MATRIX ASSISTED LASER DESORPTION IONIZATION
QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY
OF POLY(2-VINYLPYRIDINE)

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Donna M. Smith

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Thesis

Approved:  Dean of the College
Dr. Chrys Wesdemiotis  Dr. Roger B. Creel

Faculty Reader  Dean of the Graduate School
Dr. Jun Hu  Dr. George R. Newkome

Department Chair or School Director  Date
Dr. David Perry
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CHAPTER I
INTRODUCTION

The use of polymers has had a significant impact on science, business, and industry. Polymers are now used in places where metals and traditional fabrics, such as leather, cotton, and silk, were the main components. Polymers are also used to make a wide variety of manufactured products ranging everywhere from medical equipment to automobile parts to miniskirts. With the widespread use of polymers, comes a need to study polymers in terms of their properties and components. By looking at how polymers break apart, or fragment, more information can be found about how they can be formed for industrial purposes. Also, the polymers that are created today may pose problems in the future. What happens when items made of polymers start to decompose? Are the decomposed products hazardous to the environment? Will the polymers even decompose?

Mass spectrometry provides a method for studying the structural organization of polymers. Tandem mass spectrometry, in particular, provides a method of analyzing the basic structure of the polymer’s oligomers, which is achieved by fragmenting one of the oligomers of the polymer. This thesis will focus on the experimental methods and data analysis used in a tandem mass spectrometry study of the polymer poly(2-vinylpyridine). In terms of instrumentation and experimental methods, mass spectra obtained from the entire isotopic cluster of an oligomer will be compared to mass
spectra of the all $^{12}\text{C}$ isotopomer of this oligomer ion; different types of cationization (protonation vs. metalation) will also be discussed. In terms of structural studies, fragments formed upon tandem mass spectrometry by poly(2-vinylpyridine) will be compared to those formed by poly(styrene) and mechanisms and a new nomenclature will be proposed for the formation of the poly(2-vinylpyridine) fragments.

Chapter two of this thesis will describe a brief historical perspective on how mass spectrometry has been used to analyze polymers. To this time, the method used in this research, matrix-assisted laser desorption ionization (MALDI)/quadrupole time-of-flight (QTOF) mass spectrometry, has not been applied to synthetic polymers, such as the poly(2-vinylpyridine). In fact, in researching for background information, no articles were found where a MALDI-QTOF mass spectrometer was used for the purpose of polymer analysis. Most of the articles which cite the use of MALDI-QTOF mass spectrometry pertain to the research of peptides and proteins. In chapter three, experimental procedures and the MALDI-QTOF mass spectrometer will be discussed. Chapter four will focus on analysis of the mass spectra, tandem mass spectra, and proposed fragmentation mechanisms for poly(2-vinylpyridine). In the concluding chapter, chapter 5, an overall description of the project and thoughts for further study will be discussed.
CHAPTER II
LITERATURE REVIEW

2.1. Introduction to Literature Review

This literature review will provide a background on the history of polymer analysis by mass spectrometry. The type of polymer analyzed will be described, and an overview of methods of ionization and mass spectrometry used in the analysis of polymers will be provided. Different mass spectrometers will be discussed in terms of ion source, significance in the advancement of polymer studies, usefulness, and limitations. The literature review will conclude with an account of recent developments in the utilization of tandem mass spectrometry for polymer analysis.

2.2. Polymer Terminology

Since this project focuses on mass spectrometry of polymers, a brief section on polymer terminology will be provided. These terms were found in the *Compendium of Macromolecular Nomenclature*, provided by the Commission on Macromolecular Nomenclature. A polymer is characterized by the multiple repetitions of one or more species of atoms or groups of atoms. The properties of a polymer will not differ with the addition or removal of constituent atoms or groups of atoms. A monomer is a
compound consisting of molecules, each of which can provide one or more constitutional units. An oligomer will differ in physical properties with the addition or removal of one or more of its constitutional units. A block is a portion of a polymer molecule comprising many constitutional units. A block polymer will have these blocks connected linearly, with the blocks connected directly or through a constitutional unit that is not part of the block. A copolymer is derived from more than one species of monomer.

2.2.1. Poly(2-vinylpyridine)

Poly(2-vinylpyridine) or P-2VP is similar to polystyrene in structure, but contains substituted pyridine instead of substituted benzene rings. Figure 2.1 shows the structure of a polystyrene and a poly(2-vinylpyridine).

![Figure 2.1. Structure of polystyrene and poly(2-vinylpyridine)](image)

P-2VP has been investigated for its uses as an insulator. P-2VP has also been investigated as a potential conductor or semi-conductor, either by itself or complexed with a metal. The nitrogen on the P-2VP structure contributes to the thermal stability
of the polymer. Upon complexation with a metal, a resonance effect is created with the nitrogen on the pyridine ring, which then stabilizes a carbenium ion formed on the polymer chain. Complexation with the metals palladium and platinum increases the conductivity of the P-2VP to a greater degree than complexation with the metals copper and cobalt.

2.3. Mass Spectrometry in Polymer Characterization

Mass spectrometry as a tool for polymer characterization has been reviewed in recent articles. No article was found reporting on mass spectrometric studies of poly(2-vinylpyridine).

2.3.1. Ionization Methods

Most of the articles in the literature refer to the use of matrix assisted laser desorption/ionization (MALDI) combined with time-of flight mass spectrometry. With MALDI, the sample is dissolved in an appropriate solvent. This solution is then mixed with a solution of the matrix, an organic molecule that absorbs laser light. The solvent is allowed to evaporate, leaving a solid solution of the sample in the matrix. The solid solution is irradiated by the laser inside the mass spectrometer (in vacuum). The latter process leads to the desorption of the sample ions. MALDI is the predominant method of ionization at this time, but some other methods of ionization, which can be utilized, are field desorption (FD) and electrospray ionization (ESI). Field
desorption ionizes the sample by applying a high potential to an electrode which is coated with a solution of sample. In electrospray ionization, a solution containing the analyte, is sprayed at atmospheric pressure, through a potential difference between 3kV and 6kV, toward the differentially pumped entrance to the mass spectrometer. Highly charged droplets of the solution are produced and as the solvent evaporates, smaller and smaller droplets are produced by electrostatic repulsion, until the droplets become so small that sample ions desorb from their surface.

Various research groups have reported studies in which one ionization method was compared to another ionization method. For example, Hercules and his research group used both ESI-MS and MALDI-MS for the characterization of linear single nylon-6 oligomers. The dimer, tetramer, hexamer, octamer, and dodecamer of nylon-6 were investigated. The ESI and the MALDI sources were combined with a time-of-flight mass analyzer. With both techniques, end-group cleavage from the oligomer chains was observed. This study also reported that MALDI-MS gave more consistent mass spectra over a series of oligomers than ESI-MS. With ESI-MS, more doubly charged ions were found as the oligomer size increased and increased fragmentation of the end-groups was evident.

