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High-performance Fourier transform mass spectrometry: Soft ionization methods and resolution enhancement

Kim, Hyun Sik, Ph.D.
The Ohio State University, 1994
HIGH PERFORMANCE FOURIER TRANSFORM MASS SPECTROMETRY:

SOFT IONIZATION METHODS AND RESOLUTION ENHANCEMENT

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

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DEDICATION

I dedicate this work to my family: parents and brothers and sister.
ACKNOWLEDGMENTS

There are many people bring this thesis to fruition, but a few should be singled out in particular. My biggest debt is to Dr. A. G. Marshall who has been not only my academic advisor but also one of the most respectable scientist I have ever met. Also, Mrs. Marshall kindly provide legal advice and great opportunities to meet many scientists in her house.

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**FIELD OF STUDY**

Major Field: Chemistry

Studies in physical and analytical chemistry, specialized in Fourier transform ion cyclotron resonance mass spectrometry (FT/ICR/MS), performed with adviser, Dr. Alan G. Marshall.
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CHAPTER I

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

INTRODUCTION

Due to the tremendously fast repeating circular ion motion inside the magnetic field (ion cyclotron motion), the intrinsic accuracy and precision in measurement of averaged cyclotron (motion) frequency, which is reciprocally proportional to the mass of ion, provides the ultra high resolution and accuracy in mass measurement (ICR/MS). The introduction of the Fourier transform method\(^1\) enhanced the applicability of ICR/MS to various practical problems. Today the Fourier transform ion cyclotron resonance mass spectrometer (FT/ICR/MS) has become the most powerful mass spectrometer capable of ultrahigh mass resolving power, precise mass measurement, multistage MS/MS, high mass range, ion remeasurement, simultaneous detection of all ions, and long ion storage time. More than 160 FT/ICR/MS have been built worldwide and actively applied to various scientific investigations.
History

The first practical application of cyclotron motion was for generation of high speed (~$10^6$ eV) light charged particles without generation of a high electrostatic potential field. Lawrence and Livingstone used the high kinetic energy ions to strike the target neutrals for nuclear studies in 1932. After the introduction of the frequency-selective cyclotron motion acceleration by a radio frequency (rf) field, the collected current measurements of accelerated ions (Omegatron) generated a cyclotron frequency spectrum which could easily be converted as a mass spectrum (ICR/MS) and showed the analytical possibility of rf resonance detection of selected ions with respect to their mass-to-charge ratio. However, the mass selector called an omegatron mass spectrometer was never widely used because of its low mass range and low resolution. In 1963 Wobschall et al. developed a cyclotron based on marginal-oscillator detection, which responds to the power absorbed by resonant ions during a magnetic field sweep rather than to resonant ion current impinging on a collector electrode, to avoid the collisional effect of omegatron performance, but his new cyclotron was not enough to attract a chemist's attention at that time.

In mid 1960 Baldeschwieler (at Harvard and then at Stanford) and Llewellyn at Varian Associates collaborated to produce the ICR spectrometer to investigate the gas phase ion chemistry and the ICR technique become quickly recognized as a preferred tool. With the accelerated progress of ion chemistry, there were several important
instrumental developments in ICR spectrometry. First, the high and constant (and thus highly homogeneous) magnetic field of the superconducting magnet improved the resolution, mass range and ion storage efficiency. Second, the trapped ion cell having a pulsed mode of operation can control the ion-molecule reaction experiment without spatial separation of ion production and ion mass analysis region; it also accelerates the automation of the experimental procedure with computer control. Finally, the Fourier transform method added many advantages to ICR spectrometry as a powerful analytical tool including the features of fast data acquisition, ultrahigh resolution, and wide mass range. More detailed stories about FT/ICR/MS history can be found in many review articles.

**Current Trends**

After 20 years of FT/ICR/MS development, the initially predicted capabilities of FT/ICR/MS have been extended significantly. As an analytical tool, laser desorption ionization (LDI) FT/MS and electrospray FT/MS are most widely used for involatile solid samples, polymers and biologically interesting macromolecules with improved wide mass range and ultrahigh resolution. Since the first FT/ICR/MS was built, instrumentation improvement has been critical to enhancement of the chemical applicability of FT/ICR/MS. Recently ion trap design, external ion injection, ionization sources, data station, and ion optical spectroscopy are considered to be the most important
aspects of instrument development, and instrument development is
enhancing many practical capabilities especially for the structure
determination of high mass biomolecules and polymers and the
improvement of mass accuracy and resolution. For ion reaction studies,
metal ions\textsuperscript{43-45}, metal cluster ions\textsuperscript{46} and organometallic ions\textsuperscript{47-52} have
been synthesized, dissociated, and investigated with respect to ion
reaction mechanism and kinetics.

Very recently we have begun to apply what physicist have done in ion
trap development and that both the ICR ion trap and penning trap have
the same approximate physical properties, for example: quarupolar
electrostatic field, homogeneous high magnetic field and the resulting
ion motions. Even though the masses of charged particles of interest to
chemists and physicists different by in several orders of magnitude, their
experimental and theoretical treatments, already well-established, are
similar enough to be adopted to understand ion motion in an ICR trap
and to be applied to enhance the capabilities of FT/ICR/MS. One of the
most successful adaptations of the Penning trap study to FT/ICR/MS is
the cooling axialization experiment\textsuperscript{53-66}.

**BASIC INSTRUMENTATION**

To collect the time domain signal of cyclotron motion of ions, we may
generate the ions by any of several different ionization sources (electron
impact ionization\textsuperscript{67}, laser desorption ionization\textsuperscript{59,68-72}, electrospray
ionization$^{41,73-78}$, fast atom bombardment ionization$^{79-82}$, glow discharge ionization$^{34,35}$, chemical ionization$^{83,84}$, multiphoton ionization$^{85}$ etc.) and capture the ions within an ion trap by combined electrostatic and magnetic fields. The most widely used trap has cubic shape$^{86}$, assembled with six separate rectangular nonmagnetic metal plates separated by eight insulating connectors as in Fig. 1.1.

