APPLICATIONS OF MASS SPECTROMETRY TO POLY(ELECTROLYTES) AND KINETICS

A Dissertation

Presented to

The Graduate Faculty of The University of Akron

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Bethany Subel

August, 2009
APPLICATIONS OF MASS SPECTROMETRY TO POLY(ELECTROLYTES) AND KINETICS

Bethany Subel
Dissertation
ABSTRACT

The only prerequisite for studying an analyte molecule using mass spectrometry is that the sample must be ionizable. For this reason, mass spectrometry can be utilized to study many types of molecules with a wide mass range. This dissertation utilizes that characteristic to examine the metal ion thermochemistry of small biomolecules and the structures of larger synthetic polymers.

This work aims at determining the sodium binding affinity of adenine experimentally based on the metal binding location. By using derivatives of adenine to sequentially block potential binding sites of the sodium ion, mass spectrometry can measure experimentally the sodium binding affinity of adenine as a function of the binding site. Specifically, adenine, 3-methyladenine and N6-methylaminopurine were found to have different sodium binding affinities due to their different metal binding sites.

The second part of this work describes the study of larger molecules, namely polymers. The first study involving polymers determined the monomer composition of a copolymer containing styrene and allyl alcohol monomers. Using mass spectrometry, monomer composition differed from the value reported when using other methods. Tandem mass spectrometry experiments reveal internal fragments due to polystyrene and water loss from the allyl alcohol monomer.
The mass spectrum of poly(acrylic acid) revealed water loss in addition to sodium exchange with the acidic proton on the side chain of the polymer. In the positive mode mass spectrum of poly(styrene sulfonate sodium salt), Na⁺/H⁺ exchange was not observed, but occurred readily in negative mode.

The tandem mass spectrum of poly(acrylic acid) showed that H₂O loss is more abundant than previously thought. Further, the new data support that CO₂ loss does not lead to a four membered ring as previously proposed, but most likely causes a six-membered ring to form.

For poly(styrene sulfonate sodium salt), the tandem mass spectra of the tetramer in positive and negative mode showed very similar fragmentation patterns. Monomer evaporation and nominal monomer + CH₂ loss were the most abundant fragments observed. This fragmentation depends on the number of Na⁺/H⁺ exchanges that have taken place. Each SO₃Na → SO₃H exchange enables the loss of a SO₃ group from the side chain of the polymer.
DEDICATION

This dissertation is dedicated to my family: past, present and future. Without your inspiration and support, I would not be where I am today.
ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Wesdemiotis for welcoming me into his group and providing me with the support and encouragement I needed to complete my research. I would also like to thank past and present group members: Dr. Michael Polce, Dr. Ping Wang, Dr. Panthida Thomya, Dr. Edgardo Rivera-Tirado, Dr. Kittisak Chaicharoen, Dr. Sara Whitson, Alyison Leigh, David Dabney, Nilüfer Solak, Alesia Salberg, Vincenzo Scionti, Danijela Smiljanic, Madalis Casiano, Omür Celibak, Aleer Yol, Bryan Katzenmeyer and Li Xiaopeng. I would like to thank my Project SEED student, Jasmine Monson, for her efforts.

I would like to acknowledge my committee, Dr. Hu, Dr. Tessier, Dr. Zheng and Dr. Newby for their helpful suggestions.

I would like to thank my good friend, Melinda Carnahan. She was there for me through it all, and I couldn’t have done it without her. I would also like to thank my good friend Alyison Leigh for everything she has ever done for me. It’s more than she could ever imagine.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>x</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF SCHEMES</td>
<td>xiv</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. INSTRUMENTAL METHODS</td>
<td>10</td>
</tr>
<tr>
<td>2.1 Mass Spectrometry Instrumentation</td>
<td>10</td>
</tr>
<tr>
<td>2.2 Ionization Sources</td>
<td>12</td>
</tr>
<tr>
<td>2.2.1 Electrospray Ionization (ESI)</td>
<td>13</td>
</tr>
<tr>
<td>2.2.2 Matrix-Assisted Laser Desorption/Ionization (MALDI)</td>
<td>16</td>
</tr>
<tr>
<td>2.3 Mass Analyzers</td>
<td>18</td>
</tr>
<tr>
<td>2.3.1 Quadrupole Ion Trap</td>
<td>18</td>
</tr>
<tr>
<td>2.2.3 Time of Flight (ToF) Mass Analyzer</td>
<td>21</td>
</tr>
<tr>
<td>2.2.4 Quadrupole-Time-of-Flight (Q-ToF) Tandem Mass Analyzer</td>
<td>25</td>
</tr>
<tr>
<td>III. MATERIALS, METHODS AND INSTRUMENTATION</td>
<td>26</td>
</tr>
<tr>
<td>3.1 Kinetic Method</td>
<td>26</td>
</tr>
<tr>
<td>3.1.1 Polymer Materials</td>
<td>26</td>
</tr>
<tr>
<td>3.2 Methods</td>
<td>27</td>
</tr>
</tbody>
</table>
3.2.1 Kinetic Method Theory ................................................................. 28
3.2.2 Kinetic Method Preparation .......................................................... 29
3.2.3 Polymer Analysis Preparation ....................................................... 29
3.3 Instrumentation .................................................................................. 30
3.3.1 Bruker Esquire LC- ESI QiT Mass Spectrometer ......................... 31
3.3.2 Bruker Reflex III MALDI ToF Mass Spectrometer ................. 34
3.3.3 MALDI Q-ToF Mass Spectrometry .............................................. 37

IV. KINETIC METHOD RESULTS ...................................................................... 39
4.1 Background ......................................................................................... 39
4.2 Adenine ............................................................................................... 40
4.3 3-Methyladenine .................................................................................. 44
4.4 N6-MeAP .............................................................................................. 48
4.5 Kinetic Method Conclusions ................................................................. 51

V. POLY(ELECTROLYTE) ANALYSIS USING MASS SPECTROMETRY .......... 53
5.1 MALDI-ToF Mass Spectrometry of Copolymers .............................. 53
5.2 Mass Spectrometry of Poly(electrolytes) ........................................... 60
5.2.1 Poly(acrylic acid) (PAA) ................................................................. 60
5.2.2 Tandem Mass Spectra of PAA ......................................................... 63
5.2.3 Poly(styrenesulfonate sodium salt) (PSS) .................................... 75
5.2.4 Tandem Mass Spectra of PSS ......................................................... 78

VI. SUMMARY ............................................................................................................. 88
REFERENCES .................................................................................................................. 94
APPENDICES ................................................................................................................. 100
viii
APPENDIX A. ADDITIONAL DATA.................................................................101
APPENDIX B. COPYRIGHT PERMISSION....................................................103
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>27</td>
</tr>
<tr>
<td>4.1</td>
<td>51</td>
</tr>
</tbody>
</table>

3.1 Summary of Samples Studied for Copolymer Analysis Containing Styrene and Allyl Alcohol Monomers

4.1 Summary of Kinetic Method Results
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The structure of adenine with numbered atoms</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>Diagram of a polymer electrolyte fuel cell</td>
<td>8</td>
</tr>
<tr>
<td>1.3</td>
<td>Nafion is currently used as the polyelectrolyte membrane in PEFC's. (a) Nafion (b) poly(etherketone) (c) poly(styrene) (d) poly(vinyl alcohol)</td>
<td>9</td>
</tr>
<tr>
<td>2.1</td>
<td>Basic mass spectrometry set-up</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>ESI process. (a) ESI process shown with nebulization gas (b) Ion formation from solvated analyte</td>
<td>14</td>
</tr>
<tr>
<td>2.3</td>
<td>MALDI process</td>
<td>17</td>
</tr>
<tr>
<td>2.4</td>
<td>Quadrupole ion trap showing the end caps on either side of the ring electrode</td>
<td>20</td>
</tr>
<tr>
<td>2.5</td>
<td>ToF mass spectrometers (a) Linear ToF instrument (b) Reflectron ToF instrument</td>
<td>23</td>
</tr>
<tr>
<td>2.6</td>
<td>Theory of PIE</td>
<td>24</td>
</tr>
<tr>
<td>3.1</td>
<td>Diagram of the Bruker Esquire LC MS</td>
<td>31</td>
</tr>
<tr>
<td>3.2</td>
<td>Diagram of a continuous dynode electron multiplier, also known as channeltron</td>
<td>34</td>
</tr>
<tr>
<td>3.3</td>
<td>Diagram of Bruker Reflex III MALDI ToF MS</td>
<td>35</td>
</tr>
<tr>
<td>3.4</td>
<td>Diagram of a MCP detector</td>
<td>36</td>
</tr>
<tr>
<td>3.5</td>
<td>Diagram of the Waters Ultima QToF mass spectrometer</td>
<td>38</td>
</tr>
<tr>
<td>4.1</td>
<td>Structures of Ade. (a) Ade (b) 3-MeAde (c) N6-MeAP</td>
<td>40</td>
</tr>
</tbody>
</table>
4.2 Mixture between Trp, Na trifluoroacetate and Ade. (a) ESI mass spectrum of the mixture between Trp, Na trifluoroacetate and Ade (b) CAD spectrum of the sodium bound heterodimer containing Ade and Trp (m/z 361.1) ..................41

4.3 Regression line resulting from a plot of $\ln(k_{\text{Ade}}/k_{\text{AA}})$ vs. sodium binding affinity of AA ...........................................................................................................42

4.4 Regression line of the $\ln(k_{\text{Ade}}/k_{B_i})$ vs. sodium binding affinity of $B_i$ .....................43

4.5 Example of the mass and CAD spectra from a mixture of 3-MeAde and Val. (a) ESI mass spectrum of a mixture of 3-MeAde, Val and Na trifluoroacetate (b) CAD spectrum showing the sodium bound heterodimer between Val and 3-MeAde (m/z 289.1) ...............................................................................................46

4.6 Regression line resulting from heterodimers containing 3-MeAde and one reference base from set BS1 .................................................................46

4.7 CAD spectrum of the $\text{Na}^+$-bound heterodimer between 3-MeAde and DMF .................47

4.8 Regression line obtained from heterodimers between 3-MeAde and a BS2 molecule ...................................................................................................................48

4.9 Regression line obtained from heterodimers between N6-MeAP and a BS1 molecule ...................................................................................................................50

4.10 CAD spectrum of the $\text{Na}^-$-bound heterodimer between N6-MeAP and DMA........50

4.11 CAD spectra of the heterodimer between Ade and its derivatives (a) CAD spectrum of the $\text{Na}^-$-bound heterodimer between Ade and 3-MeAde (b) CAD spectrum of the $\text{Na}^-$-bound heterodimer between Ade and N6-MeAP ............52

5.1 MALDI-ToF mass spectrum of poly(styrene-co-allyl alcohol) obtained using a Bruker Reflex III instrument. (a) Entire mass region where peaks were observed (b) Mass range 1000-1400 Da where the repeat units of allyl alcohol, indicated by the blue arrow, and styrene, indicated by the red arrow, can be observed, confirming the presence of a copolymer.........................54

5.2 Tandem mass spectrum of silverated poly(styrene-co-allyl alcohol) containing seven styrene and four allyl alcohol units. (a) Entire spectrum (b) Expanded region from m/z 120 to 540. ..................................................................................58

5.3 ESI mass spectrum of PAA in positive mode ........................................................................62

5.4 ESI mass spectrum of PAA in negative mode ........................................................................62
5.5 ESI MS² spectrum of the sodiated 11-mer of PAA, distribution A using the QiT (positive mode) ..................................................................................................................64

5.6 ESI-MS² spectrum of the deprotonated 11-mer of PAA, distribution A, using the QiT (negative mode)........................................................................................................65

5.7 MALDI-MS² spectrum of the deprotonated 11-mer from PAA distribution A using the Q-ToF mass spectrometer (negative mode) ..............................................69

5.8 Positive mode ESI-MS² spectra of the sodiated pentamer of the PAA. (a) Distribution C, which contains the original polymer (b) Distribution B, which has already lost two water molecules .........................................................70

5.9 MS/MS spectra of the tetramer of the A series using different polarities. (a) positive mode (b) negative mode ..........................................................................................72

5.10 Structure of PSS analyzed ..................................................................................................................76

5.11 ESI mass spectrum of PSS in positive mode, where each ion observed is sodiated (ionization by Na⁺ adduction) ..............................................................................76

5.12 Negative mode ESI-QiT mass spectrum of PSS .................................................................78

5.13 ESI-MS² spectrum of the sodiated PSS tetramer (m/z 905).................................................80

5.14 ESI-MS² spectra of the PSS tetramer in negative mode. (a) Polymer with three Na ions (b) Polymer with two Na ions (c) Polymer with one Na ion (d) Polymer with no sodium in the sulfonated groups.......................................................85
# LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Likely compositions of major fragments in the MS/MS spectrum of silverated C₄H₉ – [CH₂CH(Ph)]₇ – [CH₂CH(CH₂OH)]₄ – H (m/z 1068)</td>
</tr>
<tr>
<td>5.2</td>
<td>PAA distributions detected in the PAA sample analyzed</td>
</tr>
<tr>
<td>5.3</td>
<td>Water loss from sodiated or deprotonated PAA to form cyclic anhydride units and consecutive CO₂ loss from the anhydride to yield a cyclic ketone</td>
</tr>
<tr>
<td>5.4</td>
<td>Dehydration of adjacent cyclic anhydride groups, formed by water losses from sodiated or deprotonated PAA</td>
</tr>
<tr>
<td>5.5</td>
<td>CO₂ loss from adjacent cyclic anhydride groups, formed by water losses from sodiated or deprotonated PAA</td>
</tr>
<tr>
<td>5.6</td>
<td>Monomer loss from the sodiated tetramer of a PAA chain containing an anhydride next to the monomer cleaved</td>
</tr>
<tr>
<td>5.7</td>
<td>Competitive and consecutive H₂O and CO₂ eliminations from the deprotonated tetramer of series A</td>
</tr>
<tr>
<td>5.8</td>
<td>Elimination of a dimer (144 Da) from the deprotonated tetramer of series A</td>
</tr>
<tr>
<td>5.9</td>
<td>Evaporation of monomer (206 Da) and monomer + CH₂ (220 Da) units from the sodiated PSS tetramer</td>
</tr>
<tr>
<td>5.10</td>
<td>Evaporation of dimer (412 Da) and dimer + CH₂ (426 Da), and formation of sodiated dimer (m/z 435) and sodiated dimer + CH₂ (m/z 449) from the sodiated PSS tetramer</td>
</tr>
<tr>
<td>5.11</td>
<td>Consecutive dissociation of m/z 685 (generated by evaporation of monomer + CH₂ from the sodiated PSS tetramer) by monomer loss to form m/z 479 (left) or by radical losses to form m/z 421 (right)</td>
</tr>
<tr>
<td>5.12</td>
<td>Acid-catalyzed SO₃ loss from PSS carrying SO₃H substituents</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION

Mass spectrometry (MS) is an important analytical technique because of its sensitivity, specificity and selectivity. MS is an inherently specific technique because it measures the mass-to-charge ratio (m/z) of an analyte molecule, which provides the corresponding molecular weight when the charge state is known. The molecular weight of a molecule generally is specific property to a specific molecule. The sensitivity is such that the detection limit of modern mass spectrometry instrumentation reaches the attomole level.\(^1\) A wide range of molecular weights can be studied by mass spectrometry; there are no lower mass limits and, depending on the instrument used, the upper mass limit can reach 1,000,000 Daltons (Da).\(^2\) Moreover, MS can be used to selectively detect trace components in a complex mixture. For these reasons, this method has become an essential analytical tool for a variety of analytes. Everything from synthetic polymers\(^3-6\) to small molecules to saccharides\(^7-9\) to large biomolecules\(^10-12\) can be studied using mass spectrometry.