In addition, the data obtained using MALDI and ESI ionization methods were compared to results found in an earlier study using TOF-SIMS, which is time-of-flight secondary ion mass spectrometry. TOF-SIMS, along with $^1$H NMR, was used to confirm the structures and molecular weights of nylon-6 oligomers. Again, fragmentation of the end groups was observed (upon TOF-SIMS), similar to that found when MALDI-MS and ESI-MS were used.
2.3.2. Applications of Mass Spectrometry in the Polymer Industry

In a study by Chen et al., MALDI-TOF-MS, ESI-MS, and tandem mass spectrometry were used to characterize complex polyol mixtures. They recognized that IR, NMR, gel permeation chromatography, and GC/MS are limited in their ability to provide deformatinal of complex polyol samples. They reasoned that an increased ability to provide analytical information about polyol samples would greatly benefit the polymer industry. Product failure analysis and deformatinal of polymer mixtures are two common problems encountered in the manufacturing of polymers. Speedy detection of impurities would allow for increased production and less shipping delays in the polymer industry. Four different samples (A-D) of ethylene oxide (EO)/propylene oxide (PO) copolymers were tested using MALDI-TOF-MS. The mass spectra collected using the MALDI-TOF-MS showed a small compositional variation in one of the four samples (sample C) that had previously gone undetected by traditional analytical methods. A linear time-of-flight mass analyzer was used in this particular case, and it was suggested that a reflectron time-of-flight mass analyzer would provide isotopic resolution. The mass spectra collected using ESI-MS were used to find variations in two separate samples, which were suspected of being EO/PO block copolymers (samples E and F). The MALDI mass spectra for both, samples E and F, showed only the matrix background peaks. The ESI mass spectra, however, proved that neither of the samples E and F had the unique distribution pattern expected for an EO/PO block polymer. Instead, both samples appeared to have a mixture of EO and PO, but information concerning the relative amounts of EO and PO could not be determined. Tandem mass spectrometry was used to identify the major components of
the sample. MS/MS spectra displayed common components and an almost identical
fragmentation pattern. By combining ESI-MS and MS/MS, it was possible to conclude
that samples E and F had different formulations, but the same components. This is
significant because sample F, which was more cost effective, could be differentiated
from sample E.

2.3.3. Chromatographic Methods Combined with Mass Spectrometry

The MALDI-TOF mass spectrometer has been combined with various
chromatographic methods, such as thin layer chromatography (TLC), liquid
chromatography at the critical point of adsorption (LCCC), and size-exclusion
chromatography (SEC).

Quantitative structural analysis of a polymer has been achieved using a
combination of liquid chromatography, electrospray ionization, and orthogonal
acceleration time-of-flight mass spectrometry, all in one instrument, as reported by
Buijtenhuijs and Nielen.13 Three liquid chromatography methods were interfaced with
mass spectrometry: size-exclusion chromatography (SEC/MS), gradient polymer elution
chromatography (GPEC/MS), and liquid chromatography at the point of adsorption
(LCCC/MS). A polydisperse poly(methyl methacrylate), PMMA, was used to obtain an
absolute mass calibration of the SEC column by SEC/MS. The calibration curve was
constructed by plotting the mass of an SEC fraction (determined by MS) vs. the
corresponding elution volume. Once this plot was established, the absolute molecular
weight distributions of other PMMA samples could be determined using the calibrated
SEC system and a refractive index (RI) detector. GPEC/MS was used for the analysis of a dipropoxylated bisphenol A/adipic acid polyester resin. Mass spectra were obtained for oligomers up to \( n = 20 \). Four different oligomer series were distinguished in terms of chemical composition. In the LCCC/MS experiments, gradient elution modes were tuned for mass-independent elution behavior. LCCC depends only on chemical heterogeneity of the polymer and is independent of mass.

Dinonylpoly(ethylene glycol), DNPEG, a water-soluble polymer with nonpolar end groups, was analyzed by LCCC/MS. In this case, a reversed phase column was used, where gradient elution occurs from aqueous toward organic solvent. The mass spectra obtained indicated three pure components that eluted in the order PEG, monononyl-PEG, and dinonyl-PEG. This successful separation and identification was followed up with an attempt to quantify the sample. The total ion currents (TIC) in the mass spectra resulting from the three baseline resolved LCCC peaks were measured (using the areas of all signals in each spectrum) and are displayed in the last column of Table 2.1. The results were compared with the composition obtained using a calibrated evaporative light scattering detector (ELSD) and raw ESI-MS peak heights (Table 2.1). The research group concluded from the data that the PEG amount is overestimated if ESI-MS is used alone, i.e. without prior LCCC separation. With LCCC preseparation, discrimination in favor of PEG is avoided, but the ESI response factors for monononyl-PEG and dinonyl-PEG are different. Because of this discrepancy, the authors underlined the need for an analog detector (ELSD) in quantitative LC/MS studies of synthetic polymers.
Buijtenhuijs and Neilen also used LCCC/ESI-TOF-MS for the analysis of a terephthalic acid/neopentyl glycol (TPA/NPG) polyester, a nonpolar polymer with polar end groups. In this case, three peaks were eluted in the order of cyclic(TPA/NPG)$_n$, linear (TPA/NPG)$_n$, and linear (TPA/NPG)$_n$TPA. Experiments using LCCC-ESI with a quadrupole as a mass analyzer yielded mass spectra for oligomers up to $n = 10$. The time-of-flight mass analyzer detected singly charged cyclic oligomers up to $n = 11$ and (smaller) doubly charged cyclics, [M + 2Na]$^{2+}$, up to $n = 15$.

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>ELSD(area %)</th>
<th>ESI-MS(height %)</th>
<th>TIC(area %)</th>
</tr>
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<tbody>
<tr>
<td>PEG</td>
<td>12</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Monononyl-PEG</td>
<td>66</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Dinonyl-PEG</td>
<td>22</td>
<td>28</td>
<td>36</td>
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2.3.4. The MALDI Ion Source and Collision Induced Dissociation

Jackson et al has used a MALDI ion source in combination with collision-induced dissociation (CID) for polymer analysis. MALDI-CID is a form of tandem mass spectrometry where an individual oligomer can be selected and is then fragmented using collisional activation. In the study reported by Jackson et al., four different samples, poly(styrene) A, poly(styrene) B, poly(styrene) C, and poly(styrene) D, underwent MALDI-CID with the intent of determining the masses of the individual end groups: sec-butyl and b-hydroxyethyl, 2-(N,N-dimethylamino) phenyl and hydrogen, 3-(N,N-dimethylamino) propyl and hydrogen, and 3-(N,N-dimethylamino) propyl and
perfluorooctyldimethylsilyl, respectively. These different end groups were introduced to the poly(styrene) samples and their structures were confirmed using nuclear magnetic resonance (NMR) spectroscopy. MALDI-CID was performed on Cu⁺ or Ag⁺ adducts of individual polystyrene oligomers; to form these adducts, copper (II) nitrate or silver nitrate were used as cationizing salts. Based on the mass to charge ratios (m/z) of the fragments present in the MALDI-CID mass spectra, fragment structures and possible mechanisms for the formation of these structures were proposed. Recurring fragmentation patterns were divided into series A through G, α, and β. The proposed structures for these series can be found in Figure 2.2. The X in each refers to either the copper or silver ion used for ionization. Mechanisms were also proposed for the formation of each of the series.
Figure 2.2. Proposed structures of the ion fragments formed by polystyrenes.
CHAPTER III
MATERIALS AND METHODS

