New Methods and Instrumentation for Electrospray Ionization – Mass Spectrometry

by

BRADLEY B. SCHNEIDER

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Department of Chemistry

We accept this thesis as conforming to the required standard

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Department of Chemistry

The University of British Columbia
Vancouver, Canada

Date Apr. 23, 2002
Abstract

A current challenge in the field of ESI-MS is the development of high-throughput methods and devices to use a mass spectrometer more efficiently. This research provides means to improve the interface between the ion source and the mass spectrometer, and insights into the mechanism involved as ions traverse the interface region. In addition, new methods and instrumentation to improve the sensitivity, stability, and throughput of an ESI-MS are developed.

This thesis deals with improving the motion of ions within the atmospheric pressure source region and the interface region of an ESI-MS. The first section describes the development of a model for the determination of the rates of collision-induced dissociation (CID) of biomolecules within the interface region of an ESI-MS. The model describes the variation of the gas number density within the first stage of the vacuum system. Various factors, such as the mass of the collision gas, the mass of the ion, the collision energy, and the properties of the collision gas are investigated. This model is used to predict the degree of fragmentation for biomolecules with various instrumental parameters. The mechanisms leading to ion fragmentation in the interface region are investigated in order to increase the degree of structural information that can be obtained from ESI-MS.

The second part of this thesis introduces a new method of focusing ions generated within the atmospheric pressure ion source region. This section describes the development of improved ESI sources based upon the incorporation of an atmospheric pressure ion lens near the tip of the electrospray capillary. The new ion sources are more stable and more sensitive than previous ones. Various parameters, such as lens potential,
lens position, sprayer orientation, and solution flow rate are examined. The new ion
sources are particularly beneficial for the analysis of protein digests because the lens
increases the ratio of multiply charged ions relative to singly charged ions. In addition,
the background noise is decreased and the positional requirements for the sprayer are
reduced.

The third part describes the extension of the ion lens technology to address issues
in high-throughput ESI-MS. This section describes the use of potentials applied to
atmospheric pressure ion lenses to enable and disable multiple sprayer ion sources. New
two and four-sprayer ion sources are developed and tested. These multiple sprayer ion
sources greatly increase the sample throughput when compared to commercial single
sprayer ion sources. The mechanical blocking devices that are currently used for
indexing of commercial multiple sprayer ion sources are no longer needed. This simple
setup is much less expensive, and can very rapidly enable and disable the individual
sprayers. In addition, these new high throughput ion sources are capable of
simultaneously sampling ions from more than one electrospray capillary.
Table of Contents

Abstract ii
Table of Contents iv
List of Tables vii
List of Figures viii
List of Abbreviations xii
Glossary xv
Preface xvii
Acknowledgements xix

Part I: Electrospray Ionization 1

Chapter 1: Electrospray Ionization - Mass Spectrometry 3
1.1 Interface Designs 3
1.2 Mass Analyzers 5
1.3 Mass Spectrometers Used in this Thesis Work 5
1.4 References 9

Part II: Ion Fragmentation within the Interface Region of an Electrospray Ionization Mass Spectrometer 12

Chapter 2: Collision - Induced Dissociation of Cyclodextrins within the Orifice - Skimmer Region of an ESI-MS 14
2.1 Purpose 14
2.2 Experimental 14
2.3 Gas Dynamics 16
2.4 Gas Number Density Within the Expansion 18
2.5 Calculation of the Number of Collisions for an Ion 19
2.6 Collision Dynamics 22
2.7 Fragmentation Data for the Cyclodextrins 28
2.8 Energy Requirement for Dissociation 31
2.9 Comparison of Theory and Experiments 31
2.10 Sources of Error 35
2.11 Conclusions 38
2.12 References 38

Chapter 3: Collision - Induced Dissociation of Bradykinin within the Interface Region of an ESI-MS 41
3.1 Purpose 41
3.2 Experimental 41
3.3 Gas Dynamics 42
3.4 Number of Collisions 43
3.5 Collision Dynamics 44
3.6 Energy Requirement for Dissociation 46
3.7 Fragmentation Data for Bradykinin 48
6.1 Purpose 102
6.2 Experimental 103
6.3 Direct Infusion Experiments 104
6.4 Nano-HPLC Data 112
6.5 Conclusions 115
6.6 References 116

Chapter 7: An Atmospheric Pressure Ion Lens Improves a Nebulizer Assisted Electrospray Ion Source 117
7.1 Purpose 117
7.2 Experimental 117
7.3 Electric Field Modeling for a Nebulizer Assisted Electrospray Ion Source 120
7.4 Optimization of the Ion Spray Source 122
7.5 Lens Sizes and Orientations 123
7.6 Ion Source Stability with the Lens 125
7.7 Sensitivity of the Ion Source with the Lens 125
7.8 Variation of Peptide Ion Signal with Lens Potential 127
7.9 Sprayer Positional Dependence with the Lens in Place
   7.9.1 Vertical Dimension 129
   7.9.2 Horizontal Dimension 131
   7.9.3 Re-optimization of the Source Potentials 133
7.10 Conclusions 135
7.11 References 136

Part IV: High Throughput Ion Sources 137

Chapter 8: A New Multiple Sprayer Ion Source for High Throughput ESI-MS 140
8.1 Purpose 140
8.2 Experimental 140
8.3 Orientation of the Sprayers 145
8.4 Prototype 1
   8.4.1 Enabling and Disabling a Sprayer 146
   8.4.2 Operation of Prototype 1 148
8.5 Prototype 2
   8.5.1 Comparisons of Signal Magnitude from One Channel of a Four-Sprayer System and a Single Sprayer 150
   8.5.2 Enabling and Disabling a Sprayer 151
   8.5.3 Operation of Prototype 2 with a Single Electrospray Power Supply 153
   8.5.4 Operation of Prototype 2 with Separate Electrospray Power Supplies 157
8.6 Conclusions 160
8.7 References 161

Concluding Remarks 164
List of Tables

Table 2.9.1 Working curves for internal energy vs. orifice – skimmer voltage difference for the cyclodextrins in the two mass spectrometers 33
Table 2.9.2 Predicted and observed orifice – skimmer voltage differences for the two mass spectrometers 34
Table 3.8.1 Predicted and observed orifice voltages for 10% dissociation of protonated bradykinin in three different mass spectrometers 55
Table 7.5.1 Dimensions of the oblong-shaped ion lenses 124
List of Figures

Figure 1.0.1 Schematic of an electrospray ion source 1
Figure 1.1.1 Schematic of an electrospray ion source with an interface incorporating a curtain plate 3
Figure 1.1.2 Schematic of an electrospray ion source with an interface incorporating a heated capillary inlet 4
Figure 1.3.1 Schematic of the prototype single quadrupole mass spectrometer 5
Figure 1.3.2 Schematic of the prototype triple quadrupole mass spectrometer 6
Figure 1.3.3 Schematic of a linear ion trap time-of-flight mass spectrometer 7
Figure 1.3.4 Schematic of a SCIEX QSTAR™ mass spectrometer 8
Figure 2.2.1 Schematic of a reduced flow rate electrospray capillary 15
Figure 2.3.1 A free jet expansion within the orifice – skimmer region of an electrospray mass spectrometer 16
Figure 2.5.1 Variation of gas number density at various distances from the start of the free jet within the orifice – skimmer region of the triple quadrupole mass spectrometer 20
Figure 2.6.1a Center of mass energy per collision for β-CD at varying orifice – skimmer voltage differences on the single quadrupole MS 25
Figure 2.6.1b Center of mass energy per collision for β-CD at varying orifice – skimmer voltage differences on the triple quadrupole MS 25
Figure 2.6.2 Center of mass energy converted to internal energy of a β-CD ion per 80 μm region from the origin of the free jet on the single quadrupole mass spectrometer 26
Figure 2.7.1 Mass spectra for β-CD in ammonium acetate solutions at various orifice - skimmer voltage differences, 30, 50, 70, and 90 V, on the triple quadrupole mass spectrometer 29
Figure 2.9.1 Energy converted to internal energy of the β-cyclodextrin ion versus the orifice – skimmer potential difference applied on the single quadrupole mass spectrometer 32
Figure 2.9.2 Energy converted to internal energy of the β-cyclodextrin ion versus the orifice – skimmer potential difference applied on the triple quadrupole mass spectrometer 32
Figure 2.10.1 Structure of β-cyclodextrin 36
Figure 3.5.1 Center of mass energy converted to internal energy of bradykinin ion per 80 μm region from the origin of the free jet on the single quadrupole mass spectrometer 46
Figure 3.7.1 Structure of the peptide bradykinin and its fragments 49
Figure 3.7.2 Fragment ion spectra of singly protonated bradykinin at varying ion collision energies in the triple quadrupole mass spectrometer 50
Figure 3.7.3 Fragmentation spectra of singly protonated bradykinin (m/z = 1061) utilizing various potential differences (62, 86, and 112 V) between the orifice and the skimmer of the single quadrupole mass spectrometer 52
Figure 3.7.4 Percent fragmentation of singly protonated bradykinin with an increase in the orifice - skimmer potential difference on the
single quadrupole (a), triple quadrupole (b), and LIT-TOF (c) mass spectrometers

Figure 4.4.1  Calculated center of mass energy input to bradykinin ions at various potential differences between the orifice and the skimmer of the triple quadrupole mass spectrometer

Figure 4.4.2  Fragmentation curves for bradykinin in the collision cell of a triple quadrupole mass spectrometer

Figure 4.5.1  Fragmentation curves for bradykinin ions versus the potential difference between the orifice and the skimmer of the triple quadrupole mass spectrometer with nitrogen (Δ), argon (o), and krypton (+) curtain gases, and an orifice - skimmer spacing of 3 mm

Figure 4.8.1  Fragmentation curves for bradykinin ions versus the potential difference between the orifice and the skimmer of the single quadrupole mass spectrometer with nitrogen (Δ), and argon (o) curtain gases, and an orifice - skimmer spacing of 1.7 mm

Figure 4.9.1  Fragmentation curves for bradykinin ions versus the potential difference between the skimmer and Q0 of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, and an orifice - skimmer spacing of 1 mm

Figure 4.10.1  Fragmentation curves for bradykinin ions versus the potential difference between the orifice and the skimmer of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice - skimmer spacing of 3 mm, and a blunt skimmer

Figure 4.10.2  Fragmentation curves for bradykinin ions versus the potential difference between the skimmer and Q0 of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice - skimmer spacing of 3 mm, and a sharp skimmer

Figure 4.11.1a  Clustering process during the gas expansion with a sharp skimmer

Figure 4.11.1b  Clustering process during the gas expansion with a blunt skimmer

Figure 4.11.2  Fragmentation curves for bradykinin ions versus the potential difference between the skimmer and Q0 of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice - skimmer spacing of 3 mm, and a sharp skimmer

Figure 4.11.3  Number density versus distance from the orifice on the triple quadrupole mass spectrometer for the monoatomic gases and nitrogen within the free jet expansion

Figure 5.2.1  Schematic of the reduced flow rate sprayer and the atmospheric pressure ion lens viewed from above

Figure 5.4.1  Schematic of a typical reduced flow rate ESI source demonstrating the defocusing nature of the equipotential lines
near the tip of the sprayer

Figure 5.4.2 Equipotentials for an alternate sprayer configuration

Figure 5.5.1 Schematic of a reduced flow rate ESI source with the incorporation of the atmospheric pressure ion lens around the tip of the sprayer

Figure 5.7.1 Mass spectra of 10 μM cytochrome c with a reduced flow rate ESI source (A) and a reduced flow rate ESI source with an ion lens (B)

Figure 5.8.1 Variation in signals for the two charge states of β-cyclodextrin with increasing ring potential

Figure 5.9.1 Variation in the degree of fragmentation of β-cyclodextrin with decreasing distance between the sprayer and the curtain plate

Figure 5.11.1 Negative ion mode mass spectra of 10⁻⁴ M glutamic acid with a reduced flow rate ion source (A) and a reduced flow rate ion source and an ion lens (B)

Figure 6.3.1 Mass spectral data acquired for a 500 fmol/μL digest of beta casein with increasing lens potential

Figure 6.3.2 Total number of ions with increasing lens potential for a 500 fmol/μL digest of beta casein

Figure 6.3.3 Mass spectrum of a beta casein digest with a low lens potential

Figure 6.3.4 Mass spectrum of a beta casein digest with a high lens potential

Figure 6.3.5 Mass spectrum of a beta casein digest using a Protana ion source

Figure 6.3.6 Mass spectrum of a beta casein digest using a Protana ion source with an atmospheric pressure ion lens

Figure 6.3.7 Total ion current and mass spectrum in the vicinity of a doubly charged peptide (m/z 1031) from a 500 fmol digest of beta casein using a Protana ion source

Figure 6.3.8 Total ion current and mass spectrum in the vicinity of a doubly charged peptide (m/z 1031) from a 500 fmol digest of beta casein using a Protana ion source with an atmospheric pressure ion lens

Figure 6.4.1 Typical TIC for a nano-HPLC-MS run of a 50 fmol digest of bovine serum albumin with the ion lens

Figure 6.4.2 Representative mass spectrum from 14.53 minutes into a nano-HPLC-MS run (a) and fragment ion spectra for a triply charged peptide with a mass to charge ratio of 480.6 (b) from a 100 fmol digest of bovine serum albumin

Figure 7.2.1 Schematic of an ion spray source with an ion lens

Figure 7.2.2 Front view of an ion lens

Figure 7.3.1 Schematic of the equipotential lines generated for a standard ion spray source

Figure 7.3.2 Schematic of the equipotential lines generated for an ion spray source with an atmospheric pressure ion lens

Figure 7.4.1 Signal for doubly protonated bradykinin versus the length of the middle concentric tube protruding from the outer tube of the ion spray source

Figure 7.7.1 Mass spectra of bradykinin demonstrating an increase in the
magnitude of the multiply charged peaks as the ion lens potential increased from 1000 V (a) to 3500 V (b)

**Figure 7.8.1** Ion signal for doubly (□) and triply (○) protonated bradykinin versus the ion lens potential

**Figure 7.9** Configuration of the ion spray capillary relative to the entrance aperture for the results presented in subsections 7.9.1 – 7.9.3.

**Figure 7.9.1** Magnitude of the ion signal for doubly protonated bradykinin as the sprayer was moved away from the optimum location for ion spray with (+) and without (o) the ion lens

**Figure 7.9.2** Magnitude of the ion signal for doubly protonated bradykinin as the sprayer was moved away from the optimum location for ion spray with (+) and without (o) the ion lens

**Figure 7.9.3** Ion signal as the ion spray source with the ion lens was moved away from the optimum location

**Figure 7.9.4** Optimized potentials applied to the sprayer and the ion lens for the data presented in Figure 7.9.3

**Figure 8.2.1** Schematic of Prototype 1 of a dual sprayer ion source

**Figure 8.2.2** Schematic of Prototype 2 of a dual sprayer ion source

**Figure 8.2.3** Schematic of an attachment piece to turn the dual sprayer ion source into a four-sprayer ion source

**Figure 8.2.4** Photograph of a four-sprayer ion source

**Figure 8.4.1** Comparison of the enabling and disabling of one sprayer from Prototype 1 of a dual sprayer electrospray ion source, using nebulizer gas flow, lens potential, and sprayer potential

**Figure 8.4.2** Destabilized signals with Prototype 1

**Figure 8.5.1** Comparison of a mass spectrum obtained using a single sprayer ion spray source (a) and one channel of a four-sprayer ion spray source (b)

**Figure 8.5.2** Data collected for repeated enabling and disabling of one sprayer from Prototype 2 of a dual sprayer electrospray ion source

**Figure 8.5.3** Data collected using Prototype 2 of a dual sprayer electrospray ion source with samples of reserpine and cytochrome c in sprayers 1 and 2, respectively

**Figure 8.5.4** Data collected using Prototype 2 of a dual sprayer electrospray ion source with sampled of bradykinin and cytochrome c in sprayers 1 and 2, respectively

**Figure 8.5.5** Data collected using Prototype 2 of a dual sprayer electrospray ion source with a sample of bradykinin and reserpine in sprayers 1 and 2, respectively

**Figure 8.5.6** Low-resolution mass spectra in the vicinity of mass to charge ratio 531 for the data presented in Figure 8.6.5
List of Abbreviations

\( \gamma \) - Ratio of heat capacity at constant pressure to heat capacity at constant volume

\( \chi_m \) - Distance from the orifice to the mach disk

\( \lambda \) - Mean free path

\( \sigma \) - Collision cross section

\( \nu \) - Vibrational frequency

\( \theta_{cm} \) - Scattering angle in the center of mass coordinate system

\( \varphi \) - Fraction of center of mass energy converted to ion internal energy

\( I^* \) - Condensation parameter

\( a \) - Speed of sound in a gas

\( A \) - Arrhenius pre-exponential factor

\( A_s \) - Aperture area

BIRD - Blackbody infrared radiative dissociation

c - Ion velocity in the gas frame of reference

\( C_D \) - Drag coefficient

CD - Cyclodextrin

CE - Capillary electrophoresis

CID - Collision – induced dissociation

CM - Center of mass

\( D_0 \) - Orifice diameter

\( E \) - Energy

\( E_A \) - Activation energy
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{cm}$</td>
<td>Center of mass energy</td>
</tr>
<tr>
<td>$E'/E$</td>
<td>Ratio of translational energy after and before a collision</td>
</tr>
<tr>
<td>$E_{gas}$</td>
<td>Translational energy in the gas reference frame</td>
</tr>
<tr>
<td>$E_{i,gas}$</td>
<td>Translational energy prior to the $(i)$th collision</td>
</tr>
<tr>
<td>$E_{int}$</td>
<td>Internal energy</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>$F$</td>
<td>Gas throughput</td>
</tr>
<tr>
<td>$G$</td>
<td>Gas flow</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IQ</td>
<td>Interquad lens</td>
</tr>
<tr>
<td>$k$</td>
<td>Unimolecular rate constant</td>
</tr>
<tr>
<td>$k_B$</td>
<td>Boltzmann's constant</td>
</tr>
<tr>
<td>LIT</td>
<td>Linear ion trap</td>
</tr>
<tr>
<td>$M$</td>
<td>Mach number</td>
</tr>
<tr>
<td>$m_1$</td>
<td>Mass of ion</td>
</tr>
<tr>
<td>$m_2$</td>
<td>Mass of target gas molecules</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>$m/z$</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>$n$</td>
<td>Number density</td>
</tr>
<tr>
<td>$p$</td>
<td>Pressure</td>
</tr>
<tr>
<td>$q$</td>
<td>Charge</td>
</tr>
<tr>
<td>Q0</td>
<td>Radiofrequency quadrupole for focusing ions</td>
</tr>
<tr>
<td>Q1</td>
<td>First mass analyzing quadrupole</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>-------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Q2</td>
<td>Quadrupole collision cell</td>
</tr>
<tr>
<td>Q3</td>
<td>Second mass analyzing quadrupole</td>
</tr>
<tr>
<td>r</td>
<td>Cross sectional radius</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RRK</td>
<td>Rice-Ramsperger-Kassel</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>S</td>
<td>Vacuum pump speed</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion current</td>
</tr>
<tr>
<td>TMP</td>
<td>Turbomolecular pump</td>
</tr>
<tr>
<td>TOF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>V</td>
<td>Potential difference</td>
</tr>
<tr>
<td>$v_{\text{flow}}$</td>
<td>Expansion gas velocity</td>
</tr>
</tbody>
</table>
# Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrel Shock</td>
<td>Disturbance that surrounds a free jet gas expansion to shield it from the background pressure within the expansion region.</td>
</tr>
<tr>
<td>Condensation Parameter</td>
<td>A number that can be used to predict the degree of clustering within a gas expansion.</td>
</tr>
<tr>
<td>Curtain Gas</td>
<td>A gas that aids in ion desolvation and prevents solvents and impurities from entering the vacuum system of a mass spectrometer equipped with a curtain plate.</td>
</tr>
<tr>
<td>Free Jet</td>
<td>A gas expansion in which the thermal enthalpy of the gas is converted into directed bulk flow kinetic energy.</td>
</tr>
<tr>
<td>Glycosidic Bonds</td>
<td>The chemical bonds between neighboring glucose molecules in sugars.</td>
</tr>
<tr>
<td>Harmonic Oscillator</td>
<td>A system in which a mass is subject to a linear restoring force, and therefore vibrates at a fixed frequency, independent of amplitude.</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Process that occurs when covalent bonds are broken by water, or by an alternative to water, such as acidic or alkaline aqueous solutions.</td>
</tr>
<tr>
<td>Interface Region</td>
<td>The area in an electrospray mass spectrometer that separates the source from the high vacuum region.</td>
</tr>
<tr>
<td>Ion Source</td>
<td>The area in a mass spectrometer where ions are generated.</td>
</tr>
<tr>
<td>Ion Spray</td>
<td>A type of electrospray ionization where a nebulizer gas is incorporated to aid in break-up of droplets from the tip of the sprayer.</td>
</tr>
<tr>
<td>Kinetic Shift</td>
<td>The energy above the bond energy, which must be put into an ion to induce fragmentation in a given measurement time.</td>
</tr>
<tr>
<td>Lewis Base</td>
<td>A chemical species that can donate a pair of electrons to form a coordinate bond.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Linear Ion Trap</td>
<td>An RF multipole rodset with gated stopping potentials on either end to confine ions.</td>
</tr>
<tr>
<td>Liquefaction Temperature</td>
<td>The temperature at which a gas becomes a liquid.</td>
</tr>
<tr>
<td>Mach Disk</td>
<td>Disturbance at the end of a free jet gas expansion where the motion of the gas re-randomizes, and the temperature heats to approximately the source temperature.</td>
</tr>
<tr>
<td>Nebulizer Gas</td>
<td>A gas which aids in break-up of droplets formed at the tip of an electrospray capillary.</td>
</tr>
<tr>
<td>((m/z)/(\Delta m/z))</td>
<td>Measure of the resolution in mass spectrometry. The mass to charge ratio of the ion ((m/z)) divided by the width of the peak at half height ((\Delta m/z)).</td>
</tr>
<tr>
<td>Quadrupole Mass Analyzer</td>
<td>A device with four parallel rods for separating ions based on their mass to charge ratios.</td>
</tr>
<tr>
<td>QSTAR™</td>
<td>A QqTOF mass spectrometer built by MDS SCIEX.</td>
</tr>
<tr>
<td>Radiolysis</td>
<td>The breakdown of molecules that results from radiation.</td>
</tr>
<tr>
<td>Reflectron</td>
<td>A device that increases the effective flight tube length for time-of-flight mass analyzers, and provides energy focusing to improve resolution.</td>
</tr>
<tr>
<td>RF multipole</td>
<td>A device for focusing ions within a mass spectrometer.</td>
</tr>
<tr>
<td>Tandem Mass Spectrometry</td>
<td>Multiple stages of mass spectrometry where precursor ion selection precedes collision – induced dissociation and a product ion scan.</td>
</tr>
<tr>
<td>Time-of-flight Mass Analyzer</td>
<td>A device for separating ions based on their flight times down a flight tube.</td>
</tr>
<tr>
<td>Total Ion Current (TIC)</td>
<td>Sum of all of the ions detected within a mass spectrometer over a period of time.</td>
</tr>
</tbody>
</table>
Preface

Portions of the following chapters have been previously published elsewhere:

Scientific Papers


United States Provisional Patents


Chapter 8 Schneider BB, Douglas DJ, Chen DDY. “Stabilization of a Multiple Sprayer Electrospray Ionization Source”, *United States

International Patent Cooperation Treaty Applications


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I wish to thank Drs. David Chen and Don Douglas for providing an environment that encouraged creative thinking, and promoted a successful research program. I would also like to thank Dr. Don Douglas for sharing his resources and laboratory space.

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I would like to thank my parents for their love and support. Without them I would not be where I am today. Finally, I would like to thank my wife Cindy for her patience, love, and support over the past four years.
Part I: Electrospray Ionization

Electrospray ionization (ESI) is a powerful technique that was first demonstrated as a source of gas phase ions by Dole et al. [1-2]. However, it was the work of Fenn and co-workers, two decades later, that demonstrated the full potential of ESI to generate multiply charged ions of macromolecules for analysis within a mass spectrometer. [3] Since these pioneering studies, ESI has been used for numerous mass spectrometry applications, including analysis of proteins [4-9], nucleotides [10-12], and other compounds. [13-17] It is commonly coupled with continuous mass analyzers, such as quadrupoles [18-20], but is also effective for pulsed mass analyzers, such as time-of-flight [21-22], and ion traps. [23-26] The benefits of ESI include the ability to multiply charge macromolecules, the small sample requirement (nL - µL), and the compatibility with high-resolution separation techniques such as capillary electrophoresis (CE) and high performance liquid chromatography (HPLC). It is generally accepted that the ions observed in the gas phase by mass spectrometry are related to the species in the analyzed solution. However, the observed gas phase charge states can be different from what is present in the aqueous phase. [5,27]

A schematic of an electrospray is presented in Figure 1.0.1.