All FT-ICR experiments are conducted in an ICR ion trap which is enclosed in a high vacuum chamber to minimize the collisional damping of ion motion. A high-performance FT-ICR/MS spectrometer is typically equipped with a horizontal-bore superconducting solenoidal magnet with a field induction of 3-7 Tesla inside the magnet bore so that the ICR trap is located in the most spatially homogeneous central magnetic field region.

The homogeneous magnetic field pushes the ions radially (in xy-plane) inward to the axis parallel to the magnetic field, and the electrostatic potential applied to the trap plates will push the ions to the middle of the trap (on z-axis). The stored ions rotate around the magnetic field axis (cyclotron motion), but the phase of the ion motion is randomly distributed and therefore, the averaged induced currents will be zero unless ions are forced to circulate with the same phase by application of a resonant radio frequency (rf) oscillatory electric field. The electric field potential difference is applied between a pair of electrodes perpendicular to the x-axis as shown in the Fig. 1.1 and this cyclotron
Figure 1.1  A typical cubic ion trap: Ions are generated by electron impact, excited by x-direction radio frequency oscillating electric field, and detected by the measurement of y-direction current amplitude induced by ion motion.
motion excitation increases the ion cyclotron radius enough to produce a large induced charge on the detection plates.

After rf excitation, the resulting coherent ion motion induces the current on the pair of electrodes perpendicular to the y-axis and the current can be converted to the voltage difference through an appropriate resistor. The amplified differential voltage produces the oscillating time domain signal and then Fourier transformation of the transient time-domain signal will produce a frequency-domain spectrum of cyclotron motions. Mass calibration\textsuperscript{87} of the frequency spectrum produces the final mass spectrum in which mass is approximately reciprocally proportional to the ion cyclotron frequency.

The typical configuration of the laser desorption ionization (LDI) FT/ICR/MS, which has generated most of spectra in this thesis, is shown in Fig. 1.2. The Nd:YAG laser operated at UV/VIS/NIR wavelength was aligned to the chamber enclosed by 3T magnet. The dual ion traps, with a conductance limit (2 mm diameter) between two consecutive cubic traps provides lowerer pressure (~10\textsuperscript{-9} torr), installed inside of the chamber and homogeneous magnetic field region, with a sample inlet system (combination of leak valve, pulse valves and reservoirs) connected to the trap. The laser and sample inlet pulses are controlled by the pulse sequence program stored in the data station.
Figure 1.2 Instrumental configuration of FT/MS composed of Nd:YAG laser and filament for laser desorption ionization, electron impact and chemical ionization.
ION MOTION

General description of ion motion within the ICR trap is one of the most important aspects in developing the application methods in FT/ICR/MS. Here the most updated description of ion motion will be discussed.

In the static magnetic field (B), ion moves circularly around the magnetic field axis (Z-axis) with the radius, \( r_c \), determined by initial ion radial velocity. In Fig. 1.3, there is no work during this motion because the Lorentz force is always perpendicular to the ion motion. The stationary circular motion (cyclotron motion) can be described by the equation of motion, Eq. 1-1.

\[
m \dot{\psi} = q \mathbf{v} \times \mathbf{B} \quad (1-1)
\]

Each Cartesian component will be described as follows:

\[
v_x = \left( \frac{qB}{m} \right) v_y \quad (1-2a)
\]

\[
v_y = - \left( \frac{qB}{m} \right) v_x \quad (1-2b)
\]

\[
v_z = 0 \quad (1-2c)
\]
The solution of the equations will describe the trajectory of ions in static magnetic field.

\[ x = \rho_c \cos(\omega_c t + \phi) \]  
(1-3a)

\[ y = \rho_c \sin(\omega_c t + \phi) \]  
(1-3b)

\[ z = v_20 t + z_0 \]  
(1-3c)

\[ \omega_c = \frac{qB}{m} \]  
(1-3d)

If we use the new coordinate, \( \rho = x + iy \), Eq. 1-3a and Eq.1-3b can be simplified as one equation:

\[ \rho = \rho_c e^{i (\omega_c t + \phi)} \]  
(1-4)

The complex form trajectory equation, Eq.1-4, shows that an ion circulates around the magnetic field axis with cyclotron frequency, \( \omega_c \), and cyclotron radius \( \rho_c \). Now if we fix the radius of ion motion, we can derive a perfect description of ion motion. If we assume the ion is at thermal equilibrium at temperature, \( T \), the ion cyclotron radius can derived by the thermal kinetic energy relation:

\[ \frac{m \dot{\rho}^2}{2} \equiv kT \]  
(1-5a)

\[ \rho_c = \frac{\sqrt{2mkT}}{qB} \]  
(1-5b)

The cyclotron radius decrease at higher magnetic field and higher ion charge. Conversely, heavier ion mass and higher temperature will increase the cyclotron radius.
Figure 1.3 The trajectory of an ion in a homogeneous magnetic field shows that the ion circulates around the magnetic field axis with constant cyclotron frequency, $\omega_c$, and constant cyclotron radius $\rho_c$. 

$$|V| = \rho_c \omega_c$$

$$\omega_c = \frac{qB}{m}$$

$\mathbf{B}$
$\mathbf{V}$
$\mathbf{F}_{\text{Lorentz}}$
$\rho_c$

$\mathbf{x}$
In a static electric field ($E$) and static magnetic field ($B$), an ion moves circularly around the magnetic field axis (Z-axis) and also oscillates in the Z-direction within a harmonic potential well which generated by DC voltage of the same sign of the trapping ions applied to both trapping electrodes to remove the z-direction freedom of ion motion as in Eq.1-3c. But intuitive thought is not deep enough to discover one more ion motion called magnetron motion. To derive these motions strictly, the equation of motion of single ion can be generated as Eq.1-1 with addition of the electrostatic field contribution term, the first term in right side of Eq.1-6. The electrostatic potential, $\Phi$, can be derived by solving the Laplace's equation, Eq. 1-7

$$m \ddot{\mathbf{r}} = q (\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

(1-6)

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

(1-7)

The trapping electrostatic field, $\mathbf{E}$, can be derived from the electric potential:

$$\mathbf{E} = -\nabla \Phi$$

(1-8)

