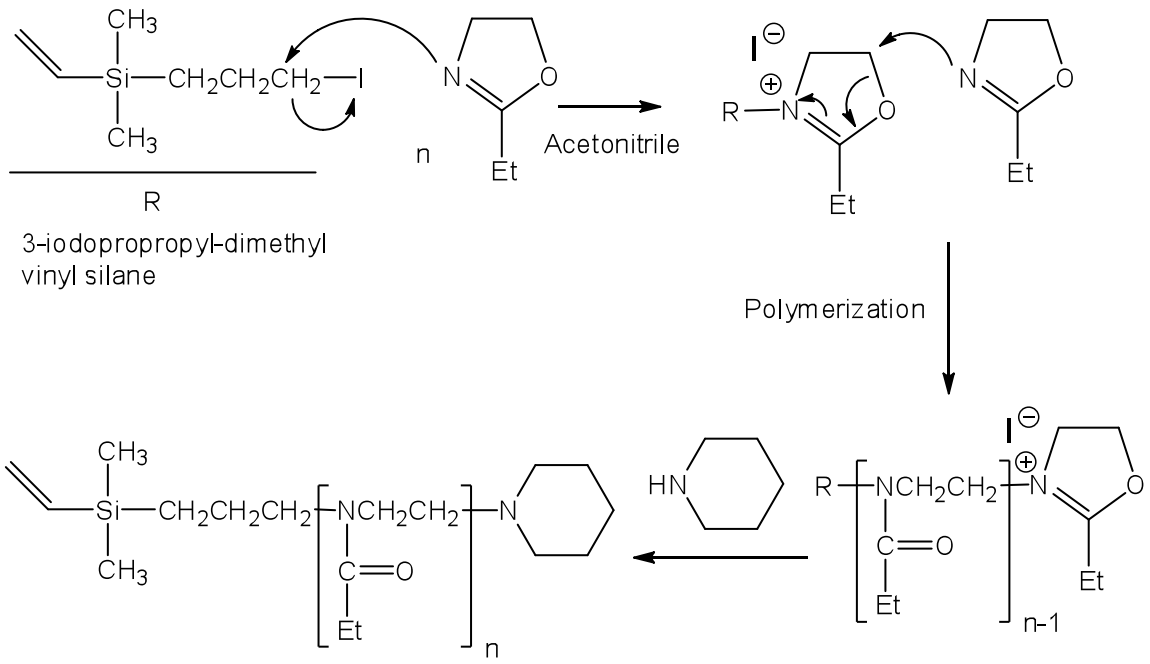
The PEOX samples were synthesized using a series of initiators chosen to produce a controlled molecular weight, narrow distribution homopolymer. The use of different times and temperatures were also explored to optimize the synthesis conditions to provide the desired product. The synthesis schemes are shown in **Figure 4.1** and are briefly described here. The PEOX samples were synthesized via cationic polymerization using one of three different initiators, methyl triflate (scheme A), methyl tosylate (scheme B) or dimethylvinylsilyl propyl idodide (scheme C). All synthetic reactions were conducted in acetonitrile and terminated with piperidine. The target molecular weight of all of the polymers was ~5,000 g/mole. Four samples (samples A-D) were synthesized using methyl triflate as the initiator (Scheme A). The reaction times and temperatures are described in Table 4.1. Sample A was synthesized for 24 hours at 80 °C and for comparison, Sample B was reacted for 4 hours at 80° C. The conversion of monomer was complete at 4 hours, so the sample that was maintained at 80 °C for 24 hours had ample time to undergo coupling and other side reactions. Sample C was synthesized at 65° C for 7 hours. Sample D was reacted at 50 °C for 24 hours. Methyl tosylate was used as the initiator for samples E and F (Scheme B). Sample E was synthesized for 3 hours at 80 °C whereas Sample F was reacted at 56 °C for 18 hours for comparison. Additionally dimethylvinylsilyl propyl idodide was used as an initiator for sample G, which was reacted for 17 hours at 60 °C (Scheme C).



Scheme A



Scheme B



Scheme C

Figure 4.1 Synthesis schemes for PEOX homopolymerization

Table 4.1 Sample table describing the initiator, time and temperature of the samples analyzed

Sample	Initiator	Time (hr)	Temperature (°C)
A	methyl triflate	24	80
B	methyl triflate	4	80
C	methyl triflate	7	65
D	methyl triflate	24	50
E	methyl tosylate	3	80
F	methyl tosylate	18	56
G	monovinyl alkyl iodide	17	60

4.2.2. Methods

Samples A-D were used for method development. The objective was to develop a method for accurately characterizing the molecular weights of the PEOX homopolymers. The parameters investigated included three different column sets, different analysis techniques, and different sample concentrations. The column sets consisted of two material types, polystyrene-divinylbenzene and crosslinked poly(hydroxyethyl methacrylate) (HEMA) porous beads. The mobile phase consisted of NMP + 0.05 M LiBr delivered at 0.5 mL/min. The sample molecular weights were analyzed using both light scattering and universal calibration.

4.3 Results and Discussion

Initially, samples A-D were analyzed on the Viscotek polystyrene-divinylbenzene columns. Three columns were connected in series with the largest pore size column connected after the guard column and the smallest pore size column last. These columns were the Viscotek LT5000L medium, LT4000L low, and LT3000L ultra low. These columns consisted of mixed pore sizes for medium, low and ultra low molecular weight cut-off ranges. The sample concentrations were in the range of 3.0 to 3.5 mg/mL. This concentration showed good separation by the columns and signal intensity by the

detectors. A lower concentration of around 1.5 mg/mL was also investigated but resulted in very low signal-to-noise ratio that was not sufficient for analysis. In sample A, the sample synthesized at 80° C for 24 hours, the chromatogram showed there was a high molecular weight shoulder. This shoulder was not evident in samples B-D. Samples B-D, which were terminated at 80-100% monomer conversion and not heated for periods following consumption of the monomer, produced symmetrical chromatograms with no tailing or shoulders. Samples A-D were analyzed via light scattering to obtain molecular weight averages. This data is presented below in **Table 4.2**. The light scattering analysis shows a higher molecular weight of M_w=9,400 g/mole for Sample A as compared to the other samples B-D which all had measured molecular weights (M_w) around 6,000 g/mole, whereas the target molecular weight (M_n) was 5,000 g/mole. This demonstrated that the high molecular weight shoulder was accounted for in the analysis by SEC for Sample A. The M_n calculated from NMR for Sample A was 5,300 g/mole, based on endgroup analysis. The NMR M_n and SEC M_n values for samples B-D were comparable indicating that molecular weights derived from endgroup analysis and SEC were in agreement.

The use of a second column set with a chemically different stationary phase was investigated for the homopolymers A-D. These columns contained polyHEMA porous beads and were more hydrophilic than the polystyrene based stationary phase. The Tosoh alpha M columns are mixed bed columns for separation of a wide range of molecular weight polymers. The broad range of molecular weights covered allows for versatility when testing a variety of polymer sizes, but limits the resolution, especially of lower molecular weight polymers or oligomers. Samples A-D were analyzed on these columns and all the samples yielded symmetrical chromatograms. This result indicated that the

high molecular weight shoulder known to be present in sample A was not evident using these columns. Therefore these columns are not as well suited for the analysis of these low molecular weight homopolymers as the separation is compromised by the lack of resolution. Both light scattering and universal calibration were used to calculate the molecular weight averages for samples A-D using the Tosoh columns and are presented in **Table 4.2**. Sample A had a molecular weight of M_w = 9,100 g/mole via light scattering analysis. Samples B-D have M_w values around 6,000 g/mole. These samples were also analyzed using universal calibration on these columns. The universal calibration showed a slightly lower M_w for Sample A than the light scattering analysis with a difference of about 1,000 g/mole between the two methods. The M_w values for Samples B-D are slightly higher by universal calibration analysis.