3.1. Sample Preparation

The poly(2-vinylpyridine) or P-2VP, molecular weight 1020, standard was purchased from Scientific Polymer Products, Incorporated, and is an amber colored solid. The solid, brittle structure of the P-2VP presented a challenge for collecting the sample to be analyzed. The small amount of sample that was needed for testing was obtained by chipping at the portion needed with a metal spatula until a small piece broke off and then by weighing the amount released with an electronic balance. Three different mixtures of the sample were prepared and tested. In the first test, P-2VP was ionized by protonation. In the second test, P-2VP was lithiated using lithium trifluoroacetate. In the third test, P-2VP was copperated using cupric acetate. Two different matrixes were tested for the MALDI process, namely trans 2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]-malonitrile, or DCTB, and 1,8,9-anthracenetriol, or dithranol. Both of these matrixes were dissolved in tetrahydrofuran (THF) solvent. Dithranol and DCTB were purchased from Fluka and THF was purchased from EMD Chemicals. Cupric acetate was purchased from Fisher Chemical and lithium trifluoroacetate was purchased from Aldrich. 0.01 g of P-2VP and 0.01 g of the salt were each dissolved in 1.0 mL of THF. 0.01 g of the matrix were dissolved in 0.5 mL of THF. To protonate the polymer, the matrix and P-2VP solutions were mixed in a 10:2 ratio, using 100 µL of the matrix and
20 µL of the P-2VP solution. To lithiate or copperate the polymer, the matrix, P-2VP, and salt solutions were mixed in a 10:2:1 ratio, using 100 µL of the matrix, 20 µL of the P-2VP, and 10 mL of the lithium trifluoroacetate or cupric acetate solution.

3.1.1. Pre-screening of the Samples

All samples were pre-screened using a Bruker Reflex MALDI-TOF mass spectrometer purchased from Bruker Daltonics Incorporated, which is operated using Unix Software. This instrument is run with an attenuated nitrogen laser with the option of using a camera to see which portion of the target plate is hit by the laser. In addition, to the protonated, lithiated, and copperated samples, a silverated sample was also pre-screened using silver trifluoroacetate as the source of the silver ion. The time base for each was set to 1.000 ns at 20 kV reflectron mode. For the protonated sample, the attenuator was set to 90, allowing 10% of the laser light to reach the sample. For the lithiated sample, the attenuator was set to 60, allowing 40% of the laser light to reach the sample. The attenuator was set to 59 for the silverated sample and 68 for the copperated sample.

3.1.2. Using the MALDI-QTOF

After the pre-screening, a MALDI hybrid quadrupole time of flight, or MALDI-QTOF, mass spectrometer was used to acquire the mass spectrum of P-2VP and tandem mass spectra of a selected P-2VP oligomer. This particular instrument is the Q-Tof
The laser on the instrument has a fixed wavelength (337 nm) and will send a pulsed beam to the moving target plate on the sample stage according to the spot selected by the instrument operator. The Q-Tof Ultima™ MALDI is operated using an unattenuated nitrogen laser. The target plate can be moved on the sample stage to have the laser directed in a linear, raster, or user-defined pattern, irradiating the sample to induce the ionization and desorption of the sample. The quadrupole in the Q-Tof Ultima™ MALDI acts as an ion transporter in single mass spectrometry experiments and as a mass selector in tandem mass spectrometry (MS/MS) experiments. Fragmentation of
the selected mass sample occurs in the argon containing collision cell during tandem mass spectrometry experiments. The pusher at the end of the collision cell “pushes” the ions out of the orthogonal acceleration cell, downward into the time-of-flight analyzer, where they travel through the reflectron lens in either V mode (shown in Figure 3.1) or W mode. A microchannel detector plate and ion counting system then detects the ions and outputs the results to the computer running the Q-Tof Ultima™ MALDI. MassLynx software is used to run the Q-Tof Ultima™ MALDI from a desktop computer and also to generate the chromatogram from which the spectra were obtained.

For each of the tests that were run on the Q-Tof Ultima™ MALDI, two rows of the target plate, as shown in Figure 3.2, were spotted with 1 µL of sample per spot. Mass spectra were obtained with an argon pressure of about 10⁻⁵ mbar, collision energies ranging from 10 eV to 90 eV, and the laser running in a linear pattern across each of the spots used on the target plate. For the collection of single mass spectra, the instrument was run with the collision energy set to 10 eV and the high mass and low mass resolution both set to 5. The instrument was calibrated using the exact mass calculated for the 10-mer ion from each of the samples tested. In calculating the mass of a P-2VP oligomer ion, the monoisotopic masses listed in Table 3.1 were used.

After single stage mass spectra were obtained, tandem mass spectrometry experiments were performed on a particular isolated oligomer. First, all isotopes of an oligomer (complete isotopic cluster) were isolated and fragmented and then the monoisotopic oligomer (the one containing only carbon-12) was isolated for fragmentation. To obtain clear spectra, the collision energy was adjusted and the low
mass resolution and high mass resolution were varied. Collision energies and other parameters will be described in Chapter 4.

Figure 3.2. Target plate of the Q-Tof Ultima™ MALDI mass spectrometer.
Table 3.1. Monoisotopic masses used to calculate mass to charge ratios of ions. By definition monoisotopic species contain only the most abundant isotope of the element contained in them.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mass(Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;N</td>
<td>105.057849229</td>
</tr>
<tr>
<td>C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>57.070425288</td>
</tr>
<tr>
<td>C</td>
<td>12.000000000</td>
</tr>
<tr>
<td>H&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.007276452</td>
</tr>
<tr>
<td>H radical</td>
<td>1.007825032</td>
</tr>
<tr>
<td>Li&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.015455953</td>
</tr>
<tr>
<td>N</td>
<td>14.003074005</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>62.929052499</td>
</tr>
<tr>
<td>Ag&lt;sup&gt;+&lt;/sup&gt;</td>
<td>106.90454444</td>
</tr>
</tbody>
</table>
CHAPTER IV
RESULTS AND DISCUSSION

4.1. Mass Spectrometry of Poly(2-vinylpyridine)

Single-stage mass spectrometry was performed on each of the samples using the Q-Tof Ultima™ MALDI mass spectrometer. See Figure 4.1(a) for the mass spectrum obtained from the protonated sample of poly(2-vinylpyridine). The starting mass to charge ratio was set to 250 and the end mass to charge ratio was set to 2200, with an average collision energy of 10 eV. The low mass resolution was set to 5 and the high mass resolution was also set to 5 in transmission mode, which is used to acquire single-stage mass spectra (5/5 = 1). 135 scans were performed on each sample and the mass spectra shown are an average of the most intense scans from the chromatogram shown on the MassLinks software. The laser was set to fire at a rate of 10 Hz. The instrument was calibrated using the calculated mass of the 10-mer of protonated poly(2-vinylpyridine) as an internal standard.