Figure 1.0.1 Schematic of a positive ion electrospray. The counter electrode is not shown.
For generation of positive ions, a capillary is maintained at a large positive potential relative to a counter electrode (for negative ions, a large negative potential is applied to the capillary relative to a counter electrode). The large potential causes an electrophoretic type of charge separation to occur within the capillary. In positive ion mode, positive ions migrate downstream towards the meniscus of a forming droplet, and negative ions are attracted towards the capillary. This leads to charge enrichment in the droplets that form at the tip of the capillary. The presence of a counter electrode generates a spray of solvated ions and charged droplets from the tip of the capillary. For electrospray ionization – mass spectrometry, there are multiple counter electrodes. These include the curtain plate, orifice, and housing of the mass spectrometer.

Electrospray ionization is amenable to a wide range of different solution flow-rates ranging from a few nL/min up to approximately 1 mL/min. At lower flow rates, the electrospray capillary may be tapered to produce a fine tip. [28] These tapered tips are typically drawn from fused silica, and may have a conductive coating [29-30], capillary junction [28], or inserted electrode [31-32] for application of the electrospray potential. For operation at higher flow rates, larger diameter capillaries are typically used, and due to the larger volumes of solvent, the capillaries are oriented at an angle to the entrance aperture of the mass spectrometer to reduce contamination of the ion optics. In some cases, the sprayer may even be positioned parallel to the entrance aperture of the mass spectrometer. A nebulizer gas may be used to aid in break-up of the droplets generated at the tip of the capillary. [16] At very high flow rates, heating devices may be incorporated into the ion source to aid in desolvation of ions. [33]
Chapter 1: Electrospray Ionization – Mass Spectrometry

1.1 Interface Designs

The interface region of an ESI-MS typically contains one or more regions of differentially pumped vacuum for transport of the ions from the atmospheric pressure source region to the high vacuum region containing the mass analyzer. It is common to have multiple stages of vacuum within the interface to reduce the cost associated with vacuum pumps with very high pump speeds, which can be extremely expensive. Two common inlet designs for mass spectrometers include a curtain plate or a heated metal capillary. [34] The gas flow from the ion source is different for these two inlets.

All of the data presented in this thesis were obtained using mass spectrometers with a curtain plate inlet. A diagram of an electrospray source attached to this type of interface is presented in Figure 1.1.1.

![Figure 1.1.1](image)

Figure 1.1.1 Schematic of an electrospray ion source with an interface incorporating a curtain plate. Electrospray capillary (1), nitrogen curtain gas flow (2), curtain plate (3), orifice plate (4), skimmer (5), RF rod set (6).

Ions and charged droplets generated in the source region enter the mass spectrometer through an aperture in the curtain plate. The region between the curtain plate and the orifice plate is flushed with nitrogen “curtain gas”. The curtain gas flows over the
orifice, and out of the aperture in the curtain plate, counter-current to the flow of ions. The curtain gas aids in desolvation of ions, and prevents solvents and impurities from entering the vacuum system. Ions and charged droplets enter the first stage of the vacuum system when they pass through the orifice. A potential difference is applied between the orifice and the skimmer to accelerate ions through the background gas. This heats the ions to desolvate them. Ions then enter the second stage of vacuum as they pass through the skimmer into an RF multipole rod set. Collisional cooling occurs here and ions are focused to the center of the rod set to improve transmission into the high vacuum region.

An alternate interface design involves the replacement of the curtain plate and orifice with a heated capillary as shown in Figure 1.1.2.

![Figure 1.1.2 Schematic of an electrospray ion source with an interface incorporating a heated capillary inlet. Electrospray capillary (1), nitrogen gas flow (2), cylindrical lens (3), heated metal capillary (4), skimmer (5), and RF rod set (6).](image)

Ions and charged droplets from the source enter the first stage of vacuum as they pass into a metal capillary. Heat is applied to the capillary to aid in desolvation of the ions. In addition, a potential difference can be applied between the exit of the capillary and a skimmer to aid in desolvation of the ions prior to their movement into further stages of
vacuum. With this type of interface, the gas flow through the capillary is in the same
direction as the ion motion. However, at higher nitrogen flow rates, the gas flow between
the sprayer and capillary may be counter-current to the ion motion.

1.2 Mass Analyzers

As mentioned in section 1.1, electrospray ion sources are compatible with many
different types of mass analyzer. The data presented in this thesis was collected with
quadrupole and time-of-flight mass analyzers. The operational details of quadrupole [35-
36] and orthogonal extraction time-of-flight [37] mass analyzers have been described in
detail.

1.3 Mass Spectrometers used in this Thesis

Four different types of mass spectrometer were used in the generation of the data
for this thesis. The simplest mass spectrometer was a prototype single quadrupole system
shown in Figure 1.3.1.

Figure 1.3.1 Schematic of the prototype single quadrupole mass spectrometer.

The gas flow through the interface region of this mass spectrometer was described in
section 1.1. Gas and ions expanded from atmospheric pressure into the first stage of
vacuum through a 0.25 mm aperture in the orifice plate. The region between the orifice and skimmer was pumped to a pressure of approximately 5 Torr. The core of the expansion was then sampled into an RF only quadrupole ion guide (Q0), through a skimmer with a 750 micron diameter aperture. The Q0 region was pumped to a pressure of approximately 7 mTorr. The short RF only rods helped to focus the ions into the mass analyzing quadrupole (Q1). Ions were detected with a Channeltron™ electron multiplier operated in ion counting mode. This mass spectrometer was used to collect some of the data presented in chapters 2-4 of this thesis.

The second mass spectrometer was a prototype triple quadrupole mass spectrometer as shown in Figure 1.3.2.

![Schematic of the prototype triple quadrupole mass spectrometer](image)

**Figure 1.3.2** Schematic of the prototype triple quadrupole mass spectrometer.

The ion source and interface region for this instrument were similar to the single quadrupole instrument. This system had a quadrupole collision cell (Q2) located after the first mass analyzing quadrupole for tandem mass spectrometry experiments. A second mass analyzing quadrupole (Q3) was located after the collision cell for analysis of fragment ions. This system was used for collection of the data presented in chapters 2-5, 7 and 8.
A third mass spectrometer was used to collect part of the data presented in chapter 3. This instrument was a linear ion trap time-of-flight mass spectrometer (LIT-TOF-MS) as shown in Figure 1.3.3. [38]

**Figure 1.3.3** Schematic of the LIT-TOF-MS.

The ion source and interface for this instrument were similar to the quadrupole instruments. This instrument had a linear ion trap followed by an orthogonal extraction time-of-flight mass analyzer. The linear ion trap was composed of an RF rod set with a trapping lens located at each end. This instrument was capable of tandem mass spectrometry, however it was not used for that purpose in this work. Ions passed straight through the trap and were mass analyzed with the time-of-flight analyzer for the collection of the data in chapter 3. The time-of-flight mass analyzer allowed for quicker data collection than the quadrupole instruments.
The final mass spectrometer used in the collection of the data presented in this thesis was a QSTAR™ from SCIEX (Concord, ON). A schematic of this instrument is presented in Figure 1.3.4 taken from [39].

Figure 1.3.4 Schematic of a SCIEX QSTAR™ mass spectrometer.

This instrument was similar to the triple quadrupole mass spectrometer described in Figure 1.3.2, however, the second mass analyzing quadrupole was replaced with a time-of-flight mass analyzer. In addition, an ion mirror (Reflectron) was incorporated to increase the mass resolution. Typical resolution \((m/z)/(\Delta m/z)\) obtained with this instrument was 10000, as opposed to approximately 1300 on the quadrupole instruments. Tandem mass spectrometry was performed in the collision cell (Q2) located after the first
mass analyzing quadrupole (Q1). This instrument was used for the collection of the data presented in chapter 6.

1.4 References


39. MDS SCIEX promotional document.
Part II: Ion Fragmentation within the Interface Region of an
Electrospray Ionization Mass Spectrometer (Chapters 2-4)

Introduction

Ions generated by electrospray ionization can remain solvated as they enter the ion sampling or interface region of the mass spectrometer. In the region between the orifice and the skimmer, final desolvation occurs due to the heating of ions by collisions as they are accelerated through the background curtain gas. It is also possible to fragment ions by application of a strong electric field between the orifice and the skimmer. This region is therefore very important in generating the analyte ions, which are presented to the mass analyzer. Due to the complexity of the processes occurring between the orifice and the skimmer, a quantitative model was not available to describe the input of energy that an ion receives due to collisions. Therefore, prior to the work described in chapters 2 – 4, it was difficult to predict what fragmentation may occur, if any, before the analyte ions are presented to the mass analyzer.

With the use of triple quadrupole mass spectrometers for tandem mass spectrometry, the role of the orifice-skimmer region in electrospray ionization has mainly been for the desolvation of desired ions. This is because a triple quadrupole mass spectrometer allows for precise control over the conditions for collision - induced dissociation (CID) of an ion in the collision cell. A constant background gas pressure can be maintained throughout the collision cell, yielding a constant mean free path, and an easily quantifiable number of collisions. Also, this pressure can be varied in a controlled manner to increase or decrease the number of collisions. The precise control
of CID parameters, such as ion energy, background pressure, and mass of the target gas in a triple quadrupole instrument makes it much more desirable for tandem mass analyses than the more complicated processes occurring in the orifice-skimmer region of a single quadrupole electrospray mass spectrometer. Also, precursor ions are mass selected prior to entering the collision cell. However, controlled fragmentation in the orifice-skimmer region of a single quadrupole mass spectrometer is also very useful, particularly when only one analyte species is present, such as in CE-MS. Collision – induced dissociation of ions within the interface region of an ESI-MS can also be important for laboratories that cannot afford a system capable of tandem mass spectrometry (MS/MS). In addition, it is imperative to understand the whole process of the production of ions in the mass spectrometer when dealing with complex biological samples, even when a triple quadrupole mass spectrometer is available.

Although electrospray is a soft ionization source, ions can be fragmented in the orifice-skimmer region, and sometimes structural information about a particular ion can be obtained. The typical method for fragmenting ions involves increasing the electric field between the orifice and skimmer until fragmentation is observed. In many instances, the actual processes occurring to produce fragmentation are poorly understood.

The increase in internal energy of an ion is proportional to the potential difference between the orifice and the skimmer, and therefore, the amount of energy transferred into an ion can be controlled. The main disadvantage of this technique is that ions can not be mass selected prior to fragmentation. All of the components of a sample experience the same conditions within the orifice-skimmer region, and this leads to complicated fragmentation spectra when complex mixtures are analyzed.
Chapter 2: Collision – Induced Dissociation of Cyclodextrins within the Orifice – Skimmer Region of an ESI-MS

2.1 Purpose

This chapter describes the first attempt to semi-quantitatively predict the electric field strengths necessary for collision-induced dissociation of ions within the orifice-skimmer region. The process of a gas expanding from atmospheric pressure into vacuum is reviewed, and a mathematical model is derived to determine the variation of background gas pressure within a free jet expansion for the two mass spectrometers used in this study. The model yields an estimate of the orifice voltages necessary for the onset of ion fragmentation.

Earlier publications have focused on determining the number of collisions occurring inside a free jet expansion within the interface region of an inductively coupled plasma mass spectrometer [1], and visualizing shock waves formed at the surface of the skimmer [2]. Neither of these studies involved electrospray ionization, but they both provided some insight into the free jet expansion within the orifice-skimmer region of a mass spectrometer.

2.2 Experimental

α and β-Cyclodextrin of 98% purity were purchased from Sigma (St. Louis, Mo). γ-Cyclodextrin of 99% purity was a gift from Beckman Instruments (Fullerton, Ca).

Cyclodextrins were prepared at concentrations of $10^{-4}$ M in 0.01 M certified ACS grade ammonium acetate from BDH Chemicals (Toronto, On). HPLC grade methanol and glacial acetic acid were from Fisher Scientific Ltd. (Nepean, On).
The instruments used for this study were a prototype single quadrupole ion spray mass spectrometer and a prototype triple quadrupole ion spray mass spectrometer both from SCIEX (Concord, On). These instruments are shown in Figures 1.3.1. and 1.3.2, respectively. A reduced liquid flow rate electrospray ion source was used for these studies. The electrospray capillary was constructed with a 50 cm long fused silica capillary having a 50 \( \mu \text{m} \) internal diameter and 150 \( \mu \text{m} \) external diameter (Polymicro Technologies, Phoenix, AZ). This capillary was connected with a 4 cm tapered tip, inside a 2 cm piece of stainless steel syringe tube with an inner diameter of 0.007 inches and an outer diameter of 0.014 inches, (Small Parts Inc, Miami Lakes, Fl). The junction was held together and sealed with epoxy glue as shown in Figure 2.2.1.

**Figure 2.2.1** Schematic of a reduced flow rate electrospray capillary. Transfer capillary (1), stainless steel syringe tubing (2), epoxy glue sealant (3), and tapered fused silica tip (4).

The tapered spray tips were pulled in house, and had an internal diameter at the tip of approximately 15 \( \mu \text{m} \). A voltage of 3 kV was applied for electrospray with a constant curtain gas flow of approximately 1 L/min as measured by a series FM-1050 gas flow meter (Matheson, Montgomeryville, PA). Medical grade nitrogen from Praxair (Mississauga, ON) was used as the curtain gas for the single quadrupole system, and ultra high purity nitrogen from Praxair (Mississauga, ON) for the triple quadrupole system. A
syringe pump (Harvard Apparatus Syringe Infusion Pump 22, South Natick, MA.) was used to generate a solution flow rate of 0.2 μL/min.

2.3 Gas Dynamics

The gas flow from atmosphere through the ion sampling orifice into the first vacuum stage of an electrospray mass spectrometer forms a free jet (Figure 2.3.1).

![Figure 2.3.1 A free jet expansion within the orifice – skimmer region of an electrospray mass spectrometer.](image)

The enthalpy of the gas in the source region of a free jet is converted into directed bulk flow kinetic energy. [3] This results in a decrease in the local gas temperature throughout the free jet as shown in eq 2.3.1:

\[ T = T_0 \left(1 + \frac{1}{2} (\gamma - 1) M^2 \right)^{-1} \]  

(2.3.1)

where, \( T \) is the temperature at a given point in the free jet, \( T_0 \) is the source temperature, \( \gamma \) is the ratio of heat capacity at constant pressure to heat capacity at constant volume
(Cp/Cv), and M is the local Mach number, or the calculated ratio of the gas flow speed to the local speed of sound. For the two mass spectrometers used in this study, the temperature in the expansion decreased from 295 K at the sampling orifice to approximately 10 K near the skimmer. When the temperature of the gas decreases, the local speed of sound in the gas also decreases, as shown in the following equation:

\[ a = \sqrt{\frac{\gamma k_B T}{m}} \]  

(2.3.2)

where \( a \) is the speed of sound in the gas, \( k_B \) is Boltzmann’s constant, \( T \) is the temperature, and \( m \) is the mass of the atom or molecule. Because of the gas acceleration, at some point downstream in the free jet, the speed of the gas molecules exceeds the local speed of sound. The Mach number in the expansion is given by:

\[ M = A \left( \frac{x - x_0}{D} \right)^{\gamma - 1} - \frac{1}{2} \left( \frac{\gamma + 1}{\gamma - 1} \right) \left( \frac{x - x_0}{D} \right)^{\gamma - 1} \]  

(2.3.3)

where \( A \) and \( x_0 \) are constants which are dependant on the value of \( \gamma \) [4], \( D \) is the orifice diameter, and \( x \) is the distance downstream from the start of the free jet. Nitrogen (\( \gamma = 1.4 \)) was used as the curtain gas in this study. A Mach number of one, where the gas speed is equal to the local speed of sound of the background gas, was achieved within approximately \( \frac{3}{4} \) of an orifice diameter. At this point, the local pressure within the free jet had dropped to 64% of its initial atmospheric pressure. At points farther downstream in the free jet, the gas speed was supersonic. The maximum Mach numbers achieved on the single and triple quadrupole mass spectrometers were 7.3 and 9.4, respectively.

The gas number density within a free jet is proportional to the inverse square of the distance from the origin. The free jet is surrounded by a barrel shock, and terminated by a shock wave known as the Mach disk [5]. In the Mach disk, the directed bulk flow of
the molecules and atoms in the free jet is re-randomized, and the temperature increases to approximately the temperature of the source. The distance from the orifice to the Mach disk ($x_m$) is given by [4]:

$$x_m = 0.67D_0\sqrt{\frac{p_0}{p_i}}$$  \hspace{1cm} (2.3.4)

where $p_0$ is the pressure in the source region, $p_i$ is the pressure within the expansion region, and $D_0$ is the diameter of the orifice.

Inside the Mach disk of a free jet expansion, it becomes difficult to model the behavior of ions and neutral molecules. For this reason it was necessary to calculate the location of the Mach disk from eq 2.3.4 to ensure that the skimmer was located within the free jet. The source pressure was approximately 760 Torr, and the pressure between the skimmer and the orifice was approximately 5 Torr and 2 Torr for the single and the triple quadrupole systems, respectively. The orifice diameters were approximately 0.25 mm on the two mass spectrometers used in this study. The distances from the orifice to the Mach disk were calculated to be 2.11 mm and 3.27 mm respectively for the single and triple quadrupole instruments. Since the measured distance from the orifice to the skimmer was only 1.7 mm for the single quadrupole and 3 mm for the triple quadrupole instrument, the area where the collisions of analyte ions and gas molecules occurred was still inside the free jet region. If a free jet is prematurely terminated at a blunt surface, the motion of the gas molecules re-randomizes, resulting in the early formation of a shock wave. A cone-shaped skimmer is intended to prevent this from occurring.

2.4 Gas Number Density within the Expansion

The theory of gas dynamics can be applied to the sampling region of an electrospray ionization - mass spectrometer. The source region is the area before the
orifice plate, where the pressure is 760 Torr, and the expansion region is the area between
the orifice and the skimmer. The gas number density along a streamline in a free jet is
given by [5]:
\[
\frac{n_0}{n_i} = \left(1 + \frac{\gamma-1}{2}M^2\right)^{-\frac{1}{\gamma-1}}
\]  
(2.4.1)
where \(n_0\) is the number density of the gas within the source region, \(n_i\) is the number
density at some point within the free jet, and \(M\) is the Mach number. Equation 2.3.3 was
solved for the Mach number by insertion of the values relevant to the two mass
spectrometers. For nitrogen (\(\gamma=1.4\)) the values of \(A\) and \(x_0\) are 3.65 and 0.40D
respectively, where D is the orifice diameter. [4] The solution of eq 2.3.3 with these
values yields eq 2.4.2.
\[
M = 3.65 \left(\frac{x - 0.01}{0.025}\right)^{0.4} - 0.8220 \left(\frac{x - 0.01}{0.025}\right)^{-0.4}
\]  
(2.4.2)
This simplified equation was substituted into eq 2.4.1 to solve for \(n_i\).
\[
\frac{n_i}{n_0} = \left(\frac{2.6645(39.2157x - 0.4)^{0.8} + \frac{0.1351}{(39.2157x - 0.4)^{0.8}} - 0.2}{0.1351 - 0.2}\right)^{2.5}
\]  
(2.4.3)
Solving this equation over small increments allowed us to determine the number density
along the axis of the free jet. Because both eqs 2.3.3 and 2.4.1 depend on \(\gamma\), different
curtain gases will lead to different equations for the number density in a free jet.

2.5 Calculation of the Number of Collisions for an Ion

Due to the complexity of eq 2.4.3, we solved for the total number of collisions
within the free jet by dividing the region between the orifice and the skimmer into 10 \(\mu m\)
increments. To obtain the average number densities, the initial \(x\) value was 0.0142 cm,
corresponding to the beginning of the region where the pressure decreased with an inverse square relationship to the distance from the orifice. The number density for the second 10 μm region was then calculated by substituting an x value of 0.0152 cm into eq 2.4.3. The average number density within each of these regions is shown in Figure 2.5.1.

**Figure 2.5.1** Variation of gas number density at various distances from the start of the free jet within the orifice-skimmer region of the single quadrupole mass spectrometer.

The free jet formed at a certain position behind the orifice, and during the expansion, the gas density was inversely proportional to the square of the distance from the orifice. There were $2.5 \times 10^{19}$ molecules per cm$^3$ under atmospheric pressure, and the number density dropped to $5.83 \times 10^{16}$ molecules per cm$^3$ in front of the skimmer cone of the single quadrupole instrument. For the triple quadrupole mass spectrometer, the density at the skimmer dropped to $1.73 \times 10^{16}$ molecules per cm$^3$. The background pressure between the orifice and the skimmer outside of the barrel shock was substantially higher than the pressure within the free jet expansion. The number density obtained from eq 2.4.3 was extremely important because it allowed the determination of the number of
collisions in the free jet region, and therefore allowed an estimate of the amount of translational energy converted into internal energy for unimolecular dissociation.

When an ion is accelerated through the free jet, the mean free path is the average distance an analyte can travel before colliding with a gas molecule, and is given by:

\[ \lambda = \frac{1}{n \sigma} \left(1 + \frac{v_{flow}}{c}\right) \]  \hspace{1cm} (2.5.1)

where \( \lambda \) is the mean free path in the lab frame of reference, \( n \) is the number density, \( \sigma \) is the collision cross section for the ion and the target gas molecule, \( v_{flow} \) is the velocity of the expansion gas flow, and \( c \) is the velocity of the ion in the gas frame of reference. The gas flow velocity was determined from eq 2.5.2:

\[ v_{flow} = Ma \]  \hspace{1cm} (2.5.2)

where \( M \) is the mach number, and \( a \) is the local speed of sound determined from eq 2.3.2 with \( T \) calculated from eq 2.3.1. The ion velocity was determined using eq 2.5.3:

\[ c = \left(\frac{2E}{m}\right)^{1/2} \]  \hspace{1cm} (2.5.3)

where \( c \) is the ion velocity in the gas frame of reference, \( m \) is the mass of the ion, and \( E \) is the translational energy of the ion. In this case, the collision cross section was calculated by:

\[ \sigma = \pi(r_1 + r_2)^2 \]  \hspace{1cm} (2.5.4)

where \( r_1 \) and \( r_2 \) were the cross sectional radii of the ion and molecule involved in the collision. The radius of a nitrogen gas molecule was estimated to be 1.49 angstroms, and
the diameters of α, β, and γ-CD have previously been determined to be 1.37, 1.53, and 1.69 nm, respectively. [6] The collision cross sections used for this model were 218.9, 262.9, and 310.9 Å², respectively. At high orifice voltages on the single quadrupole mass spectrometer, this cross section for β-CD gives an average of 371 collisions per 10 μm region at the start of the free jet, and an average of 1 collision in the 10 μm region prior to the skimmer. The number of collisions was slightly greater for γ-CD, and slightly smaller for α-CD. The collision cross sections were assumed to remain constant throughout the orifice-skimmer region. In reality, the collision cross sections decreased in magnitude throughout this region until complete ion desolvation was achieved. The decreasing ion diameter was not accounted for in this model.

Each of the 10 μm regions was then further divided to attain even smaller regions with the length of one mean free path, thus containing only one ion-gas molecule collision. This was accomplished by adding the calculated mean free path at the start of a region with the distance from the orifice to yield the start of a new region. The number densities were calculated by eq 2.4.3 for each of the new smaller regions obtained in this way. By repeating this procedure for all of the regions between the orifice and the skimmer, a large spreadsheet was obtained with each row corresponding to one collision.

2.6 Collision Dynamics

Collisions between an analyte ion and the neutral curtain gas molecules convert kinetic energy into internal energy of the ion. The simplest way to model these collisions is to use a coordinate system that moves with the center of mass of the target and the molecule. This center of mass coordinate system has been described previously. [7] The
kinetic energy of an ion prior to the \( i \)th collision, or its translational energy in the gas frame of reference is described by:

\[
E_{i,\text{gas}} = E_{i-1,\text{gas}} + q\Delta V
\]  

(2.6.1)

where \( E_{i-1,\text{gas}} \) is the ion translational energy after the \((i-1)\)th collision, \( q \) is the charge on the ion, and \( \Delta V \) is the potential difference that the ion is accelerated through. In the lab frame of reference, the kinetic energy of the ion was slightly higher by a factor of \( v_{\text{flow}} \). Since the factor \( v_{\text{flow}} \) contributed equally to the speed of both the cyclodextrin ions and the target gas molecules, it was important to model these collisions in the gas frame of reference. The energy available for conversion to internal energy, was the center of mass energy, \( E_{\text{cm}} \) given by: [7]

\[
E_{\text{cm}} = \frac{m_2}{m_1 + m_2} E_{\text{gas}}
\]  

(2.6.2)

where \( m_2 \) is the mass of the target gas molecule, and \( m_1 \) is the mass of the ion. The summation of eq 2.6.2 over all of the collisions gave the total energy available for conversion to internal energy of the analyte ion. When an analyte ion struck a nitrogen gas molecule, some of the translational energy of the complex was converted to internal energy. As many more of these low energy collisions occurred, the internal energy of the analyte ion increased, causing it to dissociate before reaching the mass analyzer. Eq 2.6.2 was used to determine how much of the lab energy of an ion was converted into its center of mass energy. Each collision was treated as an inelastic collision between a moving ion and a stationary target gas molecule, and all of the center of mass energy was assumed to be converted into the internal energy of the analyte ion. This is reasonable for macromolecules such as the cyclodextrins used in this experiment. [8-9] It is apparent
from eq 2.6.2 that heavier curtain gas molecules and higher ion energies favor more extensive fragmentation. The center of mass energy was determined for the ion in each of the increments. Plots of the center of mass energy attained by a β-CD ion per collision in the interface of the single and the triple quadrupole mass spectrometers are given in Figures 2.6.1a and 2.6.1b, respectively. These figures showed that only the single collisions occurring just before the skimmer were capable of imparting high levels of energy into the internal energy of an ion. The reason was the large mean free path an ion experienced in this region allowed it to accelerate for a much longer distance under the influence of the electric field between the orifice and the skimmer. This led to an increase in the lab energy of an ion, and thus to an increased conversion of energy to center of mass energy for each collision with the background gas. Figure 2.6.1a and 2.6.1b also showed that as a larger voltage difference was utilized between the orifice and the skimmer on the two mass spectrometers, more collisions occurred between an ion and the curtain gas. This was a result of the mean free path, because the ion velocity in the gas frame \((c)\) increased, leading to a decreased mean free path in the lab frame of reference. This also led to the surprising result that an ion experienced more collisions within the orifice-skimmer region of the single quadrupole mass spectrometer than the triple quadrupole mass spectrometer. Due to the identical orifice diameters, and atmospheric pressure upstream of the orifice, the gas flow velocity \((v_{flow})\) within the free jet was identical on both mass spectrometers. With an orifice-skimmer voltage difference of 250 V, the electric field in this region was 1470.6 V/cm on the single quadrupole system, and 833.3 V/cm on the triple quadrupole system, and as a result, the ion velocity was much greater on the former.
Figure 2.6.1a Center of mass energy per collision for β-CD at varying orifice – skimmer voltage differences on the single quadrupole mass spectrometer.