These hydrophilic columns can be used with an aqueous buffer mobile phase. However, it was found that with these polymers and a sodium sulfate buffer mobile phase, the polymers interacted with the stationary phase making them unsuitable for this analysis.

The third set of columns used consisted of Agilent PLgel 10-µm Mixed BLS columns 300x7.5mm connected in series. These columns are polystyrene-divinylbenzene porous bead columns with mixed beds. Once again the mixed bed beads allow for versatility to analyze a wide molecular weight range of polymers with some sacrifice of resolution. However, just as observed with the previously-described polystyrene columns, these columns also showed the high molecular weight shoulder on sample A. This demonstrates that it is the packing material of the columns that lead to better separation of these polymers. It was also found that the concentration of the samples run through

these columns was important. Concentrations of 3 and 5 mg/mL were compared. The 5 mg/mL samples resulted in better light scattering and refractive index signals and did not appear to result in column overloading, so this concentration was used for further analysis. Samples A-D were analyzed on these columns and the resulting chromatograms were analyzed via light scattering and universal calibration techniques. The data is presented in **Table 4.2**. Consistent with previous SEC results, Sample A had the highest M_w value of 9,400 g/mole while Samples B-D provided M_w values via light scattering analysis around 6,000 g/mole. Universal calibration analysis showed the same trend with Sample A having the highest M_w value, and all samples except Sample A had slightly higher M_w values than by light scattering analysis.

The data presented in **Table 4.2** shows that the polydispersity index (PDI) values are closest to the expected values of 1.1 using the Agilent columns. This is most likely due to improved separation of the polymers with these columns. The PDI values using the other columns are lower than expected, especially using the light scattering analysis. The M_n values when measured with light scattering tend to be overestimated, possibly due to weak signals in the lower molecular weight fractions for these polymers. This makes the PDI values smaller. One noticeable difference is the M_n value of Sample A calculated with the Tosoh columns is lower than observed with the other column types. The SEC number average molecular weights are all within 10-20% of the NMR values.

From this method development, it was noted that the Agilent columns were the best columns for this analysis. The Agilent columns separate the high molecular weight shoulder of Sample A as shown in **Figure 4.2**. **Figure 4.3** shows a representative light scattering chromatogram of samples B-D. Unlike sample A, samples B-D had a

symmetrical distribution of molecular weight. The symmetrical nature of the distribution represents better control of the chemical synthesis using lower temperatures and/or times. The lower times and/or temperatures for the synthesis limit side reactions that most likely lead to the high molecular weight shoulder observed in sample A.

Table 4.2 Molecular weight and polydispersity results of four PEOX samples by SEC using different columns compared to NMR (molecular weights in Kg/mol, LS= light scattering, UC=universal Calibration)

Sample	Mn LS Viscotek	Mw LS Viscotek	PDI LS Viscotek	Mn LS Tosoh	Mw LS Tosoh	PDI LS Tosoh	Mn UC Tosoh	Mw UC Tosoh	PDI UC Tosoh	Mn LS Agilent	Mw LS Agilent	PDI LS Agilent	Mn UC Agilent	Mw UC Agilent	PDI UC Agilent	NMR
A	**9.4**	**9.5**	**1.01**	**9.1**	**9.2**	**1.02**	**5.7**	**8.2**	**1.44**	**9.0**	**9.4**	**1.05**	**7.8**	**9.2**	**1.17**	**5.3**
B	**6.2**	**6.2**	**1.00**	**6.0**	**6.0**	**1.00**	**6.0**	**7.9**	**1.31**	**6.2**	**6.4**	**1.04**	**7.3**	**8.4**	**1.14**	**6.0**
C	**5.9**	**6.0**	**1.02**	**6.3**	**6.3**	**1.00**	**5.8**	**7.6**	**1.31**	**6.0**	**6.0**	**1.01**	**6.8**	**7.7**	**1.14**	**5.2**
D	**6.3**	**6.4**	**1.02**	**6.1**	**6.2**	**1.01**	**5.2**	**6.9**	**1.32**	**6.3**	**6.4**	**1.01**	**7.0**	**7.9**	**1.13**	**5.8**

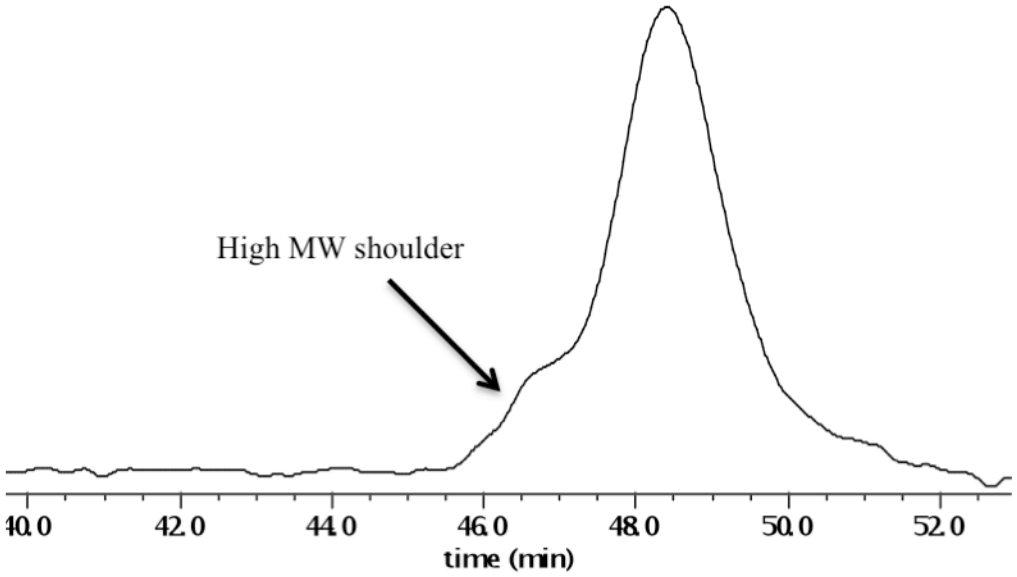


Figure 4.2: SEC light scattering chromatogram of Sample A showing the high molecular weight shoulder

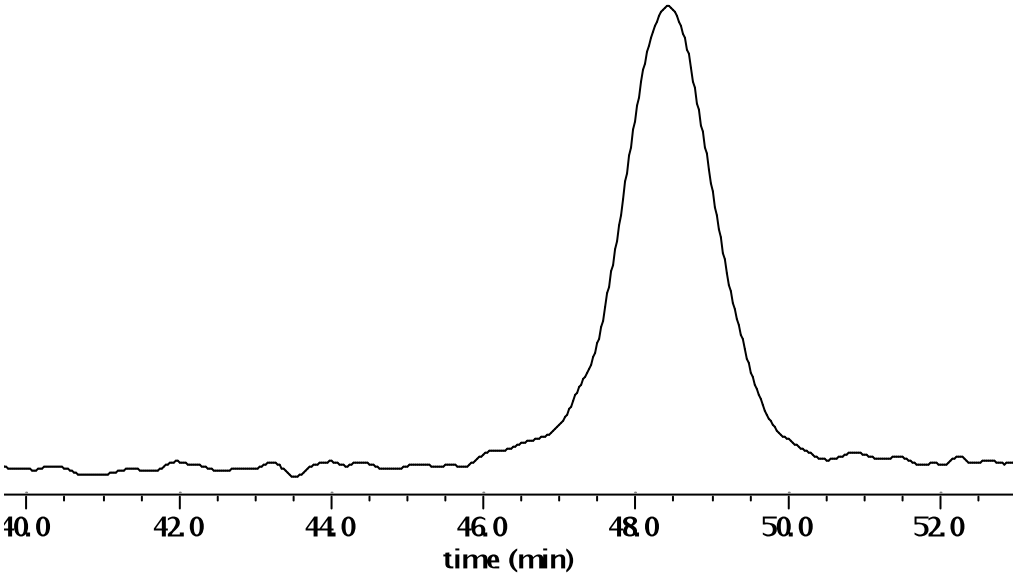


Figure 4.3: SEC light scattering chromatogram of sample B, which is representative of samples B-D, showing a symmetrical distribution of molecular weights