The boundary conditions for the cubic trap with the length, $a$, and boundary potential, $V_t$, at the trapping plates are

$$\Phi(\pm \frac{a}{2}, y, z) = \Phi(x, \pm \frac{a}{2}, z) = 0$$

(1-9a)

$$\Phi(x, y, \pm \frac{a}{2}) = V_t$$

(1-9b)
Figure 1.4 Trapping electrostatic field of cubic ICR trap including the z-direction bell shape potential well and the radial hill shape potential in xy-plane at the z=0 [Reference 1.55]
in which $V_t$ is the DC voltage applied to trapping electrodes. By the separation of variables and symmetry considerations, the solution of the Eq.1-8 can be described with the combined trigonometric functions as shown in Fig. 1.4.

$$\Phi = \frac{16V_t}{\pi^2}$$

$$\Phi = \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^m n \cosh \left( \frac{k_{mn} \pi z}{a} \right) \cos \left( \frac{2m+1}{a} \pi x \right) \cos \left( \frac{2n+1}{a} \pi y \right)}{(2m+1)(2n+1) \cosh \left( \frac{k_{mn}}{2} \right)}$$

in which, $k_{mn} = \sqrt{(2m+1)^2 + (2n+1)^2}$.

Near the center of the trap, the electrostatic potential can be approximated by a Taylor expansion as a quadrupolar potential and can be described as Eq. 1-11.

$$\Phi_q (x, y, z) = \frac{V_t}{3} - \frac{\alpha V_t}{2 a^2} (x^2 + y^2 - 2z^2)$$

in which $\alpha$ is a constant determined by trap dimension (2.77373 for a cubic trap). From Eqs. 1-6, 1-8, 1-11, the exact equation of motion can be derived for each component:

$$v_x = (\frac{\alpha q V_t}{a^2 m}) x + (\frac{q B}{m}) v_y$$

$$v_y = (\frac{\alpha q V_t}{a^2 m}) y - (\frac{q B}{m}) v_x$$

$$v_z = -2(\frac{\alpha q V_t}{a^2 m}) z$$
We can easily recognize Eq. 1-12c as a one-dimensional equation of harmonic oscillation motion and can directly write the trajectory:

\[ z = z_0 \cos(\omega_z t + \phi) \quad (1-13a) \]

\[ \omega_z = \sqrt{\frac{2\alpha q V_t}{\alpha^2 m}} \quad (1-13b) \]

From the thermal kinetic energy relation, Eq. 1-14a, the amplitude of axial oscillation can be calculated at temperature \( T \).

\[ \frac{m z^2}{2} \equiv \frac{kT}{2} \quad (1-14a) \]

\[ z_0 = \sqrt{\frac{\alpha^2 kT}{\alpha q V_t}} \quad (1-14b) \]

in which \( k \) is the Boltzman constant and \( \alpha \) and \( \alpha \) are constants determined by the trap dimensions. The trapping amplitude is a function of temperature, trapping voltage and ion charge and is independent of mass. If we simplify Eq. 1-12a and Eq. 1-12b with Eq. 1-3d, 1-13b, and new coordinate, \( \rho = x + iy \), the trajectory of xy-component can be described by Eq. 1-15.

\[ \ddot{\rho} + i\omega_c \dot{\rho} - \frac{1}{2} \omega_z^2 \rho = 0 \quad (1-15) \]

Solution of this differential equation is

\[ \rho = \rho_+ e^{-i\omega_c t} + \rho_- e^{-i\omega_c t} \quad (1-16) \]

\[ \omega_\pm = \frac{1}{2} \left( \omega_c \pm \sqrt{\omega_c^2 - 2\omega_z^2} \right) \quad (1-17) \]
Figure 1.5 Ion cyclotron and magnetron motions in the xy-plane where $\rho_+, \omega_+, \rho_-, \omega_-$ are cyclotron radius, reduced cyclotron frequency, magnetron frequency, and magnetron radius.
As for Eq.1-4, Eq.1-16 shows that the ion trajectory in the xy-plane is the superposition of two different motions of one ion. The ion rotates around the z-axis with cyclotron radius, \( \rho_+ \), which is the distance from the rotation center, and reduced cyclotron frequency, \( \omega_+ \). The center of the cyclotron motion again rotates following the equipotential line of radial electrostatic field with magnetron frequency, \( \omega_\text{m} \) and magnetron radius, \( \rho_- \), as seen in Fig. 1.5. The metastable property of the magnetron motion is well-described in a review article about Penning traps\(^8^9\). In Eq.1-17 we can find the trapping condition, because the periodic solutions require the roots to be real. From Eq.1-13b and Eq.1-3d we can define the critical mass, \( \text{m}_\text{c} \), which is the boundary values of upper mass limit.

\[
\begin{align*}
\omega_\text{c}^2 - 2\omega_\text{z}^2 &> 0 \quad \text{(1-18a)} \\
\text{m}_\text{c} &= \frac{q\alpha^2B^2}{4\alpha V_\text{t}} \quad \text{(1-18b)}
\end{align*}
\]

Now three independent ion motions can be summarized as following Eqs1-16 and shown in Fig. 1.6.

\[
\begin{align*}
\rho^+(t) &= \rho^+(0) e^{-i\omega_\text{c} t} \quad \text{(1-16a)} \\
\rho^-(t) &= \rho^-(0) e^{-i\omega_\text{m} t} \quad \text{(1-16b)} \\
z(t) &= z(0) \cos(\omega_\text{z} t + \phi) \quad \text{(1-13a)}
\end{align*}
\]
In a dynamic and static electric field (E) and static magnetic field (B), the absorbed resonant energy from the dynamic (rf) electric field will influence the ion motion. The electric field can excite any ion motion mode. There are various modes and methods of excitation. The most popular dipolar excitation will be summarized and discussed. Schweikhard et al. derive the postexcitation ion motion by equating the instantaneous single frequency power absorption by an ion (averaged...
over one cycle of its oscillation) to the time derivative of ion energy, $E$, at the ion position in a quadrupolar trapping potential field as in Eq. 1-19.

$$P = \frac{dE}{dt}$$  \hspace{1cm} (1-19)

