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To the Graduate Council:

I am submitting herewith a thesis written by Elizabeth Angela Stewart entitled "Applications of Mass Spectrometry to Synthetic Copolymers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

Kelsey D. Cook, Major Professor

We have read this thesis and recommend its acceptance:

Bin Zhao, Frank Vogt

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Accepted for the Council:

Anne Mayhew
Vice Chancellor and
Dean of Graduate Studies

(Original signatures are on file with official student records.)

APPLICATIONS OF MASS SPECTROMETRY TO SYNTHETIC COPOLYMERS

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Elizabeth Angela Stewart

December 2006

This thesis is dedicated to my parents,

Douglas and Esselin Stewart,

for their love, support, encouragement, christian education,

and most importantly prayers throughout my life.

ACKNOWLEDGEMENTS

I would like to first thank **God** who has guided me to this point in my life. He has shown me time and time again that he is "able to do exceeding abundantly above all we ask or think, according to the power that worketh in us," (Ephesians 3:20).

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I would like to sincerely thank the Measurement and Control Engineering Center (MCEC), the National Institute of Health (NIH), and the University of Tennessee Chemistry Department for financial assistance.

ABSTRACT

Over the last decade, application of mass spectrometry has grown immensely especially in polymer characterization. The focus of this thesis is to assess and extend the applicability of matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) and electrospray ionization mass spectrometry (ESI MS) in the analysis of synthetic copolymers.

Characterization of a non-ionic copolymer was attempted. The copolymer was styrene-co-maleic anhydride, which is in a class of copolymers that are used for commercial applications where low viscosity, high polarity and/or high reactivity are needed. MALDI-TOF MS was employed to obtain weight and number average molecular weight values (M_w and M_n), polydispersity index (PDI), monomer ratio, and endgroup identity. Results closer to the manufacturer's specifications were obtained for M_w, M_n and PDI using this instrument. However, suitable internal standards were not found to enable distinction between isobaric endgroup possibilities by assessment of accurate mass.

Important information about polymers can be obtained using ESI MS. In conducting studies of in-source depolymerization for copolymer analysis by ESI MS, the limit of detection of monomers in the absence and presence of polymers was determined. In doing so, differences in fragmentation were observed when comparing pure monomer and monomer derived from depolymerization. The hypothesis was then made that monomer contamination could be determined in polymers based on these differences. To validate this method, a homopolymer of styrene sulfonic acid (SSA) was used. It was

shown that the monomer-to-fragment ratio could selectively determine the amount of SSA monomer present. It was then of interest to assess whether determination was possible in a copolymer. The copolymer used to test this was poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA). SSA monomer concentrations were assessed in a 1:1 mole ratio PSSA-MA copolymer. Unknown monomer concentrations were calculated from ESI-MS data using matrix algebra. It was then assessed whether monomer ratios could be obtained simultaneously with contamination. Both a 3:1 and 1:1 mole ratio PSSA-MA copolymer were analyzed. It was found that both characteristics (contamination and monomer ratio) could be determined.

TECHNICAL ABSTRACT

Over the last decade, application of mass spectrometry methods has grown immensely especially in their significant importance in polymer characterization. The focus of this thesis is to assess and extend the applicability of matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) and electrospray ionization mass spectrometry (ESI MS) in the analysis of synthetic copolymers.

Characterization of a non-ionic copolymer was attempted. The copolymer of styrene-co-maleic anhydride is in a class of copolymers that are used for commercial applications where low viscosity, high polarity and/or high reactivity are needed. Efforts to obtain weight and number average molecular weight values (M_w and M_n), polydispersity index (PDI), monomer ratio, and endgroup identity by ESI MS provided low values of M_w and PDI. Possible contributors could be poor sampling, detection, and/or ionization efficiency for heavy oligomers. In an attempt to improve the S/N ratio, KI salt was added. The effect of this was precipitation of the polymer. There was also inadequate complexation of K^+ with the polar groups of the copolymer assumed by the low S/N ratio. Fourier transform MS with MALDI was employed but inadequate signal-to-noise (S/N) ratio was obtained because a suitable matrix could not be found. Also, substitution of KOH for KI did not improve negative ion sensitivity by promotion hydrolysis of the anhydride functionality.

In addition, high resolution was needed to distinguish between two possible sets of endgroups. The resolution needed was beyond that of the ESI MS. Fourier Transform with MALDI MS was unable to provide this as well.

To overcome the limitations of the instruments mentioned above, MALDI-TOF MS was employed to characterize this copolymer. Closer to the manufacturer's results were obtained for values such as M_w, M_n and PDI using this instrument. However, suitable internal standards were not found to determine endgroups.

In conducting studies of in-source depolymerization for copolymer analysis by ESI MS, the limit of detection of monomers in the absence and presence of polymers was determined. In doing so, differences in fragmentation were observed when comparing pure monomer and monomer derived from depolymerization. The hypothesis was then made that monomer contamination could be determined in polymers. To validate this method, a homopolymer of styrene sulfonic acid (SSA) was used. The amount of unreacted SSA monomer present in the polymer could be determined using calibration curves. It was shown that using the monomer-to-fragment ratio could selectively determine the amount of monomer present. It was then of interest whether determination was possible in a copolymer. The copolymer used to test this was polystyrene sulfonic acid-co-maleic acid (PSSA-MA). SSA monomer concentrations were assessed in a 1:1 mole ratio of PSSA-MA. Unknown monomer concentrations were calculated from ESI MS data using matrix algebra. This was achieved by treating mixture spectra of polymer with addition of monomer as linear combinations of sensitivity-weighted reference mass spectra. Monomer concentrations of approximately 1-80 micromolar (μM) SSA monomer were shown to be detectable from calibration curves. It was then assessed whether monomer ratios could be obtained simultaneously. Both a 3:1 and 1:1 mole ratio of PSSA-MA were analyzed. It was found that both could be determined. It was calculated by dividing the SSA ion (183 Daltons) intensity by the MA ion (115 Daltons)

intensity for each copolymer and the ratio of these ratios ($r_{3:1}/r_{1:1}$) were then calculated. This ($r_{3:1}/r_{1:1}$) ratio exhibited that there is three times the amount of SSA in the 3:1 copolymer relative to the 1:1 copolymer.

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CHAPTER 1

INTRODUCTION

1.1 Polymer Review

Polymers are large molecules also known as macromolecules. These molecules consist of many repeating units that are covalently linked and are terminated with units called endgroups. Polymers can be grouped into two categories: natural and synthetic. Examples of natural polymers are starch, cellulose, protein, DNA, RNA, hemoglobin and silk to name a few. Synthetic polymers, the focus of this thesis, are all around us each day and we use them often without realizing it. Table 1-1 displays various properties and applications of a few synthetic polymers. Thousands are available to us and more are being created.

If a polymer's repeating units are the same throughout the molecule, the polymer is called a homopolymer. When there is more than one kind of repeating unit in a polymer, it is a copolymer. Copolymers can be arranged in a random, alternating, block or graft fashion (Figure 1-1). Homopolymerization or copolymerization can occur based on the ability of one monomer (M_1) to react with itself or another monomer (M_2). Possibly $M_1^{\cdot}$ can react with M_1 (reaction 1.1), $M_1^{\cdot}$ can react with M_2 (reaction 1.2), $M_2^{\cdot}$ can react with M_1 (reaction 1.3) or $M_2^{\cdot}$ can react with M_2 (reaction 1.4). These four reactions have four different rate constants $k_{xy,}$ where x is the propagation end and y is the monomer attaching to it.[1] The added monomer becomes the propagating chain end. These are examples of radical chain reactions which are the most common polymerization mechanisms.

Table 1-1[2]. Properties and applications of some synthetic polymers

polymer	***properties***	***applications***
neoprene	very chemical resistant, rubbery	shoe soles, hoses, radiator hoses, wetsuits
polyamide (nylon)	fibrous, strong, durable, moisture resistant	parachutes, carpet, ropes, form-fitting skiwear, hosiery, swimwear, boat sails
polyacrylonitrile	resinous, fibrous, or rubbery, combines with butadiene and styrene to form hard, tough ABS copolymer	ABS plumbing pipe, structural panels, kettle handles, housewares; Orlon® fabric
polychloro-trifluoroethylene	can be molded by extrusion, chemically resistant	gaskets, linings for containers, parts for valves and pumps
polyester	fibers recover quickly after extension and absorb very little moisture.	filters, conveyor belts, sleeping bag insulation, coat insulation, tire cords. Brand name polymers include: Dacron® , Fortrel® , Terylene® ; Mylar® & Lexan® .
polyethylene (high-density) HDPE	can be easily formed into lightweight containers	milk, water, and juice containers; toys, liquid detergent bottles
polyethylene (low-density) LDPE	can be stretched into fine, tough, films.	bread bags, frozen food bags, grocery bags
polyethylene terephthalate (PET, PETE)	strong, easily moldable, chemically resistant, light-weight	soft drink bottles, peanut butter jars, salad dressing bottles, nonbreakable bottles
polyolefin	fiber composed of at least 85% polyethylene or polypropylene	hosiery, sportswear, undergarments, pile fabrics, upholstery, outdoor furniture, indoor carpeting, indoor-outdoor rugs and carpets, filters, marine cordage, automobile seat covers, electrical insulation, carpet backing

Table 1-1 continued

polymer	***properties***	***applications***
polystyrene	thermoplastic; resists attack by acids, alkalis, and many solvents, does not absorb water; excellent electrical insulator.	Styrofoam® cups, grocery store meat trays, fast-food sandwich containers, video cassette cases, compact disk jackets, cafeteria trays, refrigerator insulation
polysulfone	tough, strong, stiff, chemically and thermally resistant	household and plumbing items, various automotive parts, wire coatings
Polytetrafluoro -ethylene (PFTE)	strong, tough, waxy, nonflammable, chemically resistant, slippery surface, thermally stable	Viton® : gaskets, bearings, linings for containers and pipes. Teflon® : non-stick cookware, cooking utensils, pump valves, plumbing tape.
polyurethane	flexible foams, highly elastic quick drying fibers, or hard-drying films	flexible foams: upholstery material, mattresses rigid foams: cores for airplane wings fibers: spandex clothing fiber, support hosiery; Lycra® , Numa® , Spandelle® , and Vyrene. ® hard films: polyurethane varnishes
polyvinyl alcohol	colorless, water-soluble, flammable resin	component in: adhesives, emulsifiers, lacquers, coatings, and films
polyvinyl chloride	rigid when unplasticized; flexible when plasticized	unplasticized form: water pipe, plumbing fittings, phonograph records, synthetic floor tiles, credit cards plasticized form: raincoats, shower curtains, and packaging films.
polyvinyl fluoride	resistant to attack by chemicals or by weathering	protective films for: building sidings, pipes, corrosive chemical containers