One area where mass spectrometry has become very beneficial is in the structural analysis of biomolecules. The development of “soft” ionization techniques, which keep the entire molecule intact, has allowed MS studies of large biomolecules such as proteins and DNA strands to be studied.\(^13\) Although the majority of MS applications concern
structural elucidations, a significant number of studies have focused on using MS to understand the nature and strength of the intrinsic guest-host interactions that can develop between biomolecules (hosts) and small organic or inorganic guests, in particular metal ions, in order to gain a better understanding about enzyme function, drug action and biocatalysis.

Metal ions play an important role in the biological reactions taking place in the cell. For example, sodium, which is the ninth most abundant element in the human body, is involved in the synthesis, replication and structure of DNA and RNA. Sodium can act as a stabilizing metal, balancing out the negative charges of the phosphate groups of DNA. However, when the concentration of the metal ion becomes too high, it can interfere with hydrogen bonding and disrupt the double helix structure of DNA.

The nature of sodium binding to the different components of DNA or RNA, including the nucleobases, has been the subject of numerous fundamental studies aiming at shedding light on how this metal interacts with larger nucleic acid structures. Thus, a number of groups has previously published on the sodium binding affinities of the different nucleobases using theoretical calculations and different experimental methods. The first paper was published in 1996 by Blas and Wesdemiotis where a technique called Cooks’ kinetic method, along with fast atom bombardment (FAB) ionization tandem mass spectrometry, was used to measure the sodium binding affinities of the five DNA and RNA nucleobases. The dissociations of sodium bound heterodimers between the nucleobases and the amino acids glycine, alanine and valine, which served as the reference bases, were evaluated to deduce the sodium binding
affinities of uracil, thymine, adenine, cytosine and guanine, which were found to be 141, 144, 172, 177 and 182 kJ/mol, respectively.

A different experiment using threshold collision-induced dissociation (TCID)\textsuperscript{22} of sodium-nucleobase complexes came to the conclusion that the sodium binding affinities of uracil, thymine and adenine are 136, 136 and 143 kJ/mol, respectively.\textsuperscript{18} While the values for uracil and thymine compare well with the values obtained using Cooks’ kinetic method, the value for adenine is considerably lower than the one obtained using Cooks’ kinetic method.\textsuperscript{17, 18}

A study published more recently used electrospray ionization (ESI) mass spectrometry to produce sodium bound heterodimers between the various nucleobases and create a ladder of the relative sodium binding affinities based on the dissociations of these dimers.\textsuperscript{20} The relative affinities of the nucleobases were not anchored to find absolute experimental values. The ladder of relative sodium binding affinities was found to be: Gua > Cyt > Ade > 3MeAde > Thy > Ura. In addition, this study also used density functional theory (DFT) at the B3P86/6–31+G*//B3LYP/6–31G* basis level to calculate the most stable nucleobase-metal structures and corresponding sodium binding affinities.\textsuperscript{20}

In addition to these experimental approaches, several theoretical methods were used to calculate the sodium binding affinity of adenine.\textsuperscript{18-20} The structure of the neutral nucleobase and the binding site of sodium were shown to significantly influence the sodium binding affinity of adenine, seen in Figure 1.1.\textsuperscript{18, 19} At the MP2(full)/6-311+G(2d,2p) level of ab initio theory, the sodium binding affinity of adenine was predicted to have values of 139.9, 125.0 and 144.7 kJ/mol for Na\textsuperscript{+} at the N1, N3 and N7
The sodium ion binds more favorably to the imidazole nitrogen, N7, from where it can also interact with the amino group, forming a 5-membered ring complex. When the sodium binds at the N1 and N3 sites, less stable 4-membered rings are formed between the metal ion and the adenine molecule, causing a lower binding affinity. In a study done by Russo and co-workers, many different tautomers of the nucleobase were probed until the lowest three energy structures were found. The complexes between sodium and the three lowest energy tautomers were then calculated. At 29 K, using the B3LYP/6-311+G(2df,2p) level of theory, the sodium binding affinity of adenine was found to be 127, 204 and 238 kJ/mol, depending on the tautomer probed. Again the most energetically favorable complex was formed when the metal ion was bound between the N6 (amine group) and N7 atoms, forming a five-membered ring. The value of 204 kJ/mol is found when sodium binds between the N3 and N9 sites forming a four-membered ring. The highest energy value was calculated for a different tautomer of adenine where one of the amine hydrogen atoms on N6 is shifted to the N1 site, and the metal binds between the N6 and N7 sites.

![Figure 1.1. The structure of adenine with numbered atoms.](image-url)
In a different study, the most stable complexes of the nucleobase and metal were calculated to determine affinities, as opposed to first finding the most stable adenine tautomer and then complexing it to the metal.\textsuperscript{20} At the B3P86/6-31+G* //B3LYP/6-31G* level of theory, a value of 202 kJ/mol was determined for the sodium binding affinity of adenine if sodium binds between the N3 and N9 sites. This was reported to be the most stable complex at this particular level of theory.

In this dissertation, ESI MS is used, along with Cooks’ kinetic method, to provide an absolute experimental value for the sodium binding affinity of adenine. Previously, the exact binding site of the metal ion could not be determined experimentally. Here, by studying different derivatives of adenine, potential binding sites for the sodium ion can be blocked to narrow down the possible binding locations. In addition to re-evaluating the value for adenine, the sodium binding affinities of 3-methyl and 6-methyl adenine have been determined experimentally for the first time.

Whereas the first part of this dissertation deals with small biomolecules, the second part deals with the study of larger synthetic polymers. The first part of this dissertation involves experiments with instrumentation that is ideal for the study of small molecules, namely an ESI quadrupole ion trap (QiT). The second part of the dissertation employs additional types of instrumentation which are more suitable for larger molecules. Instrumentation choice is very important part for obtaining the best possible information about the sample analyzed. This dissertation describes the analysis of two differently sized samples, one of low molecular weight, described above, and a different set of analytes with molecular weights up to a few thousand Da.
The larger synthetic polymers examined in this dissertation include a copolymer of styrene and allyl alcohol in addition to poly(electrolytes). Mass spectrometry is essential to the analysis of polymers because it provides a wealth of composition and structure information. Other techniques may only solve a portion of the puzzle. Gel permeation chromatography (GPC)\textsuperscript{23} gives only molecular weight and polydispersity information about the polymer. Fourier Transform Infrared Spectroscopy (FTIR) can only give information on the functional groups which may be incorporated anywhere into the polymer.\textsuperscript{24} Nuclear magnetic resonance (NMR)\textsuperscript{23, 25, 26} can give information about the monomer and connectivity of the polymer, but mass spectrometry has the capability to furnish the combined data: it gives monomer information, end group identity and, when the polymer has a low polydispersity, molecular weight insight. All this can all be done in one analysis. In the polymer analysis experiments, a matrix-assisted laser desorption/ionization (MALDI) source was used to study higher molecular weight components of the polymers and was coupled to either a time-of-flight or quadrupole time-of-flight instrument (MALDI Q-ToF) and ESI QiT instrumentation was used to study the lower molecular weight region of the polymers. In addition to giving monomer composition of the copolymer and structure information about the polymer, mass spectrometry has the capability to determine degradation pathways of the polymer using a technique called tandem mass spectrometry (MS/MS). In MS/MS analyte ions are activated energetically by collisions with gaseous targets and dissociate. The resulting fragments provide structural information about the analyte and also reveal its intrinsically favored degradation pathways.\textsuperscript{27-30} This technique was applied to study the copolymer more in depth. The copolymer used in this study is used in coatings and inks to improve
hardness and gloss, corrosion and chemical resistance, adhesion properties and color acceptance.31

Poly(electrolytes) are a class of polymers which contain ionic or ionizable parts that are incorporated into the same polymer molecule, either at each of the monomer units or at the end groups. In solution, the charges dissociate from one another, creating negatively and positively charged species.32 These properties make it particularly interesting to study such compounds by mass spectrometry because they can be examined using both positive and negative mode. Under MS conditions, either an acidic proton or a cation can dissociate from the polymer to easily produce a negatively charged ion. For positive ions, a cation is added to the poly(electrolyte) or a cationizing agent is picked up from the environment, i.e. solvent or glass ware.

One of the reasons poly(electrolytes) have become so important recently is because they are used in polymer electrolyte fuel cells (PEFCs). This type of fuel cell is particularly important because it can provide energy immediately when needed and can be used at ambient temperatures, i.e. high temperature is not needed to run such fuel cells. The materials used in these PEFCs are a very important design characteristic of fuel cells. Currently, a highly fluorinated polymer with sulfonic acid side chains manufactured by DuPont under the name Nafion is the only commercially available poly(electrolyte) used in the design of PEFCs. Although this polymer has the proton conducting properties needed for PEFCs, it is very expensive.33 Therefore, the development of new polymeric materials for fuel cells is important, so PEFCs can become more affordable for commercial use. Polymers that contain a high level of sulfonic functional groups are suitable candidates.34, 35 They are less expensive than
Nafion, but still have the proton conducting properties in the sulfonated side chains of the polymer.36 Currently, sulfonated poly(etherketones) (PEEK) and poly(styrenes) (PS) are being tested for their use in PFECs.37 Thus far, poly(styrene sulfonate) (PSS) is used in combination with other polymers, such as poly(vinyl alcohol) (PVA), and has shown promise as a replacement for Nafion.38

Figure 1.2. Diagram of a polymer electrolyte fuel cell. The materials used for the membrane need to be developed to make more cost effective fuel cells for commercial use.
Figure 1.3. Nafion is currently used as the polyelectrolyte membrane in PEFC’s. The others are being developed for commercial use in PEFC’s. (a) Nafion (b) poly(etherketone) (c) poly(styrene) (d) poly(vinyl alcohol).

Development of novel poly(electrolytes) at an accelerated pace is expected, as fuel cells become widely used. Characterization methods for these new materials are needed to keep up with this development. Also information about the degradation pathways of poly(electrolytes) will be necessary. Preliminary MS studies about PSS have appeared that describe the analysis of higher molecular weight PSS.39, 40 This work describes the optimization of MS and MS/MS conditions for two important poly(electrolytes): PSS and poly(acrylic acid) (PAA); monomer and end group analysis, as well as degradation mechanisms are discussed.
CHAPTER II

INSTRUMENTAL METHODS

2.1 Mass Spectrometry Instrumentation

The function of a mass spectrometer is to separate ions based on their mass-to-charge ratio (m/z) from which the molecular weight of the analyte can be determined if the charge state is known. A mass spectrometer contains five main components used to find an ion’s m/z ratio: the inlet system, ionization source, mass analyzer, detector and data system. Most of the components involved in the mass spectrometer are kept under vacuum, and the diagram of this set up can be seen in Figure 2.1. The first component of the mass spectrometer, the inlet system, is a combination of syringes, lines, direct injection or solid sample introduction that allow the sample to be brought into the mass spectrometer. Once inside the mass spectrometer, the sample still needs to be ionized in order to be detected. The ionization source converts the sample into gas phase ions. Methods by which ionization can occur, will be discussed in this chapter. Once the ionized analytes are inside the mass spectrometer, they are accelerated towards the mass analyzer via a series of lens or voltage differences. The mass analyzer uses a variety of methods to separate the ions based on their m/z ratio before they are sent to the detector. The detector detects the ions and creates a signal with intensity that is proportional to the amount of each m/z value in the analyte mixture. The data system reads the signal sent
from the detector and creates the output, which is the mass spectrum. It is also the place where the information is stored and manipulated.

