INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
EXTERNAL ION INJECTION AND APPLICATIONS OF ION AXIALIZATION IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By

Jarrod A. Marto, B. S.

*****

The Ohio State University

1995

Approved by

Dissertation Committee:
Professor Alan G. Marshall
Professor Prabir K. Dutta
Professor Richard L. McCreery

Advisor
Coadvisor
Department of Chemistry
To my loving family: Mom, Dad, Kim, Mike, Daniel, and Cindy
ACKNOWLEDGMENTS

First and foremost, I wish to thank Dr. Alan Marshall for providing all the resources necessary to pursue the research presented in this thesis. I would also like to express my appreciation to the co-authors of the work presented herein: Lutz Schweikhard was extremely patient and helpful when I first started working in the Marshall group; Shenheng Guan provided advice and assistance in many aspects of my graduate research; Mike May provided programming for the SIMION ion trajectory simulation studies; Forest White worked tirelessly on phospholipid analysis; Touradj Solouki did a majority of the MALDI detection limit work. Mike Senko, Chris Hendrickson, Ljiljana Pasa Tolic, Forest White, and I worked, and argued, long and hard during construction of the electrospray FTMS. A special thanks goes to Pat Limbach, who not only contributed significantly to much of my research, but has also provided a wealth of advise and assistance, both personal and professional, since the day I began my graduate career. I am grateful for all he has done and certainly look forward to future collaborations.

Others who have made a positive contribution to my research efforts include: Tom Ricca, who provided guidance and assistance in the construction of peripheral electronics necessary for my research; Jerry
Hoff, John Herlinger, and Kevin Tewell, of the OSU chemistry department machine shop, whose patience and skill created many custom-machined components for my research. I also want to thank Kaye Craggs and Bobbie Cassity for their administrative expertise; their efforts greatly reduced my frustration in dealing with the bureaucracy. Bobbie Cassity has been especially helpful in this regard during my time in Tallahassee. Several of my classmates deserve special mention: Mike Ledney, Glenn Jordan, Beth Groeber, Chris Black, and Steve and Kayla Lee. These people helped maintain my sanity the first two years and I have missed them tremendously since moving to Tallahassee.

Finally, I thank my family; I owe everything to their endless understanding, support, and sacrifice.
VITA

Oct. 26, 1966 Born, Dayton, Ohio

1985 - 1990 B.S. in Chemistry. Centre College, Danville, Kentucky

1990 - 1993 Graduate Research Associate, The Ohio State University, Columbus, Ohio

1993 - present Graduate Research Associate, the National High Magnetic Field Laboratory, Tallahassee, Florida

PUBLICATIONS


FIELD OF STUDY

Major Field: Chemistry
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>VITA</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
</tbody>
</table>

## CHAPTER

1. FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY
   - Introduction ........................................................................... 1
   - Historical Developments ............................................ 1
   - Fundamental Ion Motion .................................................. 5
     - Ion Motion in a Static Magnetic Field .................... 5
     - Ion Motion in Static Magnetic and Electric Fields ...... 10
   - Excitation of Ion Cyclotron Motion ......................... 14
     - Single Frequency Excitation ................................ 17
     - Frequency Sweep Excitation ................................... 19
     - SWIFT Excitation .................................................. 19
     - Imperfections in the Excitation Process ............... 20
### III. AN ELECTROSTATIC WIRE ION GUIDE FOR INJECTION OF EXTERNALLY-FORMED IONS: THEORY AND SIMULATION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>92</td>
</tr>
<tr>
<td>Theory: Analytical Methods</td>
<td>96</td>
</tr>
<tr>
<td>Electrostatic Wire Ion Guide in the Absence of a Magnetic Field</td>
<td>96</td>
</tr>
<tr>
<td>Magnetic Mirror Effect in the Absence of an Electric Field</td>
<td>96</td>
</tr>
<tr>
<td>Electrostatic Ion Guide in a Magnetic Field</td>
<td>101</td>
</tr>
<tr>
<td>Analytical Expressions - EIG in the Absence of a Magnetic Field</td>
<td>102</td>
</tr>
<tr>
<td>Theory: Computational Methods</td>
<td>104</td>
</tr>
<tr>
<td>SIMION Numerical Simulations - EIG in a Magnetic Field Gradient</td>
<td>104</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>108</td>
</tr>
<tr>
<td>Ion Acceptance into the Electrostatic Ion Guide</td>
<td>108</td>
</tr>
<tr>
<td>Ion Loss Through Collision with the Central Wire</td>
<td>116</td>
</tr>
<tr>
<td>Ion Transport Through a Magnetic Field Gradient to an ICR Penning Trap</td>
<td>119</td>
</tr>
<tr>
<td>Ion Axial Kinetic Energy After Exiting the EIG</td>
<td>120</td>
</tr>
<tr>
<td>Limitations of the Present Trajectory Calculations</td>
<td>126</td>
</tr>
<tr>
<td>Conclusion</td>
<td>128</td>
</tr>
<tr>
<td>Ion Capture</td>
<td>128</td>
</tr>
<tr>
<td>Ion Transport</td>
<td>128</td>
</tr>
<tr>
<td>Ion Axial Kinetic Energy Distribution</td>
<td>128</td>
</tr>
<tr>
<td>References</td>
<td>130</td>
</tr>
</tbody>
</table>

### IV. AN ELECTROSTATIC WIRE ION GUIDE FOR INJECTION OF EXTERNALLY-FORMED IONS: INSTRUMENTATION AND EXPERIMENT

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>134</td>
</tr>
<tr>
<td>Quadrupolar Excitation for Ion Axialization</td>
<td>135</td>
</tr>
<tr>
<td>Experimental</td>
<td>138</td>
</tr>
<tr>
<td>External Source FT/ICR Mass Spectrometer</td>
<td>138</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>145</td>
</tr>
<tr>
<td>Broadband Axialization of Ions Generated from a Continuous External Cs(^+) Source</td>
<td>145</td>
</tr>
<tr>
<td>Broadband Axialization of Ions Generated from a Pulsed Source</td>
<td>147</td>
</tr>
<tr>
<td>Conclusion</td>
<td>152</td>
</tr>
<tr>
<td>References</td>
<td>154</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>58</td>
</tr>
<tr>
<td>2.2</td>
<td>85</td>
</tr>
<tr>
<td>2.3</td>
<td>86</td>
</tr>
<tr>
<td>3.1</td>
<td>110</td>
</tr>
<tr>
<td>3.2</td>
<td>118</td>
</tr>
</tbody>
</table>

## TABLE 2.1
Comparison of the design specifications and the measured values for the major dimensions of the two-electrode ion trap.

## TABLE 2.2
Mass measurements for ions derived from acetophenone and acetone, internally calibrated from perfluorotri-n-butylamine.

## TABLE 2.3
Mass measurements for ions derived from 1-bromo-4-fluorobenzene and m-xylene, internally calibrated from perfluorotri-n-butylamine.

## TABLE 3.1
Collection efficiency (i.e., solid angle of the acceptance cone divided by $4\pi$) for each of four combinations of ion initial kinetic energy, $E_{\text{ion}}$, and potential difference, $\Delta V$, between the cylinder ($r_{\text{cylinder}} = 17 \text{ mm}$) and central wire ($r_{\text{wire}} = 0.14 \text{ mm}$) of an electrostatic ion guide, for a circular disk ion source of 6 mm radius, and for a point ion source displaced radially by 6 mm. Both sources are located in the EIG entrance plane.

## TABLE 3.2
The ratio, $F_{\text{collide/capture}(r_0)}$, of the fraction of ions which is captured but collide with the EIG central wire to the total fraction of ions captured, as a function of initial ion radial displacement. $F_{\text{collide/capture}(r_0)}$ is calculated from Eqs. 3-8 and 3-11, and applies to an ion point source located at the EIG entrance plane.
4.1 Comparison of signal-to-noise ratio (S/N) and mass resolving power (m/Δm) for cesium iodide cluster cations, Cs(CsI)$_n^+$, in the presence and absence of axialization. Note that axialization produces comparable signal-to-noise ratio, but for 4000 times fewer ions.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>6</td>
</tr>
<tr>
<td>1.2</td>
<td>7</td>
</tr>
<tr>
<td>1.3</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>9</td>
</tr>
<tr>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td>1.6</td>
<td>15</td>
</tr>
<tr>
<td>1.7</td>
<td>18</td>
</tr>
</tbody>
</table>

1.1 An ion with initial velocity, \( v_{xy} \), in a homogeneous magnetic field, \( B_0 \), is bent into a circular path due to the Lorentz force, \( F_L \). ICR orbital frequency, \( \omega_c \), is inversely proportional to ion mass-to-charge ratio. 

1.2 ICR orbital frequency (Hz) plotted versus mass-to-charge ratio \((m/z)\) for 3 T, 7 T, and 9.4 T magnetic field strengths.

1.3 ICR orbital radius (mm) plotted versus mass-to-charge ratio \((m/z, \text{thermal energy})\) for 3 T, 7 T, and 9.4 T magnetic field strengths.

1.4 Ion kinetic energy (eV) for \(m/z = 1000\) plotted versus ICR orbital radius (mm) for 3 T, 7 T, and 9.4 T magnetic field strengths.

1.5 Basic configuration of a cubic ion trap, showing the trapping (T), excitation (E), and detection (D) electrodes.

1.6 Schematic representation of the cyclotron (frequency = \( \omega_+ \), radius = \( \rho_+ \)), magnetron (frequency = \( \omega_- \), radius = \( \rho_- \)), and trapping (frequency = \( \omega_T \)) motions in an ICR ion trap. Figure graciously provided by Hyun Sik Kim.

1.7 Schematic representation of various time-domain (left) and corresponding frequency-domain (right) ICR excitation waveforms. a.) Single frequency excitation; b.) Frequency sweep or "chirp" excitation; c.) SWIFT (broadband) excitation; d.) SWIFT (selective excite and eject) excitation. Figure taken with permission from ref. 44.
2.1 Three-dimensional (top) and cross-sectional views of the two-electrode ion trap, showing the ring and core electrodes, and the ceramic aligning ring. The measured trap dimensions are a.) \( \rho_{\text{core}} = 0.324 \text{ cm} \); b.) \( \rho_{\text{ring}} = 2.555 \text{ cm} \); c.) \( \rho_{\text{core,max}} = 3.633 \text{ cm} \); d.) \( \rho_{\text{ring,max}} = 3.665 \text{ cm} \); e.) \( z_{\text{core,max}} = 2.608 \text{ cm} \); f.) \( z_{\text{ring,max}} = 1.829 \text{ cm} \); g.) trap length = 7.678 cm; h.) trap diameter = 8.882 cm.

2.2 Schematic diagram of the circuit used to couple the two-electrode ion trap to the standard excitation and detection electronics of the Extrel FTMS-1000 mass spectrometer.

2.3 Effect of trapping potential on observed ion cyclotron frequency. Top: FT/ICR frequency of \( \text{N}_2^+ \) as a function of trapping potential in the two-electrode ion trap. Bottom: Normalized FT/ICR frequency shift as a function of trapping potential in the two-electrode (\( \text{N}_2^+ \)) and Penning (\( \text{C}_6\text{H}_6^+ \)) traps.

2.4 Variation of experimental ICR frequency of \( \text{C}_6\text{H}_6^+ \) with ICR orbital radius in the two-electrode trap. Ion were excited by either: varying the single-frequency resonant r.f. excitation amplitude from 0.5 to 19.0 \( V_{\text{p-p}} \) for a constant excitation interval of 100 ms; or varying the excitation interval from 60 to 2200 \( \mu\text{s} \) at a constant amplitude of 0.8 \( V_{\text{p-p}} \).

2.5 Experimental ICR frequency shift of \( \text{C}_6\text{H}_6^+ \) as a function of the amplitude times duration product in the two-electrode, cubic, and Penning ion traps. The smaller frequency variation for the two-electrode trap demonstrates its near-perfect quadrupolar electrostatic trapping potential. Note that the ordinate is magnified by a factor of 10 in Fig. 2.4 relative to Fig. 2.5.
2.6 Experimental ICR frequency of C₆H₆⁺ as a function of the relative number of ions formed (as reflected by the duration of the ionizing electron beam), for two-electrode, cubic, and Penning ion traps. The small frequency variation (≈ 8 Hz) observed in the two-electrode trap demonstrates the reduced space charge associated with off-axis ionization.

2.7 The number of ions contributing to the observed signal in the two-electrode ion trap. At a typical trapping potential of 2.0 V (top), the maximum number of ions detected is 71,000, whereas at 10.0 V (middle), the number of ions detected increases to 230,000. The bottom graph shows both sets of data re-plotted up to a signal-to-noise ratio of 100; extrapolating the best fit lines to a (magnitude-mode spectral peak height)/(baseline standard deviation noise) ratio of 3:1 yields detection limits of 500 (2.0 V) and 440 (10.0 V) singly charged ions.

2.8 Radial (top) and axial (bottom) ion ejection behavior of the two-electrode ion trap. Top: radial ion ejection profile in which the FT/ICR magnitude-mode mass spectral relative peak height is plotted as a function of ICR orbital radius. Bottom: axial ion ejection, as observed by a drop in the FT/ICR magnitude-mode mass spectral relative peak height for resonant cyclotron frequency excitation/detection following prior irradiation at the plotted abscissa frequency.

2.9 Mass discrimination in the two-electrode (top), cubic (middle), and Penning (bottom) ion traps. The relative FT/ICR magnitude-mode mass spectral peak heights for six fragment ions from PFTBA are plotted versus relative excitation amplitude-duration product.

2.10 Theoretical z-amplitudes for ions of m/z = 69, 131, 264, and 502 as a function of excitation amplitude-duration product in the two-electrode ion trap. The abscissa range includes typical experimental values used in this study; the ordinate values were calculated from Eq. 2-44 and subsequently scaled to an initial value of 1.
3.1 Potential inside the electrostatic wire ion guide (EIG) plotted as a function of relative ion radius \( r_{\text{ion}}/r_{\text{cylinder}} \). The steep potential slope focuses ions close to the central wire electrode, thereby minimizing the magnetic mirror effect. ............................... 98

3.2 Schematic representation of the magnetic mirror effect. As an ion progresses axially (middle trajectory) toward the ICR ion trap (located at 0 axial displacement in top plot), it gains radial kinetic energy at the expense of axial kinetic energy, due to interaction with the radial magnetic field (top). Eventually, the ion may be reflected away from the solenoid. Bottom: ion trajectories in the x-y plane (e.g. looking down the z-axis - see text). ..................... 100

3.3 Schematic diagram of the electrostatic wire ion guide (EIG) configuration, with dimensions shown in mm. Top: the three major electrode sets, including the source, the EIG wire and cylinder, and the ICR cylindrical ion trap. Bottom: coordinate system on which Equations 3-7 - 3-12 are based. .............................. 105

3.4 Axial (z-component) and radial (r-component) magnetic field magnitudes, \( B_z \) and \( B_r \), as input parameters to SIMION for a typical 3 T solenoid magnet. The magnetic field value between any two \( B_z \) and \( B_r \) points (entered at 5 cm increments) is obtained by linear interpolation. ................................. 107

3.5 Collection efficiency, \( \text{Eff}_{\text{point}}(r_0) \), of a point ion source located in the entrance plane of the EIG as a function of the ion source radial displacement, \( r_0 \). Note that even ions initially far off-axis are successfully captured and transported by the EIG to the ICR ion trap. ......................................................... 109

3.6 Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap vs. initial z-displacement \( (z_0) \) of the on-axis \( (r_0 = 0) \) ion source from the EIG entrance plane, for an ion of \( m/z = 1000 \), at EIG potential differences, \( \Delta V = 5, 10, 20 \) V. The ion initial velocity elevation angle, \( \theta \), is fixed at 15°. ........................................................................ 112
3.7 Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap as a function of initial ion velocity elevation angle, $\theta$, for an ion of $m/z = 1000$ with initial axial and radial displacements of 1 mm and 6 mm. The initial ion azimuthal angle, $\phi$, is fixed at $15^\circ$ ................................................................. 113

3.8 Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap vs. initial velocity azimuthal angle, $\phi$, for an ion of $m/z = 1000$ starting 6 mm off-axis and 6 mm axially displaced from the EIG entrance plane. The ion initial velocity elevation angle, $\theta$, is fixed at $15^\circ$ ...................................................... 115

3.9 Final ion cyclotron and magnetron radii, $p_+$ and $p_-$, as a function of ion initial velocity azimuthal angle, $\phi$. Ions begin with axial and radial displacements of 6 mm, and velocity elevation angle, $\theta$, fixed at $15^\circ$. Ions with $0^\circ \leq \phi \leq 180^\circ$ are "dis-rotatory" whereas those with $180^\circ \leq \phi \leq 360^\circ$ are "con-rotatory" (see text) .......................................................................................... 122

3.10 Final ion axial kinetic energy as a function of ion initial velocity elevation angle, $\theta$. Ion initial conditions are as in Figures 3.6 and 3.7, except that at each $\theta$ value, the initial velocity angle, $\phi$, is varied from $0^\circ$ to $360^\circ$ in $30^\circ$ increments. Note that the acceleration/deceleration effects of the magnetic mirror result in a wide axial kinetic energy distribution (grey region). .................................................... 123

3.11 Final ion axial kinetic energy as a function of ion initial velocity elevation angle, $\theta$, as in Figure 3.8, for $r_0 = 2$ mm (light shaded area) and $r_0 = 0$ mm (dark shaded area); note the narrowing of the final axial kinetic energy distribution as ions are focused closer to the z-axis. ................................................................. 125
4.1 Schematic diagram showing the effect of collisional damping on the relative amplitudes of ion cyclotron, magnetron, and axial motions. Note that the amplitudes of the ion cyclotron and axial motions decrease when subjected to collisional damping, while ion magnetron radius continually increases under similar conditions. Figure taken from ref. 9.... 136

4.2 Schematic layout (top view) of the mechanical components of the prototype external ion source FT/ICR mass spectrometer. .................................................. 139

4.3 Schematic diagram showing the relative placement of the probe, electrostatic wire ion guide (EIG), and open-ended cylindrical ICR ion trap. Note that ions leaving the EIG enter the ICR ion trap with relatively large initial magnetron radii (i.e., enter the trap with off-axis cyclotron orbit centers).................................. 141

4.4 FT/ICR experimental event sequence for broadband axialization of externally generated ions............... 143

4.5 Schematic diagram showing the relay circuit placed between the cell flange and excitation and detection amplifiers. In the null position (up in the above diagram), the system is in dipolar mode; a TTL signal from the data station triggers the relays (down in the above diagram) such that azimuthal quadrupolar excitation geometry is applied at the ICR ion trap..... 144

4.6 Cluster cations generated by directing a continuous beam of Cs$^+$ ions at solid CsI, followed by transport of the cluster ions through an electrostatic ion guide to the ICR ion trap. Top: FT/ICR mass spectrum of clusters generated from a 20 s primary Cs$^+$ ion beam. Bottom: FT/ICR mass spectrum based on broadband axialization of ions spanning a mass range of $2000 \leq m/z \leq 4000$, prior to conventional dipolar excitation and detection............................................................... 146

4.7 Cluster cations generated by pulsed Cs$^+$ SIMS of solid CsI. Top: For a 5 ms primary Cs$^+$ ion beam duration, no high mass clusters are observed. Bottom: Use of broadband axialization dramatically increases the signal-to-noise ratio for ions in the axialized range $2000 \leq m/z \leq 4000$. ......................... 148
FT/ICR spectra of ions produced by matrix-assisted laser desorption/ionization (MALDI) of poly(ethylene glycol) PEG-2000. Top: PEG-2000 detected without prior axialization. Middle: Mass spectrum after prior axialization of ions of \( 1500 \leq m/z \leq 2500 \). Note the greatly increased signal-to-noise ratio. Bottom: Mass spectrum after prior axialization of ions over the mass range, \( 1950 \leq m/z \leq 2120 \). Note the mass selectivity of the axialization process, leaving only those ions within the axialization window.

Structures of phospholipids in this study. Glycerol is esterified to a variety of fatty acids \( (R_1, R_2) \) at sn-1 and sn-2, whereas one of four polar head groups \( (X) \) is joined at sn-3 through a phosphodiester linkage. A given phospholipid is denoted by its polar head group \( (\text{GPE}, \text{GPC}, \text{GPS}, \text{or GPl}) \) and by the number of carbons and double bonds in the fatty acid side chains \( (\text{e.g. 16:0, 18:0, 18:1, 18:2, or 18:3}) \). Thus, \( \text{GPE-16:0, 18:1} \) indicates glycerophosphatidyl-ethanolamine, with palmitic and oleic acids esterified at sn-1 and sn-2 respectively.

Schematic layout of the major components of an Extrel FTMS-2000 FT/ICR mass spectrometer used for MALDI.

Schematic diagram showing a modified probe tip and sample plate. In addition to the standard screw hole for fastening the sample plate to the probe tip, two pins were press-fit into the probe tip, with corresponding holes in the sample plate; these in turn locked the angular position of the sample plate. The indentation spots were located at rotational positions \( (5 \text{ degrees apart - accessed via the Odyssey data station}) \) around the sample plate.
5.4 Experimental sequence used for collisional activation of phospholipids. After formation and isolation of phospholipid molecular ions in the source-side ion trap, sustained off-resonance irradiation (SORI) is used for collisional activation (in the presence of $\sim 1 \times 10^{-7}$ torr argon). Product ions are then re-focused to the central (z-) axis by use of azimuthal quadrupolar excitation. Subsequent transfer of ions to the analyzer-side ion trap allows for high-resolution mass analysis.

5.5 Experimental sequence used in ion remeasurement studies. The top segment corresponds to a single time-domain acquisition, after isolation of the mass range of interest and dipolar excitation of coherent cyclotron motion. The bottom segment shows the steps required for ion remeasurement. Relaxation of ion cyclotron motion was accomplished through either collisional damping alone (i.e. $y = 0$, $x = z$) or with a combination of collisional damping and azimuthal quadrupolar excitation (i.e. $y > 0$). Note that axialization may be applied after each detection period (i.e. $x = y$), or after several detection/collisional damping cycles (i.e. $x > y$).

5.6 Positive-ion FT/ICR magnitude-mode mass spectra for, proceeding from top to bottom, GPC-16:0,18:1, GPS-di16:0, GPE-di18:1, and GPl-16:0,18:2. In each case, the molecular ion or its sodiated adduct is observed, as well as ions indicative of the polar head group (choline, serine, ethanolamine, and inositol, respectively).

5.7 Collisional dissociation of GPC-16:0,18:1 positive ions. Following isolation of protonated molecular ions, $m/z = 760$ (top), collisional activation yields abundant phosphocholine ions, $m/z = 184$, which may be efficiently axialized and transferred to the analyzer ion trap for high-resolution mass analysis (bottom). Identification of the choline polar head group defines the phospholipid class (GPC).
5.8 Matrix (DHB) adduct anions desorbed/ionized from a room-temperature solids probe. Deprotonated GPE-16:0,18:1 molecular ions \((m/z = 716)\) are first isolated in the source-side ion trap. As the post-isolation delay increases from 0.1 - 30 s (top to bottom), the matrix adduct \((m/z = 870)\) becomes the most abundant species.......................... 174

5.9 Collisional activation of GPS-di16:0 negative ions desorbed/ionized from a room-temperature solids probe. During subsequent axialization (5-30 s following collisional activation), product anions form adducts with neutral DHB molecules subliming from the sample probe. In addition, matrix neutrals may lose a proton to palmitate anions, resulting in abundant matrix anion \((m/z = 153)\) and matrix dimer \((m/z = 307)\)................................. 176

5.10 Collisional activation of GPS-di16:0 negative ions desorbed/ionized from a liquid nitrogen-cooled probe. After isolation of deprotonated molecular ions, \(m/z = 734\) (top), collisional activation yields abundant fragment ions, corresponding to palmitate anion \((m/z = 255)\), loss of palmitic acid and serine \((m/z = 392)\), and loss of the ketene analog of palmitic acid and serine \((m/z = 409)\). Products are subsequently axialized and transferred to the analyzer ion trap for high-resolution mass analysis (bottom), in which mono-dehydrated glycerophosphatic acid is easily resolved from matrix anion (inset). Note that cryo-cooling the probe eliminates matrix adduct formation.......................................................... 177

5.11 Collisional activation of GPC-di16:0 negative ions. At a matrix-to-analyte (TCA/GPC) mole ratio of 500:1, abundant quasi-molecular ions, corresponding to loss of the trimethylamine moiety \((m/z = 673)\) are easily isolated in the source-side ion trap, axialized, and transferred to the analyzer trap (top). Collisional activation in the source trap yields abundant palmitate anions \((m/z = 255)\) which may subsequently be axialized \(^\dagger\) and transferred to the analyzer ion trap for high-resolution mass analysis (bottom).................................. 180

xx
5.12 Collisional activation of GPE-16:0,18:1 negative ions. The top spectrum shows deprotonated molecular ions detected in the analyzer ion trap, after isolation and axialization in the source trap. Following collisional activation, product ions corresponding to the palmitic ($m/z = 255$) and oleic ($m/z = 281$) acid side chains may be detected in the source-side ion trap (middle) or axialized and transferred to the analyzer ion trap (bottom). In either case, the substituent at sn-2 has approximately three times the abundance as that at sn-1. Note the increased signal-to-noise ratio and mass resolving power (compare middle and bottom spectra) for product anions axialized following CAD and transferred to the low-pressure analyzer trap.

5.13 The most abundant GPI molecular anion ($m/z = 833$) in a mixture of soybean GPI (top) is isolated (not shown) and subjected to collisional activation. Product ions are then axialized and transferred to the low-pressure analyzer ion trap for high-resolution (average $m/\Delta m_{50\%} = 145,000$) mass analysis (bottom). Fragment ions serve to identify the polar head group ($m/z = 241, 223$) as well as palmitate ($m/z = 255$) and linoleate ($m/z = 279$) anions. Loss of linoleic acid ($m/z = 553$), loss of linoleic acid and inositol moieties ($m/z = 391$), loss of both acyl substituents ($m/z = 297$), and monodehydrated glycerophosphatic anions ($m/z = 153$) are also observed.

5.14 MALDI FT/ICR single laser shot mass spectra obtained from 40 attomoles of GPC-d16:0 applied to the sample plate. Top: Single time-domain data acquisition. Bottom: Sum of time-domain data from 45 co-added remeasurements of the same ion packet. After remeasurement, ion signals corresponding to the quasi-molecular ion ($m/z = 734$), its sodium adduct ($m/z = 756$), and loss of trimethylamine (sodiated, $m/z = 697$) are readily observed at signal-to-noise ratios $\geq 3:1$.

6.1 High performance external source electrospray ionization FT/ICR mass spectrometer.
6.2 Octupole ion guide with cut-away view of aluminum support tube. The inscribed circle of the octupole is ~5 mm.......................... 197

6.3 Dual ion trap used in the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1. Figure provided by Ljiljana Pasa Tolic...... 198

6.4 Vacuum carts used in the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1................................................. 199

6.5 Mass spectrum of bovine insulin obtained on the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1. Data provided by Mike Senko................................................................. 200

6.6 Schematic diagram showing a multi-source FT/ICR mass spectrometer. ................................................................. 202
CHAPTER I

FOURIER TRANSFORM ION CYCLOTRON RESONANCE
MASS SPECTROMETRY

INTRODUCTION

Historical Developments. The ancestry of FT/ICR can be traced back to experiments done by Sommer, Thomas, and Hippie in 1949-1951.¹ ² Their ICR mass spectrometer, originally referred to as an "omegatron," consisted of a pair of opposed plates, parallel to a static magnetic field, sandwiching a series of guard (square) ring electrodes, whose interior served as the mass analyzer. An electron beam, directed through the center of the omegatron parallel to the magnetic field, continuously generated ions, which were trapped (albeit for a very short time) by application of a d.c. voltage to the guard rings. ICR motion was excited by an r.f. voltage applied to the flat plate electrodes; when the frequency of the r.f. voltage was in resonance with an ion of appropriate m/z, the ion would spiral outward, continually increasing its ICR radius until it struck a collector electrode positioned just inside the guard rings. A mass spectrum was obtained by scanning the detected ion current as a
function of the applied r.f. frequency. Wobschall and co-workers provided the next advancement in ICR instrumentation in 1963-1965.\textsuperscript{3,4} Their approach placed two long opposed plates parallel to the magnetic field. Again, an electron beam fired down the center between the two plates continuously generated ions. The parallel plates served as one capacitive arm in a fixed frequency marginal oscillator circuit. In the null condition, the circuit was balanced with no voltage output. Ions of various $m/z$ were brought into resonance by sweeping the magnetic field; as the ion's ICR orbit increased, the marginal oscillator circuit became unbalanced, and thus the resonance condition could be detected based on the voltage output of the bridge circuit. Although more sensitive than the original omegatron, Wobschall's approach still suffered from space charge effects, resulting from continuous ion formation throughout the experiment.

Baldeschwieler and co-workers made major strides in defeating the space charge problem with a three-section ICR cell.\textsuperscript{5,6} Unlike Wobschall's design, this cell was elongated in the direction perpendicular to the magnetic field and divided into three distinct sections, each consisting of two pairs of opposed plates. In the source region, ions are generated from an electron beam fired parallel to the magnetic field. A d.c. potential is applied to the source plates parallel to the magnetic field; due to the resulting $\mathbf{E} \times \mathbf{B}$ force, ions drift perpendicular to the magnetic field toward the analyzer region. Here, as in the Wobschall design, the plates parallel to the magnetic field are part of a marginal oscillator circuit. Ion cyclotron resonance is detected when the magnetic
field is swept across the $m/z$ range of interest, and the oscillator circuit becomes unbalanced. In both the source and analyzer regions, ions are confined axially by application of a d.c. voltage to the plates perpendicular to the magnetic field. The third section consisted of five plates shorted together to provide a measure of the total ion current. A unique feature of Baldeschwieler's instrument was the ability to apply a second r.f. voltage on one of the plates in the analyzer region. This allowed so-called "double resonance" experiments to be performed, in which an ion of particular $m/z$ was brought into resonance, thereby increasing its kinetic energy and leading to ion-molecule reactions, which were in turn monitored by a change in abundance of a second $m/z$ value. These early experiments are the first examples of tandem mass spectrometry, which has proven to be a powerful analytical tool for ion-molecule reaction studies as well as structural analysis.