For dipolar excitation (standard excitation mode in FT/ICR/MS), we apply an rf oscillating electric field with opposite phase to two opposed excitation plates in Fig. 1.1. The averaged power of rf excitation in a cubic trap has been derived\(^9\) and approximated as Eq. 1-20 and the energy of (reduced) cyclotron motion, $E_+$ has been derived by classical and quantum mechanics\(^9\) as Eq. 1-21.

$$P = \frac{q \beta V_{p-p} \rho^+ \omega_+}{2a} \hspace{1cm} (1-20)$$

$$E_+ = \frac{m}{2} \omega_+ \omega_+ \rho^+ \tilde{r}^2 = \left( n_+ + \frac{1}{2} \right) \frac{\hbar}{2\pi} \omega_+ \hspace{1cm} (1-21)$$

in which $\beta = 0.72167$, $\omega_p = \omega_+ - \omega_-$, and $n_+$ is the quantum number for cyclotron motion. From Eqs. 1-19, Eq. 1-20, and Eq. 1-21, we can obtain the differential equation, Eq. 1-22.

$$m \omega_p \rho^+ = \frac{q \beta V_{p-p}}{2a} \hspace{1cm} (1-22)$$

If initial cyclotron radius is negligible, $\rho^+(0) = 0$, the solution of the differential equation is

$$\rho^+(t) = \frac{q \beta V_{p-p} t}{2m \alpha \omega_p} \hspace{1cm} (1-23a)$$

$$\rho^+(t) \equiv \frac{\beta V_{p-p} t}{2 \alpha B} \hspace{1cm} (1-23b)$$
Similarly the magnetron mode can be excited by dipolar excitation by replacing the excitation frequency $\omega_+$ by $\omega_-$ and the resulting trajectory can be derived as Eq. 1-24.

$$\rho_+(t) = \rho_+(0) - \frac{q\beta v_{p-p} t}{2m a \omega_p}$$  \hspace{1cm} (1-24)

Both cyclotron and magnetron modes linearly increase with excitation duration. Because the excitation of magnetron mode will increase the magnetron radius and remove the ions from the trap, the excitation frequency range should not include the magnetron frequency.

From Eq. 1-13a, Eq. 1-16a, Eq. 1-16b, Eqs. 1-23, and the assumption of negligible preexcitation cyclotron radius, $\rho_+(0) = 0$, we can approximate the postexcitation ion motion as in Eq. 1-25 and 1-13a. and plot the ion trajectory in 3-dimensional Cartesian coordinate system (see Fig. 1.7).

$$\rho = \frac{q\beta v_{p-p} t}{2m a \omega_p} e^{-i\omega_+ t} + \rho_+ e^{-i\omega_- t}$$  \hspace{1cm} (1-25)

$$z(t) = z_0 \cos(\omega_z t + \phi)$$  \hspace{1cm} (1-13a)

**EXCITATION**

A radio frequency (rf) signal is applied to two opposing plates of the trap. If the frequency of the rf signal matches one of the natural
Figure 1.7 Ion motion during the very beginning of rf-dipolar excitation of cyclotron mode in ICR experiment.

motions of the ion, then energy is absorbed and that motion is excited. The final radius of the ion packet is proportional to the excitation amplitude and independent of ion mass and charge, Eq. 1-23b. Hence, if excitation is uniform in magnitude at each frequency, all ions will be excited coherently to the same final radius. There are three excitation
methods: single frequency, frequency sweep, and Stored Waveform Inverse Fourier Transform (SWIFT) excitation (Fig. 1.8).

Single frequency excitation or single-pulse excitation was the method used to produce the first FT/ICR mass spectrum.\(^1\) A single frequency pulse of duration, \(T\), will produce in the frequency domain a sinc function centered at the frequency of excitation with a magnitude (absolute value) mode full width at half maximum (FWHM) value of \(\approx 1.2/T\) Hz.

To excite ions over such a broad frequency range, frequency sweep excitation was introduced.\(^93\) Frequency sweep or 'chirp' excitation is the most widely used excitation method in FT/ICR/MS. The radio frequency are scanned linearly with time over the frequency bandwidth of interest, Eq. 1-26.

\[
\begin{align*}
\text{f}(t) &= e^{i\left(\omega_A t + \frac{\xi t^2}{2}\right)}, & 0 \leq t \leq T \\
\text{f}(t) &= 0, & t < 0 \text{ or } t > T
\end{align*}
\]

in which the frequency-sweep rate, \(\xi\) (in rad s\(^{-2}\)) is determined by the instantaneous angular frequency, \(\omega_A\) at \(t = 0\) and \(\omega_B\) at \(t = T\): \(\omega_B = \omega_A + \xi T\). Although excitation is improved for broadband applications, there is still the problem of non-constant excitation amplitudes over the frequency range. Variations in amplitude of \(\pm 25\%\) are observed in the frequency spectrum for linear sweep excitation, which makes quantification of ion abundances difficult. By slowing the frequency sweep, one can flatten the power spectrum and improve the frequency
Figure 1.8 Frequency-domain magnitude spectra of some standard FT/ICR time-domain excitation waveforms. (a) Rectangular pulse (encompassing a single frequency); (b) Frequency-sweep ("chirp"); (c) SWIFT for broadband excitation; (d) SWIFT for MS/MS or ion-molecule reactions. (Figure provided by Alan Marshall)
selectivity of excitation especially at the ends of the frequency range. While frequency sweep is simple, it should be used with care, especially for quantitative work.