Figure 2.1. Basic mass spectrometry set-up. Most components are held under vacuum, but the source may also be at ambient pressure.
As mentioned previously, most parts of the mass spectrometer are under a vacuum. This is needed because ions, not neutral molecules are being analyzed and detected. Ions are very reactive intermediates that need to avoid all collisions, such as those with other ions or those with any part of the mass spectrometer. Mass analyzers and detectors are always contained under a vacuum. More recently, newer ionization techniques have been developed which do not require the inlet and ionization source to be contained under a vacuum.

Most of the variation within the field of mass spectrometry instrumentation comes from different ionization sources and mass analyzers. Therefore, the different ionization sources and mass analyzers used to complete this work will be discussed more in-depth in the following sections.

2.2 Ionization Sources

The formation of gaseous ions is an integral function of the mass spectrometer because only charged molecules can be studied using MS. Ionization techniques are classified as “hard” or “soft”. “Hard” ionization techniques are those which cause extensive fragmentation to the analyte molecule. When using a “hard” ionization technique, the molecular ion peak is rarely seen, or is present in very low abundance. Electron ionization (EI) is an example of a “hard” ionization technique where little of the intact analyte peak is observed. “Soft” ionization techniques allow for the intact molecular ion peak to be formed in the gas phase and subsequently get detected and observed in the mass spectrum. From this peak, the molecular weight of the analyte can be found, which may confirm analyte identification. Electrospray ionization (ESI) and
matrix-assisted laser desorption/ionization (MALDI) are two important “soft” ionization techniques that will be discussed. They have allowed the field of mass spectrometry to grow and be used for larger macromolecules including biomolecules and synthetic polymers.

2.2.1 Electrospray Ionization (ESI)

ESI is a “soft” ionization source that occurs at atmospheric pressure. Before the development of ESI, it was difficult to introduce large molecules into the gas phase for mass analysis due to their inability to vaporize easily. The only step involved in ESI sample preparation involves dissolving the sample in a suitable solvent. A suitable solvent in ESI is one that is polar, that can promote protonation or other type of ionization of the analyte, and most importantly, one that has low surface tension and can evaporate easily. Once dissolved, the analyte goes through the four main steps in the ESI process: ion formation, nebulization, droplet disintegration and ion evaporation, as shown schematically in Figure 2.2.

The process of ion formation usually occurs in solution, prior to sample introduction into the mass spectrometer. Usually polar, volatile solvents, such as methanol, are used to facilitate the ionization and desolvation processes. In order to give the sample a positive charge, salts or acids can be added to the solution. Buffers can also be added to protonate or de-protonate the sample. When ions are not formed in solution prior to spraying, they may be formed during the spraying step.
Figure 2.2. ESI process. (a) ESI process shown with nebulization gas.\textsuperscript{2} (Copyright permission in Appendix.) (b) Ion formation from solvated analyte.\textsuperscript{3} (Copyright permission in Appendix.)
After the sample is ionized, nebulization occurs, which is the process of breaking down droplets into smaller and smaller droplets. The dissolved sample is sprayed through the nebulizing needle, which is surrounded by a nebulizing gas, usually nitrogen. A combination of the shear forces from the nitrogen gas and a strong electric field of 2-6 kV create the forces necessary to form small, charged droplets. After nebulization, desolvation occurs, where the droplet shrinks and the charged analyte accumulates around the surface of the droplet. Solvents with low surface tension and a heating gas, such as nitrogen, can be used to make desolvation occur more easily.

The last step involved in ESI is ion evaporation, to form charged analyte molecules, which eventually enter the mass analyzer. This step is believed to accompany the “Coulombic Explosion” model. As the solvent evaporates and the droplets shrink, charge repulsion within the droplets increases, and the droplets rupture. When the droplets become small, the field on their surface becomes strong enough to cause ion desorption (ion evaporation) from the droplet surface. Because there are so many charges in one droplet, it is easy to form multiply charged ions in ESI especially when there are several ionizable sites located on the analyte molecule.

Interpretation of ESI mass spectra can be complicated due to the fact that more than one peak can arise for the same analyte molecule. In addition to multiply protonated ions, it is possible to see sodiated and potassiated molecules even when salts are not added to the analyte solution. However, ESI MS is a popular technique because it is easily interfaced with separation techniques, which can be used to analyze complicated samples with matrix interferences. Its high sensitivity is important when studying low concentrations, such as those encountered in biological samples.
2.2.2 Matrix-Assisted Laser Desorption/Ionization (MALDI)

MALDI is considered to be another “soft” ionization technique where the analyte ion remains intact and is detected in high abundance. In MALDI analysis, the analyte must be mixed together with a small organic compound, called the matrix. The purpose of the matrix molecule is to absorb the energy at the appropriate wavelength from the laser and help desorb the analyte from the surface of the target, usually a stainless steel plate.

In MALDI, matrix molecules need to be prepared in much higher concentration. Molar ratios of matrix to analyte should be approximately 1000-10000:1 matrix: analyte. A cationizing agent can be added in small amounts to the analyte/matrix mixture. Once the solutions of matrix, analyte and salt are mixed together, a small amount (~0.5 μL) is spotted onto the target plate. This is referred to as the dried droplet method. The solvent quickly evaporates, leaving a solid solution of analyte and matrix, and when needed, also the cationizing agent. The target plate is inserted into the mass spectrometer. The MALDI ionization source is usually contained in a vacuum.

The solid solution is then bombarded with a laser, usually a UV laser, such as a nitrogen or Nd:YAG laser. The matrix absorbs the energy from the laser, quickly heats and vaporizes, which desorbs itself and the analyte from the surface of the target, creating a plume. Proton transfer from the photoionized matrix molecules to the analyte molecules can occur during this step, however not every analyte molecule may be ionized. The process of the MALDI ionization can be seen in Figure 2.3.

One of the disadvantages in using MALDI as the ionization source is the inability to detect low mass ions. Matrix clusters can form, ionize and be detected in relatively
high abundance which shadows the possibility to see ions in the low mass region.

Typically MALDI is not used when looking at ions below 700 Da. MALDI is a very important technique for studying polymers and proteins because it can detect ions up to 1,500,000 Da. It is also employed in the developing field of imaging mass spectrometry.

Figure 2.3. MALDI process.\textsuperscript{42} (Permission is found in Appendix B.)
2.3 Mass Analyzers

After a sample has been introduced into the mass spectrometer and gaseous ions have been formed from the analyte mixture, the analyte ions are sent through the mass analyzer, to measure their mass-to-charge ratio. There are four main characteristics of a mass analyzer: upper mass limit, transmission, resolution and fragmentation stages. The upper mass limit gives the highest mass-to-charge ratio that can be measured. The transmission is a factor which gives a ratio of ions created in the source to the ions reaching the detector. Because ions are so reactive they can easily be neutralized if they hit the side of the instrument or another molecule. Resolution is the ability of the analyzer to distinguish mass differences between two adjacent peaks; it is defined by the equation: \[ R = \frac{m}{\Delta m} \], where \( m \) is the mass and \( \Delta m \) the peak width at half maximum. The last important characteristic of mass analyzers is the ability to use them in tandem mass spectrometry experiments. Depending on the analysis being performed, tandem mass spectrometry may be needed. The three types of mass analyzers used in this dissertation will be discussed in the following sections.

2.3.1 Quadrupole Ion Trap

There are three electrodes in a quadrupole ion trap. The ions enter the trap through the inlet end cap and leave the trap through the exit end cap. The third electrode is the ring electrode, located between the two end caps. A diagram of the quadrupole ion trap can be seen in Figure 2.4. The two end caps are grounded and an RF potential (\( \Omega \)) is applied to the ring electrode to trap the ions. When the rf potential is applied, the ions
move within the trap in a hyperbolic shape determined by the Matthieu equation, whose solution leads to the dimensionless parameter:

\[ q_o = q_z = -2q_r = \frac{8\varepsilon e V}{m(r_o^2 + 2z_o^2)\Omega^2} \]  

(2.1)

where V is the amplitude of the RF potential and r_o and z_o are the trap dimensions in radial and axial directions, respectively (see Figure 2.4). The ions only remain in the trap if they have q_o values less than 0.908. An additional requirement for keeping ions inside the trap is the presence of a cooling bath gas. Helium is usually introduced at a constant pressure of 3 x 10^{-3} mbar. The helium gas helps to dissipate the kinetic energy of the incoming ions, as well as keep the trapped ions from being accelerated by the repulsive forces of the other ions in close proximity to one another. The ions can be held inside the trap anywhere from 10 \mu s to 1 second.

In order to eject the ions from the trap, and subsequently mass analyze them, the main RF potential is gradually increased in order to destabilize ion trajectories (q_o > 0.908). Ions of increasing m/z are brought into unstable trajectories as V increases, so that they are ejected axially (in the z-direction) and strike the detector. Alternatively, an auxiliary RF potential may be applied to the end caps in order to excite the ions axially. When the rf field reaches that of the secular frequency of the ion (\omega), the ion is ejected in the z-direction out of the end cap, where it hits the detector. Ions are ejected with smaller m/z ratios are ejected first. This latter method is called resonant ejection, while the former method is known as mass selective ejection at the stability limit.
Figure 2.4. Quadrupole ion trap showing the end caps on either side of the ring electrode. The ions enter and exit through the end caps.\textsuperscript{43} (Permission is found in Appendix B).

In tandem mass spectrometry experiments, all ions, except one precursor ion are ejected out of the trap. Generally, resonant ejection is used for this purpose. In order to isolate the desired precursor ion, the instrument creates a wide range of rf frequencies so that only the precursor ion remains inside the trap because its secular frequency of is not matched.

After the precursor ion has been isolated in the trap, fragmentation can be induced by resonant excitation, which adds kinetic energy to the precursor ion, so that it fragments when it collides with the He buffer gas inside the trap. This is referred to as collision induced dissociation (CID) or collisionally activated dissociation (CAD). Once
the fragments are produced, they are ejected out of the trap by increasing the auxiliary RF potential before they are detected.

One of the advantages of a (QiT) is the ability to perform MS^n studies, where additional tandem mass spectrometry experiments can be done on individual fragment ions. In practice, more than MS^3 is rarely feasible.

2.2.3 Time of Flight (ToF) Mass Analyzer

Before mass analysis by a ToF analyzer, all ions in the ionization step are accelerated by a potential, V, which gives them the same kinetic energy. The ions then travel through a field-free drift tube of a specific length, L, which separates them based on their mass-to-charge ratio. The time required for each ion to reach the detector is measured and from that the mass-to-charge ratio is calculated to create the mass spectrum. As mentioned, all ions leaving the source have the same kinetic energy.

Kinetic energy is related to mass by the equation

\[ \frac{mv^2}{2} = qV = zeV = K \]  

(2.2)

Hence, ions with a smaller m/z have a higher velocity and thus reach the detector first. Those with a higher m/z have a lower velocity and hit the detector last. Time is the actual variable being measured, which relates to mass by the following equation:

\[ t^2 = \left( \frac{m}{z} \right) \frac{d^2}{2eV} \]  

(2.3)

The ToF analyzer theoretically has no mass limit and, therefore, it is ideal for studying high molecular weight analytes, including polymers. High ion transmission is another advantage of the ToF analyzer. Only a few ions will hit the side of the mass spectrometer and neutralize before hitting the detector. Typical ToF mass spectrometers
are schematically illustrated in Figure 2.5. There are two types of ToF analyzers, linear and reflectron. The process described above is for a linear ToF mass analyzer, shown in Figure 2.5.a. A reflectron ToF analyzer is described in the following paragraphs.

In MALDI-ToF mass spectrometers, all ions may not be ionized at the same location, giving different ions a different drift tube length to travel, depending on where they were ionized. This means that ions of the same m/z may not be detected at the same time, giving them different mass-to-charge ratios in the mass spectrum. These slight differences in drift time can decrease the resolution of the mass spectrum. There are two main developments that help bring those ions with different initial kinetic energies or ionization locations but same m/z, back together to produce the same m/z in the mass spectrum. They are pulsed ion extraction (PIE), also called delayed extraction, and the use of a reflecting mirror.
In delayed extraction, the accelerating voltage, $V_1$, is applied to the ions leaving the MALDI source a short time after the laser pulse. During this delay time, the ions drift in a field-free region ($V_1 = V_2$ in Figure 2.6). After delay time, $V_2$ is dropped or $V_1$ is increased to pulse the ions out of the source and accelerate them to travel through the ToF drift tube, see Figure 2.6. The ions with a smaller initial kinetic energy “feel” more of the accelerating voltage ($V_1$) to catch up to the ions with a higher initial velocity. This allows ions of different initial kinetic energies to reach the detector at the same time. PIE
works best for smaller mass ranges. Resolution can be improved for specific mass ranges by adjusting the delay time prior to the accelerating pulse.

Figure 2.6. Theory of PIE. During the delay time, the potentials $V_1$ and $V_2$ are set the same, to briefly collect the ions before they are accelerated through the mass analyzer.\textsuperscript{45} (Permission is found in Appendix B.)

The second adjustment made to ToF analyzers to improve their resolution involves the use of reflecting mirrors. Again, ions with different initial kinetic energies are brought together in order to be detected at the same time. The ions enter the reflecting mirror after traveling through the first field free drift tube, which causes them to decelerate until they stop. Ions of different initial kinetic energies penetrate the reflectron to different depths. They are then repelled out of the reflectron and leave with the same velocity with which they enter. Ions with a higher kinetic energy travel faster and spend less time in the first field free drift region. However, this also causes them to travel deeper into the reflectron spending more time in the reflectron. The reflectron brings ions with different initial velocities and same mass-to-charge ratios to the detector.
at approximately the same time, improving resolution. Resolution can be further improved by adding more than one reflectron to the instrument.