Figure 2.6.1b Center of mass energy per collision for β-CD at varying orifice – skimmer voltage differences on the triple quadrupole mass spectrometer.
In addition, the number of collisions increased with increasing potential difference between the orifice and the skimmer.

Figure 2.6.2 illustrates the CM energy input to an ion in every 80 μm region from the start of the free jet in the single quadrupole mass spectrometer.

![Graph showing CM energy input to a β-CD ion per 80 μm region from the origin of the free jet on the single quadrupole mass spectrometer. The data pertaining to orifice – skimmer voltage differences of 250, 200, 150, 100, 50, and 0 V are illustrated.](image)

**Figure 2.6.2** Center of mass energy converted to internal energy of a β-CD ion per 80 μm region from the origin of the free jet on the single quadrupole mass spectrometer. The data pertaining to orifice – skimmer voltage differences of 250, 200, 150, 100, 50, and 0 V are illustrated.

This figure demonstrates that in each of the regions, a similar amount of internal energy was generated by the collisions. In the first 80 μm region, hundreds of low energy collisions occurred to impart approximately the same amount of internal energy to an ion as the 9 or 10 high energy collisions, which occurred in the 80 μm region prior to the
skimmer. The graphs fluctuated near the final regions due to the varying numbers of collisions within the 80 μm increments determined by the average mean free path. Some regions had a variation of one or two extra collisions, which made a greater difference near the skimmer, where there were fewer total collisions. The data for zero voltage difference between the orifice and the skimmer shows that the β-CD molecule only had thermal energy. The thermal translational energy was calculated to be 0.0375 eV by the equipartition theorem, which was based upon three degrees of freedom for the translational energy of a gas molecule. [10] As an ion with thermal translational energy traveled through the region in the free jet, very little energy was converted from translational to internal energy, preventing the occurrence of desolvation. Also, the ion signal was very weak due to the lack of electric field that was required to propel an ion past the skimmer into the Q0 region of the mass spectrometer. The ions underwent a few initial collisions, and then were swept along with the expansion gas (v_{flow}), cooling to approximately the temperature of the gas. On the other hand, with an increase in electric field, the lab energy of the complex also increased, and so did the center of mass energy. For example, with 250 V difference between the orifice and skimmer, large internal energies were acquired in all of the regions.

The ratio of the kinetic energies of an ion after and before a collision with a stationary gas molecule is given by: [7,11]

\[
\frac{E'_{\text{gas}}}{E_{\text{gas}}} = \frac{m_1^2 + m_2^2}{m^2} \frac{m_2 E_{\text{int}} + 2m_1 m_2}{m^2} \sqrt{1 - \frac{E_{\text{int}} m}{E_{\text{gas}} m_2}} (\cos \theta_{cm})
\]

(2.6.3)

where, \(E'_{\text{gas}}\) and \(E_{\text{gas}}\) represent the translational energy of the ion in the gas coordinate system after and before a collision, respectively. The sum of the masses of the ion and
the gas molecule is $m$, $E_{int}$ is the energy transferred to internal energy, and $\theta_{cm}$ is the scattering angle of the ion in the center of mass coordinate system. After a collision with a stationary gas molecule, an energetic ion loses some of its translational energy to both the ion internal energy and to the recoil of the stationary target gas molecule. [7] Eq 2.6.3 applies to both elastic and inelastic collisions. For the model used here, eq 2.6.2 and eq 2.6.3 were combined to describe an inelastic collision where all of the center of mass energy is transferred to internal energy.

$$\frac{E'_{gas}}{E_{gas}} = \frac{m_i^2}{m^2} \tag{2.6.4}$$

This equation was used to determine the ion translational energies ($E'_{gas}$) after each of the collisions. These energy values were then used as the new initial translational energy for the next collision.

### 2.7 Fragmentation Data for the Cyclodextrins

The behavior of cyclodextrin ions produced from a solution containing ammonium acetate was monitored by the mass analyzer when the orifice voltage was varied. At low orifice voltages, a single peak was obtained at m/z 991, 1153, and 1315 for the $\alpha$, $\beta$, and $\gamma$ cyclodextrins, respectively. These corresponded to the singly charged cyclodextrin complexes with an ammonium adduct. As the orifice voltage increased, protonated cyclodextrin complexes were observed. Finally, at higher orifice voltages, fragments of cyclodextrin with decreasing numbers of glucopyranose units were present as shown in Figure 2.7.1.
Figure 2.7.1 Mass spectra for β-CD in ammonium acetate solutions at various orifice-skimmer voltage differences, 30, 50, 70, and 90 V, on the triple quadrupole MS. Peak identification: β-CD•ammonium (1153), β-CD•proton (1136), and protonated fragments (488, 650, 812, 974). The mass spectra at high orifice voltages show the presence of low intensity, doubly charged dimer fragments at m/z 569, 731, 893, and 1055. The peak at m/z 1144 corresponds to a doubly charged dimer of β-CD with one ammonium and one proton adduct.
All of these fragments were observed as protonated singly charged ions. At very high orifice voltages, low intensity, doubly charged dimers were observed. For the cyclodextrins in acetic acid solutions, singly charged protonated molecular ions were observed at low voltages. Upon increasing the orifice voltage, fragmentation was induced, yielding singly charged protonated fragments. Experimental orifice voltages for proton transfer and decomposition in both ammonium acetate and acetic acid solutions were determined on both of the mass spectrometers. The formation of a proton adduct to β-CD was modeled as a proton transfer from an ammonium ion. This transfer was believed to be an intramolecular transfer because it was unlikely that two cyclodextrin ions would collide within the free jet. The gas phase proton affinity for ammonium is 205 kcal/mole or 8.89 eV/molecule. [12] The gas phase proton affinity for the cyclodextrin complexes was estimated to be 200 kcal/mole or 8.67 eV/molecule from tables of gas phase proton affinities for other organic molecules. Proton adducts were most likely associated with the oxygen atoms of the glycosidic bonds along the rim of the basket-shaped CDs as a result of the C1 conformation of the glucose sub-units. [13] The non-bonding electron pairs on these glycosidic oxygen bridges were directed towards the inside rim of the cyclodextrin cavity. These non-bonding electrons generated a high degree of electron density in this region, and consequently some Lewis base character. The calculated energy required for this proton transfer was 0.217 eV/molecule based on the difference in proton affinity for the sugar and ammonium. The bond strengths of the α-1,4 glycosidic linkages in β-CD were approximated from hydrolysis activation energies. [13] Fragments with successively fewer glucopyranose sub-units were observed, and this fragmentation pattern was in agreement with experimental
observations from both radiolysis and hydrolysis. The glycosyl bond strength used in this study was characteristic of a non-enzymatic solution phase hydrolysis value of approximately 34 kcal/mole, or 1.47 eV/molecule. [13]

2.8 Energy Requirement for Dissociation

The RRK rate equation was used to determine the kinetic shifts for the processes of proton transfer and bond dissociation. The internal energy was regarded as freely flowing between all of the harmonic oscillators in the complex. The rate constant ($k$) and the amount of energy that is necessary to cause bond dissociation has the following relationship in the RRK theory: [14]

$$k \approx \nu \left( \frac{E - E_0}{E} \right)^{s-1} \quad (2.8.1)$$

where $k$ is the RRK rate constant for dissociation of the activated complex, $\nu$ is a vibrational frequency, $E$ is the ion internal energy, $E_0$ is the bond strength, and $s$ is the number of vibrational degrees of freedom. It has been found that the results obtained from eq 2.8.1 are closer to experimental results when a reduced number of vibrational degrees of freedom is used. [14] This correction was made for the calculations in this chapter. The vibrational frequency for this analysis was that of a fast vibration ($10^{14}$ Hz). [14] The RRK rate constant used in this study was $10^3$ s$^{-1}$. This value was chosen to permit fragmentation within approximately 1 ms, or the time for an ion to travel from the interface region of the mass spectrometer to the mass analyzer, depending upon the ion energy. The total numbers of vibrational degrees of freedom were 375, 447, and 519 for the $\alpha$, $\beta$, and $\gamma$-cyclodextrin respectively.

2.9 Comparison of Theory and Experiment
Plots of the calculated internal energy acquired by a β-CD ion versus the orifice – skimmer potential difference on the single quadrupole and triple quadrupole systems are shown in Figures 2.9.1 and 2.9.2, respectively.

**Figure 2.9.1** Energy converted to internal energy of the β-cyclodextrin ion versus the orifice – skimmer potential difference applied on the single quadrupole mass spectrometer.

**Figure 2.9.2** Energy converted to internal energy of the β-cyclodextrin ion versus the orifice – skimmer potential difference applied on the triple quadrupole mass spectrometer.
The slammer was maintained at a potential of 0 V for the single quadrupole, and 110 V for the triple quadrupole system. As expected, an increase in the electric field in the orifice-skimmer region led to a linear increase in the amount of internal energy gained by the ion on both instruments. Desolvation was achieved with an approximate internal energy of 20 eV for the single quadrupole and 12 eV for the triple quadrupole instrument. The difference between instruments indicated a different efficiency in desolvation from the curtain gas for the triple quadrupole system, or an electrospray source generating ions with varying degrees of solvation. Another possibility was a slightly smaller spacing between the curtain plate and the orifice on the single quadrupole instrument. A maximum orifice voltage of 250 V was used for this study, and extensive fragmentation was possible with these electric field strengths. This maximum was limited by electrical breakdown of the gas between the orifice and the skimmer at higher voltages. This was evident by very extensive fragmentation, strong ion signals, and a small pinpoint of bright light near the orifice plate on the single quadrupole mass spectrometer.

The working curves of internal energy input versus orifice – skimmer voltage difference are presented in table 2.9.1 for the various cyclodextrins in the two mass spectrometers.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>E_{int}</th>
<th>E_{int}</th>
<th>E_{int}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Quadrupole</td>
<td>0.4190V</td>
<td>0.4196V</td>
<td>0.4197V</td>
</tr>
<tr>
<td>Mass Spectrometer</td>
<td>0.2854</td>
<td>0.3423</td>
<td>0.3741</td>
</tr>
<tr>
<td>Triple Quadrupole</td>
<td>0.4095V</td>
<td>0.4107V</td>
<td>0.4118V</td>
</tr>
<tr>
<td>Mass Spectrometer</td>
<td>0.3832</td>
<td>0.4303</td>
<td>0.5583</td>
</tr>
</tbody>
</table>

The equations are only valid for potential differences of approximately 30 – 250 V between the orifice and the skimmer of the mass spectrometers. By substituting the
calculated energies required for proton transfer and dissociation into these equations, it was possible to solve for the orifice – skimmer voltage difference required to induce dissociation and proton transfer. The predicted orifice voltages were then compared to the experimental data, and the results are shown in table 2.9.2. These values were tabulated for 10% conversion to fragments.

<table>
<thead>
<tr>
<th>Process</th>
<th>Predicted</th>
<th>Experimental</th>
<th>Predicted</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desolvation</td>
<td>50</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺</td>
<td>53.6</td>
<td>55</td>
<td>34.2</td>
<td>40</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺⁺ + Dissociation</td>
<td>81.3</td>
<td>90</td>
<td>62.5</td>
<td>70</td>
</tr>
<tr>
<td>Desolvation + Dissociation In Acetic Acid</td>
<td>77.2</td>
<td>80</td>
<td>58.4</td>
<td>55</td>
</tr>
<tr>
<td>Desolvation</td>
<td>50</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺⁺</td>
<td>54.3</td>
<td>60</td>
<td>34.9</td>
<td>45</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺⁺ + Dissociation</td>
<td>87.0</td>
<td>90</td>
<td>68.3</td>
<td>70</td>
</tr>
<tr>
<td>Desolvation + Dissociation In Acetic Acid</td>
<td>82.2</td>
<td>80</td>
<td>63.4</td>
<td>60</td>
</tr>
<tr>
<td>Desolvation</td>
<td>50</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺⁺</td>
<td>55.0</td>
<td>75</td>
<td>35.6</td>
<td>55</td>
</tr>
<tr>
<td>Desolvation + H⁺ Transfer From NH₄⁺⁺ + Dissociation</td>
<td>92.7</td>
<td>90</td>
<td>74.0</td>
<td>75</td>
</tr>
<tr>
<td>Desolvation + Dissociation In Acetic Acid</td>
<td>87.1</td>
<td>85</td>
<td>68.4</td>
<td>65</td>
</tr>
</tbody>
</table>

An orifice potential of approximately 50 V was required on the single quadrupole instrument to achieve desolvation. For the triple quadrupole system, a smaller potential difference between the orifice and the skimmer was necessary (30 V). The predicted values showed good correlation with the experimentally determined values for the
cyclodextrin complexes in both ammonium acetate and acetic acid. The predicted orifice voltage for dissociation of the cyclodextrin ions formed from acetic acid solutions was approximately 10 V lower than that for the ammonium acetate because the proton transfer energy was not required. The results obtained with the cyclodextrins in acetic acid remained constant with the addition of up to 50% v/v methanol.

2.10 Sources of Error

The effect of vibrational frequency on the predicted orifice voltages using the RRK rate equation was tested by repeating the calculations with vibrational frequencies of $10^{13}$ and $10^{15}$ Hz. This uncertainty of an order of magnitude in the vibrational frequency was found to yield a difference of 3.1% to 11.2% for the predicted orifice voltages.

For these data, one half of the total number of degrees of freedom was used in the RRK rate equation. To determine the error incorporated into this model with the use of different values for vibrational degrees of freedom, the calculations were repeated using 1/3 the number of degrees of freedom. This was found to yield decreases ranging from 5.7% up to 13.8% for the predicted orifice voltages for $\beta$-CD.

Similarly, to determine the possible error incorporated into these calculations by varying the value of the RRK rate constant, the calculations were repeated using $K$ values of $10^2$ and $10^4$ s$^{-1}$. This order of magnitude change in the rate constant resulted in 5% differences in the predicted orifice voltages.

A deviation from the calculated values was found for the voltage required to induce proton transfer to $\gamma$-CD. The predicted orifice voltage for proton transfer from ammonium to $\gamma$-CD was approximately 20 V less than that observed on both of the two
mass spectrometers. This was believed to be related to the size of the cyclodextrin cavities. The structure of β-CD is shown in Figure 2.10.1. It is a basket-shaped structure composed of seven glucopyranose molecules.

![Figure 2.10.1](image)

**Figure 2.10.1** Structure of β-cyclodextrin. For α and γ-cyclodextrin, the number of glucose subunits is 6 and 8, respectively.

α and γ-CD are composed of 6 and 8 glucose units, respectively. The cavity diameter increases with the number of glucose subunits. α-CD has a very small cavity that increases its Lewis base capability due to increased localization of the nonbonding electron pairs on the glycosidic oxygen linkages. This caused the proton transfer from ammonium to be more favorable, and as a result, it occurred at a lower orifice voltage for α-CD. γ-CD has a substantially larger cavity that was not as receptive to proton transfer. The large cavity diameter positioned the glycosidic linkages at a substantially greater distance from each other. This decreased localization reduced the Lewis base character
of γ-CD relative to both α and β-CD. As a result, more energy was necessary to drive proton transfer. This difference in proton transfer energy was not accounted for in the model, and the proton affinity used here seems to have been most applicable for α-CD.

The accuracy of the predicted orifice voltages for dissociation increased with increasing size of the cyclodextrins. This was most likely a result of the assumption that 100% of the available center of mass energy was converted to internal energy of the ion. Marzluff et al. have demonstrated that greater than 90% of the available center of mass energy was converted to internal energy with the nonapeptide bradykinin. [8] This molecule has 444 internal degrees of freedom, and is thus comparable to the β-CD used in this experiment. For smaller molecules, the conversion efficiency to internal energy has been found to be substantially less. [8] For this reason the predicted orifice voltage requirement for dissociation of α-CD, with 375 internal degrees of freedom, was expected to be low. Similarly, the above approximation was expected to be most relevant for γ-CD because it was the largest of the sugars, with 519 internal degrees of freedom.

This model does not account for collisional cooling or deactivating collisions of the ions within the first RF only quadrupole (Q0) located behind the skimmer. Collisional cooling is a process where ions lose translational energy via low energy collisions within the Q0 rods. The purpose is to focus ions to the center of the axis between the rods, thus increasing ion transmission [15]. Also, the decreased spread in the translational energy of the ions improves the mass resolution. Because an ion has been activated prior to arriving in Q0, the increase in internal energy due to these collisions was considered to be negligible. This is supported by the observation that at low voltage differences between the orifice and the skimmer, ion fragmentation was not observed.
For this reason, internal energy input to an ion must be minimal in comparison to that achieved between the orifice and the skimmer. An estimate of the energy acquired by bradykinin in Q0 can be obtained from equation 2.10.1: [16]

\[ E_{\text{int}} = \phi \frac{m_2}{m_1 + m_2} E_0 \frac{m_1}{m_2} \frac{1}{C_D} \left[ 1 - \frac{C_D m_2}{m_1} \right] \]  

where, \( \phi \) is the average fraction of center of mass energy converted to internal energy in a single collision (.90), and \( C_D \) is a drag coefficient (3.5) [17]. The energy input is calculated to be 2.46 eV, or approximately 4 – 5 % of the values listed in table 2.7.1.

2.11 Conclusions

This free jet model allowed for semi-quantitative determination of the orifice voltage necessary to induce fragmentation of ions formed by ESI, and may also be used for gas phase binding studies. Fragmentation may not be desirable under many circumstances, so it is useful to be able to predict when fragmentation will occur, and when only a parent ion will be observed by the mass analyzer. This model may prove to be useful for the multitude of single quadrupole instruments currently in operation, and for choosing the optimum conditions in triple quadrupole instruments. While these results were quite promising for large molecules, the model still needs to be tested in other systems to determine its general applicability.

2.12 References


Chapter 3: Collision – Induced Dissociation of Bradykinin Ions within the Interface Region of an ESI-MS

3.1 Purpose

In chapter 2, an equation was presented to describe the variation in the gas number density as gas molecules expand from atmospheric pressure through an orifice into the vacuum system of an ESI-MS [1]. This equation allowed semi-quantitative predictions of the orifice voltages necessary to fragment various cyclodextrin ions generated by electrospray ionization. However, due to the difficulties in determining an accurate vibrational frequency and the number of effective degrees of freedom for the cyclodextrin ions for use with the Rice-Ramsperger-Kassel (RRK) theory, it was necessary to apply this model to a system that had been characterized more extensively.

Bradykinin, a nonapeptide important in the human body’s response to trauma, was chosen for this study because its ionic form has been extensively studied in the gas phase. The Arrhenius pre-exponential factor and activation energy for bradykinin have been determined by blackbody infrared radiative dissociation (BIRD) [2-3] and thermal dissociation [4]. Its gas phase conformation [5], and the efficiency of transfer of center of mass energy into internal energy have been determined [6].

3.2 Experimental

The acetate salt of bradykinin of 99% purity was obtained from Sigma (St. Louis, MO). HPLC grade methanol and glacial acetic acid were obtained from Fisher Scientific Ltd. (Nepean On). The sample was prepared by dissolving the bradykinin salt in a solution of 59.5% water, 39.5% methanol, and 1% acetic acid, at a concentration of $10^{-4}$ M.
The instruments used for this study were a prototype single quadrupole ion spray mass spectrometer and a prototype triple quadrupole ion spray mass spectrometer from SCIEX (Concord, ON), and a linear ion trap time-of-flight mass spectrometer (LIT-TOF-MS) constructed in-house by the Don Douglas research group. These instruments are shown in Figures 1.3.1, 1.3.2, and 1.3.3, respectively. For the two quadrupole systems, a reduced flow rate (0.2 μL/min) electrospray ion source was used [1,7]. The tapered spray tips were pulled in-house, and had an internal diameter at the tip of approximately 15 μm. A voltage of 3 kV was applied for electrospray with a constant curtain gas flow of approximately 1 L/min as measured by a series FM-1050 gas flow meter (Matheson, Montgomeryville, PA). Medical grade nitrogen from Praxair (Mississauga, ON) was used as the curtain gas for both quadrupole systems, and ultra high purity nitrogen from Praxair (Mississauga, ON) for the LIT-TOF-MS. A syringe pump (Harvard Apparatus Syringe Infusion Pump 22, South Natick, MA) was used to generate a solution flow rate of 0.2 μL/min. For the LIT-TOF-MS an ion spray source was used with a potential of 5000 V. The solution flow rate was 1 μL/min, and the nebulizer gas pressure was maintained at 15 psi.

3.3 Gas Dynamics

As described in chapter one, a free jet is formed when a gas expands into vacuum through the sampling orifice of an electrospray ionization mass spectrometer. The enthalpy of the gas is converted into directed bulk flow kinetic energy, and the local temperature of the gas decreases. [8] Within the gas expansion, the Mach number is given by eq 2.3.3. [9] For the two quadrupole mass spectrometers, it was shown in chapter 2 that the skimmer was located prior to the calculated position of the Mach disk, where the
disturbance at the end of a free jet causes the temperature of the gas to rise to
approximately the temperature of the source region. The distance between the orifice and
the skimmer was 1.7 mm for the single quadrupole instrument, 2.1 mm for the LIT-TOF-
MS, and 3 mm for the triple quadrupole mass spectrometer. Eq 2.3.4 was used to verify
that the skimmer was located prior to the Mach disk for the LIT-TOF-MS as well.

The gas number density along the axis of the free jet expansion between the
orifice and the skimmer of the mass spectrometers was calculated from eq 2.4.3.
Within the free jet expansion, the gas density dropped off with roughly an inverse square
relationship to the distance from the orifice. The free jet formed at a distance of 0.0142
cm from the orifice.

3.4 Number of Collisions

The mean free path and cross section for a collision between bradykinin ions and
nitrogen gas molecules were calculated from eqs 2.5.1 and 2.5.4, respectively. The
radius of a diatomic nitrogen gas molecule was estimated to be 1.49 Å from its covalent
radius, and the diameter of protonated bradykinin was estimated to be 16.57 Å. The
diameter of protonated bradykinin was estimated from eq 2.5.4 and the work of
Wytenbach et al., who accurately determined the collision cross sections for singly and
multiply charged bradykinin colliding with helium [5]. The collision cross section used
for this study was 301 Å². This cross section was assumed to remain constant throughout
the region between the orifice and the skimmer.

A spreadsheet was generated using Microsoft Excel 2000 to model the collision
frequency between bradykinin and the nitrogen curtain gas molecules. As previously
mentioned, the origin of the free jet was taken as 0.0142 cm from the orifice. The mean
free path for a bradykinin ion was calculated at this point using eq 2.5.1 to determine how far the peptide would accelerate along a streamline in the free jet prior to a collision. This mean free path was then added to the initial position to yield the start of the next segment in the free jet. The number density and the mean free path were calculated again for this point using eqs 2.4.3 and 2.5.1 to determine the linear distance from the orifice where the second collision would occur. This process was repeated for the entire distance between the orifice and the skimmer. The calculated number of collisions ranged from 158 to 2762 for singly protonated bradykinin when the orifice-skimmer voltage was varied from 0 to 250 V in the triple quadrupole mass spectrometer. The total number of collisions was found to be slightly higher for the single quadrupole mass spectrometer and the LIT-TOF-MS at the same orifice voltages, even though the orifice-skimmer spacing was smaller on both of these systems. This was due to the higher electric field generated within the orifice-skimmer region of the single quadrupole and the LIT-TOF-MS causing the velocity of an ion in the gas frame of reference to increase substantially.