Two tosylate initiated samples were analyzed via the method established using the Agilent columns and 5.0 mg/mL sample concentrations. The SEC analysis of these samples was used to show the effects of the synthesis parameters and to measure

molecular weight. Sample E was reacted for 3 hours at 80° C, whereas sample F was reacted for 18 hours at 56° C. The chromatograms showed that a high molecular weight shoulder was present for sample E, synthesized at the higher temperature. Sample F however did not have a high molecular weight shoulder. This demonstrates for this reaction that temperature may lead to the coupling reactions that result in the high molecular weight shoulders. **Figures 4.4 and 4.5** show the chromatograms for samples E and F respectively. The molecular weight data is provided in **Table 4.3**. Light scattering analysis shows that both samples have similar average molecular weight values, with M_n of 6,800 g/mole for Sample E and 6,500 g/mole for Sample F indicating a slightly higher molecular weight for Sample E from the high molecular weight tailing. The PDI for these samples by light scattering is unrealistically low, measuring 1.02 and 1.01. The low PDI value is attributed to overestimation of M_n by light scattering. Analysis using universal calibration shows similar Mn values as those obtained by light scattering but higher Mw values by nearly 1000 g/mole. The PDI is also higher by universal calibration with a value of 1.15 and 1.17. Both light scattering and universal calibration analysis methods yielded M_n's higher than the NMR values obtained from endgroup analyses.

Table 4.3 Molecular weight and polydispersity results of Samples E and F by SEC compared to NMR (molecular weights in kg/mol, LS= light scattering, UC=universal Calibration)

Sample	M_n via LS	M_w via LS	PDI via LS	M_n via UC	M_w via UC	PDI via UC	M_n via NMR
E	**6.8**	**6.9**	**1.02**	**6.8**	**7.8**	**1.15**	**5.7**
F	**6.5**	**6.6**	**1.01**	**6.6**	**7.7**	**1.17**	**5.0**

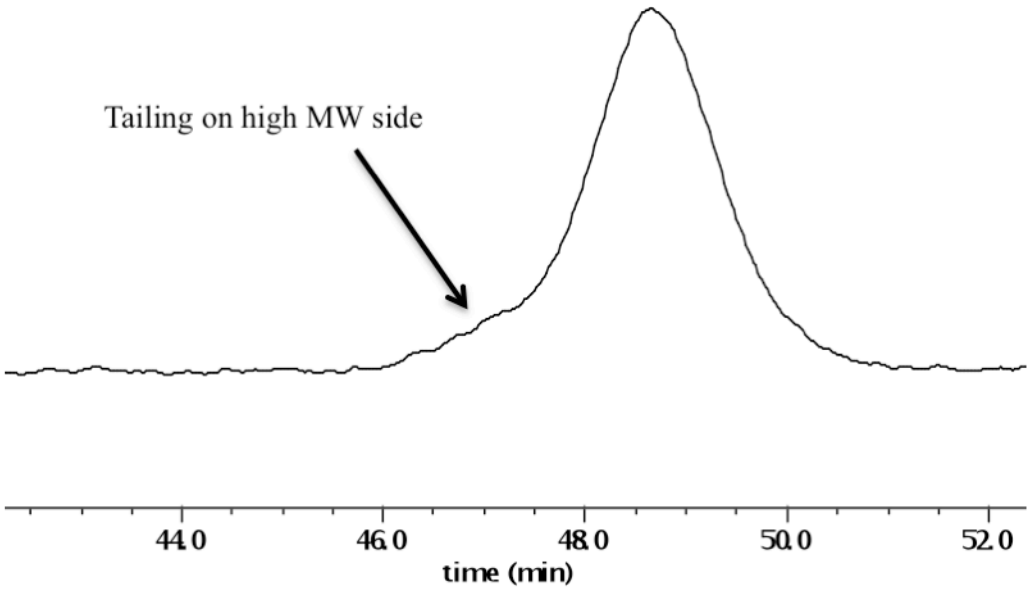


Figure 4.4: SEC light scattering chromatogram of Sample E showing tailing on the high molecular weight side

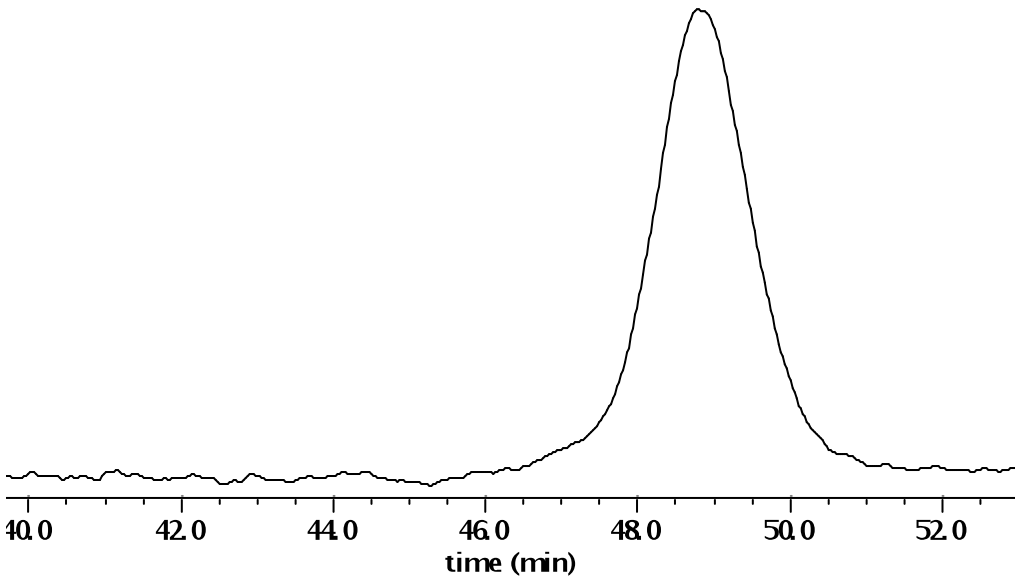


Figure 4.5: SEC light scattering chromatogram of Sample F showing a symmetrical curve without high molecular weight tailing

Lastly, a PEOX homopolymer was synthesized using dimethylvinylsilylpropyl iodide as the initiator and this was analyzed using the optimized SEC procedure (Sample G). This sample had an expected M_n value of 8,950 g/mole based on monomer conversion. NMR showed that the molecular weight was 13,750 g/mole, thus suggesting that some of the low molecular weight polymer had been fractionated out during the isolation procedure. This sample has a low molecular weight tail as shown in **Figure 4.6**, suggesting that initiation of the polymerization had occurred very slowly relative to the propagation rate. The SEC results indicated lower M_n values by both light scattering and universal calibration analysis than that calculated by NMR with M_n equal to 11,700 g/mole and 10,600 g/mole respectively. As expected the PDI was by light scattering was lower than the PDI calculated by universal calibration. It was clear that this is a low molecular weight tail as previous data has shown we can obtain symmetrical curves with PEOX homopolymer SEC.

