Abstract

ELECTROSPRAY IONIZATION TIME OF FLIGHT MASS SPECTROMETRY OF

POLY(METHYL METHACRYLATE) AND POLYSTYRENE USING SURFACE ACTIVE

QUATERNARY AMMONIUM SALTS

by

Christopher M Williams

June 2011

Chair: Dr. Rickey Hicks

Major Department: Chemistry

Electrospray ionization time of flight mass spectrometry (ESI-ToF-MS) is a powerful characterization technique for large nonvolatile compounds, but has found limited applicability in the synthetic polymer field. Two factors currently limit the utility of ESI-ToF-MS for synthetic polymer characterization: 1) Electrospray ionization often results in multiply charged species for each component in the sample. Synthetic polymers contain a distribution of many compounds with close, uniformly spaced masses. The presence of multiple m/z peaks for each mass typically results in data that is complicated and becomes increasingly complicated as the average molar mass of the sample increases. 2) Electrospray ionization is best suited for polar, easily ionized species dissolved in polar solvents. The vast majority of synthetic polymers are relatively non-polar, do not ionize easily and are not soluble in the polar solvents most often used for ESI. By adding easily ionizable, surface active quaternary ammonium salts that associate with polymer molecules during ionization, we have made significant strides in overcoming both of these limitations. By using cetyltrimethylammonium bromide (CTAB) as an additive, we have been able to produce ESI-MS spectra with only singly charged charge states for the entire

m/z range observable by our instrument (m/z = 0 – 20,000). Data will be presented for poly(methyl methacrylate) and polystyrene samples. Weight averaged molecular weight (M_w), number averaged molecular weight (M_n) and polydispersity (PD) calculations were all in good agreement with comparative analysis using Gel Permeation Chromatography (GPC) and Gel Permeation Chromatography – Multi-Angle Light Scattering (GPC-MALS). Further, for some samples, multiple distributions were identified corresponding to different end groups highlighting the utility of this approach.

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A Thesis Presented To

the Faculty of the Department of Chemistry

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Chemistry

by

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Christopher M. Williams

APPROVED BY:

DIRECTOR OF THESIS: _____
Timothy J. Romack, PhD

COMMITTEE MEMBER: _____
Paul Hager, PhD

COMMITTEE MEMBER: _____
Allison Danell, PhD

COMMITTEE MEMBER: _____
Colin Burns, PhD

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CHAIR OF THE DEPARTMENT OF CHEMISTRY:

_____
Rickey Hicks, PhD

DEAN OF THE GRADUATE SCHOOL:

_____
Paul Gemperline, PhD

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Mw   Weight Average Molecular Weight

Mn   Number Average Molecular Weight

PDI   Polydispersity

GPC   Gel Permeation Chromatography

MALS   Multi Angel Laser Light Scattering

NMR   Nuclear Magnetic Resonance

MALDI   Matrix-Assisted Laser Desorption / Ionization

ESI   Electrospray Ionization

MS   Mass Spectrometry

MS/MS   Mass Spectrometry/Mass Spectrometry

m/z   Mass to Charge Ratio

EI   Electron Impact

CI   Chemical Ionization

ToF   Time of Flight

QIT   Quadrupole Ion Trap

CRM   Charge Residue Mechanism

IEM   Ion Evaporation Model

V   Voltage

C   Celsius

μL   Microliter

μM   Micromolar

PMMA   Poly(methyl methacrylate)

PET   poly(ethylene terephthalate)

CTAB   Cetyltrimethylammonium Bromide

THF   Tetrahydrofuran

Chapter 1 Introduction

1.1 Overview

Synthetic polymers are commonly synthesized with addition or condensation reactions that result in oligomer chains of different lengths, giving rise to a molecular weight distribution. The molecular weight distribution inherent in all synthetic polymers combined with the charge state distribution, produced by electrospray ionization, results in very complex mass spectrum.

Synthetic polymers are among one of the most useful molecules in the world, and successful development of a method to produce single charge state mass spectra of synthetic polymers by Electrospray Ionization Mass Spectrometry would greatly enhance future charicterization of synthetic polymer based products.

1.2 Synthetic Polymers

1.2.1 Introduction to Synthetic polymers

Since the development of synthetic polymers in the 19th century scientists have found countless uses for synthetic polymers. Over the last two centuries polymer chemists have seen synthetic polymers become one of the most widely used molecules in the world.

The majority of synthetic polymers are typically produced from either addition or condensation reactions (chain growth or step growth). When synthetic polymers are synthesized by chain growth polymerization the end groups are determined by the initiation and termination steps. **Figure 1a** is an example of chain growth polymerization for the synthesis of PMMA. The end groups of synthetic polymers synthesized by step growth polymerization are determined by

the stoichiometric ratio of the reactants. Step growth polymerization is shown in **Figure 1b** for

PET.
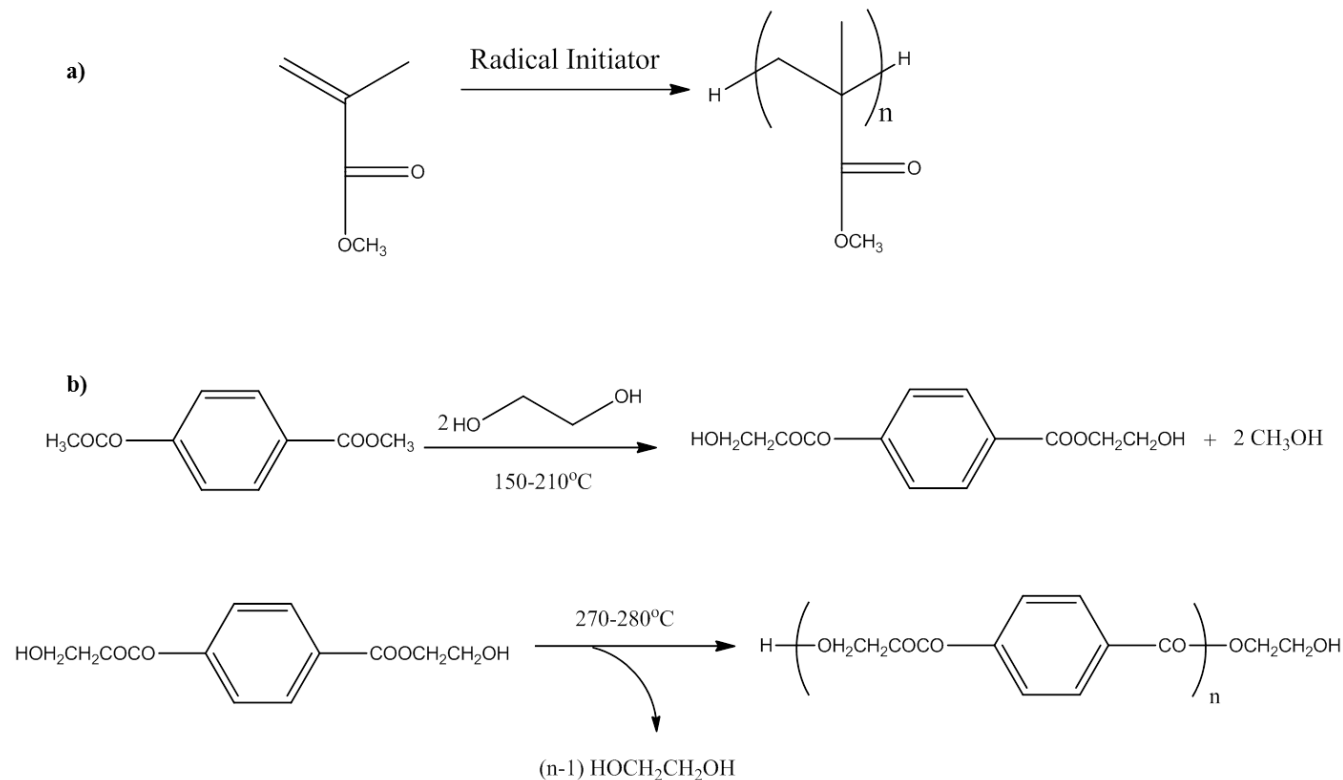
Figure 1: a) Chain growth polymerization reaction for the synthesis of PMMA. b) Step growth
polymerization reaction for the synthesis of PET.

Once the reaction has started all the polymer chains grow at different rates. Reaction rates

of each polymer chain vary based on the size/mobility of the polymer in solution and also on the

availability of the unreacted monomer near each chain. Upon termination of the reaction the

synthesized synthetic polymer sample will contain a distribution of polymer chain lengths. The

chain length distribution makes synthetic polymers polydisperse. Samples that are polydisperse

contain many molecules that are identical in all ways except for molecular weight. **Figure 2** is a

graphical depiction of how the molecular weight distribution of a synthetic polymer might

appear. The molecular weight difference between each adjacent peak is equal to the molecular weight of the monomer.
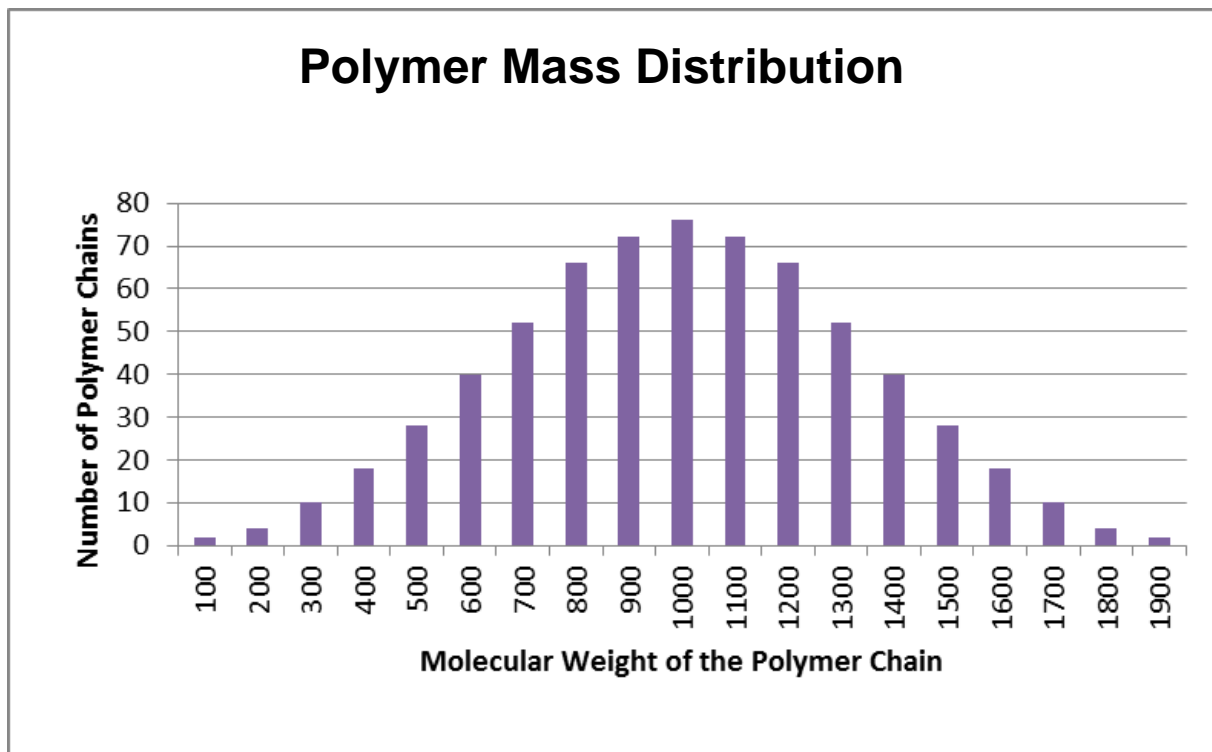
Figure 2: Graphical depiction of the polydispersity present in synthetic polymers.

The polydispersity of synthetic polymers makes molecular weight characterization no trivial task. Because there is a distribution of chain length, molecular weights are reported as averages. Calculation of number average molecular weight (M_n), weight averaged molecular weight (M_w) and polydispersity index (PDI) are the most common way to report molecular weight values for synthetic polymers.

The M_n can be calculated by **Equation 1**, and is biased toward the lower molecular weight polymer chains. Since the M_n is biased toward the lower molecular weight range of the distribution it is used to describe the chemical properties of synthetic polymers. The M_w on the

other hand is biased to larger sized polymer chains and can be calculated by **Equation 2**. M_w is used to describe the physical properties of synthetic polymers because the higher molecular weight polymer chains are primarily responsible for the physical properties of the synthetic polymer, such as tensile strength and elasticity. The polydispersity index is calculated by **Equation 3** and is used to describe the width of the molecular weight distribution of synthetic polymers. [1]

$$\mathbf{M_n} = \frac{\sum_i N_i M_i}{\sum_i N_i}$$
Equation 1: Number Averaged Molecular Weight

$$\mathbf{M_w} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$
Equation 2: Weight Averaged Molecular Weight

$$\mathbf{PDI} = \frac{\mathbf{M_w}}{\mathbf{M_n}}$$
Equation 3: Polydispersity Index

1.2.2 Techniques for Characterizing Synthetic Polymers

Completely characterizing synthetic polymers is a difficult task. Characterization of the chemical and physical properties of synthetic polymers is important because it provides insight into how the synthetic polymer can be used.

Prior to the development of electrospray ionization and matrix assisted laser desorption/ionization, mass spectrometry instruments were impractical for large synthetic polymer systems. [2] Scientists characterized synthetic polymers primarily with nuclear

magnetic resonance (NMR) and gel permeation chromatography multiangle light scattering (GPC/MALS).

With NMR, end group and chemical composition can be characterized. However, once the polymer chains reach a certain length the ability for end group determination by NMR becomes difficult because the signal intensity of the end groups is too low. One unique strength of NMR is the ability to determine the stereochemistry of synthetic polymers. [3]

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) is a chromatographic separation technique that separates molecules based on size. As shown in **Figure 3** larger molecules have shorter path lengths and therefore come through the column faster than smaller molecules. Once the sample is separated and eluted from the column, detection occurs at the multiangle light scattering detector. GPC systems are incredibly powerful instruments for determining the number average molecular weight, weight average molecular weight and

Figure 3: Separation of large and small molecules in the GPC column.

polydispersity index. However, while the characterization of molecular weight and molecular weight distribution is valuable GPC instruments are not capable of identifying end groups or determining chemical composition.

With the development of MALDI and ESI, mass spectrometry became a powerful characterization technique that could reveal a great deal of important information about macromolecules. The true power of MALDI and ESI lies in the ability to create gas phase ions from non-volatile analytes.

MALDI-MS of synthetic polymers has been shown to yield valuable characterization data. [4, 5] Using MALDI as the ionization source for mass spectrometry of synthetic polymers has the advantage of singly charging all of the analytes in the sample. There are a couple downfalls to using MALDI to characterize synthetic polymers. (Discussed further in 1.3.4.1)

ESI-MS, while not used as commonly as NMR or GPC [6] has had success when studying low molecular weight synthetic polymers. The electrospray source is capable of ionizing any size synthetic polymer with little to no fragmentation but complicated mass spectra make characterization of large synthetic polymers difficult. The two issues that make analyzing synthetic polymers by ESI-MS difficult are multiple charging and solvent limitations.

Successful characterization of synthetic polymers by coupling instruments together has been reported in the literature. [7, 8] Coupling of instruments is not an uncommon technique for acquiring better data but it is time consuming and sometimes expensive.

1.3 Mass Spectrometry

1.3.1 Introduction

Mass Spectrometry is commonly over simplified and just assumed to output molecular weight values for samples. In reality the data outputted from mass spectrometers is in units of mass over charge or m/z (m = mass and z = charge). **Scheme 1** shows the general flow of all

mass spectrometers. First molecules are charged in the ionization source, then the ions move

through the mass analyzer where they are separated based on their mass to charge ratio (m/z) and

lastly the ions are detected and info is output to a mass spectrum.