The introduction of the "trapped ion cell" in 1970 by McIver provided pulsed ion formation, thus allowing ion formation and analysis in the same cell. McIver's design looked much like a standard cubic trap (see Fig. 1.1) except it was elongated in one dimension perpendicular to the magnetic field. Nonetheless, a d.c. potential was applied to the plates perpendicular to the magnetic field while the remaining plates were biased to a somewhat lower d.c. potential; thus, ions could be trapped for up to 100 ms. As with prior designs, ion excitation relied on sweeping the magnetic field while a marginal oscillator circuit was used for detection. A true pulsed ICR experiment was not demonstrated until 1974, when Comisarow and Marshall first applied Fourier transform data
reduction techniques to an ICR time-domain response. Here, a trapped ion cell was used such that ions, generated in pulsed mode, could be confined for periods $\geq 100$ ms. A single frequency r.f. pulse excited ions in a fixed magnetic field. The voltage induced on the cell plates was then digitized and subjected to Fourier transformation to obtain the frequency-domain data. In a second experiment, Comisarow and Marshall demonstrated that ICR excitation could also be performed in broadband mode. In this case the r.f. excitation frequency was varied linearly, over a period of $\sim 2$ ms, so as to excite ions over a broad $m/z$ range. Again, Fourier transformation of the time-domain response yielded a frequency-domain spectrum containing peaks from each ion. Clearly, the advent of FT/ICR provided significant analytical advantages. Because ions over a given mass range are detected simultaneously, either a spectrum for a given mass range may be collected much faster compared to a scanning instrument, or during the time required to scan a given mass range, several FT-ICR mass spectra may be obtained, resulting in $\sqrt{N}$ improvement (where $N$ is the number of time-domain acquisitions) in observed signal-to-noise ratio. In addition, ion-molecule reaction pathways, energetics, etc. may be studied with much better temporal resolution. One last, and very significant development which cannot be overlooked in the evolution of FT/ICR as an analytical tool is Cooley and Tukey's computer algorithm which reduced the number of operations required to Fourier transform $N$ data points from $N^2$ to $N\log N$, for the case where $N$ is some power of 2.
Having touched upon some of the highlights in the historical development of FT/ICR, the rest of this chapter will focus in more detail on ion motion, formation, excitation, and detection within the ion trap.

**FUNDAMENTAL ION MOTION**

*Ion Motion in a Static Magnetic Field.* An ion moving in a spatially uniform magnetic ($\mathbf{B}$) field is subject to a Lorentz force ($\mathbf{F}_L$).

$$\mathbf{F}_L = q \mathbf{v} \times \mathbf{B} \quad (1-1a)$$

in which $q$ and $\mathbf{v}$ are the charge and linear velocity of the ion, with vector quantities in boldface. In the absence of an electrostatic trapping field, the ion follows a circular orbit, perpendicular to the magnetic field, with a natural cyclotron orbital frequency, $\omega_c$ (rad s$^{-1}$) or $\nu_c$ (Hz)

$$\omega_c = \frac{qB_0}{m} = 2\pi\nu_c \quad (1-2)$$

in which $m$ is the ion mass. Figure 1.1 illustrates ICR motion, where the magnetic field defines the z-direction: $\mathbf{B} = B_0 \mathbf{k}$. Note that $\mathbf{B}$ constrains ions only in the x-y plane (see Fig. 1.1); they are free to move along the z-axis, and thus are not yet confined for mass analysis. Nonetheless, several important trends, which nicely illustrate the potential of FT-ICR for mass analysis, may be identified from Eq. 1-2. For example, Figure 1.2 shows ICR orbital frequency plotted versus ion mass-to-charge ratio ($m/z$), for several currently used magnetic fields. Note that for a wide range of $m/z$, the ICR frequency varies from ~ 1 kHz up to ~ 100 MHz. While this bandwidth is relatively wide, there are commercially available
Figure 1.1 An ion with initial velocity, $v_{xy}$, in a homogeneous magnetic field, $B_0$, is bent into a circular path due to the Lorentz force, $F_L$. ICR orbital frequency, $\omega_c$, is inversely proportional to ion mass-to-charge ratio.

\[ F_L = qv_{xy}B_0 \]

\[ \omega_c = \frac{qB_0}{m} \]
Figure 1.2 ICR orbital frequency (Hz) plotted versus mass-to-charge ratio (m/z) for 3 T, 7 T, and 9.4 T magnetic field strengths.
Figure 1.3 ICR orbital radius (mm) plotted versus mass-to-charge ratio (m/z, thermal energy) for 3 T, 7 T, and 9.4 T magnetic field strengths.
Figure 1.4 Ion kinetic energy (eV) for \( m/z = 1000 \) plotted versus ICR orbital radius (mm), for 3 T, 7 T, and 9.4 T magnetic field strengths.
electronics (e.g. for ion excitation and detection) which cover a significant portion of this bandwidth. Next, Figure 1.3 shows ICR orbital radius for thermal energy ions, plotted versus mass-to-charge ratio, again for several commonly used magnetic field strengths. Note that even relatively large ions (e.g. \( m/z = 100,000 \)), in a 3 T magnetic field, are confined in small ICR orbits (with ICR radius of \( \sim 2.5 \text{ mm} \)). Finally, Figure 1.4 shows ion kinetic (eV) energy plotted versus ICR orbital radius (mm), for an ion of \( m/z = 1000 \). Note that ions may attain significant kinetic energy at ICR radii less than 20 mm. Since bore diameters on superconducting magnets range up to 8", it is clear that ICR has the potential to provide both mass analysis and structural information (by way of collisionally activated dissociation) from commercially available apparatus.

**Ion Motion in Static Magnetic and Electric Fields.** As mentioned above, the magnetic field serves only to confine ions in the xy-plane. In a standard cubic ion trap (see Fig. 1.5), an electrostatic d.c. potential, applied to each of the plates perpendicular to the magnetic field, provides the restoring force required to keep ions from escaping along the z-axis. The Lorentz force equation (Eq. 1-1a) becomes,

\[
\mathbf{F}_L = q \mathbf{v} \times \mathbf{B} + q \mathbf{E}
\]  

(1-1b)

in which \( q \mathbf{E} \) is the force due to the electric field. The introduction of the trapping field necessarily shifts the ion cyclotron frequency (downward—see below) from \( \omega_c \). The (shifted) ICR frequency clearly depends on the spatial distribution of the the trapping field.
Figure 1.5 Basic configuration of a cubic ion trap, showing the trapping (T), excitation (E), and detection (D) electrodes. Typical trap dimensions are 1-2” for each side.
For an ion in the \( z = 0 \) midplane of the trap, with angular velocity, \( \omega \), about the \( z \)-axis, Eq. 1-1b becomes

\[
mp\omega^2 = qB_0\rho \omega + qE
\]

(1-1c)

in which \( \rho \) is the radial displacement of the ion from the \( z \)-axis. If the electric field is proportional to \( \rho \), Eq. 1-1c shows that the ion's cyclotron frequency will be independent of its orbital radius. That (desirable) constraint, in conjunction with Laplace's equation,

\[
\nabla^2 \Phi(x,y,z) = \frac{\partial^2 \Phi(x,y,z)}{\partial x^2} + \frac{\partial^2 \Phi(x,y,z)}{\partial y^2} + \frac{\partial^2 \Phi(x,y,z)}{\partial z^2} = 0
\]

(1-3)

requires that the electrostatic potential, \( \Phi(x,y,z) \), vary as both \( \rho^2 \) and \( z^2 \), in which \( \rho^2 = x^2 + y^2 \), leading to the so-called "quadrupolar electrostatic potential," the general form of which is:

\[
\Phi_{\text{trap}}(\rho,z) = C_1 (2z^2 - \rho^2) + C_2
\]

(1-4)

Near the center of a standard cubic ion trap, the trapping potential has the form:

\[
\Phi_{\text{trap}}(\rho,z) = \frac{U_0 \alpha}{2 a^2} (2z^2 - \rho^2) + U_0 \gamma
\]

(1-5a)

where \( U_0 \) is the voltage applied to the trapping plates, \( a \) is the length of any trap edge, \( \alpha = 2.77373 \), and \( \gamma = 1/3 \). A characteristic trap dimension, \( d_0^2 \), may be defined for the cubic trap as,

\[
d_0^2 = \frac{a^2}{2 \alpha}
\]

(1-6)

and thus Eq. 1-5a becomes:
\[ \phi_{\text{trap}}(p,z) = \frac{U_0}{4d_0^2} (2z^2 - \rho^2) + \frac{U_0}{3} \]  

(1-5b)

From Eq. 1-5b, the radial electric field at the trap midplane may be calculated:

\[ E(\rho) = -\frac{\partial \phi_{\text{trap}}(p,z)}{\partial \rho} = \frac{U_0 \rho}{2d_0^2} \]  

(1-7)

Substituting for the electric field in Eq. 1-1c, and noting that the electric and magnetic forces necessarily oppose each other, yields a quadratic,

\[ m \rho \omega^2 - qB_0 \rho \omega + \frac{q U_0 \rho}{2d_0^2} = 0 \]  

(1-1d)

whose roots represent the fundamental radial ion motions observed in FT/ICR/MS,

\[ \omega_+ = \frac{\omega_c}{2} + \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \]  

(1-8a)

\[ \omega_- = \frac{\omega_c}{2} - \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \]  

(1-8b)

in which:

\[ \omega_z = \sqrt{\frac{q U_0}{m d_0^2}} \]  

(1-8c)

Inspection of Eqs. 1-8 a-c shows the effects of the electrostatic trapping field. First, the observed ICR frequency, \( \omega_+ \) (i.e. the reduced cyclotron frequency), is shifted downward from the natural or unperturbed frequency, \( \omega_c \). Second, the presence of an electrostatic field results in an
**EXCITATION OF ION CYCLOTRON MOTION**

In a typical FT/ICR/MS experiment, ions are formed, or deposited in the case of external ion injection, at or near the center of the ion trap. As is clear from Figure 1.3, even ions of relatively large \( m/z \) have ICR orbital radii of only a few mm. As will be discussed below, the detected ICR signal increases with ICR orbital radius; as a result ions in a standard cubic trap (say 1-2" in each dimension) would induce only minimal signal at these small ICR radii. Furthermore, the ions will have random phase, meaning that they are spread out equally about the circumference of their ICR orbit(s). Because the standard detection scheme is based upon the difference in image charge induced on the
Figure 1.6 Schematic representation of the cyclotron (frequency = \( \omega_+ \), radius = \( \rho_+ \)), magnetron (frequency = \( \omega_- \), radius = \( \rho_- \)), and trapping (frequency = \( \omega_o \)) motions in an ICR ion trap. Figure graciously provided by Hyun Sik Kim.

detection plates (see Fig. 1.5) as ions execute their ICR orbits, it is clear that ions with random phase, at any ICR radii, are undetectable. The net result of the above discussion is that once formed, ions must be (coherently) excited to large ICR radii for efficient detection. In standard dipole excitation, r.f. voltage is applied to one pair of opposed plates on a cubic ion trap (see excite plates - Fig. 1.5). When the frequency of the r.f. voltage matches the reduced cyclotron frequency of a given ion, the
ion will gain energy, as reflected in an increased ICR orbital radius. Note that in the case of multiple ions (of the same m/z), the entire ion packet will be excited to a larger ICR orbital radius in a coherent manner (e.g. the center of the ion packet moves along the circumference of the ICR orbit).

Note that because the observed ICR signal is proportional to ICR orbital radius, as well as the number of ions present, it is imperative that ions of varying m/z be driven to identical radii during the excitation process; only then will the observed signal magnitudes reflect the true relative ion abundances. In the case of infinitely extended excitation electrodes, the expression for ICR radius, ρ+, as a function of excitation time, T_{excite}, is:

\[ \rho_+ (T_{excite}) = \frac{V_{(p-p)} T_{excite}}{2 a B_0} \]  

(1-9a)

where \( V_{(p-p)} \) is the peak-to-peak voltage of the r.f. excitation, and a and \( B_0 \) are as defined above. However, Grosshans has shown that, for ions in the z=0 trap midplane, Eq. 1-9a greatly overestimates (by as much as 39%) the final ICR orbital radius; he calculated a correction term, \( \beta \), which varies with trap aspect ratio, provided a more accurate estimate of final ICR orbital radius,

\[ \rho_+ (T_{excite}) = \frac{\beta V_{(p-p)} T_{excite}}{2 a B_0} \]  

(1-9b)

where \( \beta = 0.72167 \) for a cubic trap (e.g. aspect ratio = 1). The lack of mass dependence in Eq. 1-9b indicates that ions of different m/z may be
excited to equal ICR radii assuming an appropriate excitation waveform is specified. In addition, Eq. 1-9b provides a means to accurately calculate post-excitation ion kinetic energy, which is important in probing various dissociation reactions. Clearly, the type of excitation waveform used will in part dictate how precisely the final ion radius and kinetic energy are defined. Grosshans and Marshall have published general treatments\textsuperscript{16-18} for accurate determination of ion post-excitation radius, and thus kinetic energy, for ICR ion traps of various geometries (e.g. cubic, elongated, and cyclindrical). In addition, Schweikhard and Marshall\textsuperscript{19} provided a comprehensive discussion of ICR orbital radius determination in various excitation geometries (e.g. dipolar, quadrature, parametric, etc.).

**Single Frequency Excitation.** Single frequency excitation is perhaps the easiest approach to understand and in fact was the type of excitation used in the first FT/ICR/MS experiment.\textsuperscript{8} Here, r.f. voltage of one frequency, corresponding to the $m/z$ of interest, is applied to two of the opposed plates of the cubic ion trap (see Figure 1-5). Ions absorb energy and are excited to larger ICR radii. Although effective for studying a specific $m/z$, single frequency excitation is not useful for analyzing ions over a wide $m/z$ range; as seen in Figure 1.7a, the excitation power applied in the frequency-domain drops rapidly at points away from the centroid. Note also that the width of the frequency-domain sinc function decreases as the length of the time-domain excitation waveform increases. Thus a very short r.f. pulse applied in the time-domain will lead to wider power profiles in the frequency-domain; unfortunately
Figure 1.7 Schematic representation of various time-domain (left) and corresponding frequency-domain (right) ICR excitation waveforms. a.) Single frequency excitation; b.) frequency sweep or "chirp" excitation; c.) SWIFT (broadband) excitation; d.) SWIFT (selective excite and eject) excitation. Figure taken with permission from ref. 44.
unreasonably high voltage amplitudes are necessary to attain any appreciable power, over a useful bandwidth, in the frequency-domain. For example, Marshall and Roe have shown that excitation of ions to a 1 cm radius, over a 2 MHz bandwidth (corresponding to $15 \leq m/z \leq 500$ in a 2 tesla magnetic field) requires a time-domain pulse of 13 kV amplitude and 30 ns duration.\textsuperscript{20}

**Frequency Sweep Excitation.** One of the more widely used approaches is frequency sweep excitation. In this case, the frequency of the applied excitation waveform is swept over the corresponding $m/z$ range of interest; Comisarow and Marshall first demonstrated this approach in 1974.\textsuperscript{9} Frequency sweep is easy to implement, requiring r.f. amplitudes of 50 - 200 volts applied across a frequency range of several kHz up to 5 MHz, with sweep rates of 10-2000 Hz/µs. As seen in Figure 1.7b, frequency sweep excitation suffers from poor selectivity at the maximum and minimum of the requested bandwidth, as well as non-uniform power across the $m/z$ range of interest. Marshall and Roe have provided theoretical studies of frequency sweep excitation.\textsuperscript{20}

**Stored Waveform Inverse Fourier Transform Excitation.** Stored waveform inverse Fourier transform excitation, or SWIFT, takes advantage of the fact that the Fourier transform works in both directions. Thus the required time-domain excitation waveform may be obtained from the inverse Fourier transform of the desired frequency-domain response. As first described by Marshall and Ricca,\textsuperscript{21-23} SWIFT offers many advantages for FT/ICR/MS. As seen in Figure 1-7c and d
power distribution in the frequency-domain is much more uniform compared to the frequency sweep technique. In addition, SWIFT provides outstanding selectivity; during a single excitation event, ions in one m/z range may be excited to a given ICR radius while ions within a second m/z window are ejected from the trap (for increased dynamic range) or not excited at all.

**Imperfections in the Excitation Process.** As discussed above, truncation of the ion trap electrodes, and the fact that they are flat (in the typical cubic trap) to begin with, leads to distortions of the quadrupolar trapping potential at positions other than near the trap center. In the case of excitation, flat electrodes are desirable, but again, the fact that they do not extend infinitely, and the presence of perpendicular trapping electrodes, leads to imperfections in the excitation process. For example, away from the trap center, the excitation electric field lines tend to "leak" towards the detection and trapping electrodes, resulting in two (undesirable) consequences: First, ions experience smaller radial excitation compared to the ideal case of infinitely extended parallel plates. Second, there is an electric field component directed axially, parallel to the magnetic field; as a result, ions experience significant axial force during radial excitation. Under the appropriate conditions, ion axial motion may be excited to the extent that they are lost to collisions with the trapping electrodes. Moreover, ion amplitudes during axial excitation are in fact mass dependent, which in turn can lead to severe discrepancies in the actual and observed ion abundances. Due to these deleterious effects, a plethora of studies have
been done on axial ejection. Note that these effects occur due to imperfect ion trap geometry and thus are observed regardless of the excitation waveform (single frequency, chirp, SWIFT) utilized. Several novel ion trap designs, all of which reduce the severity of ion axial ejection, have been described, including: elongated ion traps, shimmmed ion traps, open-ended and capacitively-coupled open-ended ion traps, and linearized ion traps.

DETECTION OF ION CYCLOTRON MOTION

The original model for detection in FT/ICR/MS was developed by Comisarow in 1978. An ion packet is modeled as a rotating electric dipole which induces charge on the ion trap detection plates. The detection plates are in turn modeled as a parallel capacitor and resistor which convert the induced charge to a sinusoidal voltage. Comisarow's model was later refined by Dunbar, Rempel, and Xiang to account for the variation in ion post-excitation radius with ion position in the trap. Grosshans has also developed a comprehensive signal model which can be applied to ion traps of various geometry.

As discussed above, ion trap geometry and non-ideal electrostatic trapping and r.f. excitation fields can lead to anomalies in the detected signal. For example, mass-dependent axial ejection results in unreliable relative signal magnitudes in the mass spectrum. Also harmonics may be observed when the ICR orbital radius approaches the maximum allowed by the ion trap. Several researchers have constructed ion traps which enhance harmonic signal strength in order to increase mass
resolving power\textsuperscript{45,46} or estimate ion post-excitation orbital radius.\textsuperscript{47} Linearized ion traps eliminate harmonics and provide accurate relative ion abundance information.\textsuperscript{40,41}

**ION FORMATION TECHNIQUES**

There are a wide variety of ionization techniques used in mass spectrometry. In this section, a brief description of those which have enjoyed substantial use in FT/ICR/MS will be presented; examples of two techniques which have generated enormous interest recently, matrix-assisted laser desorption/ionization and electrospray ionization, will be presented in later chapters.

*Electron Impact Ionization.* In this technique a beam of energetic (~10-70 eV) electrons is emitted from a heated filament and passed through a region containing neutral analyte molecules. EI is considered a "hard" ionization technique due to the extensive fragmentation which is often observed. While molecular ion information may be hard to obtain with EI, the fragmentation patterns may be useful for structure elucidation. The major drawback with EI is that the analyte must be readily volatilized; this is not typically the case with larger biomolecules, even under the ultra-high vacuum conditions used in FT/ICR/MS. Nonetheless, EI may be the most reproducible ionization technique for a given set of standard conditions. As a result, spectra for over 200,000 compounds have been compiled into libraries.
**Chemical Ionization.** Chemical ionization, or CI, utilizes a secondary reagent gas to ionize neutral analyte species through one of two mechanisms.\(^{48}\) In the charge exchange method, an excess of the reagent gas is first ionized via electron impact. The analyte is then introduced and, assuming that the recombination energy of the radical cation reagent is greater than the ionization potential of the analyte neutral, undergoes charge exchange with the reagent, forming radical cation analyte ions. For a given analyte species, the degree of fragmentation is easily controlled by choosing a reagent species with recombination energy slightly above the ionization potential of the analyte. As a result, spectra containing a high abundance of molecular ion may be obtained. Proton exchange is perhaps the more common chemical ionization technique. Again, an excess of reagent gas is first ionized via EI; proton exchange then occurs between radical cation and neutral reagent species, forming protonated reagent ions. When the analyte is introduced into the source region, proton exchange occurs between the reagent and analyte, forming protonated analyte ions. Again, the degree of fragmentation is controlled by choosing a reagent whose proton affinity is slightly lower than that of the analyte. Chemical ionization suffers from the same problem as EI, namely that it works most conveniently for volatile analytes. In addition, one may need to know something about the recombination energy or proton affinity of the analyte ahead of time.
High Energy Particle Bombardment Ionization. This general classification includes techniques in which beams of ions (Cs+, secondary ion mass spectrometry),\textsuperscript{49} atoms (Ar, fast atom bombardment),\textsuperscript{50} polyatomics (SF$_6$, fast neutral beam),\textsuperscript{51} or clusters ([glycerol]$_n^+$, massive cluster impact)\textsuperscript{52} are accelerated to high energy (~5-20 keV) and focused onto a small stage containing either solid sample or analyte dispersed in a low-volatility matrix. Secondary ion mass spectrometry was the first of these techniques developed and represented the first efficient method of transferring intact biomolecules into the gas phase for mass analysis.\textsuperscript{53} These methods all rely on momentum transfer, from the bombarding beam to the sample surface, to effect analyte ionization. Typically, higher analyte ion currents are obtained when a liquid matrix is used; unfortunately this presents a large gas load to the mass spectrometer and thus use of these sources with FT/ICR/MS requires external ionization with subsequent ion injection into the mass analyzer.\textsuperscript{54-62}

Laser Desorption Ionization. Laser desorption (LD) uses infrared laser irradiation impinging upon a mixture of analyte and alkali salt (NaCl, KBr).\textsuperscript{63} Rapid heating of the sample substrate generates a plume of neutral analyte and Na$^+$ or K$^+$ ions. Cation attachment results in quasi-molecular ions. Compared to the particle bombardment schemes, laser desorption more readily produces high $m/z$ molecular ions, without interference from low mass matrix-related species. Recently, matrix-assisted laser desorption ionization (MALDI) has become the preferred laser-based technique.\textsuperscript{64,65} Here the analyte is mixed with an excess of matrix (a small highly-UV absorbing molecule), in typical molar ratios of
The matrix absorbs ultraviolet radiation, from a nitrogen laser for example, and is lifted into the gas phase, carrying with it neutral analyte species; proton exchange between excited-state matrix and analyte molecules forms protonated molecular ions. Lower laser power densities are required for MALDI compared to LD (~$10^6$ W/cm$^2$, vs. ~$10^9$ W/cm$^2$) and it is amenable to a wider range of analytes.

In many ways, laser irradiation represents an ideal ionization source for use in FT/ICR/MS. The laser beam is unaffected by the strong magnetic field and various windows are commercially available to pass the beam into the vacuum chamber. Furthermore, samples need not be suspended in a liquid matrix (MALDI analyte/matrix mixtures are dried prior to insertion into the vacuum chamber) and thus may be positioned close to the ion trap. The radial focusing provided by the strong magnetic field increases ion trapping efficiency.

**Electrospray Ionization.** Electrospray $^{66,67}$ is currently the most promising ionization technique for high molecular weight biomolecules. A solution containing the analyte of interest is introduced, at low $\mu$l/min flowrates, through a capillary which is held at several kV of positive potential (for positive ions) with respect to a counter electrode. Charge migration occurs in the liquid at the capillary tip in response to the high electric field; the liquid surface is destabilized by the build-up of excess surface charge and begins to stretch toward the counter electrode. Eventually droplets, containing multiple charges, are released from the surface and pass through a small orifice in the counter electrode. Prior
to entering the mass spectrometer, excess solvent evaporates from the
droplet, until eventually individual multiply-charged analyte ions remain.
By lowering the $m/z$ value of large biomolecules, electrospray effectively
increases the $m/z$ range of FT/ICR mass spectrometers. However,
because electrospray operates at atmospheric pressure, it requires
external ion injection $^{68-72}$ or other specialized instrument
configurations $^{73}$ when used in conjunction with FT/ICR/MS.

**RESEARCH OBJECTIVES**

Chapter I has provided a brief history of the development of FT/ICR
mass spectrometry. In addition, the fundamental ion motions within an
ICR ion trap have been discussed, as have basic methods of ion
excitation and detection. Finally, various ionization methods have been
described. The objectives of the remaining chapters in this thesis are:
a.) demonstration of improved FT/ICR/MS instrumentation, including
novel ion trap design and ion injection devices which allow external,
high-pressure ion sources to be effectively coupled to an FT/ICR mass
spectrometer; b.) use of matrix-assisted laser desorption/ionization
FT/ICR mass spectrometry for structural characterization of
phospholipids; c.) use of ion axialization techniques for improved
performance in external ion injection systems, collisionally activated
dissociation studies, and detection limit experiments.
REFERENCES


CHAPTER II

GENERATION OF A QUADRUPOULAR TRAPPING POTENTIAL
IN A TWO-ELECTRODE FT/ICR ION TRAP

INTRODUCTION

As discussed in Chapter I, the force due the magnetic field acts only to confine ions in the radial dimension (x-y plane in Figs. 1.1 and 1.5). In order to trap ions for mass analysis, an additional "trapping" potential must be applied. In the standard cubic ion trap (Fig. 1.5) axial confinement is accomplished by application of a static d.c. voltage to the two plates perpendicular to the magnetic field. The resulting potential should be proportional to both $\rho^2$ and $z^2$ to ensure that ICR frequency is independent of ICR radius (Eq. 1-1c), and satisfy Laplace's equation (Eq. 1-3); the general form of this "quadrupolar" potential is given again below:

$$\Phi_{\text{trap}} (\rho,z) = C_1 (2z^2 - \rho^2) + C_2$$  \hspace{1cm} (2-1)

The obvious question is, how can the quadrupolar potential be generated throughout the entire trap volume, rather than only near the
trap center as in the standard cubic ion trap? Guan and Marshall have suggested that this question is best viewed as an "inversion" problem; that is, rather than specifying boundary conditions (e.g. trap walls and voltages) and then solving the Laplace equation to obtain the potential inside, the desired potential is first specified and then the necessary boundary conditions are calculated. From this line of reasoning, there are two general approaches for generating a quadrupolar potential inside an ICR ion trap. In the first, simple electrode shapes are specified (planar or cylindrical) and then segmented into many pieces such that appropriate voltages may be applied, in essence "shimming" the trap potential to quadrupolar form. Guan and Jackson have proposed a cubic ion trap in which each plate is divided into twenty five (25) pieces; this design actually optimizes all trap functions, including trapping, excitation, and detection, and certainly approaches the limit in the simple shape approach. The Penning ion trap, consisting of a ring and two end-cap electrodes, each with hyperbolic surfaces described by,

\[
\text{Ring Electrode} \quad \frac{\rho^2}{\rho_0^2} - \frac{2z^2}{\rho_0^2} = 1 \quad (2-2a)
\]

\[
\text{Endcap Electrodes} \quad \frac{\rho^2}{2z_0^2} - \frac{z^2}{z_0^2} = -1 \quad (2-2b)
\]

where \(\rho_0\) is the minimum radius of the ring electrode and \(z_0\) is the minimum distance from the center of the trap to either of the endcap electrodes, represents the opposite approach: Specify a minimum number of complex-shaped electrodes (e.g. shapes matching the desired potential contours) which in turn require a minimum number of applied
voltages. In this chapter, a two-electrode hyperbolic ion trap which extends the complex-shape approach to its limit, will be described; as the name implies, only two electrodes are required for ion trapping, excitation, and detection. This trap operates in the so-called "parametric-mode" in which ion excitation and detection signals are superimposed on the d.c. trapping voltage. A comprehensive theoretical treatment will be presented as well as extensive corroborating experimental data.

THEORY

Quadrupolar Electrostatic Potential in the Two-electrode Trap.