In the mid-1980's, Marshall, Wang and Ricca developed SWIFT excitation\textsuperscript{94,95} adapted from an earlier proposal by Tomlinson and Hill.\textsuperscript{96} If we first specify a magnitude spectrum of excitation in the frequency domain, Fig. 1.8c right, we can generate the time domain excitation signal, Fig. 1.8c left, produced by inverse Fourier transformation of magnitude spectrum and the resulting frequency domain magnitude spectrum can be improved by phase scrambling\textsuperscript{97,98} and smoothing\textsuperscript{99-101} methods. Although SWIFT has been shown to produce flat excitation so that quantification of the ion signals can be performed, perhaps its greatest advantage comes from the ability to specify unique excitation frequency profiles. To isolate ions of a single $m/z$, or multiple $m/z$'s, requires multiple excitation pulses via chirp. The experimental duration is extended and it is possible that unwanted reactions could occur during that period. Also, the frequency resolution (selectivity) of the chirp excitation events is very low. By means of SWIFT, all of the individual frequency ranges are combined into one event, thereby eliminating the need to use multiple pulses. One can also combine the ejection and excitation events into one SWIFT event by having different magnitudes at different frequency regions. By ejecting highly abundant ions, the FT/ICR dynamic range can be improved for the detection of less abundant ions.\textsuperscript{102}
DETECTION

ICR signal model

Once the ion motion has been well described, the ICR signal can be derived with several different methods\textsuperscript{103-106}. From Comisarow's signal model\textsuperscript{103} and Eq.1-25, we can derive the induced charge generated by the ion motion, which modified by trapping electrostatic field in a cubic trap, as in Eq.1-27, by assuming that the detecting plate is infinitely

\[ I_{\text{ind}} \]

\[ Q^+ \]

\[ Q^- \]

\[ \omega_+ \]

\[ q \]

\[ B \]

\[ d \]

Figure 1.9 Generation of induced current, \( I_{\text{ind}} \), by an ion circulating inside the ion trap with homogeneous magnetic field, \( B \).
extended in the xy-plane and nothing interferes with detection between ion and infinite trapping plate as in Fig. 1.9.

\[ Q(t) = -\frac{Nq}{d} \left( \rho_+ \cos(\omega t) + \rho_- \cos(\omega t) \right) \]  

(1-27)

From this approximation, we can detect the cyclotron frequency and magnetron frequency, but the signal amplitude of each mode is proportional to the postexcitation radius of the each mode. In an ICR experiment, mainly the cyclotron mode is excited and the cyclotron radius is much larger than the magnetron radius after excitation, \( \rho_+ \gg \rho_- \). Therefore, the detected signal show mainly the cyclotron resonant frequency. As mentioned previously in discussion of rf excitation. Eq. 1-24, we also can excite the magnetron motion to larger magnetron radius; however, larger magnetron radius allows the less room for cyclotron excitation and then decrease the ICR signal amplitude\(^{107}\). The conversion from induced charge to voltage has been discussed in Comisarow's signal model reference. The detailed ICR signal process will be discussed in the chapter on derivative spectra of FT/ICR/MS.

More accurately, induced differential charge for a cubic trap, Eq. 1-28\(^{104}\), is

\[ \Delta Q = -\frac{16q}{\pi^2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{x}{2} \sinh(k_{mn}a) \cos(\frac{(2m+1)\pi y}{a}) \cos(\frac{(2n+1)\pi z}{a}) \]  

\[ \frac{k_{mn}a}{(2m+1)(2n+1)\sinh\left(\frac{k_{mn}a}{2}\right)} \]  

(1-28)
In which $\Delta Q$ is the induced differential charge; $x$, $y$, and $z$ are the ion position coordinates in a laboratory frame whose origin is at the geometric center of the trap; $a$ is the length of the trap along $x$, $y$, and $z$ direction, respectively, and $-a/2 \leq x \leq +a/2$, $-a/2 \leq y \leq +a/2$, and $-a/2 \leq z \leq +a/2$; and

$$k_{mn} = \sqrt{\left(\frac{2m+1}{a}\right)^2 + \left(\frac{2n+1}{a}\right)^2}$$

If we set the trap length, $a$, equal to 1 in arbitrary units and approximate Eq.1-28 by Taylor expansion, then we can obtain a more understandable form for the induced charge as Eq.1-29.

$$\Delta Q = a_0 x + a_1 x (y^2 + z^2) + a_2 x^3 + a_3 x (y^4 + z^4) + a_4 x y^2 z^2 + a_5$$

$$x^5 (y^2 + z^2) + a_6 x^5 + a_7 x (y^6 + z^6) + a_8 x (y^4 z^2 + y^2 z^4) + a_9 x^3$$

$$(y^4 + z^4) + a_{10} x^3 y^2 z^2 + a_{11} x^5 (y^2 + z^2) + a_{12} x^7 \quad (1-29)$$

in which the values of coefficients, $a_0$ to $a_{12}$, may be found in Rempel et al.\cite{108}. From Eq.1-13a, Eq.1-25, and Eq.1-29, we can expect the signal will include multiple harmonics of the fundamental modes as well as the fundamental cyclotron, magnetron, trapping oscillation frequencies. One interesting observation is that Eq. 1-29 includes only even powered $Z$ terms multiplied by $x$-components which generate the oscillating signal of even harmonic trapping oscillation frequency detectable in (e.g.) $x$-direction dipolar detection as sidebands, as seen experimentally for benzene molecules: $v_4 \pm 2v_z$. The analysis of the resulting signal has
been well-discussed including the experimental detection and theoretical simulation of three different ion modes in Chen's thesis\textsuperscript{107}.

**Heterodyne Detection**

In the case of FT/ICR/MS we are confined to collecting all frequency components less than the Nyquist frequency and the resolution is limited by the number of data points. Therefore, the maximum allowed size of memory within the frequency range of interest will give the best resolution. But if the frequency range of interest is very narrow, we do not have to use memory to record the frequency range of empty information. Therefore we can record more accurate information about the narrow frequency range. For this purpose we use the heterodyne detection method as now described.

The time domain signal is passed through a mixer (non-linear device) with a reference frequency greater than the highest true frequency. The input to the mixture is a summation of both frequencies,

$$\text{Input}(t) = A_0 \cos \omega_{\text{true}} t + B_0 \cos \omega_{\text{ref}} t \quad (1-30)$$

The non-linear output signal may be expressed as a non-linear power series. Examination of the first two terms yields

$$\text{Output}(t) = c_1 A_0 \cos \omega_{\text{true}} t + c_1 B_0 \cos \omega_{\text{ref}} t + c_2 A_0^2 \cos^2 \omega_{\text{true}} t + c_2 B_0^2 \cos^2 \omega_{\text{ref}} t + 2c_2 A_0 B_0 \cos \omega_{\text{true}} t \cos \omega_{\text{ref}} t \quad (1-31)$$
in which \( c_x \) are constants. The original frequencies are present along with two squared terms which can be expressed as second harmonics of the original frequencies and the last term can be expressed as

\[
\cos \omega_{\text{true}} t \cos \omega_{\text{ref}} t = \frac{1}{2}[\cos(\omega_{\text{true}} + \omega_{\text{ref}})t + \cos(\omega_{\text{ref}} - \omega_{\text{true}})t] \quad (1-32)
\]

producing the sum and difference frequencies. The output signal goes through a low-pass filter, passing only the difference frequency which can be measured in narrower bandwidth and thus we can record a more detailed information for narrower bandwidth and therefore the higher resolution.