Scheme 1: Three main components of all mass spectrometers.

1.3.2 Ionization Source

In the ionization source analyte molecules are charged. There are many different types of

ionization sources but they can all be broken down into two categories, hard and soft. Hard and

soft ionization sources are distinguished by the amount of fragmentation that occurs during

ionization. Hard sources create many fragment ions while soft sources cause little to no

fragmentation. Electron ionization (EI) is the most widely used hard ionization source; many soft

ionization sources exist: ESI, MALDI, Chemical Ionization (CI) and a few others. The choice of

ionization source is largely dependent on the type of molecules that will be studied.

1.3.3 Mass Analyzer

Choice of mass analyzer for a mass spectrometer is also a decision that should be made

based partially on the mass range of analytes that need to be analyzed. There are many mass

analyzers commercially available and all have a specific mass to charge range. For example

quadrupoles and quadrupole ion traps (QIT) have m/z ranges between 0 and 5,000. However,

this does not mean that molecules with a molecular weight greater than 5,000 g/mol cannot be

analyzed in a quadrupole or QIT. (An 8,000 g/mol molecule with a charge of +2 will have a mass

to charge ratio equal to 4,001 m/z) The best mass analyzer for high molecular weight molecules where multiple charging must be limited (i.e. synthetic polymers) is time of flight (ToF). The time of flight mass analyzer has a theoretically unlimited m/z range.

1.3.4 MALDI of Synthetic Polymers

Currently the best mass spectrometry instrument for studying synthetic polymers is MALDI-MS. MALDI produces only singly charged ions and as a result generates mass spectra that are much less complex than ESI. MALDI has successfully been used to characterize synthetic polymers and has also been used to determine the mechanisms of some polymer synthesis reactions. [4-6, 9-11]

Even though MALDI is currently considered to be a better ionization source than ESI for mass spectrometry of synthetic polymers, MALDI still has a issues that make analysis of synthetic polymers tricky. The major difficulty in using MALDI as the ionization source for synthetic polymers is sample preparation. Preparing a homogenous cocrystallized sample from matrix molecules and synthetic polymers is difficult in part because of solvent compatibility issues between the polymer and matrix molecules. There has been some success in choosing matrix molecules that are compatible with synthetic polymers and some methods designed to allow for creation of almost homogenous solids. [6] However, it is our belief that ESI-MS will be better suited for synthetic polymer analysis than MALDI-MS if multiple charging can be controlled.

1.3.5 Electrospray Ionization

1.3.5.1 Introduction

Understanding how electrospray ionization, shown in **Figure 4**, works is very important when trying to develop a method for producing clean, easy to interpret mass spectra for synthetic polymers. Analyte is initially injected into the capillary in a dilute solution (1-10µM), a large potential (1-5kV) is applied to the capillary causing buildup of charge at the capillary tip. Charged droplets are sprayed from the tip of the capillary once charge repulsion exceeds the surface tension of the solvent at the Taylor Cone. Charged droplets are then pushed/pulled
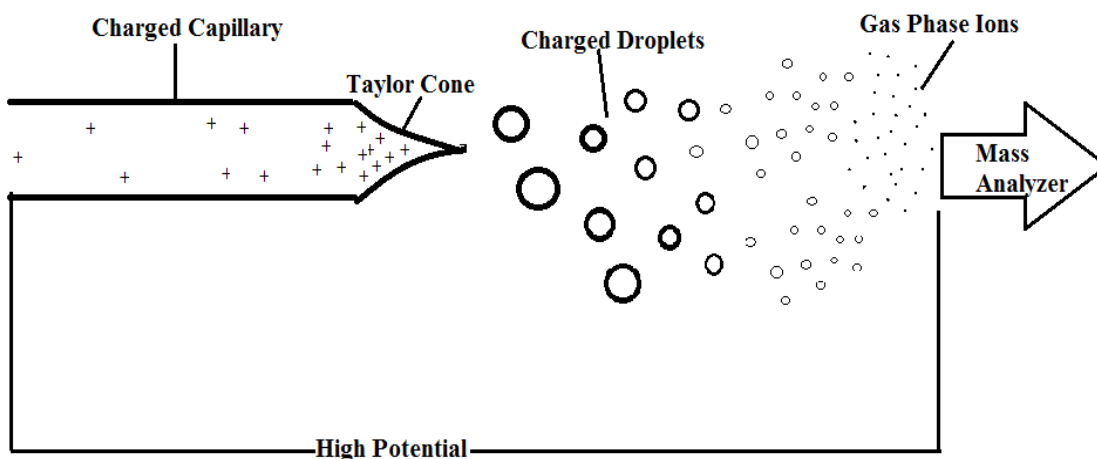
Figure 4: Illustration of electrospray ionization mechanism.

toward the entrance of the mass analyzer, undergoing solvent evaporation and droplet fission until gas phase ions are produced. There are two different mechanisms that explain how charged droplets become gas phase ions, Charge Residue Mechanism (CRM) and Ion Evaporation Model (IEM). It is possible to form cationic and anionic gas phase ions. The mode is typically chosen based on whether the analyte of a study is more likely to become positively or negatively charged. [12]

1.3.5.2   Source and Parameters

Knowing how the electrospray ionization source is affected by changing the source parameters is very important. Multiple charging, fragmentation, clustering, and aggregation are all factors that can be minimized/maximized by adjusting source parameters. **Table 1** shows all of the important source parameters and there range of values.

Table 1: Source Parameters

Parameter	Units	Range of Values
Capillary	V	1000-4000
Sample Cone	V	0-200
Extraction Cone	V	0-5
Source Temperature	˚C	0-150
Desolvation Temperature	˚C	0-400
Cone Gas	L/hr	0-300
Desolvation Gas	L/hr	0-800
Sample Flow rate	µL/min	0-50

Knowing how adjusting the source parameters will affect the ionization of analyte molecules is crucial for development of a method for electrospray ionization of synthetic polymers. **Figure 5** is provided to show where each of the parameters is applied in the source.

The first source parameter is the Capillary Voltage, for positive mode electrospray capillary voltages typically range from 2500V to 3000V. It is vital that this parameter be optimized or an unstable Taylor cone may be produced resulting in poor ionization efficiency.
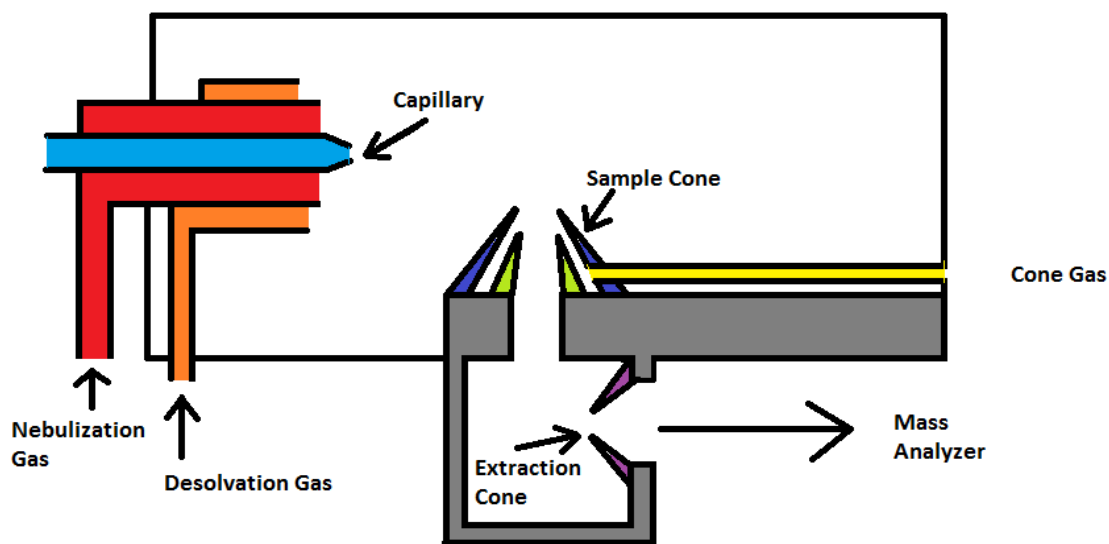
Figure 5: Electrospray ionization source diagram showing the location where all source parameters are applied.

The sample cone voltage gives energy to the analyte to accelerate it into the mass analyzer. The sample cone voltage is commonly adjusted based on the molecular weight of the analyte. For low molecular weight samples the sample cone voltage is typically between 30-50V, but as molecular weight increases the sample cone voltage must also be increased. Large molecules need more of an energy boost to get sufficient kinetic energy than do small molecules. [13] If the sample cone voltage is too high, fragmentation will occur extensively.

The extraction cone voltage is the next source parameter and has an operating range from 0-5V. High voltages can lead to fragmentation of low molecular weight ions in the source.

Source temperature is solvent dependent, for most solvents source temperature is optimized between 80-100˚C. The source temperature needs to be increased when higher flow rates are used or for solvent systems containing higher percentages of water.

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The desolvation gas is flowed past the sprayer as analyte is sprayed. Increasing the desolvation gas temperature according to the literature should result in higher charge states due to faster evaporation of the solvents. [14] Optimization of the desolvation gas temperature however contradicted reported research, increasing the desolvation gas temperature decreased amount of double charging. [15]

Cone gas flow rate is used to prevent ion adducts from forming and also to minimize solvent clustering. Turning the cone gas flow rate off increases the timescale of low surface charge density droplets, resulting in lower overall charge states.

The last source parameter to consider is the sample flow rate. Literature reported results showed that increased sample flow rate resulted in the creation of droplets with decreased surface charge density, resulting in an overall decrease in multiple charging. [16]

1.3.5.3 Gas Phase Ions

Once droplets are sprayed from the Taylor Cone there are two mechanisms that explain how gas phase ions of analyte molecules are produced, charge residue mechanism (CRM) [17] and ion evaporation model (IEM). [18] Much debate about which mechanism is correct has occurred since the invention of ESI and scientists have decided that the mechanism is dependent on the molecular weight of the analyte.
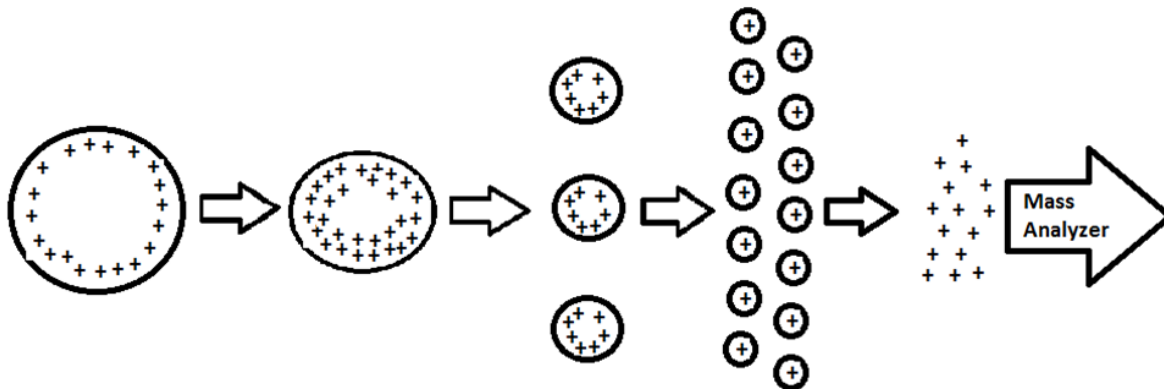
Figure 6: Charged Residue Mechanism

Charge residue mechanism, shown in **Figure 6**, is one of the two mechanisms that are believed to occur after charged droplets are sprayed from the capillary. As solvent evaporation occurs and the droplets size decreases the charge repulsion within the charged droplets increases. Once the charge repulsion inside the droplet exceeds the surface tension, or the Rayleigh Limit, of the droplet, droplet fission occurs. [19] Fission of the parent droplet results in the creation of several smaller daughter droplets, this process continues until analytes exist alone in droplets and as the rest of the solvent evaporates gas phase ions are produced. Charge residue mechanism is the mechanism for creating gas phase ions from high molecular weight analytes.
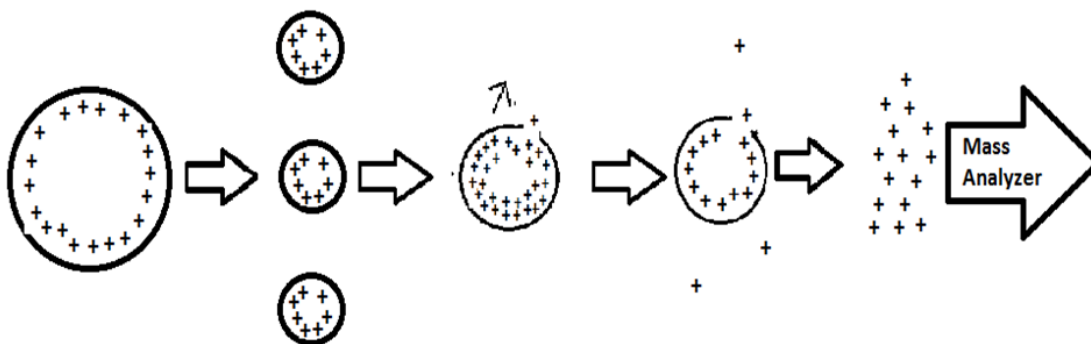
Figure 7: Ion Evaporation Model

Low molecular weight compounds go through the ion evaporation model, **Figure 7**, to become gas phase ions. IEM begins identically to CRM; droplets shrink and divide as solvent evaporation occurs. The difference between CRM and IEM however is that once the droplet reaches a critical size the charge repulsion inside the droplet is sufficient to eject and analyte ion from the droplet directly into the gas phase.

Both the CRM and IEM are important because they allow for gas phase ions of large nonvolatile compounds to be produced. For the study of synthetic polymers, CRM is of the most interest because synthetic polymers are very large molecules.

1.3.5.4   Solvent Considerations

The major issue with characterization of synthetic polymers by ESI-MS is the charge state distribution. The other hurdle that must be overcome in ESI-MS is the solvent limitation. In general more polar solvents perform better in electrospray; likewise it is easier to produce ions from more polar analytes. There are several relatively polar synthetic polymers but the majority of commercially and industrial synthetic polymers are nonpolar. So, it is important to use a solvent that will form a stable Taylor Cone and also be a good solvent for the analyte (synthetic polymers).

ESI typically works well with solvents that have a dielectric constant greater than 20. (This includes solvents like water, acetonitrile, and methanol.) A general trend in ESI solvents is the more polar the solvent the higher the charge state of the ions that will be produced. [20] With most solvents increased polarity coincides with increased surface tension which has also been observed to cause higher charge states. [21] Going back to the mechanisms of producing gas phase ions (CRM and IEM) it is clear that increasing the surface tension will cause charged

droplets to take longer to reach the Rayleigh Limit or the critical diameter which causes droplet fission and ion ejection.