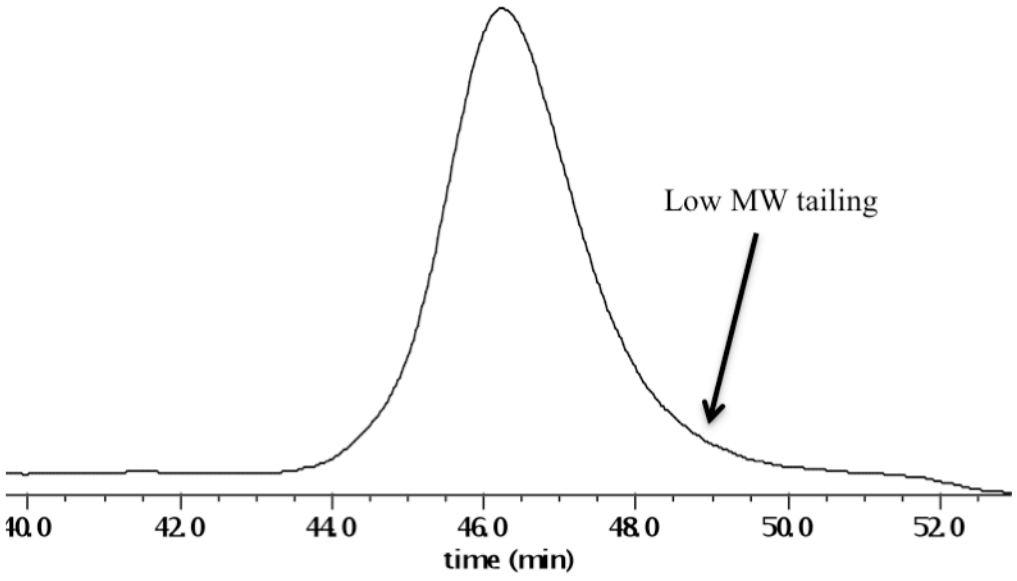


Figure 4.6: SEC light scattering chromatogram of Sample G showing low MW tailing

CHAPTER 5: Size Exclusion Chromatography of Poly(ethylene oxide)-b-Poly(2-ethyl-2-oxazoline) Diblock Copolymers

5.1 Synopsis

Both poly(ethylene oxide) (PEO) and poly(2-ethyl-2-oxazoline) (PEOX) are polymers well suited for use in biological applications. They both possess stealth-like properties that allow for use in vivo without expectation of an immune response or rapid clearance from the body[30]. Employing both of these polymers to make diblock copolymers makes good use of their combined properties. As a diblock copolymer, both the PEO block and the PEOX are hydrophilic. The ethyl side chain of the PEOX block can be modified, and this can change the properties to make it more or less hydrophobic, or the modified polymers may bind or react with other groups. Many studies have reported the use of polyoxazolines in pharmaceutical and medical applications.[32]

In this chapter the synthesis of PEO-b-PEOX diblock copolymers will be introduced. The focus is on the use of SEC to characterize the diblock copolymers and to observe any effects that may be related to aspects of the copolymerizations. The SEC molecular weight distributions in the chromatograms showed the effects of different initiators on the block copolymer structures.

5.2 Eperimental

5.2.1 Materials

PEO-b-PEOX diblock copolymers that had been prepared with different macroinitiator endgroups were analyzed by SEC (**Figures 5.1 and 5.2**). In the first case, triflic anhydride was investigated as a reagent for functionalizing the PEO macroinitiator. The reaction eliminates triflic acid, so a hindered base, 2,6-ditertbutyl pyridine, was added into the system in an attempt to trap the triflic acid by-product so that it would not

initiate homopolymerization of the ethyloxazoline monomer. In the second case, tosyl chloride was utilized to functionalize the PEO macroinitiator. The functionalization was successful.

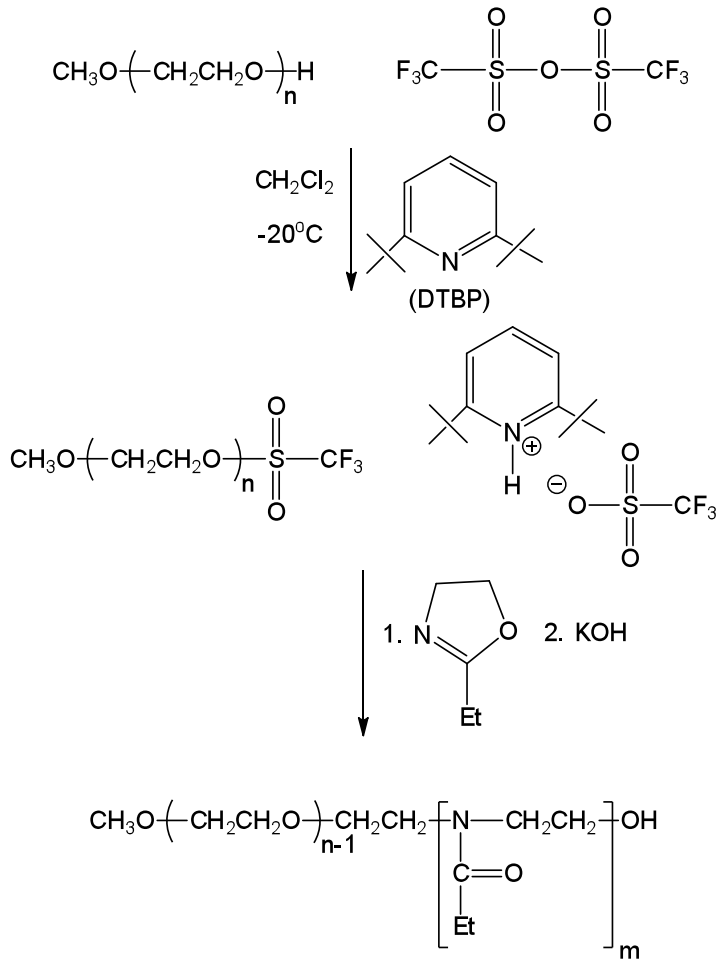


Figure 5.1: Reaction scheme showing synthesis of PEO-b-PEOX with a triflate leaving group and counterion