Schweikhard\textsuperscript{14} originally pointed out that the potential in Eq. 2-1 may also be generated by use of two electrode surfaces described by Eq. 2-2a; however, it is not immediately obvious that this combination will provide the desired potential. Knight\textsuperscript{4} has published a simple and elegant discussion of the Penning trap which demonstrates that a quadrupolar potential may be generated with arbitrary choice of $\rho_0$ and $z_0$. The analogous discussion for the two-electrode trap yields similar results. For example, consider two hyperboloids of revolution, the outer referred to as the ring electrode and the inner referred to as the core electrode, with the general form:

\begin{align*}
\text{Ring electrode} & \quad \frac{\rho^2_{\text{ring}}}{\rho_0^2} - \frac{z^2}{\alpha^2} = 1 & (2-3a) \\
\text{Core electrode} & \quad \frac{\rho^2_{\text{core}}}{\rho_0^2} - \frac{z^2}{\beta^2} = 1 & (2-3b)
\end{align*}
Note that the general form of a hyperbola in the x-y plane is,
\[
\frac{x^2}{a^2} - \frac{y^2}{b^2} = 1
\]  (2-4)

where the asymptote slope, \( m \), is defined as:
\[
m = \frac{b}{a}
\]  (2-5)

For the ring electrode, the asymptote slope, \( m_{\text{ring}} \), in the \( \rho-z \) plane is,
\[
m_{\text{ring}} = \frac{\alpha}{\rho_{\text{ring}}}
\]  (2-6a)

where \( \rho^2 = x^2 + y^2 \). Similarly, for the core electrode:
\[
m_{\text{core}} = \frac{\beta}{\rho_{\text{core}}}
\]  (2-6b)

As an initial constraint, let the ring and core electrodes have the same asymptote; this implies,
\[
\frac{\alpha}{\rho_{\text{ring}}} = \frac{\beta}{\rho_{\text{core}}}
\]  (2-7a)

or
\[
\alpha^2 = \frac{\beta^2 \rho_{\text{ring}}^2}{\rho_{\text{core}}^2}
\]  (2-7b)

Substituting for \( \alpha^2 \) in Eq. 2-3a yields,
\[
\frac{\rho_{\text{ring}}^2}{\rho_{\text{ring}}^2} - \frac{\beta^2 \rho_{\text{core}}^2}{\rho_{\text{ring}}^2} = 1
\]  (2-3c)
which may be readily solved in terms of \((2z^2 - \rho^2)\):

\[
(2z^2 - \rho^2) = \left(\frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2}\right)z^2 \rho_{\text{core}}^2 - \rho_{\text{ring}}^2
\]  

\[(2-8a)\]

Similarly, for the core electrode:

\[
(2z^2 - \rho^2) = \left(\frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2}\right)z^2 \rho_{\text{core}}^2 - \rho_{\text{core}}^2
\]  

\[(2-8b)\]

The potential on each electrode surface may now be expressed as:

\[
\Phi_{\text{ring}}(\rho, z) = C_1 \left(\frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2}\right)z^2 \rho_{\text{core}}^2 - \rho_{\text{ring}}^2 + C_2
\]  

\[(2-9a)\]

\[
\Phi_{\text{core}}(\rho, z) = C_1 \left(\frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2}\right)z^2 \rho_{\text{core}}^2 - \rho_{\text{core}}^2 + C_2
\]  

\[(2-9b)\]

Recall that the potential along each electrode surface must be constant; this implies,

\[
\frac{1}{\beta^2} = \frac{2}{\rho_{\text{core}}^2}
\]  

\[(2-10a)\]

or

\[
\beta^2 = \frac{\rho_{\text{core}}^2}{2}
\]  

\[(2-10b)\]

Thus, \(\alpha\) may be explicity defined as,

\[
\alpha^2 = \frac{\rho_{\text{ring}}^2}{2}
\]  

\[(2-7c)\]
which may then be used to explicitly define the electrode surfaces:

\[
\begin{align*}
\text{Ring electrode} & \quad \frac{\rho^2}{\rho_{\text{ring}}} - \frac{2z^2}{\rho_{\text{ring}}} = 1 \\
\text{Core electrode} & \quad \frac{\rho^2}{\rho_{\text{core}}} - \frac{2z^2}{\rho_{\text{core}}} = 1
\end{align*}
\]  
\tag{2-3d,e}

Thus, as is the case for the standard Penning ion trap, a two-electrode hyperbolic geometry may be constructed using arbitrary \( \rho_{\text{ring}}/\rho_{\text{core}} \) ratio, assuming of course that \( \rho_{\text{ring}} > \rho_{\text{core}} \). The choice of common asymptote described by \( \rho = \sqrt{2}z \) simply means that at large values of \( \rho \) and \( z \), the electrode surfaces approach the asymptote symmetrically, thereby minimizing higher-order electric field contributions.\(^4\)

The constants, \( C_1 \) and \( C_2 \) in Eq. 2-1, are easily determined from the trap operating conditions. For example, if a d.c. voltage, \( U_0 \), is applied between the ring and core electrodes, then \( C_1 \) may be found from:

\[
U_0 = \Phi_{\text{core}} - \Phi_{\text{ring}} = \\
C_1 \left( \left( \frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2} \right) z^2 \rho_{\text{core}}^2 - \rho_{\text{core}}^2 \right) + C_2 - \\
- C_1 \left( \left( \frac{2}{\rho_{\text{core}}^2} - \frac{1}{\beta^2} \right) z^2 \rho_{\text{core}}^2 - \rho_{\text{ring}}^2 \right) - C_2
\]  
\tag{2-11}

Thus

\[
C_1 = \frac{U_0}{\rho_{\text{ring}}^2 - \rho_{\text{core}}^2}
\]  
\tag{2-12a}
Typically, one of the electrodes is grounded; if the ring electrode is grounded, then Eq. 2-9a may be set equal to zero,

\[ \Phi_{\text{ring}}(\rho, z) = C_1 \left( \frac{2}{\rho_{\text{core}}} - \frac{1}{\beta^2} \right) z^2 \rho_{\text{core}}^2 - \rho_{\text{ring}}^2 + C_2 = 0 \quad (2-13a) \]

or

\[ \Phi_{\text{ring}}(\rho, z) = \frac{-U_0 \rho_{\text{ring}}^2}{\rho_{\text{ring}}^2 - \rho_{\text{core}}^2} + C_2 = 0 \quad (2-13b) \]

and solved for \( C_2 \):

\[ C_2 = \frac{U_0 \rho_{\text{ring}}^2}{\rho_{\text{ring}}^2 - \rho_{\text{core}}^2} \quad (2-12b) \]

Thus the potential inside the two-electrode trap may be expressed as,

\[ \Phi_{\text{trap}}(\rho, z) = \frac{U_0}{(\rho_{\text{ring}}^2 - \rho_{\text{core}}^2)} (2z^2 - \rho^2) + \frac{U_0 \rho_{\text{ring}}^2}{\rho_{\text{ring}}^2 - \rho_{\text{core}}^2} \quad (2-14a) \]

or

\[ \Phi_{\text{trap}}(\rho, z) = \frac{U_0}{(\rho_{\text{ring}}^2 - \rho_{\text{core}}^2)} (2z^2 - \rho^2 + \rho_{\text{ring}}^2) \quad (2-14b) \]

which clearly satisfies Laplace’s equation. Eq. 2-14b may be re-written as,

\[ \Phi_{\text{trap}}(\rho, z) = \frac{U_0}{4d_0^2} (2z^2 - \rho^2 + \rho_{\text{ring}}^2) \quad (2-14c) \]
where $d_0^2$ is the characteristic trap dimension defined by:

$$d_0^2 = \frac{1}{4} (\rho_{\text{ring}}^2 - \rho_{\text{core}}^2) \quad (2-15)$$

At this point the theoretical treatment follows that in Chapter I; the electric field at the trap midplane is determined,

$$E(\rho) = -\frac{\partial \Phi_{\text{trap}}(\rho, z)}{\partial \rho} = \frac{U_0 \rho}{2 d_0^2} \quad (2-16)$$

and substituted into the Lorentz force equation:

$$m \omega^2 - qB_0 \omega + \frac{q U_0 \rho}{2 d_0^2} = 0 \quad (2-17)$$

Solution of Eq. 2-17 gives the reduced cyclotron ($\omega_+$) and magnetron ($\omega_-$) frequencies,

$$\omega_+ = \frac{\omega_c}{2} + \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \quad (2-18a)$$

$$\omega_- = \frac{\omega_c}{2} - \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \quad (2-18b)$$

in which:

$$\omega_z = \sqrt{\frac{q U_0}{m d_0^2}} \quad (2-18c)$$

As mentioned in Chapter I, the so-called quadrupolar approximation holds only near the center of a cubic ion trap; in hyperbolic geometries, both the Penning and two-electrode traps, the potential throughout the
trap has the quadrupolar form and thus ion ICR frequency is independent of position in the trap.

**Ion Motion in the Two-electrode Trap.** In an ICR trap, the radial ion motion is a superposition of two circular motions, magnetron and cyclotron, as derived from Eq. 2-1. In addition, ions undergo a harmonic axial oscillation in the z-direction, parallel to the magnetic field. For hyperbolic geometries (either Penning or two-electrode trap), the radial and axial motions are decoupled. For orthorhombic and cylindrical geometries, the electric field may be approximated by a quadrupole only near the trap center; higher order terms in the electric field lead to perturbations such as the coupling of ion motions and the variation of ion rotation and oscillation frequencies as a function of ion position within the trap 5-10.

The resonant cyclotron frequency for ions in the two-electrode trap (or in the parametric-mode Penning trap) is easily derived by considering ion motion in the trap midplane (z = 0). In the absence of a time-varying electric field, the equations of motion for such an ion are,

\[ x(t) = x_+(t) + x_-(t) = \rho_+ \sin(\omega_+ t) + \rho_- \sin(\omega_- t) \]  
\[ y(t) = y_+(t) + y_-(t) = \rho_+ \cos(\omega_+ t) + \rho_- \cos(\omega_- t) \]

in which \( \rho_+ \) and \( \rho_- \) are the respective ion cyclotron and magnetron radii, and phase angles have been set to zero for simplicity. Thus at any time, \( t \), the radial position, \( \rho(t) \), of the ion is
\[ |\rho(t)|^2 = |x(t)|^2 + |y(t)|^2 = \rho_+^2 + \rho_-^2 + 2\rho_+\rho_- \cos(\omega_+ - \omega_-)t \quad (2-20) \]

From Eq. 2-20, it is clear that the ion radial displacement from the z-axis will vary between \( \rho_{\text{max}} = |\rho_+ + \rho_-| \) and \( \rho_{\text{min}} = |\rho_+ - \rho_-| \) at the "parametric" frequency, \( \omega_p \), given by

\[ \omega_p = \omega_+ - \omega_- \quad (2-21) \]

**Excitation of Radial Motion in the Two-electrode Trap.** In a conventional ICR ion trap, excitation (e.g., dipolar, quadrature, quadrupolar) of ion cyclotron and/or magnetron motions is produced by applying resonant rf voltage(s) to different electrode(s) than those to which the d.c. electrostatic trapping voltage is applied. However, it is also possible to excite ion cyclotron motion "parametrically", i.e., by superimposing a time-varying excitation voltage on the same electrodes to which the d.c. trapping voltage has been applied, thus producing axial quadrupolar trapping and excitation fields. Parametric excitation was first introduced by Lee et al. for ICR in a cylindrical ion trap. Shortly thereafter, Rempel and Gross applied parametric excitation for FT/ICR in a hyperbolic Penning ion trap. In both cases, excitation was performed on ions which had non-zero initial magnetron radii (i.e. off-axis ionization). Later, Schweikhard et al. demonstrated that parametric excitation could be performed on ions initially formed on-axis and subsequently shifted off-axis by excitation at their magnetron frequency. Note that the parametric (radial) excitation geometry, in which both the trapping and excitation fields are axial quadrupoles, should be distinguished from the recently reported azimuthal...
quadrupolar excitation geometry which allows axialization of ions and ion detection at the unperturbed cyclotron frequency, $\omega_c$.\textsuperscript{22-24}

The time-dependent amplitudes of each of the ion modes (cyclotron, magnetron, trapping) are readily obtained for the two-electrode trap using a method recently introduced by Schweikhard and Marshall.\textsuperscript{11} The procedure as it pertains to parametric-mode excitation will be presented here; details for other excitation geometries may be found in ref. 11. The strategy is simply to equate the instantaneous single-frequency power absorption (averaged over one oscillation period) by an ion to the time-derivative of the (known) ion total energy; the resulting differential equations are solved to yield the ion's motional amplitudes as a function of time during the excitation period.

An important (and generally valid) assumption in this approach is that the energy absorbed during one motional oscillation is small compared to the energy of the motion itself.\textsuperscript{25} Under these conditions, the instantaneous power absorption, denoted $P(t)$, is

$$P(t) = F \cdot v \quad (2-22)$$

in which $v$ is the linear ion velocity, and $F$ is the force on the ion due to the excitation electric field.

$$F = qE \quad (2-23)$$
For the parametric geometry, the excitation potential has the same spatial dependence as the trapping potential (Eq. 2-14c); therefore, the radial excitation electric field takes the form,

$$E = \left( \frac{-V_{p-p}}{2d_0^2} \sin(\omega t + \phi) \right) \mathbf{i} + \left( \frac{-V_{p-p}}{2d_0^2} \sin(\omega t + \phi) \right) \mathbf{j} \quad (2-24)$$

in which the static trapping potential, $U_0$, has been replaced by a sinusoidal rf excitation voltage of peak-to-peak amplitude, $V_{p-p}$, applied at frequency, $\omega$. The instantaneous power absorption is

$$P(t) = \left( \frac{-qV_{p-p}}{2d_0^2} \sin(\omega t + \phi) \right) x + \left( \frac{-qV_{p-p}}{2d_0^2} \sin(\omega t + \phi) \right) y \cdot \left( \dot{x}(t) + \dot{y}(t) \right) \quad (2-25)$$

Substituting for $x(t)$, $\dot{x}(t)$, $y(t)$, and $\dot{y}(t)$ from Eqs. 2-19, we obtain

$$P(t) = \frac{\rho_+ \rho_- \omega_p qV_{p-p}}{2d_0^2} \sin(\omega_p t) \sin(\omega t + \phi) \quad (2-26a)$$

For in-phase, on-resonance excitation, we substitute $\omega = \omega_p$ and $\phi = 0$, to yield

$$P(t) = \frac{\rho_+ \rho_- \omega_p qV_{p-p}}{2d_0^2} \sin^2(\omega_p t) \quad (2-26b)$$

The time-averaged power absorption, $< P >$, is found from the integral,

$$< P > = \frac{1}{T} \int_0^T P(t) \, dt \quad (2-27)$$

in which $T = 2\pi/\omega_p$. Substituting Eq. 2-26b into Eq. 2-27, we find that
the time-averaged, instantaneous power absorption is:

\[ < P > = \frac{\rho_+ \rho_- \omega_p q V (p-p)}{4 d_0^2} \]  

(2-28)

The total energy, \( E_{\text{total}} \), of the trapped ions may be written as

\[ E_{\text{total}} = E_+ + E_- + E_{\text{trapping}} \]  

(2-29)

in which \( E_+ \), \( E_- \), and \( E_{\text{trapping}} \) are the energy contributions from the cyclotron, magnetron, and trapping motions, respectively; each of these components may in turn be expressed in terms of its motional amplitude,

\[ E_+ = \frac{m}{2} \omega_p \omega_+ \rho_+^2 \]  

(2-30a)

\[ E_- = -\frac{m}{2} \omega_p \omega_- \rho_-^2 \]  

(2-30b)

\[ E_{\text{trapping}} = \frac{m}{2} \omega_z^2 z_{\text{max}}^2 \]  

(2-30c)

or in terms of the quantum numbers, \( n_+ \), \( n_- \), and \( n_z \), for each motion:

\[ E_+ = \left( n_+ + \frac{1}{2} \right) \frac{h}{2\pi \omega_+} \]  

(2-31a)

\[ E_- = -\left( n_- + \frac{1}{2} \right) \frac{h}{2\pi \omega_-} \]  

(2-31b)

\[ E_{\text{trapping}} = \left( n_z + \frac{1}{2} \right) \frac{h}{2\pi \omega_z} \]  

(2-31c)
From Eqs. 2-30 it is easily shown\(^\dagger\) that, for parametric excitation at \(\omega_p\), the rates of change of the cyclotron and magnetron energies are related by:

\[
\frac{dE_+}{dt} = -\frac{\omega_+}{\omega_-} \frac{dE_-}{dt} \tag{2-32}
\]

Substituting for \(E_+\) and \(E_-\) from Eqs. 2-30a,b into Eq. 2-32, one finds that the time rates of change in the magnetron and cyclotron radii are related by:

\[
\rho_+ \frac{dp_+}{dt} = \rho_- \frac{dp_-}{dt} \tag{2-33}
\]

From Eqs. 2-30 and 2-33, we may express the total power, \(P\), absorbed into the radial motions as a function of the change in the magnetron or cyclotron radius only (i.e., evaluate \(dE_+/dt\) and \(dE_-/dt\) from Eqs. 2-30, then substitute for either \(\rho_+(dp_+/dt)\) or \(\rho_-(dp_-/dt)\) from Eq. 2-33).

\[
P = \frac{d(E_+ + E_-)}{dt} = m \omega_p^2 \rho_+ \frac{dp_+}{dt} \tag{2-34a}
\]

\[
P = \frac{d(E_+ + E_-)}{dt} = m \omega_p^2 \rho_- \frac{dp_-}{dt} \tag{2-34b}
\]

Next, we equate Eqs. 2-34 with the time-averaged power absorption (Eq. 2-28b) in order to obtain a system of two coupled differential equations,

\[
\dot{\rho}_+ - \gamma \rho_- = 0 \tag{2-35a}
\]

\[
\dot{\rho}_- - \gamma \rho_+ = 0 \tag{2-35b}
\]

in which \(\gamma = \frac{qV_{p-p}}{4m \omega_p d_0^2}\).
Eqs. 2-35 are readily solved by use of the Laplace transform \(^{26}\) to yield

\[
\rho_+(t) = \rho_+(0) \cosh(\gamma t) + \rho_-(0) \sinh(\gamma t) \quad (2-36a)
\]

\[
\rho_-(t) = \rho_+(0) \sinh(\gamma t) + \rho_-(0) \cosh(\gamma t) \quad (2-36b)
\]

In the present experiments, the initial (thermal) ion cyclotron radius is negligibly small \([\rho_+(0) \equiv 0.1 \text{ mm for an ion of } m/z \equiv 100 \text{ at room temperature in a magnetic field of } 3 \text{ T}]\); consequently, Eqs. 2-35 reduce to:

\[
\rho_+(t) = \rho_-(0) \sinh(\gamma t) \quad (2-36c)
\]

\[
\rho_-(t) = \rho_-(0) \cosh(\gamma t) \quad (2-36d)
\]

Finally, note that since \(\omega_- \ll \omega_+\), we may approximate \(\omega_p \equiv \omega_c\), such that

\[
\gamma \equiv \frac{V_{p-p}}{4 B_0 d_0^2} \quad (2-37)
\]

**Excitation of Axial Motion in the Two-electrode Trap.** The above procedure may also be used to determine ion axial amplitude as a function of excitation time. In this case, the axial electric field has the form:

\[
\mathbf{E} = \left(\frac{V_{p-p}}{d_0^2} \sin(\omega t + \phi)\right) z \mathbf{k} \quad (2-38)
\]
Thus the instantaneous power absorption becomes,

\[ P(t) = \left( \frac{qV_{(p-p)}}{d_0^2} \right) \sin(\omega t + \phi) z(t) \cdot (z(t)) \]  \hspace{1cm} (2-39a)

where,

\[ z(t) = z_{\text{max}} \sin(\omega_z t) \]  \hspace{1cm} (2-40)

and:

\[ \dot{z}(t) = z_{\text{max}} \omega_z \cos(\omega_z t) \]  \hspace{1cm} (2-41)

Substituting for \( z(t) \) and \( \dot{z}(t) \), we obtain:

\[ P(t) = \sin(\omega t + \phi) \sin(\omega_z t) \cos(\omega_z t) \]  \hspace{1cm} (2-39b)

For in phase (\( \phi = 0 \)), on-resonance (\( \omega = \omega_p \)) excitation, Eq. 2-39b simplifies to:

\[ P(t) = \frac{z_{\text{max}}^2 q V_{(p-p)} \omega_z}{2 d_0^2} - \left( \frac{z_{\text{max}}^2 q V_{(p-p)} \omega_z}{2 d_0^2} \right) \cos(4\omega_z t) \]  \hspace{1cm} (2-39c)

The time-averaged power absorption is again calculated from Eq. 2-27:

\[ <P> = \frac{z_{\text{max}}^2 q V_{(p-p)} \omega_z}{4 d_0^2} \]  \hspace{1cm} (2-42)

Equating Eq. 2-42 with the time rate of change in ion axial energy (Eq. 2-30c) we obtain the following differential equation,

\[ \dot{z}_{\text{max}} = \frac{z_{\text{max}} q V_{(p-p)}}{2 d_0^2 \omega_z m} \]  \hspace{1cm} (2-43)
which is readily solved to yield an expression for ion axial position as a function of time during excitation:

\[ z_{\text{max}}(t) = z_{\text{max}}(0) \exp \left( \frac{q V_{(p-p)} t}{4 d_0^2 \omega_z m} \right) = \]

\[ = z_{\text{max}}(0) \exp \left( \frac{V_{(p-p)} t \omega_z}{4 U_0} \right) \quad (2-44) \]

Eq. 2-44 will be key in the discussion of mass discrimination (see below) in the two-electrode trap.

**Ion Cyclotron Signal in the Two-electrode Trap.** The ICR signal in the two-electrode trap is generated by the image charges that the ions induce on the ring and core electrodes; these are readily determined from the "reciprocity" theorem of image charges.\textsuperscript{24,27-29}

\[ \frac{Q(r)}{q} = -\frac{\Delta \Phi(r)}{\Delta U} \quad (2-45) \]

Eq. 36 says that a charge, \( q \), at position, \( r \), in the trap will induce on the detection electrodes an image charge, \( Q \), which is proportional to the potential change, \( \Delta \Phi \), at position, \( r \), resulting from the application of a voltage change, \( \Delta U \), at the detection electrodes. The image charges on the ring and core electrodes are readily determined from Eq. 36 and the potential given in Eq. 9.

\[ Q_{\text{ring}} = \frac{2z^2 + \rho_{\text{core}}^2 - \rho^2}{4 d_0^2} q \quad (2-46a) \]

\[ Q_{\text{core}} = \frac{\rho^2 - \rho_{\text{ring}}^2 - 2z^2}{4 d_0^2} q \quad (2-46b) \]
The signal generated during differential detection is:

$$\Delta Q = Q_{\text{core}} - Q_{\text{ring}} = \frac{2p^2 - 4z^2 - (\rho_{\text{ring}}^2 + \rho_{\text{core}}^2)}{4d_0^2} q \quad (2-47)$$

Substituting for $p$ and $z$ from Eqs. 2-19 and 2-40, we obtain the time-variation of these image charges:

$$Q(t) = \frac{2p^2 + 2p_{-2}^2 + 4p_{+}\rho_{-}\cos(\omega_p t) - 2z_{\text{max}}^2 - 2z_{\text{max}}^2 \cos(2\omega_p t)}{4d_0^2} q \quad (2-48)$$

As expected, we obtain a radial signal at the parametric frequency, $\omega_p$, and an axial signal at twice the trapping frequency; note that no harmonics or combination frequency signals are expected in the two-electrode trap. The signal of interest, i.e., the signal amplitude at the parametric frequency, $\omega_p$, as a function of the excitation parameters ($T_{\text{excite}}$ and $V_{(p-p)}$) is easily obtained by substituting Eqs. 2-36 into Eq. 2-48:

$$\Delta Q = 4p_{-2}(0) \sinh(\gamma T_{\text{excite}}) \cosh(\gamma T_{\text{excite}}) =
\quad = 2p_{-2}(0) \sinh(2\gamma T_{\text{excite}}) \quad (2-49)$$

in which $\gamma$ is as stated in Eq. 2-37. Thus the observed signal in the two-electrode trap is expected to increase exponentially as a function of the excitation amplitude - duration product ($T_{\text{excite}} V_{(p-p)}$), as opposed to the linear dependence observed in dipolar-mode geometries.
Scrutiny of Eq. 2-49 reveals that the maximum signal, \( \Delta Q_{\text{max}} \), is obtained when, after some excitation period, \( T_{\text{excite}} \), the magnetron radius, \( \rho^-_{\text{T}_{\text{excite}}} \), lies half-way between the ring and core electrodes, and the cyclotron radius, \( \rho^+_{\text{T}_{\text{excite}}} \), is equal to half the distance between the ring and core electrodes:

\[
\rho^+_{\text{T}_{\text{excite}}} = \frac{\rho_{\text{ring}} - \rho_{\text{core}}}{2} \quad \text{(2-50a)}
\]

\[
\rho^-_{\text{T}_{\text{excite}}} = \frac{\rho_{\text{ring}} + \rho_{\text{core}}}{2} \quad \text{(2-50b)}
\]

The excitation duration, \( T_{\text{excite}} \), corresponding to this maximum signal is readily calculated by equating Eq. 2-36c with 2-50a and Eq. 2-36d with 2-50b:

\[
\rho^+_{\text{T}_{\text{excite}}} = \rho^-(0) \sinh(\gamma T_{\text{excite}}) = \frac{\rho_{\text{ring}} - \rho_{\text{core}}}{2} \quad \text{(2-50c)}
\]

\[
\rho^-_{\text{T}_{\text{excite}}} = \rho^-(0) \cosh(\gamma T_{\text{excite}}) = \frac{\rho_{\text{ring}} + \rho_{\text{core}}}{2} \quad \text{(2-50d)}
\]

Solving for \( \rho^-(0) \) from Eq. 2-50c (or 2-50d) and substituting into Eq. 2-50d (or 2-50c) yields,

\[
\tanh(\gamma T_{\text{excite}}) = \frac{\rho_{\text{ring}} - \rho_{\text{core}}}{\rho_{\text{ring}} + \rho_{\text{core}}} \quad \text{(2-51)}
\]

From the identity

\[
\tanh^{-1}(x) = \frac{1}{2} \ln \left( \frac{1 + x}{1 - x} \right); \quad x^2 < 1
\]

\[
(2-52)
\]
\( T_{\text{excite}} \) may then be obtained as:

\[
T_{\text{excite}} = \frac{1}{2} \gamma \ln \left( \frac{\rho_{\text{ring}}}{\rho_{\text{core}}} \right)
\]  

(2-53)

Alternately, from Eqs. 2-50, the optimal initial magnetron radius may be expressed in terms of the final magnetron and cyclotron radii,

\[
\rho_{-}(0) = \sqrt{\rho_{-}^{2}(T_{\text{excite}}) - \rho_{+}^{2}(T_{\text{excite}})}
\]  

(2-54)

or as the geometric mean of the radii of the ring and core electrodes:

\[
\rho_{-}(0) = \sqrt[\rho_{\text{ring}} \rho_{\text{core}}}
\]  

(2-55)

Implicit in the calculation of the optimal magnetron radius (Eqs. 2-54 and 2-55) is the assumption that all ions are located in the \( z = 0 \) trap midplane. In practice, the ions will of course be distributed along the \( z \)-axis. In the two-electrode trap, the maximum allowed cyclotron radius decreases with axial displacement from the center plane (see Fig. 2.1); as a result, only those ions exactly in the \( z = 0 \) midplane can attain the maximum allowed cyclotron radius for a given excitation period (Eq. 2-53) and/or initial magnetron radius (Eqs. 2-54 and 2-55). Although, the optimum initial magnetron radius for a parametric-mode Penning trap is not so readily calculated, the signal detected at \( \omega_{p} \) in that case does not depend strongly on initial magnetron radius—e.g., the signal drops by only 6.25% for an initial magnetron radius of 0.5 \( \rho_{\text{ring}} \). We shall return to this point later in the discussion section.
\textbf{Number of Detected Ions.} Finally, we note that, from Eq. 2-48, the number of ions contributing to the observed signal in the two-electrode trap may be estimated using a method introduced by Comisarow \cite{31} and later refined by Limbach et al.\cite{32} Briefly, the ions which induce the observed signal are treated collectively as a current source whereas the ring and core electrodes (and the associated electrical cables) are represented by an equivalent capacitor, $C_s$, and resistor, $R_s$, in parallel, which pass the induced charge from the current source to the preamplifier. For simplicity, the switching circuit in Fig. 2.2 is included as part of the amplifier chain. For such a model, the equation of interest becomes,

\begin{equation}
\frac{V_s}{R_s} + C_s \frac{dV_s}{dt} = \frac{q_0 + \rho - \omega \rho}{d_0^2} \cos(\omega pt)
\end{equation}

in which the left-hand side represents the source (denoted by subscript "s") current in the model circuit, and the right-hand side is the current obtained from the derivative of Eq. 2-48 [note that $\cos(\omega pt)$ in Eq. 2-48 has been replaced by $\sin(\omega pt)$ to facilitate the following derivation]. Let the source voltage, $V_s(t)$, be expressed as,

\begin{equation}
V_s(t) = V_0 e^{i \omega pt}
\end{equation}

in which:

\begin{equation}
\cos(\omega pt) = \text{Re} \left( e^{i \omega pt} \right)
\end{equation}

Substitution of Eq. 2-57 into Eq. 2-56, keeping only the real signal
components, yields,

\[ \frac{V_0}{R_s} e^{i \omega t} + C_s V_0 i \omega_p e^{i \omega t} = \frac{q_{p^+p^-} \omega_p}{d_0^2} e^{i \omega t} \]  \hspace{1cm} (2-56b)

which can be solved for \( V_0 \):

\[ V_0 = \frac{q_{p^+p^-} \omega_p}{d_0^2 \left( \frac{1}{R_s} + C_s i \omega_p \right)} \]  \hspace{1cm} (2-59)

Eq. 2-57a may be re-written as:

\[ V_s(t) = \frac{q_{p^+p^-} \omega_p e^{i \omega t}}{d_0^2 \left( \frac{1}{R_s} + C_s i \omega_p \right)} \]  \hspace{1cm} (2-57b)

Multiplying Eq. 2-57b by its complex conjugate, and keeping only the real signal components yields:

\[ V_s(t) = \frac{q_{p^+p^-} \omega_p e^{i \omega t}}{d_0^2 \left( \frac{1}{R_s} + i C_s \omega_p \right)} \times \frac{1}{R_s} \frac{-i C_s \omega_p}{X} = \]

\[ \left( \frac{q_{p^+p^-} \omega_p \cos(\omega_p t)}{d_0^2 R_s} \right) + \left( \frac{q_{p^+p^-} \omega_p^2 C_s \sin(\omega_p t)}{d_0^2} \right) \]  \hspace{1cm} (2-57c)

In order to further simplify Eq 2-57c, we assume that,

\[ V_s(t) = A \cos(\omega_p t - \phi) \]  \hspace{1cm} (2-60a)
and use the trigonometric identity,

\[ A \cos(\omega_p t - \phi) = A \left( \cos(\omega_p t) \cos(\phi) + \sin(\omega_p t) \sin(\phi) \right) \] (2-61)

to obtain:

\[
A \cos(\phi) = \frac{\left( \frac{1}{R_s} \right) q_\rho \rho_- \omega_p}{d_0^2 \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right)}
\] (2-62a)

\[
A \sin(\phi) = \frac{q_\rho \rho_- \omega_p^2 C_s}{d_0^2 \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right)}
\] (2-62b)

The constant \( A \) may be expressed as:

\[
A = \sqrt{\left( \frac{1}{R_s} \right) q_\rho \rho_- \omega_p} \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right) d_0^2 \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right) + \left( q_\rho \rho_- \omega_p^2 C_s \right)^2
\]

\[
= \sqrt{\left( \frac{1}{R_s} \right) q_\rho \rho_- \omega_p} \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right)^2 d_0^4 \left( 1 + \frac{R_s^2 \omega_p^2 C_s^2}{\omega_p^2 C_s^2} \right)
\] (2-63a)

For typical values of \( R_s = 10^8 \ \Omega, \ C_s = 50 \ \text{pF}, \ \omega_p = 3.77 \times 10^6 \ \text{rad/sec}, \ \rho_+ = 0.006 \ \text{m}, \ \text{and} \ \rho_- = 0.01 \ \text{m}, \) we see that \( R_s^2 \omega_p^2 C_s^2 \gg 1 \), such that Eq. 2-62a may be re-written as:

\[
A \approx \sqrt{\left( \frac{1}{R_s} \right) q_\rho \rho_- \omega_p} \left( \frac{1}{R_s^2} + C_s^2 \omega_p^2 \right)^2 \] (2-63b)
Again, from the typical values given above, we find that \((q_{p+}p_{-} \omega_p^2 C_s)^2 >> (1/R_s) q_{p+}p_{-} \omega_p^2 C_s\)^2\), and thus Eq. 2-63b may be simplified to:

\[
A = \sqrt{\frac{(q_{p+}p_{-} \omega_p^2 C_s)^2}{(d_0^4 \omega_p^2 C_s^2)^2}} = \frac{q_{p+}p_{-}}{d_0^2 C_s}
\]  

(2-63c)

Eq. 2-60a now becomes:

\[
V_s(t) = \frac{q_{p+}p_{-}}{d_0^2 C_s} \cos(\omega_p t - \phi)
\]  

(2-60b)

From Eqs. 2-62, \(\tan(\phi)\) may be obtained:

\[
\tan(\phi) = \frac{A \sin(\phi)}{A \cos(\phi)} = R_s C_s \omega_p
\]  

(2-64)

Again, based on the typical values of \(R_s, C_s,\) and \(\omega_p\) given above, we may estimate \(\phi\) to be:

\[
\phi = \tan^{-1}(R_s C_s \omega_p) \equiv \frac{\pi}{2}
\]  

(2-65)

Thus Eq. 2-60b may be written as:

\[
V_s(t) = \frac{q_{p+}p_{-}}{d_0^2 C_s} \cos(\omega_p t - \frac{\pi}{2})
\]  

(2-60c)

or

\[
V_s(t) = \frac{q_{p+}p_{-}}{d_0^2 C_s} \sin(\omega_p t)
\]  

(2-60d)

For \(N\) ions, each of charge \(q\), we may write:

\[
V_s(t) = \frac{Nq_{p+}p_{-}}{d_0^2 C_s} \sin(\omega_p t)
\]  

(2-60e)
Finally, for a sinusoidal voltage source, \( V_s(t) = V_{(\text{p-p})} \sin(\omega pt) \):

\[
N = \frac{d_0^2 C_s V_{(\text{p-p})}}{q\rho_+ \rho_-}
\]  

(2-66)

Thus the number of ions contributing to the observed signal in the two-electrode trap may be estimated from the post-excitation cyclotron and magnetron radii (Eqs. 2-36c,d), the equivalent capacitance \( C_s \) of the detection circuit, and the rf peak-to-peak voltage, \( V_{(\text{p-p})} \) (from a known source), which gives a signal equivalent to that observed experimentally.