2.2.4 Quadrupole-Time-of-Flight (Q-ToF) Tandem Mass Analyzer

The Q-ToF mass spectrometer is an analyzer that combines a quadrupole mass analyzer with a time-of-flight mass analyzer in order to perform tandem mass spectrometry experiments. Ions first travel through the quadrupole mass filter, which is a set of four parallel rods to which rf and dc voltages are applied. The dc and rf voltages can be set to select ions of only a certain m/z to pass through; at the same time, the rf voltage focuses the ions to the center of the quadrupole and allows them to travel with a constant axial velocity. Once they are through the quadrupole analyzer, they are sent into a hexapole collision cell where collisionally activated dissociation (CAD) with argon atoms produces fragment ions. These fragment ions then travel through an orthogonal ToF tube, discussed previously, where they are mass-analyzed and subsequently detected. This mode of operation is used to perform MS/MS experiments. For MS experiments, only an rf field is applied to the quadrupole, so that it transmits all ions coming from the ion source to the ToF mass analyzer.
CHAPTER III
MATERIALS, METHODS AND INSTRUMENTATION

3.1 Kinetic Method

The materials used for the Kinetic Method study were purchased from Sigma Aldrich Corp. (Milwaukee, WI). They were used in the condition received and without further purification. Ade, its derivatives, the amino acids and acetamides were all purchased from Sigma. The HPLC grade solvents used for ESI experiments, methanol and water, were also purchased from Sigma Aldrich and used without purification.

3.1.1 Polymer Materials

The polymer materials investigated in this dissertation were obtained from a variety of sources. The matrix dithranol, was obtained from Alpha Aesar. The other matrices, including dihydroxybenzoic acid (DHB), alpha-cyano-4-hydroxycinnamic acid (α-CHCA), 2-(4-hydroxyphenylazo)-benzoic acid (HABA), 2’,4’,6’-trihydroxyacetophenone monohydrate (THAP) and sinapic acid (SA) were obtained from Sigma Aldrich. The solvents and cationizing agents used in this study, silver trifluoroacetate and sodium trifluoroacetate, were obtained from Sigma Aldrich.

There were three different copolymers containing styrene and allyl alcohol monomer units studied in this work, which were obtained from Equistar Lyondell. They
each contain different monomer compositions, different molecular weights and different polydispersities. A summary of the three samples used is shown in Table 3.1.

Table 3.1. Summary of Samples Studied for Copolymer Analysis Containing Styrene and Allyl Alcohol Monomers

<table>
<thead>
<tr>
<th>Product</th>
<th>$M_w$</th>
<th>$M_N$</th>
<th>Weight % Styrene</th>
<th>Weight % Allyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA-100</td>
<td>3400</td>
<td>1300</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>SAA-101</td>
<td>2200</td>
<td>1100</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>SAA-103</td>
<td>7100</td>
<td>2800</td>
<td>87</td>
<td>13</td>
</tr>
</tbody>
</table>

The poly(electrolytes) were supplied by two different sources. Poly(acrylic acid) (PAA) was purchased from Sigma-Aldrich and had a $M_w$ of ~ 1800 Da. End groups and polydispersity were not specified. Poly(styrene sulfonated sodium salt) (PSS) with an $M_w$ of ~ 1100 Da was obtained from Polymer Standards Service-USA.

3.2 Methods

This section describes the theory behind the kinetic method as well as the sample preparation steps involved in the kinetic method and polymer projects.
3.2.1 Kinetic Method Theory

In the kinetic method experiments, a sodium bound heterodimer was formed between adenine (or its derivative), Ade, and a base molecule of known sodium binding affinity, Bi. These heterodimers were then isolated in the ion trap and fragmented using collisionally activated dissociation (CAD) which gives either the sodiated monomer of adenine or the sodiated base molecule. The intensity ratio of the fragment ions from the heterodimer gives an approximate measure of the rate constant ratio of the competitive dissociations seen in equation (3.1); the latter ratio is related to the relative sodium binding affinity between adenine (or its derivative) and the reference base Bi.

\[
\text{Ade} - \text{Na}^+ + \text{Bi} \xleftarrow{k_i} \text{Ade} - \text{Na} - \text{Bi} \xrightarrow{k_i} \text{Ade} + \text{Na} - \text{Bi}^+ \tag{3.1}
\]

The absolute sodium binding affinity of adenine can be obtained by anchoring the experimentally measured relative affinities to those of the reference bases, Bi. The kinetic method assumes that other pathways dissociations are negligible besides formation of the metalated monomers, which is validated by the experimental results. Dissociation occurs by breaking electrostatic bonds; therefore, reverse reactions are very unlikely activation energies. In such a case, the dissociation rates of the heterodimer ion depend on thermodynamic bond dissociation enthalpies (“binding affinities”), as given by equation 3.2, where \( \Delta H_{\text{Na}} \) is the enthalpy needed to dissociate sodium from adenine or the base molecule.

\[
\ln \frac{k_i}{k_i} \approx \frac{\Delta H_{\text{Na}}(\text{Ade})}{RT_{\text{eff}}} - \frac{\Delta H_{\text{Na}}(\text{Bi})}{RT_{\text{eff}}} = \frac{\Delta(\Delta H_{\text{Na}})}{RT_{\text{eff}}} \tag{3.2}
\]

This relationship assumes that the relative entropies of the competing pathways are similar. Equation 2 relates an intensity ratio to a relative sodium binding affinity.
is the ideal gas constant and $T_{\text{eff}}$ is the effective temperature of the dissociating heterodimers (a measure of excess energy).50

A plot of the known sodium binding affinities of Bi, $\Delta H_{Na}(Bi)$, on the x-axis and the $\ln(k/k_i)$ values on the y-axis gives, according to the equation (3.2), a regression line with a slope of $1/RT_{\text{eff}}$. The value for the absolute sodium binding affinity of Ade, $\Delta H_{Na}(Ade)$, is where the line passes through x-axis (x-intercept).

3.2.2 Kinetic Method Preparation

In the kinetic method experiments, each analyte was dissolved in a 2:1 water: methanol mixture to a concentration of 1 mg/mL. The solutions of the base molecule with which adenine was prepared, sodium trifluoroacetate and adenine, or its derivative, were mixed in a ratio of 1:1:1. The final mixture was then introduced into the ESI mass spectrometer by a Cole-Palmer automatic syringe with a flow rate of 250 $\mu$L/hr. The mass spectrometer used was a Bruker Esquire LC (Bruker Daltonics, Billerica, MA). The sample was introduced into the instrument using a grounded needle spraying orthogonally to the vacuum interface. Calculations were done using excel.

3.2.3 Polymer Analysis Preparation

In order to prepare the copolymer for MALDI MS analysis, it was dissolved in THF to a concentration of 10 mg/mL. The matrix, dithranol, and cationizing agent, silver trifluoroacetate, were also dissolved in THF to concentrations of 20 and 10 mg/mL, respectively. The solutions of the sample, matrix and ionizing agent were then mixed together in a ratio of 4:10:1, respectively. The mixture was applied to the target using the
dried droplet method, prior to MALDI MS analysis using the Bruker Reflex III mass spectrometer.

A cationizing agent was not needed in the MALDI experiments of the poly(electrolytes). The polymers were investigated in positive as well as negative mode, and in neither case was a cationizing agent needed. In positive mode, the polymers had enough oxygen atoms to pick up sodium from the glassware; the identity of the sodiated ions did not change appreciably by addition of a Na\(^+\) salt. When looking at negative mode, the styrene-SO\(_3\)Na side chains of PSS easily form anions in solution, and the poly(acrylic acid) sample deprotonates at the carboxylic group. DHB and α-CHCA were the matrices of choice for these poly(electrolytes) in positive and negative mode, respectively. Both matrices were dissolved in THF to a concentration of 20 mg/mL, and the polymer samples were dissolved in water to a concentration of 10 mg/mL. The two solutions were mixed in a ratio of 10:2 matrix:sample for the optimum signal. 0.7 μL were spotted on a stainless steel target and introduced in the Waters Ultima Q-ToF mass spectrometer for MS and MS/MS analysis. For ESI mass spectra, the poly(electrolytes) in the ESI, they were dissolved in water to a concentration of 1.0 mg/mL, and a small amount of methanol was added before introducing the sample into the instrument using an automatic syringe pump at a rate of 250 μL/hr. Again, positive and negative ion spectra were acquired.

3.3 Instrumentation

The running conditions and the settings of the instruments play an important role in the experiments and are described in the following sections.
3.3.1 Bruker Esquire LC- ESI QiT Mass Spectrometer

All electrospray ionization mass spectra were obtained with a Bruker Daltonics Esquire LC mass spectrometer, which has a quadrupole ion trap mass analyzer (Figure 3.1.).

Figure 3.1. Diagram of the Bruker Esquire LC MS.\textsuperscript{43} (Permission is found in Appendix B.)

As mentioned previously, the samples are injected into the mass spectrometer via an automatic syringe pump at a rate of 250 $\mu$L/hr and travel through a metal tube into the stainless steel needle, which is grounded. As the sample flows through the needle, it is surrounded by a nebulizing gas, which in this case is nitrogen. The nebulizing gas flows concentrically at a pressure of 10 psi in these experiments and aids in forming tiny droplets from the sample solution, which contain the ionized analyte. The ions are formed at atmospheric pressure and are attracted towards the entrance of the capillary by
a -4kV potential difference. The entrance of the capillary is orthogonal to the grounded needle. This potential difference and orthogonal geometry help prevent solvent and neutral molecules from entering the capillary, which eliminates noise in the mass spectrum. The ionized droplets enter the capillary tube with the help of the drying gas, which helps to desolvate the analyte droplets with a flow rate of 8 L/min. In polymer analysis samples, the temperature of the drying gas is set to 300ºC. However, in kinetic method experiments, a temperature of 100ºC is used in order to keep the heterodimer ions intact.

The capillary tube brings the ions from atmospheric pressure of the ion source to the vacuum stage of the mass spectrometer. The entrance of the capillary tube is set to a higher potential, which allows the ions to flow through the 1mm diameter capillary toward the trap. The ions travel through the capillary by the pressure and voltage differences between its end caps and enter the skimmer region, see Figure 3.1. There are two skimmers in the skimmer region and each consecutive skimmer is set to a lower voltage to accelerate the ions through the beginning vacuum stages to the mass analyzer. The space between the skimmers is pumped to remove the final drying gas, solvent and neutral molecules from the ion beam before the beam enters the octopole. The ions are focused in the skimmer region as well as by the octopole and two subsequent lenses before entering the trap.

The ions enter the QiT through small holes in the entrance cap. They get trapped and accumulate for a set amount of time, usually 20 ms. In order for the ions to remain inside the trap, a buffer gas, typically helium, is needed to cool the trapped ions and prevent them from colliding with each other. The buffer gas also helps the ions to remain
in the center of the trap. Once a reasonable number of ions has been trapped, the RF voltage on the ring electrode can be ramped up so the ions can be ejected out of the trap one at a time, removing those with a larger m/z first. The trap is also the place where certain ions can be isolated by using resonant ejection with the help of an auxiliary RF field at the end caps, as explained in chapter II. Once isolated, a precursor ion can be subjected to collision induced dissociation (CID) with the helium gas for fragmentation.

After leaving the trap and before striking the detector, the ions must first pass through a protective skimmer, which prevents too many ions from hitting the detector during certain times of MS and MS/MS analysis. This protective skimmer helps reduce the amount of noise in the mass spectrum. The detector used in this instrument is a continuous dynode electron multiplier, and has the shape shown in Figure 3.2. An ion with a positive charge hits a negative conversion dynode from which secondary electrons are emitted and strike the entrance of a curved electron multiplier. The shape of the multiplier allows for continuous amplification of electrons, which also amplifies the peak intensities in the mass spectrum. The amplified electrons reaching the end of the multiplier produce a measurable current read by an electrometer. An ion of negative charge hits a positive conversion dynode, ejecting secondary electrons that strike the entrance of the electron multiplier, from where they continue a similar path as the electrons emitted from the negative conversion dynode.
3.3.2. Bruker Reflex III MALDI ToF Mass Spectrometer

A schematic of the MALDI ToF mass spectrometer, manufactured by Bruker (Billerica, MA), can be seen in Figure 3.3. The MALDI source described in chapter II, utilizes a pulsed nitrogen laser with a pulse width of 3 ns, a frequency of 10 Hz and a wavelength of 337 nm. This instrument uses a variable attenuator to control precisely the percentage of laser fluence hitting the target plate. Once through the attenuator, the laser beam travels through an iris, a set of lenses and finally through another iris. The lenses focus the beam into a narrow, more concentrated laser beam. The irises control the size of the laser spot hitting the target by increasing or decreasing the circumference of the beam. The size of the laser hitting the target is important because it can affect the sensitivity of the instrument. The goal is to allow the highest number of ions to be desorbed off the surface of the target from the smallest area possible. Once the laser has been adjusted properly, it enters the source and hits the target after being deflectected off by a small mirror.

Figure 3.2. Diagram of a continuous dynode electron multiplier, also known as channeltron. Permission is found in Appendix B.)
When the laser hits the target (source electrode), a plume of matrix, analyte and ionizing species (if added to the sample) is formed above the surface of the target. As explained in Chapter II, pulsed ion extraction is generally used to improve the resolution of the instrument. Whereas PIE focuses ions in the z direction (direction of pulsed beam path), other lenses focus the packet of ions, which has gone through PIE, in the x-y plane. Further, an orthogonal deflector is available to push unwanted ions out of the z direction. A 2-kV potential can be applied to the deflector and is usually turned on for only a short period to remove low-mass matrix ions from the beam. The potential is turned off to allow the ions of interest to enter the ToF tube. Then this focused packet of ions, which

---

Figure 3.3. Diagram of Bruker Reflex III MALDI ToF MS.\textsuperscript{51}
has gone through PIE, focusing lenses and the deflector is sent into the field free region of the drift tube, where mass dispersion occurs as discussed in detail in chapter II.