Therefore higher surface tension leads to greater concentration of charge and increased charging of analytes.
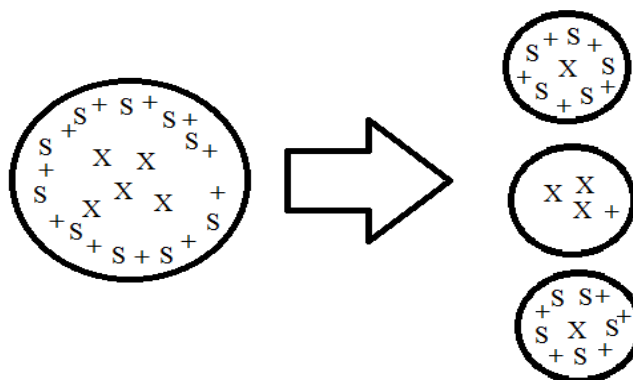
Amphiphilic molecules are easy to charge with ESI. The hydrophilic/polar portion of the molecules will facilitate charging and be very soluble in the polar ESI

Figure 8: Surface active molecules (S) have higher ionization efficiency than molecules that are found in the center of the droplets (X).

solvent. The hydrophobic section will however have high surface affinity and be in contact with the atmosphere. (Atmosphere is relatively nonpolar when compared to the polar ESI solvent.) Being on the surface of the droplet leads to higher ionization efficiency, **Figure 8** illustrates why amphiphilic molecules have higher ESI response. [22, 23]

1.3.5.5 ESI-MS of Synthetic Polymers

ESI-MS has been used to characterize molecular weight, molecular weight distribution and end groups of small synthetic polymers. [24, 25] ESI-MS can also produce reliable mechanistic and kinetic data for small polymers systems. [26] ESI-MS of synthetic polymers has had the most success with small synthetic polymers because the ESI source causes multiple charging. Multiple charging of synthetic polymers is the cause of complex mass spectra as shown in **Figure 9**.
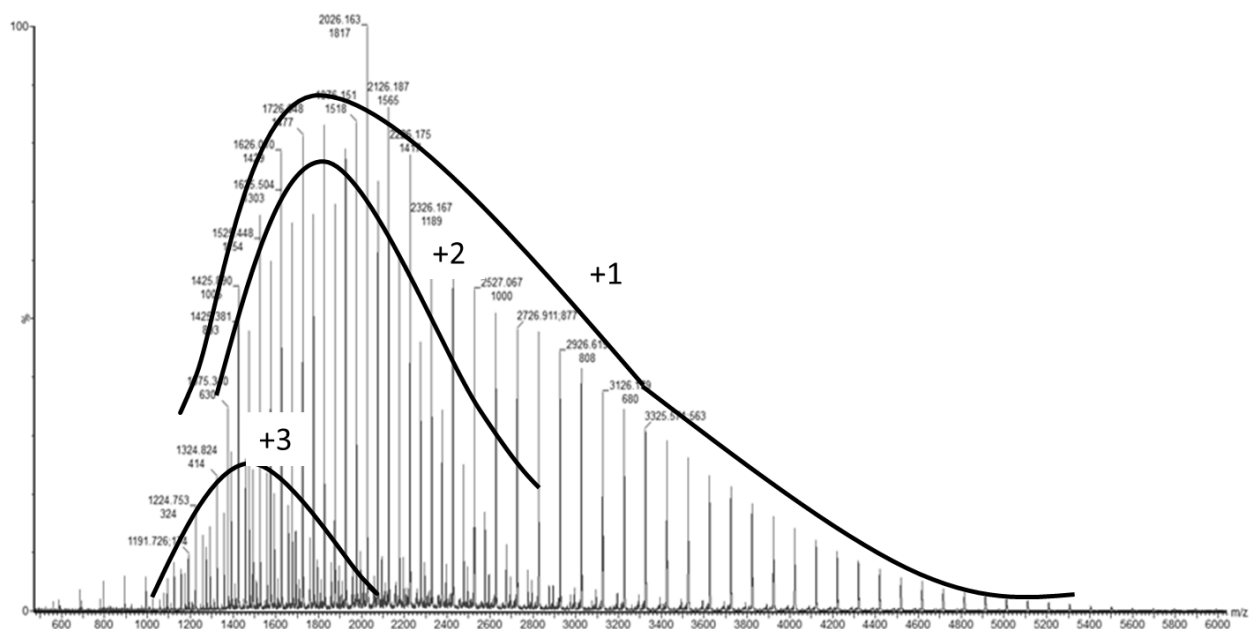
Figure 9: Mass spectrum to show how multiple charging can make spectrum interpretation difficult.

The two issues that make characterizing higher molecular weight synthetic polymers problematic are: 1) The charge state distribution of the polydisperse synthetic polymer and 2) Solvent limitations imposed by needing a polar ESI solvent and a nonpolar synthetic polymer solvent.

Several groups have reported ESI-MS studies of synthetic polymers with reduced charge states over the past few years. However, due to the complexity of these approaches the methods cannot be easily implemented. Using a QIT it was possible to react cationic poly(ethylene glycol) with perfluorocarbons and iodide anions to reduce the charge state. Though the charge state was reduced the complexity of the spectrum remained because variable amounts of fluorine, iodide, and cations were responsible for producing the +1 PEG ion. [27] Another group used a bipolar neutralizing gas (gas containing both cations and anions) to reduce the charge of ions on their way to the mass analyzer. One of the advantages of this method was the ability to use a ToF

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mass analyzer however; the system can't be easily incorporated in other ESI-MS

instruments. [28]

A few more recent approaches utilizing additives have also had success. One group reacted

15-Crown-5 with sodium charged PEG in a QIT to strip sodium ions from the synthetic polymer.

[29] Yet another approach was to use gas phase superbases to prevent excessive proton (H^+)

charging on PEG. [30] Post column addition of amines to PEG and PEGylated samples also

resulted in charge state reduction due to ammoniation of the synthetic polymer as opposed to

protonation. [31] Lastly the use of cetyltrimethylammonium bromide (CTAB) to reduce the

charge state of PEG was recently reported. [32] This last study by Nasioudis et al. is similar to

our research because we also used CTAB as our cationizing additive to prevent multiple

charging of synthetic polymers. Our research is different from Nasioudis et al. however, because

we focused on different synthetic polymers and on the production of a single charge state.

While success has been seen in the field of ESI-MS of synthetic polymers, it is important to

stress that though many of these previously mentioned methods worked, the charge states of the

synthetic polymers were not all reduced to a single charge state. The lack of the ability to

generate uniformly charged synthetic polymer samples provides a source of bias when

characterizing the molecular weight and molecular weight distribution of synthetic polymers.

(Longer polymer chains are more likely to be multiply charged than smaller ones.) Furthermore,

the analysis of a polar synthetic polymer like poly(ethylene glycol) by ESI-MS is of mild

significance to polymer chemists who are more interested in much less polar synthetic polymers.

1.4 Surfactants

Surface **Act**ive Age**nts**, are molecules that contain both hydrophilic and hydrophobic segments. [33] Typically surfactant molecules have a polar/ionic head and a nonpolar/hydrocarbon tail. Surfactants can be broken down into four classes: anionic, cationic, neutral or zwitterionic (contains both cations and anions). Surfactants are used in many commercial goods such as: laundry detergent, shampoo, hand and dish soap, and motor oils. Surfactants, apart from being commercially useful, also have many applications in analytical chemistry. Surfactants have been used to improve separation or to completely alter the way a system behaves at surface or interfacial sites. [34-36] We have previously obtained results verifing that the use of surfactants as additives to ESI samples of synthetic polymer standards can significantly reduce the multiple charging of synthetic polymers. [15] It is our belief that surfactants can be further used to generate reproducibly simple mass spectra for any synthetic polymer, regardless of size.

Chapter 2: Results and Discussion

2.0.1 Introduction to Research

As described in the previous chapter characterization of synthetic polymers by ESI-MS has not yet reached its full potential. Multiple charging and solvent limitations make analysis of synthetic polymers difficult. Many groups have developed various methods to reduce the charging of synthetic polymers but few have been simple enough to be easily implemented in other ESI-MS instruments.

Development of a simple method that allows for single charge state ionization of synthetic polymers would make ESI-MS a powerful instrument for the characterization of synthetic polymers. The addition of CTAB, shown in **Figure 10**, as the cationizing agent can prevent multiple charging of synthetic polymers. CTAB will interact with the synthetic polymer during ionization and along with charging the polymer it will also prevent the polymer from being directly charged. Additionally the use of a cosolvent system to force faster interaction of the polymer with CTAB will also reduce multiple charging.
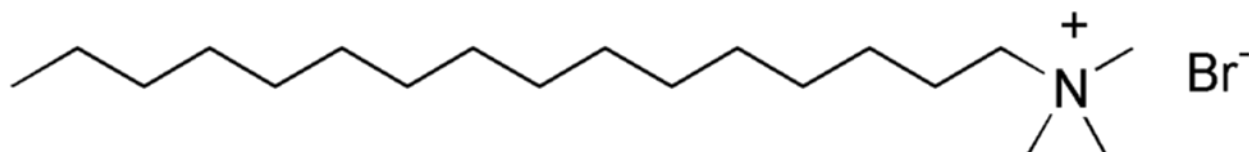
Figure 10: Cetyltrimethylammonium Bromide (CTAB)

2.1 Poly(methyl methacrylate) 4K

PMMA synthetic polymers were the first synthetic polymers studied by our research group. ESI-MS of PMMA 4000 without CTAB as the cationizing species yielded spectra with

significant multiple charging as seen in **Figure 11**. Without CTAB present as the charging

species the PMMA polymer is charged significantly with sodium ions. The charge state of the

peaks in the mass spectrum can be determined two different ways. First the spacing from one

peak to an adjacent peak is equal to the mass of the monomer divided by the charge of the ion.

For singly charged peaks the spacing is equal to the molecular weight of the monomer. The

other way to determine charging is to look at the spacing between isotope peaks. If charge

equals one (z=1) then the spacing between each carbon isotope is 1 m/z. However, if the charge

is 2 (z=2) then the spacing between the isotope peaks will be 0.5 m/z. Using these two methods

it is possible to determine how many different charge states are present in the mass spectrum.
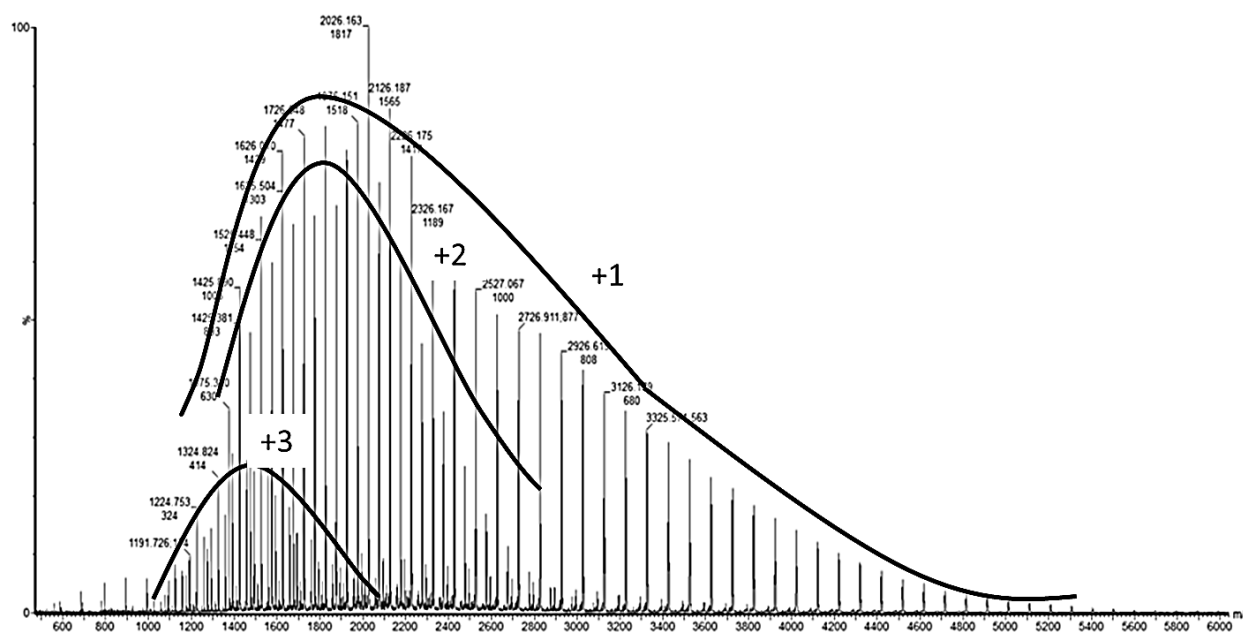
Figure 11: ESI-ToF-MS of PMMA 4000 without CTAB.

While characterization of the end group for PMMA 4000 would be possible using this

spectrum, calculation of the M_w, M_n and PDI is difficult and possibly biased. Because multiply

charged peaks overlap integration of the peaks could result in skewed values. (It is common for one peak to represent different size polymer chains with different charges.)

Addition of CTAB to the PMMA 4000 sample resulted in a mass spectrum with exclusively single charging. As can be seen in **Figure 12** there is absolutely no multiple charging present and the spectrum is dominated by only one distribution.
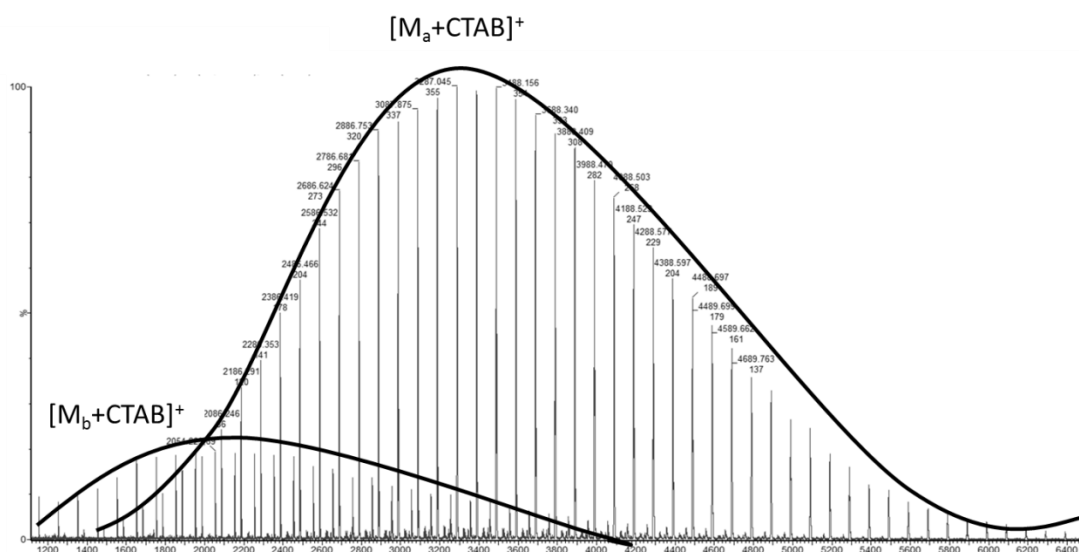
Figure 12: ESI-ToF-MS of PMMA 4000 with CTAB, M_a and M_b correspond to structures A and B seen below in Table 3.

Most importantly is that with this mass spectrum it is possible to accurately characterize the molecular weight and molecular weight distribution with **Equations 1**, **2** and **3**. **Table 2** shows the comparison of ESI-MS, GPC, and manufacturer calculated M_w, M_n, and PDI.

Table 2: PMMA 4000 molecular weight characterization, calculated from ESI-MS and GPC data.

Synthetic Polymer	Analytical Method	M_n	M_w	PDI
PMMA 4000	ESI-MS	3.46×10^3	3.68×10^3	1.06
	GPC	3.5×10^3	3.7×10^3	1.06
	Manufacturer	3.9×10^3	4.2×10^3	1.06

The percent difference between our in lab GPC and ESI-MS weight averaged molecular weight is 1.08%. This is a close result verifying the precision of characterizing synthetic polymers with our ESI-MS method. The ESI-MS and manufacturer percent difference is 13.25%, this is not nearly as close but still within reason. Calculation of M_w, M_n and PDI by GPC commonly has anywhere from 5-10% variance in value. This is because GPC instruments are calibrated by narrow synthetic polymer standards. It is assumed that any polymer analyzed with that GPC instrument behaves identically to the synthetic polymer used to calibrate the instrument. Based on our results ESI-MS of synthetic polymers appears to yield similar and possibly better molecular weight characterization than GPC.