**EXPERIMENTAL**

**Trap Construction.** The two-electrode trap (see Fig. 2.1) was constructed of oxygen-free high-conductivity copper in the O.S.U. Chemistry Department machine shop. Two-dimensional profiles for both the ring and core electrodes were cut on a computerized, wire electric discharge machine (Wire-EDM, Sodick Inc., Rolling Meadows, IL), and in turn used as templates to cut the actual electrodes on a hand-operated lathe. A coordinate measuring machine was used to measure the final electrode surfaces, ensuring that they matched the original design specifications (to within \( \pm 0.04 \) cm—see Table 2.1). A machinable ceramic ring (shown as shaded regions in Fig. 2.1) serves to align the ring electrode with respect to the core electrode. The initial magnetron radius was defined by a 2 mm diameter hole drilled through the core electrode and offset by approximately 1 cm from the trap axis. An electron ionizing beam was produced by passing current through a rhenium
Figure 2.1 Three-dimensional (top) and cross-sectional views of the two-electrode ion trap, showing the ring and core electrodes, and the ceramic aligning ring. The measured trap dimensions are a.) $\rho_{\text{core}} = 0.324 \text{ cm}$; b.) $\rho_{\text{ring}} = 2.555 \text{ cm}$; c.) $\rho_{\text{core max}} = 3.633 \text{ cm}$; d.) $\rho_{\text{ring max}} = 3.665 \text{ cm}$; e.) $z_{\text{core max}} = 2.608 \text{ cm}$; f.) $z_{\text{ring max}} = 1.829 \text{ cm}$; g.) trap length = 7.678 cm; h.) trap diameter = 8.882 cm.
TABLE 2.1 Comparison of the design specifications and the measured values for the major dimensions of the two-electrode ion trap.

<table>
<thead>
<tr>
<th>Dimension (See Fig. 2.1)</th>
<th>Design Specification (cm)</th>
<th>Measured Value (cm)</th>
<th>Error (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho_{\text{core}}$</td>
<td>0.318</td>
<td>0.324</td>
<td>+0.006</td>
</tr>
<tr>
<td>$\rho_{\text{ring}}$</td>
<td>2.540</td>
<td>2.555</td>
<td>+0.015</td>
</tr>
<tr>
<td>$\rho_{\text{core, max}}$</td>
<td>3.653</td>
<td>3.633</td>
<td>-0.020</td>
</tr>
<tr>
<td>$\rho_{\text{ring, max}}$</td>
<td>3.653</td>
<td>3.665</td>
<td>+0.012</td>
</tr>
<tr>
<td>$z_{\text{core, max}}$</td>
<td>2.573</td>
<td>2.608</td>
<td>+0.035</td>
</tr>
<tr>
<td>$z_{\text{ring, max}}$</td>
<td>1.854</td>
<td>1.829</td>
<td>-0.025</td>
</tr>
</tbody>
</table>

filament mounted 0.64 cm above the 2 mm hole; electron emission current was measured on a collector plate mounted on the core face opposite the filament.

**Trapping, Excitation and Detection Connections.** Trapping (d.c.) and excitation (r.f.) voltages were delivered to the trap as shown in the circuit in Figure 2.2. With the switch in the closed position, the excitation rf voltages from the FTMS-1000 cell controller are delivered through the 500 pF capacitors to the ring and core electrodes. During the detection event, the switch is open; the induced image current difference between the ring and core electrodes passes through the 2 kΩ resistors, and the resulting voltages are sent directly to the FTMS-1000 pre-amplifier. The switch requires a separate TTL to 24-volt level converter which in turn is triggered by the excite gate from the FTMS-1000. In all experiments
reported here, a minimum delay of 5 ms between excitation and detection eliminates noise associated with the switching event. A positive trap voltage was applied to the core electrode across a 1 MΩ resistor, and the ring electrode was grounded across the second 1 MΩ resistor.

**FT/ICR/MS Excitation and Detection Parameters.** All experiments were performed with an FTMS-1000 mass spectrometer (Extrel FTMS Millipore, Inc., Madison, WI) operated at an applied magnetic field of 3.046 tesla (obtained from y-intercept of Fig. 2.3, top). A base pressure of $1.0 \times 10^{-9}$ Torr was maintained in the vacuum chamber by a CTI CryoTorr 8 cryopump (CTI Corporation, Waltham, MA). Gaseous samples were introduced into the vacuum chamber through a leak valve (No. 951-5100, Varian Associates, Walnut Creek, CA). Unless otherwise noted, electron ionization was performed at 70 eV electron energy, and all mass spectra are reported as 32K magnitude-mode data, produced by discrete Fourier transform of a single unapodized 32K time-domain digitized transient signal (padded with an additional 32K zeroes before FFT). Various ionization, excitation and detection conditions are reported in the appropriate Results and Discussion sections.

**RESULTS AND DISCUSSION**

**Shift in ICR Orbital Frequency with Trapping Potential.** Although the reduced ICR frequency, $\omega_\alpha$, is constant with respect to position in a purely quadrupolar potential (Eq. 2-18a), it nonetheless varies with the
Figure 2.2 Schematic diagram of the circuit used to couple the two-electrode ion trap to the standard excitation and detection electronics of the Extrel FTMS-1000 mass spectrometer.
applied trapping potential:

\[
\omega_p = \omega_+ - \omega_- = \omega_c \sqrt{1 - \frac{2qU_0}{m\omega_c^2d_0^2}}
\] (2-67a)

Taylor expansion of the square root in Eq. 2-67a reveals that \(\omega_p\) varies linearly with trapping potential, provided that the ion mass is well below the "critical" mass at which \(\omega_+ = \omega_- = \omega_c/2 = \omega_z/\sqrt{2}\).

\[
\omega_p \equiv \omega_c - \frac{qU_0}{m\omega_c d_0^2} = \omega_c - \frac{U_0}{B_0 d_0^2}
\] (2-67b)

The derivative of Eq. 2-67b,

\[
\frac{d\omega_p}{dU_0} \equiv \frac{-1}{B_0 d_0^2}
\] (2-68a)

or

\[
\frac{dv_p}{dU_0} \equiv \frac{-1}{2 \pi B_0 d_0^2}
\] (2-68b)

predicts a parametric frequency shift (downward) of \(-325.3\) Hz per volt of trapping potential, based on \(d_0^2 = 1.606 \times 10^{-4}\) m\(^2\) calculated from the measured values given in Table 2.1. The uncertainty in the predicted magnetron shift is \(\pm 1\) Hz/V (0.3 % relative), assuming \(\pm 0.001\) cm in the measured values in Table 2.1 and \(\pm 0.001\) T in the magnetic field value.

To determine the shift in ICR orbital frequency as a function of trapping voltage, we excited \(\text{N}_2^+\) ions with a resonant single-frequency rf pulse (5.2 V\(_{p-p}\), 200 \(\mu\)s), and detected the resulting time-domain transients in heterodyne-mode with a 40 kHz Nyquist bandwidth. The
trapping voltage was varied from 0.75 to 10.0 V in 0.25 V increments. The vacuum chamber pressure was held constant at 3.0 \times 10^{-9} \text{ Torr}. As seen in Figure 2.3 (top), the experimental frequency shift of -325.0 Hz/volt agrees to within 0.092\% of the expected value (i.e., well within experimental uncertainty).

The ICR frequency shifts for different hyperbolic traps are readily compared by correcting for the different characteristic dimension, $d_0^2$, of the respective traps. In Figure 2.3 (bottom) the two-electrode trap ($d_0 = 1.267 \text{ cm}$, calculated from the measured values in Table 2.1) is compared with a Penning trap ($d_0 = 0.778 \text{ cm}$) from Marshall's laboratory.$^{33}$ Because the Penning trap was operated in dipolar mode, its ICR frequency shift should be one-half that observed in the parametric-mode two-electrode trap (after scaling with respect to $d_0^2$ for each trap).

\[
\omega_+ = \omega_c - \omega_- \quad \text{(dipolar mode)} \tag{2-69a}
\]
\[
\omega_p = \omega_+ - \omega_- = \omega_c - 2\omega_- \quad \text{(parametric mode)} \tag{2-69b}
\]

The observed ICR frequency shift (-441 Hz/V) as a function of trapping voltage for the Penning trap agrees to within 0.5\% of the predicted value (-439 Hz/V).$^{33}$ As expected, the observed slope (normalized) for the two-electrode trap (-521.9 Hz cm$^2$/V) is approximately twice the normalized slope (-269 Hz cm$^2$/V) observed for the Penning trap (Fig. 2.3, bottom).

**Exactness of Quadrupolar Electrostatic Potential in Two-electrode, Cubic, and Penning Traps.** As noted earlier, one benefit of a pure quadupolar trapping potential is that the ICR orbital frequency is
Figure 2.3 Effect of trapping potential on observed ion cyclotron frequency. Top: FT/ICR frequency of $\text{N}_2^+$ as a function of trapping potential in the two-electrode ion trap. Bottom: Normalized FT/ICR frequency shift as a function of trapping potential in the two-electrode ($\text{N}_2^+$) and Penning ($\text{C}_6\text{H}_6^+$) traps.
independent of ion position in the trap. As a measure of the exactness of the quadrupolar potential in our two-electrode trap, we recorded the variation in the ICR frequency of C₆H₆⁺ ions as a function of ICR orbital radius (see Fig. 2.4). Ions generated by electron ionization [75 nA emission current (measured on a collector on the opposite side of the trap from the electron filament) electron beam of 10 ms duration] were excited by either: (a) varying the single-frequency resonant rf excitation amplitude from 0.5 to 19.0 V(p-p) for a constant excitation interval of 100 μs, or (b) varying the excitation interval from 60 to 2,200 μs at a constant excitation amplitude of 0.8 V(p-p). In both cases, the trapping d.c. voltage was held at 2.0 V and the pressure in the vacuum chamber was 5.0 x 10⁻⁹ Torr. Figure 2.4 shows that for either excitation method, the maximum shift in (parametric) cyclotron frequency is less than ~8 Hz [598.517 kHz ≤ ωₚ(C₆H₆⁺) ≤ 598.525 kHz] over an ion cyclotron radius range of 0.025 - 1.15 cm (cyclotron radii calculated from Eq. 2-36a, based on d₀² calculated from the measured values in Table 2.1).

For comparison, the (dipolar) cyclotron frequency in a 2.54 cm cubic trap shifts by up to 64 Hz over an ICR radius range of 0.18 - 1.42 cm (see Fig. 2.5). Although there is no simple expression relating ICR orbital radius to excitation voltage in the Penning trap, the cyclotron radius is nevertheless proportional to the product of resonant single-frequency excitation amplitude and excitation period, V(p-p) Texcite. Over a comparable range of V(p-p) Texcite, the (dipolar) cyclotron frequency in the Penning trap (see Fig. 2.5) shifts by up to 55 Hz (Note that the
\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure2.4}
\caption{Variation of experimental ICR frequency of $C_6H_6^+$ with ICR orbital radius in the two-electrode trap. Ions were excited by either: varying the single-frequency resonant r.f. excitation amplitude from 0.5 to 19.0 V (p-p) for a constant excitation interval of 100 $\mu$s; or varying the excitation interval from 60 to 2200 $\mu$s at a constant amplitude of 0.8 V (p-p).}
\end{figure}
Figure 2.5 Experimental ICR frequency shift of $\text{C}_6\text{H}_6^+$ as a function of the amplitude times duration product in the two-electrode, cubic, and Penning ion traps. The smaller frequency variation for the two-electrode trap demonstrates its near-perfect quadrupolar electrostatic trapping potential. Note that the ordinate is magnified by a factor of 10 in Fig. 2.4 relative to Fig. 2.5.
ordinate is magnified by a factor of ten in Fig. 2.4 compared to Fig. 2.5). The results shown in Figures 2.4 and 2.5 clearly establish that the two-electrode trap exhibits a highly quadrupolar electrostatic trapping potential.

**Shift in ICR Orbital Frequency due to Space Charge.** Coulomb repulsions between trapped ions on average produce a net radial outward force which, like the radial outward force due to the applied trapping potential, shifts the observed ion cyclotron frequency downward.\(^{34-36}\) In the two-electrode trap, ions are necessarily formed off-axis; consequently, if the ionization event is long in duration compared to one period of the magnetron frequency, ions prior to cyclotron excitation will be distributed about a "ring of charge" in the annulus between the core and ring electrodes. As a result, ion-ion Coulomb repulsions are expected to be much weaker than for conventional dipolar-detection arrangements in which ions are tightly packed along the z-axis.

The effect of space charge in the two-electrode trap may thus be evaluated by measuring the ICR frequency shift as a function of the number of trapped ions. The number of C\(_6\)H\(_6^+\) ions was varied by electron ionization at 50 nA emission current for 5 - 3,000 ms of benzene at 1.0 \times 10^{-8}\) Torr. Single-frequency resonant rf excitation (4.2 V\(_{p-p}\), 200 \(\mu\)s) induced an ICR signal detected in heterodyne-mode (Nyquist bandwidth of 40 kHz, 50 transients co-added prior to FFT). In Figure 2.6 the observed ICR frequency of C\(_6\)H\(_6^+\) ions is plotted versus ionizing
Figure 2.6 Experimental ICR frequency of C\textsubscript{6}H\textsubscript{6}\textsuperscript{+} as a function of the relative number of ions formed (as reflected by the duration of the ionizing electron beam), for two-electrode, cubic, and Penning ion traps. The small frequency variation (\approx 8 Hz) observed in the two-electrode trap demonstrates the reduced space charge associated with off-axis ionization.
electron beam duration (which in turn determines the number of ions formed and trapped) for two-electrode, cubic, and Penning traps. Note that for the data acquired from the dipolar-mode cubic and Penning traps, the ions were formed initially on axis \(^{33}\) at similar pressure, beam duration, and electron emission current. Over an ion formation period of 5 - 3000 ms, the observed ICR frequency shifts by \(~ 8\) Hz, 171 Hz, and 54 Hz, for the two-electrode, cubic, and Penning traps, respectively. It is clear that for comparable experimental conditions, the space charge-induced shift in cyclotron orbital frequency is reduced by an order of magnitude in the two-electrode trap relative to the cubic or Penning traps.

**Trap Capacity and Dynamic Range.** A corollary of off-axis ionization is that if space charge is reduced, then it should be possible to fill the trap with more ions. For on-axis ionization, for example, the detected signal increases approximately linearly with the number of ions formed (i.e. beam duration), but then levels off to a near-constant value, as the trap is "saturated." \(^{32,37}\) In contrast, for similar experiments carried out under the conditions of Figure 2.6, in the two-electrode trap, the detected signal increases approximately linearly with electron beam duration up to 1 s, and then continues to increase somewhat more slowly up to \(~5\) s beam duration, after which the signal begins to level off.

The number of ions contributing to a given signal in the two-electrode trap was calculated as follows: First, the equivalent
capacitance of the detection circuit ($C_s$ in Eq. 2-66) was determined by inserting a home-built capacitance bridge in parallel with the detection lines, between the switch circuit (Fig. 2.2) and the preamplifier. The FT/ICR magnitude-mode peak height, determined by 3-point quadratic interpolation, for benzene molecular ions (at $7.5 \times 10^{-8}$ torr) was recorded as a function of the capacitance (18-1000 pF) added to the bridge. The experimental sequence consisted of a 5 ms quench pulse to remove all ions from the trap, electron beam duration of 50 ms at an emission current of 100 nA to form new ions, single-frequency, on-resonance excitation (4.2 V_{pp}, 230 μs), and heterodyne detection (4K data points, 400 kHz bandwidth). The sequence was repeated 2000 times and the time-domain transient was zero-filled to 32K data points prior to fast Fourier transformation. With the capacitance inserted in parallel with the detection circuit, the data should take the form,

$$C_{\text{bridge}} = \frac{\text{constant}}{\text{ion signal}} - C_s \quad (2-70)$$

in which $C_{\text{bridge}}$ is the measured capacitance of the bridge, $C_s$ is the capacitance of the detection circuit, and the FT/ICR magnitude-mode peak height serves as the ion signal. The y-intercept from a plot (not shown) of $C_{\text{bridge}}$ vs. (ion signal)$^{-1}$ gave a value of $C_s = 104.7$ pF, which was used (in Eq. 2-66) for all subsequent calculations of the number of ions in the two-electrode trap.

Next, a frequency generator was used to determine the equivalent peak-to-peak voltage ($V_{\text{pp}}$ in Eq. 2-66) resulting from a given ion signal. The output (5 mV, 600 kHz) from the generator was fed into a differential
amplifier; the resulting 180° out-of-phase signals were attenuated (60–22 dB) and inserted between the trap and switching circuit. The observed FT/ICR magnitude-mode peak height was then plotted as a function of rf peak-to-peak voltage. The experimental sequence was similar to that used for the capacitance measurement, except that single transients were collected at each voltage. From the resulting plots (not shown), the equivalent peak-to-peak voltage was determined and used in Eq. 2-66 to calculate the number of ions in the two-electrode trap. Note that the post-excitation cyclotron and magnetron radii (\(\rho_+\) and \(\rho_-\) in Eq. 2-66) were calculated from Eqs. 2-36, whereas \(d_0^2\) was calculated from the measured values in Table 2.1.

The number of ions contributing to the observed signal in the two-electrode trap was determined both at typical (2.0 V) and high (10.0 V) trapping potentials. In either case, the experimental sequence consisted of a 10 ms quench pulse, an electron beam duration of 20 s at 100 nA, followed by single-frequency, on-resonance excitation (4.2 V\(_{\text{p-p}}\), 230 \(\mu\)s). Under these excitation conditions, the post-excitation ion radius is (from Eq. 2-36c) 0.524 cm, just slightly less than one-half the maximum allowed radius of 1.116 cm (determined from the measured values in Table 2.1). Single 5 ms transients were collected in heterodyne mode (400 kHz bandwidth, 4K data points) and padded with 7 x 4K zeros prior to discrete Fourier transformation. To obtain the maximum number of trapped ions, the benzene neutral pressure was set to the highest level (~1.0 x 10\(^{-8}\) Torr) at which no damping of the ICR signal occurred during the detection period. The number of ions was then varied by reducing
the benzene neutral pressure to successively lower values. For each spectrum, the noise level was obtained from a program which fits the magnitude-mode baseline noise, for a signal-free baseline segment, to a Rayleigh distribution histogram.\textsuperscript{38} The noise level used to define the experimental signal to noise ratio is the standard deviation of the magnitude-mode baseline noise, which is equal to one-half the magnitude-mode root-mean-square baseline noise.\textsuperscript{39}

Figure 2.7 shows the linear response, plotted as the number of ions contributing to the observed signal (ordinate) versus the magnitude-mode peak height to baseline standard deviation noise ratio (abscissa). At a typical trapping potential of 2.0 V (top), the maximum number of observed ions is 71,000, whereas at higher trapping potential (10.0 V, middle), the maximum ion number increases to 230,000. The detection limit, defined here as the minimum number of ions which gives a magnitude-mode spectral peak height to baseline standard deviation noise ratio of 3:1, was determined by re-plotting the data in Fig. 2.7 (top and middle) up to a signal-to-noise ratio of 100:1 (Fig. 2.7, bottom). Extrapolating the best-fit lines for each data set to a 3:1 signal-to-noise ratio gave detection limits of 500 (2.0 V trapping potential) and 440 (10.0 V trapping potential) singly-charged ions. Based on the maximum and minimum number of ions observed, the linear dynamic range for the two-electrode trap is 140 (2.0 V trapping potential) or 520 (10.0 V trapping potential). Extrapolating the above data to previously defined parameters for the FT/ICR detection limit (single scan, undamped 1 s detection period, ions excited to one-half their maximum cyclotron
**Figure 2.7** The number of ions contributing to the observed signal in the two-electrode ion trap. At a typical trapping potential of 2.0 V (top), the maximum number of ions detected is 71,000, whereas at 10.0 V (middle), the number of ions detected increases to 230,000. The bottom graph shows both sets of data re-plotted up to a signal-to-noise ratio of 100; extrapolating the best fit lines to a (magnitude-mode spectral peak height)/(baseline standard deviation noise) ratio of 3:1 yields detection limits of 500 (2.0 V) and 440 (10.0 V) singly charged ions.
Figure 2.7.
radius),\textsuperscript{32} yields theoretical detection limits of 35 (2.0 V trapping potential) and 31 (10.0 V trapping potential) singly-charged ions in the two-electrode trap.

Previous work \textsuperscript{32} showed that a 4.76 cm cubic cell can trap (and detect) as many as $5.6 \times 10^6$ singly-charged ions, or ~2.5 orders of magnitude greater than that obtained for the present two-electrode trap. However, note that the available trapping volume in the two-electrode trap (annulus volume $\approx 25 \text{ cm}^3$) is significantly smaller than that in a 4.76 cm cubic trap ($\approx 108 \text{ cm}^3$). Thus it is more appropriate to compare the calculated number of ions trapped (and detected) per unit volume in each trap. By that criterion, the two-electrode trap can detect $\sim 9200$ ions/cm$^3$ versus $\sim 52,000$ ions/cm$^3$ for the cubic trap. It is reasonable to assume that whereas the two-electrode cell may indeed trap a large number of ions, a significant fraction of these is not detected; as noted earlier, the maximum allowed cyclotron radius decreases with increasing displacement from the $z=0$ midplane. For any given magnetron and cyclotron radii, ions will have a maximum axial displacement, beyond which they will be ejected by collision with the core electrode [provided that $\rho_- < (\rho_{\text{ring}} + \rho_{\text{core}})/2$]. For the excitation conditions cited above, the post-excitation cyclotron and magnetron radii are 0.524 cm and 1.15 cm respectively. The minimum displacement of an ion from the $z$-axis will be $1.15 - 0.524 = 0.626$ cm; substituting this value for $\rho$ in Eq. 2-3e gives a maximum allowed axial displacement of 0.379 cm; all ions with a $z$-amplitude greater than or equal to the maximum will be ejected
(presumably by contact with the core electrode) during excitation and will thus not contribute to the observed ICR signal from the two-electrode trap.

**Radial Ejection in the Two-electrode Trap.** Radial ejection in the two-electrode trap may be evaluated from a plot (Fig. 2.8, top) of magnitude-mode FT/ICR relative mass spectral peak height of C$_6$H$_6^+$ ions, excited as in Figure 2.4 (e.g. vary $T_{\text{excite}}$), as a function of ICR orbital radius. The maximum observed ion cyclotron radius (calculated from Eq. 2-36c) was 1.114 cm, in excellent agreement with the maximum allowed radius of 1.116 cm determined by the (measured) radial separation between the waists of the ring and core electrodes. Note that the ejection profile deviates from that typically observed; that is, rather than a somewhat symmetric increase and decrease about the maximum signal, the profile is skewed such that ejection is not complete until considerably after the maximum signal is observed. We account for this atypical profile by noting that only those ions located in the trap $z = 0$ midplane during excitation can attain the maximum ICR orbital radius of 1.116 cm. For example, in Figure 2.8 (top) we observe the maximum ICR signal at an ICR radius of 0.452 cm; under those conditions ($V_{\text{pp}} T_{\text{excite}} = 840$ V $\mu$s), the magnetron radius will be 1.12 cm as determined by Eq. 2-36d. Therefore, from Eq. 2-3e, we see that all ions with a $z$-amplitude greater than or equal to 0.413 cm have been ejected (presumably into the core) when the largest signal is observed. As the excitation amplitude $\times$ duration product increases further, both the cyclotron and magnetron radii increase, with the cyclotron radius
Figure 2.8 Radial (top) and axial (bottom) ion ejection behavior of the two-electrode ion trap. Top: radial ion ejection profile in which the FT/ICR magnitude-mode mass spectral relative peak height is plotted as a function of ICR orbital radius. Bottom: axial ion ejection, as observed by a drop in the FT/ICR magnitude-mode mass spectral relative peak height for resonant cyclotron frequency excitation/detection following prior irradiation at the plotted abscissa frequency.
increasing faster.\textsuperscript{11} As a result, the range of allowed z-amplitudes continually decreases during radial excitation until only those ions exactly in the $z = 0$ midplane remain, and then are finally ejected.

**Axial Ejection in the Two-electrode Trap.** Generally speaking, the electric field in a hyperbolic trap is spatially anisotropic; specifically, the a.c. excitation field (except at the $z=0$ midplane) has a significant $z$-component. This condition is exacerbated for parametric-mode relative to dipolar-mode, because the parametric excitation voltage is applied between the ring and core (or "end-cap") electrodes, rather than between two opposed ring segments. Consequently, in the two-electrode trap, all ions except for those in the $z = 0$ midplane experience a significant axial force during excitation. A short argument \textsuperscript{11,40,41} shows that axial motion is excited by parametric excitation at $2\omega_z$ (i.e., twice the trapping frequency). Moreover (see Eq. 2-44), ion z-amplitude increases exponentially with the applied excitation amplitude - duration product.

Axial ejection (z-ejection) at twice the trapping frequency can be demonstrated for C$_6$H$_5^+$ ions by use of a double-resonance experimental event sequence (Fig. 2.8, bottom). The first single-frequency rf excitation (8 mV(p-p), 1 ms) is applied at each of a series of frequencies between 2–90 kHz; the second single-frequency rf excitation (238 mV(p-p), 1 ms) is then applied at the parametric frequency, $\omega_p$, to excite the ICR motion of the remaining ions. If the first excitation frequency is at or near $2\omega_z$, ions will be axially ejected, and their abundance following the second excitation will decrease. Thus, axial ejection at $2\omega_z$ is evidenced by a
minimum (at 37.5 kHz) in Figure 2.8 (bottom), which agrees to within 5.1% of the value, $2\omega_z = 39.5$ kHz, calculated from Eq. 2-18c. Note that significant axial ejection results from parametric excitation of only 8.0 $V_{\text{p-p}}$ μs, compared to 840 $V_{\text{p-p}}$ μs required to produce radial ejection.

**Mass Discrimination in the Two-electrode Trap.** From Eq. 2-44, we see that the parametrically-excited ion $z$-amplitude in the two-electrode trap increases exponentially with trapping frequency $\omega_z$, which in turn varies inversely with ion mass-to-charge ratio. Consequently, low-mass ions should be preferentially $z$-ejected.

To determine the mass-dependence of axial ejection in the two-electrode trap, the FT/ICR magnitude-mode relative mass spectral peak heights of six ionic fragments of perfluoro-tri-$n$-butylamine (PFTBA) were recorded as a function of ICR orbital radius. Ions were excited by a frequency sweep from low (5 kHz) to high (1 MHz) frequency, at a sweep rate of 600 Hz/μs. The rf excitation voltage was varied over an amplitude range of 2.1 - 26.7 $V_{\text{p-p}}$. Signal detection was performed in direct-mode over a 1 MHz bandwidth, with 300 transients co-added prior to discrete FFT. The sample pressure was held constant at $5.0 \times 10^{-9}$ Torr and the ions were produced by a 70 eV electron beam of 15 ms duration and 250 nA emission current.

Figure 2.9 shows the FT/ICR magnitude-mode relative mass spectral peak heights (scaled with respect to the CF$_3^+$ peak ($m/z = 69$) as 100 at all radii) of six fragment ions from PFTBA versus relative excitation amplitude - duration product, $[V_{\text{p-p}} T_{\text{excite}}]/[V_{\text{p-p}} T_{\text{excite max}}]$, for two-
Figure 2.9 Mass discrimination in the two-electrode (top), cubic (middle), and Penning (bottom) ion traps. The relative FT/ICR magnitude-mode mass spectral peak heights for six fragment ions from PFTBA are plotted versus relative excitation amplitude-duration product.
electrode, cubic, and Penning ion traps. $[V_{\text{p-p}} T_{\text{excite}}]_{\text{max}}$ is the amplitude - duration product corresponding to the largest absolute FT/ICR magnitude-mode peak height for the m/z = 502 fragment. In the cubic trap, the CF$_3^+$ (m/z = 69) fragment ion maintains the highest relative abundance throughout the entire excitation range. Direct comparison of the hyperbolic traps (or more generally, comparison of dipolar- and parametric-mode geometries) is difficult, because ion radius increases linearly with excitation amplitude - duration product for dipolar excitation (Penning trap), whereas ion radius increases exponentially for parametric excitation (two-electrode trap). For both dipolar- (Penning) and parametric-mode (two-electrode) hyperbolic traps, Fig. 2.9 shows that the relative abundance of the lowest m/z fragment decreases with increasing excitation amplitude $\times$ duration. For the two-electrode (parametric-mode) trap, complete (presumably) axial ejection of CF$_3^+$ occurs well before $[V_{\text{p-p}} T_{\text{excite}}]/[V_{\text{p-p}} T_{\text{excite}}]_{\text{max}}$ reaches 1.0—thus, the remaining fragment ions, although not yet completely ejected, cannot be displayed in Fig. 2.9 once the CF$_3^+$ ions have been ejected (all peak heights are scaled to CF$_3^+$ = 100 at all radii). For the Penning (dipolar-mode) trap, complete ejection of CF$_3^+$ ions does not occur until after the m/z = 502 fragment has reached its maximum relative abundance. Figure 2.9 thus shows that the parametric-mode two-electrode trap exhibits pronounced mass-dependent axial ejection, compared to dipolar-mode cubic and Penning traps.
The observed mass discrimination in the two-electrode trap can be predicted from a plot of the theoretical ion z-amplitude versus excitation amplitude - duration product (see Fig. 2.10), according to Eq. 2-44. Although Figure 2.10 is based on single-frequency parametric excitation at $2\omega_z$, the results should still apply qualitatively to frequency-sweep experiments: low-$m/z$ ions will be preferentially z-ejected in the two-electrode trap.