Once through the ToF mass analyzer, the ions travel to the detector, which is a multi-channel plate (MCP). The MCP detector is an electron multiplier detector which multiplies the signal of the entering ions. (Figure 3.4) The MCP detector is positioned orthogonally to the ion beam. It consists of a series of parallel cylindrical channeltrons. MCP detectors are frequently used for ToF instruments because their surface area can accommodate the wide spatial distribution of ions exiting the analyzer. When an ion hits the semi-conducting surface of the plate, secondary electrons are produced which are multiplied in the microchannels. And this produces a signal which has been intensified by a factor of $10^5$ and, if two connected plates are used, by as much as $10^8$. These signals are then sent to the SUN station for data manipulation.

Figure 3.4. Diagram of a MCP detector. (Permission is found in Appendix B.)
3.3.3. MALDI Q-ToF Mass Spectrometry

A MALDI Q-ToF mass spectrometer from Waters/Micromass (Manchester, UK) was used when MS/MS experiments were needed, which cannot be performed on a Bruker Reflex III instrument. A diagram of the MALDI Q-ToF mass spectrometer can be seen in Figure 3.5. Again, a nitrogen laser is used to desorb the sample from the target plate. However, in this instrument, there is no attenuating wheel, so the entire laser power hits the target plate.

In MS mode, the quadrupole is used in RF only mode to transmit all ions produced upon MALDI. However, in MS/MS mode, the quadrupole is utilized to mass select the precursor ion and transport only this ion into the collision cell. The collision cell is filled with argon gas and used to fragment the precursor ion via collisions with the neutral gas. After the fragments are formed, they are orthogonally accelerated into the ToF mass analyzer where they can travel through the tube in either a V or W path before they are detected. The W mode allows for a longer ToF path, which increases the resolution in the mass spectrum. All experiments run utilized the V mode of the instrument. An MCP detector is used to detect the ions exiting the ToF analyzer.
Figure 3.5. Diagram of the Waters Ultima QToF mass spectrometer\textsuperscript{52} (Permission is found in Appendix B.)
CHAPTER IV
KINETIC METHOD RESULTS

4.1 Background

Two different sets of reference bases (B_i) were used to find the sodium binding affinities of adenine and its derivatives. The first set of reference bases (base set 1, BS1), includes the amino acids: glycine, alanine, valine, serine, proline, tyrosine, tryptophan, glutamine, histidine and the dipeptide, glycine-leucine with sodium binding affinities of 161, 167, 173, 192, 196, 201, 210, 222, 228 and 212 kJ/mol, respectively.\textsuperscript{53, 54} These values are all experimental and were obtained by the kinetic method in studies aiming at creating a ladder of relative sodium binding affinities.\textsuperscript{53} The second set of reference bases (BS2) includes dimethylformamide (DMF), methylacetamide (MA), dimethylacetamide (DMA) and pyridazine (P) whose sodium binding affinities are 157, 158, 163 and 159 kJ/mol respectively.\textsuperscript{55, 56} The values for BS2 were all obtained using theoretical calculations. All three molecules studied, adenine (Ade), 3-methyladenine (3-MeAde) and N6-methylaminopurine (N6-MeAP), which can be seen in Figure 4.1, were paired with both base sets.
Figure 4.1. Structures of Ade. (a) Ade (b) 3-MeAde (c) N6-MeAP.

4.2 Adenine

The specific base molecules used to obtain $\Delta H_{Na}(Ade)$ include Tyr, Trp, and GlyLeu, with which Na$^+$-bound dimers with Ade could be formed. Many other reference bases were probed, but would not form Na$^+$-bound dimers with Ade. Therefore, each experiment was done in triplicates at multiple collision energies. An example of the mass spectrum and CAD spectrum of the mixture between Ade, Na salt and Trp is shown in Figure 4.2. In the mass spectrum, the most intense peak is for sodiated Trp. Note the low intensity of the heterodimer in the mass spectrum, indicating the difficulty of forming such heterodimers, even under conditions of lower drying gas temperature. This was also the case for all other heterodimers used in the kinetic method experiments. Despite the low intensity of the heterodimer, it could still be isolated and fragmented, and the tandem mass spectrum shows dominant peaks from the dissociation of the heterodimer to either Ade-Na$^+$ or Trp-Na$^+$. This dissociation behavior indicates that the ligands are weakly bound to the central metal ion, which is a requirement of the kinetic method. The
fragment peak present in the CAD spectrum with higher intensity results from the molecule with higher sodium binding affinity. In this case, Trp has a slightly higher sodium binding affinity than Ade.

![Figure 4.2. Mixture between Trp, Na trifluoroacetate and Ade. (a) ESI mass spectrum of the mixture between Trp, Na trifluoroacetate and Ade (b) CAD spectrum of the sodium bound heterodimer containing Ade and Trp (m/z 361.1).](image)

This experiment was repeated using Tyr and GlyLeu as the base molecules within BS1 to determine $\Delta H_{\text{Na}}(\text{Ade})$. Although all of the amino acids were probed as reference base molecules, only GlyLeu, Trp and Tyr produced heterodimers that were intense enough to be isolated and fragmented. This base set is symbolized by AA. The natural
logarithm of the intensity ratio of the sodiated peaks in the CAD spectra was is related to the relative affinity of the dimer ligands, as explained in Chapter III (see equation 3.1). The intensity ratio is a measure of the corresponding rate constant ratio, $k_{Ade}/k_{AA}$, of the dissociations leading to sodiated Ade ($k_{Ade}$) and sodiated AA ($k_{AA}$). The $\ln(k_{Ade}/k_{AA})$ values for AA = Tyr, Trp and GlyLeu are plotted versus the known sodium binding affinities of the AA molecules in Figure 4.3. The points on the graph lead to a regression line with the equation: $y = -0.3466x + 72.155$. The $x$-intercept of this equation, gives a sodium binding affinity for Ade of 208 kJ/mol. Combining standard deviation of the $x$-intercept ($\pm 1.2$ kJ/mol) with the uncertainty in $\Delta H_{Na}(AA)$ ($\pm 8$ kJ/mol)$^{53,54}$ gives an overall error limit of $\pm 9.2$ kJ/mol.

Figure 4.3. Regression line resulting from a plot of $\ln(k_{Ade}/k_{AA})$ vs. sodium binding affinity of AA. The base molecules used to probe this line were Tyr, Trp and GlyLeu with sodium binding affinities of 201, 210 and 212 kJ/mol, respectively.
Since BS1 gave a higher value for $\Delta H_{Na}(Ade)$ compared to previous experimental values,\textsuperscript{17,18} a different set of base molecules was employed to probe the lower affinity found by others. This time BS2, a base set with lower binding affinities was used, which should be more suitable for probing a lower affinity.\textsuperscript{21} The BS2 members have sodium binding affinities that range from 156.7 to 162.6 kJ/mol. Adenine formed heterodimers with all of the molecules in BS2. The corresponding spectra give rise to a regression line with the equation: $y = -0.4107x + 68.121$ (Figure 4.4). This equation yields a sodium binding affinity of adenine of 166 (± 1.3) kJ/mol.

![Figure 4.4](image-url)

Figure 4.4. Regression line of the $\ln(k_{Ade}/k_{B_i})$ vs. sodium binding affinity of $B_i$. The $B_i$ molecules used in these experiments were DMF, MA, P and DMA with sodium binding affinities of 157, 158, 159 and 163 kJ/mol, respectively.

In order to better understand the results from the current study, it is helpful to summarize and compare the previous results. The first experimental data were reported by Cerda and Wesdemiotis who found a value of 172 (± 4) kJ/mol, when Ade was paired
with Gly, Ala and Val using Cooks’ kinetic method and a tandem mass spectrometer with a FAB (fast atom bombardment) source and EiBE2 geometry. Rodgers and Armentrout used the threshold collision-induced dissociation method to derive an experimental value of 139.6 (± 4.2) kJ/mol. In addition to these experimental values, computational predictions for the sodium binding affinity of adenine also exist. The predicted values range from 124 – 202 kJ/mol depending on the level of theory used and the temperature of the Ade-Na+ complex (typically either 0 K or 298 K). The current study has found experimental values of 166 kJ/mol using BS2, and 208 kJ/mol using BS1. These differences can be explained by looking at the metal-adenine tautomer probed. When sodium binds between the N3 and N9 sites of adenine, theoretical binding affinities of 201 and 202 kJ/mol are predicted. This structure is most likely probed in the heterodimer with BS1 which yield an experimental affinity of 208 kJ/mol. Conversely, when sodium binds between the N6 and N7 sites on adenine, theoretical values between 129 and 132 kJ/mol have been found. Therefore a mixture of tautomers is most likely being probed when a value of 166 kJ/mol is found with BS2. When using BS2, the sodium atom is binding between the N3 and N9 sites in ~50% of the heterodimers, and between the N6 and N7 sites in the remaining ~50% of the heterodimers, giving a ΔH_{Na}(Ade) of 166 kJ/mol.

4.3 3-Methyladenine

For more information about the binding site of sodium in Ade, 3-MeAde was investigated. In adenine derivatives, potential binding sites of the sodium ion can systematically be blocked. By adding a methyl group to the N3 site on adenine, the
sodium ion is unable to bind at the N3 position and it is forced to attach elsewhere. The expectation is that a lower sodium binding affinity will be found because the sodium will now bind between the N6 and N7 site, which had a theoretical value that was much lower than the N3/N9 binding site.\textsuperscript{18-20}

With BS1, which mainly contains amino acids, 3-MeAde did not form heterodimers with the same amino acids as Ade. Already this is an indication that Ade and 3-MeAde do not bind Na\textsuperscript{+} at the same location. The BS1 molecules used to probe 3-MeAde were Gly, Ala, Val, Ser and Pro that have sodium binding affinities in the range 161 – 196 kJ/mol. An example of the mass and CAD spectra from a mixture of 3-MeAde and Val can be seen in Figure 4.5. The dissociations of heterodimers between 3-MeAde and these amino acids render a regression line of $y = -0.2237x + 38.98$ with an $r^2$ value of 0.997, which gives a sodium binding affinity of 174 (±1.0) kJ/mol, see Figure 4.6. When compared to the amino acids, Ade, on the other hand, had a sodium binding affinity of 208 (± 9.2) kJ/mol. Thus, by blocking the N3 site on Ade, which has a calculated sodium binding affinity of 202 kJ/mol, the sodium binding affinity of Ade decreases. Since N3 is not available, the sodium ion must bind at a different site, namely between the N6 and N7 atoms, where the sodium binding affinity is indeed predicted to be lower.
Figure 4.5. Example of the mass and CAD spectra from a mixture of 3-MeAde and Val. (a) ESI mass spectrum of a mixture of 3-MeAde, Val and Na trifluoroacetate (b) CAD spectrum showing the sodium bound heterodimer between Val and 3-MeAde (m/z 289.1).

Figure 4.6. Regression line resulting from heterodimers containing 3-MeAde and one reference base from set BS1.
In order to more accurately compare the sodium binding affinity of Ade and its derivative, 3-MeAde, BS2 was also used with 3-MeAde. In the tandem mass spectra of all heterodimers with a BS2 molecule, the peak of sodiated 3-MeAde is always lower in intensity, suggesting that 3-MeAde has a lower sodium binding affinity than every BS2 molecule used. The CAD spectrum with DMF, which has the lowest sodium binding affinity, can be seen in Figure 4.7. Using BS2, a regression line with the equation \( y = -0.3949x + 60.857 \) is obtained, which can be seen in Figure 4.8. This regression line provides a value of 154 (± 0.9) kJ/mol for the sodium binding affinity of 3-methyladenine, compared to the value of 174 kJ/mol found when amino acids served as the reference bases.

![CAD spectrum of the Na\(^+\)-bound heterodimer between 3-MeAde and DMF. 3-MeAde must bind with a structure that has a lower sodium binding affinity than DMF.](image-url)
Figure 4.8. Regression line obtained from heterodimers between 3-MeAde and a BS2 molecule.

The data show that there are two different values for the sodium binding affinity of both Ade (166 and 210 kJ/mol), as well as 3-MeAde (154 and 175 kJ/mol). It is interesting to note that consistently each molecule binds sodium more strongly if it is paired with BS1. In order to unveil the cause of this phenomenon, a second derivative of Ade was studied, N6-MeAP, whose structure can be seen in Figure 4.1. In this derivative, the N6 binding site is now blocked; hence, this molecule should have the highest sodium binding affinity according to the theoretical predictions.

4.4 N6-MeAP

The BS1 reference bases used to deduce the sodium binding affinity of N6-MeAP were those of higher sodium binding affinity, namely Trp, Gln, and His with sodium binding affinities of 210, 222 and 228 kJ/mol, respectively. Other amino acids were probed, but would not form a heterodimer with N6-MeAP. Plotting the $\ln(k/k_i)$, the

\[
y = -0.3949x + 60.857
\]

$R^2 = 0.9307$
amino acids mentioned previously against $\Delta H_{\text{Na}}(B_i)$ created a line with the equation $y = 0.2085x - 44.874$, as seen in Figure 4.9. From the x-intercept of this equation, a value of 215 (± 2.3) kJ/mol is derived for the sodium binding affinity of 6-methylaminopurine; this is 5 kJ/mol higher than that of adenine and 29 kJ/mol higher than that of 3-methyladenine, when using the same base set.