Table 3: Assigned structures for peaks of PMMA 4000 spectrum shown in Figure 12.

Assigned Structure	$[M+CTAB]^+$	$[M+Na]^+$
A	3287.921 n=30	N/A
B	2055.265 n=15	N/A

It is also possible to determine the end group of the synthetic polymer from the mass spectrum obtained for PMMA 4000 with CTAB. **Table 3** shows the two different end groups believed to be present in the PMMA 4000 sample; the molecular weight of these structures matches up exactly with peaks seen in the mass spectrum. Structure **A** represents what poly(methyl methacrylate) synthetic polymers are synthesized to be, the primary distribution of peaks are this structure. However, there is a lower distribution of peaks identified as poly(methyl methacrylate) synthetic polymers that have been terminated by backbiting (Structure **B**).

The reaction used to synthesize this PMMA 4000 standard was confirmed to be group transfer polymerization (GTP) by the manufacturer. GTP reactions have initiation, propagation, and termination steps. The initiation step starts by activating the catalyst and attaching the trimethylsilyl group to a monomer, the group transfer then occurs to another monomer forming the new carbon-carbon bond this reaction happens many times resulting in long polymer chains. The reaction normally continues until all of the monomers are reacted of the terminating species is added. [37-39] In some cases the growing polymer chain reacts back on one of the already attached monomers resulting in an early termination by backbiting.

Similar results for PMMA 4000 were previously obtained by our group, characterizations of molecular weight distribution for PMMA 4000 are in close agreement. Peak assignment and end group determination however, were much different for the lower lying distributions. [15] This is likely due to the calibration issues resolved just prior to this PMMA 4000 analysis. Also it should be noted that ion intensity was improved from prior experiments. Increased ion intensity is attributed to more surfactant charging, producing a clean easy to interpret mass spectrum for PMMA 4000.

2.2 Poly(methyl methacrylate) 8K

PMMA 8000, similar to PMMA 4000, was previously studied by our group and yielded good results. [15] Herein we report similar finding and corrections to previous results.
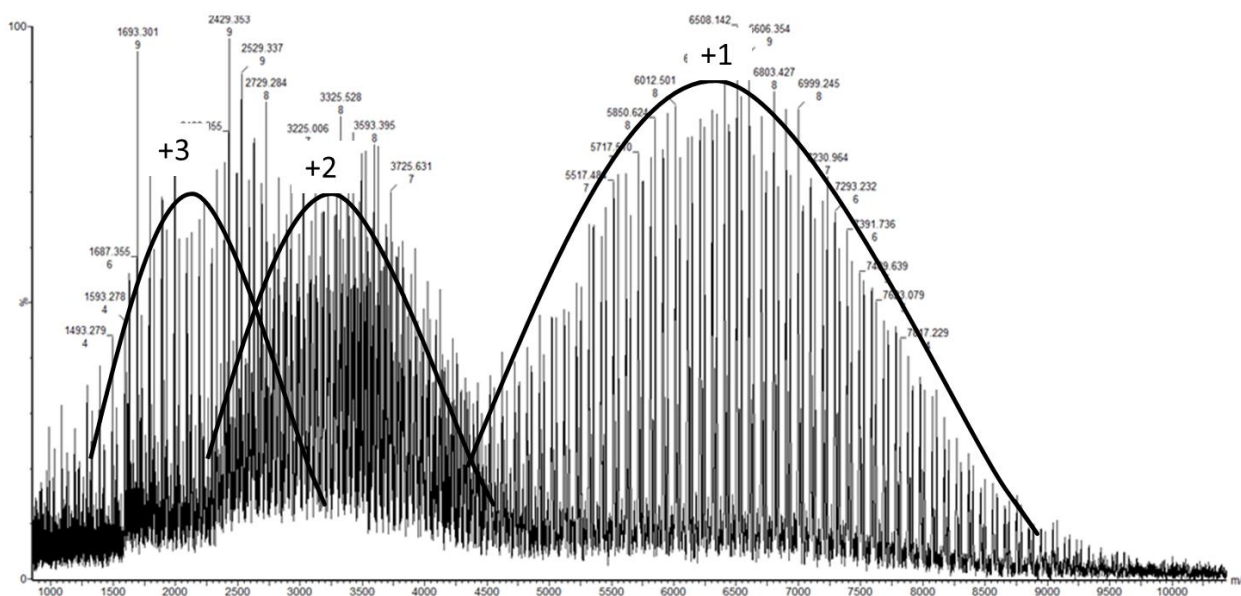
Figure 13: ESI-ToF-MS of PMMA 8000 without CTAB

Initial attempts to produce clean singly charged mass spectrum for PMMA 8000 without the cationizing surfactant molecules resulted in the complex mass spectrum displayed in **Figure 13**. Similar to PMMA 4000 the mass spectrum for PMMA 8000 without CTAB is congested and difficult to analyze. Three charge states (+1, +2, and +3) can clearly be seen and are overlapping making it is hard to tell where the mass distribution of PMMA 8000 begins.

As with PMMA 4000, the addition of cetyltrimethylammonium bromide to the ESI-MS PMMA 8000 sample resulted in a clean single charge state distribution of the synthetic polymer, seen in **Figure 14**. Unlike **Figure 13**, in the exclusively singly charged mass spectrum of PMMA 8000 a second distribution from 1,500 m/z to 4000 m/z is visible. This bimodal

24

distribution for PMMA 8000 was unexpected and due to the spacing of the peaks, multiple charging is not the cause of the low m/z range distribution.
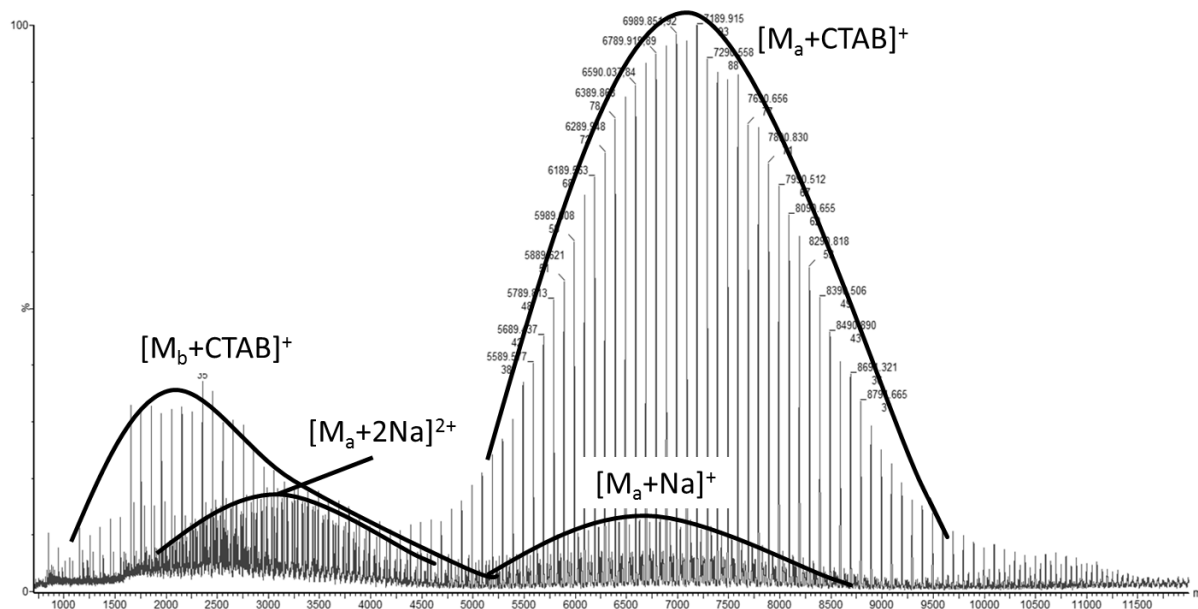
Figure 14: ESI-ToF-MS of PMMA 8000 with CTAB, M_a and M_b correspond to structures A and B seen below in Table 5.
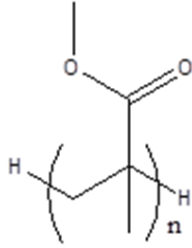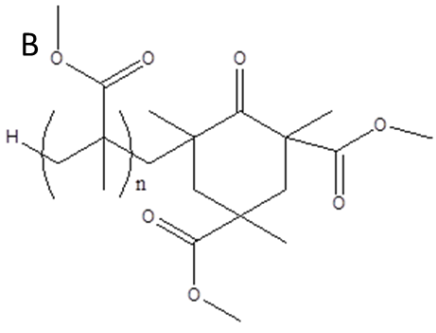
Molecular weight characterization was performed using **Equations 1, 2** and **3.** M_w, M_n and PDI calculated for ESI-MS and GPC data are reported in **Table 4,** along with manufacturer provided values for PMMA 8000.
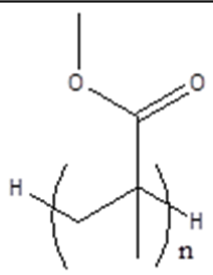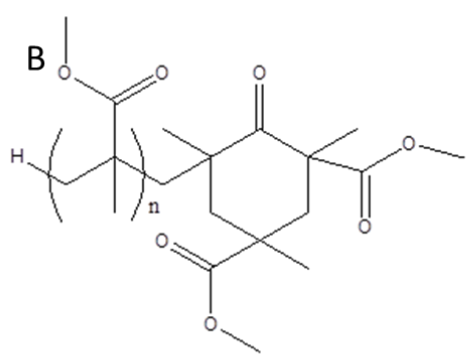
Table 4: PMMA 8000 molecular weight characterization, calculated from ESI-MS and GPC data.

Synthetic Polymer	Analytical Method	M_n	M_w	PDI
PMMA 8000	ESI-MS	6.20×10^3	8.84×10^3	1.10
	GPC	6.18×10^3	6.51×10^3	1.05
	Manufacturer	7.92×10^3	7.95×10^3	1.04

Percent difference again showed reasonable correlation between our in lab GPC results and ESI-MS molecular weight data. Percent difference for the weight averaged molecular weight was 5.01%. Percent difference between ESI-MS and manufacturer provided values was larger but still within reason 14.89%. The increase in the percent difference can possibly be attributed to the bimodal distribution. Our calculations included both distributions present in the spectrum, it possible that the manufacturer did not include the lower distribution if they were able to separate the two distributions in their GPC. If this is the case our calculations would be expected to be lower than the manufacturer provided values.

After characterizing the molecular weight of PMMA 8000, structural assignments for the peaks present in **Figure 14** is necessary. Structural assignments for PMMA 8000 are shown in **Table 5.** As with PMMA 4000, the most intense distribution belongs to the primary structure of PMMA (structure **A**) with hydrogen end groups charged by CTAB. The lower lying distribution between 5,000 m/z and 9,500 m/z is the same structure (**A**) charged by sodium. The smaller distribution seen between 1,500 m/z and 4,000 m/z is (structure **B**) backbitten PMMA charged by CTAB. PMMA 8000 was synthesized with the same reaction as PMMA 4000 which also produced some backbitten polymer chains. The difference between PMMA 4000 and PMMA 8000 is that instead of have backbiting occurring occasionally throughout the entire synthesis of PMMA 8000, it appears that a spike in backbiting occurred early in the GTP synthesis of PMMA 8000.

Table 5: Assigned structures for peaks of PMMA 8000 spectrum shown in Figure 14.

Assigned Structure	[M+CTAB]+	[M+Na]+
A	6789.919 n=65	6828.454 n=68
B	2054.139 n=15	N/A

In an attempt to verify the presence of back biting, a mass spectrometry/mass spectrometry (MS/MS) experiment was performed. The goal of the MS/MS experiment was to observe the fragmentation pattern of PMMA polymers with and without backbiting. The presence of backbiting could be confirmed two ways: 1) A fragment with mass equal to the molecular weight of the backbitten end group. 2) A clear fragmentation pattern with 31 Da (molecular weight of $^-OCH_3$) difference between the backbitten and non-backbitten polymer chains. MS/MS spectra for PMMA 8000 are shown in **Figure 15.**
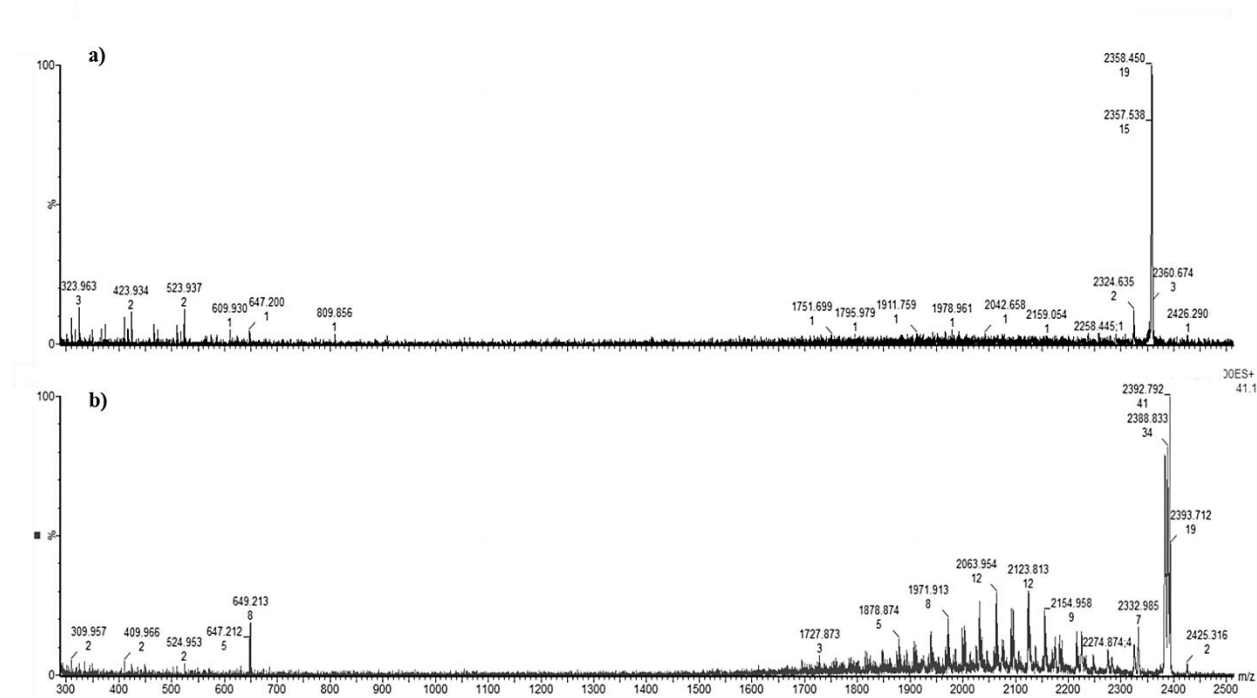
Figure 15: a) MS/MS spectrum for backbitten PMMA. b) MS/MS spectrum for PMMA without backbiting.

MS/MS experiments yielded no valuable results because our QToF Micromass instrument is only capable of producing first generation fragments. Since further fragmentation of the fragments is not possible we were unable to see a fragment with mass equal to the backbitten end group or a clear pattern 31Da different when comparing the non-backbitten PMMA spectrum to the backbitten PMMA spectrum. Even though the MS/MS experiment failed to verify the presence of backbiting, the molecular weight of backbitten PMMA fits the peaks of the bimodal distribution. Cross contamination of PMMA 4000 and 8000 samples was ruled out by purchasing another PMMA 8000 sample (from the same batch). Mass spectra for both PMMA 8000 standards were identical. For these reasons we are confident that backbiting is responsible for the bimodal distribution observed in the mass spectrum.