A polymer pattern is apparent with successive oligomers appearing at 105 Da intervals, which is the repeat unit for the poly(2-vinylpyridine). For each of the oligomer peaks, the mass of the oligomer can be deduced by subtracting the mass of the ion added during MALDI. For example, the 10-mer of the protonated sample is observed at mass to charge ratio (m/z) 1109.6639 and thus has a mass of 1109.6639 Da. If the mass of the added proton is subtracted from 1109.6639 Da, the mass of
C₄H₉(C₇H₇N)₁₀H is deduced. The experimentally derived mass agrees excellently with the mass calculated for the 10-mer using the data of Table 3.1:

Experimentally measured mass of 10-mer:

\[1109.6639 \text{ Da} \text{ protonated } C_4H_9(C_7H_7N)_{10}H - 1.0073 \text{ Da} \text{ H} = 1108.6566 \text{ Da}\]

Calculated mass of 10-mer:

\[57.0705 \text{ Da} \text{ C}_4\text{H}_9 + 10(105.0578 \text{ Da} \text{ C}_7\text{H}_7\text{N}) + 1.0078 \text{ Da} \text{ H} = 1108.6563 \text{ Da}\]

The mass spectra of the lithiated and copperated species are shown in Figures 4.1(b) and Figure 4.1(c), respectively. The experimental parameters set on the Q-Tof Ultima™ MALDI for the lithiated and copperated species were similar to the parameters used for the protonated species of the poly(2-vinylpyridine). The mass spectra of the protonated, lithiated, and copperated sample contain oligomer peaks every 105 Da; the mass spectra of each oligomer increases by about 1 Da (protonated), 7 Da (lithiated), and 63 Da (copperated), after MALDI. The 10-mer peaks appear at 1109.6639 Da with the protonated sample, 1115.6716 Da with the lithiated sample, and 1171.5852 Da with the copperated sample. Calculation:

Mass of ionized 10-mer – mass of ion attached = mass of 10-mer

Protonated 10-mer \[1109.6639 \text{ Da} - 1.0073 \text{ Da} = 1108.6566 \text{ Da}\]

Lithiated 10-mer \[1115.6716 \text{ Da} - 7.0155 \text{ Da} = 1108.6561 \text{ Da}\]

Copperated 10-mer \[1171.5852 \text{ Da} - 62.9291 \text{ Da} = 1108.6561 \text{ Da}\]
Figure 4.1. Mass Spectra of P-2VP
(a) Protonated P-2VP
(b) Lithiated P-2VP
Mass spectra for the protonated, lithiated, and copperated samples were also obtained using the Bruker MALDI-TOF mass spectrometer. This was done to first determine the feasibility of running these samples using a time-of-flight mass analyzer, and also to determine the best matrix and solvent for P-2VP. After testing with both DCTB and dithranol, dithranol was chosen as the matrix to be used. There was no evidence that one matrix offered an improvement over the other. A silverated sample was also run using the Bruker MALDI-TOF mass spectrometer with silver trifluoroacetate as the source of the silver ion. Ag⁺ ionization was thought to be problematic, since silver’s mass of 107 Da is similar to that of the repeating unit for P-2VP, which is 105 Da. The presence of a protonated species in the silverated sample could cause difficulties in the interpretation of the data. In comparing the results found with the MALDI-TOF mass spectrometer to those found using the MALDI-QTOF mass spectrometer, there was a distinct improvement in resolution when the MALDI-QTOF instrument was used. In particular, the baseline resolution of the MALDI-QTOF mass spectra was much clearer.

4.1.1. Isotopic Patterns in the Mass Spectra

Several isotopomers are observed for each ionized oligomer. At the low mass end of the spectrum, the all ¹²C isotopomer is the most intense peak among the isotope peaks of an oligomer. At the higher mass end of the spectrum, the carbon-13 satellite (i.e. the isotopomer containing one ¹³C atom) becomes the most intense isotope peak. Based on the composition of P-2VP, C₄H₉(C₇H₇N)ₓH, the intensities of all ¹²C isotopomer and ¹³C-
satellite should become approximately equal between the 12-mer and 13-mer. These carry 88 and 95 carbon atoms, respectively; hence, their $^{13}$C-satellites would have $88 \times 1.1\% = 97\%$ or $95 \times 1.1\% = 105\%$ of the intensity of the corresponding all $^{12}$C isotopomers. In accord with this expectation, in the spectrum for the protonated species, the carbon-13 satellite starts to dominate at the 12-mer and for the lithiated and copperated species, the carbon-13 satellite starts to dominate at the 13-mer. Figure 4.2(a-c) shows an enlarged portion of the original mass spectra where the oligomers in question are enhanced and compared. In the mass spectra for the copperated species, isotopes of the copper become evident in the clustering of four abundant peaks at each oligomer point.

4.2. Tandem Mass Spectrometry of Poly(2-vinylpyridine)

After obtaining the single mass spectra for each of the samples, the 12-mer was selected for fragmentation on the MALDI Q-TOF mass spectrometer. This oligomer was selected because of its high relative abundance. The instrument was adjusted to obtain tandem mass spectra from the complete isotopic cluster of the 12-mer and then adjusted to select out the corresponding all $^{12}$C isotopomer only. A fragmentation pattern became apparent in the spectra.
Figure 4.2. Comparison of isotopic clusters. The first abundant peak in the cluster contains only $^{12}$C atoms. The following peaks contain one, two, three, four, or five $^{13}$C atoms.

(a) 11-mer, 12-mer, and 13-mer of protonated P-2VP
(b) 11-mer, 12-mer, and 13-mer of lithiated P-2VP
(c) 11-mer, 12-mer, and 13-mer of copperated P-2VP
4.2.1. Tandem Mass Spectra of the 12-mer from Protonated P-2VP

CID tandem mass spectra were acquired for both the complete isotopic cluster of [12-mer + H\(^+\)] (see Figure 4.2) as well as the all \(^{12}\)C isotope of the oligomer ion. For obtaining the spectrum of the complete isotopic clusters, the precursor ion mass was set at 1319.79 Da and the start and end masses of the range to be scanned were set to 50 and 1350 Da, respectively. The low mass resolution was adjusted to 5.0 and the high mass resolution was set to 5.0. These parameters were determined at a collision energy of 10 eV. After these parameters were determined, the collision energy was increased to 70 eV to measure the CID tandem mass spectrum shown in Figure 4.3(a). This spectrum was obtained by averaging high intensity scans in the chromatogram calculated by the MassLinks software during spectral acquisition.

After the CID spectrum of the entire isotopic cluster of the protonated 12-mer was obtained, the MALDI Q-ToF was further adjusted to narrow in on the monoisotopic all \(^{12}\)C peak. As was done with the entire isotopic cluster, the precursor ion mass was set at 1319.79 Da. The instrument parameters were then adjusted to narrow the selected mass range to just the 1319.79 Da peak. This was achieved by setting the low mass resolution to 6.0 and the high mass resolution to 16.0, with a collision energy of 68 eV. The low mass was set to 50 Da and the high mass was set to 1350 Da. The mass spectrum resulting by averaging the scans obtained at 68 eV is shown in Figure 4.3(b).