**STANDARD EXPERIMENTAL EVENT SEQUENCE**

We can summarize the principles of FT/ICR/MS by describing the standard procedure of data collection to produce a mass spectrum. The simplified experimental procedure is the sequential events along the time axis as shown in Fig. 1.10.

*Figure 1.10* Typical experimental procedure as a function of time.
Initially ionized ions may have high kinetic energy and wide spatial distribution as in laser desorption ionization or low kinetic energy and narrow distribution as in electron impact ionization. For cooling the high velocity ions, delay between ionization and excitation usually increases the spectral resolution. These ions will be simultaneously excited by rf excitation of wide range of frequency (chirp\textsuperscript{109} or SWIFT\textsuperscript{94-102,110-118}). The excited cyclotron motion will produce an induced charge (current) on the detection plate during the detection events. The current is converted to voltage and amplified to be recorded as a digital transient signal. The Fourier transformed time-domain signal can be calibrated to produce a mass spectrum with ultra high resolution and accuracy (usually the uncalibrated spectrum is accurate enough to read the nominal mass).

**CONCLUSIONS**

The fundamental principles and recently updated aspects of ion motion have been discussed in this introductory chapter and they are the essential theories to be extended in following chapters. Now we are getting close to perfectly understanding the single ion motion in ICR trap and applying the basic theories to the analytical method development in practical problems. Recently, extensive instrumental developments are underway; however, the instrumental development of the detection part is still staying in the early FT/MS invention time. In addition, new ionization sources are still under development. Therefore, the
instrumental development will be still the most urgent and critical problem in FT/ICR/MS field for practical applications. In addition, we also need to find out the information in signals generated by a well-developed instrument with the most efficient method. To enhance the advantage of the ultra-high resolution of FT/ICR/MS, we also need to manipulate the data to improve the mass spectrum. The following chapters describe the theoretical, instrumental and computational improvement of FT/ICR/MS capability. In addition, the ion spectroscopy of ions of selected mass is introduced in this thesis for the first time.
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CHAPTER II

AXIALIZATION OF AN ION CLOUD BY AZIMUTHAL QUADRUPOLAR EXCITATION AND COLLISIONAL COOLING

INTRODUCTION

The wide radial distribution of ions within an ICR ion trap restricts the many advantages of FT/ICR/MS in various experiments. Ion axialization, introduced as a new experimental technique in FT/ICR experiments, can solve the wide radial distribution problem and thus improve the resolving power and precision in mass measurement and enhance the capabilities of multistage MS/MS, high mass ion detection, ion remeasurement and longer ion storage time. In this chapter, the theoretical development will be discussed and experimental applications will be described. In later chapters, Chaps. 5, 8, 11, more applications will be shown.

Trapping of ions in the presence of a strong magnetic field and a three-dimensional quadrupolar electrostatic potential is the foundation of virtually all FT-ICR experiments. As shown in Fig.1.4, in the (axial) direction of the magnetic field, the potential is parabolic with a minimum.
at the center of the trap. However, on the transverse midplane (z=0), the potential is an inverted parabola with a maximum at the center of the trap. In the absence of a time-varying electric excitation field, ion motions may be considered to be a combination of three independent motions, namely cyclotron, magnetron, and axial motions (as shown in Figs. 1.5,6).\(^1\) The cyclotron frequency of an ion in a quadrupolar electrostatic trapping potential is shifted downward relative to \(v_c\), and the center of the cyclotron motion drifts along an iso-potential contour with a drift velocity, \(\mathbf{E}x\mathbf{B}/B^2\), in which \(\mathbf{E}\) is the electrostatic trapping field and \(\mathbf{B}\) is the magnetic field induction. The drift circular motion, or magnetron motion, is nearly independent of \(m/z\) ratio of the ion and (except at very high \(m/z\)) is much lower in frequency than \(v_c\). Ion axial motion is a simple linear harmonic oscillation whose frequency is inversely proportional to \(\sqrt{m/z}\).

If energy dissipation mechanisms are not provided, these three motions are bounded and ions remain trapped. However, ions can lose energy in many ways in real FT-ICR experiments. Collisional damping is the most common way for ions to lose energy. The initial amplitudes of ion cyclotron and axial motions (Fig. 2.1. top left and right) decrease with time because a smaller cyclotron radius corresponds to lower kinetic energy as shown in Eq.1-21 and a smaller z-amplitude corresponds to lower kinetic and potential energy which can be derived from Eq.1-13a. Although the magnetron orbit is stable in the absence of collisions or other mechanisms for ion energy loss, collisional damping causes the
Figure 2.1 Effect of collisional damping on the three natural ion motions in an ICR ion trap. Top left: exponential damping of cyclotron radius. Top middle: exponential growth of magnetron radius. Top right: exponential damping of z-oscillation amplitude. Bottom: Relative time rates of change of the three motional amplitudes—note that cyclotron radius is damped about twice as fast as axial oscillation, and that both are damped at rates much faster than the rate of increase of magnetron radius.2
magnetron radius to expand since a large magnetron radius corresponds to lower (trapping) potential energy (Fig. 2.1, top middle). Ions gradually migrate radially toward the electrodes parallel to the magnetic field and are eventually lost from the trap.