Similar to PMMA 4000, past ESI-MS experiments with PMMA 8000 have been conducted and molecular weight distribution characterization along with end group analysis has previously been reported. [15] However, due to errors that occurred during peak assignment it is important to report our findings once again. The low molecular weight distribution of the bimodal distribution seen in PMMA 8000 has been identified as backbiting. Molecular weight characterization from previous experiments and those reported in **Table 4** show that our ESI-MS of synthetic polymers method is reproducible. Even more telling of the potential that ESI-MS offers to synthetic polymer characterization, is that the bimodal distribution of the PMMA 8000 sample would have gone unnoticed (not observed in GPC) if not for the single charge state ESI-MS spectrum seen in **Figure 14**.

2.3 Poly(methyl methacrylate) 12K

PMMA 12000 was our choice for a higher molecular weight synthetic polymer. PMMA 12000 has a molecular weight distribution ranging from 9,000 m/z to 20,000 m/z. Though PMMA 12000 is not considered to be a high molecular weight synthetic polymer, its molecular weight distribution reaches almost to the maximum of our ESI-MS instrument. This is not an instrument maximum as much as it is a software maximum.

Figure 16 shows the mass spectrum for PMMA 12000 without any added cationizing species; this spectrum was obtained with optimized conditions for generating low charge states.
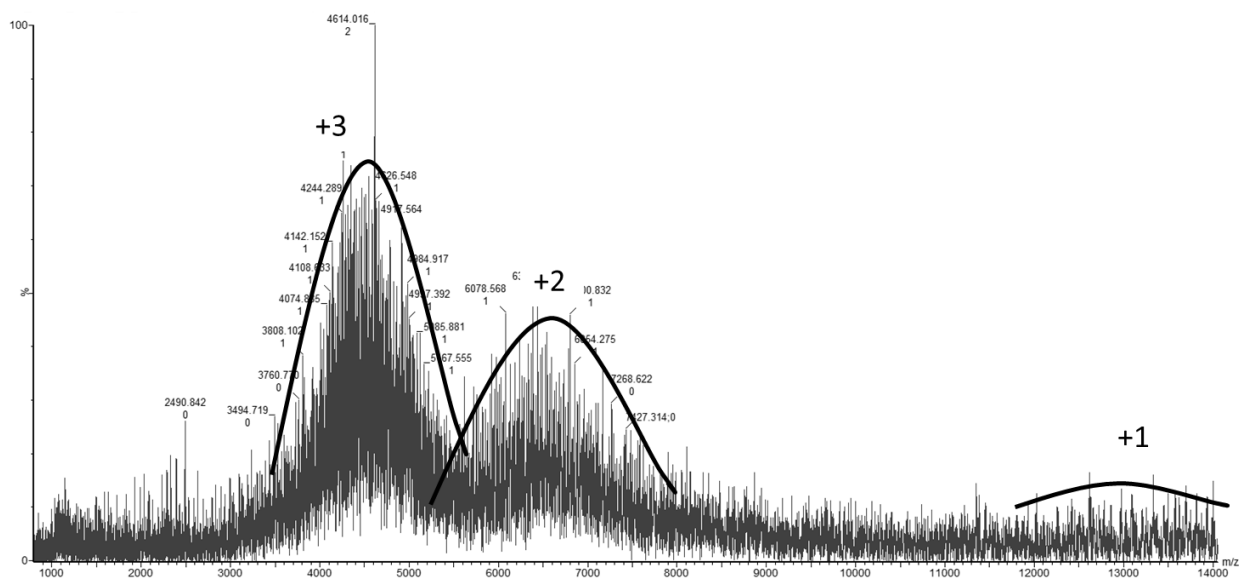
Figure 16: ESI-ToF-MS of PMMA 12000 without CTAB

In **Figure 16**, there is a lot of multiple charging and very little single charging. Again as with both PMMA 4000 and 8000 this complex spectrum makes characterization of PMMA 12000 difficult. With the addition of CTAB as our cationizing species we obtained a much different mass spectrum, **Figure 17**.

In **Figure 17** we have only the single and double charge states present. Even though this mass spectrum is much cleaner and easier to pull data from than **Figure 16**, it still was not sufficient for full characterization of PMMA 12000. We did find that increasing the CTAB concentration further resulted in slight suppression of double charging. For both PMMA 4000 and 8000 the addition of CTAB eliminated the multiple charging seen in mass spectrum without CTAB. For PMMA 12000, however, addition of CTAB alone was not enough to completely eliminate multiple charging.
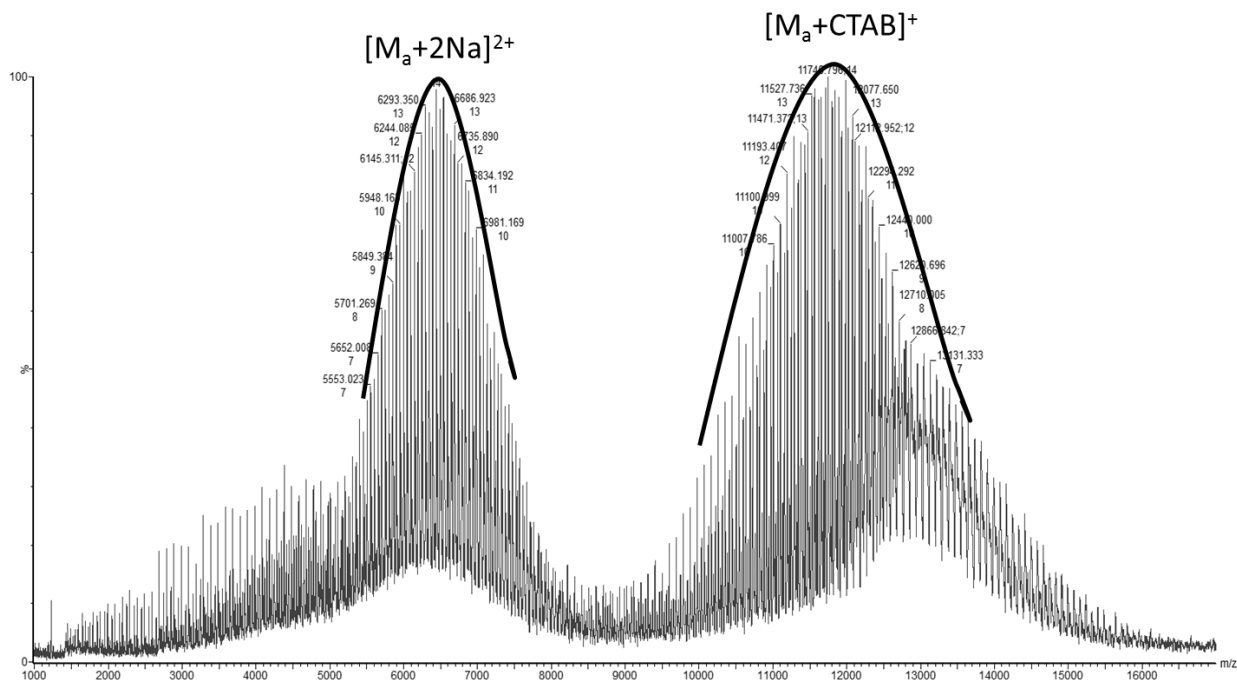
Figure 17: ESI-ToF-MS of PMMA 12000 with CTAB.

2.3.1 Two Solvent System

Since the addition of CTAB to the PMMA 12000 sample was only partially successful in reducing the charged state, we decided to determine what results could be obtained by adjusting the solvents present in the PMMA 12000 sample.

For all of the PMMA polymer series we used acetone as our solvent. Acetone was chosen as our solvent because it has a dielectric constant just high enough to be considered a reasonable ESI solvent. Acetone is also a very good solvent for PMMA. As described in the previous chapter ESI systems have solvent limitations. If the solvents dielectric constant is too low then a stable Taylor Cone cannot be formed and ionization of analyte molecules will not occur. This solvent limitation is not a problem for many analytes but for synthetic polymers it is an issue.

Most synthetic polymers used commercially are relatively nonpolar and therefore difficult to solvate with polar "ESI Solvents."

Our cosolvent system is designed to take advantage of the fact that PMMA and other nonpolar synthetic polymers are not soluble in common ESI solvents. Using a mixture of acetonitrile (a good ESI solvent with a higher boiling point than acetone) and acetone, the synthetic polymer can be forced toward the surface of the ESI droplets. The synthetic polymer is then more likely to be charged by CTAB and less likely to be directly charged (See Section 1.3.5.4).

To understand how the cosolvent system will force the synthetic polymer to the surface of the droplet, it is important to remember how CRM produced gas phase ions. In CRM solvent evaporation caused charge repulsion inside droplets to exceed the Rayleigh Limit of the droplet causing droplet fission. This process continued until gas phase ions were produced. For the cosolvent system instead of having one solvent we now have two.This does not change the mechanism that produces gas phase ions but it does significantly alter how the analytes will arrange themselves within the droplets. The two solvents that were used in this experiment were acetonitrile and acetone. It is important to note that these two solvents are miscible, PMMA is not soluble in acetonitrile and acetone has a higher vapor pressure than acetonitrile.

After the cosolvent droplets are sprayed from the Taylor Cone solvent evaporation begins. Since acetone has a higher vapor pressure than acetonitrile, it can be assumed that the surface of the droplet will lose acetone faster than acetonitrile. This creates a situation where the surface of the droplet will be composed of mostly acetonitrile. Since PMMA is not soluble in acetonitrile the synthetic polymer will precipitate onto the surface of the droplet as the acetone

carries PMMA to the surface and then evaporates. Once the charge repulsion of the droplet reaches the Rayleigh Limit droplet fission occurs and the process of solvent evaporation and PMMA precipitation continues. Having the synthetic polymer on the surface of the droplet resulted in has higher ionization efficiency and reduction of the charge state, seen in **Figure 18**.
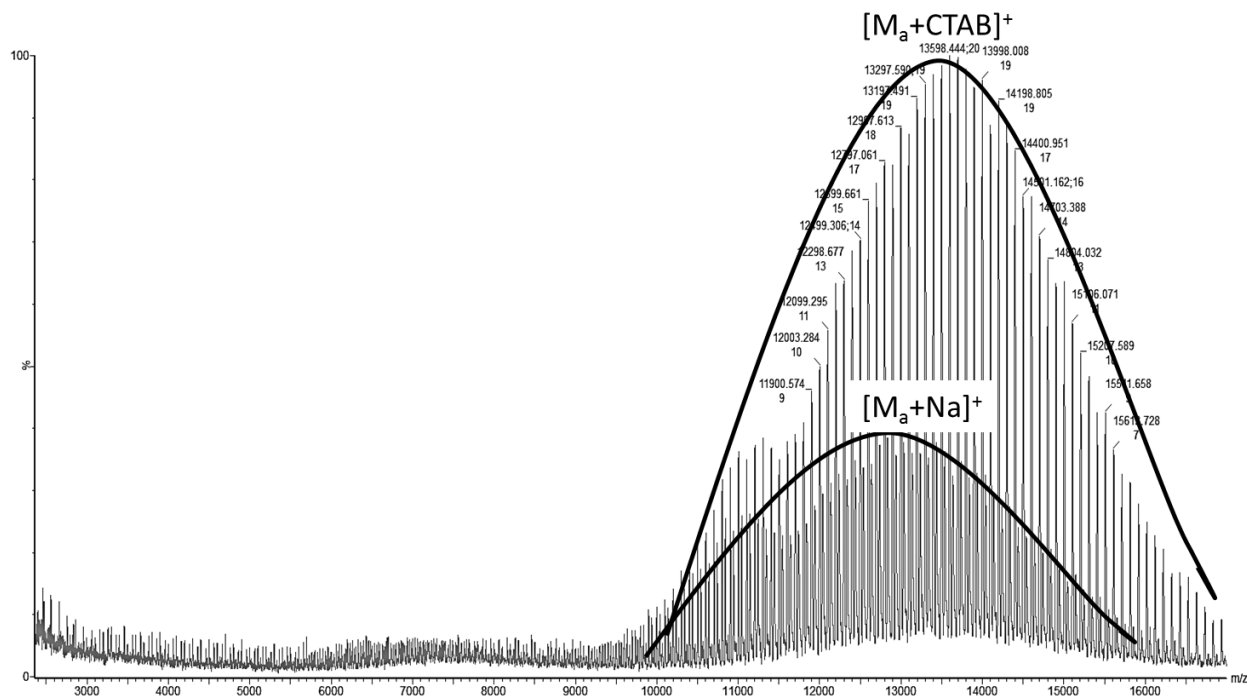
Figure 18: ESI-ToF-MS of PMMA 12000 with CTAB in 70% acetone and 30% acetonitrile. M_a and M_b correspond to structures A and B seen below in Table 7.

Using CTAB and the cosolvent system a single charge state mass spectrum for PMMA 12000 was produced. In the mass spectrum we see only singly charged ions making it is possible to accurately characterize both molecular weight and end group. The M_w, M_n, and PDI were calculated using **Equations 1, 2,** and **3** and are presented in **Table 6**.
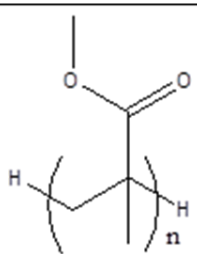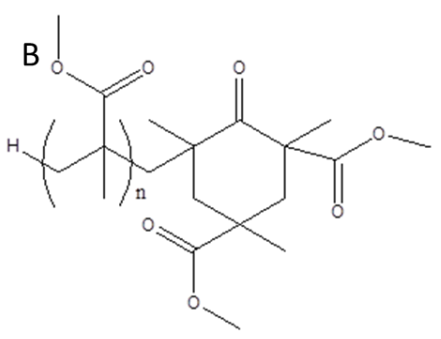
Table 6: PMMA 12000 molecular weight characterization calculated from ESI-MS and GPC data, compared to manufacturer provided values.

Synthetic Polymer	Analytical Method	M_n	M_w	PDI
PMMA 12000	ESI-MS	1.30×10^4	1.32×10^4	1.01
	GPC	1.20×10^4	1.27×10^4	1.06
	Manufacturer	1.14×10^4	1.2×10^4	1.04

The percent difference between the weight averaged molecular weight (M_w) calculated from in lab GPC and ESI-MS data was 3.9%. Again as with PMMA 4000 and 8000, the characterization of molecular weight distribution by both GPC and ESI-MS correlate well. The percent difference between ESI-MS M_w and manufacturer provided M_w was 9.5%. The percent difference for PMMA 12000 is lower than the percent difference for either PMMA 4000 or 8000; this is significant because it shows that our method is not getting less accurate with increase in molecular weight.

End group characterization of PMMA 12000 was also possible. Unlike PMMA 4000 and 8000, no backbitten polymer chains were observed. There are two distributions present in the mass spectrum shown in **Figure 18** the largest being PMMA charged by CTAB, structure **A Table 7**. The lower lying distribution is also structure **A** charged by sodium.

Table 7: Assigned Structures for Peaks of PMMA 12000

Assigned Structure	[M+CTAB]$^+$	[M+Na]$^+$
A	13397.151 n=131	13436.241 n=134
B	N/A	N/A

PMMA 12000 did not appear to have any backbiting present. This can be attributed to the synthesis of the standard because the PMMA 12000 standard was bought from a different supplier than both PMMA 4000 and PMMA 8000. It is likely that it was synthesized under different conditions and possibly a different reaction.