Table 1-1 continued

polymer	***properties***	***applications***
polyvinylacetate	water-insoluble resin	carpet backings; film-forming ingredient of water-based (latex) paints, adhesives, lacquers, and cements

-ABBAAABABABBAABABAABBABA-
Random

-ABABABABABABABABABABABAB-
Alternating

-AAABBBBAAAABBBBBAABBB-
Block

```
-AAAAAAAAAAAAAAAAAAAAAAAA-
        B         B         B
        B         B         B
        BBBB      BBBB      BBBB
```

Graft

Figure 1-1. Different arrangements of two repeating units A and B within a copolymer

$$M_1^{\cdot} + M_1 \xrightarrow{k_{11}} \sim\sim\sim M_1^{\cdot} \qquad \text{reaction 1.1}$$

$$M_1^{\cdot} + M_2 \xrightarrow{k_{12}} \sim\sim\sim M_2^{\cdot} \qquad \text{reaction 1.2}$$

$$M_2^{\cdot} + M_1 \xrightarrow{k_{21}} \sim\sim\sim M_1^{\cdot} \qquad \text{reaction 1.3}$$

$$M_2^{\cdot} + M_2 \xrightarrow{k_{22}} \sim\sim\sim M_2^{\cdot} \qquad \text{reaction 1.4}$$

A polymer's molecules can be created with different arrangement of units. A chain can be in a linear, branched, or network form (Figure 1-2).

There are different ways of calculating molecular weight (MW) averages for a polymer. Most synthetic polymer samples will have MW distributions. This is why these averages are important and why there are different ways of calculating them. Two common average molecular weights are used. The number average molecular weight (M_n) equation is:

$$\bar{M}_n = \frac{\sum N_x M_x}{\sum N_x}$$

where N_x is the number of molecules having a molecular weight (M_x). Using various means such as endgroup analysis, membrane osmometry, freezing point depression, boiling point elevation or osmotic pressure can determine M_n.[1] The weight average molecular weight (M_w) of a polymer is a molecular weight average that is biased towards its larger molecules. The equation is:

$$\bar{M}_w = \frac{\sum N_x M_x^2}{\sum N_x M_x}$$

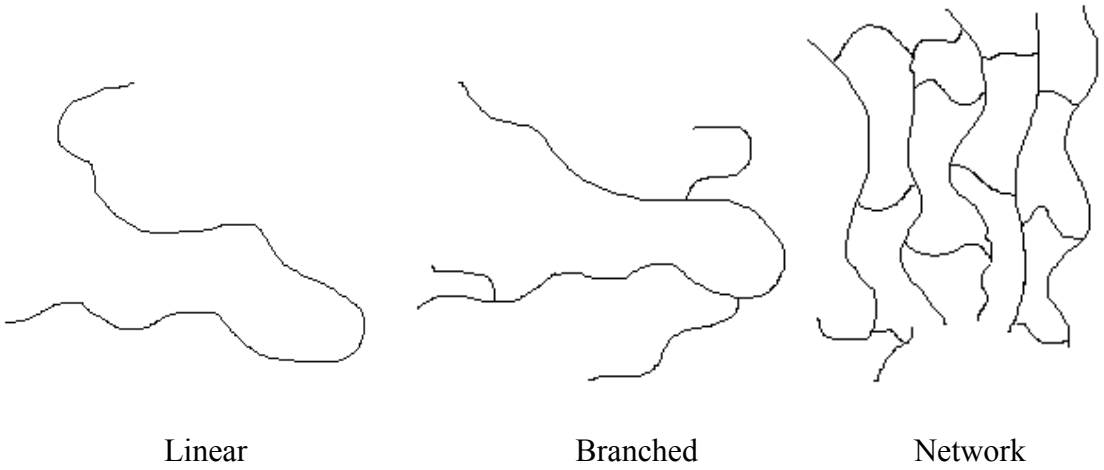


Figure 1-2. This is an assortment of possible polymer architectures.

M_w can be determined by methods such as light scattering, ultracentrifugation, and neutron scattering.[1] Most of these methods estimate M_n and M_w directly without separately determining N_x and M_x for all values of x present. However, when analysis is done by mass spectrometry (MS) and gel permeation chromatography (GPC), N_x and M_x are usually determined. A polymer's polydispersity index (PDI) is a ratio of M_w to M_n. This ratio basically shows how the polymer's molecules present range in molecular weight. When M_n=M_w, PDI =1 then the polymer is monodisperse. This ratio of 1 implies that all molecules in a particular polymer sample have the same molecular weight otherwise, PDI>1. It is important to note that M_n, M_w and PDI are specific to a polymer sample, whereas M_x refers to each molecule. A polymer's molecular weight can range from several thousand to several million Daltons.

Synthetic polymers can be characterized by its structure, MW, endgroups, PDI, monomer contamination and monomer ratio to name a few. Monomer ratio is assessed within a polymer with more than one repeating unit. The structure of a molecule affects the appropriate MW of a polymer for a particular application. For instance, a polymer that has molecules which have hydrogen bonding can exhibit high mechanical strength at low MW. On the other hand, for a polymer that has molecules that have weak interactions such as London dispersion forces, a larger MW will be required in order for that polymer to exhibit high strength. Structure and endgroup information can be probed using methods such as nuclear magnetic resonance (NMR) [3], and mass spectrometry (MS). Often sample clean up is necessary for NMR or MS to obtain accurate information. There are methods of attaining MW other than those described above. GPC, also called size exclusion chromatography (SEC), is a well-known method of determining

MW distribution. This technique separates based on molecular weight. Higher molecular weight compounds elute first and lower molecular weight compounds last. GPC is a time consuming process and not accurate for high mass oligomers. GPC can resolve oligomers below 3000 but distributions turn into broad bands for higher MWs. Little to no information is obtained on chemical composition.[4] Fractional precipitation as well as thin-layer chromatography (TLC) may be used to obtain MW also.[1] However, these two methods do not offer the same resolution as MS at high MW.

Monomer contamination can be assessed using the hyphenated technique of Pyrolysis-Gas Chromatography (Py-GC). This technique is fast, has good sensitivity, and can analyze a wide range of polymers. However, this method has many drawbacks. GC is not an efficient chromatographic method in the separation of degradation products. Also, chromatograms produced are difficult to interpret. It was found that GC was useful if hyphenated with techniques such as MS and Fourier Transform Infrared (FTIR).[4] Infrared spectroscopy (IR) was shown to determine monomer contamination within a polymer.[10] Nevertheless, this method described was sample specific. Carbonyl groups had to be present in the polymer in order to quantitate. GPC is another technique to determine monomer contamination. Also, nuclear magnetic resonance (NMR) has also been used to assess monomer contamination.[11] This method is also very time consuming as sample clean up is essential and often long acquisition times are necessary.

NMR[3] has been used to determine monomer ratios but as noted above, it has its drawbacks. IR has been used to determine ratios. Fourier transform (FT) can be coupled with IR as well. This technique uses a small amount of sample and information can be gained within seconds. However, the data produced from this method is very challenging

to interpret.[12] As shown, MS can be used for many aspects of characterizing polymers may be and used over several other methods.

1.2 MALDI-TOF MS

1.2A MALDI-TOF MS Instrumentation

Matrix-assisted laser desorption ionization (MALDI) is a "soft" ionization method. This means that during the ionization process (Figure 1-3), intact molecular ions are produced. This allows for identification of the MW of the compound under study. Ionization occurs when the matrix in the analyte mixture absorbs a pulsed laser beam. This mixture is of an organic matrix and the analyte. In cases where a non-ionic analyte is being sampled, complexation with a metal ion might be necessary for ion formation. The solvent used to make these solutions should dissolve matrix, analyte and salt. The organic matrix used should be able to absorb the wavelength of the laser. The analyte mixture is usually dried on a metal plate before being introduced into a high vacuum.

The use of a vacuum is to prevent collision of ions when produced. Under vacuum, the laser irradiates the analyte mixture, the matrix absorbs the energy of the laser, and this energy is transferred to the analyte molecules. When this phenomenon occurs, analyte molecules are ionized. These ions are then accelerated by an electric field to the mass analyzer.

The mass analyzer used is usually a time-of-flight (TOF). This analyzer (Figure 1-4) is composed of a drift tube, detector, reflectron and reflectron detector. A TOF resolves ions by their different velocities at constant energy. In the field-free region of

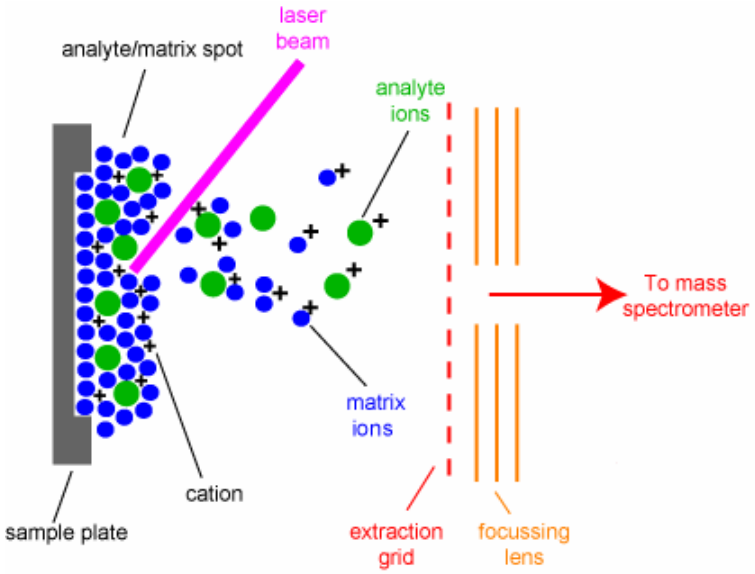


Figure 1-3[13]. A schematic diagram of MALDI is shown. Figure used with permission of Dr. Paul Gates, School of Chemistry, University of Bristol.