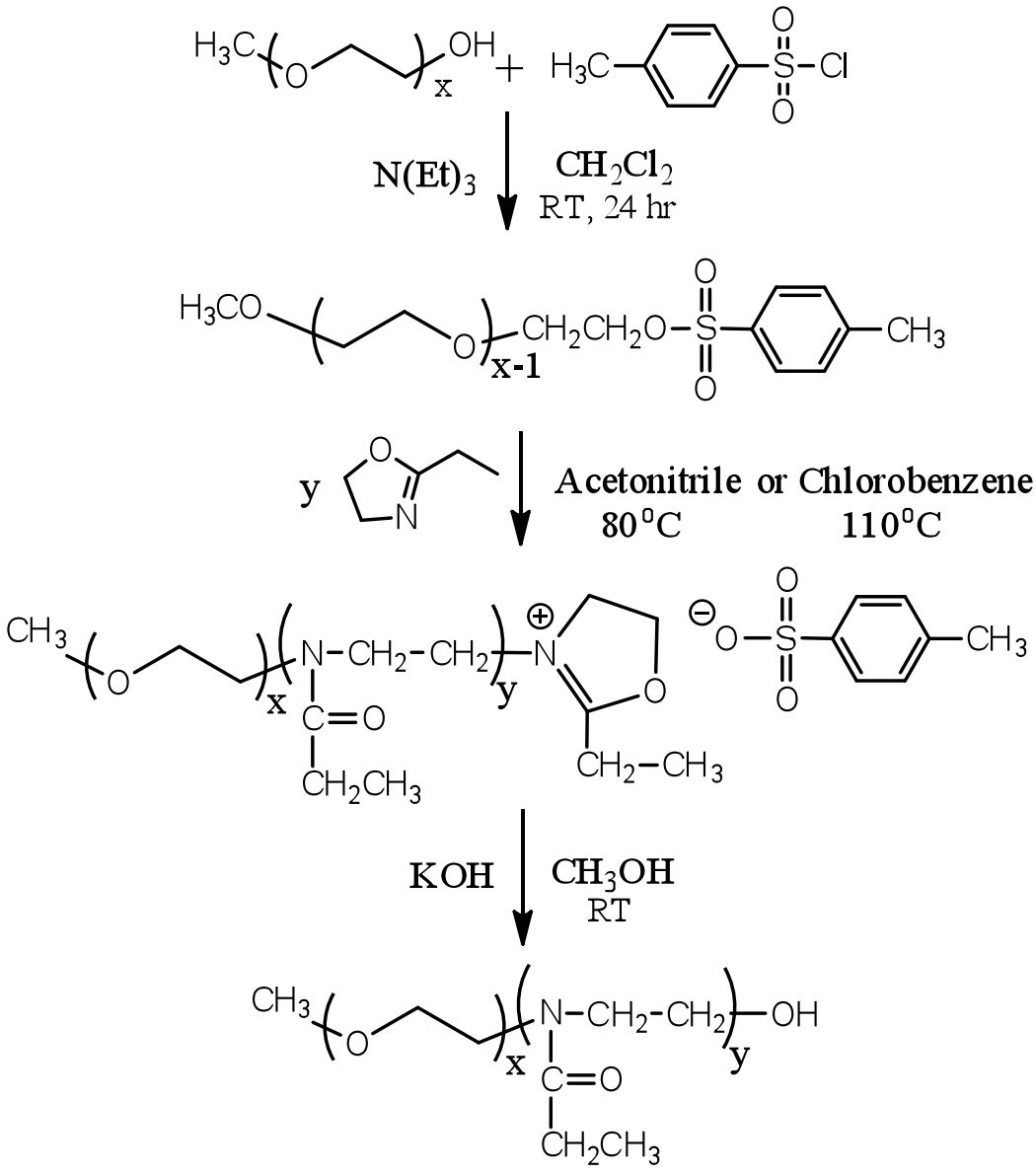


Figure 5.2: Reaction scheme showing synthesis of PEO-b-PEOX with a tosylate leaving group

The copolymers were vacuum dried at 60° C for 2-3 hours before SEC analysis and stored in a desiccator between runs. *N*-methylpyrrolidone was purchased from Fisher Scientific and distilled and filtered before use as the SEC mobile phase. The mobile phase also contained 0.05 M LiBr purchased from Sigma Aldrich that was pre-dried prior

to addition to the mobile phase. Two sets of columns were investigated. The first set consisted of three Viscotek PLgel mixed bed columns connected in series. The Viscotek LT5000L medium, LT4000L low and LT3000L ultra low were polystyrene-divinylbenzene based columns. The second set investigated consisted of three Agilent PLgel 10-µm Mixed BLS columns 300x7.5mm connected in series and these were also polystyrene-divinylbenzene columns. An isocratic pump (Agilent 1260 Infinity, Agilent Technologies, USA) with an online degassing system (Agilent 1260), autosampler and column oven was used for mobile phase delivery and sample injection. A system of multi-detectors connected in series was used. These included a multiangle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology Corporation, USA) operating at a wavelength of 658 nm, a viscometer detector (Viscostar, Wyatt Technology Corporation, USA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, USA). The SEC system was corrected for interdetector delays, band broadening, and the chromatograms were normalized using a 21,720 g/mole polystyrene standard from Agilent Technologies as described in previous chapters. Data acquisition and analysis utiized Astra 6 software from Wyatt Technology Corporation, USA.

5.2.2 Methods

The objective of this study was to measure molecular weight of the copolymers via SEC analysis. In addition we wanted to investigate effects of different initiators on the copolymer structure. Both universal calibration and light scattering were employed to calculate molecular weights. Sample concentrations were in the range of 3-5 mg/mL. The mobile phase used was NMP + 0.05 M LiBr.

5.3 Results and Discussion

In order to establish a method for analyzing these diblock copolymers, we initially analyzed the PEO macroinitiator and a commercial PEOX homopolymer to observe the detector responses. **Figure 5.3** shows a chromatogram for the PEO. The PEO had a very low differential refractive index signal indicating a low *dn/dc* in NMP + 0.05 M LiBr. Therefore the molecular weight could not be calculated by light scattering due to low signal-to-noise. The intensity of the light scattering signal is proportional to the $(dn/dc)^2$ and molecular weight as illustrated in **Equation 2.3**. Because of this proportionality there was only a very slight light scattering signal for this low molecular weight 5,000 g/mole PEO. The signal from the viscometric detector is not affected by the refractive index increment, and thus this detector provided a prominent signal.

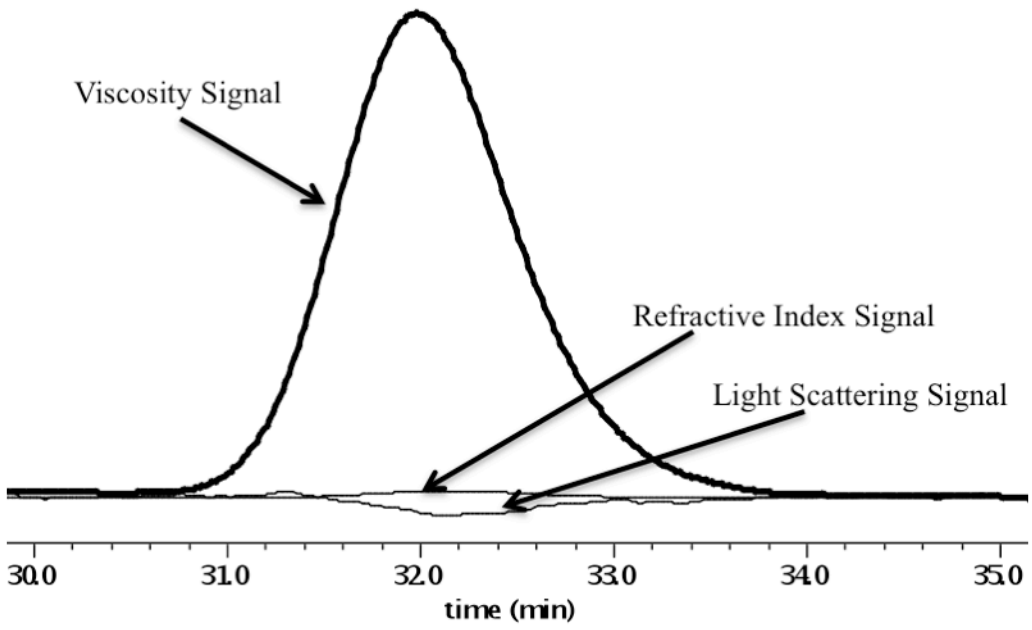


Figure 5.3: SEC chromatogram showing the three detector curves of the PEO macroinitiator

Figure 5.4 depicts chromatograms generated with the three detectors for a PEOX homopolymer. PEOX has a higher dn/dc of 0.0500 and the molecular weight of this sample was high, ~50,000 g/mole. The detector response was evident from all three detectors. The measured molecular weight by light scattering gave a M_n value of 53,300 g/mole and a M_w of 75,400 g/mole with a PDI of 1.41.