With BS2, heterodimers were formed in much lower abundance than previous experiments. Heterodimers that could be fragmented did not show any sodiated BS2 base in their CAD spectra. Only the sodiated N6-MeAP peak was observed. In the CAD spectra where methylacetamide and dimethylformamide were the base molecules, a peak representing the loss of water was observed in addition to the sodiated N6-MeAP peak, but no sodiated base molecule was present (see Figure 4.10). When the relative intensities of the sodiated fragment ions can not be compared, a sodium binding affinity can not be measured. The base molecule with the highest sodium binding affinity in BS2 was dimethylacetamide, with a value of 163 kJ/mol. Using this information, it can only be concluded that N6-MeAP has a sodium binding affinity that is much higher than 163 kJ/mol.
Figure 4.9. Regression line obtained from heterodimers between N6-MeAP and a BS1 molecule.

\[ y = 0.2085x - 44.874 \]
\[ R^2 = 0.9706 \]

Figure 4.10. CAD spectrum of the Na\(^+\)-bound heterodimer between N6-MeAP and DMA. N6-MeAP must bind with a structure that has a much higher sodium binding affinity than DMA.
4.5 Kinetic Method Conclusions

A summary of results is presented in Table 4.1, which shows the sodium binding affinities of Ade and its derivatives deduced by using two different base sets. When compared to BS1 (amino acids and a peptide), Ade and 3-MeAde had a higher sodium binding affinity. In the case of N6-MeAP, no conclusion could be made because the CAD spectra only contained sodiated N6-MeAP peaks. The experimental results confirm the theoretical prediction that the N3 site of Ade should have a higher sodium binding affinity. The difference in sodium binding affinities using two different base sets for the same molecule indicate that different structures of sodiated Ade (or 3-MeAde) are probed with the two different reference base sets. The exact reason for this observation remains unclear. It is possible that the formation of the less stable complexes (those with lower affinities) is kinetically favored. Finally it is worth noting that Ade was also compared to 3-MeAde and N6-MeAP; the CAD spectra revealed that Ade did have a higher sodium binding affinity than 3-MeAde, but a lower affinity when compared to N6-MeAP (Figure 4.11).

<table>
<thead>
<tr>
<th></th>
<th>Ade (kJ/mol)</th>
<th>3-MeAde (kJ/mol)</th>
<th>N6-MeAP (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS 1</td>
<td>208 ± 1.2</td>
<td>175 ± 1.0</td>
<td>215 ± 2.3</td>
</tr>
<tr>
<td>BS 2</td>
<td>165 ± 1.3</td>
<td>154 ± 0.9</td>
<td>n.d.*</td>
</tr>
</tbody>
</table>

Table 4.1. Summary of Kinetic Method Results
Summary of the results from Cooks’ Kinetic Method experiments with Na+-bound dimers of Ade (or its derivatives) and two different reference base sets. *n.d. – could not be determined.
Figure 4.11. CAD spectra of the heterodimer between Ade and its derivatives. (a) CAD spectrum of the Na$^+$-bound heterodimer between Ade and 3-MeAde. Ade binds with a structure that has a much higher sodium binding affinity than 3-MeAde (b) CAD spectrum of the Na$^+$-bound heterodimer between Ade and N6-MeAP. N6-MeAP binds with a structure that has a higher sodium binding affinity.
5.1 MALDI-ToF Mass Spectrometry of Copolymers

The analysis of copolymers using mass spectrometry can give information regarding monomer composition. This particular study looked at the copolymer between styrene (S) and allyl alcohol (A) monomers which have repeat units of 104 and 58 Da, respectively. The MALDI-ToF mass spectrum, shown in Figure 5.1, obtained using the Bruker Reflex III mass spectrometer shows peaks at every 10 or 12 mass units. From any peak in the mass spectrum, another peak is observed both at +58 Da and -58 Da. For example, when starting the peak at m/z 1113.4, a peak is observed at m/z 1171.5 (58 Da higher) as well as at m/z 1055.3 (58 Da lower; see Figure 5.1b) confirming that the polymer does contain the allyl alcohol repeat unit. Taking this same peak, m/z 1113.4, a peak is observed 104 Da higher at m/z 1217.5 in addition to a peak at 1009.2, which is 104 Da lower in mass. These observations indicate that the polymer also contains the styrene repeat unit. The isotope pattern in the inset of Figure 5.2b shows that the added silver cationizing agent ionized the sample, leading to ions containing $^{107}\text{Ag}$ or $^{109}\text{Ag}$. 


Figure 5.1. MALDI-ToF mass spectrum of poly(styrene-co-allyl alcohol) obtained using a Bruker Reflex III instrument. (a) Entire mass region where peaks were observed  (b) Mass range 1000-1400 Da where the repeat units of allyl alcohol, indicated by the blue arrow, and styrene, indicated by the red arrow, can be observed, confirming the presence of a copolymer.
Only lower molecular weight oligomers are observed in the spectrum, indicating a high polydispersity (PD) for the polymer. Polydispersity is defined as $M_w/M_N$ and indicates the range of molecular weights in the sample. If a polymer has high polydispersity (> 1.2), only the lower molecular weight components are ionized and observed. This creates what is called the Schultz distribution. If a polymer has a high polydispersity its true molecular weight distribution can not be obtained using mass spectrometry.

In addition to the information given above, the monomer composition of individual oligomers was determined. Because the end groups of the polymer were known, the exact co-polymer composition of each peak could be calculated. The initiator used for the polymerization was t-butyl peroxide, which upon heating leaves a t-butyl group as the initiating end group of the polymer. The polymer chain was terminated by a H. The combination of initiating and terminating end groups of the polymer gives a combined end group mass of 58 Da.

In order to find the composition of the polymer, the mass of the cation must be subtracted from the mass observed in the mass spectrum because only singly charged ions were observed, mass and m/z ratio have identical values. For example, when looking at the monoisotopic ion at m/z 1217.5 Da, 107 units must first be subtracted to account for the silver ion, which leaves a mass of 1110.5 Da for the polymer. From this mass, the masses of the end groups must also be subtracted, giving a mass of 1052 Da, which matches a combination of nine styrene units and two allyl alcohol units. The empirical formula for the ionized polymer with this composition is $C_{82}H_{94}O_2Ag$, having a calculated mass of 1217.63, which is close to the experimental value of 1217.5,
confirming the composition assigned to the polymer. With the help of excel, this calculation can be done for each peak in the mass spectrum to determine all of the different possible comonomer combinations in the copolymer. In addition to the copolymer, a homopolymer of polystyrene was found as a minor product in the mass spectrum.

Once the styrene content is found for each copolymer observed in the mass spectrum, the average styrene content of the product can be calculated. For example, the peak mentioned above corresponds to a copolymer with nine styrene units having a mass of 936 Da. The mass percentage of styrene in this copolymeric 11-mer is 84%. Also for the 10-mer, an average styrene content of 84% was found. This number is slightly higher than the one found using the hydroxyl number, which was 78%.

Sequence information about a copolymeric macromolecule can be obtained by tandem mass spectrometry. For this, the polymer must break along the backbone. Tandem mass spectrometry experiments on the copolymer with seven styrene and four allyl alcohol units were completed on the MALDI-Q/ToF mass spectrometer (see Figure 5.2). In this case, the polymer did not form enough fragments from backbone cleavages for complete sequence determination. Water loss from the allyl alcohol repeat unit gave rise to the most intense peak in the tandem mass spectrum. When ionized alcohols are subjected to tandem mass spectrometry experiments, water loss is often the most abundant fragment ion.\textsuperscript{58} In addition to water loss, internal polystyrene and copolymer fragments were observed in the lower molecular weight region, marked by blue stars (m/z 198, 211, 268, 302 and 419) and purple pound signs (m/z 256 and 373), respectively. Such fragments have been observed previously in tandem mass spectra of silver-
cationized homopolymers. A few small copolymer fragments from the terminating chain end (yn series) were also observed. Scheme 5.1 provides possible structures of all these fragments. The relatively high allyl alcohol content of fragments from the terminating chain end points out that most allyl alcohol is incorporated near the end of the polymerization, consistent with its lower reactivity as compared to styrene. Based on fragments observed, allyl alcohol diads and possibly triads are present near the ω chain end.
Figure 5.2. Tandem mass spectrum of silverated poly(styrene-co-allyl alcohol) containing seven styrene and four allyl alcohol units. (a) Entire spectrum (b) Expanded region from m/z 120 to 540. The blue stars indicate internal polystyrene fragments and the purple pound signs internal copolymer fragments (Scheme 5.1); 46 Da is the decrease in mass by replacing a styrene with an allyl alcohol unit.
Scheme 5.1. Likely compositions of major fragments in the MS/MS spectrum of silverated C₄H₉ – [CH₂CH(Ph)]₇ – [CH₂CH(CH₂OH)]₄ – H (m/z 1068). All these fragments contain Ag⁺. (not shown with the structures).

In this experiment, mass spectrometry proved helpful in determining the composition of a copolymer. However, only limited sequence information about the copolymer could be obtained because the tandem mass spectrum was dominated by the
water loss from the allyl alcohol comonomer, and did not show abundant backbone fragments across the entire mass range.

5.2 Mass Spectrometry of Poly(electrolytes)

The study of poly(electrolytes) has been divided into the different polymers mentioned previously, poly(acrylic acid) and poly(styrene sulfonate sodium salt).

5.2.1 Poly(acrylic acid) (PAA)

PAA was used to find the optimum running conditions for poly(electrolytes) and it was found that adding NaTFA and to run the polymer using both polarities gave the highest S/N ratio in the ESI spectra. Additionally, adding 0.1% acetic acid to the spraying solution and processing the polymer without cations was also tested under ESI conditions. Although the addition of acetic acid increased the intensity of the spectrum, it made the S/N ratio much worse. Running the polymer without the addition of a cation did not improve the S/N ratio or intensity of the spectrum.

Under MALDI conditions, salt addition did not improve spectral quality. In order to obtain the best S/N ratio in positive mode, DHB was used as the matrix and mixed with the analyte in a ratio of 10:2. In negative mode, α-CHCA was the optimum matrix and was mixed with the analyte in a ratio of 10:4, which gave the highest ion intensities using the same number of shots. Several graphs which summarize the optimization of running conditions can be seen in the appendix.

Five different series could be identified in the ESI mass spectrum of the polymer, as can be seen in Figure 5.3. In both positive and negative mode, similar polymer distributions were observed with similar relative abundances. In positive mode, the ions
are sodiated, appearing 24 Da higher in mass than in negative mode, where ionization involves proton loss. Each series contains the 72 Da repeat unit, consistent with the presence of PAA. Upon closer examination, sodium exchange and water losses are the reason for observing five different series. The polymer was prepared by radical polymerization in isopropanol, with the (CH₃)₂C·(OH) radical acting as initiator. The expected end groups from this reaction (60 Da) are observed in series C (Figure 5.3 and Scheme 5.2). Lactone formation at the initiating chain end via H₂O loss gives the most intense series A, which has a nominal end group mass of 42 Da (Scheme 5.2). Series C is present in low abundance and is the least abundant series in the region above m/z 800 in the ESI positive mode mass spectrum. The second most abundant series, B, arises from a second water loss, from A, yielding a nominal end group mass of 24 Da (60 - 2x18), see Scheme 5.2. Series D has an end group with a nominal mass of 64 Da, which is 22 Da higher than that of series A. Therefore series D must arise by the exchange COOH $\rightarrow$ COONa in one repeat unit of series A. Series E, which has a nominal end group of 12 Da, results from the esterification of one COOH group by isopropanol. Since isopropanol was not used in the MS analysis, series C, A and E must have been present in the polymer. Series B and D, on the other hand, were probably generated during the MS experiment. Very similar results were obtained using negative mode (Figure 5.4).
Figure 5.3. ESI mass spectrum of PAA in positive mode. The spectrum was obtained after addition of Na trifluoroacetate in the sprayed sample.

Figure 5.4. ESI mass spectrum of PAA in negative mode. The spectrum was obtained after sample dissolved in water, with methanol added to the sprayed sample.
5.2.2 Tandem Mass Spectra of PAA

The 11-mer was fragmented in both positive and negative mode in the QiT (Figures 5.5 and 5.6, respectively). Positive and negative mode tandem mass spectra show similar degradation products but different fragmentation patterns. Eight consecutive water losses take place in positive mode (Figure 5.5), while only six occur in negative mode (Figure 5.6). It has been shown that water and carbon dioxide losses are the dominant losses in the thermal degradation of PAA, but the MS/MS fragmentation behavior of this polymer has not previously been investigated. The 11-mer of series A

Scheme 5.2. PAA distributions detected in the PAA sample analyzed.59
has ten COOH groups (see Scheme 5.2). A water molecule can easily be lost from two
consecutive repeat units to form a six-membered ring anhydride, as shown in Scheme 5.3.
Such a reaction has been observed upon CAD of deprotonated and sodiated
poly(methacrylic acid).\textsuperscript{30, 60} This mechanism can account for only five water losses from
the 11-mer of A series, which contains ten COOH groups. The additional water losses
could be due to the rearrangement shown in Scheme 5.4. After water losses have
occurred to form six-membered anhydride rings, carbon dioxide eliminations begin
(Scheme 5.3).

Figure 5.5. ESI MS\textsuperscript{2} spectrum of the sodiated 11-mer of PAA, distribution A using the
QiT (positive mode).

64
Figure 5.6. ESI-MS² spectrum of the deprotonated 11-mer of PAA, distribution A, using the QiT (negative mode).

Scheme 5.3. Water loss from sodiated or deprotonated PAA to form cyclic anhydride units and consecutive CO₂ loss from the anhydride to yield a cyclic ketone.⁵
Scheme 5.4. Dehydration of adjacent cyclic anhydride groups, formed by water losses from sodiated or deprotonated PAA.