Figure 4.4 shows the precursor ion region after precursor ion isolation. For the spectrum of monoisotopic [12-mer + H\(^+\)], only the peak at m/z 1319.78 (all \(^{12}\)C isotopomer) was selected, while for that of the entire isotopic cluster of [12-mer + H\(^+\)], all isotopes (and a few adjacent minor peaks) were selected. Comparison of the isotopic
patterns in Figure 4.2 (center) and Figure 4.4 (top) reveals that turning on the precursor ion isolation potentials and increasing the collision energy distorts the isotopic pattern. In the CID spectrum of the entire isotopic cluster, the fragment peaks also contain isotopomers and, thus, are not as well resolved as the fragment ion peaks in the CID spectrum of monoisotopic [12-mer + H]^+.

4.2.2. Tandem Mass Spectra of the 12-mer from Lithiated P-2VP

From the lithiated sample of P-2VP, the 12-mer was again selected for CID. Specifically, the precursor ion mass was set at 1325.8026 Da. To transmit the complete isotopic cluster, the low mass resolution was set to 5.0 and the high mass resolution was set to 5.0. The low mass was set to 50 Da and the high mass was set to 1350 Da. A collision energy of 10 eV was used for these adjustments; later it was increased to 90 eV to acquire the MS/MS spectrum. Figure 4.5(a) shows the fragmentation pattern from the complete isotopic cluster of [12-mer + Li]^+; this spectrum is an average of the high intensity scans identified in the CID chromatogram.

For the CID spectrum of the all ^12C isotopomer of [12-mer + Li]^+, the low mass resolution was adjusted to 6.0 and the high mass resolution was adjusted to 16.0. All other parameters remained the same as for the complete isotopic cluster. The fragmentation pattern generated from monoisotopic [12-mer + Li]^+ is shown in Figure 4.5(b).
4.2.3. Tandem Mass Spectra of the 12-mer from Copperated P-2VP

Also from the copperated sample of P-2VP, the 12-mer peak was selected for fragmentation. In this case, the precursor ion mass was set at 1381.6874 Da. For selection of the entire isotopic cluster, the low mass resolution was set to 5.0 and the high mass resolution was set to 5.0. The low mass was set to 50 Da and the high mass was set to 1400 Da. The collision energy was held at 10 eV for these adjustments and then increased to 90 eV to acquire the MS/MS spectrum. Figure 4.6(a) shows the fragmentation pattern obtained when the complete isotopic cluster is isolated.

For the selection of the monoisotopic [12-mer + $^{13}$Cu]$^+$ (all $^{12}$C isotopomer) the low mass resolution was adjusted to 6.0 and the high mass resolution was adjusted to 16.0. All other parameters remained the same as for the complete isotopic cluster. The resulting fragmentation pattern is shown in Figure 4.6(b).
Figure 4.3. Tandem mass spectra of the protonated 12-mer from P-2VP
(a) Fragmentation pattern from CID of the complete isotopic cluster of [12-mer + H]^+
(b) Fragmentation pattern from CID of the all $^{12}$C isotopomer, i.e. of monoisotopic [12-mer + H]$^+$
Figure 4.4. Precursor ion isolation for MS/MS. Complete isotopic cluster (top) and all $^{12}$C isotopomer (bottom) of $[12\text{-mer} + H]^+$ from P-2VP
Figure 4.5. Tandem mass spectra of the lithiated 12-mer from P-2VP
(a) CID on complete isotopic cluster of [12-mer + Li]⁺
(b) CID on all $^{12}\text{C}$ isotopomer of $[12\text{-mer + }^{7}\text{Li}]^{+}$
Figure 4.6. Tandem mass spectra of the copperated 12-mer from P-2VP
(a) CID from complete isotope cluster of [12-mer + Cu]
(b) CID on all $^{12}$C isotopomer of $[12\text{-mer} + {^{63}\text{Cu}}]^+$
4.3. Fragmentation Series of the P-2VP 12-mer

To make spectral interpretation easier to understand, each of the tandem mass spectra was broken down into 12 sections. The breakdown for the spectra acquired from the all $^{12}\text{C}$ isotopomer of the protonated, lithiated, and copperated 12-mer is shown in Figure 4.7. Section one of the spectrum contains the smallest fragments formed. Section twelve in each spectrum includes the isolated monoisotopic precursor ion. Sections two through eleven include fragments with two through eleven units, respectively. The same division was applied to the spectra acquired from the corresponding complete isotopic clusters of the 12-mer.

Figure 4.7. Regions 1-12 in the CID tandem mass spectra of three different 12-mer ions from P-2VP. The monoisotopic (all $^{12}\text{C}$) 12-mer ions were selected for CID.
The different P-2VP ions fragment in a unique pattern. Figure 4.8 shows the fragmentation pattern for the middle mass range (sections five and six) for each of the three samples. The fragment ion series observed in Figure 4.8 have been labeled following the nomenclature explained in Figure 4.9. The P-2VP polymer chains contain two types of backbone bonds, marked I and II in Figure 4.9. Cleavage of bond I with charge retention on the piece carrying the initiating end group produces series a; if the charge is retained on the piece carrying the terminating chain end, series b is generated.

Similarly, cleavage of bond II gives rise to series c (carrying the initiating chain end) and d (carrying the terminating chain end). Homolytic scission of bonds I and II initially leads to radical ions $a_n^\bullet$, $b_n^\bullet$, $c_n^\bullet$, and $d_n^\bullet$, where the $^\bullet$ superscript denotes the unpaired electron and the $n$ subscript the number of repeat units contained in the fragment. Hydrogen atom rearrangements can take place in these fragments, as will be discussed later, to produce the closed-shell fragment ion series dominating the middle and higher mass regions of the MS/MS spectra. The structures resulting from such rearrangements from CID of the protonated polymer are given in Figure 4.10.

The protonated sample only forms the fragment ion series depicted in Figure 4.10, which are also the predominant fragments from the lithiated and copperated precursor ions. Additionally, the Li$^+$ and Cu$^+$ adducts dissociate to yield several minor fragment distributions, whose likely structures are included in Figure 4.11.
Figure 4.8. Expanded middle range (sections 5 and 6) of the tandem mass spectra acquired from the all $^{12}$C isotopomer of the protonated, lithiated, and copperated P-2VP 12-mer.
Figure 4.9. Symbols for fragment ions in the CID mass spectra of P-2VP oligomers. The Roman numbers denote the different types of backbone bonds in the polymer.
Figure 4.10. Proposed structures of the fragment ion series generated from protonated P-2VP.
Figure 4.11. Proposed structures of the minor fragment ion series generated from lithiated and copperated P-2VP (X = Li or Cu). \( \phi^\bullet \) designates the pyridyl radical, \( \text{C}_5\text{H}_4\text{N}^\bullet \) (78 Da).
4.3.1. Start of Series (Fragments of Lowest Mass)

The lower mass range in the MS/MS spectrum of the protonated sample is different from the corresponding range in the spectra of the lithiated or copperated sample. Figure 4.12(a) shows section one from the CID tandem mass spectra for the monoisotopic protonated, lithiated, and copperated samples. The fragment at m/z 223.15, an internal [bc]o ion (see next section) dominates the spectrum of the protonated sample, but is much less intense in the spectra of the lithiated (m/z 229.16) and copperated (m/z 285.08) samples. This particular fragment appears to be formed very efficiently from the protonated sample. The d1+ fragment (a radical ion) is particularly abundant in the spectra of both the lithiated and copperated samples. This ion does not appear in the spectrum of the protonated P-2VP. Fragment d1+ has a mass of 204.15 Da when it is lithiated and a mass of 260.07 Da when it is copperated. It also should be noted that no other member of the d0+ series is formed from either the Li+ or the Cu+ adduct of P-2VP. Other radical ions observed in the MS/MS spectra of the lithiated and copperated samples are a1+ from the Li+ adduct and a0+ -16, a0+ -2, and a1+ from the Cu+ adduct, see Figures 4.12(a) and 4.13; the latter Figure contains section two of the CID tandem mass spectra.