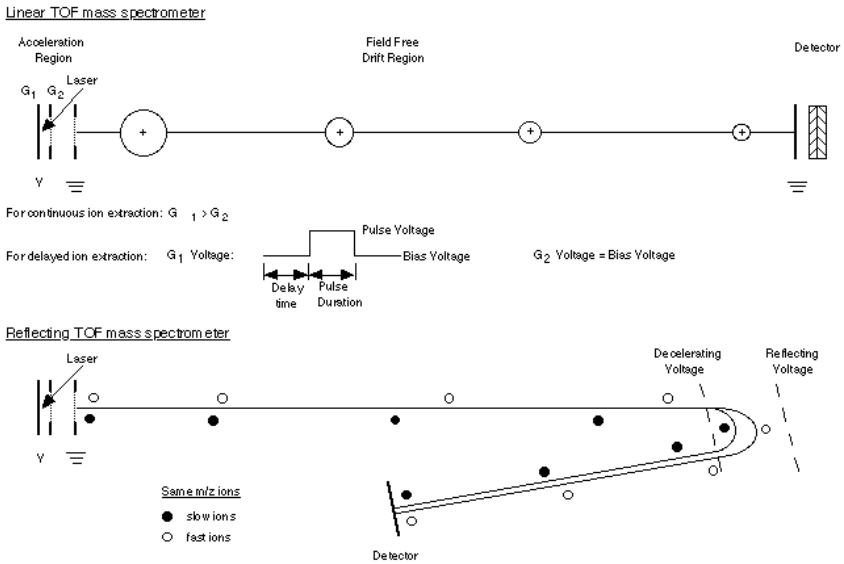


Figure 1. Basic components of a linear (upper) and reflecting (lower) TOF mass spectrometer.

Figure 1-4[14]. The basic components of a linear and reflecting TOF.

the drift tube which is under high vacuum, lower mass ions travel faster and higher mass ions travel slower. This causes ions with similar mass-to-charge ratio (m/z) to reach the detector essentially at the same time. This is known as the linear mode. This mode is preferred where higher sensitivity is needed, especially at high MW. It is usually used when molar mass distributions of high molecular polymers are desired. Resolution attained is usually 200-400. Resolution of an instrument is the instrument's ability to resolve two closely spaced peaks. The equation used to determine the resolution needed for two adjacent peaks is $\Delta m/m$ where Δm is the mass difference and m is the average. A reflectron can be used to increase resolution further by ~10 fold. The reflectron acts like a mirror which furthers the flight of the ions. This compensates for the ions spread in kinetic energies of the same m/z. This greatly resolves ions of same m/z by causing them to reach the detector simultaneously. Accurate mass and endgroup information can be obtained using this mode. In this mode, isotopic peaks are resolved giving accurate mass thereby allowing specific identification of endgroups. Delayed ion extraction can be used in both modes to improve resolution. This also allows for adequate time for complexation of cations.

The detector used is a Microchannel Plate (MCP) detector. The detector is well suited for a TOF MS which requires high temporal resolution. This detector consists of three parts: a converter, MCPs, and a readout device. A converter transforms ions from the analyzer into photons or electrons. These particles are then amplified into an electron pulse by MCPs. A readout device then detects this electron pulse when it exits the MCPs.

1.2B Characterization of Polymers using MALDI-TOF MS

MALDI MS has shown to be the most widely used MS method in characterizing polymers[4]. This method, in addition to composition, is sometimes able to aid in investigating reaction kinetics[15], polymerization mechanisms[16] and degradation[17]. This method has many advantages in the analysis of polymers. Since mainly intact molecular ions are produced, identification of the MW of the polymer being analyzed can be determined. In addition, singly charged ions are mainly produced which greatly simplifies the interpretation of spectra. In recent years, a MALDI method has been made available to analyze insoluble polymers. This "solvent-free" method is an approach used to analyze polymers with very low or no solubility. Polymer and matrix are ground together finely and molded into a pellet[18]. In another sample preparation method, polymer and matrix were ground together and cooled with liquid nitrogen[19]. The use of liquid nitrogen allows the analyte and matrix to be more compressed together. In both cases, the matrix is mixed in its dry form along with the analyte before compacted and sampled. MALDI MS has proven to be a more rapid technique in comparison to chromatographic techniques such as GPC in determination of molecular masses of oligomers.[20] MALDI MS can obtain this information within a few minutes. A limitation to the MALDI MS method is that it is not easily interfaced online with separation methods. However, in recent years on and off-line coupling of MALDI with separation methods have been developed. TLC fractions have been analyzed by MALDI MS[21] and capillary electrophoresis eluent was directly deposited onto a MALDI target plate[22]. Other MS methods have been shown to be more readily interfaced with separation methods. MALDI MS is also incapable of providing correct molecular mass values for

polydisperse polymers if not interfaced with a high resolution analyzer.[23] A high resolution analyzer is able to do this by the fact that it can separate ions efficiently according to oligomer size.

The endgroups of a polymer determines the characteristics of the polymer. For instance, if a polar endgroup terminates the polymer chain of a nonpolar polymer, this will have an effect on its overall polarity. Endgroups are also important in attaining a needed molecular weight. In the case where the polymer's molecules are terminated with reactive endgroups, they may or may not react with other monomers present. This can limit or exceed the preferred molecular weight. In addition, the instability or stability of these endgroups can contribute to the polymer's toxicity if they can react or degrade. For these reasons, it is essential to find a suitable means to assess the endgroups present within a polymer.

A Poly(styrene-co-maleic anhydride) (Poly(S-MAn)) sample was analyzed by MALDI-TOF MS for endgroup identification. The purpose of this research is to determine whether this instrument offers high resolution in order to determine correct endgroups from two different isobaric possibilities. Two samples of Poly(S-MAn) having different endgroups were analyzed previously. For one sample cumene was used as the initiator therefore it was assumed that it would also be the endgroup. The same assumption was made for the second sample in which butyl peroxide was used as initiator but it was suspected the endgroups were two butyl groups based on its mass spectrum. It was also a possibility that since both copolymers were synthesized in cumene solvent, the initiator could have formed a radical with the solvent which then in effect started a chain transfer with the monomers. This is the reason a high resolution instrument is needed to

distinguish between the two endgroups. It was noticed that corresponding isobaric masses only differed in exact mass by 110 mDa. Because of the low resolution that other instruments offered, MALDI-TOF MS was employed to characterize poly(styrene-co-maleic anhydride) (Poly(S-MAn)). Adequate resolution or accurate mass is needed in order to distinguish between two isobaric possibilities. These possibilities have two different sets of endgroups. However, it was not possible to determine this because suitable internal standards were not found. Information such as M_n, M_w and PDI will be compared to the manufacturer's specified values.

1.3 ESI Quadrupole MS

1.3A ESI Quadrupole MS Instrumentation

Electrospray ionization (ESI) is a "soft ionization" process. A general apparatus for ionization is shown in Figure 1-5. A sample solution is passed through a silica capillary needle and then through a stainless steel capillary at a rate of μL/min. An electrospray of the solution is produced with the aid of a strong electric field to the liquid passing through the steel capillary. This electric field is produced by applying a potential difference of several kilovolts between the capillary and the counter electrode at atmospheric pressure. The liquid exiting the capillary accumulates a charge at the surface. This occurs by reduction of the solution at the capillary tip for negative ions and oxidation for positive ions. This depends on ion concentration, mobility and polarization effects. The solution then becomes a charged spray with the aid of a nebulizing gas. Once this spray passes through the counterelectrode, it is subject to a heated inert gas

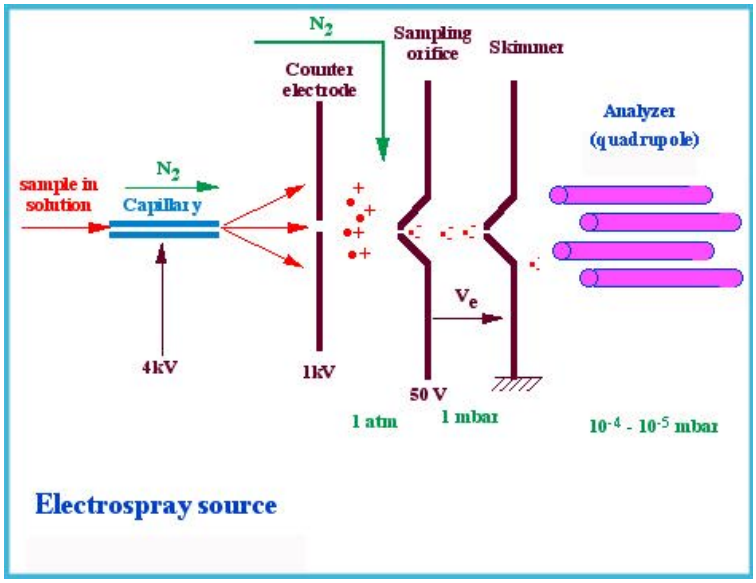


Figure 1-5[24]. The process of Electrospray Ionization (ESI)

where evaporation of solvent takes place. This evaporation of solvent leaves charged ions. These ions enter through a sampling orifice then skimmer and pass into a mass analyzer.

The mass analyzer used is a quadrupole (Figure 1-6). A quadrupole is composed of four cylindrical or hyperbolic metal rods that function like a mass filter. Two of the rods, which oppose one another, are attached to the positive end of a direct current (dc) source and the other two opposing rods are attached to the negative end. Ions enter the center of the four rods. Depending on the alternating current (ac) and dc potential voltage given to these rods, the ions are filtered based on their m/z. Different m/z ions respond to various ac and dc potentials differently. The potentials are set in such a way to neutralize all but selected ions by causing them to collide with rods. This is how these rods behave as a mass filter. The ions that are not eliminated flow to the end of the rods and pass to the detector.

The detector used in these studies is a photomultiplier. This detector can detect positive and negative ions. Consequently, there are two types of conversion dynodes, one that carries a positive potential and the other a negative potential. If in positive ion mode, for example, the ion beam hits the photoemissive cathode then the positive ions are attracted to the dynode that carries a negative potential. Secondary ions are produced from the dynode and these ions then travel to the phosphorescent screen. There they are converted to photons and are then detected by the photomultiplier where amplification can reach 10^5.