If the observed mass discrimination were due solely to axial ejection at $2\omega_z$, then a possible solution would be to set the low-frequency excitation limit well above $2\omega_z$ of the lowest-mass ion of interest. However, depending on the $m/z$ range of interest, it is quite possible that $2\omega_z$ for the lowest-$m/z$ ions is higher than the cyclotron frequency of the highest-$m/z$ ions; in such a case, one might propose to use a SWIFT excitation waveform tailored so as to generate zero amplitude at $2\omega_z$ corresponding to each $m/z$ of interest. To test this hypothesis, we generated a SWIFT excitation waveform,\(^{42-45}\) with zero-amplitude "notches" at $2\omega_z$ corresponding to each of the six major fragment ions from PFTBA, with flat frequency-domain excitation magnitude over the rest of the 2.667 MHz excitation bandwidth. However, we observed only marginal improvement in mass discrimination under these SWIFT excitation conditions. z-ejection is a complicated problem \(^{11}\) which deserves further scrutiny for parametric-mode operation in a two-electrode trap.
Figure 2.10 Theoretical z-amplitudes for ions of \( m/z = 69, 131, 264, \) and 502 as a function of excitation amplitude-duration product in the two-electrode ion trap. The abscissa range includes typical experimental values used in this study; the ordinate values were calculated from Eq. 2-44 and subsequently scaled to an initial value of 1.
**Mass Accuracy in the Two-electrode Trap.** Independent of ion relative abundance accuracy, we may nevertheless hope for high mass accuracy in the two-electrode trap, considering the demonstrated exactness of the quadrupolar potential (Fig. 2.4) and its decreased space charge effects (Fig. 2.6). It is important to note that the appropriate mass calibration law for an ICR trap operated in parametric mode

\[ v^2 = \frac{\alpha}{m^2} + \frac{\beta}{m} \]  

(2-71)

differs from the mass calibration law for dipole-mode operation.

\[ m = \frac{A}{v} + \frac{B}{v^2} \]  

(2-72)

Perfluoro-tri-n-butylamine (PFTBA) was chosen as an internal standard for determining the masses of the major singly-charged ionic fragments from acetophenone, 1-bromo-4-fluorobenzene, and m-xylene. In each case, sample pressure in the vacuum chamber was held constant at 6.0 x 10^{-9} Torr, and an electron beam (400 ms, 250 nA emission current) produced ions which were subsequently excited with a frequency sweep from 70 kHz to 2.667 MHz, at a sweep rate of 750 Hz/μs. The resulting time-domain transients (100 co-added transients per FFT) were detected in direct-mode over a 1 MHz Nyquist bandwidth for 32.77 ms to yield 64K data, to which another 64K zeros were added (without apodization) prior to discrete Fourier transformation.

Tables 2.2 and 2.3 show the measured masses and corresponding errors (in parts per million) for the major ionic fragments of
**TABLE 2.2** Mass measurements for ions derived from acetophenone and acetone, internally calibrated from perfluorotri-n-butylamine

<table>
<thead>
<tr>
<th>Calibrant ion</th>
<th>True mass (m/z)</th>
<th>Measured mass (m/z)</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF$_3^+$</td>
<td>68.994663</td>
<td>68.994713</td>
<td>+0.73</td>
</tr>
<tr>
<td>C$_3$F$_5^+$</td>
<td>130.991471</td>
<td>130.991515</td>
<td>+0.33</td>
</tr>
<tr>
<td>C$_4$F$_9^+$</td>
<td>218.985087</td>
<td>218.985429</td>
<td>+1.56</td>
</tr>
<tr>
<td>C$<em>5$F$</em>{10}$N$^+$</td>
<td>263.986555</td>
<td>263.986771</td>
<td>+0.82</td>
</tr>
<tr>
<td>C$<em>8$F$</em>{16}$N$^+$</td>
<td>413.976989</td>
<td>413.977649</td>
<td>+1.59</td>
</tr>
<tr>
<td>C$<em>9$F$</em>{20}$N$^+$</td>
<td>501.970605</td>
<td>501.971698</td>
<td>+2.18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte ion</th>
<th>True mass (m/z)</th>
<th>Measured mass (m/z)</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_6$H$_5^+$</td>
<td>77.038576</td>
<td>77.038680</td>
<td>+1.35</td>
</tr>
<tr>
<td>C$_7$H$_5$O$^+$</td>
<td>105.033491</td>
<td>105.033716</td>
<td>+2.14</td>
</tr>
<tr>
<td>C$_8$H$_8$O$^+$</td>
<td>120.056966</td>
<td>120.057840</td>
<td>+7.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Background ion</th>
<th>True mass (m/z)</th>
<th>Measured mass (m/z)</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_3$H$_6$O$^+$</td>
<td>58.041865</td>
<td>58.041492</td>
<td>-6.43</td>
</tr>
</tbody>
</table>
TABLE 2.3  Mass measurements for ions derived from 1-bromo-4-fluorobenzene and \( m \)-xylene, internally calibrated from perfluorotri-\( n \)-butylamine

<table>
<thead>
<tr>
<th></th>
<th>True mass (m/z)</th>
<th>Measured mass (m/z)</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrant ion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{CF}_3^+ )</td>
<td>68.994663</td>
<td>68.994713</td>
<td>+0.73</td>
</tr>
<tr>
<td>( \text{C}_3\text{F}_5^+ )</td>
<td>130.991471</td>
<td>130.991552</td>
<td>+0.62</td>
</tr>
<tr>
<td>( \text{C}_4\text{F}_9^+ )</td>
<td>218.985087</td>
<td>218.985665</td>
<td>+2.64</td>
</tr>
<tr>
<td>( \text{C}<em>5\text{F}</em>{10}\text{N}^+ )</td>
<td>263.986555</td>
<td>263.986305</td>
<td>-0.95</td>
</tr>
<tr>
<td>( \text{C}<em>8\text{F}</em>{16}\text{N}^+ )</td>
<td>413.976989</td>
<td>413.976783</td>
<td>-0.50</td>
</tr>
<tr>
<td>( \text{C}<em>9\text{F}</em>{20}\text{N}^+ )</td>
<td>501.970605</td>
<td>501.969495</td>
<td>-2.21</td>
</tr>
<tr>
<td>Analyte ion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{C}_6\text{H}_4\text{F}^+ )</td>
<td>95.029703</td>
<td>95.029277</td>
<td>-4.48</td>
</tr>
<tr>
<td>( \text{C}_6\text{H}_4\text{F}^{79}\text{Br}^+ )</td>
<td>173.948039</td>
<td>173.947866</td>
<td>-1.00</td>
</tr>
<tr>
<td>( \text{C}_6\text{H}_4\text{F}^{81}\text{Br}^+ )</td>
<td>175.945992</td>
<td>175.945996</td>
<td>+0.02</td>
</tr>
<tr>
<td>Analyte ion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{C}_7\text{H}_7^+ )</td>
<td>91.054226</td>
<td>91.054270</td>
<td>+0.48</td>
</tr>
<tr>
<td>( \text{C}<em>8\text{H}</em>{10}^+ )</td>
<td>106.077010</td>
<td>106.077937</td>
<td>+8.74</td>
</tr>
</tbody>
</table>
acetophenone (Table 2.2, with acetone as a background ion), 1-bromo-4-fluorobenzene (Table 2.3), and \( m \)-xylene (Table 2.3). In each case, the spectra were calibrated from six major ionic fragments of PFTBA; the magnitude-mode spectral peaks were located by 3-point parabolic interpolation, and the measured cyclotron frequencies were then fitted to Eq. 2-72 by use of a linear least-squares algorithm. The best fit \( \alpha \) and \( \beta \) coefficients were then used in conjunction with the measured frequencies of the above "sample" fragment ions to determine their measured \( m/z \) values. Tables 2.2 and 2.3 show that the two-electrode trap provides excellent mass accuracy; all calibrant fragment ions are within 3 ppm of their true values, whereas the mass errors for our "sample" species were less than 9 ppm.

**CONCLUSION**

The two-electrode trap exhibits a near-perfect quadrupolar potential as demonstrated by the very small cyclotron frequency shift with increasing cyclotron radius. Furthermore, the use of off-axis ionization results in an order-of-magnitude reduction in space charge because the ions are spread around the trap annulus prior to excitation. In these respects, the two-electrode trap is superior to dipolar-mode cubic and Penning traps; however, no increase in ion-holding capacity (either absolute or relative, compared to a 4.76 cm cubic trap) was observed. As expected, the radial ejection characteristics observed in the two-electrode trap are similar to those observed in other trap geometries. The atypical radial ejection profile for the two-electrode trap is attributed to a
decrease in the maximum allowed ICR orbital radius with increasing ion
displacement from the $z = 0$ midplane. Mass discrimination (predicted by
Eq. 2-44) is more pronounced for the two-electrode trap than for dipolar-
mode cubic and Penning traps. Finally, the two-electrode trap offers
excellent mass accuracy; for example, the average mass errors for the
calibrant ions in Tables 2.2 and 2.3 are $\sim 1.20$ and $1.28$ ppm respectively.
Corresponding errors for dipolar cubic and Penning ion traps are $\sim 2.63$
and $1.62$ ppm respectively.\textsuperscript{33} Not surprisingly, accurate mass
measurements, specifically involving one (or two closely-spaced) $m/z$
species, represent the most appropriate use of the two-electrode ion trap.
Unfortunately, the excessive mass discrimination observed in the two-
electrode trap precludes its use in experiments covering any significant
$m/z$ range.
REFERENCES


(3) Penning, F. M. *Physica (Utrecht)* **1936**, *3*, 873.


(27) Shockley, W. J. Appl. Phys. 1938, 9, 635.


(30) Rempel, D. L., Personal communication.


CHAPTER III

AN ELECTROSTATIC WIRE ION GUIDE FOR INJECTION
OF EXTERNALLY-FORMED IONS: THEORY AND
SIMULATION

INTRODUCTION

The performance advantages of Fourier transform ion cyclotron resonance mass spectrometry (FT/ICR/MS), including high mass resolution, high mass accuracy, and long ion storage period, $^{1-13}$ are optimized by high magnetic field strength ($> 3$ T) and low pressure ($< 10^{-8}$ torr) in the ICR ion trap. Because some useful ionization sources (e.g., fast atom bombardment, glow discharge, and electrospray) do not operate conveniently, if at all, under those conditions, optimal FT-ICR performance may require "external" ion generation with subsequent ion injection into the ICR ion trap. There are two main obstacles to overcome when coupling high-pressure ionization sources to a FT/ICR mass spectrometer. The first, pressure incompatibility between ion source and mass analyzer, may be remedied by use of differential pumping; the ion source is separated from the mass analyzer by several
(up to six) vacuum stages, each isolated from its neighbor by a small (few mm) conductance limit. The second impediment to ion injection is the so-called "magnetic mirror effect" in which an ion's axial kinetic energy is reduced, and possibly reversed, as it traverses the magnet's divergent fringe field. As will be discussed below, the mirror effect results mainly from coupling between the radial components of both the ion velocity and magnetic field. Thus some type of ion optics are needed both to guide ions through the small conductance limits separating each vacuum stage and to reduce the ion's interaction with the radial magnetic field. Several different ion guides have been used in this capacity.

Hunt and co-workers used two (or more) sets of quadrupole rods to guide ions from an external SIMS source, through two stages of differential pumping, and into an ICR ion trap. In these experiments, the first set of quadrupole rods (e.g. the set located closest to the ion source) was operated in "mass selective" mode such that only those ions in the \( m/z \) range of interest were transmitted to the ICR trap. Similarly, McIver and co-workers used r.f.-only quadrupole rods to transport ions through the magnetic fringe field; this work pushed the high-mass detection limit (for singly-charged ions) up to \( m/z \approx 32,000 \). Recently, McIver has coupled matrix-assisted laser desorption/ionization to a FT/ICR mass spectrometer via r.f.-only quadrupole rods, and demonstrated high-mass accuracy (~5 ppm), high-mass resolving power (up to \( m/\Delta m = 24,000 \)), and low limits of detection (~10 fmol) for several representative peptides. McLafferty has also injected ions formed via SIMS into an ICR ion trap using a quadrupole
ion guide. More recently, this group, along with Smith and co-workers, have achieved impressive results from external electrospray ionization sources.

Kofel and co-workers used a series of Einzel lenses to focus ions of a given energy range close to the central z-axis and into the ICR ion trap. Ions formed by both electron and chemical ionization were injected through the magnetic fringe field. Barshick and Eyler have used a similar approach to transport ions from an externally-located glow discharge source. Finally, Smalley has used electrostatic lenses to inject cluster ions formed in a supersonic beam by laser ablation of a metal target.

Each of these approaches requires sophisticated instrumentation. For example, when using quadrupole guide rods, an r.f. voltage of approximately 800-1000 V_{p-p} is required in order to generate a pseudo-potential deep enough to capture ions leaving the source region. The static d.c. voltage applied to Einzel lenses may also be on the order of several hundred volts; in this case ions tend to be accelerated axially prior to penetrating the magnetic fringe field. As a result, decelerating lenses must be placed before the ICR cell to increase ion trapping efficiency. As an alternative to external injection of electrospray ions, Laude and co-workers developed a system of concentric tubes which provides differential pumping in the radial rather than axial dimension. The electrospray source itself is actually located near the homogeneous region of the magnet. As ions exit the needle, they are
necessarily confined radially by the strong magnetic field, pass (axially) through small orifices at the end of each concentric tube, and are then trapped in the ICR ion trap. While this approach provides "automatic" ion radial confinement (due to the magnetic field), it does require a complex and relatively inefficient pumping scheme. Moreover, many ion sources will not operate in strong magnetic fields.

Limbach and Marshall have previously proposed and demonstrated experimentally the use of an electrostatic wire ion guide (EIG), consisting of cylinder and central wire electrodes held at different d.c. potentials, for external ion injection. In this simple device, the force produced by the radially-inward directed electric field captures ions in Lissajous-type orbits about the central wire electrode such that they experience minimal interaction with the radial magnetic field (and hence minimal mirror effect). An EIG was first used in FT/ICR/MS to couple a thermionic emitter source external to a solenoidal 3 T magnet. In Chapter IV of this thesis, preliminary results obtained on a prototype external source FT/ICR mass spectrometer, utilizing both matrix-assisted laser desorption/ionization (MALDI) and Cs⁺ secondary ion (SIMS) sources, will be discussed.

The principles of operation of an EIG in the absence of a magnetic field have been described previously. For ion injection in FT/ICR/MS, the EIG equations of motion are further complicated by the presence of a magnetic field gradient. Here, the equations of motion for a infinitely extended EIG in the absence of a magnetic field are
discussed. Next, numerical trajectory calculations are employed to characterize transport by an electrostatic ion guide of ions (1-200 eV initial kinetic energy) axially through the strong magnetic field gradient (0 to 3 T) of an FT-ICR mass spectrometer. Specifically, the effects of ion kinetic energy, EIG voltages (absolute and difference), ion source placement, and initial ion angular distribution on both ion transport efficiency and ion axial kinetic energy are considered.

**THEORY: ANALYTICAL METHODS**

The intended function of the EIG is to transport ions from an externally located ion source into an ICR ion trap without any additional acceleration, focusing, and/or deceleration lenses. It is assumed that an ion source can generate a large flux of ions at the EIG entrance plane and that the ions have kinetic energies between $1 \text{ - } 200 \text{ eV}$ (see Figure 3.3 for coordinate system and ion guide configuration and dimensions).

**Electrostatic Wire Ion Guide in the Absence of a Magnetic Field.**

The electromagnetic force acting on an ion of mass, $m$, and charge, $q$, is given by the Lorentz force equation:

$$\mathbf{F} = m \mathbf{a} = q \mathbf{E} + (q \mathbf{v} \times \mathbf{B}) \quad \text{(S.I. units)}$$

(3-1)

in which $\mathbf{a}$ is ion acceleration, $\mathbf{v}$ is ion velocity, $\mathbf{E}$ is electric field, and $\mathbf{B}$ is magnetic induction. The axial and radial electric field magnitudes, $E_z$ and $E_r$, between two infinitely extended concentric cylinders (i.e., an idealized electrostatic ion guide) may be expressed (in cylindrical coordinates) as,
\[ E_z = 0 \]  
\[ E_r = -\frac{\Delta V}{r \ln(r_{wire}/r_{cylinder})} \]

in which \( E_z \) is the axial electric field, \( E_r \) is the radial electric field, \( r \) is the radial displacement of the ion from the central axis, \( \Delta V = V_{cylinder} - V_{wire} \) is the potential difference, between the outer and central cylinders, \( r_{cylinder} \) is the radius of the outer cylinder, and \( r_{wire} \) is the radius of the central wire. The potential inside the EIG is plotted as a function of relative ion radius in Figure 3.1. Clearly, ions are focused close to the \( z \)-axis by the steep potential slope. As a result, they experience little interaction with the radial magnetic field and thus minimal magnetic mirror effect.

**Magnetic Mirror Effect in the Absence of an Electric Field.** The magnitude of the axial magnetic field, \( B_z \), along the central axis of a solenoidal electromagnet may be approximated as,

\[
B_z \approx \frac{\mu_0 N I r_{solenoid}^2}{2} \int_{-L/2}^{L/2} \frac{(dz)}{(r_{solenoid}^2 + z^2)^{3/2}}
\]

in which \( B_z \) is the magnetic field near the \( z \)-axis, \( \mu_0 \) is the magnetic permittivity of a vacuum, \( N \) is the number of turns of the solenoidal conductor per unit length, \( I \) is the current in a single loop of the solenoid, \( L \) is the solenoid length, \( z \) is the axial displacement of the ion with respect to the center of the solenoid, and \( r_{solenoid} \) is the solenoid radius. Because the radial and axial magnetic field components, \( B(r) \)
**Figure 3.1** Potential inside the electrostatic wire ion guide (EIG) plotted as a function of relative ion radius ($r_{ion}/r_{cylinder}$). The steep potential slope focuses ions close to the central wire electrode, thereby minimizing the magnetic mirror effect.

and $B(z)$, are related to each other by Maxwell's equation, $\nabla \cdot \mathbf{B} = 0$, the magnitude of the radial magnetic field, $B_r$, may be also be estimated near the solenoid central axis:

$$\frac{1}{r} \frac{\partial}{\partial r} [r B_r] + \frac{\partial B_z}{\partial z} = 0 \quad (3-4)$$
(Eq. 3-5 is approximately valid in the limit that dBz/dz does not vary strongly with r.) Thus, the radial magnetic field magnitude is strongest when the axial magnetic field gradient is strongest—for a typical 3 T magnet, at z = 25 cm from the center of the solenoid (see Figures 3.2 and 3.4). The radial magnetic field, Br, of Eq. 3-5 gives rise to the well-known "magnetic mirror" effect. An ion which has a velocity component, v⊥, perpendicular to the z-axis (and thus perpendicular to Br, see below) will experience a force whose axial magnitude, Fz, is:

$$F_z = q v_L B_r$$  \hspace{1cm} (3-6)

Since Br reaches a maximum when Bz is about half of its maximum value, v⊥ at that z-position is essentially perpendicular to Br, because v⊥ is dominated by ion cyclotron motion about Bz, and the (q v⊥ Br) force is therefore always in the negative z-direction (i.e., a retarding force which reduces the ion z-velocity - Note that this argument applies whether Bz points along the positive or negative z-direction, because the sign of $\left[ \frac{dB_z}{dz} \right]_{r=0}$ changes if the direction of Bz changes). Thus, the "magnetic mirror" slows (off-axis) ions as they approach a region of strong magnetic field. The mirror effect is pictured in Figure 3.2; the top plot shows the axial (Bz) and radial (Br) magnetic field magnitudes as a function of axial displacement from the solenoid center (e.g. center of ICR ion trap). The middle trajectory in Figure 3.2 corresponds to an ion with initial radial displacement of ~1 cm, moving toward the ICR ion trap (the black circles
Figure 3.2 Schematic representation of the magnetic mirror effect. As an ion progresses axially (middle trajectory) toward the ICR ion trap (located at 0 axial displacement in top plot), it gains radial kinetic energy at the expense of axial kinetic energy, due to interaction with the radial magnetic field (top). Eventually, the ion may be reflected away from the solenoid. Bottom: ion trajectories in the x-y plane (e.g. looking down the z-axis - see text).
in the middle and bottom trajectories indicate the ion's initial position). As the ion encounters the radial magnetic field, it experiences a force perpendicular to the central axis (directed upward in Figure 3.2 - middle) and thus begins to spiral about the z-axis. The ion continues to gain radial kinetic energy as both the radial and axial magnetic field magnitudes increase; however, because the total ion kinetic energy is fixed, the increase in radial kinetic energy comes at the expense of ion axial kinetic energy. Just past the point of maximum radial magnetic field, the ion in Figure 3.2 (middle) reaches zero axial kinetic energy and is reflected in the negative z-direction, away from the ICR ion trap. The bottom plots in Figure 3.2 show the ion trajectory in the x-y plane (e.g. looking down the central axis, indicated by the cross in each plot). The left plot in Figure 3.2 (bottom) shows the ion's trajectory as it penetrates into the solenoid, towards the ICR ion trap; the second half of the trajectory is pictured on the right, where the magnetic mirror reverses the ion's axial velocity.

**Electrostatic Ion Guide in a Magnetic Field.** Substitution of Eqs. 3-2, 3-3, and 3-5 into Eq. 3-1 yields the equations of motion for an ion inside an infinitely extended electrostatic ion guide and near the axis of a solenoidal magnet. Unfortunately, an analytical solution to Eq. 3-1 is not possible, since there is no analytical expression for the electric or magnetic field over the course of ion travel from the ion source to the FT-ICR ion trap (see Figure 3.3). Therefore, numerical calculations (see Results and Discussion) are used to establish the approximate initial
conditions which favor ion capture and transport through the magnetic field gradient and into an ICR ion trap.

**Analytical Expressions - EIG in the Absence of a Magnetic Field.**

Typically, an external ion source is located far (> 1 m) from the center of the solenoid of an FT-ICR mass spectrometer; the magnetic field at the source is thus negligible and ion behavior in this region (e.g., at the entrance of the EIG) may be estimated from analytical expressions (see Eqs. 3-7 - 3-12 below). Let the EIG axis be colinear with the solenoidal magnetic field symmetry z-axis, and let the ion (point) source be displaced radially (by r₀) and axially (by z₀) from the center of the EIG entrance plane (see Figure 3.3). Let the initial ion velocity, \( \mathbf{v} \), define an "elevation" angle, \( \theta \), relative to a line containing the initial position coordinates of the ion and parallel to the z-axis; in addition, there is an "azimuthal" angle, \( \phi \), between the projection of \( \mathbf{v} \) onto the xy-plane and a plane containing both the ion initial position and the z-axis. Based on the above coordinate system, with \( z_0 = 0 \) (i.e., ion source in the entrance plane of the EIG), Oakey and Macfarlane derived the following expression for the ion maximum "acceptance cone angle" for ions entering an infinitely extended EIG in the absence of a magnetic field \(^{46}\).

\[
\theta_{max}^2 = \frac{q|\Delta V|}{E_{ion}} \left[ \frac{\ln(r_0/r_{cylinder})}{\ln(r_{wire}/r_{cylinder})} \right] \left[ 1 - \frac{r_0^2/r_{cylinder}^2 \sin^2 \phi}{1 - (r_0^2/r_{cylinder}^2) \sin^2 \phi} \right]
\]

in which \( \theta_{max} \) is the maximum "elevation" angle at which ions may still enter and successfully traverse the EIG, and \( E_{ion} \) is the ion kinetic energy. \(^{46}\) Oakey and Macfarlane have derived the equations for the "collection efficiency" of either a point source or a circular disk source,
both located at the entrance plane of the EIG. The collection efficiency, \( \text{Eff}_{\text{point}}(r_0) \), for a point source at \( r_0 \) in the EIG entrance plane is defined as the solid angle of the acceptance cone divided by \( 4\pi \): \( \text{Eff}_{\text{point}}(r_0) = \frac{0.25 \frac{q |\Delta V|}{E_{\text{ion}}} \ln(r_0/r_{\text{cylinder}}) / \ln(r_{\text{wire}}/r_{\text{cylinder}})}{[1 - (r_0^2/r_{\text{cylinder}}^2)]^{1/2}} \) \( \text{(3-8)} \)

Similarly, the collection efficiency from a circular disk-shaped source of radius \( r_{\text{disk}} \) (for \( r_{\text{disk}} \gg r_{\text{wire}} \)) is:

\[
\text{Eff}_{\text{disk}}(r_{\text{disk}}) = \left( \frac{0.5 \frac{q |\Delta V|}{E_{\text{ion}}} r_{\text{cylinder}}^2}{\ln(r_{\text{wire}}/r_{\text{cylinder}}) r_{\text{cylinder}}^2} \right) \times \]

\[
\times \left[ (1 - \ln(r_{\text{disk}}/r_{\text{cylinder}})) \left( 1 - \frac{r_{\text{disk}}^2}{r_{\text{cylinder}}^2} \right)^{1/2} \right] - \ln \left( 1 + \left( 1 - \frac{r_{\text{disk}}^2}{r_{\text{cylinder}}^2} \right)^{1/2} \right) \frac{r_{\text{cylinder}}}{r_{\text{disk}}} - 0.3069 \right) \]

\( \text{(3-9)} \)

As seen in Eq. 3-7, the potential difference between the wire and cylinder, \( \Delta V \), and initial kinetic energy, \( E_{\text{ion}} \), determine whether an ion attains a stable orbit about the central wire; thus, for a fixed EIG geometry, ions of widely varying energy may be transported successfully, simply by adjusting the potential difference between the wire and cylinder electrodes. Conversely, mono-energetic ions of different mass should be readily captured by the EIG at a given voltage. Furthermore, lower-energy ions have a larger acceptance cone, making the EIG particularly well-suited for typical ion sources used in FT-ICR mass spectrometry.
Also, the direct dependence of maximum acceptance cone angle and collection efficiency on ion charge means that multiply-charged ions (as from an electrospray ion source) should be captured more readily by the EIG for transport into the ICR trap.

**THEORY: COMPUTATIONAL METHODS**

**SIMION Numerical Simulations - EIG in a Magnetic Field Gradient.**

EIG performance is now examined systematically as a function of $\Delta V$, $E_{\text{ion}}$, $r_0$, $\theta$, $\phi$, and z-displacement of the ion (point) source away from the entrance plane of the EIG, for a particular ion mass-to-charge ratio, $m/z$. Figure 3.3 shows the geometry and nominal dimensions of an EIG, on which all numerical simulations were based. The guide cylinder is 838 mm long, with 34 mm inside diameter. The central wire is 838 mm long and 0.28 mm in diameter, and is aligned coincident with the central symmetry (z-) axis of the solenoidal magnet. A cylindrical ion source electrode biased at +5V potential was positioned at various points in, or displaced axially from, the ion guide entrance plane. A three-section open-ended cylindrical ICR ion trap was centered in the solenoidal magnet, 76 mm axially displaced from the ion guide exit. Each ICR trap section was 50 mm long and 50 mm in diameter, and each was centered on the z-axis. The vacuum chamber surrounding the ion source, ion guide, and ICR ion trap was set to ground potential for all calculations. In all numerical simulations (e.g. Figures 3.6 - 3.11), ion initial conditions are designated with respect to a point source electrode, where $r_0$ still denotes the radial displacement of the source probe. (Note that
**Figure 3.3** Schematic diagram of the electrostatic wire ion guide (EIG) configuration, with dimensions shown in mm. Top: The three major electrode sets, including the source, the EIG wire and cylinder, and the ICR cylindrical ion trap. Bottom: Coordinate system on which Equations 3-7 - 3-12 are based.
Eqs. 3-7 - 3-12, Tables 3.1 and 3.2, and Figure 3.5 refer to an ion source in the entrance plane of the EIG.

Ion trajectory simulations were performed with SIMION PC/PS2 (Version 5.0, Idaho National Engineering Laboratory, Idaho Falls, ID). All calculations were based on a two-dimensional electric potential array of 600 x 40 points; a three-dimensional array was then generated by assuming cylindrical symmetry. Magnetic field plots for a 3 T superconducting magnet were provided by Oxford Instruments Inc. (Scientific Research Division, Concord, MA). Discrete values for both $B_z$ and $B_r$ were supplied at 5 cm axial increments (0-110 cm axial displacement from the solenoid center) and 0.5 cm radial increments (0-3 cm radial displacement from the solenoid central axis). Best-fit lines for $B_z (r=0 \text{ cm})$, $B_r (r=1 \text{ cm})$, $B_r (r=2 \text{ cm})$, and $B_r (r=3 \text{ cm})$ are plotted versus axial displacement ($z$) from the solenoid center in Figure 3.4.

Discrete values of $B_z (r=0 \text{ cm})$ and $B_r (r=3 \text{ cm})$ were entered into a magnetic potential array in SIMION at 5 cm axial increments, beginning at the ion trap center and extending to the ion source probe tip ($0 \leq z \leq 110 \text{ cm}$). During trajectory calculations SIMION used linear interpolation to obtain $B_z$ and $B_r$ at ion positions other than those corresponding to the discrete magnetic field entries. Note that values for $B_z (r=1-3 \text{ cm})$ agree very well with those for $B_z (r=0 \text{ cm})$, indicating that, in fact, $dB_z/dz$ does not vary strongly with radius (up to 3 cm). Thus Eq. 3-5 holds, and our use of linear interpolation to estimate $B_r$ at radii between 0-3 cm introduces only minimal error. Likewise, linear
Figure 3.4 Axial (z-component) and radial (r-component) magnetic field magnitudes, $B_z$ and $B_r$, as input parameters to SIMION for a typical 3 T solenoid magnet. The magnetic field value between any two $B_z$ and $B_r$ points (entered at 5 cm increments) is obtained by linear interpolation.
interpolation will slightly over-estimate $B_z$ at large axial displacements ($\geq 40$ cm, see Figure 3.4) while slightly under-estimating $B_z$ at axial displacements of $\sim 10-22$ cm (e.g. between the regions of maximum axial and radial magnetic fields - see Figure 3.4). More important, in the region of maximum $B_T$ (e.g. maximum magnetic mirror), $dB_z/dz$ is relatively constant, so that linear interpolation provides accurate estimates of $B_z$.