The most abundant fragments from lithiated and copperated P-2VP 12-mer appear at m/z 322.2 and 378.14, respectively and correspond to b2 ions; b2 is also quite intense with the protonated sample (m/z 316.20). Also b1 is a significant fragment from all three P-2VP precursor ions. The high relative intensity of b1 and, especially, b2 is partly due to the fact that they overlap with internal fragments of the same structure,
Figure 4.12. Section one of the MS/MS spectra from monoisotopic precursor ions
(a) Comparison of fragmentation patterns from protonated, lithiated and copperated P-2VP. See Chapter
4.3.2 for the mechanisms leading to these fragments and a more detailed discussion of the
nomenclature used.
namely [ab]₁ and [ab]₂, respectively (see schemes later in thesis). Additionally, b₂ (or [ab]₂) may overlap with an isomeric internal ion, [ba]₂, originating from consecutive decompositions, as will also be explained later. The structures of fragments d₁⁺ and [ba]₂ are provided in Figure 4.12(b).

(a) Proposed structures for fragments d₁⁺ and [ba]₂.
Figure 4.13. Section two of the MS/MS spectra from the monoisotopic protonated, lithiated, and copperated P-2VP 12-mer. See chapter 4.3.2 for the mechanisms leading to these fragments and a more detailed discussion of the nomenclature used.
4.3.2. Decomposition Mechanisms of the P-2VP Oligomer Ions

P-2VP oligomers have not been studied previously by MS/MS, but the fragments identified in their spectra are similar to those detected by Jackson et al. in the MS/MS spectra of Ag$^+$ cationized polystyrene (PS) oligomers.$^{14}$ The nomenclature used to describe the PS fragments (Figure 2.2) is less systematic than the one used in this thesis, which is explained in Figures 4.9, 4.10, 4.11, and 4.12(b). The mechanisms proposed by Jackson et al. to rationalize the fragmentations of silverated PS oligomers involved H- rearrangements in closed-shell systems via (mainly) 6-membered and 4-membered rings.$^{14}$ Such reactions are not observed upon the thermal degradation of PS, which is rather dominated by typical radical reactions, including homolytic bond scissions, H$^\bullet$ rearrangements via (mainly) 6-membered rings, $\beta$-scissions, disproportionations, recombinations, and radical additions.$^{16,17,18,19,20}$

Protonated species with stable charge sites as well as molecules ionized by alkali metal ions or d$^{10}$ transition metal ions have been shown to decompose upon CID by charge-remote fragmentations, i.e. reactions in which the charge does not directly participate.$^{21}$ Although such reactions were originally believed to proceed predominantly via concerted H-rearrangements, similar to those proposed by Jackson et al. for the [PS + Ag]$^+$ fragmentations, there has been increasing evidence that charge-remote gas-phase reactions proceed through radical intermediates, especially when promoted by low-energy CID under multiple collision conditions.$^{21,22,23}$ The mechanisms presented in this thesis involve such radical intermediates to account for the observed fragments.
The dissociations of the X⁺ (X = H, Li, Ag) adducts of the P-2VP 12-mer (1) are proposed to begin with homolytic C-C bond cleavage to produce two radicals that are held loosely together by X⁺. Depending on which backbone bond is broken in this process, two different radical combinations are possible, 2 and 3, as explained in Scheme 4.1. Interligand H⁺ transfer reactions can take place in the X⁺ bound radical complexes 2 and 3. Alternatively, these assemblies may dissociate to yield the incipient radical ions aₙ⁺, bₙ⁺, cₙ⁺, and dₙ⁺, which can rearrange intramolecularly and dissociate further if sufficient internal energy is available.

In the radical complexes 2 and 3, benzylic H⁺ atoms can be transferred from one ligand to the other to form saturated or olefinic chain ends. Dissociation after such intermolecular (interligand) H⁺ rearrangement provides a route to fragments aₙ + 2 and bₙ from 2 and to fragments cₙ and dₙ + 2 from 3 (see Scheme 4.2). Consecutive decomposition of these initial fragments according to the same mechanisms produces smaller fragments of the same kind or internal fragments, i.e. fragments that do not contain any of the original end groups. Scheme 4.3 illustrates such a case, involving a bₙ ion (formed by type-I bond cleavage) that decomposes sequentially via a type-II bond cleavage. From the original precursor ion 1, the latter cleavage would have led to cₙ and dₙ + 2 fragment ions, containing the initiating and terminating chain ends, respectively. From bₙ (which lacks the initiating chain end), the same bond cleavage generates an internal [bc]ₙ ion or a smaller dₙ + 2 ion, instead. Note that [bc]ₙ ions can also arise from type-I bond cleavage in cₙ. Because P-2VP does not have a unique terminating chain end (i.e. a chain end other than -H), several types of internal fragments have the same structures as bₙ or dₙ + 2 ions (Figure 4.14).
The interligand H⁺ transfers presuppose that the X⁺ bound radicals 2 and 3 are bound strongly enough to survive for the time needed for such reactions. If these complexes do not have the necessary lifetimes for intermolecular rearrangement, dissociation to the radical ions aₙ⁺, bₙ⁺, cₙ⁺, or dₙ⁺ would take place. Intramolecular H⁺ transfers in these radical ions, followed by β scissions provide alternative pathways to some of the MS/MS fragments of the P-2VP ions and account for the fragment ion series that cannot be generated via the interligand H⁺ rearrangements discussed thus far.

A 1,5-H⁺ rearrangement in aₙ⁺ (back-biting) yields isomeric ion 4 (Scheme 4.4), which can undergo β C-C bond scissions to generate a terminal cₙ ion (top left) or shorter aₙ⁺ radical ion (bottom left). These dissociations release small, internal fragments, which may also retain the added charge X⁺. The internal fragments, arising from aₙ⁺ are the radical ion [ad]₁⁺, and the closed-shell species [ab]₂ (Scheme 4.4). The acronyms give the sequence of bond cleavages leading to the internal fragments as well as the number of repeat units in the fragments. The common first letter in [ad]₁⁺ and [ab]₂ designates that both fragments originate from an a-type ion. The second letter indicates the type of bond broken in the a-type ion to yield the internal fragments; cleavage of a type-II bond (see Figure 4.9) leads to [ad]₁⁺, while cleavage of a type-I bond leads to [ab]₂. Because the P-2VP studied has an –H terminating end group, [ad]₁⁺ and [ab]₂ are identical with the terminal fragments d₁⁺ and b₂, respectively.