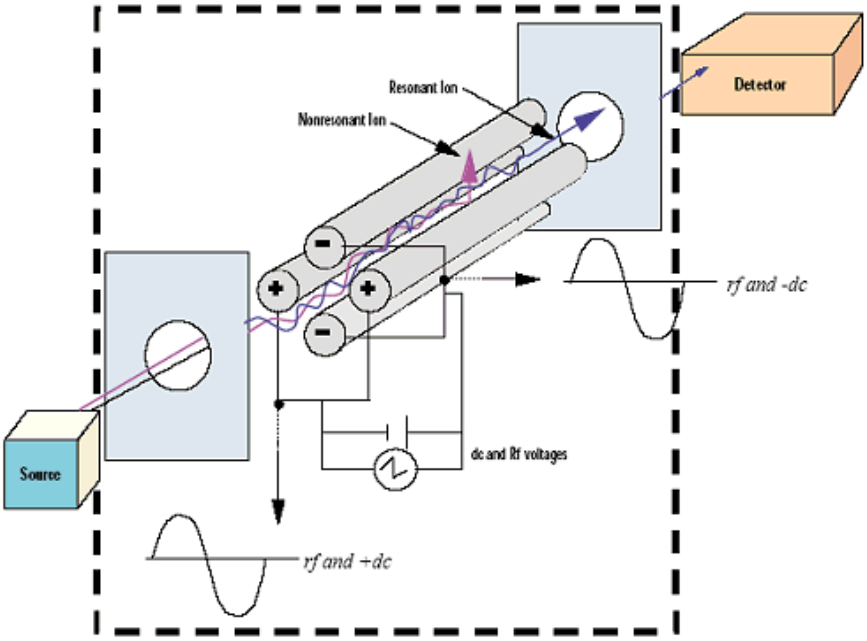


Figure 1-6[25]. Quadrupole analyzer enclosed in dotted line

1.3B Quantitation of Polymers using ESI Quadrupole MS

Electrospray ionization (ESI) MS is also a well-known method in the analysis of polymer systems.[4] This method is complementary to MALDI MS in characterization of polymers. ESI MS is better suited to the analysis of more polar polymers. However, ESI MS has its limitations. Multiply charged ions are usually produced with the exception of polymers with <1,000 Da molecular weight. A spectrum that has multiply charged ions is more complicated therefore increasing difficulty of extracting needed information. This ionization method is more suitable in the analysis of proteins or peptides as multiply charged ions are usually produced. Another factor that limits the types of polymers ionizable by ESI MS is insolubility. Polymers have to be dissolved in a volatile solvent before being analyzed. Some polymers are restricted to a very narrow range of suitable solvents. This ionization method is never used on insoluble polymers. ESI MS has several strengths. Since this is a "soft" ionization method little or no fragmentation of ions is usually seen. Separation methods such as gas and liquid chromatography[26] are easily interfaced online with MS. This is easily done because of compatible flow rates of chromatography and ESI. Capillary electrophoresis (CE) is also readily interfaced as well. Another advantage is the ease of MS^n to other fragmentation methods to determine structure and sequence information. Collision induced dissociation (CID), for example, is usually done in a quadrupole analyzer. This analyzer is ideally suited and used with ESI. For example, polyol mixtures were analyzed by using ESI and tandem MS[27] and sequence distributions in bacterial copolyesters were determined using ESI and multistep MS^n [28]. Also, insight into the fragmentation pathways of four hyperbranched synthetic polyesteramides was gained[29].

Monomer contamination may adversely affect the chemical and physical properties of polymers. Properties to be considered are for example the polymer's thermal stability, reactivity, mechanical stability, viscosity, and polarity. Furthermore, many monomers may also be highly toxic if not incorporated into the polymer. If the monomers used in production or storage of a polymer are not properly evaporated or extracted away it can be a serious problem in such instances as food and pharmaceutical packaging. In these situations, monomers may enter during manufacturing of products and their storage up to the consumption time[5]. Polymers have the tendency to degrade over long periods of time. Degradation may also occur during the heating process to fabricate the end product shape causing residual monomers to be produced[6-9]. Therefore, it is important to monitor monomer contamination. Assessment of monomer ratios within a copolymer is necessary to see if actual ratios were incorporated. This can lead to the discovery of the polymerization mechanism used in the synthesis of this polymer and to determine its properties.

In studies for developing a method for monomer contamination, the assessment of limit of detection was assessed in the absence and presence of polymers using ESI MS. Differences in the fragmentation were observed when sampling pure monomer relative to monomer derived from polymer. [30] In light of this observation, it was of interest to assess whether collision-induced dissociation (CID) of polymers in an electrospray ion source might offer a rapid method for assessing monomer contamination. In a single measurement, both monomer contamination and monomer ratios may be determined within a copolymer once optimum conditions within ESI MS are achieved. The purpose of this research is to determine whether ESI MS can be a fast, relatively easy method of

determining both in copolymers. The two copolymers, which were used to validate this method, were both polystyrene-co-maleic acid having mole ratio of 3:1 and 1:1 each.

CHAPTER 2

EXPERIMENTAL

2.1 MALDI-TOF MS

2.1A MALDI-TOF MS Chemicals

Polystyrene-co-maleic anhydride (Poly(S-MAn)) MW 1000 (Figure 2-1) was received from Elf Atochem®. The following chemicals were purchased from Sigma-Aldrich: dithranol as matrix, sodium trifluoroacetate (NaTFA) as the ionizing agent and tetrahydrofuran (THF) as the solvent. All chemicals were mixed separately in THF with the following concentrations: 10 mg/ml Poly(S-MAn), 20 mg/ml dithranol, and 10 mg/ml NaTFA. Copolymer, matrix and ionizing agent were mixed 1:10:1 (v/v/v), then spotted twice (2 μL) per sample well on a stainless steel plate and allowed to air dry. This procedure was adapted from a study of benzyl and cumyl dithiocarbamates.[31]

2.1B MALDI-TOF MS Instrumental Parameters

MALDI-TOF MS was used to characterize polystyrene-co-maleic anhydride (Poly(S-MAn)). The instrument is a Voyager-DE™ PRO Biospectrometry Workstation. Positive ion reflectron mode was used to acquire spectra. The accelerating voltage used was 25 kV. 300-ns delayed-extraction was used to improve resolution. The acquisition

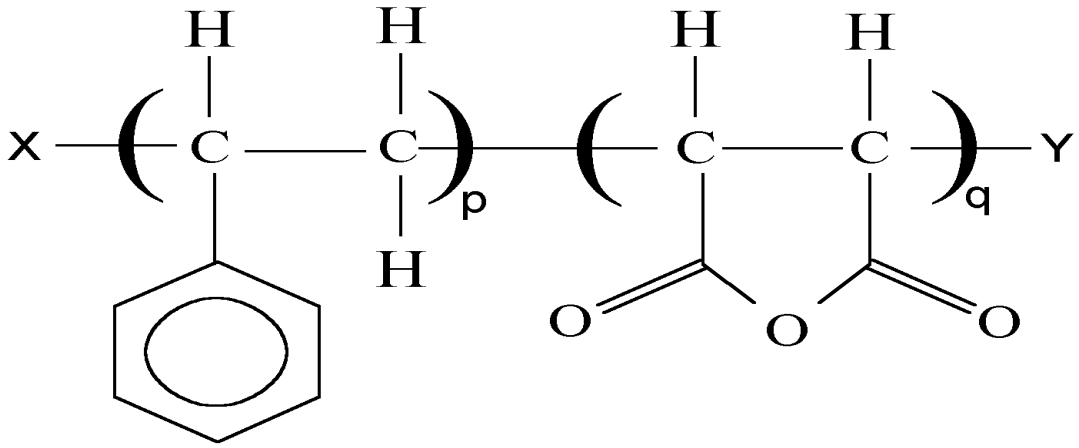


Figure 2-1. The structure of Polystyrene-co-maleic anhydride (Poly(S-MAn)). p represents the number of styrene units and q represents the number of maleic anhydride units. X and Y represent unknown endgroups. Sequence information is not implied here.

mass range was 500-5000 Daltons since this is a polydisperse polymer. The laser power was 1747 microjoules which was just above threshold. 100 laser shots were accumulated to obtain each spectrum.

2.2 ESI Quadrupole MS

2.2A ESI Quadrupole MS Chemicals

The following reagents were obtained from Sigma-Aldrich (Milwaukee, WI): HPLC-grade methanol and water, reagent grade NaOH, reagent grade styrene sulfonate sodium salt, maleic acid sodium salt, homopolymer poly(acrylic acid) (poly-AA) (Figure 2-2 (a)), and 3:1 and 1:1 mole ratio copolymers Poly(styrene sulfonic acid-co-maleic acid) (Figure 2-3) 20,000 Da each. Homopolymer poly(styrene sulfonate) (Figure 2-2 (b)), sodium salt (poly-SSA; 1600 and 6500 nominal molecular weight) was obtained from Pressure Chemical Company (Pittsburgh, PA). 5 μM monomer and 2 μM polymer samples were prepared (except as noted for calibration curves) in 50/50 (v/v) methanol/water. Calibration curves were acquired over the range 1.0 nM to 80 μM for SSA by dilution of a ~1000 μM stock solution. The copolymer solutions were 2 μM 3:1 or 1:1 PSSA-MA in methanol/water 50/50 (v/v). To each copolymer solution 0, 0.001, 0.01, 0.1, 1, 10, and 80 μM of SSA monomer was added.