Because ionization sources produce ions with both low (e.g. glow discharge, electrospray) and high (e.g. MALDI) kinetic energies, trajectories are computed for ions having initial kinetic energies in the (broad) range, $1 - 200$ eV. The partitioning of that ion energy in different directions is then fixed by specifying the initial angular divergence in our ion trajectory simulations.

**RESULTS AND DISCUSSION**

**Ion Acceptance into the Electrostatic Ion Guide.** If an ion is to pass through the EIG, it is obvious that it cannot collide with either of the two electrodes. An ion entering the EIG at greater than a maximum allowable elevation angle, $\theta_{\text{max}}$, will collide with the outer electrode. Moreover, an ion entering the guide must have a minimum angular momentum to avoid collision with the central wire.

Figure 3.5 is a plot of the collection efficiency (computed from Eq. 3-8) as a function of ion initial off-axis displacement, $r_0$, for four combinations of ion initial kinetic energy and EIG potential difference,
Figure 3.5 Collection efficiency, $\text{Eff}_{\text{point}}(r_0)$, of a point ion source located in the entrance plane of the EIG as a function of the ion source radial displacement, $r_0$. Note that even ions initially far off-axis are successfully captured and transported by the EIG to the ICR ion trap.
ΔV, for a point ion source in the entrance plane of the EIG. As expected, the collection efficiency for ions to be captured in a stable orbit in the EIG for transport to the ICR ion trap increases with increasing ratio of potential difference to ion initial kinetic energy, ΔV/E_{ion}. It is encouraging to find that a significant fraction of ions entering the EIG significantly off-axis (large $r_0$) is captured by the guide (recall that Eq. 3-8 applies to a point source emitting ions over $4\pi$ geometry). Table 3.1 shows that expanding the ion source from a point to an on-axis circular disk of 6 mm radius increases the absolute collection efficiency still further.

**TABLE 3.1** Collection efficiency (i.e., solid angle of the acceptance cone divided by $4\pi$) for each of four combinations of ion initial kinetic energy, $E_{ion}$, and potential difference, ΔV, between the cylinder ($r_{cylinder} = 17$ mm) and central wire ($r_{wire} = 0.14$ mm) of an electrostatic ion guide, for a circular disk ion source of 6 mm radius, and for a point ion source displaced radially by 6 mm. Both sources are located in the EIG entrance plane.

<table>
<thead>
<tr>
<th>ΔV (volts) (volts)</th>
<th>$E_{ion}$ (eV)</th>
<th>$Eff_{disk}$ ($r_{disk} = 6$ mm)</th>
<th>$Eff_{point}$ ($r_{point} = 6$ mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.0</td>
<td>0.413</td>
<td>0.290</td>
</tr>
<tr>
<td>10.0</td>
<td>2.5</td>
<td>0.331</td>
<td>0.232</td>
</tr>
<tr>
<td>10.0</td>
<td>5.0</td>
<td>0.165</td>
<td>0.116</td>
</tr>
<tr>
<td>5.0</td>
<td>5.0</td>
<td>0.083</td>
<td>0.058</td>
</tr>
</tbody>
</table>

However, the ratio, $ΔEff/r_{disk}/Δr_{disk}$, decreases with increasing disk radius. In other words, for a circular disk ion source, each incremental
increase in ion source surface area results in a smaller incremental increase in collection efficiency. These simulations indicate that the energy dependence (Eq. 3-7) for successful ion capture also holds for ions formed outside the EIG; for example, when the source probe is displaced by 6 mm with respect to the EIG entrance plane, mono-energetic ions over the range $50 \leq \text{m/z} \leq 100,000$ ($r_0 = 2 \text{ mm}, \theta = 15^\circ, \phi = 45^\circ, \Delta V = 10 \text{ V}, E_{\text{ion}} = 20 \text{ eV}$) all had maximum Lissajous orbital radii (e.g., ion apogee radius inside the EIG) of ~4.4 mm and were transported to the ICR ion trap.

As the axial displacement of a point ion source from the EIG increases, the maximum allowed ion kinetic energy decreases. Figure 3.6 shows that an ion which begins axially near the EIG with an elevation angle, $\theta = 15^\circ$, has an increased probability of attaining a stable trajectory inside the EIG, mainly because a smaller initial axial displacement results in a smaller initial radial displacement, $r_0$, at the entrance plane: in Eq. 3-8, $\ln(r_0/r_{\text{cylinder}})/\ln(r_{\text{wire}}/r_{\text{cylinder}}) \to 1$ as $r_0 \to r_{\text{wire}}$ (i.e., as the ion initial radial displacement approaches the central wire). Stated another way, if the ion source is moved closer to the EIG, then the same number of ions can be collected by an EIG of smaller outer cylinder radius, $r_{\text{cylinder}}$. Figure 3.6 also confirms that a higher cylinder-wire potential difference increases the probability of a stable orbit inside the guide.

Figure 3.7 shows how the maximum initial kinetic energy for which ions of m/z 1000 may still be captured by the EIG depends on initial
Figure 3.6  Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap vs. initial z-displacement ($z_0$) of the on-axis ($r_0 = 0$) ion source from the EIG entrance plane, for an ion of $m/z = 1000$, at EIG potential differences, $\Delta V = 5, 10, 20$ V. The ion initial velocity elevation angle, $\theta$, is fixed at $15^\circ$. 
Figure 3.7: Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap as a function of initial ion velocity elevation angle, $\phi$, for an ion of $m/z = 1000$ with initial axial and radial displacements of 1 mm and 6 mm. The initial ion azimuthal angle, $\theta$, is fixed at 15°.

Maximum Kinetic Energy (Ions to the Cell, eV)

Elevation Angle ($\phi$)
polar ("elevation") entry angle, θ, for fixed initial axial and radial displacements, again for each of three values of EIG potential difference. For example, for an EIG potential difference of 10 V, an ion with an initial elevation angle of 20° and up to ~43 eV initial kinetic energy will still be captured by the guide and transported to the ICR Penning trap. The maximum kinetic energy decreases to ~18 eV at 30° and ~8 eV at 40°, in qualitative agreement with Eq. 3-7, which predicts that E_{ion} should vary as (θ_{max})^{-2}.

Eq. 3-7 also predicts how ion capture depends on the azimuthal entry angle, ϕ (see Figure 3.3). In fact, it has been pointed out previously that for a given radial displacement (r_0) of an ion (point) source at the entrance (xy) plane of the EIG, the maximal allowed initial elevation angle, θ, results from ϕ = π/2. Alternatively, for a given value of r_0 and θ, the maximum allowed kinetic energy for which an ion may still be captured in a stable orbit about the wire electrode increases with increasing ϕ (up to ϕ = π/2, i.e., ion velocity component perpendicular to the z-axis is also perpendicular to the direction of r_0). Figure 3.8, plotted for an ion starting with r_0 = 6 mm, θ = 15°, and z_0 = 6 mm, shows that ion initial kinetic energy is maximized for 130° ≤ ϕ ≤ 230°. In addition note that as the ion source is moved away from the guide entrance plane, the kinetic energy maximum is achieved at higher azimuthal angle. In fact, with the ion source displaced by ~15 mm (and other conditions as in Figure 3.8), the maximum kinetic energy (not shown) shifts to ϕ = 180° (i.e., ion velocity component perpendicular to z points radially inward).
Figure 3.8 Maximum ion kinetic energy for EIG capture and ion transport to an ICR ion trap vs. initial velocity azimuthal angle, $\phi$, for an ion of $m/z = 1000$ starting 6 mm off-axis and 6 mm axially displaced from the EIG entrance plane. The ion initial velocity elevation angle, $\theta$, is fixed at $15^\circ$. 
Comparison of the theoretical and observed maximum ion kinetic energies illustrates the importance of locating the ion source close to the EIG entrance plane. For example, under the same conditions as Figure 3.8 ($r_0 = 6\, \text{mm}, \theta = 15^\circ, \phi = 0^\circ, \Delta V = 10\, \text{V}, E_{\text{ion}} = 20\, \text{eV}$), Eq. 3-7 yields a maximum ion kinetic energy of 31.6 eV. As seen in Figure 3.8, the maximum kinetic energy of an ion starting 6 mm axially displaced from the entrance plane is ~21 eV, or ~33% less than that of an ion starting at the EIG entrance plane ($z_0 = 0$). Fortunately, the EIG cylinder diameter can be relatively large (34 mm in our case) so that any of several types of ion sources can be located conveniently at its entrance in an optimal geometric configuration.

**Ion Loss Through Collision with Central Wire.** A second advantage of injecting ions with large $\phi$ is the decrease in the number of ions which collide with the central wire electrode. Oakey and MacFarlane have derived an expression for the minimum azimuthal angle, $\phi_{\text{min}}$, below which an ion injected from a point source with a given $r_0$ and $\theta$ will be lost by collision with the wire:  

$$\phi_{\text{min}} = \left( \frac{r_{\text{wire}}}{r_0} \right) \left[ 1 + \left( \frac{q|\Delta V|}{E_{\text{ion}}\theta^2} \right) \frac{\ln(r_{\text{wire}}/r_0)}{\ln(r_{\text{wire}}/r_{\text{cylinder}})} \right]^{1/2} \quad (3-10)$$

The fraction of ions hitting the wire as a function of ion initial radial displacement, $F_{\text{collide}}(r_0)$, is:  

$$F_{\text{collide}}(r_0) = \left( 1 - \frac{r_{\text{wire}}}{r_0} \right) \left[ 1 + \left( \frac{q|\Delta V|}{E_{\text{ion}}\theta^2} \right) \frac{\ln(r_{\text{wire}}/r_0)}{\ln(r_{\text{wire}}/r_{\text{cylinder}})} \right]^{1/2} \quad (3-11)$$
\[ F_{\text{collide}}(r_0) = \left( \frac{r_{\text{wire}} q |\Delta V|}{2\pi r_0 E_{\text{ion}}} \right) \times \]

\[ \times \left\{ \left( \frac{\ln(r_{\text{cylinder}}/r_0)}{\ln(r_{\text{cylinder}}/r_{\text{wire}})} \right)^{1/2} + \left( \frac{\ln(r_0/r_{\text{wire}})}{\ln(r_{\text{cylinder}}/r_{\text{wire}})} \right) \times \right. \]

\[ \times \ln \left( \frac{(\ln(r_{\text{cylinder}}/r_0))^{1/2} + \ln(r_{\text{cylinder}}/r_{\text{wire}})^{1/2}}{(\ln(r_0/r_{\text{wire}}))^{1/2}} \right) \right\} \quad (3-11) \]

Combining Eqs. 3-8 and 3-11 yields the ratio, \( F_{\text{collide/capture}}(r_0) \) of the fraction of ions which are captured inside the EIG but collide with the central wire to the total fraction of ions captured, as a function of ion initial radial displacement:

\[ F_{\text{collide/capture}}(r_0) = \left( \frac{2r_{\text{wire}}}{\pi r_0} \right) \times \]

\[ \times \left\{ \left( \frac{\ln(r_{\text{cylinder}}/r_{\text{wire}})}{\ln(r_{\text{cylinder}}/r_0)} \right)^{1/2} + \left( \frac{\ln(r_0/r_{\text{wire}})}{\ln(r_{\text{cylinder}}/r_0)} \right) \times \right. \]

\[ \times \ln \left( \frac{(\ln(r_{\text{cylinder}}/r_0))^{1/2} + \ln(r_{\text{cylinder}}/r_{\text{wire}})^{1/2}}{(\ln(r_0/r_{\text{wire}}))^{1/2}} \right) \right\} \times \]

\[ \times \left( 1 - \left( \frac{r_0^2}{r_{\text{cylinder}}^2} \right) \right)^{1/2} \quad (3-12) \]
Based on the EIG dimensions given above, Table 3.2 lists values of $F_{\text{collide}/\text{capture}}(r_0)$, calculated from Eq. 3-12, for initial ion radial displacements of 1-10 mm. Note that for a given set of ion initial conditions (e.g. $\Delta V$, $E_{\text{ion}}$, $\phi$, and $\theta$), the fraction of ions which is lost to collision with the wire decreases with increasing initial radial displacement; however, even for relatively small initial ion radial displacement (e.g. $r_0 = 2$ mm), ion loss is only $\sim 10\%$.

**TABLE 3.2** The ratio, $F_{\text{collide}/\text{capture}}(r_0)$, of the fraction of ions which is captured but collide with the EIG central wire to the total fraction of ions captured, as a function of initial ion radial displacement. $F_{\text{collide}/\text{capture}}(r_0)$ is calculated from Eqs. 3.8 and 3.11, and applies to an ion point source located at the EIG entrance plane.

<table>
<thead>
<tr>
<th>$r_0$ (mm)</th>
<th>$F_{\text{collide}/\text{capture}}(r_0)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.18</td>
</tr>
<tr>
<td>2.0</td>
<td>0.11</td>
</tr>
<tr>
<td>3.0</td>
<td>0.084</td>
</tr>
<tr>
<td>4.0</td>
<td>0.070</td>
</tr>
<tr>
<td>5.0</td>
<td>0.060</td>
</tr>
<tr>
<td>6.0</td>
<td>0.053</td>
</tr>
<tr>
<td>7.0</td>
<td>0.048</td>
</tr>
<tr>
<td>8.0</td>
<td>0.044</td>
</tr>
<tr>
<td>9.0</td>
<td>0.040</td>
</tr>
<tr>
<td>10.0</td>
<td>0.036</td>
</tr>
</tbody>
</table>
Note that Eqs. 10-12 apply for ions beginning at (or inside) the EIG entrance plane; thus the values in Table 3.2 are estimates for ions which start out axially displaced from the entrance plane. Also, as discussed below, the initial ion radial displacement also affects the ion final axial kinetic energy distribution at the ICR ion trap. The low number of ion-wire collisions coupled with a very large acceptance angle makes the EIG well suited for capturing and transporting a significant fraction of ions initially formed at the source.

**Ion Transport Through a Magnetic Field Gradient to an ICR Penning Trap.** As an ion passing through the EIG begins to enter the strong magnetic field region of the mass spectrometer, the ion velocity parallel to the magnetic field becomes, 34

\[
v(z) = v_0 \left[ 1 - \frac{B(z)}{B(0)} \sin^2 \theta \right]^{1/2}
\]

in which \(v_0\) is the ion initial velocity, \(v(z)\) is the ion axial velocity at position \(z\), \(B(0)\) is the (axial) magnetic induction at the ion source, \(B(z)\) is the magnetic induction at position \(z\), and \(\theta\) is the ion initial elevation angle (see Figure 3.3) between \(v_0\) and \(B(0)\). In order for ions to pass through the magnetic mirror, an ion must maintain axial velocity greater than zero, (i.e., \(v(z) > 0\), so that the ion continues to move toward the ICR ion trap). Kofel et al. showed that, in the absence of an electric field, ions will overcome the magnetic mirror effect provided that: 34

\[
\theta < \arcsin \left[ \frac{B(0)}{B(z)} \right]^{1/2}
\]
For values of $\theta$ above this critical limit, the mirror force dominates and ions are reflected away from the trap (i.e., $v(z) < 0$). In the present experiments, $B(0)$ at the source and $B(z)$ at the ICR ion trap are approximately 0.008 T and 3 T respectively, requiring that $\theta < 3^\circ$ for transmission of ions leaving the source in the absence of the EIG. Thus a major advantage of the EIG lies in its large acceptance cone; even ions with large initial elevation angle at the ion source (see Fig. 3.7) are held close to the z-axis and transported through the magnetic field gradient to the ICR cell.

**Ion Axial Kinetic Energy After Exiting the EIG.** Ions formed at the source with initial conditions, $(E_{\text{ion}}, r_0, \theta, \phi)$, have no rotational preference about the central wire because the magnetic field is negligible at the source. Conversely, once ions have passed through the magnetic field gradient to reach a homogeneous magnetic field, ions undergo cyclotron rotation in only one sense according to the right-hand rule for the Lorentz magnetic force, $F = q v \times B$. Between the source and the homogeneous magnetic field region, ions which rotate inside the EIG in the same sense ("con-rotatory") as the final ion cyclotron motion at the EIG exit will be axially decelerated (e.g., the mirror force, $F_z = q v_\perp B_r$, is directed along the negative z-axis) as they traverse the transition region between Lissajous-type motion (e.g. determined by the EIG potential difference) and cyclotron motion (e.g. determined strictly by the magnetic field). Alternatively, ions initially rotating inside the EIG in the opposite sense ("dis-rotatory") as their final cyclotron motion will be axially accelerated in the $+z$ direction in the region of high radial magnetic field.
Since ion total kinetic energy must be conserved, any change in ion axial kinetic energy will be reflected in a corresponding change in the final ion cyclotron and magnetron radii.

Figure 3.9 shows the final ion cyclotron and magnetron radii, \( \rho_+ \) and \( \rho_- \) (estimated from SIMION trajectory plots) as a function of ion initial azimuthal angle, \( \phi \) (with other initial conditions as in Figure 3.8). Ions having \( 0^\circ \leq \phi \leq 180^\circ \) are dis-rotatory with respect to their final ICR motion. Moreover, note that the maximum \( z \)-acceleration and thus minimum cyclotron radius occurs at \( \phi = 90^\circ \), which corresponds to the maximum initial angular momentum (see Eq. 3-7) with which an ion may be injected. Conversely, when an ion is injected with maximum con-rotatory angular momentum (\( \phi = 270^\circ \)), the final magnetron radius reaches a minimum (see Figure 3.9). Overall, the cyclotron and magnetron radii vary over the range \(-1\text{-}6 \text{ mm} \) and \(-0.5\text{-}5 \text{ mm} \) respectively. The corresponding ion final axial kinetic energy, estimated by subtracting the radial kinetic energy (calculated from \( \rho_+ \) in Figure 3.9) from the total kinetic energy (provided by SIMION), varied over the range \(-9\text{-}25 \text{ eV} \).

In Figures 3.8 and 3.9, the final distribution in ion axial kinetic energy corresponds to ions for which \( \theta = 15^\circ \); in fact, ion sources will produce ions having a range of \( \theta \)-values. Thus one might expect an even wider range of final axial kinetic energies for ions formed with a wide range of initial \( \theta \) and \( \phi \) values. Figure 3.10 provides an estimate of the axial kinetic energy distribution for monoenergetic ions having initial
Figure 3.9 Final ion cyclotron and magnetron radii, $\rho_+$, and $\rho_-$, as a function of ion initial velocity azimuthal angle, $\phi$. Ions begin with axial and radial displacements of 6 mm, and velocity elevation angle, $\theta$, fixed at $15^\circ$. Ions with $0^\circ \leq \phi \leq 180^\circ$ are "dis-rotatory" whereas those with $180^\circ \leq \phi \leq 360^\circ$ are "con-rotatory" (see text).
Figure 3.10 Final ion axial kinetic energy as a function of ion initial velocity elevation angle, $\theta$. Ion initial conditions are as in Figures 3.8 and 3.9, except that at each $\theta$ value, the initial velocity azimuthal angle, $\phi$, is varied from 0 to $360^0$ in $30^0$ increments. Note that the acceleration/deceleration effects of the magnetic mirror result in a wide axial kinetic energy distribution (grey region).
conditions as in Figure 3.8. For each elevation angle (0 → 20°, in 2° increments), the azimuthal angle was varied from 0 → 360°, in 30° increments. The shaded region represents an envelope encompassing all of the data points. As expected, the acceleration/deceleration effects of the radial magnetic field result in a large axial kinetic energy distribution of approximately 20 eV. Thus, whereas the EIG can transport a large fraction of ions which begin with their trajectories radially displaced from the z-axis, subsequent mass analysis is obviously complicated by the wide range of final ion axial kinetic energies. For example, the highest trapping efficiency is expected for narrow axial kinetic energy distributions, especially for accumulated trapping 29.55, in which ions are collected for periods of up to several seconds prior to excitation and detection. Fortunately, the axial kinetic energy distribution of ions exiting the EIG may be narrowed simply by decreasing the ion initial radial displacement. Figure 3.11 displays results compiled as in Figure 3.10, for initial ion radial displacements of 2 mm (light shaded area) and 0 mm (dark shaded area). The axial kinetic energy distributions drop to ~6 eV and ~3 eV for initial radial displacements of 2 mm and 0 mm respectively. Clearly, ions which begin their trajectories closer to the central wire will be held closer to the z-axis, thereby encountering a smaller radial magnetic field and hence less axial acceleration or deceleration; furthermore, ions exiting the EIG close to the central z-axis will have smaller cyclotron and magnetron radii, facilitating subsequent mass analysis. Experimentally, enhanced ion trapping efficiency and reduced post-injection ion cyclotron and magnetron radii have been
Figure 3.11 Final ion axial kinetic energy as a function of ion initial velocity elevation angle, $\theta$, as in Figure 3.10, for $r_0 = 2$ mm (light shaded area) and $r_0 = 0$ mm (dark shaded area); note the narrowing of the final axial kinetic energy distribution as ions are focused closer to the z-axis.
obtained by use of azimuthal quadrupolar excitation during ion injection on a prototype EIG system. Nevertheless, ion sources do not produce monoenergetic ions, and therefore the experimentally observed axial kinetic energy distribution will represent a convolution of the axial kinetic energy distribution inherent in the EIG (e.g. Figure 3.10 or 3.11) and the energy distribution of the ion source. Note that for ions with large initial radius, \( r_0 \), increasing the EIG potential difference, \( \Delta V \), will also reduce the axial kinetic distribution because ions will then execute Lissajous orbits closer to the wire electrode.

**Limitations of the Present Trajectory Calculations.** Although the present results are for ions of a single mass-to-charge ratio (\( m/z=1000 \)) and a maximum magnetic field of 3 tesla, several inferences may nonetheless be made for other experimental conditions. For example, in matrix-assisted laser desorption, ion kinetic energy is expected to scale linearly with mass because ion velocity is nearly mass-independent (for ions of \( m/z \geq 1000 \)).\(^5\) Based on an average ion velocity of 750 m/s,\(^5\) our simulations (not shown) indicate that ions up to \( m/z \approx 22,000 \) (\( r_0 = 2 \text{ mm}, z_0 = 6 \text{ mm}, \theta = 15^\circ, \phi = 45^\circ, E_{\text{ion}} = 3-65 \text{ eV} \)) are transported to the cell for a wire-cylinder potential difference of 10 V; under these conditions, the maximum Lissajous orbital radius increased quadratically from 2.5 mm (\( m/z = 1000 \)) to 15 mm (\( m/z = 22,000 \)). As discussed above, ions having larger Lissajous radii will experience higher radial magnetic field and thus greater mirror effect. However, when the EIG potential difference was increased to 45 V, the transmitted \( m/z \) range extended above 100,000 (other conditions as above, with \( E_{\text{ion}} \))
extending to \( = 300 \text{ eV} \) whereas the maximum Lissajous radius was reduced to \( \leq 5 \text{ mm} \) for \( m/z \leq 40,000 \) (again the Lissajous radius increased quadratically to \( 15 \text{ mm} \) for \( 40,000 \leq m/z \leq 100,000 \)). Use of an EIG at higher magnetic field will also require larger wire and cylinder voltages because the radial magnetic field will be greater than in the present simulations. Note that other options exist for minimizing the Lissajous orbital radius (and thus the deleterious effects of the radial magnetic field): For example, a smaller diameter wire would allow for smaller ion orbital radius during transport through the EIG. The use of a cylinder electrode which tapers from a relatively large diameter at the source region to a smaller diameter prior to the magnetic fringe field is a second intriguing approach; in this case collection efficiency should be maximized at the EIG entrance whereas ions would be squeezed close to the central axis prior to entering the region of high radial magnetic field.

The present analysis is predicated on assumed point or circular disk ion sources, each with uniform angular distribution. In fact, an actual ion source will have a non-uniform angular distribution; e.g., a \( \cos^2 \theta \) distribution for SIMS sources. Moreover, the discrete nature of SIMION for both electric potential and magnetic field values necessarily limits the precision of the data. Finally, SIMION is limited to single-ion trajectories, so that ion-ion Coulomb forces and ion-neutral collisions are neglected. The presently reported EIG collection efficiencies thus probably represent upper limits in practice.
CONCLUSION

The present SIMION trajectory calculations predict that an electrostatic ion guide can transport, from an external ion source and through a magnetic field gradient into an ICR Penning trap, ions having a wide range of initial kinetic energy and angular divergence. Three general aspects of EIG performance have been evaluated:

**Ion Capture.** The maximum kinetic energy for ion capture by the EIG is achieved by placing the ion source close to the EIG entrance plane and close to the EIG central axis. In addition, ion collection efficiency increases with increasing ratio of EIG potential difference to ion initial kinetic energy, $\Delta V/E_{\text{ion}}$.

**Ion Transport.** Successful ion transport requires minimal ion interaction with the radial magnetic field. In this respect, small initial ion radial displacement and/or large $\Delta V$ will focus ions closer to the central axis, thereby reducing the magnetic mirror effect. However, ion collisions with the central wire electrode increase with decreasing $r_0$. Fortunately, Table 3.2 shows that only about 10% of ions collide with the central wire when the initial ion radial displacement is as small as ~2 mm.

**Ion Axial Kinetic Energy Distribution.** Independent of the efficiency of ion transport to the ICR Penning trap, efficient ion trapping requires that ions arrive with a narrow range of axial kinetic energies. The axial acceleration/deceleration effects of the magnetic field gradient may be
minimized by small initial ion radial displacement (i.e., placing the ion source near the magnet z-axis). Specifically, our trajectory simulations predict that an EIG with a cylinder-wire potential difference as low as 10 V should transport a large fraction of ions having initial kinetic energies in the 40-50 eV range, based on readily achievable ion source placement of \( r_0 = 2 \) mm and \( z_0 = 6 \) mm, and ion initial angular divergence, \( \theta \leq 20^\circ \). Thus, the electrostatic ion guide appears to represent an attractive alternative to multipole guides and electrostatic lens systems.
REFERENCES


(22) Li, Y.; McIver, R. T. RCMS 1994, 8, 743-749.


CHAPTER IV

AN ELECTROSTATIC WIRE ION GUIDE FOR INJECTION OF EXTERNALLY-FORMED IONS: INSTRUMENTATION AND EXPERIMENT

INTRODUCTION

Chapter III presented computer simulation studies which indicate that an electrostatic wire ion guide may be an effective means for injecting externally-formed ions, having a wide range of initial energy and angular distribution, through a magnetic fringe field and into an ICR ion trap. Based on these studies, a prototype FT/ICR mass spectrometer was constructed; in this chapter the instrument design and initial data will be presented. In addition, azimuthal quadrupolar ion axialization is shown to provide dramatic improvements in overall system performance. Chapter V will discuss the use of ion axialization in collisionally induced activation studies as well as ion remeasurement experiments.

One of the key trends identified in Chapter III was the potentially wide (radial) spatial distribution of ions exiting the EIG. It is well
known that ion manipulation inside the ICR ion trap is facilitated for "spatially localized" ion packets (preferably at the trap center). Thus, while an electrostatic wire ion guide may efficiently transport externally-formed ions to an ICR mass analyzer, overall performance will nonetheless suffer if ions have large magnetron radii and random magnetron phase prior to (dipolar) excitation and detection. Clearly, a technique which could "cool" injected ions down to the trap center would be beneficial, especially when used in conjunction with an EIG.

QUADRUPOLAR EXCITATION FOR ION AXIALIZATION

As discussed in Chapters I and II, ions in an ICR trap undergo three fundamental motions (see Figure 1.6). Cyclotron motion is a circular oscillation, perpendicular to the magnetic field. Ion magnetron motion is a slow (circular) drift of the cyclotron orbit center (again perpendicular to the magnetic field) about equipotential lines created by the static trapping potential and driven by the $\mathbf{E} \times \mathbf{B}$ force. In the direction parallel to the magnetic field, ions undergo simple harmonic motion, oscillating between the two trap plates. In the absence of energy dissipation mechanisms, the three motions are stable and ions will remain trapped indefinitely. However, in real FT/ICR/MS experiments, collisional damping is always present and serves as the main mechanism for energy dissipation. Figure 4.1 shows the effect of collisional damping on the relative ion motional amplitudes. As seen in the top plot, both the ion cyclotron and axial amplitudes decrease with time in the presence of collisional damping. As a result, the ions tend to relax back to the
**Figure 4.1** Schematic diagram showing the effect of collisional damping on the relative amplitudes of ion cyclotron, magnetron, and axial motions. Note that the amplitudes of the ion cyclotron and axial motions decrease when subjected to collisional damping, while ion magnetron radius continually increases under similar conditions. Figure taken from ref. 9.

Center of the ion trap (see Figure 4.1 bottom left and right). Conversely, the ion magnetron radius increases with time during collisional damping. Figure 4.1 (bottom - middle) shows that unless ions are formed exactly on-axis (e.g. zero initial magnetron radius), they will ultimately be lost due to radial expansion of the magnetron radius.
Brown and Gabrielse first proposed "cooling" the magnetron radius by coupling it to ion cyclotron motion. The rationale behind such a proposal is clear from Figure 4.1. To a first approximation, the three ion motions are independent in a standard ICR cubic ion trap. Thus converting ion magnetron motion to cyclotron motion, in the presence of collisional damping, should provide an effective means for reducing the radial distribution of the ion packet. The axial distribution will also decrease, as the ion z-motion remains unaffected by the coupling between the radial modes. In effect, the ion packet is "squeezed" to the ICR trap center. Bollen and co-workers first discussed the use of azimuthal (x-y) quadrupolar excitation to couple the magnetron and cyclotron ion motions is a Penning trap. Subsequently, Savard and co-workers provided the first experimental demonstration of what has become known as "axialization." Application of axialization to FT/ICR mass spectrometry has progressed rapidly since Savard's initial work. Schweikhard and Marshall first used azimuthal quadrupolar excitation on a standard FT/ICR cubic ion trap to confine ions for several seconds at background pressures of ~1 x 10^-5 torr. Marshall and co-workers have demonstrated marked improvements in laser desorption and tandem FT/ICR mass spectrometry experiments by use of ion axialization. In addition, Speir et al. showed that ion axialization could be used in ion remeasurement studies to increased observed signal-to-noise ratio; they obtained remeasurement efficiencies of greater than 99%. Note that all the above experiments were based on single-frequency axialization; that is, azimuthal quadrupolar excitation is applied at the resonant frequency
which couples the magnetron and cyclotron motions of a single \( m/z \) value. While this approach is highly selective, it is somewhat restricted in terms of real analytical utility. Guan \textit{et al.} have developed extensive theoretical treatments of the axialization process,\textsuperscript{8,9} and based on this work have demonstrated that Stored Waveform Inverse Fourier Transform (SWIFT) azimuthal quadrupolar excitation may be used for axialization of ions over a given \( m/z \) range.\textsuperscript{10,11}

All the above work was done on ions formed inside an ICR ion trap; however, axialization has also been used to improve performance in external ion injection experiments. For example, Hasse \textit{et al.} employed quadrupolar excitation to accumulate a series of pulsed externally generated cluster ions injected into an ICR ion trap.\textsuperscript{12} Similarly, Bruce, \textit{et al.} used quadrupolar excitation to selectively axialize continuously injected ions generated from an electrospray source.\textsuperscript{13} Although both of these experiments increased the efficiency of ion accumulation, both were based again on resonant single-frequency excitation (i.e., single \( m/z \) ratio). In this Chapter, application of broadband ion axialization will be demonstrated for external ion sources operating in both pulsed and continuous modes.