Back-biting and subsequent β C-C bond scissions in bₙ⁺ produce a shorter bₙ⁺ ion (Scheme 4.5, top right) or a terminal dₙ ion (bottom right). Here, the internal, small fragments coproduced are the [ba]₂ ion and the [bc]₀ radical ion, respectively. The
Scheme 4.1. Initial step in the fragmentation of P-2VP oligomer ions, involving hemolytic C-C bond scission in the polymer backbone.
Scheme 4.2. Intermolecular H⁺ rearrangements in X⁺ bound radical complexes 2 and 3, leading to the fragment ion series an + 2 and bn (from 2) or cn and dn + 2 (from 3).
Scheme 4.3. Consecutive dissociation of $b_n$ via cleavage of one of its type-II bonds, yielding an internal $[bc]_n$ and a terminal $d_n + 2$ fragment according to the mechanisms outlined in Schemes 4.1 and 4.2.
latter ion is not detected in the MS/MS spectra. Unlike d1\textsuperscript{•}, which contains a stable benzyl radical, [bc]0\textsuperscript{•} is a primary alkyl radical and, thus, reactive towards further fragmentation, reducing the m/z value below the detectable limit for the instrument used (~10-15 % of the precursor ion’s m/z value).

In b\textsubscript{n}\textsuperscript{•}, the aromatic substituent is in β position to the unpaired electron. This enables an alternative β scission, involving the aromatic substituent, as depicted in Scheme 4.6. The resulting product ions carry the terminating end group and a vinyl group at the other chain end. A very similar process is possible in c\textsubscript{n}\textsuperscript{•} radical ions, which also contain an aromatic substituent β to the radical site (Scheme 4.7). Homolytic bond cleavage of this substituent creates product ions that still carry the initiating end group and an allyl group at the other chain end.

Figure 4.14. Internal (a) b-type and (b) d-type ions generated by sequential type-I or type-II bond cleavages according to Schemes 4.1 and 4.2.

\[ b_n = [ab]_n, [ba]_n, [dc]_n \quad d_n + 2 = [ad]_n + 2, [da]_n + 2, [cd]_n + 2 \]
Scheme 4.4. Intramolecular 1,5-H* rearrangement (back-biting) followed by β C-C bond scission in initial radical ion fragment \( a_n \). Because P-2VP does not contain a unique terminating end group, the internal fragments \([ad]_1\) and \([ab]_2 \) match the terminal fragments \( d_1 \) and \( b_2 \), respectively.
Scheme 4.5. Intramolecular 1,5-H\(^+\) rearrangement (back-biting) followed by \(\beta\) C-C bond scission in initial radical ion fragment \(b_n^+\). The internal ion \([ba]_2\) is an isomer of the \([ab]_2\) ion shown in Scheme 4.4.
Scheme 4.6. Dissociation of initial radical ion fragment $b_n^\bullet$ via $\beta$ bond scission in the backbone substituent.

Scheme 4.7. Dissociation of initial fragment ions $c_n^\bullet$ via $\beta$ bond scission in the backbone substituent.
Scheme 4.8. Intramolecular 1,5-\(\text{H}^\bullet\) rearrangement (back-biting) followed by \(\beta\) C-C bond scission in initial radical ion fragment \(c_n^\bullet\). The internal ions \([\text{cd}]_2^\bullet\) and \([\text{cb}]_0^\bullet\) are identical with the internal ions \([\text{ba}]_2^\bullet\) and \([\text{bc}]_0^\bullet\), respectively, depicted in Scheme 4.5.
The back-biting and $\beta$ C-C bond scission reactions ensuing from $c_n^*$ are summarized in Scheme 4.8. They produce a terminal $a_n$ ion (top left) or a shorter $c_n^*$ radical ion (bottom left). The small, internal fragment coproduced with the former is $[cb]_0^*$ and that coproduced with the latter is $[cd]_2$. The radical ion $[cb]_0^*$ is identical with the $[bc]_0^*$ fragment arising from $b_n^*$ (Scheme 4.5). Similarly, the $[cd]_2$ fragment is identical with the internal $[ba]_2$ fragment generated from $b_n^*$, and both are isomers of the terminal $b_2$ fragment shown in Scheme 4.4.

Finally, the back-biting and $\beta$ scission reactions possible from $d_n^*$ (Scheme 4.9) give rise to a shorter $d_n^*$ radical ion (top right) or a terminal $b_n$ ion (bottom right) and coproduce internal $[ab]_2$ ions (top left) or $[da]_1^*$ radical ions (bottom left). It has been mentioned that $[ab]_2$ has the same structure as a terminal $b_2$ ion. Analogously, $[da]_1^*$ has the same structure as a terminal $d_1^*$ ion, which was also true for the internal $[ad]_1^*$ radical ion generated from $a_n^*$ (Scheme 4.4).

It is evident from Schemes 4.4, 4.5, 4.8, and 4.9 that intramolecular $H^*$ rearrangements and consecutive $\beta$ scissions in $a_n^*$, $b_n^*$, $c_n^*$, or $d_n^*$ partly regenerates such radical ions (with shorter chain length), which can undergo anew the same reactions. The closed-shell products $a_n$, $b_n$, $c_n$, or $d_n$ arising from the back-biting/$\beta$ scission sequences also can fragment consecutively, if still energetically excited; the latter consecutive fragmentations provide a route to larger internal fragment ions, as outlined in Scheme 4.10.
Scheme 4.9. Intramolecular 1,5-H* rearrangement (back-biting) followed by β C-C bond scission in initial radical ion fragment dₙ*. The internal radical ions [da]₁* is identical with the internal radical ion [ad]₁* shown in Scheme 4.4 and with terminal ion d₁*. 
Scheme 4.10. Formation of a terminal $b_n$ ion via homolysis, back-biting, and $\beta$ scission, and consecutive fragmentation of this $b_n$ to internal $[bc]_n$ ion via a second sequence of homolysis, back-biting, and $\beta$ scission.
4.3.3. Rationalization of the MS/MS Fragmentation Patterns

The proportion of P-2VP oligomer ions dissociating through intermolecular H⁺ rearrangements in the X⁺ bound complexes (Schemes 4.2 and 4.3) depends on the stability of the latter complexes, which in turn is mainly determined by the X⁺ affinity of the two radical ligands. The most likely attachment sites of the cationizing species X⁺, namely H⁺, Li⁺, or Cu⁺, are the pyridine substituents of P-2VP. Based on the reported proton and lithium ion affinities of pyridine, which are 930 kJ/mol²⁴ and 183 kJ/mol²⁵ respectively, H⁺ is bound much more strongly than Li⁺. The copper (I) affinity of pyridine is unknown. That of benzene is 218 kJ/mol,²⁶ while the Li⁺ affinity of benzene is 164 kJ/mol.²⁷ Assuming a similar difference in Li⁺ and Cu⁺ affinities for pyridine, the copper (I) affinity of the latter is estimated at 230-240 kJ/mol. Thus, Cu⁺ is bound more strongly by pyridine than Li⁺, but both metal ions form significantly weaker bonds compared to H⁺. With these affinity estimates at hand, it is concluded that proton-bound radical complexes are thermodynamically more stable and, hence, have longer lifetimes than the corresponding Li⁺ or Cu⁺ bound analogs.