2.2B ESI Quadrupole MS Instrumental Parameters

ESI MS was used to quantitate two copolymers of polystyrene sulfonic acid-co-maleic acid having 3:1 and 1:1 mole ratio. The instrument used for mass spectrometry

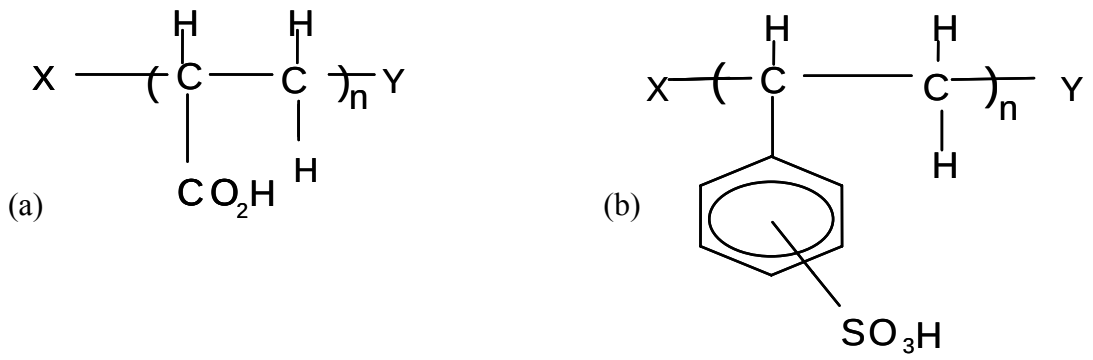


Figure 2-2. The structures of the homopolymers studied. X and Y represent endgroups. n represents the relative monomer content. (a)Poly-AA (b) Poly-SSA.

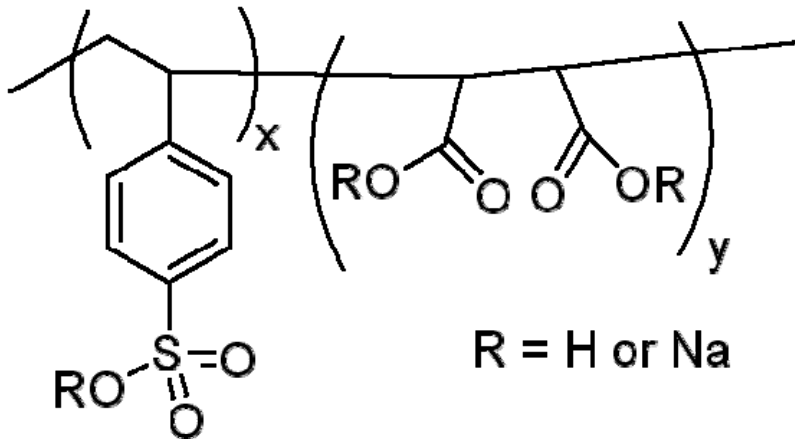


Figure 2-3. The structure of Poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA). X represents the styrene sulfonic acid unit and Y represents the maleic acid unit. Sequence information is not implied here.

was the Quattro II ® (Micromass, Manchester, UK) triple quadrupole mass spectrometer. This MS has a standard Z-source and a coaxial probe. All data were acquired in multi-channel acquisition (MCA) mode. Preliminary scans of the entire mass range (up to 4,000 Da) were used to select the appropriate ranges for subsequent multi-channel acquisition (MCA) scans. MCA spectra were acquired over the selected region (20 – 30 scans per spectrum) 20-500 m/z. Because of the acidic nature of the monomers, experiments employed the negative ion mode. The capillary voltage was set to –2.5 kv and the counterelectrode voltage was –500 kv. Nitrogen gas flow rates were 20 L/hr (drying) and 300 L/hr (nebulizing). Source temperature was maintained at 80°C for all experiments. All ions were subject to 10-100 cone volts. Solutions were each infused at a rate of 5µL/min into the MS using a 250 µL SGE® syringe and a Harvard Apparatus ® model 22 syringe pump.

2.3 Gel Permeation Chromatography (GPC)

To purify polymers, stock solutions of each polymer sample were prepared for GPC by dissolving about 500 mg of polymer in 1 ml of Millipore® water separately. Millipore® water is purified by removing most organic and inorganic contaminants. A 2.5 cm x 32 cm column with a fritted disk to retain stationary phase was packed to a height of about 30 cm with BioGel® P-2 media from Bio-Rad Labs (Hercules, CA). The flow rate of the column was about 1.4 ml/min. After the void volume (which was found to be about 60 ml) had passed, 20 5ml fractions were collected in vials. The fractions were freeze dried using an ethanol dry ice solution and a Virtis® Freezemobile (Biopharma Process Systems Ltd.). For both samples there was over 85% recovery.

2.4 High Performance Liquid Chromatography (HPLC)

Isocratic reversed phase HPLC is done to corroborate with the MS data of determination of monomer contamination in PSSA-MA copolymers. A Hewlett Packard 1100 HPLC system was used with a C-18 column as stationary phase. The mobile phase was 50:50 methanol: water. All samples were run at 1ml/min. The UV detector was set at 254 nm and thermostat at 25◦C for the duration of these experiments.

Samples run were SSA, MA, and pure 1:1 and 3:1 mole ratio PSSA-MA copolymers. Monomer samples were run at a concentration of 1mg/ml and each copolymer sample was run at 2mg/ml. The solvent used to prepare all samples was 50:50 methanol: water.

2.5 Nuclear Magnetic Resonance (NMR)

Proton NMR spectra were acquired using a Varian® (Palo Alto, CA) Mercury 300 MHz NMR. Purified polymer samples were dissolved at ~5mg/ml in D_2O containing 0.03% tetramethylsilane (TMS) Sigma-Aldrich (Milwaukee, WI). This was a 90° single pulse experiment. Spectral width and pulse width were 4506.5 Hz and 7.0 µsec respectively for each sample. The acquisition time was ~50 seconds for each sample. The number of scans was 16 which were 2 seconds /scan and the receiver delay was 1 second.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Characterization of Copolymers using MALDI-TOF MS

Figure 3-1 shows a section of a MALDI-TOF mass spectrum of one of the two copolymers. The peak at 1258.8 Da for example, which was assigned as 6 styrenes, 5 maleic anhydrides, Na+ and cumyl + H endgroups has a calculated exact mass of 1257.44 Da. The same peak could have the composition of 7 styrenes, 4 maleic anhydrides, Na+, and two butyl endgroups with a calculated mass of 1257.55 Da. To obtain accuracy needed to distinguish the endgroups, internal mass standards are needed. It was attempted to internally calibrate this mass spectrum but efforts to do so were unsuccessful.

Values such as number average (M_n) and weight average (M_w) molecular weights as well as polydispersity (PDI) are compared to that obtained using an ESI Quadrupole MS (Table 3-1). As shown, the calculated values of M_w, M_n, and PDI from the MALDI-TOF MS data were closer to the manufacturer's values than of that calculated from ESI Quadrupole MS data. The MALDI-TOF MS values are still far from the manufacturer's actual values. This suggests that using these instruments is not the best method for analysis of high PDI polymers (>1).

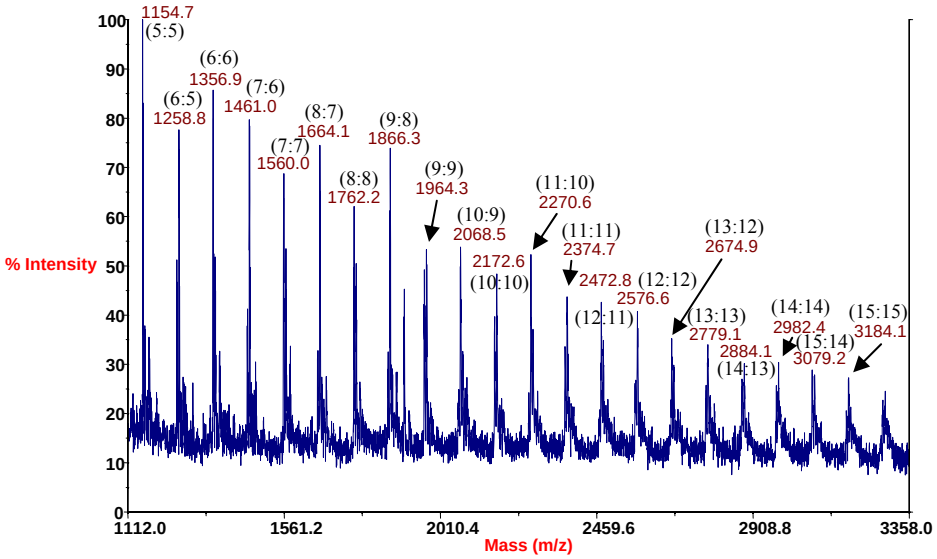


Figure 3-1. The reflectron mode spectrum of Poly(styrene-co-maleic anhydride. The parenthetic numerals indicate the S:MAn ratio.

Table 3-1. Comparison of M_w, M_n, and PDI for Poly(S-MAn) data from different instruments to manufacturer's results. [1]Data obtained from ESI Quadrupole MS, [2]data from MALDI-TOF MS and [3]data provided from manufacturer.

Data	**M_w**	**M_n**	**PDI**
Poly(S-MAn)[1]	1600	1538	1.04
Poly(S-MAn)[2]	1835	1611	1.14
Poly(S-MAn)[3]	5200	2000	2.60

3.2 Quantitation of Copolymers using ESI Quadrupole MS

Dependence of Monomer and Polymer Fragmentation on cone voltage (CV)

The CV is used to produce ions. Ions are usually produced if CV is between 25-70 V. CV can be raised to a higher voltage than that of sample ion voltage to induce fragmentation. CV for production of sample and fragment ions is sample dependent. At low CV (-CV < 20 V), no ions from poly-AA are apparent. As the CV is increased from -20 to -100 V, the monomer peak (71 m/z) increases and small oligomers are present (Figure 3-2 (b)). It is known that from previous studies [32], that it takes more energy to depolymerize terpolymers than it does for copolymers and less for homopolymers. Also, the polymer's acidity plays a large part in the amount of energy it takes to depolymerize. In contrast to what is expected because of AA's weak acidity, the monomer fragments easily at high CV. Figure 3-2 (a) is a spectrum of AA at high CV. It is evident that it is a weak acid as no peak is shown at 71 m/z above solvent background. Figure 3-3 presents a typical ESI mass spectrum obtained at low cone voltage (CV=-20 V, little or no CID) from Poly SSA (1600 M_w). It is noticed for the low CV spectrum of poly-SSA, different charge states are present as shown from several overlapping series. For example, if you examine the series labeled A_n in the figure, ions at m/z [(184n + 58 – 4)/4] corresponds to the quadruply charged oligomer ions. This series has a degree of polymerization n= 4 to 9. Furthermore, all cations present in this series are protons instead of sodium ions. This is proven by the fact that 184 m/z is the mass of styrene sulfonic acid rather than sodium styrene sulfonate. Also, included in each oligomer peak is the endgoup mass of 58 m/z (H + butyl group). It was found that nearly all of the total ion current for figure 3-3 can