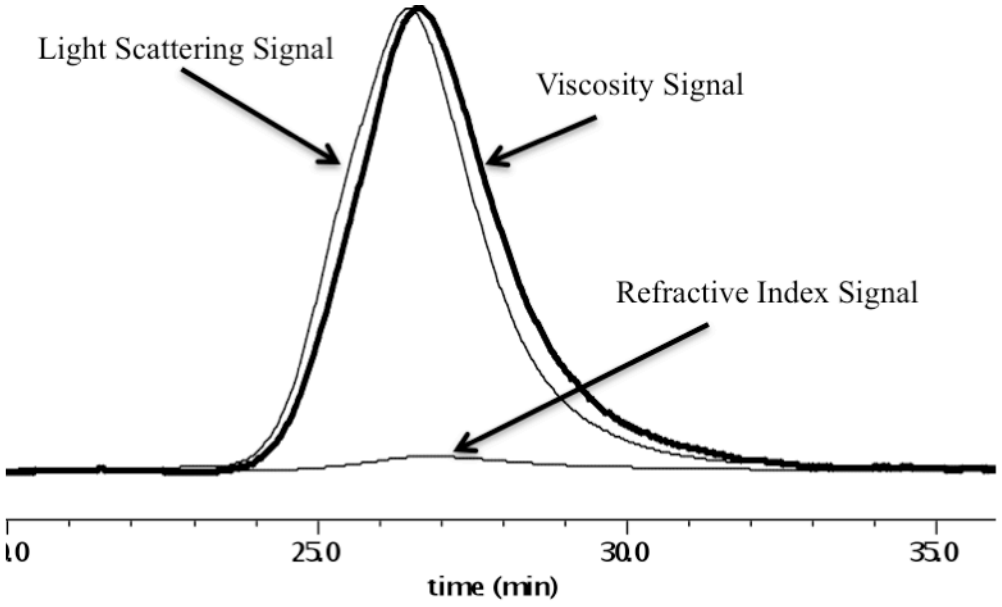


Figure 5.4: SEC chromatogram showing the three detector curves of a high molecular weight PEOX homopolymer

The first PEO-b-PEOX copolymer set that was analyzed was prepared using a tosylated PEO macroinitiator with tosylate as the leaving group. The expected molecular weight of the copolymer (copolymer A) was 5,800 g/mole for the PEO block and 8,900 g/mole for the PEOX block based on analysis by proton NMR. The chromatogram is shown in **Figure 5.5**. The chromatogram shows only signals from the viscosity and the refractive index detectors since the molecular weight and *dn/dc* were not high enough to produce a sufficient light scattering signal. The refractive index chromatogram shows a

unimodal peak whereas the viscosity signal is bimodal. It was also noted that under the low molecular weight shoulder of the viscosity peak, there was no refractive index signal.

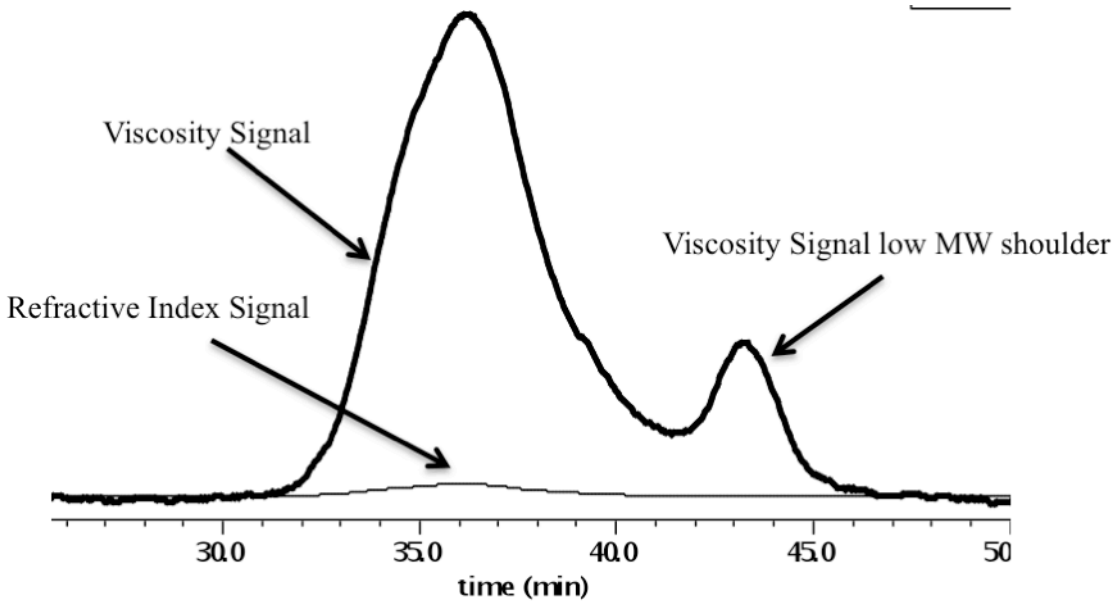


Figure 5.5: Chromatogram of a PEO-b-PEOX diblock copolymer (Copolymer A) showing a bimodal viscosity peak

It was hypothesized that the low molecular weight shoulder on the viscosity peak was due to uninitiated PEO macroinitiator. Thus, a sample of Copolymer A was "spiked" with the PEO macroinitiator to investigate whether the shoulder peak on the viscosity signal intensified. The resulting chromatogram is shown in **Figure 5.6**. The low molecular weight shoulder increased as expected with addition of PEO to the copolymer in solution, confirming that this peak was due to the PEO macroinitiator. The spiked Copolymer A chromatogram was overlayed with the PEO chromatogram (**Figure 5.7**). Due to the differences in *dn/dc*, the shoulder peak, and low light scattering signal the molecular weight data could not be calculated for this copolymer.

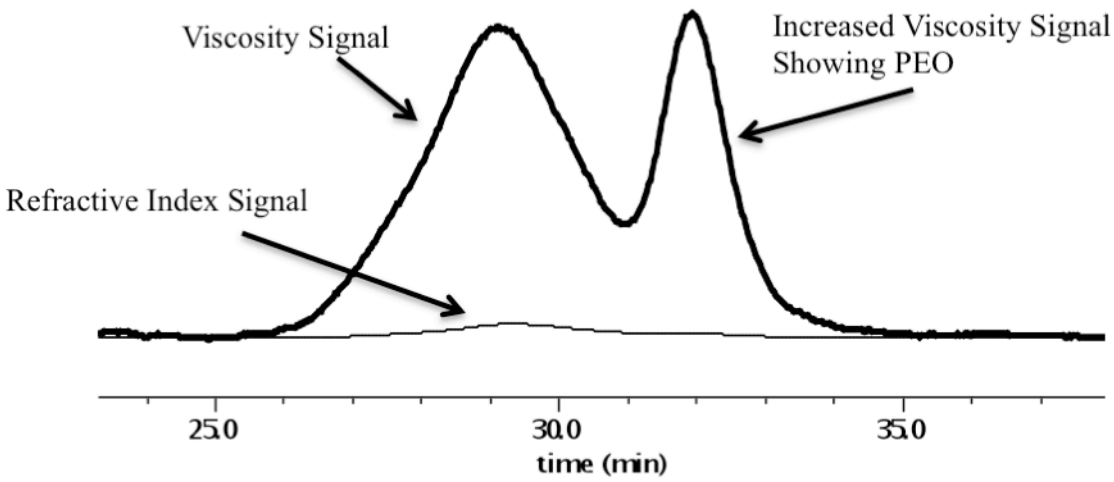


Figure 5.6: Chromatogram of Copolymer A "spiked" with the PEO macroinitiator showing an increase in the viscosity signal resulting from the increased concentration of PEO macroinitiator