3.5 Collision Dynamics

The collisions between bradykinin ions and the neutral gas molecules converted the ion translational energy into internal energy of the bradykinin ions. The center of mass coordinate system was used to model the energy conversion in these collisions. [10] The kinetic energy of an ion prior to the (i)th collision, \( E_{i,\text{gas}} \), which was also its translational energy in the gas frame of reference was calculated from eq 2.6.1. The calculated energy available for conversion to internal energy of the ion was the center of mass energy given in eq 2.6.2. The center of mass energy was determined for each
collision. All collisions were treated as inelastic involving a moving ion and a stationary gas molecule. Marzluff et al. have demonstrated from trajectory calculations that greater than 90% of the calculated center of mass energy was converted into internal energy for bradykinin [6]. Recent studies suggested that this conversion was slightly less efficient when averaged over all impact parameters and peptide orientations [11-12]. Studies demonstrating high efficiencies of transfer of center of mass energy into internal energy have been reviewed [13]. For the data presented in this chapter, a 90% conversion rate of center of mass energy into internal energy was assumed.

The ratio of the kinetic energy of an ion after and before an inelastic collision with a stationary gas molecule was given in eq 2.6.4. This equation accounted for translational energy losses due to conversion to internal energy of the ion, as well as recoil of the neutral gas molecule [14]. The equation was used to determine that approximately 95% of the lab translational energy of the bradykinin ions was maintained after each of the collisions. The calculated ion translational energy after the collision \( E'_{\text{gas}} \) was then used as the new initial translational ion energy at the start of the next single collision region on the spreadsheet. The total energy converted to internal energy of the bradykinin ions was determined by summing the contributions from all of the collisions with the nitrogen gas molecules. These calculations were carried out at various orifice-skimmer potential differences to yield a working curve of ion internal energy versus orifice-skimmer potential difference. This working curve allowed us to predict the necessary orifice voltage to fragment ions with various bond strengths. Figure 3.5.1 illustrates a plot of the kinetic energy converted to internal energy at various distances from the orifice on the single quadrupole mass spectrometer. The free jet region was
divided into 80 µm segments to illustrate that similar amounts of internal energy were generated in all regions of the free jet expansion. In the first region, over a thousand low energy collisions occurred, at an orifice-skimmer potential difference of 100 V, which imparted the same internal energy as the 5 or 6 high energy collisions in the final region. The difference in energy between these collisions was due to the larger mean free path for ion acceleration close to the skimmer.

![Graph](image)

**Figure 3.5.1** Center of mass energy converted to internal energy of bradykinin ion per 80 µm region from the origin of the free jet on the single quadrupole mass spectrometer. The data for orifice-skimmer voltage differences of 250, 200, 150, 100, 50, and 0 V are illustrated.

### 3.6 Energy Requirement for Dissociation

The internal energy necessary for dissociation of bradykinin molecules was determined using the Arrhenius equation with activation energies and pre-exponential factors determined from BIRD. The pre-exponential factor for singly protonated
bradykinin was $10^{12}$ s$^{-1}$ and the activation energy was 1.3 eV [2-3]. Although these values were measured in the zero-pressure limit, they can be applied to the high pressure regime in the front of an electrospray mass spectrometer for large molecules. Large biomolecules absorb and emit blackbody radiation at a rate much higher than the rate of dissociation. In this regime, where the internal energy distribution of the biomolecule can be characterized by a Boltzmann distribution, the pre-exponential factor and activation energy determined by BIRD approach the values of the high pressure limit [15-16]. Simple first order kinetics was used to predict the necessary rate constant for bond dissociation on the order of the time frame for the bradykinin ions to travel through the mass spectrometers. The equation for this was the following:

$$\ln \frac{[A]}{[A_0]} = -kt$$  \hspace{1cm} (3.6.1)

where [A] was the concentration of the parent ion at time $t$, [A$_0$] was the initial parent ion concentration, and $k$ was the unimolecular rate constant. This equation was used to determine the rate constant necessary for 10% dissociation of the bradykinin ions. The calculated rate constant was 105.4 s$^{-1}$ assuming that it took approximately 1 ms for an ion to travel from the skimmer to the mass analyzer of the mass spectrometers. The Arrhenius equation was used to determine the necessary ion internal temperature to generate this unimolecular reaction rate constant. The equation is given by: [17]

$$k = Ae^{-E_A/RT}$$  \hspace{1cm} (3.6.2)

where $k$ is the rate constant, $A$ is the pre-exponential factor, $E_A$ is the activation energy, $R$ is the gas constant, and $T$ is the temperature. Utilizing the rate constant calculated above, the necessary internal temperature was calculated to be 656 K. To determine the energy needed to heat the ions to this temperature, it was assumed that the ion internal
temperature was equilibrated to room temperature, or 295 K, prior to heating within the orifice-skimmer region. Thus, the temperature difference was 361 K. Assuming most of the internal energy of the ion was present as vibrational energy, the internal energy necessary to achieve 10% dissociation was estimated by:

\[ \Delta E = nk_B \Delta T \]  

(2.7.3)

where \( n \) was the number of degrees of freedom, \( k_B \) was the Boltzmann constant, and \( \Delta T \) was the temperature change. Bradykinin has 150 atoms, and thus has 444 internal degrees of freedom. For singly charged bradykinin ions, it was estimated that 13.83 eV of internal energy was necessary to cause 10% fragmentation of the ion within the mass spectrometers. Integration of the heat capacity of bradykinin as a function of temperature would be required if a more accurate energy were needed. This procedure of giving all of the ions the energy calculated from the model is approximate because it does not account for a Boltzmann distribution of ion internal energies.

### 3.7 Fragmentation Data for Bradykinin

The structures of bradykinin and its fragments are shown in Figure 3.7.1. The peptide is composed of nine amino acids, and has two terminal arginine groups. Bowers et al. demonstrated from ion mobility studies that singly protonated bradykinin has a tightly folded conformation in the gas phase [18]. Other research groups provided qualitative support for the formation of a salt-bridge structure where a proton is transferred from the carboxylic acid to one of the arginine groups [19]. Figure 3.7.1 shows the possible b and y fragment ions using the notation of Roepstorff [20].
Mass spectra of $10^{-4}$ M bradykinin solution showed the presence of singly, doubly, and triply protonated ions. This analysis was confirmed by resolving the isotopic peak spacing. To determine the fragments observed for each of these ions, the triple quadrupole mass spectrometer was first used to conduct tandem mass spectrometry. Each ion was separately mass selected in Q1; followed by collisional heating in Q2. The fragments were then observed by scanning a wide range of masses in Q3.

The MS/MS spectra of triply protonated bradykinin ions contained peaks corresponding to $(P+3H)^{3+}$, $(P+3H-H_2O)^{3+}$, $y_2^+$, $b_4^+$, $y_3^+$, $y_4^+$, $b_5^+$, $(P+2H)^{2+}$ and other fragments. Fragments from the triply charged ion included doubly protonated bradykinin making it difficult to quantitatively determine the behavior of the doubly charged peak as the orifice-skimmer potential was increased. For this reason, and because doubly charged bradykinin exhibited extensive fragmentation, only singly protonated bradykinin ions were used for the calculations in this chapter.
The results obtained for MS/MS of singly protonated bradykinin with the triple quadrupole mass spectrometer are shown in Figure 3.7.2 with 1 mTorr of nitrogen in Q2.

**Figure 3.7.2** Fragment ion spectra of singly protonated bradykinin at varying ion collision energies in the triple quadrupole mass spectrometer. The ion energy ranges from 51 eV to 161 eV, and the main daughter peaks correspond to $b_8^+$ ($m/z = 887$), $y_8^+$ ($m/z = 905$), ($P+H-NH_3$)$^+$ ($m/z = 1044$), and ($P+H-60$)$^+$ ($m/z = 1001$). The ion translational energies at the entrance to Q2 were 51 eV, 101 eV, and 161 eV, respectively.
At low energies, the singly charged ion fragmented to yield predominantly the $y_8^+$ fragment. At higher fragmentation energies, the $b_8^+$ peak and the $(P+H-NH_3)^+$ peak became evident as well as a peak corresponding to $(P+H-60)^+$.

Utilizing the potential difference between the orifice and the skimmer of the three mass spectrometers, the predominant fragments observed for singly protonated bradykinin were the $y_8^+$ fragment and the $b_8^+$ fragment. At higher orifice-skimmer potential differences, the $(P+H-60)^+$ fragment was observed. The sum of the fragments was used to determine the extent of fragmentation of the parent peak. The skimmer was maintained at ground on the single quadrupole mass spectrometer, 110 V on the triple quadrupole mass spectrometer, and 15 V on the LIT-TOF-MS. Typical data for orifice-skimmer dissociation of singly charged bradykinin ions are shown in Figure 3.7.3 for the single quadrupole mass spectrometer. The orifice potentials were 62.2, 86.1, and 112 V for the three runs, respectively, as measured with a voltmeter between the power supply boards and the orifice plate of the instrument. The fragments produced by CID in the interface region were similar to those produced in Q2. This similarity has been noted previously for other peptides [21-22], however for proteins, the formation of dimers and multimers in the source can make direct comparisons difficult [23]. A significantly higher degree of ion fragmentation was possible when ions were heated within the orifice-skimmer region as shown in Figure 3.7.3. The fragmentation in Q2 could be increased with higher collision gas pressures and ion translational energy, however, higher ion translational energies resulted in a loss of sensitivity.
Figure 3.7.3 Fragmentation spectra of singly protonated bradykinin (m/z = 1061) utilizing various potential differences (62, 86, and 112 V) between the orifice and the skimmer of the single quadrupole mass spectrometer. The main fragment ion peaks correspond to b₈⁺ (m/z = 887), y₈⁺ (m/z = 905), and (P+H−60)⁺ (m/z = 1001).
The orifice - skimmer fragmentation curves for the single quadrupole (a), triple quadrupole (b), and LIT-TOF-MS (c) are shown in Figure 3.7.4.

**Figure 3.7.4** Percent fragmentation of singly protonated bradykinin with an increase in the orifice - skimmer potential difference on the single quadrupole (a), triple quadrupole (b), and LIT-TOF (c) mass spectrometers.
Each of the points represented an average of three replicates. The fragmentation curves were reproducible, with a relative standard deviation of approximately 5% for each measurement taken at the 10% dissociation level. The curves looked very similar for the three instruments, demonstrating an increase in the degree of fragmentation with increased orifice – skimmer potential difference. Typical orifice-skimmer potential differences used to achieve desolvation were 30 V, 50 V, and 70 V for the single quadrupole, triple quadrupole, and, time of flight mass spectrometers respectively. Ions can be heated very rapidly between the orifice and the skimmer of an ESI-MS. The heated ions then enter the Q0 region of the instrument, where collisional cooling occurs. In this region, the ions can gain a small amount of internal energy from the collisions with the gas molecules, but also can lose a small amount of internal energy via collisional deactivation and emission of radiation. The rate of input of internal energy to the ions between the skimmer and Q0 was minimized by maintaining a small potential difference (7 V) between the two lens elements. The LIT-TOF-MS was found to require a larger desolvation voltage than either of the two quadrupole instruments. This may have been due to the fact that the gas pressure within Q0 of the LIT-TOF-MS (1.8 mTorr) was lower than it was in the quadrupole systems (7 mTorr). As a result, an ion experienced fewer collisions to maintain its internal temperature within Q0, necessitating an increased orifice - skimmer voltage difference. However, from equation 2.10.1, it can be shown that the lower pressure in Q0 of the LIT-TOF-MS reduced the internal energy acquired by the ions by only 5%. Another reason for the large orifice-skimmer voltage difference necessary for desolvation on the LIT-TOF-MS may be the larger droplet size formed by the ion spray source as opposed to the reduced flow rate ion source. This may have
caused the ions to enter the mass spectrometer with a higher degree of solvation. Finally differences in the spacing of the curtain plate to the orifice may have contributed as well because smaller spacing gave less time for ion desolvation.

3.8 Comparisons of the Predicted and Experimental Dissociation Voltages

The predicted orifice voltages for fragmentation of singly protonated bradykinin on the single and triple quadrupole mass spectrometers, and the LIT-TOF-MS were compared with the experimental results in table 3.8.1.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Predicted Voltage Using the Arrhenius Equation with BIRD Values (V)</th>
<th>Observed Orifice Voltages (V) (+/- 2V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Quadrupole Mass Spectrometer</td>
<td>86</td>
<td>85</td>
</tr>
<tr>
<td>Triple Quadrupole Mass Spectrometer</td>
<td>177</td>
<td>177</td>
</tr>
<tr>
<td>Linear Ion Trap-Time of Flight-Mass Spectrometer</td>
<td>121</td>
<td>123</td>
</tr>
</tbody>
</table>

The predictions from the Arrhenius equation were similar to what was observed experimentally. The predictions were accurate for all three instruments, demonstrating the general applicability of this model.

3.9 Assessment of Errors

Typical orifice – skimmer potential differences are 60 – 150 V. Therefore, a semi-quantitative model for unimolecular dissociation is useful if it can predict potential differences to within approximately 5 – 10%. Due to the rapid increase in fragmentation near the dissociation threshold energy, the calculated error in the experimental orifice
voltages for dissociation was approximately 2V. The similarity of these results with the predicted values supports the premise that ions are heated in a series of collisions in the interface region in ESI-MS, and the total internal energy acquired by an ion can be regarded as the sum of the energy generated in each of the individual ion/neutral collisions. Further evaluation of this model is necessary to determine its general applicability.

3.10 Conclusions

The work in this chapter demonstrated that the rate of unimolecular dissociation of ions can be predicted based upon the internal energy an ion acquires through collisions with background gas molecules, and that the magnitude of the acquired energy can be controlled by adjusting the orifice-skimmer potential difference on an electrospray ionization mass spectrometer. Predicted values for 10% dissociation of bradykinin were similar to the experimental values when the Arrhenius equation was used to determine the necessary ion internal energy for fragmentation. Useful predictions by this method required knowledge of appropriate values of the Arrhenius preexponential factor, and activation energy. Useful predictions were made for bradykinin when the degree of fragmentation was chosen to be below approximately 20% because extensive fragmentation at higher energies made it difficult to unambiguously assign the daughter peaks to the singly charged parent ion.

3.11 References


Chapter 4: Ion Fragmentation using Different Gases within the Interface Region of an ESI-MS

4.1 Introduction

Electrospray ionization (ESI) is an effective method for generating gas phase ions for mass spectral analysis that provides direct information about the mass to charge ratio of ions in the gas phase. However, particularly in multi-component samples, this may not be sufficient to unambiguously identify an analyte. In these situations it is common to carry out multiple stages of mass spectrometry, or MS/MS. The two most common methods of MS/MS involve the use of either an ion trap [1-3] or a triple quadrupole mass spectrometer. [4-6] Both of these instruments allow the user to mass select a particular ion prior to fragmentation by CID with a neutral collision gas. The mass selection step allows one to obtain a dissociation spectrum for a pure ion.

As demonstrated in chapters 2 and 3, another method for obtaining structural information from an ion in ESI-MS is to apply a potential difference within the first differentially pumped vacuum stage between the orifice and the skimmer. Some instruments use a capillary instead of an orifice, as shown in section 1.1; fragmentation can then be induced by a potential difference applied between the capillary exit and skimmer. A curtain gas is often utilized to prevent solvents and impurities from entering the vacuum system via the aperture in the orifice plate. [7] Ions can be accelerated through this gas as it expands into vacuum. This causes the ions to acquire internal energy, leading to fragmentation. This method of CID has been utilized in the dissociation of the heme group from myoglobin, [8] and the dissociation of other organic molecules. [9] The processes of declustering and fragmentation within the source region have been examined by qualitative comparison with results obtained in Q2 of a triple
quadrupole instrument. Also, attempts have been made to determine the energy
distribution of ions generated by an electrospray source. In chapters 2 and 3, a
collision model for semi-quantitatively predicting the potential difference between the
orifice and the skimmer required to fragment ions of cyclodextrin, and bradykinin
was described. These studies focused on the use of nitrogen as the curtain gas
because it was inexpensive and inert.

4.2 Purpose

This chapter compares the observed dissociation yields for the peptide bradykinin
with three different curtain gases, nitrogen, argon, and krypton. The collision model
developed in chapters one and two predicted that the degree of fragmentation should
increase with increasing mass of the gas. The opposite trend was observed. It is
proposed that this is due to condensation of argon and krypton in the free jet expansion.
Modifications to the interface, which minimized condensation, resulted in the observation
of the predicted trend.

4.3 Experimental

The acetate salt of bradykinin of 99% purity was from Sigma (St. Louis, MO).
HPLC grade methanol and glacial acetic acid were from Fisher Scientific Ltd (Nepean,
On). Bradykinin salt was dissolved in a solution of 59.5% water, 39.5% methanol, and
1% acetic acid (v/v), at a concentration of $10^{-4}$ M.

The instrument used in this study was a triple quadrupole mass spectrometer,
shown in Figure 1.3.2. As described in section 1.1, ions formed by ESI passed through a
curtain gas as they entered an aperture in the curtain plate of the mass spectrometer.
They entered the first vacuum stage through an orifice, and the second vacuum stage
through a skimmer. In some experiments, the spacing between the orifice and the skimmer was varied from the original distance of 3 mm down to approximately 1 mm. This required replacement of the TMP 50 turbomolecular pump (50 L/s pump speed) evacuating the Q0 region with a TMP 361 turbo pump (360 L/s pump speed), both from Leybold Vacuum Products Inc. (Export, PA). The region between the orifice and the skimmer was pumped by a model S25B rotary vane pump (7.14 L/s pump speed) (Leybold Vacuum Products Inc, Export, PA) to a background pressure of approximately 2.0 Torr. The Q0 region was pumped to a pressure of approximately 7 mTorr. Some experiments were done on a single quadrupole mass spectrometer with a front end similar to that of the triple quadrupole except for a 1.7 mm spacing between the orifice and the skimmer.

Two electrospray ion sources were used in this study. The first was a reduced liquid flow rate ESI source, \([15,13]\) with a 0.2 \(\mu\)L/min flow rate. The second was an ion spray source constructed in-house, with a 1 \(\mu\)L/min flow rate. The curtain gas flow rate was measured with a series FM-1050 gas flow meter (Matheson, Montgomeryville, PA), and was verified for argon and krypton with a model 822-13-0V1-PV1-V4 flowmeter from Sierra Instruments, Inc. (Monterey, CA) calibrated for 0-2 standard Litres per minute (SLPM) of argon. Ultra high purity nitrogen, argon, neon, and research grade krypton from Praxair (Mississauga, ON) were used as the curtain gases. A syringe pump (Harvard Apparatus Syringe Infusion Pump 22, South Natick, MA) was used to control the solution flow rate. For the ion spray source, medical grade compressed air (Praxair, Mississauga, ON) was used as the nebulizer gas.
4.4 Collision Dynamics

The application of a potential difference between the orifice and the skimmer of an ESI-MS causes ions to be accelerated through this region. As described in chapters 2 and 3, the total energy available for conversion to internal energy for an ion that is activated between the orifice and the skimmer is the sum of $E_{cm}$ generated in each of the collisions. For the case where all of $E_{cm}$ is transferred to internal energy of the ion, the ratio of the LAB ion translational energy after an inelastic collision ($E'_{gas}$) to that before ($E_{gas}$) is given by eq 2.6.4. [13] Heavier curtain gas molecules generate a larger $E_{cm}$ per collision, but ions lose more LAB translational energy per collision as well. The calculated energy transferred to internal energy of a bradykinin ion from a series of collisions with nitrogen, argon, and krypton between the orifice and the skimmer of the triple quadrupole mass spectrometer, using the model described in chapters 2 and 3, is given in Figure 4.4.1.

![Figure 4.4.1](image)

**Figure 4.4.1** Calculated center of mass energy input to bradykinin ions at various potential differences between the orifice and the skimmer of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases.
This model predicted that heavier curtain gases should yield higher internal energies and thus a greater degree of ion fragmentation for a given orifice - skimmer voltage difference. Supporting experimental results can be obtained by fragmenting the peptide in the collision cell on a triple quadrupole mass spectrometer as shown in Figure 4.4.2.

![Fragmentation curves for bradykinin in the collision cell of a triple quadrupole mass spectrometer with 1 mTorr of krypton (+), argon (o), nitrogen (Δ) and neon (◇) collision gases, respectively. The percent fragmentation was calculated by dividing the intensity of fragment ions by the sum of the intensities of fragment ions and surviving parent ions, and multiplying by 100.](image)

**Figure 4.4.2** Fragmentation curves for bradykinin in the collision cell of a triple quadrupole mass spectrometer with 1 mTorr of krypton (+), argon (o), nitrogen (Δ) and neon (◇) collision gases, respectively. The percent fragmentation was calculated by dividing the intensity of fragment ions by the sum of the intensities of fragment ions and surviving parent ions, and multiplying by 100.

The gas pressure within the collision cell was 1 mTorr, corresponding to approximately 15-19 collisions per bradykinin molecule. As expected, the heavier gases were most effective for fragmenting the peptide.

### 4.5 Ion Fragmentation within the Interface Region

Ions of bradykinin were fragmented by CID between the orifice and the skimmer of the triple quadrupole mass spectrometer. As shown in chapter 3, the predominant
fragment ions corresponded to \([b_8^+]\) (m/z = 887), \([y_8]^+\) (m/z = 905), \([P+H-NH_3]^+\) (m/z = 1044), and \([P+H-60]^+\) (m/z = 1001), where P denotes the neutral peptide. Very extensive fragmentation of the peptide was possible at high orifice - skimmer potential differences. Figure 4.5.1 demonstrates the fragmentation yields for bradykinin with dissociation in the interface of the triple quadrupole mass spectrometer for nitrogen, argon, and krypton.

**Figure 4.5.1** Fragmentation yields for bradykinin ions versus the potential difference between the orifice and the skimmer of the triple quadrupole mass spectrometer with nitrogen (Δ), argon (○), and krypton (+) curtain gases, and an orifice-skimmer spacing of 3 mm.

Here the percent fragmentation was the sum of the fragment ion intensities divided by the sum of fragment and surviving parent ion intensities. Neon could not be used due to arcing between the curtain plate and the orifice. These curves are interesting because they demonstrated the opposite of the fragmentation trend predicted based upon the gas
masses. Nitrogen was a much more effective curtain gas for inducing dissociation of ions between the orifice and the skimmer than argon or krypton.

4.6 Cluster Theory

To understand the observations shown in Figure 4.5.1, further details of the processes occurring as the curtain gas expands from atmospheric pressure through the aperture in the orifice plate to vacuum were needed. Within a free jet gas expansion, the temperature of the gas, \( T \), is given by eq 2.3.1. [16] The temperature of the source region was approximately 295 K, and the temperature became very low as the gas expanded. This led to cluster formation in the expansions when using curtain gases other than nitrogen.

Condensation and clustering of gases within a free jet expansion is a research area, which has had extensive growth in the past forty years, after the discovery of clusters in molecular beams of hydrogen, nitrogen, and argon. [17] Extensive work has been carried out by Hagena, [18-21] and others, [22-24] using primarily monatomic gases. Becker et al. demonstrated that high source pressures and low source temperatures were both favorable conditions for the formation of clusters. [17] Hagena demonstrated that another important parameter was the nozzle diameter for the expansion, with a larger diameter favoring clustering. [25] The tendency of a gas to form clusters in a molecular beam can be described by a condensation parameter \( \Gamma^* \). [20] This parameter is useful because it can be used to compare beams produced under different conditions. The mean cluster size is expected to be the same for beams with the same value of \( \Gamma^* \). A simplified form of the condensation parameter was given by Moller et al.: [26]

\[
\Gamma^* = \frac{\kappa p_0 (d)^{0.85}}{(T_0)^{2.2875}}
\]  

(4.6.1)
where $\kappa$ is a constant dependent upon the molar enthalpy of the gas at 0 K and the density of the solid, $p_0$ is the source pressure in mbar, $d$ is the orifice diameter in $\mu$m, and $T_0$ is the source temperature in degrees Kelvin. The values of $\kappa$ are 1646 and 2980 for argon and krypton, respectively. [26] This equation is valid for the monatomic gases only, and requires some modification for diatomics. For condensation parameters less than 200, no clustering is expected. For values between 200 and 1000, a mixture of clusters and gas will be present in the expansion, and for values greater than 1000, extensive clustering with cluster sizes greater than 100 atoms is expected.

For the gas expansion through the orifice of the triple quadrupole mass spectrometer in this study ($p_0 = 1013$ mbar, $T_0 = 295$ K, $d = 250$ $\mu$m), the condensation parameter was calculated to be 408 for argon and 739 for krypton. These data suggested that clusters should form in both the argon and krypton expansions, but to a lesser extent within the argon expansion. The free jet terminates in a Mach disk, which is a distance, $\chi_m$, from the orifice given by eq 2.3.4. [16] As demonstrated in chapters 2 and 3, the skimmer was located 3 mm from the orifice, truncating the expansion prior to the Mach disk. The condensation parameter gives no information about the location of the onset of clustering within these expansions.