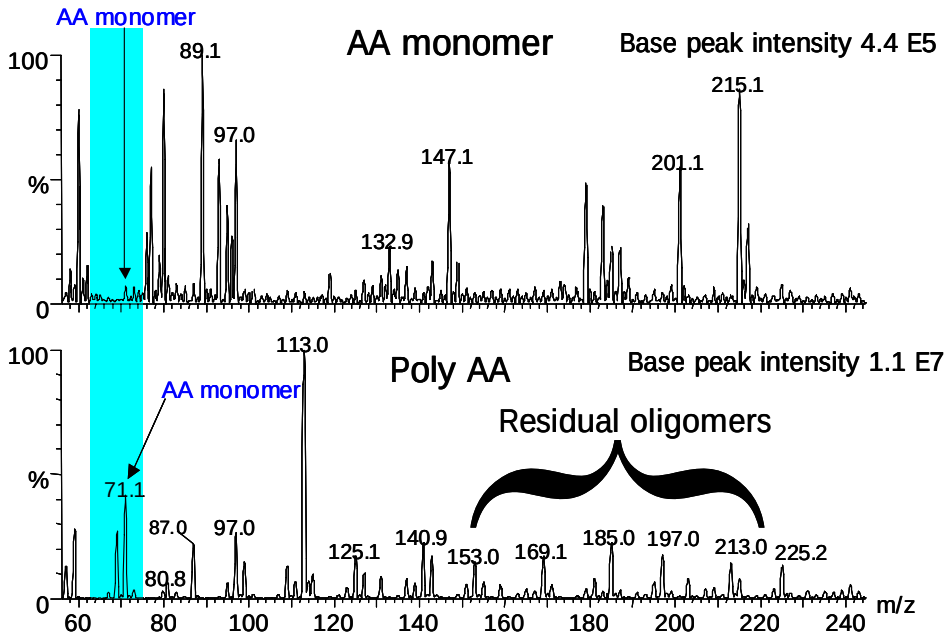


Figure 3-2[33]. ESI mass spectra of (a) AA monomer and (b) poly-AA (nominal molecular weight 5000) obtained with CV = –100 V.

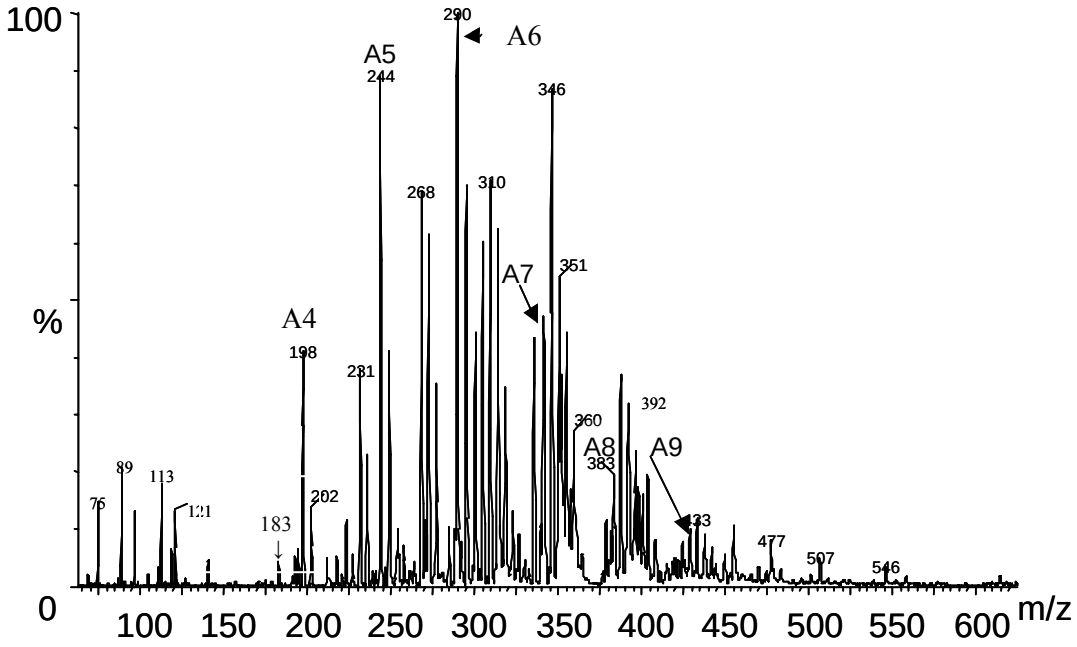


Figure 3-3[33]. ESI mass spectra of poly-SSA obtained with CV = –20. Peaks labeled A_n represent a series of oligomers with the subscripted degree of polymerization, combined endgroup mass = 58, and charge state z = -4.

be assigned to series with degree of polymerization ranging from 3 to 13. Charges of these series range from -3 to -6 with retention of up to 6 sodium atoms (Table 3-2).

At -20 CV, there is a signal at 183 m/z, which indicates the presence of monomer contamination or degraded SSA. It is noticed that when CV is changed to -100 V (Figure 3-4), the poly-SSA polymer envelope disappears and the peak at 183 m/z increases. From calibration curves, this corresponds to a concentration of ~14 μM SSA.[32] Also, an SSA related fragment at 80 m/z becomes the base peak. It is noted that by changing the CV from -20 to -100, the intensity at 183 m/z is no longer prominent. This intensity decreased by ~63±2% and both related monomer ion fragment intensities (80 and 119 m/z) increase. This MS data at low (-20) and high (-100) CV, indicates that the source of monomer is identifiable. It suggests that the signal at183 m/z in Figure 3-4 (high CV) is from polymer.

Use of Fragment Ratios to Indicate Monomer Contamination in Homopolymers

From previous studies on homopolymers[33], it was proven that at low and high CV the monomer:fragment intensity ratio (i_{183}/i_{80}) is different. Therefore, the source of these ions can be determined. For example, the poly-SSA when sampled gives a ratio of ~ 1.74 ± 0.05 and the SSA monomer gives a ratio of ~ 0.02 ± 0.01. Figure 3-5 shows the breakdown diagrams of the monomer and the polymer intensities. These diagrams indicate that the absolute intensities for monomer are substantially higher for polymer when CV is between -50 and -100 whereas at for these CV's the fragments are more prominent for the monomer. It was found that in adding SSA monomer to 2 μM of

Table 3-2[33]. Assignment of ions in Figure 3-3 to series of the general formula $[184n + 22m - z + 58]^{-z}$. %TIC refers to the fraction of the total ion current represented by the indicated series.

z (charge)	n (SSA)[1]	m (Na)[2]	n_{max} (Relative Intensity, %)	% of TIC
3	3-4	0	3 (7.9)	0.4
3	4-7	1	5 (24.8)	1.8
3	6-7	2	6 (15.5)	0.6
4	4-7	0	5 (56.6)	4.5
4	5-10	1	6 (89.4)	5.0
4	6-10	2	6 (43.4)	3.2
4	7-10	3	7 (43.6)	2.2
4	8-11	4	8 (26.5)	1.2
4	9-11	5	9 (10.6)	0.5
5	5-8	0	6 (100.0)	6.9
5	6-10	1	6 (83.6)	8.4
5	7-10	2	7 (69.2)	5.5
5	8-12	3	8 (53.2)	3.8
5	9-13	4	9 (41.2)	2.3
5	10-13	5	10 (15.3)	1.2
5	11-13	6	11 (6.0)	0.4
6	6-9	0	7 (99.4)	8.8
6	7-12	1	9 (63.2)	7.6
6	8-12	2	10 (69.9)	5.8
6	9-13	3	11 (22.3)	4.9
6	10-13	4	11 (11.6)	1.0
6	11-13	5	11 (13.9)	0.7
3-6	3-13	0-6		89.2% of TIC

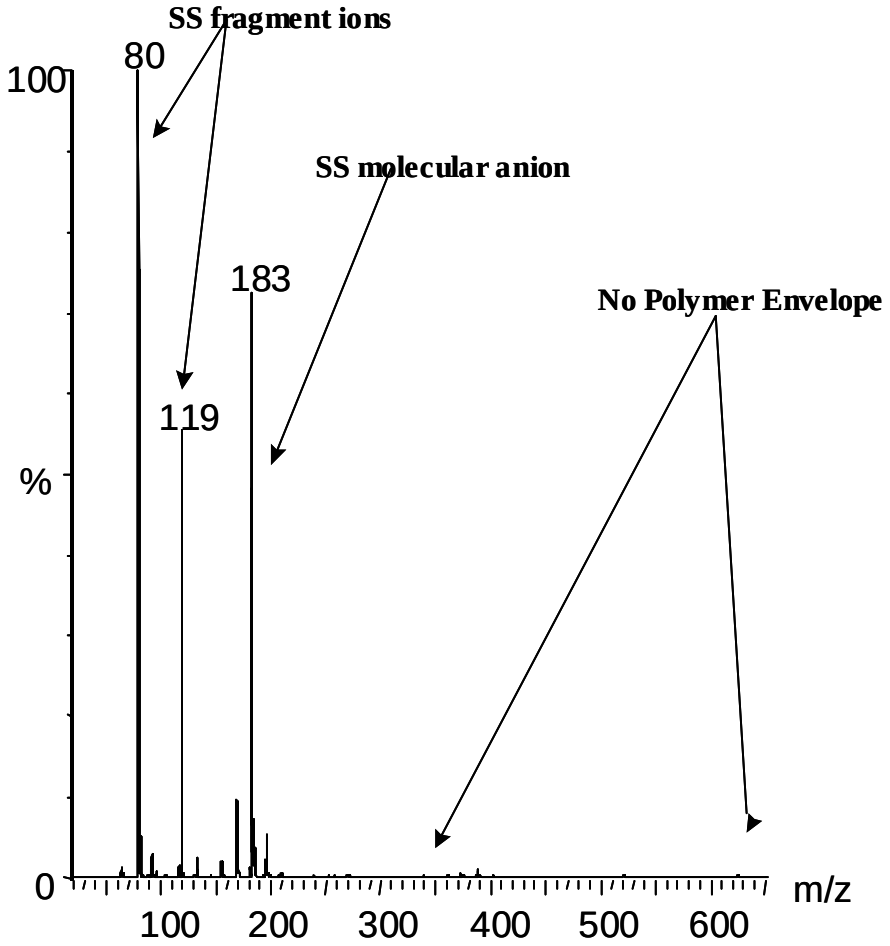
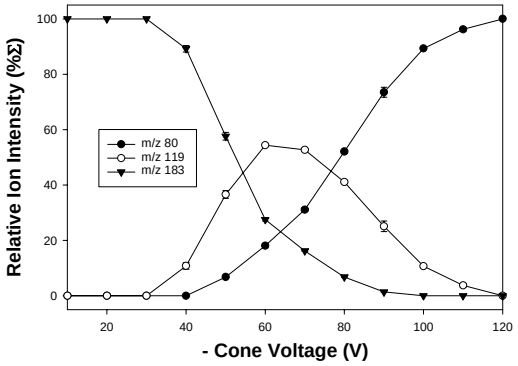


Figure 3-4[33]. ESI mass spectra of poly-SSA obtained with CV = –100. Note the disappearance of the polymer envelope and the generation of monomer and fragment ions.