**EXPERIMENTAL**

**External Source FT/ICR Mass Spectrometer.** Figure 4.2 shows a schematic view of our FT-ICR mass spectrometer, equipped with a 3 tesla superconducting magnet (Oxford Instruments Inc., Concord, MA). The vacuum chamber is pumped by two CTI Cryo-Torr 8 cryopumps (CTI
Figure 4.2 Schematic layout (top view) of the mechanical components of the prototype external ion source FT/ICR mass spectrometer
Cryogenics, Mansfield, MA), which maintain a base pressure of ~8.0 x 10^{-9} torr. Samples are introduced into the ionization region by means of a solids insertion probe [Model 665012, MDC Vacuum Products Corp., Hayward, CA]; as shown in Figure 4.3, either Cs+ secondary ion mass spectrometry (SIMS) or matrix-assisted laser desorption/ionization (MALDI) may be selected simply by rotating the probe by 180°. A homebuilt cesium ion gun and associated electronics provide the primary ion beam for the SIMS source; the beam duration is controlled by an Extrel FTMS 1280 data station. For SIMS experiments, the primary Cs+ ion current was 100 nA measured on the probe tip. MALDI was performed with a PTI PL 2300 nitrogen laser (Photon Technology International, Inc., London, Ontario Canada) with a maximum output energy of 1.58 mJ per pulse (800 ps). The laser beam was focused onto the probe tip by a 5.08 cm convex lens (focal length = 150 mm). Both the Cs+ primary ion beam and nitrogen laser beam are triggered from the data system which also controls d.c. trapping potential, generates excitation waveforms, and digitizes and Fourier transforms the time-domain ICR signal.

The externally-formed ions were transported to a three-section open-ended, cylindrical ICR in trap via an electrostatic ion guide (EIG);^14 consisting of a 3.81 cm O.D. x 83.8 cm long stainless steel tube with a 0.028 mm O.D. stainless steel wire strung down the center. The EIG extends from 2.54 cm from the probe tip to 25.4 cm inside the superconducting solenoid. The distance from the end of the guide to the center of the 5.08 cm diameter open-ended
Figure 4.3 Schematic diagram showing the relative placement of the probe, electrostatic wire ion guide (EIG), and open-ended cylindrical ICR ion trap. Note that ions leaving the EIG enter the ICR ion trap with relatively large initial magnetron radii (i.e., enter the trap with off-axis cyclotron orbit centers).
cylindrical cell is approximately 14 cm (see Fig. 4.3). d.c. voltages of -20 and -8 V were applied to the EIG wire and tube.

Broadband axialization and cooling of the injected ions was performed by simultaneously applying pulsed azimuthal quadrupolar excitation and introducing argon collision gas through two pulsed valves. During the interval (typically 60-100 s) between ionization and conventional dipolar excitation/detection, a TTL axialization signal is sent from the data station to a Macintosh II personal computer equipped with an NB-DIO-24 digital I/O board (National Instruments, Austin, TX). A short program in THINK C was written to trigger repeated quadrupolar excitation SWIFT pulses and collision gas pulses by alternately opening and closing the two pulsed valves. The SWIFT waveforms are generated by a home-built SWIFT module. The program allows the user to control the length of each SWIFT axialization waveform, the delay between each waveform, the number of waveforms output between each pulse of collision gas, and the total number of collision gas pulses. The SWIFT excitation waveforms are typically 30-150 ms long and the delay between each waveform is 500-1500 ms. The pulsed valves open after each 3-5 SWIFT waveforms, with a total of 10-20 gas pulses per experiment. The argon pressure in the gas reservoir (1 L volume) is set to 500-1,000 mtorr such that during the quadrupolar excitation period, the pressure inside the vacuum chamber stabilizes at 3.0-6.0 x 10^{-7} torr. Typically, the last collision gas pulse arrives after ~2/3 of quadrupolar excitation period, to allow ample time for the system to pump down to 1.0 -2.5 x 10^{-8} torr prior to dipolar excitation/detection. Note that the
Figure 4.4 FT/ICR experimental event sequence for broadband axialization of externally generated ions.
Figure 4.5 Schematic diagram showing the relay circuit placed between the cell flange and the excitation and detection amplifiers. In the relay null position (up in the above diagram) the system is in dipolar mode; a TTL signal from the data station triggers the relays (down in the above diagram) such that azimuthal quadrupolar excitation is applied at the ICR ion trap.

SWIFT quadrupolar excitation waveforms are clocked out during the entire axialization period, after which there is a 10 s interval prior to dipolar excitation/detection. The experimental event sequence is shown in Figure 4.4. An Extrel excitation amplifier (maximum amplitude $\approx 27$ V$_{p-p}$) was used for both the quadrupolar and dipolar excitation events. Figure 4.5 shows the relay circuit located between the excitation
amplifier and the cell flange, which serves to switch between quadrupolar and dipolar excitation modes, so that both excitations may be performed in a single ICR ion trap.

RESULTS AND DISCUSSION

**Broadband Axialization of Ions Generated from a Continuous External Cs⁺ SIMS Source.** Figure 4.6 (top) illustrates the current upper-mass limit for externally generated ions in our system. This spectrum was acquired by bombarding Csl, evaporated onto the probe tip from a saturated solution, with 7.5 keV Cs⁺ ions. The primary beam duration was 20 s, during which time one 5 ms pulse of argon was introduced into the FT-ICR ion trap. To facilitate ion accumulation during the beam event, the front trap plate, e.g. the trap plate closest to the electrostatic ion guide (EIG), was held at +5.0 V while the rear trap plate was set to +5.4 V. After the ionization period, both trap plates were set to +5.0 V. Next, a (dipolar) chirp pulse (25 V\textsubscript{p-p}, 58.6 ms) ejects all ions in the mass range, 100 ≤ m/z ≤ 2000. After a 30 s delay to allow for both pump-out of neutral collision gas and relaxation of the remaining ions along the axial potential well, a second (dipolar) chirp pulse (25 V\textsubscript{p-p}, 1.24 ms) excites the ICR motion of the high mass cluster ions. Note that the most abundant cluster species are readily observed, up to Cs(Csl)\textsubscript{37}⁺ (m/z = 9746).

The spectrum shown in Figure 4.6 (bottom) was obtained by use of the experimental event sequence shown in Figure 4.4. The Cs⁺ ion beam and the axialization waveform/collision gas pulses are triggered
Figure 4.6 Cluster cations generated by directing a continuous beam of Cs\(^+\) ions at solid CsI, followed by transport of the cluster ions through an electrostatic ion guide to the ICR ion trap. Top: FT/ICR mass spectrum of clusters generated from a 20 s primary Cs\(^+\) ion beam. Bottom: FT/ICR mass spectrum based on broadband axialization of ions spanning a mass range of 2000 ≤ m/z ≤ 4000, prior to conventional dipolar excitation and detection.
simultaneously. The ionization period is 5 s while the axialization waveforms are clocked out for a total of 80 s. Each (quadrupolar) SWIFT waveform consisted of 4096 data points, covering a frequency range of 11.6 kHz - 23.3 kHz (corresponding to 2000 ≤ m/z ≤ 4000), with a duration of 32.8 ms (25 V_{p-p}). A 500 ms delay between each waveform and a 5 ms pulse of argon was introduced after every third SWIFT pulse (total of 10 pulses of collision gas). After the axialization period, a 1 s delay prior to dipolar excitation was inserted to allow for ions to collisionally relax axially to the z = 0 trap midplane. Note that the resulting time-domain signal damped only slightly after ~ 650 ms, in contrast to complete damping of the signal in Fig. 4.6 (top) after only ~ 50 ms in the absence of axialization. Axialization clearly produces dramatic enhancements in both signal-to-noise ratio and mass resolving power: e.g., for the Cs(Csl)_{13}^{+} cluster (m/z = 3510), axialization increases the observed signal-to-noise ratio from 38 to 581 and increases mass resolving power from 202 to 4810 in the spectra shown in Figure 4.6.

**Broadband Axialization of Ions Generated from a Pulsed Source.**

Figure 4.7 (top) shows FT-ICR mass spectra obtained from pulsed-mode operation of the primary Cs^+ SIMS beam. For the top spectrum, a 5 ms pulse of collision gas was introduced just prior (~10 ms) to the ionization period (5 ms Cs^+ beam). During the beam event, the front trap plate is dropped to 0 V while the back plate is maintained at +5.0 V. After the ionization period, both trap plates are held at +5.0 V. A 10 s delay prior to dipolar excitation (25 V_{p-p},20 ms) allows for pump-out of
Figure 4.7 Cluster cations generated by pulsed Cs$^+$ SIMS of solid CsI. Top: For a 5 ms primary Cs$^+$ ion beam duration, no high mass clusters are observed. Bottom: Use of broadband axialization dramatically increases the signal-to-noise ratio for ions in the axialized mass range $2000 \leq m/z \leq 4000$. 

Ionization Duration = 5 ms
No Axialization

$Cs(Csl)_n^+$

Ionization Duration = 5 ms
Axialization

$2000 < m/z < 4000$

$Cs(Csl)_n^+$
the collision gas; the detection period was 273 ms. The resulting spectrum is virtually devoid of signal, with Cs(CsI)$_n^+$ clusters at m/z = 2471 and m/z = 3510 barely visible above the baseline noise. However, on application of (quadrupolar) SWIFT excitation waveforms and collision gas pulses during a 100 s interval between the ionization and dipolar excitation events (Fig. 4.7, bottom), with conditions otherwise the same is for Fig. 4.7 (top), all of the cluster cations within the axialized m/z range (i.e., from Cs(CsI)$_8^+$ at m/z = 2211 to Cs(CsI)$_{13}^+$ at m/z = 3510 ) become clearly visible. Although the ionization period decreases by a factor of 4000 in Fig. 4.7 (bottom) compared to Fig. 4.6 (top), the signal-to-noise ratio is actually higher in Fig. 4.7 (bottom) for some of the clusters and mass resolving power increases by a factor of 3-8. These results are tabulated in Table 4.1, below.

Finally, Figure 4.8 shows spectra obtained for poly(ethyleneglycol) (PEG average molecular weight = 2000) ions generated by means of external-source matrix-assisted laser desorption (MALDI). A 500 µL solution containing dihydroxybenzoic acid, fructose, and PEG-2000 in 2500/100/1 mole ratio, plus 20 µL of saturated KBr solution, was prepared; 10 µL of the stock solution, containing 5 nmole PEG-2000, was applied to the probe tip and allowed to dry completely prior to inserting into the ionization source.

In the top spectrum in Figure 4.8, the front trap plate is at 0 V and the rear plate is held at +2.5 V to decelerate injected ions during a 400 µs
TABLE 4.1 Comparison of signal-to-noise ratio (S/N) and mass resolving power (m/Δm) for cesium iodide cluster cations, Cs(Csl)$_n^+$, in the presence and absence of axialization. Note that axialization produces comparable signal-to-noise ratio, but for 4000 times fewer ions.

<table>
<thead>
<tr>
<th>n</th>
<th>S/N</th>
<th>m/Δm</th>
<th>S/N</th>
<th>m/Δm</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>N/A</td>
<td>N/A</td>
<td>27</td>
<td>2420</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>334</td>
<td>80</td>
<td>2130</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>305</td>
<td>6</td>
<td>1660</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>443</td>
<td>12</td>
<td>1690</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>378</td>
<td>16</td>
<td>1570</td>
</tr>
<tr>
<td>13</td>
<td>38</td>
<td>302</td>
<td>32</td>
<td>1580</td>
</tr>
</tbody>
</table>

interval inserted immediately following the laser shot. Prior to dipolar excitation (16 V$_{p-p}$, 8 ms), a 5 s period (trap plates at + 2.0 V) allows axial relaxation of the ion cloud. The middle spectrum in Fig. 4.8 was obtained with the same experimental event sequence and parameters as the top spectrum, with the addition of broadband SWIFT axialization waveforms and collision gas pulses during a 100 s period between the ionization/gated trapping event and axial relaxation. Each axialization waveform consisted of 8,192 data points, spanning a frequency range of 18.4 kHz – 30.7 kHz (corresponding to a mass range of 1500 ≤ m/z ≤ 2500) and was 32.8 ms (3.1 V$_{p-p}$) in duration. A 500 ms delay between each SWIFT waveform allows for damping of the cyclotron motion, and
Figure 4.8 FT/ICR mass spectra of ions produced by matrix-assisted laser desorption/ionization (MALDI) of poly(ethylene glycol) PEG-2000. Top: PEG-2000 detected without prior axialization. Middle: Mass spectrum after prior axialization of ions of $1500 < m/z < 2500$. Note the greatly increased signal-to-noise ratio. Bottom: Mass spectrum after prior axialization of ions over the mass range $1950 < m/z < 2120$. Note the mass selectivity of the axialization process, leaving only those ions within the axialization window.
one 5 ms pulse of collision gas was introduced after every third axialization waveform (total of 15 collision gas pulses). As before, broadband axialization results in increased signal-to-noise ratio (factor of ~3) and mass resolving power (~20%). Note that the same 234 ms acquisition period was used for all of the spectra in Figure 4.8, and that in each case, the spectrum represents data accumulated from 3 consecutive laser shots (from top to bottom) on the same sample spot. We estimate that the laser spot covers approximately 1/10 of the probe surface area; in addition, ion signal is readily observed for a minimum of 10 laser shots on the same sample spot. Thus, each spectra in Figure 4.8 represents roughly 50 pmoles of sample desorbed. Next, the (quadrupolar) SWIFT axialization waveforms were modified to cover a mass range of 1950 \( \leq \frac{m}{z} \leq 2120 \) (4096 data points, 32.8 ms, 3.1 V_p-p). Fig. 4.8 (bottom) illustrates the excellent mass selectivity of the broadband axialization method: the only polymer species yielding detectable signal are those within the axialization window (m/z = 1963, 2007, 2050, 2093, and 2136). Both the signal-to-noise ratio and mass resolving power are virtually unchanged in the middle and bottom spectra of Figure 4.8.

**CONCLUSION**

An electrostatic ion guide provides provides a simple and effective means for injection of externally-formed ions in FT/ICR mass spectrometry. For low wire-cylinder potential differences (10-20 V) a mass transmission range of ~10,000 is easily obtained. Note that this
likely represents a worst-case upper mass limit as the excitation and detection electronics used on the prototype spectrometer have low-frequency cut-offs near 5 kHz.

Broadband axialization of ions generated (in either continuous or pulsed mode) external to the FT-ICR ion trap dramatically increases both signal-to-noise ratio and mass resolving power. This technique should prove especially valuable for any external ion injection technique (such as an electrostatic ion guide) from which ions enter the ICR ion trap off-axis (i.e., with finite initial magnetron radii).
REFERENCES


CHAPTER V

STRUCTURAL CHARACTERIZATION OF PHOSPHOLIPIDS
BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION
FOURIER TRANSFORM ION CYCLOTRON RESONANCE
MASS SPECTROMETRY

INTRODUCTION

Phospholipids are the principal component of biological cell bilayer membranes and of various subcellular organelles. The basic phospholipid structure (see Figure 5.1) consists of a glycerol backbone joined to an alkyl chain at sn-1, through either an ester or ether linkage, and a second alkyl group esterified at sn-2. A phosphate diester at the sn-3 position joins one of four typical polar head groups, which in turn define the particular phospholipid class: phosphatidyl-choline (GPC), -ethanolamine (GPE), -serine (GPS), or -inositol (GPI). The alkyl chain length(s) and degree of unsaturation further specify a particular phospholipid within each class. (Other phospholipid classes, such as sphingomyelins and cardiolipins, are not considered here.) The biological role of phospholipids in fact extends beyond that of membrane building.
Figure 5.1 Structures of phospholipids in this study. Glycerol is esterified to a variety of fatty acids (R₁, R₂) at sn-1 and sn-2, whereas one of four polar head groups (X) is joined at sn-3 through a phosphodiester linkage. A given phospholipid is denoted by its polar head group (GPE, GPC, GPS, or GPI) and by the number of carbons and double bonds in the fatty acid side chains (e.g. 16:0, 18:0, 18:1, 18:2, or 18:3). Thus, GPE-16:0,18:1 indicates glycerophosphatidylethanolamine, with palmitic and oleic acids esterified at sn-1 and sn-2 respectively.

blocks. For example, glycerophosphatidyl-ethanolamine and -inositol¹⁻³ have been identified as key elements in anchoring various proteins to cell membranes. In addition, glycerophosphatidylserine has been shown to stimulate histamine secretion from mast cells.⁴⁻⁶ Finally, glyrophosphatidylcholines containing arachidonic acid yield, upon hydrolysis,
free arachidonic acid which is in turn converted to oxygenated species such as prostaglandins, thromboxanes, and leukotrienes.\textsuperscript{7-9}

Mass spectrometric analysis of phospholipids has previously been attempted by electron ionization (EI),\textsuperscript{10-12} field desorption,\textsuperscript{13-17} chemical ionization (CI),\textsuperscript{18-22} plasma desorption,\textsuperscript{23} thermospray/EI,\textsuperscript{24,25} laser desorption,\textsuperscript{26-28} and fast-atom bombardment (FAB).\textsuperscript{29-33} To date, FAB has been the most successful ionization method for phospholipid mass analysis; much of that work has been thoroughly reviewed by Murphy and Harrison.\textsuperscript{34} Recently, Kim and co-workers\textsuperscript{35} and Han and Gross\textsuperscript{36} have applied electrospray ionization to phospholipid mass analysis.

From its introduction in 1988,\textsuperscript{37,38} matrix-assisted laser desorption/ionization (MALDI) has provided a versatile and sensitive means for producing singly-charged gas-phase biomolecules for mass analysis.\textsuperscript{39,40} In particular, the combination of MALDI and Fourier transform ion cyclotron resonance mass spectrometry (FT/ICR/MS) offers ultrahigh mass resolution, unparalleled mass accuracy, and MS\textsuperscript{n} capabilities. Recent advances in FT/ICR instrumentation, most notably ion axialization,\textsuperscript{41} allow for all of these advantages to be realized simultaneously. For example, Huang \textit{et al.}\textsuperscript{42} have recently shown that simultaneous application of dipolar and azimuthal quadrupolar excitation in the source side ion trap of a dual trap mass analyzer allows for MS\textsuperscript{n} up to n = 4; at each stage, product ions formed during collisional activation may be refocused along the central axis of the electrostatic trapping potential and passed through a conductance limit.
to a second low-pressure analyzer ion trap for analysis at ultrahigh mass resolving power. To date the most successful mass analyses at low biomolecule sample quantities (e.g., ≤ 10^{-15} moles) have employed other types of mass analyzers.^{43-45} However, Marshall et al. have shown that as few as ~200 singly-charged ions may be detected in a 3 T FT/ICR mass spectrometer,^{46} indicating that attomole detection limits are attainable.

Smith and co-workers have demonstrated femtomole detection limits for electrospray ionization FT/ICR/MS,^{47} while McLafferty et al. have reached the attomole level.^{48} In each case, larger sample quantities are loaded; the sample consumed (and thus the detection limit) was estimated based on the flow rate and ionization/injection period. Recently, McIver and Li reported femtomole detection limits for several polypeptides obtained from an external MALDI source FT/ICR mass spectrometer.^{49} Here, several femtomoles of sample were loaded onto a probe, and time-domain signals, corresponding to several laser shots, were co-added.

In this Chapter, the as yet unrecognized potential of MALDI FT/ICR/MS for mass analysis of phospholipids is demonstrated. Tandem mass spectrometry, based on sustained off-resonance (dipolar) irradiation (SORI)^{50} of both positive and negative molecular ions, generates structurally informative fragment ions, which may be axialized by broadband azimuthal quadrupolar irradiation in the presence of argon collision gas.^{42,51} The fragment ions may then be transferred to a second low-pressure analyzer ion trap for high-resolution mass analysis.
Preliminary results for a variety of matrices are discussed. In addition, a sample cryo-cooling technique is found to minimize matrix adduct formation. Finally, modifications of a commercial sample probe are described which allow accurate and reproducible attomole-level sample deposition. Ion remeasurement techniques are then used to improve observed signal-to-noise ratios for these low sample quantities.

**EXPERIMENTAL**

**Instrumentation.** All FT-ICR mass spectra were acquired at 3 tesla with an Extrel FTMS-2000 FT-ICR mass spectrometer (Extrel-Waters, Madison, WI), equipped with a dual ion trap mass analyzer, automatic insertion probe, and Odyssey data station. A schematic diagram of this instrument is shown in Figure 5.2. A UV laser beam from either a frequency-tripled 355 nm Nd:YAG (Surelite II, Continuum, Inc, Santa Clara, CA) or a 337 nm nitrogen laser (VSL-337, Laser Science, Inc., Newton, MA) was directed through a window in the analyzer side of the main vacuum chamber. The beam was focused by a telescope lens assembly (Nd:YAG laser) or a 1 m focal length lens (nitrogen laser) through the conductance limit separating the source and analyzer traps and onto the sample probe. When inserted, the sample plate at the end of the probe rests ~0.5 cm from the source-side trap plate. The relay for switching between dipolar and quadrupolar excitation was similar to that described in Chapter 4 (see Figure 4.5). Due to the high capacitive load presented by this circuit, a low-power (~27 V_{p-p}) Nicolet excitation
**Figure 5.2** Schematic layout of the major components of an Extrel FTMS-2000 FT/ICR mass spectrometer used for MALDI.
amplifier was used in place of the high-power amplifier supplied with the FTMS-2000 mass spectrometer.

To reduce the amount of sample deposited on the stainless steel probe plate, a modified plate was constructed with a series of small indentations such that attomoles of analyte could be placed on a spot size of \( \leq 1 \text{ mm}^2 \) (see Figure 5.3). First, the stainless steel probe plate was mechanically polished to a mirror shine. The surface was ground with a series of silicon carbide abrasive papers from 600-1200 grit at medium speed (300 rpm) on a Rotopol-2 (Struers, Waterlake, OH) disk grinder followed by a low speed (150 rpm) polishing disk with a broadcloth covering. Finish polishing was performed by applying a polishing abrasive (6-1 \( \mu \text{m} \) diamond suspension) on a Buehler's Vibromet (Lakebluff, IL). A Vicker's microhardness tester (series 200 Wilson-Tukon) confirmed that the indentations on the polished plate were aligned with the laser beam.

To mark the sample plate at the laser focal point, a thin film of 4-nitroanaline was applied to the probe plate, and several laser shots then removed the organic layer at different probe angular positions; these laser spots were identified and marked under the diamond-tipped microhardness tester with a 1000 kg load. The probe plate plate with indentation marks (~100 \( \mu \text{m} \) deep \( \times \) ~5x10^{-3} \text{ mm}^2 \) area) are shown schematically in Figure 5.3; typically four indentation spots were required to completely mark the laser spot (laser spot size ~200 \( \mu \text{m} \) x 200 \( \mu \text{m} \)). Two pins locked the angular position of the probe plate onto
Figure 5.3 Schematic diagram showing a modified probe tip and sample plate. In addition to the standard screw hole for fastening the sample plate to the probe tip, two pins were press-fit into the probe tip, with corresponding holes in the sample plate; these in turn locked the angular position of the sample plate. The indentation spots were located at rotational positions (5 degrees apart - accessed via the Odyssey data station) around the sample plate.
Extrel's standard solids autoprobe. Results were highly reproducible from one marked spot to another, confirming the accuracy of the laser alignment and spot placement (see Figure 5.14 and associated text).

**Sample Preparation.** For structural characterization of phospholipids (see Figures 5.6 - 5.13), appropriate aliquots of stock solutions, 1 mM phospholipid (Sigma Chemical Co., St Louis, MO) in either chloroform or 50/50 (v/v) chloroform/methanol and 0.5-1 M matrix in methanol acidified with 0.1% (v/v) trifluoroacetic acid (TFA), were combined to make sample solutions, each containing (unless otherwise noted) matrix:analyte at 5,000:1 mole ratio. 20 μL of sample solution, containing ~3.5 nmol of sample, was then applied over the entire surface of a stainless steel sample plate (see Figure 5.3) and allowed to dry completely before insertion into the mass spectrometer. Based on the relative size of the sample probe and laser spot, and assuming even sample distribution on the probe surface, it is estimated that ~1.5 pmols of lipid are desorbed per laser shot.

For detection limit studies (see Figure 5.14) 1 μL of 1 mM lipid solution was diluted to a total volume of 1 mL with 1 M 2,5-dihydroxybenzoic acid (DHB, acidified with 0.1% TFA). One μL of this solution was again diluted to 1 mL with 1 M acidified DHB to give a final matrix-to-analyte ratio of 1x10⁹:1, and a lipid concentration of 1 fmol/μL. 0.4 μL (containing ~40 attomoles of phospholipid) was applied by microsyringe onto an indentation in the probe plate (see Figure 5.3). For the spectra shown in Figure 5.14, six sample deposits, each ~1 mm
in diameter and centered on a spot radius of ~7 mm, were placed at well-spaced positions on the probe plate.

**FT/ICR Experimental Event Sequence.** The experimental sequence for structural characterization of phospholipids is shown in Figure 5.4. Ion trapping was facilitated by biasing the conductance limit plate to +/- 9 V (positive ion/negative ion), the source trap plate to ground potential, and the sample probe to +/- 2 V, for 50-75 μs during the laser fire event (laser pulse width was ~7 ns (Nd:YAG) or ~3 ns (nitrogen)). After ionization, the source trap and conductance limit plates were set to +/- 2 V, and the ions were allowed to relax to the central trap axis for 2-4 s. For broadband excitation and detection [e.g. no isolation event - Figs. 5.6, 5.8, and 5.13 (top)] standard chirp excitation (1-500 kHz at a sweep rate of 100 Hz/μs at ~27 Vp-p amplitude) and dipolar detection (256K time-domain data acquired with a 1 MHz Nyquist bandwidth) were then carried out in the source-side ion trap. The time-domain signal was Fourier transformed without zero-filling or apodization, and displayed in magnitude-mode.

For collisional activation (Figs. 5.7, 5.9 - 5.13 (bottom)), the molecular ion of interest was first isolated by use of SWIFT (dipolar) radial ejection of undesired ions,\(^{52,53}\) followed by sustained off-resonance (dipolar, single-frequency) irradiation (~ 9.5 Vp-p for 300-500 ms).\(^{50}\) 100 ms later, a series of broadband SWIFT azimuthal quadrupolar excitation waveforms (spaced 250 ms apart) served to axialize the product ions.\(^{41,51}\) Pressure in the source-side ion trap was
Figure 5.4 Experimental sequence used for collisional activation of phospholipids. After formation and isolation of phospholipid molecular ions in the source-side ion trap, sustained off-resonance irradiation (SORI) is used for collisional activation (in the presence of $\sim 1 \times 10^{-7}$ torr argon). Product ions are then re-focused to the central ($z$-) axis by use of azimuthal quadrupolar excitation. Subsequent transfer of ions to the analyzer-side ion trap allows for high-resolution mass analysis.
held constant at \( \sim 1.0 \times 10^{-7} \) torr during collisionally activated dissociation and axialization. Following transfer of ions through a conductance limit to the analyzer ion trap, dipolar frequency-sweep excitation (1-500 kHz at a sweep rate of 1000 Hz/\( \mu \)s at \( \sim 78 \) V\(_{p-p}\) amplitude) and detection (1 M time-domain data acquired with a 1 MHz Nyquist bandwidth) were performed. The time-domain signal was Fourier transformed without zero-filling or apodization, and displayed in magnitude-mode.

The experimental sequence for detection limit determination is shown in Figure 5.5. The single (top) and multiple (bottom) segments of the event sequence are shown separately. As before, ion trapping was facilitated by biasing the conductance limit plate to +/- 9 V, the source trap plate to ground potential, and the sample probe to +/- 2 V, for 50-75 \( \mu \)s during the laser fire event. The trapped ions were allowed to relax, in the presence of \( \sim 4 \times 10^{-8} \) torr argon, to the central trap axis for \( \sim 4 \) s. Unwanted background ions (such as matrix-related species) were ejected from the ion trap by use of SWIFT dipolar excitation. Coherent ICR motion was excited by frequency-sweep dipolar excitation (\( \sim 78 \) V\(_{p-p}\), 1-500 kHz at a sweep rate of 1000 Hz/\( \mu \)s). Fourier transformation of the resulting time-domain signal (32 K time-domain data points over a 1 MHz Nyquist bandwidth), without zero-filling or apodization, yielded the magnitude-mode mass spectrum corresponding to a single data acquisition. Following the initial detection event, damping of the ICR motion was achieved by collisional damping only, or by combining collisional damping and axialization. For example, after every few
**Single Acquisition**

Ionization | Isolation (Dipolar) | Excitation (Dipolar) | Detection | Collisional Damping

**Multiple Acquisitions**

Excitation (Dipolar) | Detection | Collisional Damping | Product Ion Axialization (Quadrupolar)

**Figure 5.5** Experimental sequence used in ion remeasurement studies. The top segment corresponds to a single time-domain acquisition, after isolation of the mass range of interest and dipolar excitation of coherent cyclotron motion. The bottom segment shows the steps required for ion remeasurement. Relaxation of ion cyclotron motion was accomplished through either collisional damping alone (i.e. $y = 0, x = z$) or with a combination of collisional damping and azimuthal quadrupolar excitation (i.e. $y > 0$). Note that axialization may be applied after each detection period (i.e. $x = y$), or after several detection/collisional damping cycles (i.e. $x > y$).