In positive mode MS/MS of the 11-mer (Figure 5.5), two water molecules are lost before a CO₂ loss is observed, even though only one water loss is necessary to consecutively lose CO₂ according to the mechanism in Scheme 5.3. Similarly, CO₂ loss is significant in the negative mode MS/MS spectrum after the elimination of two H₂O molecules (Figure 5.6). Based on these observations, an alternative mechanism is proposed for the CO₂ eliminations, outlined in Scheme 5.5. The latter pathway creates a
six-membered ring, which is energetically more favorable than a four-membered ring. In total, only three CO₂ losses were observed in the positive mode tandem mass spectra, which is fewer than the five losses possible via Scheme 5.3., indicating preference for the dissociation pathway in Scheme 5.5.

Scheme 5.5. CO₂ loss from adjacent cyclic anhydride groups, formed by water losses from sodiated or deprotonated PAA.
When the MALDI Q-ToF instrument was employed for MS/MS in the negative mode, more than two consecutive water losses were not observed. CO$_2$ elimination proceeds now with considerable yield already after one H$_2$O loss (see m/z 771.3 in Figure 5.7), pointing out that the extent of decarboxylation via Scheme 5.3 increased. In addition to the water and CO$_2$ losses, MS/MS in the Q/ToF instrument also leads to smaller molecular weight fragments which were not observed in the QiT tandem mass spectrum. Generation of energetically more excited polymer ions are available in MALDI (on the Q/ToF instrument) than in ESI (available in the QiT) and also by the deposition of higher internal energies upon CAD in Q/ToF vs. QiT instrumentation. The inability to attenuate the laser intensity of the Q/ToF MALDI source can also cause additional fragmentation to occur. The low molecular weight fragments are smaller oligomers missing the initiating and/or terminating chain end (similar to those formed from poly(methylacrylate)$^{27}$ and with various degrees of H$_2$O and CO$_2$ losses.

The positive mode fragmentation patterns of the B and C series were compared using the pentamer (Figure 5.8). Series C is the intact original polymer with one OH and five COOH pendants and series B is the polymer resulting after two water losses. Oligomer C mainly shows elimination of two water molecules in the MS/MS spectrum. Additionally, a peak arising from a (monomer + water) loss is seen at m/z 353.
Figure 5.7. MALDI-MS$^2$ spectrum of the deprotonated 11-mer from PAA distribution A using the Q-ToF mass spectrometer (negative mode). The blue stars indicate water losses.
Figure 5.8. Positive mode ESI-MS$^2$ spectra of the sodiated pentamer of the PAA. (a) Distribution C, which contains the original polymer. (b) Distribution B, which has already lost two water molecules (see Scheme 5.2). The spectra were acquired on the QiT.
What is important to note is that in the B oligomer, which has already lost two water molecules and in which two COOH groups remain (Scheme 5.5), CO₂ loss occurs with measurable yield from B itself (m/z 363) as well as after dehydration (m/z 345). In the MS/MS spectrum of the C oligomer, no CO₂ loss was observed beyond noise level. (Figure 5.8) These data show that fragmentation behavior that occurs is significantly influenced by the substitution pattern of the PAA chain.

A smaller oligomer from PAA series A was also investigated, in both positive and negative modes, for comparison with series B and C and the larger oligomer A discussed earlier. The MS/MS spectra of the sodiated and deprotonated tetramer of A are depicted in Figure 5.9. Based on these spectra, the deprotonated tetramer produces more fragments than the sodiated tetramer. In the positive mode MS/MS spectrum, one water loss is the most abundant fragment ion. The tetramer from series A carries three COOH groups and, thus can only lose one H₂O molecule according to the pathway in Scheme 5.3. The other fragments are very minor, about one hundredth of the intensity of the initial water loss, and are formed by consecutive and competitive water and CO₂ losses and monomer evaporation. Monomer evaporation to m/z 260 is attributed to contamination of the A tetramer (see structure in Scheme 5.2) with an isomeric anhydride, originating by H₂O loss from the C tetramer (see Scheme 5.6). In the negative mode MS/MS spectrum, water loss is again the most dominant fragment ion (m/z 311). The difference in the negative mode is that two consecutive CO₂ losses occur, whereas only one, barely detectable, CO₂ loss took place in positive mode. Finally, a neutral dimer (but no monomer) is lost in negative mode. A possible mechanism is shown in Scheme 5.7.
Figure 5.9. MS/MS spectra of the tetramer of the A series using different polarities. (a) positive mode (b) negative mode.
CO\textsubscript{2} loss after initial H\textsubscript{2}O loss is most abundant; the energetically most favorable pathway would involve H\textsubscript{2}O loss to form an anhydride ring next to the lactone ring (Scheme 5.7), so that CO\textsubscript{2} loss can ensue by the mechanism outlined in Scheme 5.5. Surprisingly, CO\textsubscript{2} loss is also observed directly from the precursor ion. This minor fragmentation, as well as the second (also minor) CO\textsubscript{2} loss are ascribed to charge-directed fragmentations, rationalized in Scheme 5.7.

Scheme 5.6. Monomer loss from the sodiated tetramer of a PAA chain containing an anhydride next to the monomer cleaved. The precursor ion (m/z 353) is isomeric with the sodiated A tetramer (see Scheme 5.4 for the A series structure.)
Scheme 5.7. Competitive and consecutive H$_2$O and CO$_2$ eliminations from the deprotonated tetramer of series A.
Scheme 5.8. Elimination of a dimer (144 Da) from the deprotonated tetramer of series A. This fragmentation is not observed from larger oligomers (see Figures 5.6 and 5.7), which primarily dissociate via charge-remote pathways.

5.2.3 Poly(styrenesulfonate sodium salt) (PSS)

In positive mode, PSS (see Figure 5.10) was dissolved in H₂O and run using the ESI QiT, without the addition of a cation. The sodium atom in the backbone was not exchanged under these conditions and remained incorporated into the polymer during MS analysis, giving the polymer a repeat mass of 206 Da, as seen in the spectrum of Figure 5.11. Oligomers were observed up to 9-mer in the ESI mass spectrum, when instrument parameters were optimized for higher molecular weight. The main series, series A, has a nominal end group mass of 58 Da, which agrees well with the t-butyl initiating and H terminating group of the purchased compound.
Figure 5.10. Structure of PSS analyzed.

Figure 5.11. ESI mass spectrum of PSS in positive mode, where each ion observed is sodiated (ionization by Na\(^+\) adduction). The numbers indicate the size of the sodiated n-mer.
There is also a second series, B, present in lower abundance, which appears 102 Da lower than series A. The 102 Da deficit comes from one repeat unit losing the SO$_3$Na substituent (styrene unit). Independent of the oligomer size, only one SO$_3$Na substituent is missing. Because series B is not observed in negative mode (vide infra), it is presumably produced in the ion trap by ion molecule reactions with background moisture.

In the negative mode mass spectrum, seen in Figure 5.12, the repeat mass is also 206 Da, verifying that each repeat unit contains a SO$_3$Na salt moiety. The monomer anion without end groups or sodium, observed at m/z 183, gives rise to the most abundant peak in the mass spectrum; in contrast, the sodiated trimer is the base peak in positive mode. The negative mode mass spectrum also indicates an end group of 58 Da, and it does not contain ions missing the SO$_3$Na substituent, as did the positive mode spectrum. The negative mode MS shows consecutive Na$^+/H^+$ exchanges, the number of which depends on the size of the n-mer. For example, five peaks are observed for the pentamer anion: one for the polymer with one SO$_3^-$ and four SO$_3$Na groups, and four subsequent peaks, arising from consecutive Na$^+/H^+$ exchanges, each causing a 22 Da decrease in mass. Only for the pentamer and dimer, the peak corresponding to the completely sodiated anion is the most abundant. Within the singly charged polymers, the pentamer is the largest oligomer observed. However, the doubly charged distribution extends to the heptamer. The mass difference between adjacent doubly charged oligomers is 103 Da, indicating the incorporation of sodium in the doubly charged ions. Na$^+/H^+$ exchanges are also detected, each leading to 11 Da mass decrease. But in this case, the extent of sodium/proton exchanges is low.
5.2.4 Tandem Mass Spectra of PSS

The sodiated tetramer (positive mode; m/z 905 Da) was fragmented by CAD in the QiT mass spectrometer (Figure 5.13). The dominant fragmentation of the tetramer is evaporation of up to two monomer units. In addition, peaks representing the loss of the elements of the monomer plus CH₂ are seen in the MS/MS spectrum indicating that fragmentation occurs at all points along the backbone. End group loss was seen after the loss of the dimer molecule. The fragmentation pattern of this polymer differs from the structurally similar polystyrene, where fragments containing one of the end groups (but not both) dominate the tandem mass spectrum.²⁸
The fragments observed can be rationalized by homolytic cleavages along the PSS backbone to create radicals that are held together in dimeric Na⁺-bound complexes (Scheme 5.9).<sup>28, 61</sup> The sodium sulfonated groups and the cationizing Na⁺ build a salt bridge network that keeps the newly formed radicals interconnected, as shown in Scheme 5.9. Within this complex, radical reactions can take place that generate the observed fragments. Schemes 5.9 – 5.11 summarize the reactions possible after initial C-C bond cleavage next to the first repeat unit. Monomer losses from the resulting complex give rise to m/z 699 and m/z 493 (Scheme 5.9). Rearrangement of the primary radical by 1,2-phenyl shift to a more stable secondary radical enables the loss of a 220 Da unit (monomer + CH₂), leading to m/z 685 or, after consecutive monomer loss, to m/z 479.

The primary radical can alternatively rearrange to a more stable benzylic radical by 1,4-H atom transfer (Scheme 5.10). The benzylic radical can lose a dimer (412 Da) or a dimer + CH₂ (426 Da) to generate m/z 479 and 449, respectively, in one elimination.

The dimer and dimer + CH₂ units may also keep the Na⁺ charge instead of being eliminated as neutral losses; this generates m/z 435 and 449, respectively. Note that the monomer and monomer + CH₂ units carry only one SO₃Na group, which prevents them from effectively competing for the Na⁺ charge; moreover, the m/z values of the latter fragment ions are < 1/3 of the m/z of the precursor ion (m/z 905), which compromises their trapping in the QiT.
Figure 5.13. ESI-MS² spectrum of the sodiated PSS tetramer (m/z 905). See Schemes 5.9 – 5.11 for plausible pathways to the major fragments observed.
Scheme 5.9. Evaporation of monomer (206 Da) and monomer + CH$_2$ (220 Da) units from the sodiated PSS tetramer.
Scheme 5.10. Evaporation of dimer (412 Da) and dimer + CH2 (426 Da), and formation of sodiated dimer (m/z 435) and sodiated dimer + CH2 (m/z 449) from the sodiated PSS tetramer.
Although dimer less CH₂ unit (398 Da) is not lost, the corresponding sodiated species (m/z 421) is observed in the MS/MS spectrum (Figure 5.13). Scheme 5.11 provides a likely route to this fragment, involving consecutive dissociation of m/z 685.

Analogous reactions, leading to the same fragment ions, can occur after initial cleavage of the other backbone C-C bonds of PSS.

The fragmentation of the polymer becomes more interesting in the negative mode. Each tetramer ion observed in the mass spectrum (Figure 5.12) was fragmented in the QiT. The tetramer with one SO₃⁻ and three SO₃Na substituents, as well as the tetramers that underwent one, two or three SO₃Na/SO₃H exchanges were subjected to CAD. The resulting four tandem mass spectra are included in Figure 5.14.

The spectrum of the precursor ion having three SO₃Na groups and no acidic proton (m/z 859) shows the same fragmentation pattern as the tetramer in positive mode (of Figures 5.14a and 5.13). In both cases, the dominant fragmentations involve evaporation of monomer and evaporation of monomer plus CH₂ units. In negative mode, the most intense fragment arises by monomer loss, whereas the most abundant fragment in positive mode is the loss of a dimer plus CH₂ (or a monomer and a monomer + CH₂).

Fragmentation pathways similar to those given in Schemes 5.9 – 5.11 account for all major fragments.
Scheme 5.11. Consecutive dissociation of m/z 685 (generated by evaporation of monomer + CH$_2$ from the sodiated PSS tetramer) by monomer loss to form m/z 479 (left) or by radical losses to form m/z 421 (right).
Figure 5.14. ESI-MS² spectra of the PSS tetramer in negative mode. (a) Polymer with three Na ions  (b) Polymer with two Na ions  (c) Polymer with one Na ion  (d) Polymer with no sodium in the sulfonated groups. 

The fragmentation pattern changes dramatically when one Na+/H+ exchange occurs. The precursor ion (m/z 837 Da) now mainly loses SO$_3$ (Figure 5.14b). After the SO$_3$ has been lost, peaks representing consecutive monomer evaporation (m/z 551) and
the dimer with no end groups (m/z 389) appear just above noise level. This example shows that the presence of an acidic proton can significantly affect the fragmentation pattern of the polymer. The SO₃H group enables a charge-directed fragmentation, promoted by the SO₃H proton (Scheme 5.12). Such reactions have lower energy requirements than charge-remote, homolytic bond cleavages and, thus, process much more efficiently during the time window available for fragmentation (MS time scale in the QiT).

Scheme 5.12. Acid-catalyzed SO₃ loss from PSS carrying SO₃H substituents.

In the negative mode tandem mass spectrum of the tetramer with only one sodium ion (Figure 5.14c), the fragmentation pattern changes again. In this case, two consecutive SO₃ losses are the main fragments in the tandem mass spectrum, as expected from the presence of two SO₃H groups in this tetramer. In addition to these SO₃ losses, the dimer and dimer less CH₂ units with no end groups are also seen in the tandem mass spectrum as minor products.
Lastly, the tetramer without any sodium was fragmented using CAD. Because there are three SO$_3$H groups in this polymer, three consecutive SO$_3$ losses are observed in the tandem mass spectrum (Figure 5.14d). This time no dimer ion is present in the tandem mass spectrum. The dimer (and dimer less CH$_2$) must be generated via Na$^+$-bound dimer radicals, as explained before. The absence of Na$^+$ consequently disables their formation. When comparing the tandem mass spectra of the tetramers with three SO$_3$Na versus three SO$_3$H groups, they show completely different fragmentation patterns. The former reveals monomer unit and backbone sequence, while the latter the number of sulfonated substituents present. They also reveal that the presence or absence of acid plays an important role in the degradation of the polymer.
CHAPTER VI

SUMMARY

The applications of mass spectrometry are so wide reaching because it is able to study a wide range of samples with a wide range of molecular weights. Depending on the instrument used, there is no lower limit on the molecular weights that can be detected. With the help of multiple charging, molecular weights around 1 million Da can also be studied. This work utilizes that characteristic of mass spectrometry to study many different types of ions.