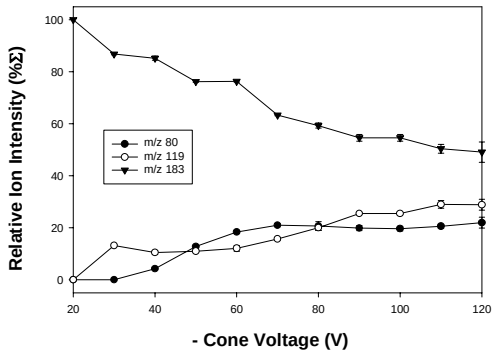


Figure 3-5[33]. SSA monomer (5 μM) and polymer (2 μM) breakdown diagrams.

polymer that there is a noticeable change in the monomer:fragment ratio between addition of ~0.1 to 80 μM from -60 to -100 CV. Beyond the addition of ~80 μM, the ratio is as 5μM pure monomer (Figure 3-6). This was also the case when the (i_{119}/i_{80}) ratio was assessed. Table 3-3 shows the slopes of the calibration curves from -60 to –100 CV. It is shown that at the lowest CV, the slope is the highest indicating higher sensitivity. Fragments of 183 m/z are not produced below -50 V indicating this energy cannot break apart the polymer envelope.

At the higher energy needed for analysis of monomer ratios in copolymers[32], simultaneous analysis of monomer contamination and ratio seems possible. Additionally, these studies indicate that monomer contamination up to ~ 0.1 μM will have little influence the monomer: fragment ion ratio of homopolymers.

It is common for polymer samples to be contaminated with residual monomers. The monomers detected in Figure 3-4 may have been present as contaminants undetected in Figure 3-3 because of low sensitivity at low cone voltage. From simple calibration curves for the individual monomers obtained at high CV, it can be estimated that roughly 1.6 ± 0.2 μM SSA would be necessary to account for the signals at m/z 183 in Figure 3-4. This is well above the 2σ limit of detection (LOD; concentration at which the signal from monomer exceeds that from a polymer blank by twice the standard deviation of the blank) for these monomers at low CV in the presence of poly-SSA (24 nM for SSA). If monomer had been present at the μM level, it should have been detected in Figure 3-3.

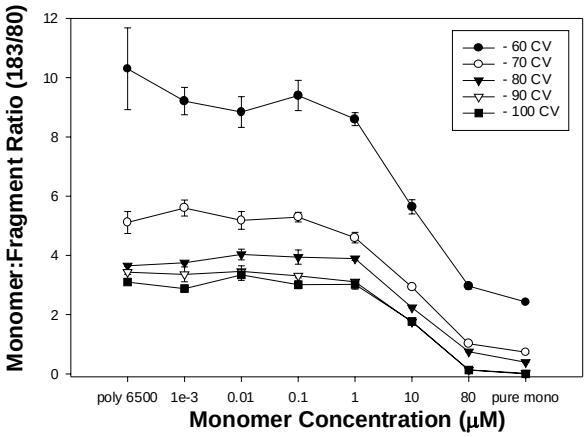


Figure 3-6[33]. Monomer: fragment ratios from 2 μM poly-SSA (M_w 6500) with addition of up to 80 μM SSA monomer.

Table 3-3[33]. Slopes from "calibration curves" (Fig. 3-6) data considering only from 1-80 μM SSA monomer addition to poly-SSA (nominal molecular weight 6500) are displayed.

- CV (V)	Poly- SSA (x10 4)	
	i_{183}/i_{80}	i_{183}/i_{119}
60	-6.9	-6.6
70	-4.5	-3.3
80	-3.6	-2.6
90	-3.5	-2.3
100	-3.4	-1.9

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Use of Fragment Ratios to Indicate Monomer Contamination and Ratios in Copolymers

SSA and AA copolymer was not commercially available therefore poly(styrenesulfonic acid-co-maleic) acid was analyzed. Maleic acid (MA) is a weak acid like AA and it was thought that similar results would be found. In addition, polymaleic acid was not commercially available and it was determined that it could not be synthesized. Synthesis of this homopolymer is impossible because of the structure of its monomer. MA has two carboxylic acid groups attached to the two carbons adjacent to the double bond. 1, 2 disubstituted alkenes are known not to be homopolymerized.

Before both copolymers could be run by ESI-MS or NMR for assessment, sample clean up by GPC was essential. These copolymers had been purchased several years prior and had shown to degrade by ESI-MS. Monomer: Fragment ratio of the purified 1:1 copolymer was 7 ± 1 compared to the 0.52 ± 0.03 for the unpurified 1:1 copolymer. The monomer: fragment ratio was low proving a high amount of monomer present. From calibration curves, more than 80 μM of SSA monomer is present.

It was found that at 60 CV, monomer contamination as well as monomer ratios could be determined. Unknown concentrations of SSA monomer were calculated using matrix algebra. From calibration curves (Figure 3-6), it was estimated that approximately 1-80 μM of SSA monomer could be detected in the copolymers of SSA and MA. Concentrations were obtained by treating mixture spectra of polymer with addition of monomer as linear combinations of sensitivity-weighted reference mass spectra. Sensitivities are determined by using a normalized pure spectra polymer and monomer with a sample spectrum containing known amount of polymer and monomer.

Sensitivities are then used with the same normalized pure spectra to calculate the concentration of a mixture of unknown concentrations of monomer and polymer. All spectra were normalized to the base peak in order to avoid shifts in absolute intensity measurements before averaging triplicate spectra. The equation used for these calculations is shown in figure 3-7. This was a method used in our lab in a previous publication[33] to calculate unknown concentrations of each gas in a gaseous mixture. Table 3-4 displays calculated unknown SSA concentrations and unknown 1:1 copolymer concentrations in mixture solutions.

When determining the monomer ratios, it was found that the ratio for the 3:1 copolymer was calculated to be 66 ± 6 and for the 1:1 copolymer it was 20 ± 2. The ratio of these ratios ($r_{3:1}/r_{1:1}$) is 3.3 ± 0.4 exhibiting the amount of SSA in the 3:1 copolymer relative to the 1:1 copolymer.

3.3 HPLC assessment of monomer contamination

Isocratic reversed phase HPLC was used to quantitate the amount of SSA and MA monomer present in PSSA-MA copolymers. Table 3-5 shows the calculated degraded amount of SSA and MA combined in each PSSA-MA sample. Instrumental response was assumed linear for all samples because the same conditions were used for all experiments. Both monomers have approximately the same retention times seen from individual runs of both monomers. Therefore, the combined amount of degraded monomer can be determined in a copolymer sample. Also, no other peaks were present after running polymer samples for a little over 60 minutes. Both 1mg SSA and 1mg of

$$\begin{bmatrix} a_1 & b_1 \\ a_2 & b_2 \\ a_3 & b_3 \end{bmatrix} \begin{bmatrix} S_a & 0 \\ 0 & S_b \end{bmatrix} \begin{bmatrix} C_a \\ C_b \end{bmatrix} = \begin{bmatrix} I_1 \\ I_2 \\ I_3 \end{bmatrix}$$

a-pure monomer intensities
b-pure polymer intensities
S_a-pure monomer sensitivity
S_b-pure polymer sensitivity
C_a-monomer concentration
C_b-polymer concentration
I-intensities in polymer sample with added monomer
Peak 1-183 m/z
Peak 2-80 m/z
Peak 3-119 m/z

Figure 3-7. Matrix algebra equation used to calculate unknown monomer and polymer concentrations in a sample.

Table 3-4: Comparison of example calculated monomer (M) and 1:1 copolymer (P) concentrations in mixture sample containing 1 μM SSA monomer and 2 μM polymer using (a) 183 m/z and 119 m/z peaks and (b) 183 m/z, 119 m/z and 80 m/z.

	Experimental Concentrations (uM)	**Calculated Concentrations (uM)**
(a)183 m/z, 119 m/z	M=1	M=0.65±0.2
	P=2	P=1.84 ±0.5
(b)183 m/z,119 m/z, 80 m/z	M=1	M=1.5±0.01
	P=2	P=2.4±0.02

Table 3-5. Estimated total degraded monomer values using HPLC data in (a) sampled "pure copolymers" compared to 1 mg SSA and (b) sampled "pure copolymers" compared to 1 mg MA.

Sample	**(a)Degraded monomer (mg)**	**(b)Degraded monomer (mg)**
Pure 1:1 PSSA-MA	0.25±0.08	0.25±0.08
Pure 3:1 PSSA-MA	0.14±0.03	0.13±0.03

MA was used as a reference to calculate the total amount of monomer present. The "t-test" was used to determine whether these two methods could be used to calculate the amount of monomer. It was found that the calculated t is less than the t value listed for the 95% confidence and 1 degree of freedom. The two techniques are not significantly different at the 95% confidence level.

The purified copolymers had degraded from the time of purification by GPC. The copolymers were sampled and the integrated area comparable to the 1 mg SSA or MA displayed how much degraded monomer was present. For the 1:1 mole ratio pure PSSA-MA, it was calculated to have ~0.25mg (12.5%) degraded monomer. The 3:1 mole ratio pure PSSA-MA, it was estimated to have ~0.14mg (7%) degraded monomer.