Remeasurements (denoted by the first brackets, subscripted by $x$ in Figure 5.5), azimuthal quadrupolar excitation, consisting of a series of SWIFT waveforms covering the mass range of interest in the presence of collisional cooling (second brackets, subscripted by $y$ in Figure 5.5)
refocused the ions close to the center of the ICR trap. As with the CAD experiments a relay, described in Chapter 4 (see Figure 4.5), was used for switching between dipolar and quadrupolar excitation. The co-added time-domain signals from multiple detections were also Fourier transformed without zero-filling or apodization.

RESULTS AND DISCUSSION

Matrix Selection. Initially, a frequency-tripled Nd:YAG laser (355 nm) with 2,5-dihydroxybenzoic acid (DHB) as the MALDI matrix was used. The optimum matrix-to-analyte ratio was found to be ~5000:1 for a variety of phospholipids. Abundant positive and negative ions were observed, although matrix adduct peaks frequently appeared in the negative-ion mass spectra (see below). For the same matrix-to-analyte ratio, trans-4-hydroxy-3-methoxy cinnamic acid (TCA) gave abundant negative ions, but little or no positive ion signal. Among several other matrices (5000:1 matrix-to-analyte ratio), including 4-hydroxy-3-methoxy benzoic acid (HMBA), 3,4-dihydroxy cinnamic acid (DCA), 3-hydroxy piccolinic acid (HPA), 4-nitroanaline (NA), 3,5-dimethoxy-4-hydroxy cinnamic acid (DHCA), and alpha-cyano-4-hydroxy cinnamic acid (CHCA), only DCA and NA yielded phospholipid ions, albeit much smaller than for either DHB or TCA. It is possible that optimization of matrix-to-analyte ratio could improve the ion yield for those matrices.

GPC, GPS, GPE, GPI Positive-ion Mass Spectra. Figure 5.6 shows results obtained with a nitrogen laser; those mass spectra were acquired with a DHB matrix, in a 5000:1 molar ratio with respect to the lipid
Figure 5.6 Positive-ion FT/ICR magnitude-mode mass spectra for, proceeding from top to bottom, GPC-16:0,18:1, GPS-di16:0, GPE-di18:1, and GPI-16:0,18:2. In each case, the molecular ion or its sodium adduct is observed, as well as ions indicative of the polar head group (choline, serine, ethanolamine, and inositol, respectively).
analyte. Figure 5.6 (top) shows a positive-ion FT-ICR mass spectrum for GPC-16:0,18:1. Both protonated ($m/z = 760$) and sodiated ($m/z = 782$) molecular ions are observed, as well as sodiated fragments corresponding to loss of trimethylamine ($m/z = 722$) and loss of polar head group ($m/z = 599$). In addition, fragments corresponding to the polar head group ($m/z = 184$) and loss of palmitic acid ($m/z = 504$) are also observed. The next spectrum in Figure 5.6 shows positive ions from GPS-di16:0, for which the most abundant fragment is a sodium adduct, corresponding to loss of the polar head group ($m/z = 573$). Sodiated molecular ion is also present ($m/z = 758$), as well as sodium adducts of dipalmitoylglycerophosphatic acid ($m/z = 671, 693$), formed by loss of serine.

Proceeding downward to the third spectrum in Figure 5.6, the most abundant positive ion observed for GPE-di18:1 is the loss of the polar head group ($m/z = 603$), along with the corresponding sodium adduct ($m/z = 625$). The sodiated molecular ion ($m/z = 766$) is also observed. Finally, the lowermost spectrum in Figure 5.6 is from GPI-18:2,16:0, for which the most abundant fragment is the sodiated polar head group ($m/z = 283$), along with the sodiated molecular ion ($m/z = 857$) and an ion formed by neutral loss of the polar head group ($m/z = 597$). Note that for positive ions from each phospholipid class, there is an abundant ion serving to identify the polar head group: phosphocholine ($m/z = 184$) for GPC, sodiated phosphoinositol ($m/z = 283$) for GPI, and ions corresponding to the neutral loss of the polar head group for GPS and GPE.
**Collisional Activation of GPC Positive Ions.** Although the ions observed in Figure 5.6 allow in principle for assignment of phospholipid class, unambiguous identification of a specific lipid present in an unknown mixture requires tandem mass spectrometric analysis. For example, Figure 5.7 shows MS/MS of GPC-16:0,18:1 positive ions. First, the protonated molecular ion \((m/z = 760)\) is isolated by SWIFT (dipolar) ejection of ions of other \(m/z\) values. Next, a series of azimuthal quadrupolar SWIFT (broadband) waveforms axializes the remaining ions. After transfer to the analyzer-side ion trap, standard dipolar excitation and detection yields the spectrum shown in Figure 5.7 (top). Clearly, isolation of protonated molecular ions, \((M+H)^+\), is quite efficient, and high mass resolving power is easily obtained after axialization and transfer to the analyzer ion trap. To collisionally activate the molecular ion, sustained off-resonance irradiation is applied between the isolation and axialization events. The average (lab frame) ion kinetic energy, calculated by the method of Huang et al.,\(^{42}\) attained during off-resonance irradiation is \(-7.5\) eV. Figure 5.7 (bottom) shows that CAD yields abundant ions of \(m/z = 184\), corresponding to the phosphocholine head group. CAD efficiency is high, as is the collection efficiency due to axialization of product ions after dissociation.

**Collisional Activation of GPS Negative Ions: Matrix Adducts.**

Although each phospholipid class yielded negative ions in abundance, matrix adduction proved problematic, especially for DHB matrix (see the GPE-16:0,18:1 negative ion FT-ICR mass spectra in Figure 5.8). Each spectra was acquired from the source-side ion trap
Figure 5.7 Collisional dissociation of GPC-16:0,18:1 positive ions. Following isolation of protonated molecular ions, \( m/z = 760 \) (top), collisional activation yields abundant phosphocholine ions, \( m/z = 184 \), which may be efficiently axialized and transferred to the analyzer ion trap for high-resolution mass analysis (bottom). Identification of the choline polar head group defines the phospholipid class (GPC).
Figure 5.8 Matrix (DHB) adduct anions desorbed/ionized from a room-temperature solids probe. Deprotonated GPE-16:0,18:1 molecular ions \( m/z = 716 \) are first isolated in the source-side ion trap. As the post-isolation delay increases from 0.1 - 30 s (top to bottom), the matrix adduct \( m/z = 870 \) becomes the most abundant species.

After isolation of the deprotonated molecular ion \( m/z = 716 \) and a variable time period prior to standard (dipolar) excitation and detection. No matrix adduction was observed when excitation/detection followed promptly (100 ms) after isolation of the deprotonated molecular ions (Fig. 5.8, top). However, after a 5 s post-isolation delay, the matrix
adduct \((m/z = 870)\) was nearly as abundant as the molecular ion (Fig. 5.8, middle), and after a 30 s delay, the matrix adduct was the only ion present (Fig. 5.8, bottom).

Matrix adduction is especially problematic for collisional activation. Although off-resonance irradiation is applied promptly (i.e., within 500 ms) after deprotonated molecular ion isolation, so that matrix adducts are not formed initially, the post-dissociation product axialization process can require from 5-30 s to achieve efficient focusing of ions along the central z-axis of the ion trap. As a result, matrix adducts can dominate the mass spectrum. For example, Figure 5.9 shows product ions observed after CAD of GPS-di16:0. The experimental event sequence here consists of ionization, 4 s ion cooling period, isolation of deprotonated molecular ion \((m/z = 734, \text{not shown})\), 100 ms delay, collisional activation (SORI at average parent ion kinetic energy of \(~6.2\) eV), 100 ms delay, product ion broadband axialization, and ion transfer to the analyzer trap for standard (broadband) dipolar excitation and detection. Matrix \((m/z=153, 307)\) and matrix adduct species \((m/z=546, 563)\) clearly dominate the mass spectrum and complicate its interpretation.

**Collisional Activation of GPS Negative Ions: Sample Cryo-cooling.**

The automatic insertion solids probe of the FTMS-2000 instrument is equipped with a small port, through which cryogenic gas may be fed to the probe tip for effective sample cooling under vacuum. Figure 5.10 shows collisional activation of GPS-di16:0 while the sample probe is kept
Figure 5.9 Collisional activation of GPS-di16:0 negative ions desorbed/ionized from a room-temperature solids probe. During subsequent axialization (5-30 s following collisional activation), product anions form adducts with neutral DHB molecules subliming from the sample probe. In addition, matrix neutrals may lose a proton to palmitate anions, resulting in abundant matrix anion (m/z = 153) and matrix dimer (m/z = 307).
GPS = 735 Da

Source Isolation
Broadband Axialization
Analyzer Detection
Cryo-cooled Probe

Source CAD (SORI, 4.9 eV)
Broadband Axialization
Analyzer Detection
Cryo-cooled Probe

Figure 5.10 Collisional activation of GPS-d16:0 negative ions desorbed/ionized from a liquid nitrogen-cooled probe. After isolation of deprotonated molecular ions, \( m/z = 734 \) (top), collisional activation yields abundant fragment ions, corresponding to palmitate anion \( (m/z = 255) \), loss of palmitic acid and serine \( (m/z = 392) \), and loss of the ketene analog of palmitic acid and serine \( (m/z = 409) \). Products are subsequently axialized and transferred to the analyzer ion trap for high-resolution mass analysis (bottom), in which mono-dehydrated glycerophosphatic acid is easily resolved from matrix anion (inset). Note that cryo-cooling the probe eliminates matrix adduct formation.
cold with liquid nitrogen. The top spectrum shows the isolated GPS deprotonated molecular ions \((m/z = 734)\) after broadband axialization and transfer to the analyzer ion trap. Collisional activation of this ion (SORI at an average parent ion kinetic energy of \(-4.9 \text{ eV}\)) followed by broadband axialization and transfer of product ions to the analyzer trap, yields the bottom spectrum; note the absence of matrix adduct ions. Now, abundant fragment ions corresponding to palmitate anion \((m/z = 255)\), neutral loss of palmitic acid and serine \((m/z = 391)\), and neutral loss of the ketene analog of palmitic acid and serine \((m/z = 409)\) are readily observed.

The inset in Figure 5.10 (bottom) provides a mass-scale expansion of the region near \(m/z = 153\). The lower-mass ion \((m/z = 152.996)\), a mono-dehydrated glycerophosphatic acid resulting from loss of serine, palmitic acid, and the corresponding ketene of palmitic acid, differs by only 0.023 u from the matrix anion \((m/z = 153.019)\); nevertheless, these species are easily resolved (mass resolving power, \(m/\Delta m_{50\%} = 228,000\) for the matrix anion, where \(\Delta m_{50\%}\) is the full peak width at half-maximum peak height).

At room temperature (Figure 5.9), the matrix dimer is the most abundant species, even though initial isolation of the GPS molecular ion effectively removes matrix anions from the ion trap. Figure 5.C also shows no signal for either palmitate anion \((m/z = 255)\) or its matrix adduct for the probe at room temperature. Since palmitate anions are clearly present in abundance from the cold probe, it appears that DHB
neutrals subliming from the room-temperature probe transfer a proton to palmitate anions, thus forming abundant matrix anions at the expense of the palmitate anions. It was concluded that cryo-cooling the probe tip greatly reduced matrix sublimation. In support of that view, note that the neutral-loss species involving loss of palmitate (m/z 392, 409 in Figure 5.10 for the cryo-cooled sample) appear with added matrix in the room-temperature sample (m/z 546, 563 in Figure 5.9). Finally, Nelson et al.\textsuperscript{54} have previously obtained laser desorption/ionization time-of-flight mass spectra from frozen aqueous solutions of DNA and protein, but their experiments were performed at a laser wavelength (~580 nm) designed to match the minimum absorbance of the (water) matrix, whereas the present experiments are conducted at a laser wavelength designed to maximize matrix absorption.

**Collisional Activation of GPC Negative Ions.** Abundant negative ions were also observed with trans-4-hydroxy-3-methoxy cinnamic acid (TCA) as the matrix. Matrix adduction was not as severe with TCA as for DHB, and cryo-cooling the sample probe was thus not necessary. Figure 5.11 shows collisional activation for GPC-d16:0 formed from TCA matrix. Because of the trimethylamine moiety, deprotonated molecular ions are not observed in negative-ion GPC FAB mass spectra; rather, quasi-molecular species corresponding to loss of a methyl group [M-15], loss of quaternary amine [M-60], and loss of choline [M-86] are typically seen.\textsuperscript{31,55} At a matrix-to-analyte ratio of 5000:1, we obtained roughly equal abundances of the [M-15] and [M-60] anions (not shown). As reported for MALDI analysis of other biomolecules,\textsuperscript{56} the degree of
Figure 5.11 Collisional activation of GPC-di16:0 negative ions. At a matrix-to-analyte (TCA/GPC) mole ratio of 500:1, abundant quasi-molecular ions, corresponding to loss of the trimethylamine moiety ($m/z = 673$) are easily isolated in the source-side ion trap, axialized, and transferred to the analyzer trap (top). Collisional activation in the source trap yields abundant palmitate anions ($m/z = 255$) which may subsequently be axialized and transferred to the analyzer ion trap for high-resolution mass analysis (bottom).
fragmentation may be adjusted by changing the matrix-to-analyte ratio. For a matrix-to-analyte ratio of 500:1, loss of trimethylamine became the most abundant species in the negative-ion FT-ICR mass spectrum of GPC-di16:0. Figure 5.11 (top) shows the quasi-molecular anion (m/z = 673) after isolation (source-side ion trap), axialization, and transfer to the analyzer ion trap. Upon collisional activation (SORI at average parent ion kinetic energy of ~5.4 eV), the quasi-molecular ion dissociates, yielding abundant palmitate anions (m/z = 255). The bottom spectrum in Figure 5.11 shows the dissociation product ions after axialization and transfer to the analyzer side of the dual ion trap. Again, both the dissociation and axialization processes are extremely efficient, yielding product ions in high abundance with high mass resolving power.

**Collisional Activation of GPE Negative Ions.** Deprotonation of the terminal amine results in abundant [M–H]– anions for glycerophosphatidylethanolamines. Figure 5.12 shows results obtained from collisional activation of GPE-16:0,18:1 [M–H]– anions, with TCA matrix at a 5000:1 molar ratio relative to the analyte. The top spectrum shows GPE deprotonated molecular anion (m/z = 716) after isolation in the source ion trap, broadband axialization, and transfer to the analyzer trap. Collisional activation of the molecular ion was then performed in the source side trap followed by either source side (dipolar) excitation and detection (Figure 5.12, middle), or broadband axialization and transfer to the analyzer trap for (dipolar) excitation and detection (Figure 5.12, bottom). In each case, collisional activation was achieved
Figure 5.12 Collisional activation of GPE-16:0,18:1 negative ions. The top spectrum shows deprotonated molecular ions detected in the analyzer ion trap, after isolation and axialization in the source trap. Following collisional activation, product ions corresponding to the palmitic ($m/z = 255$) and oleic ($m/z = 281$) acid side chains may be detected in the source-side ion trap (middle) or axialized and transferred to the analyzer ion trap (bottom). In either case, the substituent at sn-2 has approximately three times the abundance as that at sn-1. Note the increased signal-to-noise ratio and mass resolving power (compare middle and bottom spectra) for product anions axialized following CAD and transferred to the low-pressure analyzer trap.
by off-resonance dipolar excitation at \( \sim 1 \text{ kHz} \) below the reduced cyclotron frequency, \( \omega_+ \), whereas other parameters (SORI excitation voltage amplitude and duration) were optimized for maximum product ion signal magnitude. Collisional activation results in formation of palmitate (\( m/z = 255 \)) and oleate (\( m/z = 281 \)) anions. Comparison of middle and bottom mass spectra in Figure 5.12 clearly establishes the much greater observed ion abundance and higher mass resolving power resulting from product ion axialization and ion transfer to the low-pressure analyzer trap following collisional activation. For example, the signal-to-noise ratio for the oleate anion (\( m/z = 281 \)) increases by a factor of at least 5, whereas the mass resolving power increases 15-fold (\( m/\Delta m_{50\%} = 10,000 \) for source trap detection vs. \( m/\Delta m_{50\%} = 155,000 \) for analyzer trap detection).

Note that for either source or analyzer ion trap detection, the observed ratio of carboxylate anions from the sn-2 and sn-1 positions is approximately 3:1; in good agreement with prior reports with other ionization methods.\(^{31,58-60}\) A 6-membered intermediate is proposed to involve the fatty acid acyl group, the corresponding carbon (at either sn-1 or sn-2), and proton located at the \( \gamma \) position (relative to the acyl group). Formation of a cyclic intermediate and subsequent loss of the carboxylate at sn-2 is facilitated by the presence of four \( \gamma \)-protons (at sn-1 and sn-3 respectively). However, in the case of substituents at sn-1, only the \( \gamma \)-proton at sn-2 may participate.
Collisional Activation of GPI Negative Ions: Mixture Analysis. An obvious long-term goal of the present line of experiments will be to analyze biological mixtures of phospholipids, as from biological cell extracts. Toward that end, a GPI mixture, consisting of phospholipids of a common class (i.e., the same inositol polar head group) but with various possible fatty acid side chains was analyzed. The sample is a soybean extract (Sigma Chemical Co., 98% pure) reported to contain mainly palmitic (16:0) and linoleic (18:2) acids. TCA was used as the matrix, at a 5000:1 molar ratio relative to GPI. Figure 5.13 (top) shows a standard broadband FT-ICR mass spectrum obtained in the source-side ion trap. Of the various observed possible GPI molecular ions, varying widely in abundance, GPI-16:0,18:2 was assigned based on CAD (see below), whereas other molecular ions were assigned based on prior reports.\textsuperscript{32,61} The inset is a mass-scale expansion of the molecular ion region (830 \textless m/z \textless 870). The most abundant ions (m/z = 833) were isolated in the source ion trap (not shown) and subjected to collisional activation (SORI at average parent ion kinetic energy of ~5.5 eV). Product ions were axialized following CAD and transferred to the analyzer ion trap where standard (dipolar) excitation and detection yielded the spectrum shown in Figure 5.13 (bottom). The many structurally informative anions include: palmitate (m/z = 255) and linoleate (m/z = 279) anions, loss of both acyl groups from the molecular ion (m/z = 297), loss of water from the polar head group (m/z = 241, 223), loss of both linoleic acid and inositol moieties (m/z = 553, 391) from the molecular ion, and mono-dehydrated glycerophosphatic
The most abundant GPI molecular anion ($m/z = 833$) in a mixture of soybean GPI (top) is isolated (not shown) and subjected to collisional activation. Product ions are then axialized and transferred to the low-pressure analyzer ion trap for high-resolution (average $m/\Delta m_{50\%} \approx 145,000$) mass analysis (bottom). Fragment ions serve to identify the polar head group ($m/z = 241, 223$) as well as palmitate ($m/z = 255$) and linoleate ($m/z = 279$) anions. Loss of linoleic acid ($m/z = 553$), loss of linoleic acid and inositol moieties ($m/z = 391$), loss of both acyl substituents ($m/z = 297$), and mono-dehydrated glycerophosphatic anions ($m/z = 153$) are also observed.
acid ($m/z = 153$). Again, axialization of product anions after
dissociation, and subsequent transfer to the analyzer ion trap provides
high mass resolving power (average $m/Δm_{50\%} ≈ 145,000$ for GPI product
ions). From CAD analysis, the most abundant species in the GPI
mixture was identified as GPI-16:0,18:2. In this case, the relative
positions (sn-1 or sn-2) for the palmitic and linoleic acyl groups cannot
be determined from the present data; the structure shown in Figure 5.13
(bottom) is based on prior work by Myher and Kuksis.61

**Detection Limit for GPC: Positive Ions.** Figure 5.14 shows the current
detection limit for GPC-d16:0. As discussed previously, six (6) sample
depositions of 40 attomoles each were placed, well separated from one
another, on the modified probe plate. In the single-measurement
spectrum shown in Figure 5.14 (top) the only recognizable species is a
matrix cluster ion at $m/z 699$. After a 3.6 s collision period to allow for
damping of the cyclotron motion, the same ion packet was again excited
by frequency-sweep excitation. Following dipolar detection, a 4.5 s
collision period allowed for damping of the cyclotron motion. The
sequence of chirp excitation, 1 ms delay, dipolar detection, and 4.5 s
cyclotron relaxation was repeated a total of 45 times (e.g., $x = 45$, $y = 0$, $z
= 1$ in Figure 5.5, bottom); co-addition of the time-domain data yielded
the bottom spectrum in Figure 5.14. After multiple remeasurement,
several peaks of signal-to-noise ratio $≥ 3:1$ are observed: protonated
molecular ion ($m/z 734$), its sodium adduct ($m/z 756$), and the sodium
adduct of the species corresponding to loss of trimethylamine ($m/z 697$).
Figure 5.14 MALDI FT/ICR single laser shot mass spectra obtained from 40 attomoles of GPC-di16:0 applied to the sample plate. Top: Single time-domain data acquisition. Bottom: Sum of time-domain data from 45 co-added remeasurements of the same ion packet. After remeasurement, ion signals corresponding to the quasi-molecular ion (m/z = 734), its sodium adduct (m/z = 756), and loss of trimethylamine (sodiated, m/z = 697) are readily observed at signal-to-noise ratios ≥ 3:1.
It is worth noting that the spectra are highly reproducible—spectra comparable to those shown in Figure 5.14 were obtained for four of the six sample spots applied to the probe plate. At present 8 attomole of substance P (not shown) represents the best detection limit obtained using this method. In this case 40 remeasurements were co-added, with axialization applied between each detection period (corresponding to \( x = y = 1, z = 40 \) in Figure 5.5, bottom).

**CONCLUSION**

Matrix-assisted laser desorption/ionization produces abundant molecular or quasi-molecular phospholipid ions, which are readily identified by Fourier transform ion cyclotron resonance mass spectrometry. The 2,5-dihydroxybenzoic acid (DHB) matrix yields both positive and negative ions, whereas trans-4-hydroxy-3-methoxy cinnamic acid (TCA) provided mainly negative ions. Cryo-cooling the sample probe effectively eliminated matrix adduction for negative ions produced from a DHB matrix. For either matrix, abundant ion signal, suitable for structural analysis, was observed for phospholipids in the low picomole range.

Collisional activation of quasi-molecular ions yielded product ions from which the polar head group and fatty acid side chains could be identified. Azimuthal quadrupolar excitation following dissociation efficiently focuses product ions close to the central axis; subsequent transfer to the low-pressure analyzer ion trap provides high-resolution \((m/\Delta m_{50\%} > 100,000)\) mass analysis.
Compared to prior FAB ionization with triple-quadrupole MS/MS, phospholipid analysis with MALDI FT/ICR/MS/MS provides much higher mass resolving power, particularly in the second MS stage, thus facilitating identification of acyl groups differing by only two mass units (e.g. one double bond). Furthermore, MALDI minimizes extraneous matrix peaks, clusters and adducts. Finally, the combination of attomole-quantity sample deposition and ion remeasurement provides detection limits superior to those obtained by FAB Quadrupole MS and comparable to those obtained by ESI-FT/ICR/MS.
REFERENCES


(49) Li, Y.; McIver, R. T. *RCMS* **1994**, 8, 743-749.


CHAPTER VI

FUTURE/CURRENT EXPERIMENTS IN FT/ICR MASS SPECTROMETRY

A HIGH PERFORMANCE ELECTROSPRAY IONIZATION FT/ICR MASS SPECTROMETER

As discussed briefly in Chapter I, electrospray ionization (ESI) is the most exciting ionization technique for mass spectral analysis of high mass biomolecules. By producing multiply-charged ion, ESI effectively brings high mass species down to an \( m/z \) range amenable to most mass analyzers. In the case of FT/ICR/MS, lower \( m/z \) values translate into higher resolution and higher mass accuracy mass spectra. Moreover, the instrumentation required for external injection of electrospay ions is continually advancing. For example, McLafferty et al., have demonstrated mass resolving power (\( m/\Delta m \)) in excess of 500,000 for carbonic anhydrase (29,025 Da), with sub-ppm mass errors. In MS/MS experiments, mass resolving power approaching \( 1 \times 10^6 \) and mass accuracy better than 1 ppm were obtained for product ions formed by collisional activation of ubiquitin (8.6 kDa).
Here, the first high performance electrospray FT/ICR mass spectrometer installed in the ICR User Facility at the National High Magnetic Field Laboratory will be described. The system, built around a 6 T, 6" horizontal bore superconducting magnetic, is shown in Figure 6.1. A modified Vestec (two-stage) electrospray source is mounted at stage 1, which is evacuated by a 1500 L/s turbo-drag hybrid pump. Ions are transported through stages 2 and 3, each pumped by 1500 L/s cryo-pumps, by a two-piece octupole ion guide, pictured in Figure 6.2. The octupole consists of eight titanium rods (~3.2 mm dia.) arranged with an inscribed circle of ~5 mm. Two titanium supports sandwich a Macor ceramic spacer and serve to hold each set of four octupole rods. The ceramic rings, spaced approximately every 20 cm, in turn support the octupole ion guide inside a perforated aluminum tube. Ion trajectory simulation studies by Huang et al., indicate that an r.f.-only octupole guide can transmit ions of \( m/z \leq 2000 \), with kinetic energy of 10-30 eV, through a magnetic field gradient of a 9.4 T magnet with efficiency in excess of 95%.

The octupole is split near the bellows shown in Figure 6.1. When the bellows is fully extended, the gate valve, mounted on stage 2, may be closed; this allows the source area to be serviced without breaking vacuum in stages 2-4. Ions are delivered to a home-built dual ion trap assembly, pictured in Figure 6.3. The stainless steel 0.25" dia. tubes which serve as electrical feedthurs and provide mechanical support, are welded at the opposite end (not shown) to an 8" O.D. conflat flange, separating stages 2 and 3 (see Figure 6.1). The conductance limit plate extends out to the I.D. of the main vacuum
Figure 6.1  High performance external source electrospray ionization FT/ICR mass spectrometer.
Figure 6.2 Octupole ion guide with cut-away view of aluminum support tube. The inscribed circle of the octupole is ~5 mm.
Figure 6.3 Dual ion trap used in the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1. Figure provided by Ljiljana Pasa Tolic

chamber; a 2 mm dia. hole located at the center of the common trap plate separates the source and analyzer traps.

The entire system was constructed in a modular fashion, such that various external users could re-configure various components for their particular needs. Each pumping stage is mounted on stainless steel square-frame carts, as shown in Figure 6.4. These carts ride on 1" dia. shaft on the lab floor. The associated pumps and vacuum crosses mount on a custom-built 10" O.D. conflat nipple, which in turn have tripod leveling mounts and also ride on smaller rails oriented perpendicular to the floor race. A spectrum of bovine insulin (5734 Da) is shown in
Figure 6.4 Vacuum carts used in the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1.
Figure 6.5 Mass spectrum of bovine insulin obtained on the high-performance electrospray FT/ICR mass spectrometer shown in Figure 6.1. Data provided by Mike Senko.

Figure 6.5 and is representative of current system performance. Approximately 5 pmol of analyte was injected into the source-side ion trap and subjected to azimuthal quadrupolar axialization for several seconds. The data in Figure 6.5 was obtained after transfer of ions to the analyzer-side trap. Mass resolving power for the $5^+$ charge state of bovine insulin is greater than $1 \times 10^5$. Much of the existing system will
soon be transferred to a 9.4 T superconducting magnet, providing the highest-field FT/ICR mass spectrometer in the world.

**A HIGH PERFORMANCE MULTI-SOURCE FT/ICR MASS SPECTROMETER**

The data presented in Chapters III and IV demonstrate that an electrostatic wire ion guide is a simple and effective means for injection of externally-formed ions in FT/ICR mass spectrometry. Moreover, its capability to capture and transport ions having a wide range of initial kinetic energy and angular distribution indicates that it may be an appropriate choice for a FT/ICR/MS equipped with various ionization sources. A multi-source system, currently under construction at NHMFL is pictured (top view) in Figure 6.6. Stage 1 is a 9-way cross, pumped by a 1400 L/s diffusion pump below, which allows multiple ionization sources to be installed simultaneously. As seen in Figure 6.6, there is a glow discharge source, pumped by a separate 210 L/s hybrid turbo-drag pump, mounted co-axially with the central axis and a solids insertion probe for use during MALDI studies. Ions are transported through the fringe field of a 6 T superconducting magnet by an electrostatic ion guide. Note that the entrance of the guide tapers in the source region; this allows for maximum ion collection efficiency while maintaining a pressure difference between neighboring vacuum stages. In this system the bellows serves to translate the mouth of the EIG across the source chamber. Stages 2 and 3 are pumped by 1500 L/s cryo-pumps and a dual
Figure 6.6 Schematic diagram showing a multi-source FT/ICR mass spectrometer.
ion trap, similar to that described above, will be installed at the center of the main vacuum chamber.

ION AXIALIZATION FOR MS\textsuperscript{n} EXPERIMENTS: CHARGE-REMOTE FRAGMENTATION IN PHOSPHOLIPID ANALYSIS

Chapter V illustrated the effectiveness MALDI-FT/ICR/MS for phospholipid analysis. The use of azimuthal quadrupolar axialization subsequent to collisional activation re-focuses product ions (either polar head group or fatty acid side chain) back to the trap center. Clearly, this approach should provide for additional MS stages. After each successive dissociation, the product ion of interest would be isolated using SWIFT and then subjected to additional stages of collisional activation.

Charge-remote fragmentation, in which the charge site does not play an active role in the observed fragmentation pattern, has been observed in the dissociation of unsaturated fatty acids\textsuperscript{2}. In negative ion analysis, where the charge is localized on the carboxylate group, remote-site fragmentation results in loss of successive -CH\textsubscript{2} groups except at points of unsaturation, in which -C\textsubscript{2}H\textsubscript{2} is lost. In the resulting spectrum, double bonds are identified a peak separation of 26 m/z units. Wysocki \textit{et al.}, have shown that the onset of charge-remote fragmentation can occur at ion kinetic energies as low as several hundred eV\textsuperscript{3,4}. As shown in Figure 1.4, this kinetic energy regime is readily accessible in FT/ICR/MS at magnetic field strengths of 7 or 9.4 T. Thus, in the case of phospholipids, MS\textsuperscript{3} in negative ion-mode would provide location of
double bonds within the fatty acid side chains through charge-remote fragmentation.
REFERENCES


LIST OF REFERENCES


206


Kelleher, N. L.; Senko, M. W.; Little, D. P.; O’Connor, P. B.; McLafferty, F. W. JAMS 1995, 6, 220-221.


Klein, R. A. J. Lipid Res. 1971, 12, 628-634.


Li, Y.; McIver, R. T. RCMS 1994, 8, 743-749.


Penning, F. M. Physica (Utrecht) 1936, 3, 873.

Rempel, D. L., Personal communication.