4.7 Capture of Ions into Clusters

Work conducted with helium clusters suggests that the collision of a molecule with a cluster frequently results in the capture of that molecule by the cluster, [27-30] although sometimes the molecule may pass through the cluster and continue onward. When a foreign ion or molecule such as bradykinin ion is incorporated into a cluster, it deposits its kinetic energy, internal energy, and linear momentum into the cluster; [27] the
energy is then dissipated by evaporation of atoms from the cluster. Capture of neon in helium clusters has been observed in crossed beam experiments. [31] The velocity of the neon beam relative to the helium beam was 500 m/s. This corresponds to a translational energy of 0.051 eV, or a center of mass collision energy of less than 0.01 eV. This is comparable to the ion/neutral collision energies between the orifice and the skimmer. This mechanism helps to explain the data of Figure 4.5.1. Ions were internally heated between the orifice and the skimmer, and sometime later, prior to the mass analyzer they dissociated. If a bradykinin ion encountered clusters prior to dissociation, the internal energy of the ion was dissipated, and no fragmentation occurred. Thus, the voltage needed to induce fragmentation was higher than expected. This hypothesis is further supported by work involving the incorporation of SF$_6^+$ ions into helium clusters. [32] The dissipation of internal energy for SF$_6^+$ ions was found to be extremely rapid in the clusters, as illustrated by a greatly decreased rate of unimolecular fragmentation after electron impact ionization of the beam. For the second scenario, where an ion passes through a cluster, it would also dissipate some of its energy into the cluster. This decrease in translational and internal energy once again requires a higher energy input to produce the same degree of fragmentation. It was also possible the ion acted as a “seed” to initiate cluster formation. Regardless of the exact mechanism of the interaction of an ion such as bradykinin with free jet clusters, it was apparent that for a given orifice-skimmer voltage difference, the expected degree of dissociation was smaller with clustering.
4.8 CID of Bradykinin on the Single Quadrupole (1.7 mm orifice-skimmer spacing)

Experiments were conducted on the single quadrupole mass spectrometer with source conditions similar to the triple quadrupole instrument to try to determine the onset of clustering of argon. As described in the previous two chapters, the two machines differed in the distance between the orifice and the skimmer; 1.7 mm in the single quadrupole instrument and 3.0 mm in the triple quadrupole instrument. The data for CID of bradykinin with nitrogen and argon is shown in Figure 4.8.1.

![Fragmentation curves for bradykinin ions versus the potential difference between the orifice and the skimmer of the single quadrupole mass spectrometer with nitrogen (Δ), and argon (○) curtain gases, and an orifice-skimmer spacing of 1.7 mm.](image)

The shorter gas expansion in the single quadrupole instrument prevented the gas from reaching as low a temperature as was reached within the triple quadrupole instrument. The gas temperature at the tip of the skimmer was calculated to be 26 K for nitrogen and
6.5 K for argon in the single quadrupole mass spectrometer, and 16 K for nitrogen and 3.0 K for the monatomic gases in the triple quadrupole mass spectrometer. Thus, the final temperature was calculated to be approximately two times higher in the single quadrupole instrument. By comparing the data in Figure 4.5.1 and Figure 4.8.1, it can be seen that the relative potential difference necessary to fragment the peptide with nitrogen and argon decreased for the instrument with the shorter gas expansion region. The relative potential difference decreased from approximately 25 V for 20% dissociation to approximately 10 V, which was most likely due to the reduced clustering occurring on the single quadrupole instrument within the truncated expansion. Presumably if the gas expansion could be truncated prior to the region of the free jet where the clusters initially started to form, the dissociation trends would revert to those predicted from the collision model.

4.9 CID in the Interface with 1 mm Spacing between the Orifice and the Skimmer

In an attempt to prove this, a skimmer with a smaller orifice diameter (430 μm) was installed on the triple quadrupole instrument, and the gap between the orifice and the skimmer was decreased to approximately 1 mm. Since the gas number density scaled inversely as the square of the expansion distance, the vacuum pump speed for the Q0 region was increased from 50 L/s to 360 L/s. The gas flow (G) into Q0 (s\(^{-1}\)) is given by:

\[ G = n_s v_s A_s \]  

where \( n_s \) is the number density at the skimmer tip, \( v_s \) is the flow speed of the gas, and \( A_s \) is the aperture area. With the 1.7 times decrease in the aperture diameter, and the 9 times
increase in the number density, the gas flow into the Q0 region was expected to increase by a factor of 5.3. The Q0 pressure (P) is given by:

\[ P = \frac{F}{S} \]  

(4.9.2)

where F is the gas flow (TorrL/s) into Q0, and S is the Q0 pumping speed. The increase in throughput was countered by the increase in pump speed, resulting in a pressure of 8 mTorr in Q0 and 1.1 \times 10^{-5} \text{Torr} in the main chamber.

The dissociation studies on bradykinin ions were repeated with this configuration on the triple quadrupole mass spectrometer. It was found that increasing the orifice to skimmer potential difference did not lead to dissociation of bradykinin ions. The ion transport was dominated by the gas expansion rather than the electrostatic potentials within this region. Even increasing the skimmer potential to 20 V above the orifice potential had very little effect on the signal intensity. Ions could be fragmented by application of a potential difference between the skimmer and Q0 as shown in Figure 4.9.1. The onset of fragmentation of bradykinin occurred with the smallest potential difference for krypton, followed by argon, and then nitrogen. This was the expected trend in the absence of clusters, and was similar to dissociating ions in Q2. The increased center of mass energy with heavier gases (eq 2.6.2) gave dissociation at lower ion translational energies. The background pressure between the orifice and the skimmer was approximately 2 Torr. By treating the gas temperature as approximately the source temperature (295 K) prior to expansion into Q0, the condensation parameters were calculated to be 1.7 and 3.1 for argon and krypton, respectively.
Thus, clustering was not predicted within this gas expansion. Unfortunately, with this configuration, it was not possible to ensure that the temperature of the gas was 295 K prior to expansion into Q0. Also, it was possible that clusters formed prior to the skimmer may be sampled through the sharp skimmer into Q0. A blunt “skimmer” was utilized to further study this phenomenon.

4.10 Experiments with a Blunt Skimmer

A blunt skimmer, spaced 3 mm from the orifice, was installed on the triple quadrupole instrument while using the larger TMP 361 Q0 pump. The orifice diameter of the blunt skimmer was 500 μm, and this resulted in a pressure of 2.25 mTorr within
the QO region and $4.2 \times 10^6$ Torr in the main chamber. The purpose of the blunt skimmer was to deliberately induce formation of a shock wave in front of the skimmer. Within a shock wave, the directed bulk flow of the gas stops, and to conserve energy, the gas reheats to approximately the temperature of the source region. This is shown in eq. 4.10.1, [34]

$$\frac{T_s}{T_i} = \left(1 + \frac{\gamma - 1}{2} \frac{M^2}{\gamma - 1} \frac{2\gamma}{\gamma + 1} \frac{M^2 - 1}{\gamma - 1} \frac{(\gamma + 1)^2}{2(\gamma - 1)^2} \right)^{\frac{\gamma}{\gamma - 1}}$$

(4.10.1)

where $T_s$ is the temperature within the shock, and $T_i$ is the gas expansion temperature immediately prior to the shock. For the monatomic gases ($M = 17$), the calculated temperature within the shock was 274 K, or 92.9% of the initial source temperature, and for nitrogen ($M = 9.41$), the calculated temperature was 287 K, or 97.3% of the initial source temperature. These higher temperatures served to evaporate any clusters formed within the orifice - skimmer gas expansion prior to the expansion into the QO region. The fragmentation curves of bradykinin for the three different curtain gases with the blunt skimmer are shown in Figure 4.10.1. Due to the thickness of the shock wave formed prior to the skimmer, the expansion was truncated, and the gas reheated within the shock. This negated the clustering effects for argon, as shown by the correction of the dissociation trends for bradykinin. The bradykinin dissociation curve for krypton was even more interesting. The rate of dissociation was independent of orifice-skimmer potential difference up until approximately 60 V. It is possible that krypton was still undergoing quite extensive clustering prior to the shock wave. Above 60 V, the degree of dissociation increased with a roughly linear relationship.
Figure 4.10.1 Fragmentation curves for bradykinin ions versus the potential difference between the orifice and the skimmer of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice-skimmer spacing of 3 mm, and a blunt skimmer.

The peptide was partially dissociated even with a negligible potential difference between the orifice and the skimmer. The reason for this is shown in Figure 4.10.2. Figure 4.10.2 shows the dissociation curves for the peptide at various potential differences between the blunt skimmer and Q0. The shock prior to the skimmer heated the gases enough to remove clusters. Consequently, the peptide dissociation threshold trends occurred in the expected order of krypton, argon, and then nitrogen. The pressure within the shock, \( P_s \), is given by: [34]

\[
\frac{P_s}{P_i} = \left( \frac{2\gamma}{\gamma+1} \right) - \left( \frac{\gamma-1}{\gamma+1} \right)
\]

(4.10.2)

where \( P_i \) is the pressure immediately prior to the shock.
Figure 4.10.2 Fragmentation curves for bradykinin ions versus the potential difference between the skimmer and Q0 of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice-skimmer spacing of 3 mm, and a blunt skimmer.

The calculated pressure prior to the shock was 8.3 mTorr for the monatomic gases, and 28.1 mTorr for the nitrogen curtain gas. From Eq 4.10.2, the calculated pressure within the shock was 3.0 Torr for the monatomic gases and 2.9 Torr for the nitrogen curtain gas. From Eq. 4.6.1, the condensation parameter for re-expansion through the skimmer was calculated to be 3.4 for argon, and 6.1 for krypton, demonstrating that there should be no new clusters formed in the re-expansion.

4.11 CID between the Skimmer and Q0 with a Sharp Skimmer

Repeating the above peptide fragmentation using a sharp skimmer spaced at 3 mm, an interesting difference was noted. With the sharp skimmer, a shock wave was no
longer expected to be present in front of the skimmer, and therefore, clusters formed between the orifice and the skimmer may be sampled into Q0 as shown in Figures 4.11.1a and b. As demonstrated in Figure 4.11.1b, the blunt skimmer induces the formation of a shock wave that reheats the gas at its surface. The shock wave is caused by scattering of the gas as it strikes the blunt surface of the skimmer. Due to the elevated temperature within the shock region, clusters would evaporate as shown in Figure 4.11.1b.

Figure 4.11.1a Clustering process during the gas expansion with a sharp skimmer. Clusters formed between the orifice and the skimmer may be sampled into Q0.

With the sharp skimmer, the pressure within Q0 was 1 mTorr, and the pressure in the main chamber was $3 \times 10^{-6}$ Torr. The skimmer-Q0 dissociation trends were once again in the "proper" direction as shown in Figure 4.11.2, but no peptide fragmentation was observed with argon and krypton until greater than approximately 7 V difference was applied to the skimmer-Q0 region with the sharp skimmer.
Figure 4.11.1b Clustering in the gas expansion with a blunt skimmer. Clusters formed between the orifice and the skimmer evaporate within the shock wave (shown in black) prior to transit into Q0.

This supported the premise that some of the clusters formed along the central core of the expansion, prior to the skimmer, expanded into the Q0 region. Because of the presence of these clusters, an increased potential difference was necessary to fragment the ions. The dissociation curves for the peptide with nitrogen as the curtain gas were very similar with or without the shock wave prior to the skimmer. This suggested that clustering did not occur with nitrogen, and that the difference in fragmentation with the monatomic curtain gas was most likely not due to the decreased pressure in Q0 alone. The condensation parameter for nitrogen is expected to be less than that of argon due to its lower liquefaction temperature, [35] and the fact that the gas expansion started slightly later for diatomic gases, as shown in Figure 4.11.3. If any clustering was occurring for nitrogen, it had little effect on the collision-induced dissociation of the peptide ions.
Figure 4.11.2 Fragmentation curves for bradykinin ions versus the potential difference between the skimmer and Q0 of the triple quadrupole mass spectrometer with krypton (+), argon (o), and nitrogen (Δ) curtain gases, an orifice - skimmer spacing of 3 mm, and a sharp skimmer.

Figure 4.11.3 Number density versus distance from the orifice on the triple quadrupole mass spectrometer for the monoatomic gases and nitrogen within the free jet expansion.
4.12 Conclusions

In this chapter, it was shown that for collision – induced dissociation of ions within the orifice to skimmer region of an ESI-MS, a heavier curtain gas was not necessarily an effective means for increasing the degree of ion fragmentation. Qualitative evidence was presented for the formation of clusters within expansions of this type for both argon and krypton in two electrospray ionization – mass spectrometers. These data were supported by calculations based upon the condensation parameter for a monatomic gas. When the gas expansion processes were manipulated to decrease the value of the condensation parameter for argon and krypton, the heavier gases did result in more extensive ion fragmentation.

As suggested by eq. 4.6.1, either the diameter of the orifice or the source pressure can be decreased to increase the degree of ion dissociation when monoatomic curtain gases are used. However, a smaller orifice leads to decreased sensitivity, and a lower source pressure leads to increased cost. The most practical way should be to increase the source temperature to reduce cluster formation, and therefore increase the degree of ion fragmentation. The elimination of clusters by increasing the source temperature should increase the fragmentation yields with argon and krypton curtain gases relative to that observed with nitrogen curtain gas.

4.13 References


Part III: Atmospheric Pressure Ion Lenses (Chapters 5 – 7)

Introduction

ESI can be operated over a wide range of solution flow rates. In general, these can be divided into three main classes; high flow rate (≥ 1μL/min), reduced flow rate (80 nL/min – 1 μL/min), and very low flow rate ion sources (< 80 nL/min). Wilm and Mann described the first ESI source for operation at very low liquid flow rates (≈20 nL/min) [1]. Stabilization of electrospray at this flow rate required the use of tapered glass capillary tips, with internal diameters of 1-2 μm. A thin layer of gold was deposited on the capillary tips for application of the electrospray potential. This technique was termed nanoelectrospray ionization. Sampling efficiency was increased, however operation with the tapered tips was more difficult than with electrospray at higher flow rates.

The first description of electrospray operating as a reduced flow rate ion source was by Smith et al. [2]. In this work a capillary electrophoresis system was coupled with a mass spectrometer. Shortly thereafter, Emmett et al. described a packed electrospray needle for interfacing chromatography with mass spectrometry at low flow rates (300 nL/min and above) [3]. Following these initial experiments, there have been many studies with electrospray operating in this flow regime for applications involving direct infusion [4-5], or the coupling of CE [6-7] and LC [8-9] with ESI-MS. Typically these reduced flow rate ESI sources provide improved signal stability and sampling efficiency when compared to the higher flow rate ESI sources. However they require the use of tapered capillaries with a metal coating [1], in-capillary electrode [10], or some other type of junction [4, 11-12] for applying the electrospray potential.
High flow rate electrospray ion sources are easy to operate, but typically suffer from a lower sampling efficiency, and poorer signal stability when compared with reduced flow rate electrospray ion sources. This problem is accentuated when spraying samples with high aqueous content. A nebulizer gas can be used to help break up droplets formed at the sprayer tip [13]. This technique of nebulizer assisted electrospray ionization, or “ion spray” helps to stabilize the ESI process for flow rates up to approximately 1 mL/min.

ESI-MS has, in general, a very low ion sampling efficiency. Attempts to increase the sampling efficiency have lead to the development of nanoelectrospray ionization [1] and other reduced flow rate electrospray ion sources, [4] as well as ion spray. [13] Reduced flow-rate ion sources make use of a tapered sprayer with an internal diameter (10-50 μm) smaller than typical ESI sources (50-200 μm). For the same solution concentration of analyte, the signal is typically the same or higher than that of full electrospray sources even though lower flow rates are used. This is a result of a substantial increase in the sampling efficiency.

Another approach used to increase the sampling efficiency involves modifying the mass spectrometer to which the electrospray source is attached. The diameter of the entrance aperture to the ion optics region can be increased in an attempt to draw more ions into the vacuum system. [14] This requires a higher speed pump on the vacuum system to maintain the low pressure within the spectrometer. [15] Provided the ion to gas ratio remains constant, increases in ion signal with this technique are proportional to the increases in the gas flow into the system. The drawback with this technique is the expense associated with a substantial increase in the pump speed.
Few studies to date have focused on methods of improving the trajectories of the ions in the region between the sprayer and the mass spectrometer entrance aperture. Smith et al. showed a focusing ring located downstream from an ESI sprayer connected to a home-built capillary electrophoresis instrument. [16-17] The ring was located in front of the ion sampling orifice, but no discussion was given as to whether it increased the ion signals. Franzen used an atmospheric pressure ring electrode on the inside wall of a heated capillary inlet for electrospray ionization mass spectrometry. [18] The ring was intended to help draw ions into the inlet capillary of the mass spectrometer, although no discussion was given as to whether an appreciable signal increase was observed. Gulcicek et al. showed an interface for ESI-MS with a heated capillary as a desolvation tube, where they mentioned the presence of an end plate lens element and a cylindrical lens. [19] These lenses were located a substantial distance from both the sprayer and the inlet aperture of the heated capillary. Finally, Agnes et al. demonstrated that wire lenses located downfield from a droplet levitation ion source led to increased ion currents within a mass spectrometer. [20] All of these examples of atmospheric pressure ion focusing were aimed at improving the trajectories of ions immediately prior to entry into a mass spectrometer. Prior to the use of electrospray as an ion source for mass spectrometry, Beavis et al. demonstrated the use of an atmospheric pressure focusing ring with electrospray for sample preparation of large involatile organic molecules. [21] The ring was located downstream from the sprayer needle, and was found to decrease the diameter of spots generated with an electrospray coating device.

Chapters 5 – 7 describe the use of an ion lens at atmospheric pressure to increase and stabilize the flux of ions into various mass spectrometers.
References


Chapter 5 – An Ion Lens to Improve Electrospray Ionization at Low Solution Flow Rates

5.1 Purpose

The research described in this chapter demonstrates a new method for improving ion trajectories within the region between an electrospray capillary and a mass spectrometer inlet aperture. Electric field modeling with MacSimion 2.0 illustrated the defocusing nature of the electric field in the vicinity of the sprayer tip for an electrospray ion source. A ring-shaped electrode was incorporated near the tip of a tapered electrospray capillary in an attempt to improve the shape and distribution of the electric field lines within this region. Although the electrode is not strictly a lens with a well-defined focal point, it is referred to as an atmospheric pressure ion lens because it changes the direction of ion motion.

5.2 Experimental

Cytochrome c of 95% purity (from horse heart), β-cyclodextrin of 98% purity, reserpine of 99% purity, and glutamic acid of 99% purity were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Samples of β-cyclodextrin were prepared by dissolution of the sugar in water with 0.01 M certified ACS grade ammonium acetate from BDH chemicals (Toronto, ON, Canada). Cytochrome c was prepared at 10 μM in 90% water/10% methanol (Fisher Scientific, Nepean, ON, Canada, HPLC grade). Reserpine was prepared at 10^{-5} M in 10% water/90% acetonitrile (Sigma Chemical Co., St. Louis, MO, USA) with 1 mM ammonium acetate. Glutamic acid was prepared at 10^{-4} M in 99% methanol/1% water.

The instrument used in this study was a prototype triple quadrupole ion spray mass spectrometer from SCIEX (Concord, ON, Canada) as shown in Figure 1.3.2. [1]
For some experiments, the ion spray needle and mount were replaced with a home-built reduced flow rate electrospray ion source as shown in Fig 5.2.1.

**Figure 5.2.1** Schematic of the reduced flow rate sprayer and the atmospheric pressure ion lens viewed from above. Transfer capillary (1), sprayer (2), capillary butt junction (3), high voltage arm (4), ion lens (5), mounting block (6), set screw (7), tapered capillary tip (8), sprayer high voltage lead (9), mounting hole (10), ion lens high voltage lead (11), machined groove (12), and location of the sprayer tip relative to the ion lens (13).

The sprayer assembly was composed of a transfer capillary (1) (Polymicro Technologies, Phoenix, AZ, USA) attached to a 4 cm long sprayer (2) to form a capillary butt junction (3) within a piece of stainless steel hypodermic tubing (Small Parts Inc., Miami Lakes, FL, 28 gauge). The fused silica capillary had an internal diameter of 50 μm and an external diameter of 150 μm. The electrospray potential was applied directly to the stainless steel tubing through a conductive arm (4). An ion lens (5) was attached to the Plexiglas mount (6) by a set screw (7). The lens (5) was located approximately 2 mm from the tapered tip (8) of the reduced flow-rate electrospray source. The inner diameter of the tapered tip was approximately 15 μm, and the solution flow rate was 200 nL/min, as controlled by a syringe infusion pump (Model 22, Harvard Apparatus, South Natick,
The lens was composed of an oblong-shaped aperture cut into a rectangular piece of stainless steel, 1 mm thick. The lens was placed around the tapered tip with an asymmetrical orientation in the horizontal plane (shown end-on at 13 in Fig. 5.2.1). The distance from the tapered glass capillary to the right side of the oblong-shaped ring was approximately 2 mm. The distance to the left side of the ring was approximately 6-7 mm. In the vertical direction, the spacing between the fused-silica capillary tip and the stainless steel ring was approximately 2.5 mm. The optimum sprayer position was slightly to the right hand side of the aperture in the curtain plate. Favorable results have also been achieved with the tapered tip centered within the ion lens. To achieve good results, it was very important that the shape of the tapered capillary tip was as uniform as possible. Ultrahigh purity nitrogen (Praxair, Mississauga, ON, Canada, manufacturer’s stated purity 99.9995%) was used as a curtain gas.

For experiments in negative ion mode, the polarity of the ion path elements was reversed. For detection of negative ions, the front edge of the ion counting detector (Channeltron™) was maintained at a positive potential, and the back of the continuous dynode was maintained at a larger positive potential. The solution flow rate was 1 μL/min for operation in negative ion mode.

5.3 Ion Motion at Atmospheric Pressure

In the presence of an electric field and at high pressure, ions and small charged droplets have trajectories orthogonal to the equipotential lines. Ions drift with a velocity ($\mathbf{v}$) given by;

$$\mathbf{v} = k\mathbf{E} + \mathbf{G}$$  \hspace{1cm} (5.3.1)
where $E$ is the electric field, $k$ is the mobility, and $G$ is the gas velocity. In the absence of severe space charge effects, the motion of ions can be controlled at atmospheric pressure provided suitable electric fields can be formed.

5.4 Electric Field Modeling for Typical Reduced Flow Rate Electrospray Ion Sources

Figure 5.4.1 illustrates the equipotentials (calculated with MacSIMION 2.0, version 2.0) obtained when the optimum potentials were applied to a reduced flow rate ESI source.

Figure 5.4.1 Schematic of a typical reduced flow rate ESI source demonstrating the defocusing nature of the equipotential lines near the tip of the sprayer. All areas shown in the figure are at atmospheric pressure. A counter-flow of nitrogen curtain gas flows through the region between the curtain plate and the orifice plate. Gas and ions expand into the first stage of vacuum through the aperture in the orifice plate. Qualitative ion trajectories are shown. The contour interval is 250 V.
The sprayer was centered in front of the inlet aperture of the mass spectrometer, and the equipotentials were defocusing at the tip of the sprayer. The potentials in this figure were 3000 V, 1000 V, 190 V, and 0 V for the sprayer, curtain plate, orifice, and housing, respectively. The nitrogen curtain gas flows out from the aperture in the curtain plate, countercurrent to the ion motion within this region as shown in Figure 1.1.1. This aids in desolvation of ions as they enter the mass spectrometer. The gas flow speed at the aperture in the curtain plate was calculated to be 95 cm/s. The electric field at this point was about 5000 V/cm (Figure 5.5.1) and therefore the ion drift velocity \( kE \) in eq 5.3.1 of cyclodextrin for a mobility of 1 cm\(^2\)/Vs [3] is calculated to be 5000 cm/s. The gas streamlines are oriented in the direction opposite to the ion flow near the tip of the sprayer, however due to the large ion drift velocity, it is the electric field that controls the ion motion, rather than the gas flow. The ion motion was orthogonal to the equipotentials. Qualitative ion trajectories (drawn by hand) suggested that a wide plume of ions was generated, resulting in a relatively low efficiency of transfer to the mass spectrometer. With this arrangement, the spatial spread of the ions formed at the tip of the sprayer increased as the ions traveled towards the curtain plate. This causes a large fraction of the ions to strike the curtain plate rather than to enter the mass spectrometer.

Approximately a two times increase in ion signal was generated by positioning the sprayer off to the side of the aperture in the curtain plate instead of having it centered in front of the aperture. This source configuration is shown in Figure 5.4.2. With this sprayer configuration, only ions or charged droplets generated on the edge of the spray were directed into the entrance aperture of the mass spectrometer.
Figure 5.4.2 Equipotentials for an alternate sprayer configuration. The contour interval is 250 V.

5.5 Electric Field Modeling for a Reduced Flow Rate Electrospray Ion Source with an Atmospheric Pressure Ion Lens

To increase the ion current directed into a mass spectrometer, ion “lenses” operating at atmospheric pressure were used to improve the shape and distribution of the equipotential lines. Figure 5.5.1 illustrates the improvement in the shape of the equipotentials generated with a reduced flow rate ESI source when a ring-shaped ion lens is placed near the tip of the sprayer. The equipotentials near the tip of the ESI source are flattened, reducing the defocusing effect. Ions are pushed towards the curtain plate and inlet orifice of the mass spectrometer, and the spread in ion trajectories is greatly reduced. The voltages utilized in this figure were 5100 V, 3500 V, 2000 V, 190 V, and 0 V for the ion lens, sprayer, curtain plate, orifice, and housing, respectively. Thus qualitative ion trajectory modeling suggested that the ion lens would increase the number of ions arriving at the inlet aperture of the mass spectrometer.
Figure 5.5.1 Schematic of a reduced flow rate ESI source with the incorporation of an ion lens around the tip of the sprayer. The equipotential lines at the tip of the sprayer were less defocusing. The contour interval is 250 V.