3.4 NMR assessment of monomer ratios

Proton NMR spectra acquired on the Varian® 300 MHz NMR were assessed for monomer ratio to corroborate with ESI-MS data. Proton NMR estimation (Figure 3-8) for the copolymer was done using CS Chemdraw® Ultra Version 6.0. This allowed us to see where chemical shifts characteristic of this copolymer would occur. Methyl protons (0.96 shift), methane protons (1.65 shift), and benzene protons (7.54 and 7.93 shifts) from the SSA monomer were used for quantitation. The two backbone methane protons (2.35 and 2.76 shifts) from the MA were used. For the 3:1 copolymer spectrum (Figure 3-9), after integration the monomer ratio was calculated to be 2.72:1 SSA: MA respectively and for the 1:1 copolymer spectrum (Figure 3-10), after integration the ratio was calculated to be 1.19:1 SSA: MA.

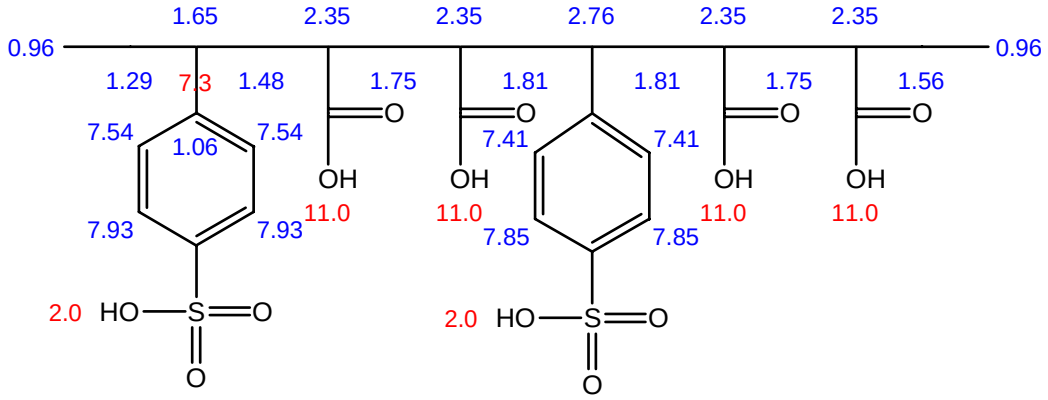

Figure 3-8. Proton estimation of PSSA-MA copolymer

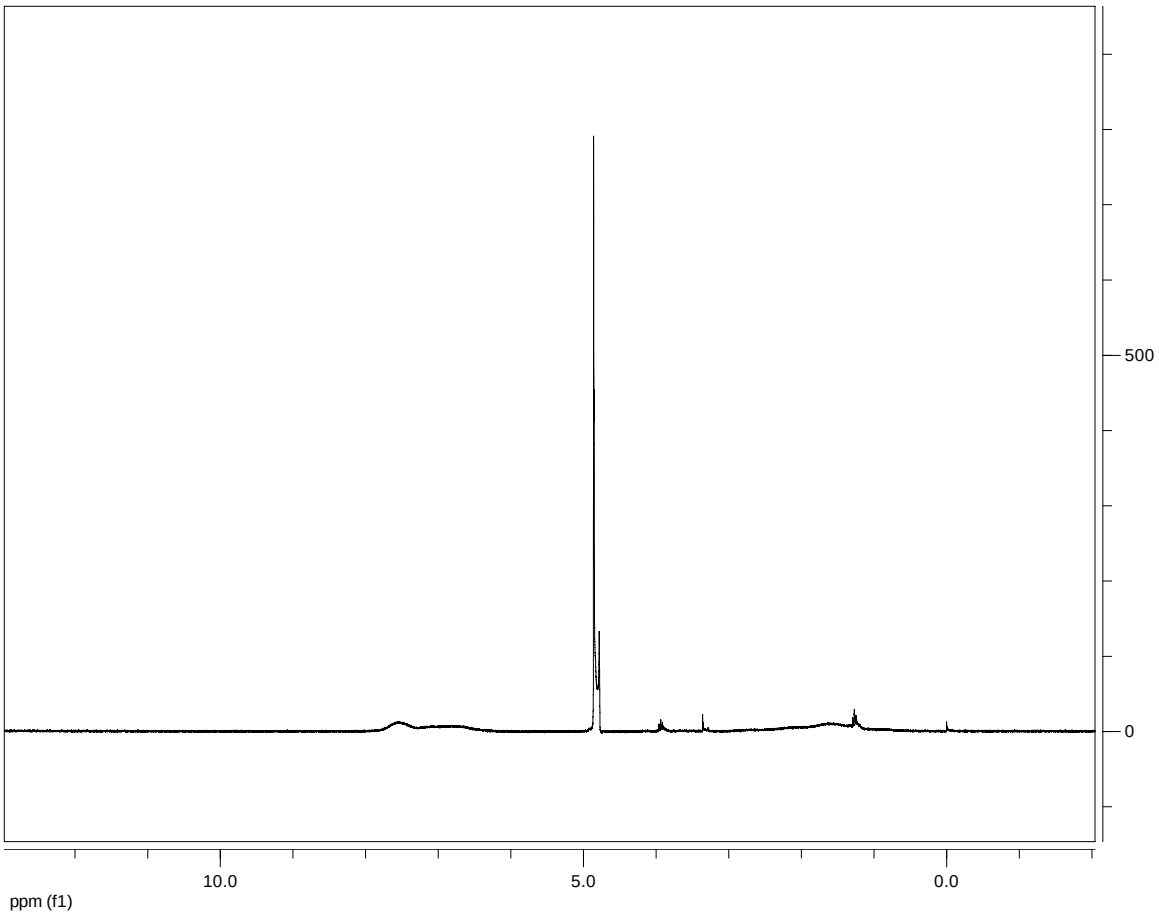

Figure 3-9. Proton NMR of 3:1 PSSA-MA copolymer

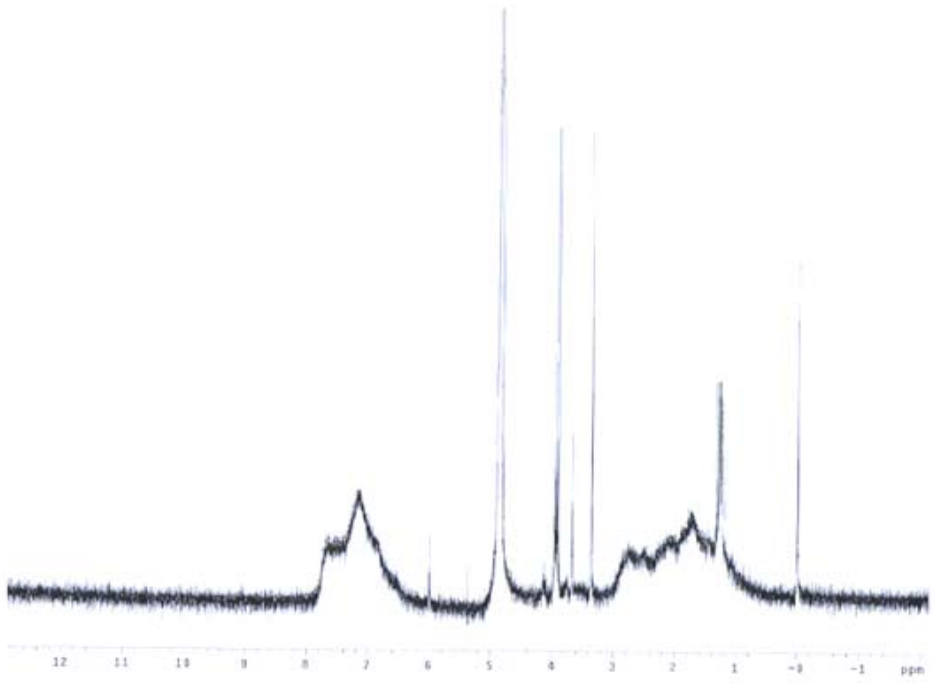

Figure 3-10. Proton NMR of 1:1 PSSA-MA copolymer

CHAPTER 4

CONCLUSIONS AND FUTURE WORK

4.1 Applications of MALDI-TOF MS to Copolymers

Internal standards need to be identified in order to calibrate the MALDI-TOF MS spectrum for Poly(S-MAn). Compounds chosen should be structurally compatible with the copolymer. Proteins or peptides are ideal for standards as usually one peak is shown. Two standards should be chosen to give two peaks surrounding oligomer peaks of the polymer. One standard may be adequate if the molecular weight (MW) spacing between oligomers is known. The MW of the standard and the peak spacing can be used to determine accurate mass of each oligomer. Therefore, endgroups can be distinguished. Values such as M_w, M_n, and PDI should be determined using another instrument. Mass spectrometry, in general is rarely accurate if PDI $\geq$ 1.2.

4.2 Applications of ESI Quadrupole MS to Copolymers

It was proven that under controlled ESI MS conditions monomer contamination as well as monomer ratios can be assessed. In order to fully validate this method, other factors need be considered. For instance, how dependence on molecular weight and analyte concentrations affects the choice of ESI MS conditions. Properties such as a polymer's PDI, viscosity, polarity and/or reactivity should be factored in. For other copolymers chosen to determine these characteristics, it is important to note the limitations of ESI and Quadrupole.

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VITA

Elizabeth Angela Stewart was born in Toronto, Ontario, Canada to Douglas and Esselin Stewart. She has two older siblings: Sandra S. Stewart and Maurice A. Michelin. Elizabeth attended high school at C. W. Jefferys Colligate Institute in September of 1994. Upon graduation from high school in June of 1998, she decided to attend Oakwood College in Huntsville, Alabama August of that year. She received a B.S in chemistry in September of 2002. Elizabeth moved to Knoxville, Tennessee to enter the graduate program in Chemistry at the University of Tennessee (UTK) in August 2002. She successfully defended her master's thesis on August 8th, 2006 and received her mater's degree in Chemistry December 2006.

While at UTK, she held a teaching assistantship position in General Chemistry for four years of which four semesters she was the head teaching assistant. In June of 2005, she volunteered as a teaching assistant for the annual Chemistry camp for grades 6-8. The theme was, "Fun with Forensic Chemistry".

Elizabeth did several poster presentations on her research work at conferences. In April 2005, she was awarded 3rd prize in a poster contest on her research. She was also honored to be nominated for a merit award in March of 2006. Elizabeth is currently working on a manuscript to be submitted to JASMS.