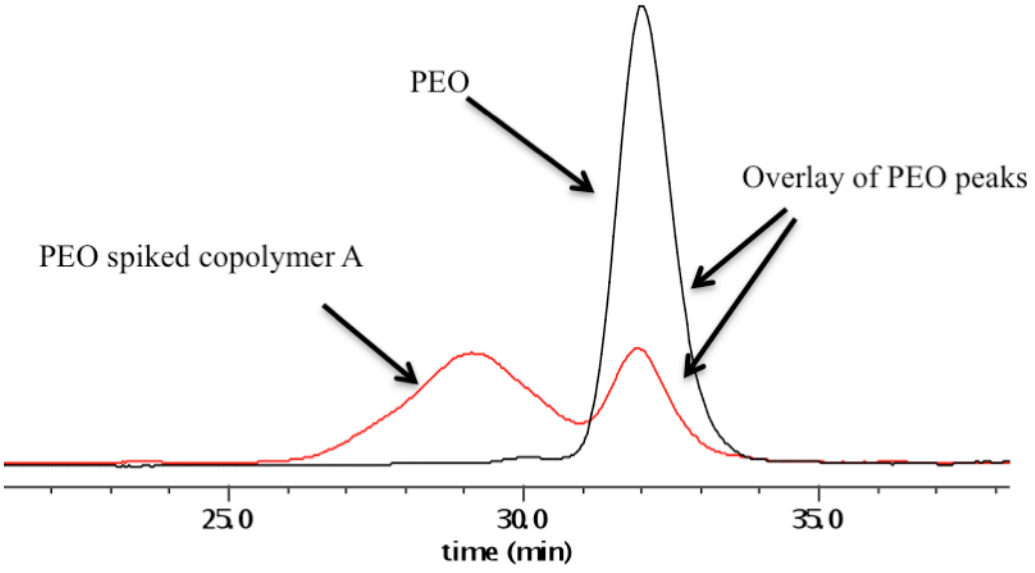


Figure 5.7 Overlay of the viscometric chromatograms of PEO-spiked Copolymer A and PEO macroinitiator

The PEO macroinitiator in addition to the diblock copolymer is due to slow initiation of the tosylated PEO. The chromatogram for the copolymer synthesized with

the PEO-triflate macroinitiator is shown in **Figure 5.8** and noted as Copolymer B. There is also a low molecular weight shoulder on the viscosity curve. However, unlike the previous case with PEO-tosylate, it was noted that this peak also exhibited reasonable refractive index and light scattering signals, and it had been established that PEOX homopolymers had a high refractive index and light scattering signal in this mobile phase (unlike PEO). Therefore, it was hypothesized that this sample contained PEOX homopolymer in addition to the desired PEO-b-PEOX diblock copolymer. The PEOX homopolymer would have been initiated from the triflic acid byproduct that was generated during macroinitiator synthesis. A second PEO-triflate initiated copolymer was synthesized and the chromatogram is shown in **Figure 5.9** and noted as Copolymer C. In this chromatogram one also can observe a low molecular weight shoulder peak on the viscometric chromatogram that was accompanied by a high refractive index and light scattering signal, again indicating that this was due to PEOX homopolymer. In Figure **5.10** the viscometric chromatograms of both copolymer C and the PEO macroinitiator are overlayed. This shows that the low molecular weight shoulder peak is not due to residual PEO macroinitiator since the peaks do not overlay. Because of the broad and bimodal peaks, together with the heterogeneity in sample composition across the molecular weight distribution, the molecular weight data of these copolymers could not be calculated by SEC. This was also true for the other copolymers C and D.

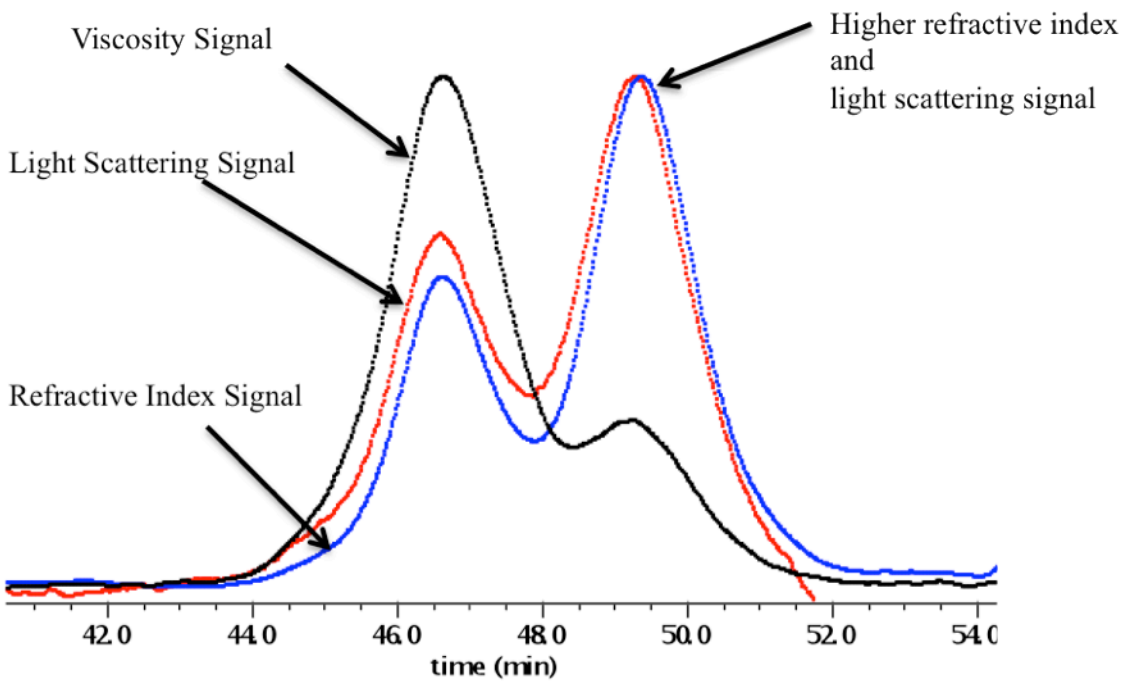


Figure 5.8: Chromatograms of Copolymer B that was nominally a PEO-b-PEOX diblock initiated with PEO-triflate

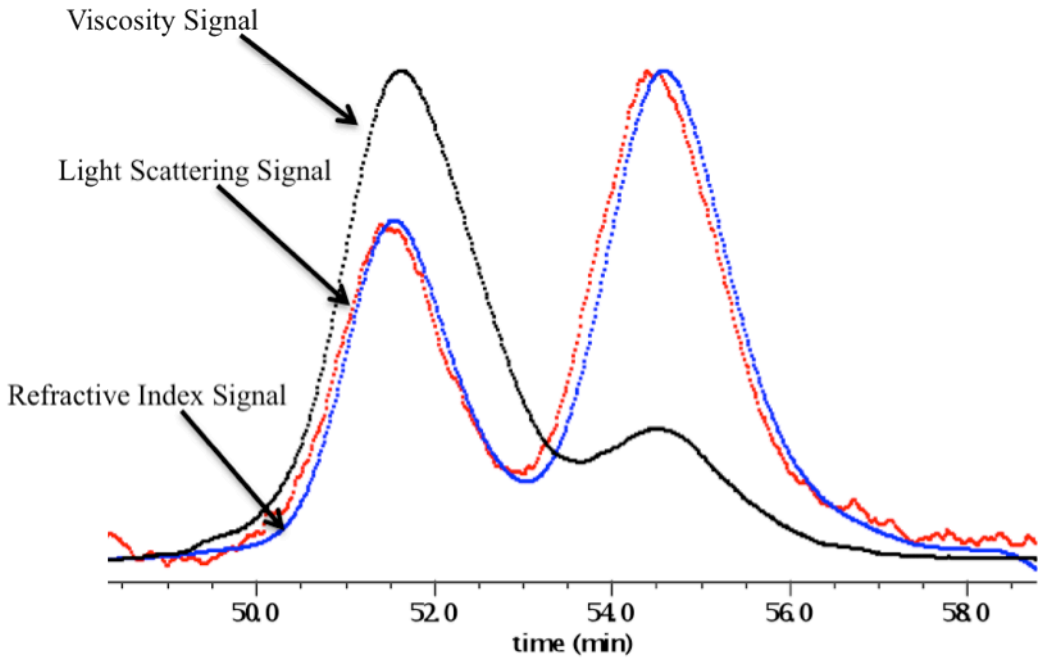


Figure 5.9: Chromatograms of Copolymer C that was nominally a PEO-b-PEOX diblock initiated with PEO-triflate