2.4 PMMA 4000, 8000 and 12000 Combined Spectra

The last PMMA sample was made containing all three PMMA standards and CTAB. We did not perform a run without any cationizing agent because after seeing the complexity generated by each PMMA standard alone. It is clear that the low m/z range of the mass spectrum would be severely congested if the sample were run without CTAB. **Figure 19** shows

the spectrum obtained when all three PMMA standards were mixed together with CTAB and run through the ESI-MS.
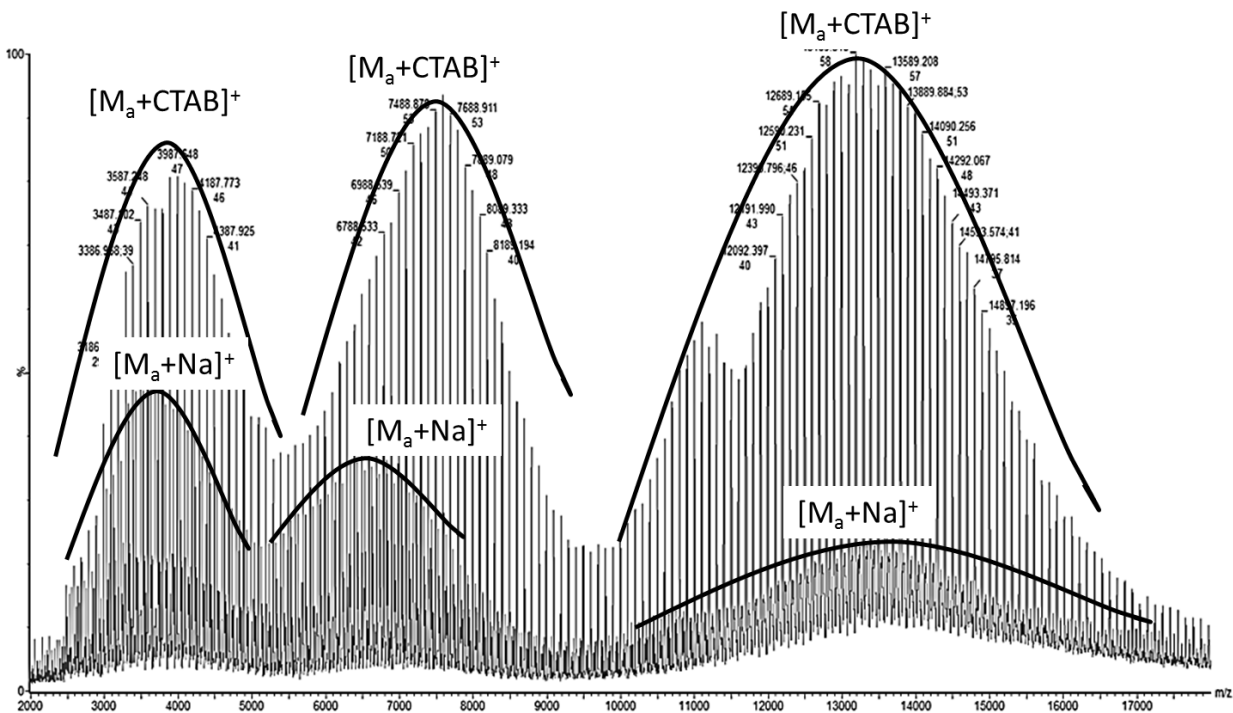
Figure 19: ESI-ToF-MS of PMMA 4000, 8000, and 12000 combined in the presence of CTAB.

Even though the distributions overlap all three of the PMMA standards can be identified. It is possible to produce a mass spectrum where all three of these standards can be distinguished from one another if CTAB is included in the sample. This spectrum also shows that we get strong ionization efficiency over the entire range of our ESI-MS instrument. **Figure 20** shows an overlay of this mass spectrum with our GPC results for a sample containing all three PMMA standards.
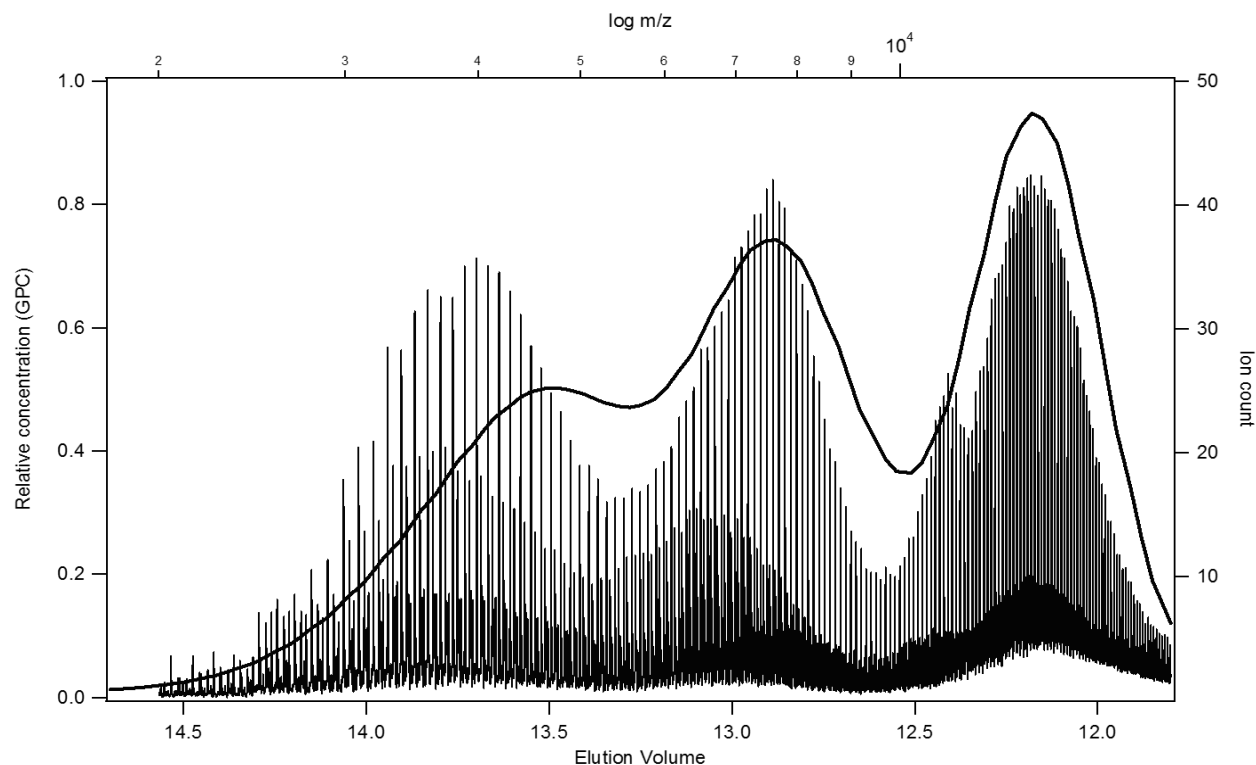
Figure 20: GPC ESI-ToF-MS overlay of PMMA 4000, 8000, and 12000 standards combined.

This GPC and ESI-MS overlay shows that ESI-MS has the potential to be a better instrument for characterization of synthetic polymers than GPC. As can be seen in the PMMA 4000 region of this overlay the separation is slightly worse for the low molecular weight standard. It is common for GPC instruments to have less separation efficiency for molecules at lower molecular weight. The mass spectrum of these three standards however shows very clear separation of all three standards present in the sample. **Figure 20** shows that ESI-MS has better resolution and less mass bias than GPC.

2.5 Polystyrene 10290

Results for the three different PMMA standards are promising and show that the use of surfactants as cationizing agents can greatly reduce the complexity of mass spectra obtained for

synthetic polymers. Due to the success of CTAB and the cosolvent system at eliminating

multiple charging, we next chose to analyze polystyrene

10290. Polystyrene shown in **Figure 21** is a much less

polar synthetic polymer than PMMA and as stated before

most industrial and commercially used synthetic polymers

are relatively nonpolar. By using CTAB and an 85%

acetone 15% acetonitrile solvent mixture we were able to

obtain the mass spectrum shown in **Figure 22**.
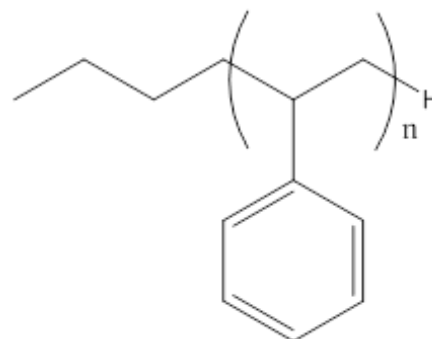
Figure 21: Polystyrene
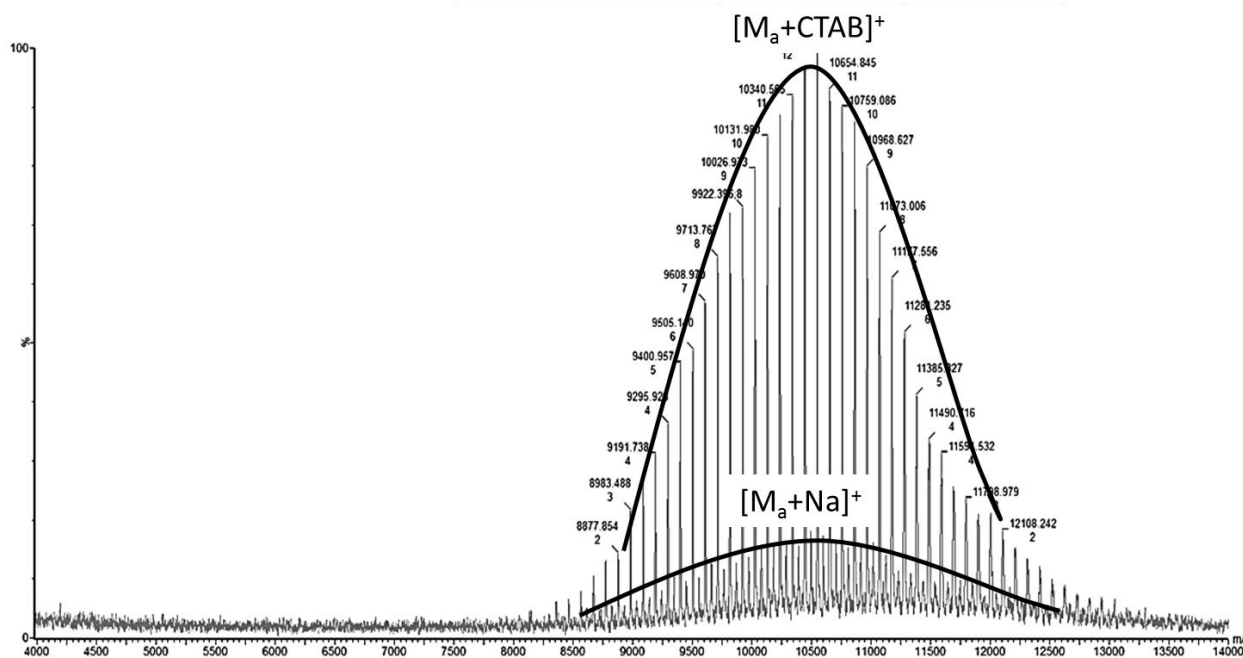
Figure 22: ESI-ToF-MS of Polystyrene 10290 with CTAB in 85% acetone 15% acetonitrile. M_a corresponds with structure A seen in Table 9.

Immediately, it is clear that we were able to singly charge the polystyrene molecules

without having any multiple charging. While at least one other research group has successfully

used ESI-MS to produce mass spectra for polystyrene [40], this is the first reported ESI mass

spectrum of a polystyrene sample in excess of 5,000 Da. Also to our knowledge this is the first

ESI-MS of polystyrene without the presence of an alkali salt (i. e. NaI) to increase ionization

efficiency. The use of such salts commonly contributes to spectrum complexity due to the

presence of salt cluster ions, particularly in the low m/z region.
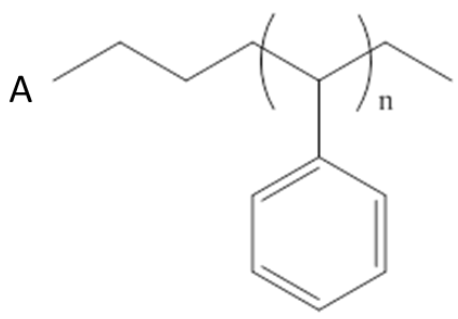
Table 8: Molecular weight distribution calculated from ESI-MS and Manufacturer
provided data for Polystyrene 10290

Synthetic Polymer	Analytical Method	M_n	M_w	PDI
PS 10290	ESI-MS	1.02×10^4	1.03×10^4	1.01
	Manufacturer	1.01×10^4	1.03×10^4	1.02

From **Figure 22** molecular weight characterization was done and is presented in **Table 8**.

The percent difference for M_w between ESI-MS calculated and manufacturer provided data is

0.17%. This result shows that the method used to generate this mass spectrum for polystyrene

10290 has almost identical molecular weight distribution characterization to those obtained by

the manufacturer of the standard. This is a very significant result because polystyrene samples

are nonpolar and difficult to ionize efficiently with ESI. The use of CTAB and the cosolvent

system allowed for sufficient ionization to characterize the polystyrene sample.

The mass spectrum shown in **Figure 22** also allows for end group determination. There

are only two distributions present in the mass spectrum and both of them are the same

polystyrene structure (**A**) with different charging species **Table 9**.

Table 9: Assigned Chemical Structure for Polystyrene 10290 Peaks.

Assigned Structure	[M+CTAB]$^+$	[M+Na]$^+$
A	10759.086 n=99	10496.313 n=99

Successful molecular weight and end group characterization of polystyrene 10290 is an important result. To our knowledge this is the first ESI-MS characterization of polystyrene with molecular weight greater than 10,000 Da.

2.6 Synthesis of Surfactant

While optimizing the parameters of the ESI-MS and choosing the correct solvent can go a long way to producing single charge state mass spectra of synthetic polymers, we believe that using surfactant molecules that will have stronger interactions with the synthetic polymer will further increase ionization efficiency. In order for the molecular structure of the surfactant to become another optimizable parameter it is important for the synthesis to be quick and relatively high yielding. For these reasons we chose to synthesize a quaternary ammonium salt.

The quaternary ammonium salt chosen is shown in **Figure 23**; this quaternary ammonium salt was chosen because the benzene ring should interact with the benzene rings of polystyrene making it more likely to charge polystyrene than CTAB, due to its higher affinity for polystyrene.
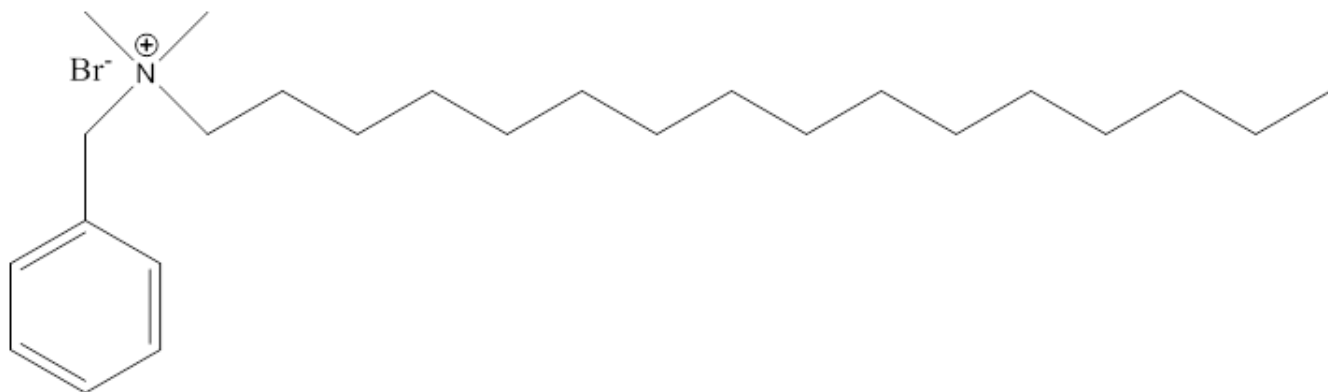
Figure 23: Synthesized Quaternary Ammonium Salt

Synthesis of this quaternary ammonium salt was straight forward. [41, 42] The reactants shown in **Figure 24** were added in equilmolar amounts to a round bottom flask containing THF. The reaction vessel was then heated to reflux for 24 hours. Product was precipitated into hexanes and recrystallized twice with ethyl ether.