5.6 Effect of the Lens on the Total Ion Count Rate for a Sample of Cyclodextrin

Experiments showed that a significant increase in the total ion count rate was achieved with the ion lens located around the sprayer. Samples of β-cyclodextrin (1.0 x 10^{-3} M in 10.0 x 10^{-3} M aqueous ammonium acetate) and reserpine (1 x 10^{-5} M in 90% acetonitrile/10% water) were tested. For β-cyclodextrin, the total ion current (TIC) was measured for all ions, including (M+H)^+, (M+2H)^{2+}, clusters, and fragments, using an ion spray source (1 μL/min) without the lens, and a reduced flow rate ESI source (0.2 μL/min) with the ion lens. The total ion count rate for the sample using an ion spray source was approximately 1.3 x 10^5 counts per second (cps), compared to approximately 5.5 x 10^5 cps for the reduced flow rate interface with the ring electrode. The voltages were 4800 V and 1100 V for the sprayer and the curtain plate, respectively for the ion
spray source. The voltages were 4421 V, 4350 V, and 1800 V for the reduced flow rate sprayer, lens, and curtain plate, respectively. For the experiments with the reduced flow rate interface, the sprayer was located extremely close to the curtain plate (within 1 mm). Without the ion lens in place, the reduced flow rate sprayer had to be positioned farther from the curtain plate (approximately 2 mm) to maintain a strong signal. The use of the ion lens with the reduced flow rate ESI source increased the total number of ions entering the mass spectrometer by factors of 3.5 and 4.3 compared with the reduced flow rate source without the ion lens, and the ion spray source, respectively.

5.7 Effect of the Lens on the Count Rate for Ions

Figure 5.7.1 demonstrates the optimized signals for a 10 μM sample of cytochrome c using a reduced flow rate electrospray ion source (A) and a reduced flow rate electrospray ion source with the ion lens (B). The applied potentials were 3374 V and 1560 V for the sprayer and the curtain plate (A), and 4000 V, 2000 V, and 4200 V for the sprayer, curtain plate, and ion lens, respectively (B). The source potentials were optimized for the +9 peak of cytochrome c (m/z = 1374). The lens increased the ion signal by a factor of approximately 3. It is important to note that there was no increase in the background continuum noise in the vicinity of the +9 peak. Therefore, the signal to noise ratio was increased by a factor of 3. Ion signals were increased by a factor of approximately 2 for all of the non-optimized protein peaks as well. Signal increases of 2.0 to 2.5 times have been achieved for protonated reserpine (m/z = 609) and cyclodextrin ions (m/z = 1136).
Figure 5.7.1 Mass spectra of 10 μM cytochrome c with a reduced flow rate ESI source (A) and a reduced flow rate ESI source with an ion lens (B). The peaks correspond to the +8, +9, +10, and +11 charge states of the protein at m/z 1546, 1374, 1237, and 1125, respectively.

5.8 Effect of the Lens on the Charge States of Ions

By varying the potential applied to the ion lens, and the position of the sprayer relative to the curtain plate, it was possible to vary the observed charge states of various ions. For a cyclodextrin sample, the potentials could be optimized separately for both the singly and doubly charged ions, as shown in Figure 5.8.1. For 5.8.1A and 5.8.1B, the sprayer potential was 3000 V and the curtain plate potential was 1580 V. The ion lens
potential was increased from 1600 V (A) to 2850 V (B). Higher lens potentials were effective for generating higher charge states for sugars and proteins. The optimum lens potentials were 1600 V and 2850 V for singly and doubly protonated cyclodextrin, respectively. The large increase in signal for doubly protonated cyclodextrin at higher lens potentials was achieved with only a minor decrease in the signal for the singly protonated ion.

**Figure 5.8.1** Variation in signals for the two charge states of β-cyclodextrin with increasing ring potential. The singly charged ion with an ammonium adduct is at m/z 1153, and the doubly charged ion with two ammonium adducts is at m/z 586. The ion lens potentials were 1600 and 2850 V in A and B, respectively. For both spectra, the sprayer potential was 3000 V and the curtain plate potential was 1580 V.
It is unclear whether the increase in the signal of higher charge states is due to a change in the mechanism of the electrospray process, or an increased focusing of higher charge states into the mass spectrometer. Experiments conducted with bradykinin demonstrate the potential of the ion lens to substantially increase the ion signal for the higher charge states of the peptide (2+ and 3+), while at the same time decreasing or maintaining the signal for the singly charged background solvent peaks (data not shown). This can lead to substantial increases (3 – 6 times) in the signal to noise ratio for multiply charged peptides.

5.9 Variations in the Degree of Fragmentation for Analytes with Changes in the Ion Lens Potential

The installation of the ion lens around the sprayer may result in a variation in the degree of fragmentation of analytes in the interface region (described in chapters 2 – 4), as shown in mass spectra of β-cyclodextrin in Figure 5.9.1. The voltages were 3100 V, 1000 V, 190 V, and 110 V for the reduced flow rate sprayer, curtain plate, orifice plate, and skimmer, respectively for Figures 5.9.1A – 5.9.1C. The lens potentials were 3750 V, 5100 V, and 4500 V, respectively for Figures A – C. For runs A and B, the lens was positioned 2 mm behind the sprayer tip, and the sprayer was positioned 3 mm and 1 mm from the curtain plate, respectively. For run C, the lens was 4 mm behind the sprayer tip to prevent arcing to the curtain plate, and the sprayer tip was even with the aperture in the curtain plate. As the spray was stabilized closer to the curtain plate to increase the ion current, more highly solvated ions were generated. Ions were desolvated as they acquired internal energy through collisions with neutral curtain gas molecules within the first vacuum stage between the orifice and the skimmer. [Chapters 2 – 4, 4-5] More highly
solvated ions require more energy for desolvation, so less energy is available to induce fragmentation.

**Figure 5.9.1** Variation in the degree of fragmentation of β-cyclodextrin with decreasing distance between the sprayer and the curtain plate. The distance from the sprayer tip to the curtain plate was 3 mm, 1 mm, and 0 mm for 5A – 5C. Peaks correspond to cyclodextrin with an ammonium adduct (m/z = 1153), protonated cyclodextrin (m/z = 1135), and fragments (m/z = 326, 488, 650, 812, and 974).
The fragment ion peaks decreased in magnitude as the electrospray was stabilized closer to the entrance aperture of the mass spectrometer. In addition, the protonated cyclodextrin peak (m/z = 1136) decreased as the sprayer was positioned closer to the entrance aperture. This decrease is consistent with a decreased ion internal energy, [6] even though the potential difference between the orifice and skimmer was constant (80 V) for all of the data in Figure 5.9.1. These data demonstrate that the degree of fragmentation can be varied by adjusting the position of the sprayer tip relative to the entrance aperture of the mass spectrometer, and setting an appropriate lens potential.

5.10 Effect of the Lens on the Stability of Ion Signals in ESI-MS

The atmospheric pressure ion lens also improved the stability of the signal for reduced flow rate electrospray sources. The relative standard deviation (RSD) for repeat 10 ms measurements of protonated cyclodextrin measured using the reduced flow rate electrospray ion source and the reduced flow rate source with the ion lens were approximately 2.5% and 1.4%, respectively (0.55% expected from count statistics). In addition, the ion lens made the reduced flow rate electrospray ion source easier to use, by generating a larger ion signal in a broader range of positions around the entrance aperture of the mass spectrometer (discussed in more detail in Chapter 7 for a higher flow rate electrospray ion source).

5.11 Effect of the Lens on Signals in Negative Ion Mode

Experiments demonstrated that the ion lens was also useful for increasing signals in negative ion electrospray ionization. Figure 5.11.1 shows mass spectra obtained for a sample of $10^{-4}$ M glutamic acid with a reduced flow rate electrospray ion source (A) and
a reduced flow rate electrospray ion source with an ion lens (B). The voltages were
-3853 V and -520 V for the sprayer and curtain plate in Figure 5.11.1A.

Figure 5.11.1 Negative ion mode mass spectra of $10^{-4}$ M glutamic acid with a reduced
flow rate ion source (A) and a reduced flow rate ion source and an ion lens (B). Peaks
corresponding to deprotonated glutamic acid (m/z = 146), and its isotopic peaks (m/z =
147, 148, and 149) were present.
Further increases in the sprayer or curtain plate potential decreased and destabilized the ion signal in the absence of the lens. The voltages for Figure 5.11.1B were $-6332\, \text{V}$, $-3000\, \text{V}$, and $-1500\, \text{V}$ for the sprayer, lens, and curtain plate, respectively. The addition of the lens increased the ion signal by a factor of 2. This improvement was achieved with a substantial increase in the potentials applied to the curtain plate and the sprayer, similar to the results obtained in positive ion mode. This similarity between lens operation in positive and negative mode suggests that the lens may be particularly effective for operation with multiply charged negative ions.

5.12 Ion Lens Limitations

Under some circumstances, the voltage applied to the ion lens could not be increased above the voltage on the sprayer capillary because the effective electric field at the tip of the sprayer decreased to the point where it was insufficient to overcome the surface tension of the droplet. In this case, electrospray ceased, and a growing droplet was observed at the tip of the reduced flow rate capillary. It is common to use a small fraction of methanol or other organic solvent in samples that are analyzed by mass spectrometry to decrease the surface tension of the forming droplet. Further increases to the organic content of solutions may lead to increases in the maximum potential that can be applied to the ion lens, and thus to further increases in ion signal.

5.13 Conclusions

It was demonstrated that atmospheric pressure ion lenses substantially increased the stability, ease of use, and sampling efficiency of electrospray ion sources operating at low solution flow rates. The charge states and degree of fragmentation of analyte ions were varied by changing the potential applied to the ion lens. It is believed that the
potential on the ion lens generates more favorable equipotential lines at the tip of the sprayer. Further optimization may lead to even larger improvements. This technique may be the most economical method available to significantly increase the signal to noise ratio of an ESI-MS. Atmospheric pressure ion lenses can in principle be utilized for ESI-MS as well as for CE-MS, LC-MS, and nanospray ion sources.

5.14 References


Chapter 6: Analysis of Protein Digests Using Reduced Flow Rate Electrospray Ionization with an Atmospheric Pressure Ion Lens

6.1 Purpose

Mass spectrometry is a key enabling technique for the field of proteomics, the comprehensive study of the protein content expressed in cells. Although encoded in the genome, many proteins are not activated until they undergo post-translational modification. In addition, many important proteins are present in very small amounts within living cells. For this reason, more sensitive methods are required for the analysis of dilute concentrations of proteins.

A key emerging technique for proteomic analysis is nanolitre – flow rate HPLC. Emmett et al. [2] and Davis et al. [3] were among the first to describe interfaces between ESI and chromatography with the stationary phase packed into tapered capillary tips. These systems were capable of operation with flow rates of less than 1 μL/min. Alexander and co-workers used a similar system to demonstrate that the stability of nanoflow HPLC was improved by operating in the flow regime of 100 – 200 nL/min as opposed to 50 nL/min and lower. [4] For these experiments, the tapered tips were attached to a separation column. More recently, an inexpensive method for generation of an HPLC gradient has been demonstrated for operation at low flow rates. [5] The coupling of liquid chromatography with mass spectrometry has been the subject of several reviews. [6-7]

The research described in this chapter focuses on the analysis of protein digests using a reduced flow rate electrospray ion source and an atmospheric pressure ion lens, to improve the sensitivity and stability for analyses by ESI-MS and nano-HPLC-ESI-MS.
In particular, the most important benefits of the ion lens for this application were reduced background noise, increased ion signals, and improved stability with a reduced flow rate electrospray ion source operating in the flow regime of 100 – 400 nL/min.

6.2 Experimental

A tryptic digest of bovine beta casein was supplied by Dev Pinto from NRC Halifax. MDS Proteomics (Toronto, ON) supplied a nano-HPLC and tryptic digests of bovine serum albumin. The proteins were digested overnight at 37° C with trypsin (enzyme:protein ratio of 1.50). The digested proteins were diluted to various concentrations with water containing 10% acetonitrile/0.1% formic acid. The nano-HPLC and autosampler were from LC Packings (San Francisco, CA). The mass spectrometer for these studies was a QSTAR™ provided by MDS SCIEX (Concord, ON). The experiments were done at MDS SCIEX and MDS Proteomics. A schematic of this instrument was provided in Figure 1.3.4. As mentioned in section 1, this instrument is similar to the triple quadrupole used in chapters 2 – 5, however the second mass analyzing quadrupole is replaced with a time-of-flight mass analyzer. The original concave curtain plate was replaced with a flat curtain plate to reduce the occurrence of arcing between the lens and the mass spectrometer entrance. In addition, a separate external power supply (0 – 6 kV) was installed to replace the original curtain plate power supply. This modification was necessary because the original curtain plate power supply was fixed at 1 kV (non-adjustable). The commercial ion source was replaced with a nanoelectrospray ion source from Protana (Odense, Denmark). This ion source was similar in design to the one described in Figure 5.2.1, however the tapered capillary tips were removable to simplify replacement in the event of breakage. The necessary bracket
for incorporation of an ion lens was built in-house at MDS SCIEX (Concord, ON). The tapered tips (New Objective, Woburn, MA) had an inner diameter of approximately 15 microns. For direct infusion experiments, the tapered tips contained a glass frit near the tip. For nano-HPLC experiments, approximately 5 cm of the tapered tips were packed with S5 ODS2 Spherisorb C18 stationary phase from Waters (Milford, MA). The diameter of the packing was 5 μm. A packing slurry (10% w/v in methanol) was forced into the tapered needle tips using a pressure of 2000 psi. Ultra-high purity nitrogen was used as the curtain gas for the QSTAR™ instruments. Nano-HPLC experiments were conducted with gradient elution. The gradient consisted of a water rinse (100% aqueous), followed by a gradient of approximately 10 minutes, and terminated with an acetonitrile rinse (100% organic). The majority of the peptides eluted between 11 and 20 minutes after the start of the run.

6.3 Direct Infusion Experiments

For the direct infusion experiments, the mass spectrometer was used to monitor the total number of ions (TIC) and the count rate for specific doubly and triply protonated protein fragments. The addition of an atmospheric pressure ion lens to the Protana ion source substantially improved its performance. Multiply charged peptide ion signals increased concurrently with decreases in the singly charged background chemical noise peaks. This phenomenon is demonstrated in Figure 6.3.1 for the doubly (m/z = 1031) and triply (m/z = 688) protonated ions corresponding to a hydrophilic phosphopeptide fragment (FQpSEEQQQTEDELQDK) from a β-casein digest. The ion lens potential was initially 500 V, and was increased in 500 V increments to 3000 V. For this data, the
sprayer potential was approximately 4000 V and the curtain plate potential was approximately 1600 V.

Figure 6.3.1 Mass spectral data acquired for a 500 fmol/µL sample of beta casein digest with increasing lens potential. Pane A shows the total ion current, or sum of all ions in the mass spectrum vs. time, pane B shows the signal for one of the triply charged peptides (m/z = 688.3), and pane C shows the signal for one of the doubly charged peptides (m/z = 1031.4). The labels correspond to local maxima in the ion count rate. Increasing the lens potential to 1500 V and 2500 V maximized the ion signal for the doubly charged peptide and the triply charged peptide, respectively. At the same time,
the total number of ions decreased due to a decrease in the number of unwanted singly charged background noise ions.

Figure 6.3.2 shows an expanded view of the total ion current from Figure 6.3.1A.

![Figure 6.3.2](image)

Figure 6.3.2 Total number of ions with increasing lens potential.

From the total ion current, the total number of ions detected within the mass spectrometer decreased by a factor of two as the lens potential increased from 500 V to 3000 V. The mass spectrum corresponding to time point one (0.33 minutes) is shown in Figure 6.3.3. The data in Figure 6.3.3 demonstrate that it was very difficult to find any peaks corresponding to multiply charged peptides.
Figure 6.3.3 Mass spectrum of a beta casein digest with a low lens potential.

The doubly charged (m/z = 1031) and triply charged (m/z = 688) peptides from Figures 6.3.1B and 6.3.1C could not be differentiated from the background noise continuum. The large total ion signal was generated predominantly by background noise. This type of spectrum was generated frequently when using this ion source with New Objective capillary tips on this instrument in the absence of an ion lens. The mass spectrum corresponding to time point two (2.2 minutes) from Figure 6.3.2 (high lens potential) is shown in Figure 6.3.4. The mass spectrum for the beta casein digest was much cleaner with a higher lens potential applied to the ion source. The atmospheric pressure ion lens helped to increase the ratio of multiply charged peaks relative to singly charged noise, thereby increasing the sensitivity for multiply charged ions. At high lens potentials, signals for the doubly charged (m/z = 1031) and triply charged (m/z = 688) peptide were discernable from the background noise.
Figure 6.3.4 Mass spectrum of a beta casein digest with a high lens potential.

This trend is further demonstrated in Figures 6.3.5 and 6.3.6. for the beta casein digest. The data in Figure 6.3.5 was collected using the Protana ion source with no ion lens, and the data in Figure 6.3.6 was collected using the same ion source after an atmospheric pressure ion lens was installed. In both cases, the ion source parameters were adjusted to optimize the ion signal for a triply charged phosphopeptide (m/z = 688). Analysis of the isotopic peak distribution showed that the predominant peaks in Figure 6.3.5 corresponded to singly charged background peaks, even though the source parameters were optimized for one of the multiply charged peptide peaks.
Figure 6.3.5 Mass spectrum for a beta casein digest using a Protana ion source. The ion source parameters were optimized for the triply charged peptide peak at m/z 688.3. The large peaks at m/z 749.4, 780.5, and 830.5 were singly charged background peaks.

Figure 6.3.6 Mass spectrum for a beta casein digest using a Protana ion source with an atmospheric pressure ion lens. The source parameters were optimized for the triply charged peptide peak at m/z 688.3. The ratio of the multiply charged peptides to the singly charged background peaks was substantially improved relative to Figure 6.3.5.
In addition, there was a substantial background in the vicinity of the multiply charged peptide peaks (m/z = 660 – 740). The mass spectrum in Figure 6.3.6 was much cleaner with the use of the ion lens. The background was reduced, and there was approximately a 3 times increase in the signal for the triply charged peptide. Increases of 2 – 2.5 times were observed for the other peptides in the region of m/z 660 – 740, even though the source potentials were not specifically optimized for them. The ratio of multiply charged ions to singly charged ions increased by approximately four times. The combination of increases in the ion signal and decreases in the background noise levels led to large improvements in the signal to noise ratio when an atmospheric pressure ion lens was incorporated into this reduced flow rate ion source. One way to simplify mass spectral analyses of protein digests is to ignore singly charged ions, and only consider multiply charged ions for further analysis steps such as tandem mass spectrometry (MS/MS). The ion lens is ideally suited for these types of analyses because the ratio of multiply charged ions to singly charged ions can be substantially increased.

Figures 6.3.7 and 6.3.8 demonstrate the total number of ions and the mass spectra within the vicinity of a doubly charged phosphopeptide (m/z = 1031.3) collected for a 500 fmol digest of the protein beta casein. In both cases, the ion source parameters were optimized to yield the maximum signal for the doubly charged peptide. The total number of ions (all m/z ratios) was similar for the data presented in Figures 6.3.7 and 6.3.8, however, there was a substantial increase in the magnitude of the peptide ion signal with the lens in place. In the absence of the lens (Figure 6.3.7), the peptide ion signal was near the limit of detection. The count rate for the peptide was 20 cps with a background count
rate of 4 cps. With the lens in place (Figure 6.3.8), the peptide signal was easily
differentiated from the background noise.

**Figure 6.3.7** Total ion current and mass spectrum in the vicinity of a doubly charged
peptide (m/z = 1031) from a 500 fmol digest of beta casein using a Protana ion source.

**Figure 6.3.8** Total ion current and mass spectrum in the vicinity of a doubly charged
peptide (m/z = 1031) from a 500 fmol digest of beta casein using a Protana ion source
with an atmospheric pressure ion lens.
The count rate for the peptide improved to 89 cps with a background of approximately 4 cps. The increase in peptide ion signal was achieved with no increase in the background noise. The ion lens improved the signal to noise ratio by a factor of 4.5.

The source parameters were re-optimized for a different peptide from the 500 fmol beta casein digest. Repeating the above procedure for a new doubly charged peptide (LLYQEPVLGPVR, m/z = 692) from a 500 fmol beta casein digest, similar results were obtained. The ion count rates were 1229 cps and 644 cps with and without the lens on the reduced flow rate ion source, respectively. These intensities were calculated by integration of the area under the peptide peaks. By summation of the total count rate for ions within a 1 m/z window adjacent to the peptide peak, the noise level was calculated to be approximately 40 cps and 80 cps for the ion source with and without the ion lens, respectively. The data demonstrated an improvement of 3.8 in the signal to noise ratio for this peptide. For peptides from this sample, signal to noise improvements of 3 – 6 times were observed with the addition of the ion lens.

6.4 Nano-HPLC Data

A typical nano-HPLC run with the ion lens in place is shown in Figure 6.4.1. A 50 fmol sample of bovine serum albumin digest was injected onto the C18 packing in the tapered sprayer tip. The column was initially washed with pure water, followed by a gradient, which terminated in 100% organic phase. Potentials of approximately 4200 V, 1800 V, and 1800 V were applied to the sprayer, lens, and curtain plate, respectively. The majority of the peptides eluted after 12 minutes, or towards the end of the gradient. The peptide ion signals were strong, as shown in the TIC in Figure 6.4.1, and tandem
mass spectrometry was carried out on the two most intense peptide ion signals detected in each scan.

![Typical TIC for a nano-HPLC-MS run of a 50 fmol digest of bovine serum albumin with the ion lens in place.](image)

**Figure 6.4.1** Typical TIC for a nano-HPLC-MS run of a 50 fmol digest of bovine serum albumin with the ion lens in place.

The MS/MS data was matched with a protein database, and bovine serum albumin was correctly identified with a certainty of 300 orders of magnitude above random. The ion lens was an invaluable addition to this nano-HPLC system because it was impossible to maintain a stable electrospray in its absence. When no ion lens was used with this ion source, the electrospray process ceased to operate during the aqueous portion of the gradient due to the increased surface tension of water relative to the organic phase. A large droplet formed at the tip of the tapered sprayer, and it was impossible to regenerate a stable spray. The counter-current flow of curtain gas compounded this problem by exerting a force on the droplet to push it backwards, away from the tapered tip. The
droplet moved back from the tip of the sprayer and increased in size. This prevented a stable electrospray from being generated after droplet formation. Even with a 10 times more concentrated sample of bovine serum albumin (500 fmol digest), it was difficult to complete a single run. With the lens in place, the spray process was uninterrupted, even during the 100% aqueous portion of the gradient.

Figure 6.4.2 shows a representative scan, and the fragment ion spectrum collected for one of the peptides from a 100 fmol digest of bovine serum albumin.

![Representative scan from 14.53 minutes into run](image)

**Figure 6.4.2** Representative mass spectrum from 14.53 minutes into a nano-HPLC-MS run and fragment ion spectra for a triply charged peptide with a mass to charge ratio of 480.6 from a 100 fmol digest of bovine serum albumin.

The ion source parameters were the same as those given above for Figure 6.4.1. The representative scan from 14.53 minutes showed that the most intense ion in the mass spectrum corresponded to the triply charged peptide at m/z 480.6. The system software
was programmed to carry out MS/MS on the most intense doubly and triply charged ions present in each scan. The fragment ion spectrum for ions of m/z 480.6 exhibited extensive fragmentation, while a small number of parent ions remained in the fragment ion spectrum. The MS/MS data was matched to a protein database that correctly identified the sample as bovine serum albumin. The ion lens generated fragment ion spectra with clean backgrounds, and strong peptide ion signals as demonstrated in Figure 6.4.2. It is important to note that this data could not be generated with this ion source in the absence of the ion lens because the sprayer did not tolerate the aqueous portion of the gradient.

6.5 Conclusions

An atmospheric pressure ion lens substantially improved the sensitivity and stability for direct infusion ESI-MS and nano-HPLC-MS analysis of protein digests. The lens increased the ratio of multiply to singly charged ions, and provided a substantial increase in the signals of peptide ions. In many cases, there was also a decrease in the level of the background chemical noise compared to the spectra generated in the absence of the lens. In addition, the ion lens was very useful in stabilizing the spray for reduced flow rate electrospray ion sources when aqueous samples were sprayed. In the absence of the ion lens it was difficult to obtain a successful nano-HPLC-MS run for a 500 fmol digest of bovine serum albumin. With the lens in place, the detection limits of peptides were determined to be in the low fmol range. The ion lens provided a substantial improvement to low flow rate ion sources for the analysis of multiply charged biomolecules for the study of proteomics.
6.6 References


Chapter 7: An Atmospheric Pressure Ion Lens to Improve a Nebulizer Assisted Electrospray Ion source

7.1 Purpose

In chapters 5 and 6, a device for stabilizing and increasing the ion signals from reduced flow rate electrospray ion sources was described [1]. The signal improvement was obtained by placing an atmospheric pressure ion lens around the tapered tip of the electrospray capillary, leading to an improvement in the shape of the equipotentials near the sprayer tip. The work presented in this chapter is an extension of the ion lens technology to the higher flow rate regime. The lens was found to improve the stability of the ion signals substantially, while also increasing the signal for an ion spray source with flow rates of 1 – 5 μL/min. In addition, the ion spray source was much easier to operate when the lens was in place because changes in the ion signal with different sprayer positions were greatly reduced. The lens was also found to increase the ratio of multiply charged to singly charged ions. The performance enhancements generated by the ion lens should be applicable to even higher flow rates.