Consistent with the above prediction, all fragment ions in the CID spectra of protonated P-2VP oligomers can be rationalized by dissociation via interligand H⁺ transfers within H⁺ bound radical complexes, as explained in Scheme 4.2. Protonation makes the pyridine ring a strong electron-withdrawing substituent, which should weaken the nearest backbone C-C bonds, promoting their homolytic cleavage to form proton-bound complexes of the resulting radicals (2 and 3 in Schemes 4.1 and 4.2 with X = H). Interligand H⁺ rearrangements in the complexes lead to the complementary
fragment ion series $a_n + 2$ and $b_n$ or series $c_n$ and $d_n + 2$ (Scheme 4.2). Throughout the
MS/MS spectra, series $b_n$ and $c_n$, which contain olefinic chain ends, are more intense
than the corresponding complementary series $a_n + 2$ and $d_n + 2$ (see top parts in Figures
4.8, 4.12, and 4.13). This trend is attributed to a higher proton affinity for the P-2VP
chains containing olefinic end groups.

Further fragmentation of the dominant $b_n$ and $c_n$ series by the same mechanisms
leads to the internal $[bc]_n$ series (Scheme 4.3), which becomes more abundant in the
lower m/z region of the MS/MS spectra (compare Figures 4.12 and 4.13 vs. Figure 4.8),
in agreement with its generation by consecutive dissociations. Note that $[bc]_n$ ions
(from $b_n$ ions fragmenting by type-II bond cleavage, as shown in Scheme 4.3) and $[cb]_n$
ions (from $c_n$ ions fragmenting via type-I bond cleavage) have the same structure.

At a first glance, the MS/MS spectra of lithiated and copperated P-2VP look
similar to that of protonated P-2VP (see Figures 4.7 and 4.8). Closer inspection
however, reveals several differences:

(a) the metalated ions ($X = Li, Cu$) produce additional fragment ion series with
closed-shell structures, specifically $a_n$, $d_n$, $[b_n^\bullet - \phi^\bullet]$, and $[c_n^\bullet - \phi^\bullet]$, as indicated in
Figure 4.8;

(b) the metalated ions also give rise to abundant $d_1^\bullet$ radical ions and to less
abundant $a_1^\bullet$ and $a_0^\bullet$ ($X = Li$) or $a_0^\bullet - 2$ ($X = Cu$) radical ions. Figures 4.12 and 4.13
attest that radical ions are not observed from the corresponding protonated oligomers;

(c) the dominant fragment from $[12-mer + H]^+$ is $[bc]_0$ (Figures 4.7 and 4.13). In sharp contrast, $[bc]_0$ has very low relative abundance from the metalated precursor
ions, whose spectra are dominated by $b_2$ and $d_1^\bullet$ ions (Figures 4.7 and 4.12).
The described differences clearly show that the metalated precursor ions fragment, at least partly, through pathways that are not traversed by the protonated precursor ions upon CID. The back-biting/β scission mechanisms discussed in the previous section (Schemes 4.4 – Schemes 4.10) can reconcile the unique MS/MS characteristics of the metalated systems. Because of their lower thermodynamic stabilities, the Li^+ and Cu^+ bound radical complexes arising from initial backbone C-C bond cleavages (Scheme 4.1) dissociate readily to the isolated radical ions a\textsubscript{n}^•, b\textsubscript{n}^•, c\textsubscript{n}^•, and d\textsubscript{n}^•, which can ultimately generate, through sequential back-biting and β scission pathways, the new fragment ion series a\textsubscript{n} (Scheme 4.8), d\textsubscript{n} (Scheme 4.5), [b\textsubscript{n}^• - φ^•] (Scheme 4.6), and [c\textsubscript{n}^• - φ^•], (Scheme 4.7). With all four initial radical ions (a\textsubscript{n}^•, b\textsubscript{n}^•, c\textsubscript{n}^•, and d\textsubscript{n}^•), the intramolecular H\textsuperscript{+} rearrangements and β C-C bond scission steps coproduce smaller radical ions of the same kind, which can repeat the same steps (Schemes 4.4, 4.5, 4.8, and 4.9). This explains why the only radical ions actually observed in the MS/MS spectra are d\textsubscript{1}^•, a\textsubscript{1}^•, and a\textsubscript{0}^•, in which back-biting is either impossible or sterically constrained. The particularly high abundance of d\textsubscript{1}^• must originate from production of this radical ion (or an identical internal ion) during the decomposition of both a\textsubscript{n}^• as well as d\textsubscript{n}^• ions (see Schemes 4.4 and 4.9). Further, all terminal radical ions (a\textsubscript{n}^•, b\textsubscript{n}^•, c\textsubscript{n}^•, d\textsubscript{n}^•) can yield internal ions that have the b\textsubscript{2} or an isomeric structure (see Schemes 4.4, 4.5, 4.8, and 4.9). This nicely accounts for the high intensity of this fragment (basepeak) in the MS/MS spectra of lithiated and copperated P-2VP.

Both, the Li^+ and the Cu^+ adduct of P-2VP dissociate to yield a\textsubscript{n} + 2 and d\textsubscript{n} + 2 fragment ions (Figure 4.8), although to a lower extent than protonated P-2VP.
Fragments $a_n + 2$ and $d_n + 2$, which contain saturated chain ends, cannot be formed through back-biting/β scission reactions. Hence, a significant fraction of lithiated and copperated P-2VP must dissociate via the interligand $H^\bullet$ rearrangements, which can lead to $a_n + 2$ and $d_n + 2$ ions (see Scheme 4.2). Overall, the MS/MS results provide evidence that protonated P-2VP exclusively dissociates via interligand $H^\bullet$ rearrangements, whereas lithiated and copperated P-2VP dissociate via both interligand $H^\bullet$ rearrangements and intramolecular $H^\bullet$ rearrangements (back-biting) combined with β C-C bond scissions.
MALDI-QTOF mass spectrometry proved to be an effective method for analyzing poly(2-vinylpyridine). Both, clear mass spectra as well as clear tandem mass spectra of the polymer were obtained. By using different ions, such as hydrogen, copper, and lithium cations, the overall MS/MS fragmentation patterns could be elucidated and the specific dissociations favored by each type of precursor ion could be explained.

Further studies could be performed on the P-2VP using MALDI-QTOF mass spectrometry. One study might include isolating one of the peaks occurring between the main oligomer peaks in the mass spectra. A study of these smaller peaks may help to identify the end groups and structure of the corresponding oligomers, which in turn may provide more insight into the properties of this polymer or into the capabilities of the MALDI-QTOF instrumentation.

A second study might focus on a different oligomer of the main distribution, besides the 12-mer used in this research. The fragmentation pattern should be similar for a different oligomer, but a comparison could be made of the intensities of the fragment peaks that are formed. Also, the proposed mechanisms for fragmentation of the P-2VP ions could be either reinforced or amended by the characteristics found with other oligomers. The quadrupole time-of-flight mass spectrometer has been used very
little for analysis of polymers, so far. Further studies should be performed to maximize
the potential uses of the instrument. As for the proposed mechanisms, further studies on
other types of polymers are necessary in order to determine whether they are generally
valid or specific to the P-2VP connectivity.
BIBLIOGRAPHY