1-Bromohexadecane

N,N-Benzyldimethylamine
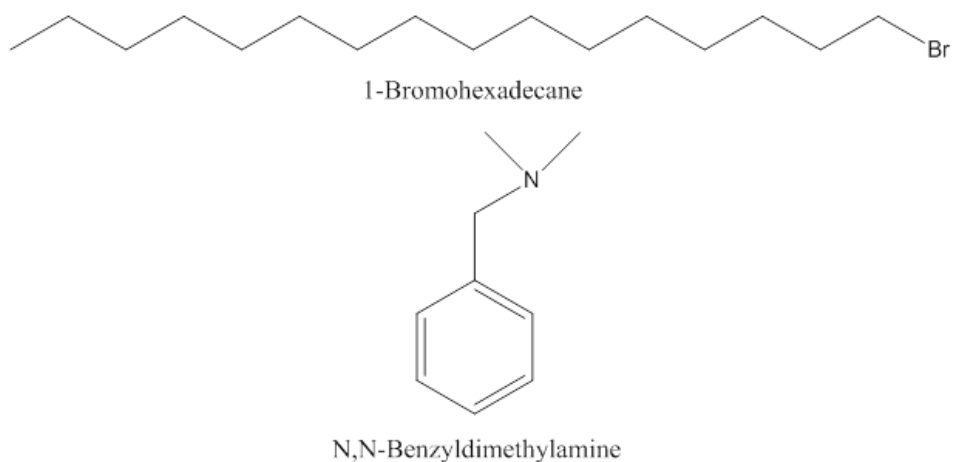
Figure 24: Reactants used to synthesize the Quaternary Ammonium Salt in Figure 23.

ESI-MS was used to confirm that the quaternary ammonium salt was successfully synthesized. (NMR for the synthesized quaternary ammonium salt is shown in appendix B.) **Figure 25** and **Figure 26** show two different sets of peaks present in the mass spectrum that verify the existence of our target quaternary ammonium salt. The peak shown at 360.369 m/z is the expected peak for our quaternary ammonium salt after the bromide counter ion was lost during ionization. ($C_{25}H_{46}N$ = 360.363 m/z) In **Figure 26** a set of four peaks starting at 799.049 m/z is the set of peaks we expect when our quaternary ammonium salt is clustered with another quaternary ammonium salt. This is because the isotopic abundance of bromine is 50.7% ^{79}Br and 49.3% ^{81}Br, combined with carbon isotopes four peaks should be present. The first peak at 799.049 m/z is $C_{50}H_{92}N_2Br$ (799.644 g/mol).
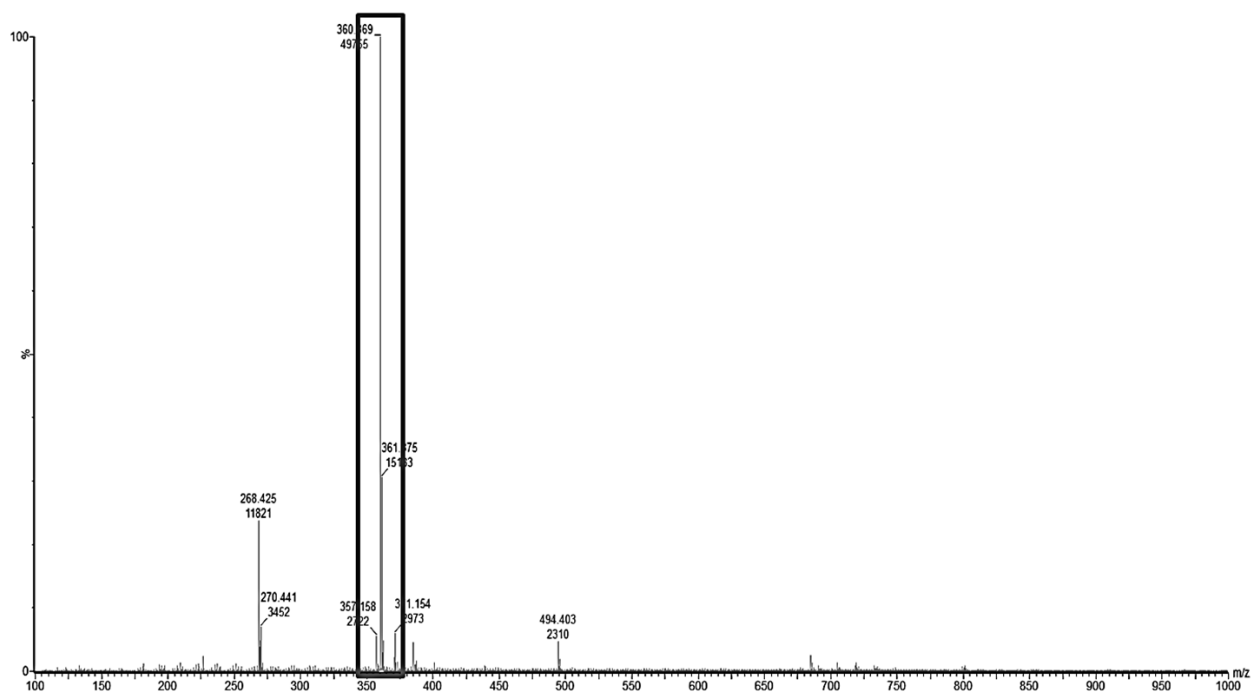
Figure 25: ESI-ToF-MS of synthesized quaternary ammonium salt.
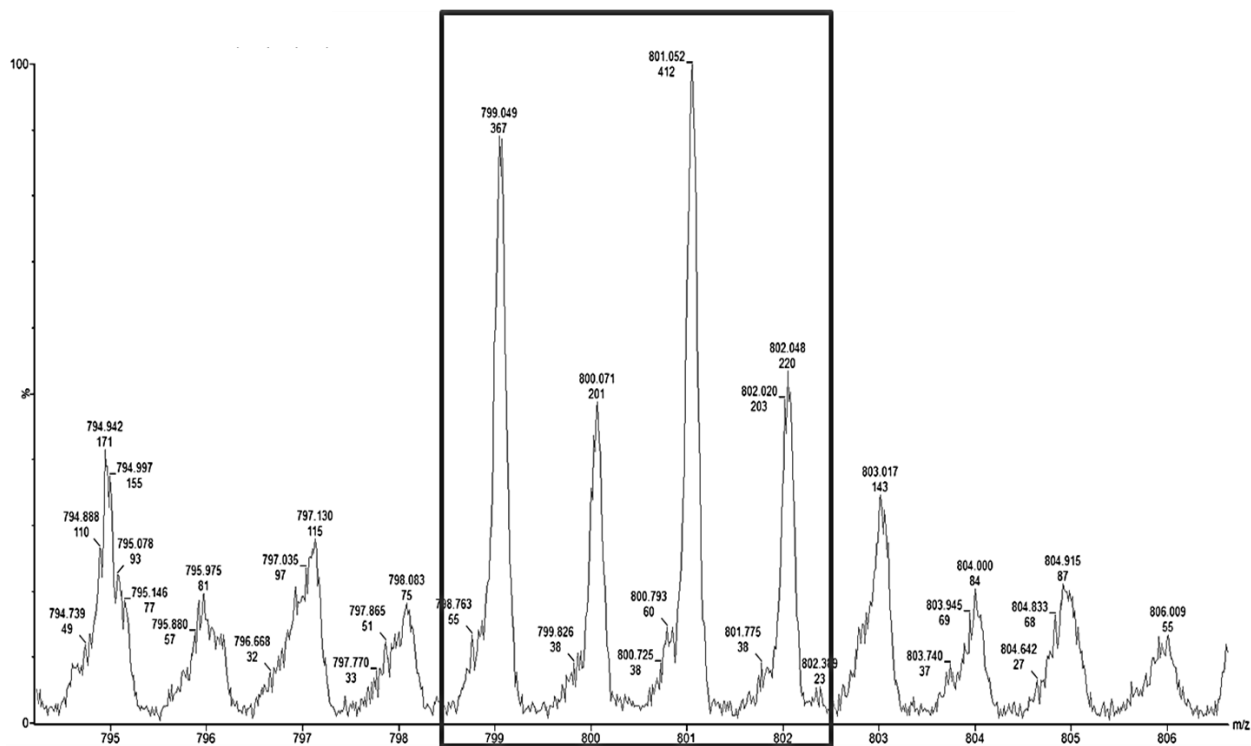
Figure 26: ESI-ToF-MS of synthesized quaternary ammonium salt, zoom in on double surfactant cluster.

After successful synthesis of the surfactant was verified the next step was to use the quaternary ammonium salt as the cationizing agent for the polystyrene 10290 sample. **Figure 27** shows the mass spectrum obtained using the synthesized surfactant. The parameters used for **Figure 27** were exactly the same as the parameters used for **Figure 22**. The polystyrene 10290 samples were made identically except for the surfactant that was used.
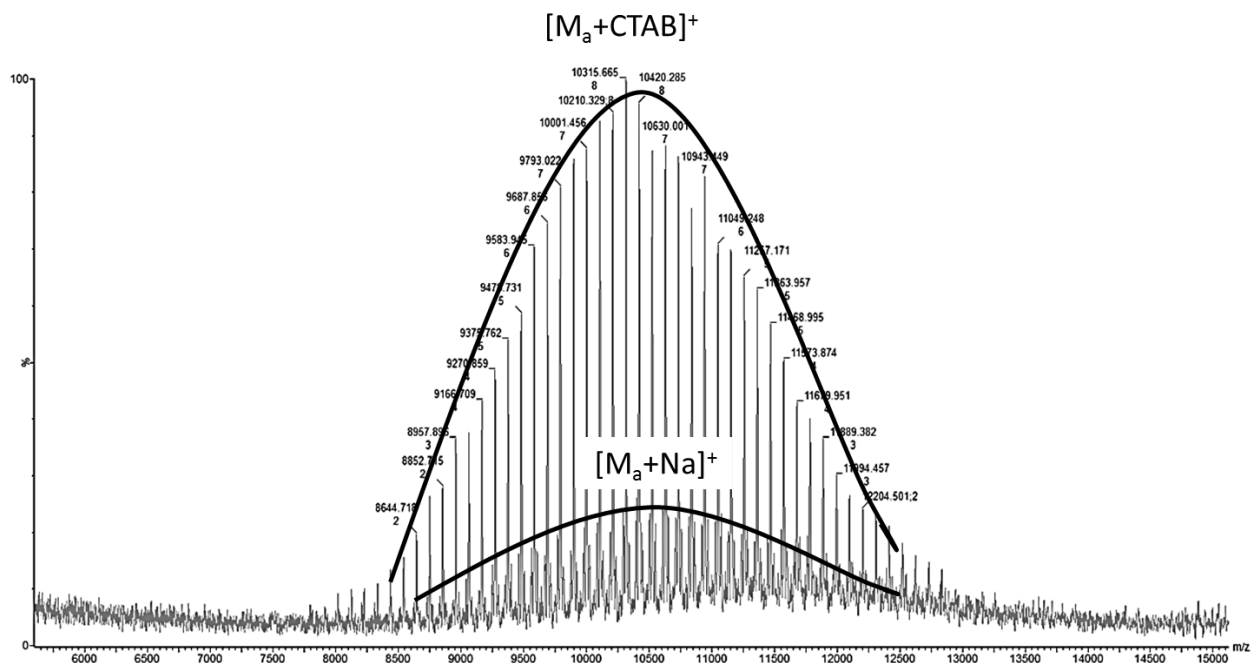
Figure 27: ESI-ToF-MS of Polystyrene 10290 with synthesized quaternary ammonium salt.

The polystyrene mass spectra obtained with our synthesized quaternary ammonium salt and CTAB are very similar. The shape of the distribution and the ion intensity of the two spectra are almost identical. Characterization of the molecular weight distribution also yielded similar results to those obtained using CTAB, **Table 10** shows values calculated from **Figure 27**. Percent difference between the polystyrene 10290 and manufacturer provided values was again very low. These results show that our method for the characterization of synthetic polymers with ESI -MS works very well. Even though the ionization efficiency was not improved it has been shown that other quaternary ammonium salts work well with our method. This is promising for future studies of different synthetic polymers such as fluoropolymers that may be charged using a perfluorcarbon based quaternary ammonium salt.

Table 10: Molecular weight distribution calculated from ESI-MS of polystyrene 10290 with synthesized quaternary ammonium salt.

Synthetic Polymer	Analytical Method	M_n	M_w	PDI
PS 10290	ESI-MS	1.01×10^4	1.01×10^4	1.01
	Manufacturer	1.01×10^4	1.03×10^4	1.02

Chapter 3: Conclusions and Future Experiments

In conclusion we report an update to previously characterized structural assignments for PMMA 4000 and 8000, due in large part to calibration of our instrument. This is the first reported 1 to 20,000 m/z range calibration using synthetic polymers, and was achieved by mixing three PMMA standards together with CTAB. We also report for the first time successful generation of single charge state ESI-MS for PMMA synthetic polymers with a molecular weight distribution greater than 10,000 g/mol. Molecular weight characterization of PMMA 4000 and PMMA 8000 were in close agreement with previously calculated values by earlier ESI-MS experiments. Good correlation between ESI-MS calculated values and GPC obtained results were also seen.

Single charge state mass spectra were also produced for polystyrene samples with molecular weight distribution greater than 10,000 g/mol. To our knowledge this is the first successful ESI-MS of polystyrene synthetic polymers larger than 5,000 g/mol. Molecular weight and end group characterization of the polystyrene samples were in excellent agreement with manufacturer provided values.

The production of exclusively singly charged ions is attributed to the use of surfactant molecules. CTAB is a much larger charging species than common ESI-MS instrument charging atoms (Na^+, H^+, or K^+). The fact that the surfactant is highly surface active and interacts with the synthetic polymer during ionization allows for exclusively single charging of the synthetic polymer. Large synthetic polymers or synthetic polymers with poor ESI solvent solubility were analyzed with a cosolvent system. The cosolvent system was successful in eliminating any multiple charging that was occurring in the single solvent systems. The cosolvent system also increased ionization efficiency for nonpolar synthetic polymers like polystyrene.

Future studies will be done on different synthetic polymers using different surfactant molecules. Successful characterization of a fluoropolymer using a perfluorocarbon quaternary ammonium salt would be of great scientific importance. Lastly the use of a multicationic (+2, +3, +4, etc.) surfactant molecule and production of single charge state ions would allow for much larger synthetic polymers to be studied and characterized in the lower m/z range.

Chapter 4: Experimental

4.1 Materials

The LC/UV grade acetone was manufactured by ChromAR (Lot # H14B00) was not altered before use. The HPLC grade acetonitrile was made by Burdick and Jackson (Lot # C0614) and was not altered before use. The HPLC grade tetrahydrofuran was made by T.J. Baker (Lot # G35804) and also used without being altered. The ethyl ether (Lot # 47026) was manufactured by EMD Chemicals and used without alteration. The hexanes (Lot # 066378) was manufactured by Fisher Scientific and used without alteration. Anhydrous toluene (Batch # 15486EH) was purchased from Sigma Aldrich and used without being altered.