The first project involved in this work includes the study of smaller molecular weight molecules, namely adenine and its derivatives. Mass spectrometry was an important part of this analysis due to its specificity where the mass of adenine could be differentiated from its base molecules. By studying the derivatives of adenine, Cooks’ kinetic method was used for the first time to find out how the binding location of the sodium ion affects the absolute value for the sodium binding affinity.

Two different base sets were used to probe the sodium binding affinity of adenine and its derivatives. At first the amino acids were used because they have been studied thoroughly and their values are readily available. When using glycine, valine, alanine, serine, proline, tyrosine, tryptophan, glutamine, histidine and glycine-leucine, which have sodium binding affinities that range from 161 – 212 kJ/mol as the reference bases, a
sodium binding affinity of 208 kJ/mol was found for ade; 175 kJ/mol for 3-MeAde and 215 kJ/mol for N6-MeAP. The value for ade was significantly higher than previously reported experimental results, which were 172 and 136 kJ/mol using the kinetic method and threshold collision-induced-dissociation, respectively. The values for the sodium binding affinity of 3-MeAde and N6-MeAP had not previously been determined experimentally.

In order to account for the lower values found previously, a base set was probed which had lower sodium binding affinities, which included: dimethylformamide, methylacetamide, pyridazine and dimethylacetamide, with sodium binding affinities of 157, 158, 159 and 163 kJ/mol, respectively. When these molecules were used as the base set, values of 165 and 154 kJ/mol were found for the sodium binding affinities of Ade and 3-MeAde, respectively. N6-MeAP was unable to form heterodimers with these base molecules, indicating that its sodium binding affinity is not near the range 157 – 163 kJ/mol. It is expected that its sodium binding affinity is higher than this range. When paired with the amino acids, it had the highest sodium binding affinity, a value of 215 kJ/mol. Additionally, Ade was paired separately to 3-MeAde and N6-MeAP to compare relative sodium binding affinities to each other. Based on these two comparisons, a ladder of the three molecules can be made where $\Delta H_{Na}(\text{N6-MeAP}) > \Delta H_{Na}(\text{Ade}) > \Delta H_{Na}(\text{3-MeAde})$.

This experiment is important, because for the first time, Cooks’ kinetic method was used to find the sodium binding affinity based on binding location of the metal ion. It was also found that the base molecules used in the experiment had an affected the experimental value for the sodium binding affinity of adenine and its derivatives.
In addition to studying these lower molecular weight molecules, poly(electrolytes) were studied because of their importance to the field of fuel cells. In order to find more commercial applications for fuel cells, new proton conducting materials need to be developed that are less expensive and can be used at ambient temperatures. The current material used for fuel cells is too expensive to use for wide range commercial use.

It was found the best technique for studying these poly(electrolytes) was to dissolve the sample in a suitable solvent, which in this case was water, and add Na TFA to ionize the sample for ESI analysis. This procedure worked best for both positive and negative mode. In terms of MALDI preparation, DHB was the best matrix to use in positive mode and the optimum matrix to sample ratio was 10:2. In negative mode, α-CHCA was the matrix of choice but more matrix was needed and the matrix to sample ratio used was 10:4. The addition of salt in either polarity only made the spectrum difficult to interpret.

Water loss was the dominant series observed in the mass spectrum of poly(acrylic acid), which forms a lactone ring between the initiator and the first monomer unit of the polymer. The second most abundant series in the mass spectrum contained another water loss from the side chains of two adjacent monomer units in the polymer. The series containing the intact polymer and the appropriate end groups was actually one of the least abundant series in the mass spectrum. Additionally, a series representing sodium exchange was seen.

The tandem mass spectra confirm previous results that water loss as the most abundant degradation product. However, this study reveals more water losses than previously thought to give a new structure after the fragmentation of poly(acrylic acid).
In addition to these water losses, carbon dioxide was also a dominant neutral loss from the polymer. As fewer CO$_2$ losses were observed than previously thought from proposed mechanisms, a new mechanism and structure is shown to account for the loss of CO$_2$ from the polymer, which involves a six-membered ring instead of the less favorable four-membered ring. Due to the higher deposition of higher internal energies in the MALDI Q/ToF instrument, more fragments were observed in the tandem mass spectrum of the A series of the 11-mer, as compared to the CAD experiments using the QiT. The number of water molecules still incorporated into the pentamer of PAA affected the fragmentation of the oligomer. When looking at the intact oligomer molecule, water losses were the only measureable fragments observed in the tandem mass spectrum, as compared to series B, which had already lost two water molecules prior to fragmentation and produced CO$_2$ fragments as well. The negative mode tandem mass spectrum of the tetramer showed a greater number of fragments than the same polymer sodiated in positive mode.

The other class of poly(electrolytes) studied was poly(styrene sulfonated sodium salt) and produced spectra in both positive and negative mode. The 206 Da repeat unit of the polymer, shows that sodium exchange did not occur in the positive mode MS analysis. The positive mode spectrum also showed a small series due to the loss of only one SO$_3$Na substituent of the styrene side chain, independent of the oligomer observed. The negative mode mass spectrum was more complicated where Na$^+$/H$^+$ exchange was observed in addition to multiply charged oligomers. In the negative mode mass spectrum, the number of Na$^+$/H$^+$ exchanges corresponds to the oligomer observed for
singly charged oligomers. Sodium exchange is also seen in the case of doubly charged molecules, but does not correspond to the oligomer observed.

The tandem mass spectra of PSS revealed a range of fragment ions depending on the number of sodium exchange in the polymer. In the case of the positive mode MS$^2$ spectrum of the tetramer, monomer and monomer + CH$_2$ evaporation were the most abundant fragments observed. Additionally, the end groups were lost after the loss of two monomer units. The same peaks were observed in the negative MS$^2$ spectrum where only one sodium atom was lost to create the negative charge on the polymer. When Na$^+$/H$^+$ exchange occurs, the fragmentation pattern begins to change, so that for every Na$^+$/H$^+$ exchange, an SO$_3$ group is lost. After two Na$^+$/H$^+$ exchanges, no monomer loss is observed in the tandem mass spectrum.

A copolymer between styrene and allyl alcohol was studied in order to determine the exact composition of the copolymer. Results of the study show that the copolymer composition differed from the results obtained using a different method, hydroxyl number. When the tandem mass spectrometry experiment was completed, it was found that the sequence of the polymer could not be determined due to the chemistry of the polymer. The alcohol side chain of the allyl alcohol caused water loss to be the most abundant fragment ion of the polymer. Internal polystyrene fragments were also observed.

Mass spectrometry shows great promise in its applications to both small and large molecule analysis. This work showed that for the first time, mass spectrometry can be used to find the location of metal binding to a small molecule and its absolute binding affinity can be measured. Additionally, larger polymers were analyzed for monomer
composition of a copolymer and the optimum running conditions for poly(electroytes) were found, in addition to the fragmentation patterns of these polymers.
REFERENCES


37. Fu, Y.; Manthiram, A.; Guiver, M. D., Blend membranes based on sulfonated poly(ether ether ketone) and polysulfone bearing benzimidazole side groups for proton exchange Membrane fuel cells. *Electrochemistry Communications* **2006**, *8*, (8), 1386.


APPENDICES
APPENDIX A
ADDITIONAL DATA

Table A.1. Average Values for the ln \( \frac{k_{Ade}}{k_{B_i}} \). These values were used to create the regression lines found in Chapter IV.

<table>
<thead>
<tr>
<th></th>
<th>Ade</th>
<th>3MeAde</th>
<th>N6MeAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>(161 kJ/mol)</td>
<td>2.98379</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>(167 kJ/mol)</td>
<td>1.78780</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>(173 kJ/mol)</td>
<td>0.10568</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>(192 kJ/mol)</td>
<td>-4.16253</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>(196 kJ/mol)</td>
<td>-4.64259</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>(201 kJ/mol)</td>
<td>2.43851</td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>(210 kJ/mol)</td>
<td>-0.30070</td>
<td>-1.21940</td>
</tr>
<tr>
<td>Gly Leu</td>
<td>(212 kJ/mol)</td>
<td>-1.57816</td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>(222 kJ/mol)</td>
<td></td>
<td>1.78547</td>
</tr>
<tr>
<td>His</td>
<td>(228 kJ/mol)</td>
<td></td>
<td>2.40745</td>
</tr>
<tr>
<td>DMF</td>
<td>(157 kJ/mol)</td>
<td>3.70382</td>
<td>-1.17335</td>
</tr>
<tr>
<td>MA</td>
<td>(158 kJ/mol)</td>
<td>3.48017</td>
<td>-1.04529</td>
</tr>
<tr>
<td>P</td>
<td>(159 kJ/mol)</td>
<td>2.71680</td>
<td>-2.23166</td>
</tr>
<tr>
<td>DMA</td>
<td>163 kJ/mol</td>
<td>1.35017</td>
<td>-3.30701</td>
</tr>
</tbody>
</table>
Figure A.1. Optimization of ESI conditions for poly(electrolytes) using PAA. Although adding acetic acid produced the most intense spectrum, it did not produce the best S/N. Therefore, Na trifluoroacetate addition was used for the analysis of poly(electrolytes).

Figure A.2. MALDI optimization conditions for both positive and negative mode, respectively. DHB was the matrix of choice for the positive mode when used in a ratio of 10:2 matrix:poly(electrolyte). In negative mode, α-CHCA was the optimum matrix and mixed in a ratio of 10:4 matrix:poly(electrolyte).
APPENDIX B
COPYRIGHT PERMISSION

GMail - Figure Permission

Bethany Subel <bethany.subel@gmail.com>

To: Vic Fursey <vf@bcai.com>

Fri, Jul 10, 2009 at 4:26 PM

Hello Vic,

I hope all is well and you get to enjoy some of your summer!

I am writing to request permission to use figures from the Bruker Manuals in my dissertation. I would like to use figures from the "Requires UC manual and the Reflex III manual.

Thank you for your time! I hope to catch up soon, which should happen after my defense is over on July 20th.

Best Regards,

Beth Subel

Graduate Research Assistant
The University of Akron
Department of Chemistry
Akron, OH
bethany.subel@gmail.com
440-238-3133

Vic Fursey <vf@bcai.com>

To: Bethany Subel <bethany.subel@gmail.com>

Thu, Jul 16, 2009 at 9:54 AM

Hi Beth,

Good luck with the defense! I'm sure you will do great.

You have our permission to use the figures from the two instrument manuals in your dissertation. Please just let me know which ones you eventually move into the document so we have it on file.

Hopefully we will catch up soon.

Best regards,

Vic Fursey

Fursey, Vic

Asst. Vice President, Marketing and Sales
Broker DataCom Inc.
4D Naming Road
Bilicance, WA - 98201
Tel: 316 663-3290 x 1250
Email: vf@bcai.com
[Contact information]

http://mail.google.com/mail/?ui=1&ik=6e3d620903&view=pt&sho=1283476b209a4a&sr... 7/16/2009
This e-mail confirms Elsevier approval of your order request and references a corresponding order number. Your payment for this order is Copyright Clearance Center.

Your order is not complete and the license to reuse has not been granted until you complete the order by clicking on the link below and accepting the terms and conditions of the license. PLEASE READ THE TERMS AND CONDITIONS CAREFULLY AS SPECIFIC COPYRIGHT LINE REQUIREMENTS MAY BE APPLIED.

Click here to complete your order:
http://e105.copyright.com/CustomerAdmin/PC.jsp?macID=9996754793084752&printName=K.S

Please visit https://myaccount.copyright.com and log in using your Rightslink User ID and Password if you would like to view/edit your account information.

Order Details
Licensee: Bethany Suhel
Order Date: Jun 16, 2009
Order Number: 500538337
Publication: TRAC Trends in Analytical Chemistry
Type of Use: Thesis / Dissertation
Total: $50 USD

Please take a moment to complete our customer satisfaction survey:
http://www.surveymonkey.com/s.asp7u=100021201336

If you have any comments or questions, please contact Rightslink:

Copyright Clearance Center
Rightslink
Tel (toll free): 1-877-422-594
Tel: 1-978-646-2777
E-mail: mailto:customerscare@copyright.com
Web: http://www.copyright.com

https://webmail.uakron.edu/horde/imps.php?actionID=print_message&index=126... 7/16/2009
Date: Sun, 12 Jul 2009 07:40:41 -0700 (PDT) [07/12/2009 10:40:41 AM PDT]
From: siuzak@scips.edu
To: b624@ultron.edu
Subject: Re: Permission to use figure

Hi Bethany,

You are welcome to use it.

Gary

> Hello Professor Stuzak,
> 
> I am a student in Professor Bound's lab at the University of Akron and I am writing my dissertation. I am writing to ask your permission to use your ESI figure from your book, The Expanding Role of Mass Spectrometry in Biotechnology, in my dissertation. I had trouble locating the publisher's website, so I thought this would be the best place to start. Please let me know if there is someone else I should contact instead.
> 
> Thank you for your time,
> 
> Bethany Subel

https://webmail.ultron.edu/harde.imp/messages.php?actionID=print_messages&index=129... 7/16/2009