7.2 Experimental

The acetate salt of bradykinin of 99% purity and β-cyclodextrin of 98% purity were from Sigma (St. Louis, MO). Various concentrations of β-cyclodextrin were prepared by dissolution of the sugar in water with 0.01 M certified ACS grade ammonium acetate from BDH chemicals (Toronto, ON, Canada). HPLC grade methanol and glacial acetic acid were from Fisher Scientific Ltd. (Nepean, ON, Canada). Bradykinin was prepared by dissolution of the peptide in a solution of 59.5% water, 39.5% methanol, and 1% acetic acid at concentrations ranging from 10^{-4} to 10^{-6} M.
The instrument used in this study was a prototype triple quadrupole mass spectrometer from SCIEX (Concord, ON, Canada) shown in Figure 1.3.2. The ion spray needle and mount shown in Figure 7.2.1 were built in-house.

**Figure 7.2.1** Schematic of an ion spray source with an ion lens. The ion spray mount (1) was attached to the mass spectrometer with a stud inserted through the mounting hole (2). The electrospray potential is applied to a stainless steel tee (3) through the conductive mount (1). Two concentric stainless steel capillaries (4) were used for the sample and the nebulizer gas flowing through the nebulizer gas line (5). The sample was introduced through a fused silica capillary (6) to the inner stainless steel tube (7). The ion lens (8) was located near the tip of the inner stainless steel tube (7). The ion lens had a mounting bracket (9) and an adjustable arm (10). Pivots (11 and 12) allowed the lens to be positioned in different locations. The length of protrusion of the inner stainless steel tube from the outer tube is labeled “X”.

Sample was delivered to the sprayer through a glass capillary (Polymicro Technologies, Phoenix, AZ) with a 150 μm outer diameter and a 50 μm inner diameter. The fused silica capillary was inserted into two concentric stainless steel tubes (Small Parts Inc., Miami Lakes, FL) with standard wall thickness for 19 and 27 gauge, respectively (referred to as...
ion spray configuration 1). For some experiments, the 19 gauge tube was replaced with a smaller diameter 21 gauge stainless steel tube (referred to as ion spray configuration 2). A stainless steel tee (Valco Instruments, Houston, TX) was used to hold the sprayer in place, and allow attachment of the nebulizer gas flow line. The nebulizer gas was compressed air (Praxair, Mississauga, ON, medical grade). The electrospray potential was applied through the mounting bracket to the stainless steel tee. The ion lens was composed of an oblong-shaped aperture cut into a rectangular piece of stainless steel with 1 mm thickness. An adjustable arm on the mounting bracket was used to adjust the position of the ion lens (Figure 7.2.2) relative to the sprayer.

Figure 7.2.2 Front view of an ion lens (8) demonstrating the location of the stainless steel sprayer tube (7) within the lens. The vertical (A) and horizontal (B) dimensions of the aperture in the lens (8) are shown.

For these experiments, the lens was set back 2 mm from the tip of the sprayer. The lens was oriented perpendicular to the axis of the stainless steel sprayer as shown in Figure 7.2.1. Different lens sizes were constructed by varying the aperture diameter in the vertical dimension (A) and the horizontal dimension (B). A Spellman CZE 1000R (Hauppauge, NY) power supply provided the voltage to the lens. Ultrahigh purity
nitrogen from Praxair (Mississauga, ON, manufacturer’s stated purity 99.9995%) was used as the curtain gas for the triple quadrupole system, and the solution flow rate was controlled by a syringe infusion pump (Model 22, Harvard Apparatus, South Natick, MA).

7.3 Electric Field Modeling for a Nebulizer Assisted Electrospray Ion Source

The mechanism of formation of ions from charged droplets in ESI is unresolved. Two mechanisms have been proposed. The first, proposed by Iribarne and Thomson, involves ion evaporation from small microdroplets with radii less than 1 μm [2]. The microdroplets may be formed by Coulombic fissions of larger droplets with an excess of charge above the Rayleigh limit of instability [2]. The second mechanism, proposed by Rollgen and co-workers, involves the release of single ions from very small droplets (diameter = 1 nm) by solvent evaporation [3]. Other research groups have also attempted to describe the mechanisms involved in the formation of ions by electrospray [4-7]. Although the proposed mechanisms have slight differences, the one similarity is that a mixture of ions and charged droplets are generated in the atmospheric pressure region between the ESI sprayer and the entrance aperture to the mass spectrometer.

In the absence of any gas flow, ions and small charged droplets at atmospheric pressure have trajectories orthogonal to the electric equipotential lines. As described in eq 5.3.1, the ion drift velocity ($\vec{v}$) is related to the electric field and the mobility [8]. The use of a nebulizer to aid in break-up of the charged droplets produced by an ion spray source generates an additional velocity vector to complicate the description of the motion of small droplets. Figure 7.3.1 shows the equipotential lines for a typical nebulizer assisted electrospray ion source.
Figure 7.3.1 Schematic of the equipotential lines generated for a standard ion spray source. Potentials are listed in the figure. The contour interval is approximately 450 V.

The potentials in this figure were 5000 V, 1000 V, 190 V, and 0 V for the sprayer, curtain plate, orifice plate, and housing, respectively. Qualitative ion trajectories (drawn by hand) illustrate that a wide plume of ions is generated, resulting in a low efficiency of transfer of ions to the mass spectrometer. Figure 7.3.2 demonstrates the improvement in the shape of the equipotential lines near the tip of the ion spray source when an atmospheric pressure ion lens is incorporated. The potentials in this figure were 5000 V, 5000 V, 1000 V, 190 V, and 0 V, for the sprayer, ion lens, curtain plate, orifice plate, and housing, respectively. The equipotential lines near the tip of the ion spray source are flattened, reducing the ion defocusing effect.
Figure 7.3.2 Schematic of the equipotential lines generated for an ion spray source with an atmospheric pressure ion lens. Potentials are listed in the figure. The contour interval is approximately 450 V.

Qualitative ion trajectories (drawn by hand) suggest that a greater number of ions is directed toward the inlet aperture of the mass spectrometer.

7.4 Optimization of the Ion Spray Source

Prior to testing of the ion lens, the configuration of the ion spray tip was optimized for the two different sprayers by adjustment of the length with which the innermost stainless steel tube protruded from the outer tube ("X" in Figure 7.2.1). This parameter was found to be most important for signal magnitude and stability with ion spray configuration 2 (21 gauge middle tube). The length, X, was varied from 1 mm to 5 mm, and the sprayer, lens, and curtain plate voltages, as well as the nebulizer gas flow were optimized to produce the maximum ion signal for the doubly charged peak of the peptide bradykinin with both ion spray configurations. The results are shown in Figure 7.4.1.
Ion spray configuration 1 was found to yield a stronger and more stable ion signal than configuration 2. The signal for configuration 1 was independent of X over the range 1 – 5 mm with a nebulizer pressure of 5 psi. For configuration 2, the maximum ion signal was obtained with X adjusted to the range between 2 and 3 mm with a nebulizer pressure of 45 psi. Shorter or longer values resulted in attenuation of the ion signal by a factor of approximately 2. For the data presented in this chapter, X values of 3 mm and 2 mm were used to optimize the signal magnitude for configuration 1 and 2, respectively.

7.5 Lens Sizes and Orientations

Various lens dimensions were used in these studies as listed in table 7.5.1. The best results were achieved with oblong-shaped ion lenses.
Table 7.5.1. Dimensions of the Oblong-Shaped Ion Lenses.

<table>
<thead>
<tr>
<th>Lens</th>
<th>A (mm)</th>
<th>B (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.90</td>
<td>12.40</td>
</tr>
<tr>
<td>2</td>
<td>10.20</td>
<td>14.10</td>
</tr>
<tr>
<td>3</td>
<td>11.10</td>
<td>14.92</td>
</tr>
<tr>
<td>4</td>
<td>13.00</td>
<td>17.60</td>
</tr>
<tr>
<td>5</td>
<td>15.00</td>
<td>19.30</td>
</tr>
</tbody>
</table>

It is important to note that lenses with dimensions smaller than lens 1 were susceptible to arcing between the lens and the sprayer. The lenses were positioned perpendicular to the sprayer. For incorporation of lenses 1 through 3 into the ion source, the sprayer was oriented at approximately a 45° angle to the curtain plate. For lenses 4 and 5, the sprayer had to be turned on a 90° angle (perpendicular to the face of the curtain plate) to prevent arcing between the edge of the ion lens and the curtain plate during optimization of the sprayer position. Similar signal magnitudes and stabilities were obtained with all of the oblong-shaped lenses.

The optimum position for the lens with both ion spray configurations was approximately 2 mm behind the sprayer tip. As the lens was moved closer to the sprayer tip, there was an attenuation of the ion signal, most likely due to a decrease in the magnitude of the electric field near the sprayer tip. This is similar to what was previously observed with a reduced flow rate electrospray ion source, except that, in this case, the nebulizer gas blew the solution away from the sprayer tip regardless of the magnitude of the electric field [1]. The effects of the lens were also attenuated as it was moved farther back from the sprayer tip, even when the lens potentials were re-optimized. For the results presented in this paper, the lens was positioned 2 mm behind the sprayer tip with both ion spray configurations.
7.6 Ion Source Stability with the Lens

The ion lens provided a substantial improvement in the stability of the ion signal for an ion spray source. Data collected for repeat measurements of the magnitude of the singly charged peak for a sample of $10^{-4}$ M β-cyclodextrin were compared. The potential applied to the detector was adjusted to obtain similar signal magnitudes with and without the ion lens. The RSD of either 6 or 20 individual measurements was calculated. Each measurement of the signal was determined by the addition of 10 scans with a 10 ms dwell time, and the peak height was used. The signal RSD was approximately 1.4% and 3.0 % for the ion spray source with and without the lens, respectively. For comparison, the RSD expected from count statistics was 0.5%. The signal RSD was reduced by a factor of approximately two with the addition of the ion lens. The signal stability improvement with the ion lens can be important for analyses such as determination of isotopic peak ratios for ions.

7.7 Sensitivity of the Ion Source with the Lens

The ion lens increased the magnitude of ion signals detected within a mass spectrometer. Typical signal increases ranged from a minimum of approximately 1.5 times up to a maximum of approximately 4 times. To achieve these results, it was found that elevated curtain plate potentials were important to maximize the ion signals. Typical potentials applied to the curtain plate were 1600 – 2000 V. There was no indication of arcing in the front end of the mass spectrometer with these elevated curtain plate potentials.

The sensitivity gains with the ion lens were greater for higher charge states. Figure 7.7.1 shows the results for analysis of a $10^{-6}$ M sample of bradykinin.
Figure 7.7.1 Mass spectra of bradykinin demonstrating an increase in the magnitude of the multiply charged peaks as the ion lens potential increased from 1000 V (a) to 3500 V (b). The magnitude of the multiply charged analyte peaks increased, but the background did not.

The mass spectrum in Figure 7.7.1a showed the signal obtained for doubly (m/z = 531) and triply protonated (m/z = 354) peptide with 6627 V, 1000 V, 1790 V, and 140 V applied to the sprayer, ion lens, curtain plate, and orifice, respectively. The mass spectrum in Figure 7.7.1b was obtained with the same sample, and the same potentials
except that 3500 V was applied to the ion lens. The increase in lens potential resulted in an increase in the magnitude of all of the doubly and triply charged peptide peaks in the mass spectrum, however, the background chemical noise was unaffected. In some cases, the background signal from singly charged ions decreased with higher lens potentials. This charge state effect is similar to that described in chapter 6 for a reduced flow rate electrospray ion source.

7.8 Variation of Peptide Ion Signal with Lens Potential

The variation of signal for doubly and triply protonated bradykinin with increasing lens potential is presented in Figure 7.8.1 for a $10^{-4}$ M sample of the peptide. The maximum ion signal obtained with ion spray was approximately $1.00 \times 10^6$ cps for the doubly protonated ion and $1.82 \times 10^5$ cps for the triply protonated ion. The addition of the ion lens increased the number of doubly and triply protonated ions detected in the mass spectrometer. The increase to the doubly charged ion was only a factor of 1.4, but the increase to the triply charged ion was a factor of approximately seven. The doubly and triply charged ion signals had maxima at different potentials. Ions of higher charge states were optimized with an increased lens potential. It is interesting to note that the significant increase in the magnitude of the signal for the triply charged ion was observed with only a minor decrease in the magnitude of the signal for the doubly charged ion. This showed that more ions were focused into the entrance aperture of the mass spectrometer, most likely due to the improved shape of the equipotential lines.
It was also possible the potential from the lens somehow changed the spray process so that more highly charged ions were formed. At very high lens potentials (above or close to the potential applied to the sprayer) the signal from all ions was completely eliminated.

**7.9 Sprayer Positional Dependence with the Lens in Place**

The following three subsections (7.9.1 – 7.9.3) describe the variation in ion signal intensity as the position of the ion spray capillary relative to the entrance aperture of the mass spectrometer is adjusted. The initial position of the sprayer (1 mm to the right hand side of the entrance aperture in the curtain plate) is labeled A in Figure 7.9. In subsection 7.9.1, the ion signal is monitored as the sprayer height is increased from position A to a height of approximately 15 mm above A with and without the ion lens. In subsections
7.9.2 and 7.9.3, the ion signal is monitored as the sprayer’s horizontal position is varied from position A to position B, a horizontal distance of Y mm.

**Figure 7.9** Configuration of the ion spray capillary relative to the entrance aperture for the results presented in subsections 7.9.1 – 7.9.3. In the initial sprayer position (A), the capillary is pointed 1 mm off to the right hand side of the aperture in the curtain plate. The data in subsection 7.9.1 compares the ion signal as the vertical height of the sprayer is increased relative to the entrance aperture with and without the ion lens. The data in subsections 7.9.2 and 7.9.3 compare the ion signal as the horizontal position of the sprayer is adjusted with respect to the entrance aperture with and without the ion lens. In the final sprayer position (B), the capillary has been moved a distance of Y mm.

### 7.9.1 Vertical Dimension

The ion signal exhibited a reduced dependence on sprayer position with the lens in place. The dependence of the signal magnitude on the vertical position of the sprayer is demonstrated in Figure 7.9.1 for ion spray with and without the atmospheric pressure ion lens.
Successful operation of this ion spray source required careful positioning of the probe in the vertical direction. For the data in Figure 7.9.1 the optimum position of the sprayer tip, with a $10^{-4}$ M sample of bradykinin, was approximately 1.5 cm from the curtain plate, and 1 mm off to the right hand side of the aperture in the curtain plate. The voltages for the sprayer, ion lens, and curtain plate were 6000 V, 2400 V, and 1970 V, respectively. For the runs without the ion lens, the voltages for the sprayer and curtain plate were 6000 V and 1100 V, respectively. A vertical position of 0 mm is defined as the point where the sprayer tip was the same height as the middle of the aperture in the curtain plate. The signal with and without the lens was normalized to 100 at this point. Positions above the optimum location were defined as positive, and positions below the optimum location
were defined as negative. The maximum range of adjustment available with this housing was \(-5.5\) mm to \(+15.5\) mm. Without the ion lens, movement of the sprayer 3 mm up or down from the optimum position attenuated the ion signal by a factor of slightly greater than 2. Elevation of the sprayer by 6 mm completely eliminated the signal for doubly protonated bradykinin using the ion spray source. The installation of the ion lens led to a dramatic decrease in the dependence of the ion signal on vertical position. The ion signal was attenuated by a factor of two only after the sprayer was raised 14 mm from the optimum position. Greater than 35\% of the initial ion signal was maintained at a sprayer position 15.5 mm above the optimal location. The ion lens provided a much broader range of useful sprayer positions.

7.9.2 Horizontal Dimension

Moving the sprayer a few millimeters in the horizontal direction from the optimum location also made a substantial difference in the magnitude of the observed ion signal with this ion spray source. The use of the ion lens near the tip of the sprayer diminished this effect. A broader range of sprayer positions was available for maintaining strong ion signals. Figure 7.9.2 demonstrates this phenomenon for movement of the sprayer off to the right hand side of the aperture in the curtain plate with a sample of \(10^{-4}\) M β-cyclodextrin in water with 5 mM ammonium acetate. The voltages and initial position of the sprayer were the same as those for the data in Figure 7.9.1. The point defined as 0 mm was the edge of the curtain plate to the right of the aperture. The signals with and without the lens were normalized to 100 at this point. The sprayer was moved in 1 mm increments to the right of the aperture in the curtain plate.
The sprayer was positioned approximately 1.5 cm from the curtain plate. Initially, with the sprayer in the optimum location, there was approximately 1.5 times more signal obtained for singly protonated cyclodextrin with the ion lens in place. When the sprayer was moved off to the right hand side, the signal decreased both with and without the lens. The signal was completely eliminated for the ion spray source when it was moved 8 mm from the optimum location. For the sprayer with the ion lens, signal was maintained for distances up to 13 mm from the optimum location. It is important to note that the signal with the lens in place was greater than the maximum ion spray signal up to approximately 4 mm from its optimum location. As the sprayer was positioned closer to the curtain plate, the effect became even more dramatic. The ion signals increased as the sprayer
was positioned closer to the entrance aperture of the instrument. However, with the ion spray source, the loss of signal was much more dramatic as the sprayer was moved to the right hand side.

The decreased dependence of ion signal on the sprayer position has important implications in the areas of high throughput analysis and separations integrated with mass spectrometry. Sprayer position can be important in situations where capillary electrophoresis or liquid chromatography are interfaced to ESI-MS. Currently, success in these types of analyses requires proper location of the sprayer. The lens provides an inexpensive performance enhancement for any electrospray source, and may be particularly useful in reducing the sprayer positional requirements for analyses involving separation techniques interfaced to ESI-MS.

7.9.3 Re-optimization of the Source Potentials

The data in Figures 7.9.1 and 7.9.2 demonstrate that the ion lens provided a much wider range of useful positions for generating strong ion signals with an ion spray source, however, this data was gathered without trying to re-optimize the source potentials at each of the positions. Figure 7.9.3 demonstrates the signal for doubly protonated ions from a $10^{-4}$ M sample of bradykinin for the ion spray source with an ion lens as the sprayer was moved off to the right hand side of the aperture in the curtain plate. In this experiment, the source potentials were either re-optimized (+) or not re-optimized (o) at each position. Greater than 90% of the initial ion signal was maintained as the sprayer was moved 8 mm from the optimum location when the source potentials were re-optimized, compared to slightly less than 50% of the original ion signal without re-optimization of the source potentials.
Figure 7.9.3 Ion signal as the ion spray source with the ion lens was moved away from the optimum location. The sprayer was moved to the right hand side of the aperture in the curtain plate, and the source potentials were re-optimized (+) or not re-optimized (o) at each position.

When the source potentials were re-optimized at each position, greater than 50% of the initial ion signal was maintained even though the sprayer was moved 13 mm from the optimum position. Optimization of the source potentials involved increasing the sprayer and ion lens potential as the sprayer was moved farther from its initial location. The curtain plate potential was approximately constant. Figure 7.9.4 shows the optimized sprayer and ion lens potential for each of the sprayer positions with respect to the aperture in the curtain plate. At sprayer potentials above 8200 V, arcing was observed in the source region. This limitation led to the dramatic decrease in the ion signal as shown in Figure 7.9.3 at a position 14 mm to the right of the aperture in the curtain plate.
Figure 7.9.4 Optimized potentials applied to the sprayer and the ion lens for the data presented in Figure 7.9.3.

7.10 Conclusions

The incorporation of an ion lens near the tip of a nebulizer assisted electrospray ion source improved its performance substantially. The lens increased and stabilized the ion signals, particularly for ions of higher charge states. In addition, the lens made an ion spray source easier to use by reducing the positional dependence of the ion signal. A strong ion signal could still be observed with the sprayer positioned at surprisingly large distances (> 1 cm) from the optimum location. The reduced sprayer positional dependence may be important for applications such as the stabilization of multiple sprayer electrospray ion sources (Part IV). In addition, the ability to turn off a sprayer
with high lens potential (Figure 7.8.1) can be important for electronic control of multiple sprayers as demonstrated in chapter 8.

7.11 References


Part IV: High Throughput Ion Sources

Introduction

Electrospray ionization – mass spectrometry (ESI-MS) has become an invaluable tool for the analysis of many types of analytes, including small molecules [1-3] and biomolecules [4-6], partly because of the commercial availability of ESI-MS coupled with separation techniques such as high performance liquid chromatography (HPLC) [7-8]. Traditionally, the separation process has been the rate-limiting step in these kinds of analyses. However with the development of new technologies such as multiple extraction column HPLC [9-10], and multichannel microfluidic devices [11], it has become increasingly important to generate more information from a single mass spectrometer in a shorter time. This can be accomplished through the use of a multiple sprayer ESI ion source.

The first description of a multiple sprayer ESI-MS system was by Smith et al. [12-13]. This system incorporated a Y-shaped interface between two electrospray ion sources and the various stages of differentially pumped vacuum. The two ion sources were used to generate ions of opposite polarity for the investigation of ion-ion chemistry. This system was not used in an attempt to increase the throughput of the mass spectrometer.

Rulison et al. were the first to describe the operation of an array of sprayers in parallel [14]. It was shown that the throughput of liquid was substantially increased when an array of six capillaries was placed in close proximity to a plate counter electrode. This study showed that the sprays generated at the tip of the four central
capillaries were similar in appearance, however end effects caused a deflection of the sprays generated on the two end capillaries.

Kostiainen et al. incorporated two and four sprayers into an ion source to increase the maximum possible solution flow rate into the ion source [15]. Shia et al. described a similar source design for the direct coupling of HPLC effluent to an ESI-MS [16]. These multiple sprayer systems were useful for increasing the total number of charged droplets generated for a particular solution flow-rate, leading to higher ion signals. Later, Tang and co-workers demonstrated improved sensitivity for ESI-MS when multiple sprayers were used to analyze a single sample [17]. None of these systems was used in an attempt to increase the sample throughput for ESI-MS analyses.

Kassel and coworkers coupled a dual sprayer ESI source to a multi-column HPLC system in an attempt to increase sample throughput [18]. The two sprayers were operated in parallel. While this system provided a substantial increase in sample throughput, a major limitation was that the analytes in the two sprayers had to produce ions of different mass to charge ratios. It was impossible to tell which sprayer the ions were generated from without a priori knowledge of the particular analytes. A similar system was used for high throughput quantitation of compounds from biological fluids [19]. Others have applied multiple sprayer technology to improve mass accuracy measurements on time-of-flight [20] and sector [21] instruments by introducing mass calibrants.

Multiple sprayer ion sources where one flow stream could be differentiated from another were first described by Kassel et al. [22] and separately by de Biasi et al. [23]. Each of these systems involved the placement of multiple electrospray tips within the source region. A rotatable plate or rotor was used to align an aperture with a particular
sprayer. In this way, it was possible to sequentially sample from each sprayer for a given
time, but it was not possible to sample from multiple sprayers simultaneously. In
addition, there was a short period of time between sprayer sampling that was required for
the movement of the aperture. This type of multiple sprayer system is commercially
available, and has been described in detail [24-27]. Sampling with the rotor system has
been shown to decrease the sensitivity by a factor of approximately three when compared
to that of a single sprayer [26].

An alternate method for generating multiple discrete sample sprays from an ESI
source has been described recently [28-29]. Multiple sprayer systems where each sprayer
was contained in a separate source region were described. With these systems it was
possible to sample from multiple sprayers simultaneously. A lens, or other ion optic
device located downstream from the source regions was used to deflect an ion beam away
from a downstream mass analyzer to shut off a particular sprayer. The performance of
these types of systems should be similar to the mechanical blocking systems described
above, with the added benefit of removal of the mechanical blocking device. A drawback
with these types of systems is that the installation of multiple separate entrance apertures
in the interface of an ESI-MS dramatically increases the vacuum pump speed required to
maintain the reduced pressure within the instrument, and this significantly increases the
cost of the mass spectrometer.
Chapter 8: A New Multiple Sprayer Ion Source for High Throughput ESI-MS

8.1 Purpose

This chapter describes a new multiple sprayer electrospray ion source that is capable of producing ions from multiple samples simultaneously or separately. This multiple sprayer system makes use of atmospheric pressure ion lenses that have been described in part 3 for reduced flow rate [30] and ion spray [31] sources. The ion lenses were located slightly behind the tip of each sprayer of the two or four sprayer systems used in the studies in this chapter. It was shown in part 3 that the incorporation of an atmospheric pressure ion lens improved the shape of the equipotential lines near the tip of a sprayer, leading to improved sensitivity, reduced dependence of the ion signal on sprayer position, and improved measurement precision (lower RSD). [30-31] In addition, it was shown that increasing the lens potential relative to a sprayer reduced the effective electric field near the tip of the sprayer to the point where the sprayer ceased to operate. In this chapter, the feasibility of using ion lens technology to build a simple and reliable multiple sprayer ion source is demonstrated. Sprayers were enabled or disabled simply by changing the potential applied to the ion lenses.