For various FT-ICR/MS experiments, however, it is important to be able to trap ions at high neutral gas pressure for an extended period. For example, tandem mass spectrometry is conducted most effectively by use of collision-induced dissociation which requires a high collision gas pressure for ion activation. A unique feature of FT-ICR/MS is its capability to remeasure high-mass ions, an experiment which requires many collisions to relax the ions back to the center of the trap for re-excitation. Ion internal energy needs to be reduced prior to accurate measurement of ion-molecule reaction rate constants: a large number of collisions may be needed to remove vibrational energy of an ion. High-mass ions of great biological interest may be generated by matrix-assisted laser desorption ionization (MALDI) in which ions have high kinetic energy, or electrospray ionization in which ions exhibit lower (but wider distribution in) kinetic energy. High and/or a distribution in ion kinetic energy is undesirable for high-resolution ICR detection. Collisional damping is the most efficient method for reducing ion kinetic energy. It is apparent that a method that could prevent or even reverse the magnetron expansion process would be highly desirable for extending the utility of FT-ICR/MS.
The ion axialization method originated in the field of atomic physics, in which confinement of charged particles in a small volume in space is of great interest for precise measurements of fundamental properties of the particles. One of the most successful methods is use of a Penning trap to isolate one or a few electrons or atomic ions for a prolonged period (hours to months). Although the electrodes of a Penning trap may be machined near-perfect hyperboloids of revolution, the electrostatic potential nevertheless deviates significantly from a pure quadrupolar potential when ions move away from the center of the trap, due to truncation of the trap (to finite dimensions) and because of holes in the electrodes to admit or expel ions. It is therefore necessary to confine the ions near the center of the trap. Brown and Gabrielse proposed that cooling of magnetron motion by coupling to cyclotron motion in a Penning trap could be achieved by splitting the ring electrode and applying a quadrupolar \( xy \)-excitation potential at frequency, \( \omega_+ + \omega_- \), in which \( \omega_+ \) is the cyclotron frequency. The coupling of magnetron and cyclotron motions was later studied in more detail by Bollen et al. For cooling of heavy ions, collisional damping is the most efficient method. Magnetron cooling by quadrupolar excitation in the presence of collisional damping was first demonstrated experimentally by Savard et al. for ions of a single \( m/z \) ratio.

Later, broadband axialization was introduced by Schweikhard et al. The first ion axialization with broad-band ICR detection was carried out by use of a dual trap FT-ICR mass spectrometer. Ions generated in the
source compartment of the dual trap were subjected to azimuthal quadrupolar excitation at the unperturbed cyclotron frequency, $\omega_c = \omega_+ + \omega_-$, of ions of the $m/z$ of interest in the presence of a high pressure of buffer gas. In this way, the magnetron radius of the ions could be reduced to less than the radius of the conductance limit separating the source trap from the analyzer compartment, so that axialized ions could be transferred to the analyzer for detection at much lower pressure. With the ion axialization method, prolonged trapping at high buffer gas pressure, enhanced mass resolving power, and improved mass selectivity under high space charge conditions for laser-desorbed ions were demonstrated.\textsuperscript{16} Speir et al. later showed that ion remeasurement efficiency in a single-compartment ICR trap could be improved by pulsing a collision gas during the ion axialization interval.\textsuperscript{17} For optimal ion axialization, a relatively high buffer gas pressure stable for tens of seconds is required. However, ions should be detected at the lowest pressure possible to attain high mass resolving power. Although the dual trap configuration allows separation of ion axialization and detection, the high pressure in the source trap causes a non-negligible increase in pressure in the analyzer trap where ions are detected. We resolved that problem by use of multiply pulsed buffer gas,\textsuperscript{18} making it possible to axialize ions at nearly constant pressure and detect them at the lowest possible pressure. Mass resolving power for laser-desorbed ions may be further improved by axialization and enhanced by a "frequency drift correction method" (see chapter 3).
Another important aspect in development of the ion axialization method is extension of single-frequency resonant axialization of ions of just one $m/z$ ratio to broadband axialization over wide $m/z$ range(s). The broadband axialization can easily be realized by use of repeated low-amplitude SWIFT azimuthal quadrupolar excitation. Each of the identical SWIFT excitation pulses converts a fraction of magnetron amplitude to cyclotron amplitude which is in turn collisionally damped before the next pulse. The axialization range is defined solely by the magnitude spectrum of the SWIFT waveform, so that ions of arbitrary $m/z$ range(s) may be axialized simultaneously.

Optimal instrumental technique development typically requires deep understanding of the underlying theoretical principles. In this chapter, the comprehensive analytical treatment of the theory of resonant azimuthal quadrupolar excitation in the presence of collisional damping will be discussed. Starting from the quadrupolar electrostatic ("trapping") and quadrupolar time-varying ("excitation") electric potentials in an ICR ion trap, the equations of ion motions are solved analytically in the presence of azimuthal quadrupolar excitation and collisional damping: the ion trajectory depends on collisional damping rate and excitation amplitude. Finally, in the Applications section, examples illustrating the various advantages of the new axialization technique will be shown.
QUADRUPOLAR EXCITATION POTENTIAL AND EQUATIONS OF ION MOTION

The classical motion of an ion of mass, \( m \), and charge, \( q \), moving at velocity, \( \mathbf{v} = \mathbf{i} \dot{x} + \mathbf{j} \dot{y} + \mathbf{k} \dot{z} \), in a magnetic field, \( \mathbf{B} \), and electric field, \( \mathbf{E} \), in the presence of collisional damping force proportional to ion velocity is described by the modified Lorentz equation (S.I. units)\(^{21,22}\)

\[
m \ddot{\mathbf{v}} = q (\mathbf{E} + \mathbf{v} \times \mathbf{B}) - m \gamma \mathbf{v}
\]

(2-1)

In most FT-ICR experiments, \( \mathbf{B} \) is constant and spatially homogeneous, and its direction may be used to define the \( z \)-axis (\( \mathbf{B} = -B \mathbf{k} \)). \( \gamma \) is the collision damping rate constant (s\(^{-1}\)). The electric field, \( \mathbf{E} \), may be determined from the potential, \( \Phi \), inside the trap

\[
\mathbf{E} = -\nabla \Phi
\]

(1-8)

In the absence of Coulomb interactions, the electric potential is determined by the Laplace equation

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

(1-7)

subject to boundary conditions defined by the ICR ion trap geometry (see below).
Electrostatic trapping potential

For the cubic trap, the electrostatic trapping potential has been described previously in Eq. 1-10 and Eq. 1-11 and Fig. 1.4. To derive the simpler analytical solution of ion motion during the axialization, the approximate quadrupolar potential, Eq. 1-11, will be used through this chapter.