PMMA standards PMMA 4000 and PMMA 8000 were purchased from Sigma Aldrich and used without being altered (PMMA 4000 Lot # WA22833 and PMMA 8000 Lot # 0001428483). Both PMMA 4000 and 8000 were manufactured by Fluka. PMMA 12000 (Lot # 510978) was purchased from manufactured by Polymer Sciences Incorporated and was used without alteration. Polystyrene samples were all pulled from the Varian Incorporated polystyrene standards kit (Lot # 101). The polystyrene standards kit was manufactured by Polymer Labs and used without being altered. The 1-Bromohexadecane (Lot # 05026TR) was manufactured by Aldrich Chemical Company and used without alteration. N,N-Benzyldimethylamine was manufactured by Polysciences Incorporated and used without being altered. The cetyltrimethylammonium bromide (Lot # 019K0024) was manufactured by Sigma and used as purchased.

4.2 GPC

4.2.1 Columns

The columns used in our GPC system were placed in the following order: PLgel Mixed-C, PLgel Mixed-E, Waters HR 2, and Waters HR 0.5. Data was extracted from Millennium and imported to Cirrus. Cirrus was calibrated with polystyrene standards and all GPC data was calculated in Cirrus.

4.2.2 Sample Preparation

0.0051 g of PMMA 4000 was added to a GPC vial followed by approximately 1.5 mL of THF and 2.0 μL of toluene (flow rate marker). 0.0047 g of PMMA 8000 was added to a GPC vial followed by approximately 1.5 mL of THF and 2.0 μL of toluene. 0.0051 g of PMMA 12000 was added to a GPC vial followed by approximately 1.5 mL of THF and 2.0 μL of toluene. Analysis of GPC data was performed on Cirrius, molecular weight results were calculated using polystyrene calibration curve.

4.3 ESI

4.3.1 Calibration

Previous work by our research group was plagued by calibration issues. The QToF Micromass instrument had a calibration up to 2,000 m/z. While this is sufficient for many users, the calibration was insufficient for characterization of synthetic polymer standards with masses anywhere from 1,600 Da to 19,000 Da. In order to get reliable molecular weight data for synthetic polymers the instrument needed to be calibrated out to 20,000 m/z.

Calibration over large mass ranges by clusters of alkali metal salts was reported in the literature. [43, 44] Calibration attempts with salt clusters (NaI, Na(Rb)I and CsI) failed to

provide adequate ion intensity beyond 10,000 m/z. Due to the failure of salt clusters, calibration with synthetic polymers was attempted. Small synthetic polymers like poly(ethylene glycol) are commonly used to calibrate the low mass range of ESI-MS instruments. A sample containing three poly(methyl methacrylate) standards with molecular weight ranging from 1,200 g/mol to 20,000 g/mol was used as calibrant. As seen in **Figure 28**, with the use of cetyltrimethylammonium bromide (CTAB) as the cationizing agent it was possible to calibrate the QToF Micromass from 0-20,000 m/z. (Reference file used for provided in Appendix A.)
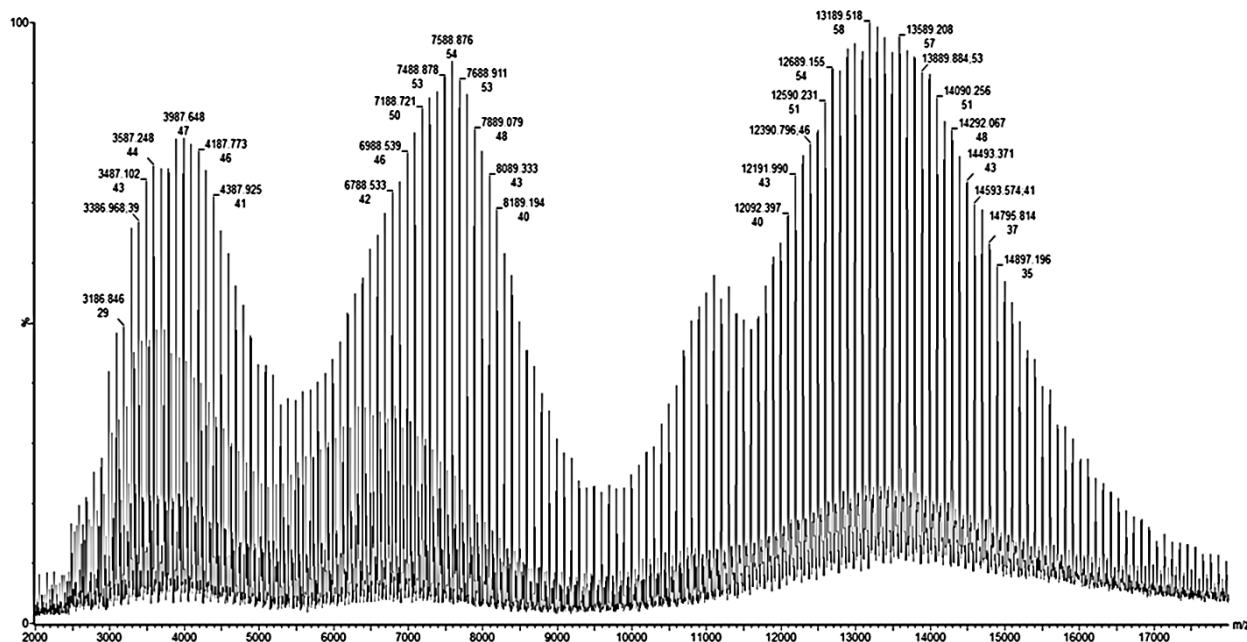
Figure 28: Mass spectrum of the sample containing poly(methyl methacrylate) 4000, 8000, and 12000 with cetyltrimethylammonium bromide (CTAB) as the charging species.

4.3.2   Bulk Sample Preparation

PMMA 4000 bulk solution was prepared by adding 0.0208 g of PMMA 4000 to a 20 mL scintillation vial followed by 10.00 mL of acetone. PMMA 4000 bulk sample concentration was 0.00052 M.

PMMA 8000 bulk solution was prepared by adding 0.0407 g of PMMA 8000 to a 20 mL scintillation vial followed by 10.00 mL of acetone. PMMA 8000 bulk sample concentration was 0.00051 M.

PMMA 12000 bulk solution was prepared by adding 0.0208 g of PMMA 12000 to a 20 mL scintillation vial followed by 20.00 mL of acetone. PMMA 12000 bulk sample concentration was 0.00025 M.

Polystyrene 10290 bulk solution was prepared by adding 0.0199 g of polystyrene 1290 sample to a 20 mL scintillation vial and then adding 10.00 mL of acetone. The concentration of the polystyrene 10290 bulk sample was 0.00019 M.

CTAB bulk solution 1 was prepared by adding 0.0036 g of CTAB to a 20 mL scintillation vial followed by 10.00 mL of acetone. CTAB bulk solution concentration was 0.00099 M.

CTAB bulk solution 2 was prepared by adding 0.0029 g of CTAB to a 20 mL scintillation vial followed by 20.00 mL of acetone. CTAB bulk solution concentration was 0.00040 M.

Synthesized quaternary ammonium salt bulk solution was prepared by adding 0.0054 g of our synthesized surfactant to a 20 mL scintillation vial followed by the addition of 10.00 mL of acetone. The concentration of the sample was 0.00122 M.

4.2.1 Sample Preparation

The PMMA 4000 spectrum shown in **Figure 12**, was prepared by adding 60 μL of the PMMA 4000 bulk sample and 250 μL of the CTAB bulk solution 2 to a 20 mL scintillation vial diluted with 10.00mL of acetone. The PMMA 4000 concentration of the sample was 3.12 μM and the CTAB concentration was 9.95 μM. The parameters were identical for both the mass spectrum of PMMA 4000 with (**Figure 12**) and without (**Figure 11**) CTAB. The parameters for the instrument are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	100V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	500 L/hr
Sample Flow Rate	10 μL/min
Number of Spectrum Combined	268

The PMMA 8000 spectrum shown in **Figure 14**, was prepared by adding 100 μL of the PMMA 8000 bulk sample and 250 μL of the CTAB bulk solution 2 to a 20 mL scintillation vial diluted with 10.00 mL of acetone. The PMMA 8000 concentration of the sample was 5.09 μM and the CTAB concentration was 9.95 μM. The parameters were identical for both the mass spectrum of PMMA 8000 with (**Figure 14**) and without (**Figure 13**) CTAB. The parameters for the instrument are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	140V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	250 L/hr
Sample Flow Rate	10 μL/min
Number of Spectrum Combined – **Figure 13**	321
Number of Spectrum Combined – **Figure 14**	535

The PMMA 12000 spectrum shown in **Figure 18**, was prepared by adding 80 μL of the

PMMA 12000 bulk sample and 750 μL of the CTAB bulk solution 2 to a 20 mL scintillation

vial diluted with 10.00 mL of acetone and acetonitrile in a 70% acetone 30% acetonitrile

mixture. The PMMA 12000 concentration of the sample was 2.16 μM and the CTAB

concentration was 29.84 μM. The parameters were identical for all three the mass spectra of

PMMA 12000 with (**Figure 17**) and without (**Figure 16**) CTAB and also with the cosolvent

system (**Figure 18**). The parameters for the instrument are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	200V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	200 L/hr
Sample Flow Rate	10 μL/min
Number of Spectrum Combined – **Figure 16**	498
Number of Spectrum Combined – **Figure 17**	208
Number of Spectrum Combined – **Figure 18**	798

The combined mass spectrum (**Figure 19**) was prepared by adding 40 µL of the PMMA 4000 bulk solution, 100 µL of the PMMA 8000 bulk solution, 600 µL of the PMMA 12000 bulk solution, and 1000 µL of the CTAB bulk solution 2. The sample was then diluted with 10.00 mL of a 70% acetone 30% acetonitrile solvent mixture. The concentrations of PMMA 4000, 8000, 12000 and CTAB in this sample were 1.73 µM, 4.24 µM, 12.6 µM, and 33.16 µM respectively. The parameters for the instrument are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	175V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	200 L/hr
Sample Flow Rate	10 µL/min
Number of Spectrum Combined – **Figure 19**	804

The polystyrene 10290 sample used to produce the mass spectrum shown in **Figure 22** was prepared by adding 38.6 µL of the polystyrene 10290 bulk solution and 125 µL of the CTAB bulk solution 2 to a 20 mL scintillation vial. Next 10.00 mL of an 85% acetone and 15% acetonitrile solvent mixture were added to the vial. The concentration of polystyrene 10290 in the sample was 2 µM and the concentration of CTAB was 5 µM. The parameters used for this sample are shown below:

54

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	170V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	500 L/hr
Sample Flow Rate	15 μL/min
Number of Spectrum Combined – **Figure 22**	535

The polystyrene 10290 sample used to produce the mass spectrum shown in **Figure 27** was prepared by adding 38.6 μL of the polystyrene 10290 bulk solution and 40.8 μL of the synthesized quaternary ammonium salt bulk solution to a 20 mL scintillation vial. Next 10.00 mL of an 85% acetone and 15% acetonitrile solvent mixture were added to the vial. The concentration of polystyrene 10290 in the sample was 2.0 μM and the concentration of synthesized quaternary ammonium salt was 5.0 μM. The parameters used for this sample are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	170V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	500 L/hr
Sample Flow Rate	15 μL/min
Number of Spectrum Combined – **Figure 27**	534

4.4 Synthesis of Quaternary Ammonium Salt

Synthesis of the quaternary ammonium salt used for the ESI-ToF-MS of polystyrene 10290 was performed by the following steps. First 2.52 mL of 1-Bromohexadecane was added to a

50 mL round bottom flask. Next 2.83 mL of Benzyldimethylamine was added to the reaction

vessel. Lastly 15.0 mL of THF was added to the flask. The flask was then hooked up to a

condenser and allowed to react at reflux for 24 hours. After 24 hours the reaction was stopped

and the reaction flask was precipitated into 200 mL of hexanes. After filtration the solid crude

sample was then recrystallized twice in 200 mL of ethyl ether.

Successful synthesis was confirmed as described above and shown in **Figures 25** and **26**.

The ESI-ToF-MS of the quaternary ammonium salt was performed on a dilute sample of

product in acetone. The parameters used to obtain the mass spectrum are shown below:

Parameter Name	Setting
Capillary Voltage	2900V
Sample Cone Voltage	40V
Extraction Cone Voltage	2.0V
Source Temperature	90˚C
Desolvation Temperature	180˚C
Cone Gas Flow Rate	0 L/hr
Desolvation Gas Flow Rate	500 L/hr
Sample Flow Rate	5 μL/min
Number of Spectrum Combined – **Figure 25**	102
Number of Spectrum Combined – **Figure 26**	102

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APPENDIX A. Reference File used for Calibration of ESI-MS

m/z values were calculated for PMMA peaks with hydrogen end groups and CTAB as

charging species

n=	m/z
[CTA]$^+$	284.332
[CTAB$_{79}$+CTA]$^+$	647.582
[CTAB81+CTA]$^+$	649.581
10	1286.872
11	1386.924
12	1486.977
13	1587.029
14	1688.085
15	1788.138
16	1888.190
17	1988.242
18	2088.295
19	2188.347
20	2288.400
21	2388.452
22	2488.505
23	2588.557
24	2688.610
25	2788.662

26	2888.714
27	2988.767
28	3088.819
29	3189.875
30	3289.927
31	3389.980
32	3490.032
33	3590.085
34	3690.137
35	3790.189
36	3890.242
37	3990.294
38	4090.347
39	4190.399
40	4290.452
41	4390.504
42	4490.557
43	4590.609
44	4690.662
45	4791.717
46	4891.769
47	4991.822
48	5091.874
49	5191.927
50	5291.979

51	5392.032
52	5492.084
53	5592.137
54	5692.189
55	5792.242
56	5892.294
57	5992.346
58	6092.399
59	6192.451
60	6293.507
61	6393.559
62	6493.612
63	6593.664
64	6693.717
65	6793.769
66	6893.821
67	6993.874
68	7093.926
69	7193.978
70	7294.031
71	7394.083
72	7494.136
73	7594.188
74	7694.241
75	7794.293

76	7895.349
77	7995.401
78	8095.454
79	8195.506
80	8295.559
81	8395.611
82	8495.663
83	8595.716
84	8695.768
85	8795.821
86	8895.873
87	8995.925
88	9095.978
89	9196.031
90	9296.083
91	9397.139
92	9497.191
93	9597.244
94	9697.296
95	9797.348
96	9897.401
97	9997.454
98	10097.505
99	10197.558
100	10297.611

101	10397.662
102	10497.715
103	10597.768
104	10697.821
105	10797.872
106	10898.928
107	10998.981
108	11099.034
109	11199.085
110	11299.138
111	11399.191
112	11499.243
113	11599.295
114	11699.348
115	11799.400
116	11899.453
117	11999.505
118	12099.557
119	12199.610
120	12299.662
121	12399.714
122	12500.771
123	12600.823
124	12700.875
125	12800.928

126	12900.980
127	13001.033
128	13101.085
129	13201.137
130	13301.190
131	13401.243
132	13501.294
133	13601.347
134	13701.400
135	13801.453
136	13901.504
137	14002.560
138	14102.613
139	14202.665
140	14302.717
141	14402.770
142	14502.823
143	14602.874
144	14702.927
145	14802.980
146	14903.032
147	15003.084
148	15103.137
149	15203.190
150	15303.242

151	15403.294
152	15503.347
153	15604.403
154	15704.454
155	15804.507
156	15904.560
157	16004.612
158	16104.664
159	16204.717
160	16304.769
161	16404.823
162	16504.873
163	16604.926
164	16704.979
165	16805.032
166	16905.084
167	17005.137

APPENDIX B, NMR of the Synthesized Quaternary Ammonium Salt