8.2 Experimental

Cytochrome c of 95% purity (from horse heart), β-cyclodextrin of 98% purity, and reserpine of 99% purity were purchased from Sigma Chemical Co. (St. Louis, MO, USA). β-Cyclodextrin was prepared at $10^{-4}$ M in water with 0.01 M certified ACS grade ammonium acetate from BDH chemicals (Toronto, ON, Canada). Cytochrome c was prepared at 10 μM in a solution of 90% water / 10% methanol (Fisher Scientific, Nepean,
Reserpine was prepared at $10^{-5}$ M in 10% water / 90% acetonitrile (Sigma Chemical Co., St. Louis, MO, USA) with 1 mM ammonium acetate.

The instrument used in this study was the prototype triple quadrupole ion spray mass spectrometer from SCIEX (Concord, ON, Canada) [32] shown in Figure 1.3.2. For some experiments, the ion spray needle and mount were replaced with a dual sprayer ion source (referred to as Prototype 1) shown in Figure 8.2.1.

![Figure 8.2.1 Schematic of Prototype 1 of a dual sprayer ion source. Sprayer mount (1), mounting hole (2), sprayer tees (3), nebulizer gas lines (4), shielded Teflon spacer (5), ion lenses (6), curtain plate (7), and sample transfer capillaries (8).](image)

Briefly, the mount (1) was fabricated from PVC with a mounting hole (2) near the back. Sprayer tees (3) were mounted in close proximity at the front of the mount. The nebulizer gas was provided through lines (4) to the tees (3). A shielded Teflon spacer (5) was inserted into the small gap between the tees to prevent arcing. The lenses (6) were located approximately 2 mm from the tips of the sprayers. The sprayers were approximately 2 cm from the curtain plate (7) during operation. The sprayer tips were
pointed approximately 5 mm to the sides of the aperture in the curtain plate. Each sprayer and lens had a separate power supply to control its potential. Potentials were applied to the lenses using Spellman CZE 1000R (Hauppauge, NY) power supplies (0 – 30 kV). Ultrahigh purity nitrogen from Praxair (Mississauga, ON, Canada, manufacturer’s stated purity 99.9995%) was used as the curtain gas for the mass spectrometer. The solution flow-rate was controlled by a syringe infusion pump (Model 22, Harvard Apparatus, South Natick, MA).

For some experiments, an alternate arrangement of a dual sprayer source (referred to as Prototype 2), shown in Figure 8.2.2, was used.

Figure 8.2.2 Schematic of Prototype 2 of a dual sprayer ion source. Sprayer mount (1), mounting hole (2), high voltage wire from electrospray power supply (3), conductive stud (4), insulated wires (5), sprayer tees (6), nebulizer lines (7), high voltage wires from lens power supplies (8), insulated conductive brackets (9), sprayers (10), slot (11), ion lenses (12), sprayer tips (13), and sample transfer capillaries (14).
Briefly, the sprayer mount (1) was fabricated from poly (vinyl chloride) (PVC). There was a mounting hole (2) in the back, and two round holes in the front for holding sprayer tees (6). A slot (11) was incorporated between the sprayer tees (Valco Instruments, Houston, TX) for addition of the four-sprayer attachment shown in Figure 8.2.3.

Figure 8.2.3 Schematic of an attachment piece to turn the dual sprayer ion source into a four-sprayer ion source. Insulated wires (5), sprayers tees (6), nebulizer lines (7), high voltage wires from lens power supplies (8), insulated conductive brackets (9), sprayers (10), ion lenses (12), sprayer tips (13), transfer capillaries (14), and slot (15).

The slot (15) on the attachment slipped into the slot (11) on the mount to generate a four-sprayer system. The high voltage wires (5) from the two or four sprayers were attached to a conductive stud (4), which was attached to a high voltage supply for application of
the electrospray potential. For some experiments, separate electrospray power supplies were attached to each sprayer. The sprayers were composed of three concentric stainless steel tubes (Small Parts Inc., Miami Lakes, FL) with standard wall thickness for 17, 19, and 27 gauge, respectively. The sample solution was delivered to the sprayers through a glass capillary (Polymicro Technologies, Phoenix, AZ) with a 150 µm outer diameter and a 50 µm inner diameter. A nebulizer gas flow of compressed air (Praxair, Mississauga, ON, Canada, medical grade) was delivered to the two or four sprayer tees through separate lines (7). An ion lens (12) was directly fastened to each sprayer tee with a screw-on bracket. The lenses were built in-house, and had aperture dimensions of 10.3 mm in the vertical direction and 13 mm in the horizontal direction. The lenses were located 2 mm back from the tips (13) of the sprayers, and a potential was applied to them through high voltage wires (8) attached to the shielded conductive brackets (9). A photograph of the four-sprayer system is shown in Figure 8.2.4. The sprayer tips were centered within the lenses, and were oriented symmetrically with respect to the aperture in the curtain plate of the mass spectrometer. During operation, the sprayers were approximately 1.5 cm from the curtain plate, and the tips of the sprayers were oriented so the spray plumes were directed in front of the aperture in the curtain plate. For results obtained using a single sprayer, a home built single sprayer and mount were used. The single ion spray source was constructed in the same fashion as each of the sprayers used in the multiple sprayer prototypes, but without an ion lens.
8.3 Orientation of the Sprayers

The orientation of the sprayers with respect to the entrance aperture of a mass spectrometer can be important for stable operation of multiple sprayer electrospray ion sources. Two different sprayer orientations were investigated in this chapter. In Prototype 1, the sprayers were parallel to each other, orthogonal to the curtain plate. The sprays were not directed at the entrance aperture of the mass spectrometer. The sprayer tips were pointed approximately 5 mm to the sides of the aperture in the curtain plate.

In Prototype 2, the sprayer tips were oriented at approximately 45 degree angles to the curtain plate to direct the sprays across the inlet aperture of the mass spectrometer. The sprayer tips were pointed approximately 1 mm from the aperture in the curtain plate.
8.4 Prototype 1

8.4.1 Enabling and Disabling a Sprayer

With this sprayer orientation and location, it was not possible to maintain ion signals within the mass spectrometer if a nebulizer gas was supplied. The extra velocity towards the curtain plate, supplied by the nebulizer, apparently prevented the ions from entering the aperture in the curtain plate. It is also possible that the high velocity nebulizer gas flow caused solvent vapor to penetrate into the curtain gas region, resulting in the formation of clusters that were too big to be observed within the mass to charge range of the instrument. However, a stable signal was obtained with the nebulizer gas turned off. Figure 8.4.1 demonstrates data collected using Prototype 1 of the dual sprayer system. The mass analyzer was fixed on a mass to charge ratio of 531, corresponding to doubly protonated bradykinin ions. Figure 8.4.1 demonstrated the ion signal (log scale) versus the scan number. The dwell time for the mass analyzer was 50 ms, so the horizontal axis is also a time axis. Initially, a strong signal was obtained for the peptide with no nebulizer gas. Potentials of 6268 V, 4300 V, 1835 V, and 190 V were applied to the sprayer, lens, curtain plate, and orifice, respectively. At measurement 577, the nebulizer gas was turned on at approximately 40 psi. The signal for the peptide was eliminated within 150 ms, demonstrating that the nebulizer gas flow can be used to disable a sprayer with this configuration. However, when the nebulizer gas was turned off, at approximately measurement number 735, the ion signal was slow to re-stabilize (requiring approximately 8 seconds), even though only 3 – 4 seconds was required to drain the residual gas from the nebulizer line. For comparison purposes, an increase in
lens potential to 8000 V was used to shut off the sprayer at approximately measurement 1360.

![Graph showing comparison of enabling and disabling of one sprayer from Prototype 1 of a dual sprayer electrospray ion source, using the nebulizer gas flow, the lens potential, and the sprayer potential. The mass analyzer was fixed on a mass to charge ratio corresponding to doubly protonated bradykinin (m/z = 531). The peptide signal was completely eliminated within 50 ms. The signal was regenerated within 50 ms when the lens potential was decreased to re-enable the sprayer at measurement 1445. The sprayer potential was decreased, at scan 1610, to eliminate the ion signal within approximately 100 ms. By turning on the sprayer potential, the signal was regenerated within approximately 150 ms. These data demonstrate that turning the nebulizer gas flow on or off is not an effective method for enabling or disabling a sprayer. The sprayer potential can be used, however it is at least 3 – 5 times slower than using the lens potential. The lens power supply was manually controlled for the]
collection of this data, requiring approximately 1 s to change from enabling to disabling, or vice versa. Since the signal stabilized within 50 ms, it occurred during the ramping of the lens potential.

8.4.2 Operation of Prototype 1

Difficulties were encountered with Prototype 1 when trying to simultaneously sample ions from both sprayers. Although a strong signal could be generated from either one of the sprayers in this system, when the neighboring lens potential was set to the optimum potential for that sprayer alone, the signals for both sprayers destabilized as shown in Figure 8.4.2.

![Destabilized signals with Prototype 1](image)

**Figure 8.4.2** Destabilized signals with Prototype 1. Initially, a strong signal was present for reserpine (m/z = 609), and no signal was present for bradykinin (m/z = 531).

A sample of reserpine and bradykinin was provided to sprayers 1 and 2, respectively. Initially the potentials were 6725 V, 3000 V, 0 V, 0 V, and 1800 V for sprayer 1, lens 1, sprayer 2, lens 2, and the curtain plate, respectively. A strong signal was present for protonated reserpine from sprayer 1, and no signal was present for bradykinin ions from
sprayer 2. At approximately measurement 410, the potential applied to sprayer 2 was increased to 6381 V. The signal at m/z 609 from sprayer 1 destabilized briefly, and then returned to its previous magnitude and stability. At approximately measurement 640 the potential applied to lens 2 was increased to 3000 V. The signal at m/z 531 increased briefly, and then the ion signals at m/z 531 and 609 both destabilized. The ion signals could not be regenerated. The potential applied to one ion lens was believed to repel the ions generated from a neighboring sprayer. This was supported by equipotential modeling with MacSimion 2.0 that showed a detrimental effect on ion trajectories when a large positive potential (in this case the neighboring lens) was located on the opposite side of an inlet aperture. For this reason, only one of the sprayers could be operated at a time, however, a substantial improvement in sample throughput could still be achieved with this system. Each sprayer can be connected to separate HPLC systems, permitting a rinsing step to be performed in one sprayer while the other is operating. This prototype could still be operated in a fashion analogous to an ion source with a mechanical blocking device, because it is not possible to simultaneously sample ions from multiple sprayers in this type of ion source either.

8.5 Prototype 2

The main limitation with Prototype 1 seemed to be the orientation of the sprayers. The sprays were directed off to the side of the inlet aperture of the mass spectrometer. Therefore, as discussed above, a large potential applied on the opposite side of the aperture repelled the ions and charged droplets generated in the spray. To alleviate this problem, Prototype 2 was developed. With this prototype, the sprayers were oriented so they directed ions across the inlet aperture of the mass spectrometer. This
was found to eliminate the loss of ion signal when a neighboring lens potential was increased.

8.5.1 Comparisons of Signal Magnitude from one Channel of a Four-Sprayer System and a Single Sprayer

Initial tests were performed using the four-sprayer version of Prototype 2. Direct comparisons were made with the signals obtained from a sample of bradykinin using a single sprayer ion spray source without a lens and using one channel of the four-sprayer source with a lens. The mass spectra are shown in Figure 8.5.1. With the single sprayer source, 5200 V and 1000 V were applied to the sprayer and curtain plate, respectively. For the four-sprayer system, potentials of 5500 V, 1000 V, and 1000 V were applied to the sprayers, lens 1, and the curtain plate, respectively. No potential was applied to the other three ion lenses in this experiment (i.e. they were floating). All other settings of the mass spectrometer were constant for these two mass spectra. It is important to note that the two mass spectra were almost identical. There was a slight increase in the ratio of the triply charged peptide peak (m/z = 354) and its fragment peak (loss of water m/z = 348) with the sprayer and lens of the four-sprayer system, however, it was a minor difference. Similar results could be obtained from the other sprayers by separately optimizing the voltages associated with their operation. These data are important, because they show that the four-sprayer device can be used as a direct replacement for a single ion spray source. If only one sprayer is required, a single channel of the system can be used. This is a significant advantage over other systems, where the incorporation of multiple sprayers degrades the sensitivity in comparison to a single sprayer source [26].
Figure 8.5.1 Comparison of a mass spectrum obtained using a single sprayer ion spray source (a) and one channel of a four-sprayer ion spray source (b).

8.5.2 Enabling and Disabling a Sprayer

For simplicity, and due to a lack of additional power supplies, the rest of the data presented in this chapter were collected using only the dual sprayer system of Prototype
2. Figure 8.5.2 demonstrates the effect of lens potential on ion signal for prototype two. These data were collected by fixing the mass analyzer on mass to charge ratios corresponding to doubly protonated bradykinin (m/z = 531) and triply protonated bradykinin (m/z = 354). The vertical axis is a logarithmic scale. Each data point was collected with a 50 ms dwell time, so the horizontal axis is also a time axis. Initially, a sample of bradykinin was provided to sprayer one, and no solution was provided to sprayer two.

![Figure 8.5.2](image)

**Figure 8.5.2** Data collected for repeated enabling and disabling of one sprayer from Prototype 2 of a dual sprayer electrospray ion source. The mass analyzer was fixed on mass to charge ratios corresponding to doubly (m/z = 531) and triply (m/z = 354) protonated bradykinin. The signal for doubly protonated bradykinin was approximately 100 times greater than the signal for triply protonated bradykinin.

Voltages of 6000 V, 600 V, and 1961 V were applied to the sprayers, lens one, and the curtain plate, respectively. Lens two was floating (i.e. not connected to a power supply).
A strong signal was present for doubly protonated bradykinin, and a smaller signal was present for triply protonated bradykinin. At approximately measurement 50, the potential applied to lens one was increased to 8000 V to disable sprayer one. The signals at mass to charge ratios 531 and 354 dropped off to 0 cps within approximately 200 – 300 ms of the increase in lens potential. It is important to note that the lens power supply was controlled manually, and 1 s was required to change the potential from enabling to disabling, or vice versa. Therefore, the signal was completely eliminated prior to the lens potential arriving at 8000 V. On measurement number 154, the potential applied to lens one was decreased to 600 V to re-enable sprayer one. The ion signals returned to their previous magnitudes and stabilities within approximately 200 – 300 ms of the change in lens potential. The sprayer was disabled and re-enabled repeatedly to demonstrate the reproducibility of this effect.

8.5.3 Operation of Prototype 2 with a Single Electrospray Power Supply

Initial experiments with Prototype 2 of the dual sprayer system were conducted using a single electrospray power supply to apply the same potential to both of the sprayers. The use of one electrospray power supply decreased the cost associated with this multiple sprayer system, however there were some limitations (discussed in detail later). Figure 8.5.3 shows data collected with a sample of reserpine in sprayer one and cytochrome c in sprayer two. Initially both sprayers were enabled with potentials of 6500 V, 4000 V, 2500 V, and 1800 V applied to the sprayers, lens 1, lens 2, and the curtain plate, respectively. Figure 8.5.3a demonstrates that a strong ion signal was observed for various charge states of cytochrome c (+9 to +18) and for singly protonated reserpine (R+H⁺), (m/z = 609), as well as some solvated ion signals.
Figure 8.5.3 Data collected using Prototype 2 of a dual sprayer electrospray ion source with samples of reserpine and cytochrome c in sprayers 1 and 2, respectively. Both sprayers were enabled in Figure 8.5.3a. Sprayers 1 and 2 were disabled in Figures 8.5.3b and 8.5.3c, respectively. A single power supply was used to apply the same electrospray potential to each sprayer.
The potential applied to lens 1 was increased to 8000 V to disable sprayer one, as shown in Figure 8.5.3b. The count rate at mass to charge ratio 609 decreased to the level of the background noise from sprayer two, and the cytochrome c signals were relatively unaffected (approx. 96% of the initial signal). The large potential applied to lens 1 did not affect the signal generated from sprayer 2 because ion lens 2 helped to shield sprayer 2 from the other sprayer and lens. Figure 8.5.3c shows the mass spectrum obtained with sprayer one re-enabled (lens one potential = 4000 V), and sprayer two disabled (lens two potential = 8000 V). The signals for various charge states of cytochrome c were eliminated, leaving only reserpine peaks, and a residual background noise from sprayer one. In this case, the signal for reserpine (sprayer 1) was attenuated by a factor of 2.

Figure 8.5.4 shows data similar to that in Figure 8.5.3, with a sample of $10^{-4}$ M bradykinin in sprayer 1, and a sample of $10^{-6}$ M cytochrome c in sprayer 2. The potentials were 6500 V, 4000 V, 2500 V, and 1800 V applied to the sprayers, lens 1, lens 2, and the curtain plate, respectively. The predominant peak in Figure 8.5.4a corresponded to doubly protonated bradykinin ($m/z = 531$), however, there were also peaks corresponding to various bradykinin fragments, and various charge states for cytochrome c (+9 to +17). Figure 8.5.4b shows the mass spectrum obtained with sprayer 1 disabled (lens 1 potential = 8000 V). Only peaks corresponding to various charge states of cytochrome c remained. The ion signals were attenuated by approximately 10% for the protein. Figure 8.5.4c shows the data obtained with sprayer 1 re-enabled (lens 1 potential = 4000 V) and sprayer 2 disabled (lens 2 potential = 6000 V). Once again, the signal from sprayer 1 was attenuated by a factor of two with a large potential applied to lens 2.
Figure 8.5.4 Data collected using Prototype 2 of a dual sprayer electrospray ion source with samples of bradykinin and cytochrome c in sprayers 1 and 2, respectively. Both sprayers were enabled in Figure 8.5.4a. Sprayers 1 and 2 were disabled in Figures 8.5.4b and 8.5.4c, respectively. A single power supply was used to apply the same electrospray potential to each sprayer.
As described above, the use of a single electrospray power supply to apply the same potential to multiple sprayers decreased the expense associated with a multiple sprayer ion source. However, the use of a single electrospray power supply resulted in a compromise for the potential applied to the sprayers. The potential applied to each of the sprayers could not be optimized separately, although in some cases, the lenses could be used to compensate for this. It seems logical that if the sprayers were oriented completely symmetrically with respect to the inlet aperture of the mass spectrometer, the optimum potentials for the two sprayers would be the same. Experimentally, it was found that different sprayers required different electrospray potentials even when they appeared to be symmetrically oriented. A contributing factor may be the difficulty associated with building two sprayers that are completely identical. The electrospray potential seemed to be more optimized for sprayer 2, as shown by the 2-fold decrease in signals observed from sprayer 1 in Figures 8.5.3c and 8.5.4c. It was found that separate power supplies must be used to have different sprayers behave similarly.

8.5.4 Operation of Prototype 2 with Separate Electrospray Power Supplies

Figure 8.5.5 demonstrates data obtained with Prototype 2 with a sample of bradykinin in sprayer 1 and a sample of reserpine in sprayer 2, with separate electrospray power supplies for each sprayer. For these data, the mass analyzer was fixed on a mass to charge ratio of 531 (corresponding to doubly protonated bradykinin) and 609 (corresponding to protonated reserpine). The vertical axis has a logarithmic scale, and the dwell times were 50 ms. Initially, signals were present for both singly protonated reserpine and doubly protonated bradykinin with 6000 V, 6918 V, 1800 V, 3000 V, and 1835 V applied to sprayer 1, sprayer 2, lens 1, lens 2, and the curtain plate, respectively.
At approximately measurement 360, the potential applied to lens 1 was increased to 6500 V to disable sprayer 1. The signal for doubly protonated bradykinin was eliminated, however, the count rate at m/z = 531 did not decrease to 0 cps. It decreased to the level of the background noise from sprayer 2.

![Graph](image)

**Figure 8.5.5** Data collected using Prototype 2 of a dual sprayer electrospray ion source with samples of bradykinin and reserpine in sprayers 1 and 2, respectively. Both sprayers were enabled initially. At scan 360, sprayer 1 was disabled. At scan 820, sprayer 1 was re-enabled. Sprayers 2 and 1 were disabled at scans 1200 and 1620, respectively. The signal for doubly protonated bradykinin was approximately 10 times greater than the signal for protonated reserpine.

At the same time, there was a slight increase in the signal for singly protonated reserpine (m/z = 609). At approximately measurement 820, sprayer 1 was re-enabled (lens one potential decreased to 1800 V), and the signal for doubly protonated bradykinin and singly protonated reserpine returned to their initial magnitudes and stabilities. At approximately measurement 1200, the potential applied to lens 2 was increased to 8000 V to disable sprayer 2. The signal for singly protonated reserpine was eliminated, the count rate at m/z = 609 decreased to the level of the residual background noise from sprayer 1.
At the same time, the signal for doubly protonated bradykinin increased and stabilized slightly. At approximately measurement 1620, the potential applied to lens 1 was increased to 6500 V to disable it. With both sprayers disabled, the count rates (signal and background ions) at m/z = 609 and 531 dropped to 0 cps. Figure 8.5.6 shows low-resolution mass spectra near mass to charge 531 with sprayer 1 enabled (a) and disabled (b).

**Figure 8.5.6** Low-resolution mass spectra in the vicinity of mass to charge ratio 531 for the data presented in Figure 8.5.5. Spectra were obtained with sprayer one enabled (a) and disabled (b).
The vertical scale is 1000 times smaller in Figure 8.5.6b (sprayer 1 disabled). Initially, there was a very strong signal for doubly protonated bradykinin (approximately $1.2 \times 10^6$ cps). The increase in potential applied to lens 1 completely eliminated the signal for bradykinin leaving only noise in the vicinity of $m/z = 531$. The data in Figure 8.5.5 demonstrate that the two sprayers behave similarly when separate electrospray power supplies are used with the dual sprayer electrospray system.

8.6 Conclusions

This chapter provided proof in principle for a new type of multiple sprayer ion source that can overcome many of the limitations associated with the present commercially available systems using mechanical blocking devices for control of sprayers. The sprayers were enabled or disabled simply by application of appropriate potentials to ion lenses. The lenses helped to shield the sprayers from the potentials applied to neighboring sprayers. The key advantages of this type of system in comparison to mechanical devices include lower cost, mechanical simplicity, faster enabling/disabling of sprayers, no moving parts, independent adjustment of the sprayers to optimize ion signals, and no decrease in sensitivity with increased numbers of sprayers. In addition, there was no evidence of memory effects with this apparatus.

This ion source presents an inexpensive alternative for high throughput ESI-MS. Unlike other commercially available high throughput ion sources, multiple sprayers may be enabled simultaneously for infusion of mass calibrants or multiple sample streams. In addition, there are no moving parts to wear out, and the signal generated from the sprayers is not attenuated. Even with manual lens potential control (approximately 1 s to
adjust the power supply), the sprayers can be enabled or disabled as quickly as commercially available ion sources based upon mechanical blocking devices (approximately 50 ms). The addition of high speed switching devices to each lens should reduce this time substantially.

8.7 References


Concluding Remarks

This thesis describes new methods and instrumentation for electrospray ionization mass spectrometry. Section 1 focused on the physical and chemical processes in the interface region of an ESI-MS. Future research in this area should focus on application of the model developed in chapters one and two to other interface designs, such as the heated capillary described in Figure 1.2.2. In these types of interfaces, the gas dynamic processes are different than those described in chapters 2-4. New equations would need to be derived to describe the variation in gas number density in this type of interface. Also, condensation studies could be performed on a mass spectrometer with a heated capillary interface. Since the temperature of the heated capillary (source temperature) can be accurately controlled, further testing of the equation for the condensation parameter could be performed. In addition, various other types of curtain gases could be used, including sulphur hexafluoride and gas mixtures.

In part two of this thesis, new electrospray and ion spray sources were developed. Future research would involve the development of these ion sources from the proof of principle stage to a marketable product. This would involve further modeling to determine the best geometries for the lenses relative to the ion source. Work would also be required to fit these ion sources to various commercial mass spectrometers and develop effective software control.

Part three of this thesis provided proof of principle for new multiple sprayer ion sources for high throughput ESI-MS. Future research would lead to commercialization of these ion sources. Work would involve automation of the ion source control with computer software, as well as modifications to fit into commercial mass spectrometers.
In addition, high speed switches could be added to each of the lens power supplies to permit the sprayers to be enabled and disabled much faster than commercially available high throughput ion sources. Another future direction would be the addition of more sprayers to these ion sources. Four separate four-sprayer ion sources could be mounted in the source region of a mass spectrometer without major source redevelopment. This would greatly increase the sample throughput with this system because 16 sprayers would be available. Finally, future research initiatives could focus on the placement of other atmospheric pressure ion lenses within the ion source between the sprayers to further isolate each capillary from its neighbor.