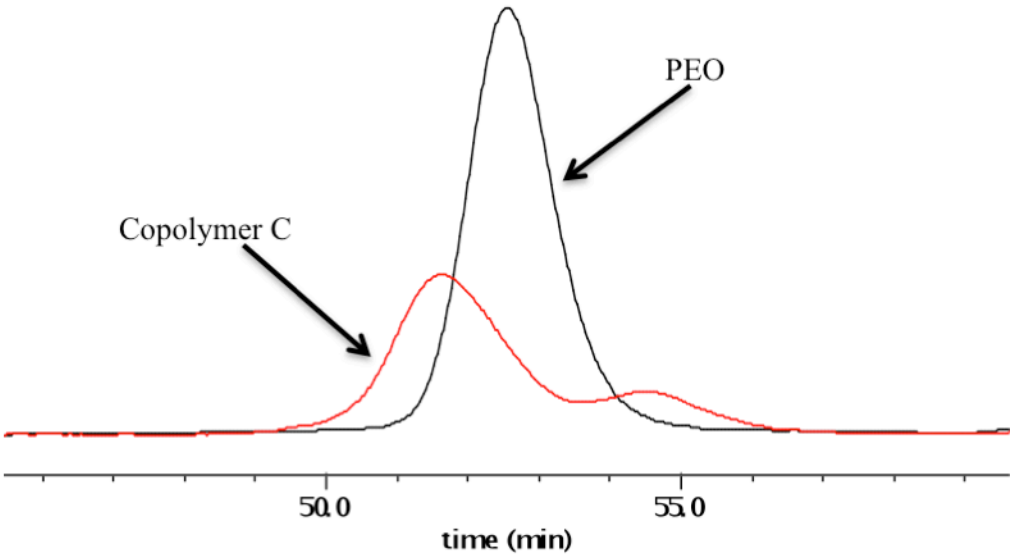


Figure 5.10: Viscometric chromatograms of Copolymer C and the PEO macroinitiator

A third attempt was made to synthesize a PEO-b-PEOX copolymer in the presence of a bulky base with methyl triflate as the initiator. The base used was diisopropylethylamine. It was rationalized that this was a stronger base than the previously utilized ditertbutylpyridine, and that it might be able to trap the proton from the byproduct triflic acid (from the macroinitiator preparation) to prevent the PEOX homopolymer from forming during the reaction. **Figure 5.11** shows the chromatograms of this copolymer noted as Copolymer D. However, as observed in the chromatograms, there was still a slight shoulder peak on the viscometric chromatogram that was accompanied by high refractive index and light scattering signals on the low molecular weight side of the curve. This indicated that PEOX homopolymer had still formed in addition to the desired copolymer.

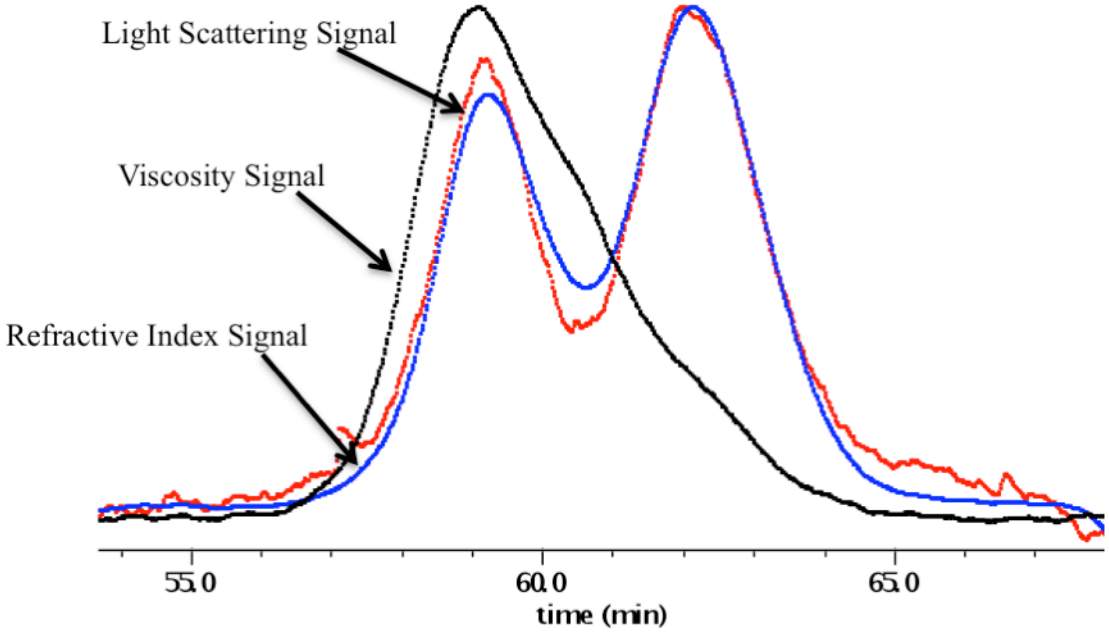


Figure 5.11: Chromatograms of Copolymer D showing a low molecular weight shoulder on the viscometric curve

CHAPTER 6: Conclusions and Recommendations for Future Work

A suitable SEC method was developed to analyze PEOX homopolymers and PEO-b-PEOX diblock copolymers. SEC showed effects of synthesis conditions and molar mass averages for the polymers analyzed.

Chapter 4 discussed the development of a method to characterize PEOX homopolymers. These polymers were analyzed using various columns and two different techniques, which included light scattering and universal calibration. The analysis showed there were differences in the chromatograms for different column packing materials and this also affected the molar mass averages. This resulted in a method using polystyrene-divinylbenzene columns as the best choice for PEOX homopolymer analysis. SEC was also able to show the effects of time and temperature of the synthesis conditions on the homopolymers. It was found that homopolymers initiated with methyl triflate and maintained at 80° C for too long a time (24 hours) resulted in chromatograms with a high molecular weight shoulder, likely formed by coupling reactions once the monomer was depleted. By controlling the time and temperature of the reactions, homopolymers initiated with methyl triflate were produced without these high molecular weight fractions.

Chapter 5 described the use of SEC to investigate effects of reaction conditions on the structures of PEO-b-PEOX diblock copolymers. A method was established for analyzing these copolymers by initially analyzing the individual components. PEO and PEOX homopolymers were analyzed and it was found that the PEO macroinitiators had a very low *dn/dc* in NMP + 0.05 M LiBr. In contrast, the PEOX produced a sufficient signal in both the RI and light scattering chromatograms. It was found that diblock

synthesis initiated with PEO-tosylate resulted in a bimodal viscosity peak, indicating the presence of significant amounts of PEO macroinitiator in addition to the desired diblock. To increase the rate of initiation, a PEO-triflate was prepared by reacting monohydroxy-PEO with triflic anhydride in the presence of hindered amine acid acceptors. However, this resulted in low molecular weight shoulders that were identified as being due to the polymerization of PEOX homopolymers in addition to the diblocks. This was indicated by the strong RI and light scattering signals under the low molecular weight shoulder peaks. It had been hypothesized that using a bulky base in addition to the triflic anhydride in the macroinitiator synthesis step would prevent PEOX homopolymer from forming during the diblock synthesis. Unfortunately, this did not work and the low molecular weight shoulder was still present in the chromatograms. Future work is needed to synthesize PEO-b-PEOX diblock copolymers with unimodal distritbutions. Once these parameters have been established, SEC can be used to determine molar mass averages of these copolymers. This will require additional work to collect fractions of the distributions to analyze how the *dn/dc* changes across the distribution. Future work will also include the modification of the PEOX side chains to create a polyelectrolyte. A SEC method will need to be established to accurately analyze these copolymers.

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