\[ \Phi_q(x,y,z) = \frac{V_t}{3} - \frac{\alpha V_t}{2 \alpha^2} (x^2 + y^2 - 2z^2) \quad (1-11) \]

Quadrupolar excitation potential in a cubic ICR ion trap

As previously pointed out for azimuthal quadrupolar excitation,\(^\text{23}\) we need to solve a Laplace problem subject to the boundary conditions

\[ \Phi_{xy} = \begin{cases} V_{xy}(t) & x = \pm \alpha/2 \\ -V_{xy}(t) & y = \pm \alpha/2 \end{cases} \quad (2-2) \]

in which \(V_{xy}(t)\) is the excitation voltage applied to each "side" electrode. The potential for this boundary problem may be expressed as the superposition of two potentials

\[ \Phi_{xy}(x, y, z) = \Phi_1(x, y, z) + \Phi_2(x, y, z) \quad (2-3) \]

with boundary conditions:

\[ \Phi_1(x,y,z) = \begin{cases} 0 & y = \pm \alpha/2 \text{ or } z = \pm \alpha/2 \\ V_{xy}(t) & x = \pm \alpha/2 \end{cases} \quad (2-4a) \]
and

$$\Phi_2(x,y,z) = \begin{cases} 0 & x = \pm a/2 \text{ or } z = \pm a/2 \\ -V_{xy}(t) & y = \pm a/2 \end{cases}$$

(2-4b)

$\Phi_1$ and $\Phi_2$ are anti-symmetric with respect to reflection through the $x = y$ plane. Thus, to obtain the potential for the original geometry, we need only solve the Laplace equation for $\Phi_1(x,y,z)$, Eq. 1-7. Fortunately, the above boundary problem is equivalent to that for the electrostatic potential in a cubic trap (Eq. 1-11) with interchange of the $z$-axis for the $x$- or $y$-axis.

$$\Phi_1(x,y,z) = V_{xy}(t) \left[ \frac{1}{3} - \frac{\alpha}{\alpha^2} (z^2 + y^2 - 2x^2) \right]$$

(2-5a)

and

$$\Phi_2(x,y,z) = -V_{xy}(t) \left[ \frac{1}{3} - \frac{\alpha}{\alpha^2} (z^2 + x^2 - 2y^2) \right]$$

(2-5b)

Addition of Eqs. 2-5a and 2-5b gives

$$\Phi_{xy}(x, y, z) = \Phi_1(x, y, z) + \Phi_2(x, y, z) = \frac{3\alpha V_{xy}(t)}{\alpha^2} (x^2 - y^2)$$

(2-6)

Fig. 2.2 shows equipotential surfaces for three different potential configurations of an ICR ion trap of square cross-section. The electrostatic potential for a cubic trap is the familiar three-dimensional hyperbolic equipotential surfaces common to ICR and quadrupole ion traps.24 For a tetragonal ion trap extended infinitely along the axial ($z$-) direction, the dipolar potential used for excitation of ion cyclotron
Figure 2.2 Symmetry of each of three different electrical potentials in an ICR ion trap. Top: electrostatic trapping potential is approximately three-dimensional quadrupolar, resonant at $\omega_x$ (and $\omega_-$ and $\omega_z$). Middle: time-varying electric excitation for magnetron/cyclotron interconversion is approximately azimuthal two-dimensional quadrupolar, resonant at $\omega_C$. Right: Azimuthal dipolar excitation is one-dimensional spatially uniform. [From reference 2]
motion is approximately one-dimensional and the azimuthal quadrupolar excitation potential of Eq. 2-6 is two-dimensional. All three pictures represent approximate representations which become exact at the center of the trap.

**Equations of ion motion under azimuthal quadrupolar excitation**

During azimuthal quadrupolar excitation, the electric field is the superposition of the trapping and the excitation fields. From Eqs. 1-11 and 2-6,

\[
E = -\nabla [\Phi_q + \Phi_{xy}]
\]

\[
= -\nabla \left[ V_t \left( \frac{1}{3} - \frac{\alpha}{a^2} (x^2 + y^2 - 2z^2) \right) + \frac{3 \alpha V_{xy}(t)}{a^2} (x^2 - y^2) \right]
\]

\[
= \frac{m}{2q} \omega_z^2 [xi + yj] - \frac{m}{q} \omega_z^2 z k - \frac{3 \alpha V_{xy}(t)}{a^2} [x i - y j] \quad (2-7)
\]

in which

\[
\omega_z = \left( \frac{2 \alpha q V_t}{m a^2} \right)^{1/2} \quad (1-13b)
\]

is the axial or z-oscillation frequency. Eq. 2-7 may be analyzed into an equation for axial motion and one for azimuthal motion

\[
\dddot{z} + \gamma \dot{z} + \omega_z^2 z = 0 \quad (2-8)
\]
\[ \ddot{\rho} - \omega_c \mathbf{k} \times \dot{\rho} + \gamma \dot{\rho} - \frac{1}{2} \omega_z^2 \rho + \phi_{xy}(t) [x \mathbf{i} - y \mathbf{j}] = 0 \]  \hspace{1cm} (2-9)

in which

\[ \phi_{xy}(t) = \frac{3 q a V_{xy}(t)}{m \alpha^2} \]  \hspace{1cm} (2-10)

and

\[ \rho = x \mathbf{i} + y \mathbf{j} \]  \hspace{1cm} (2-11)

is the radial position vector.

It is algebraically convenient to express the azimuthal motion of Eq. 2-9 in complex coordinates \((x, iy)\)

\[ \ddot{\rho} + i \omega_c \rho + \gamma \dot{\rho} - \frac{1}{2} \omega_z^2 \rho + \phi_{xy}(t) \rho^* = 0 \]  \hspace{1cm} (2-12)

in which

\[ \rho = x + iy \]  \hspace{1cm} (2-13a)

\[ \rho^* = x - iy \]  \hspace{1cm} (2-13b)
V-vector representation of equations of motion for magnetron/cyclotron interconversion

Brown and Gabrielse\textsuperscript{1} originally suggested that the above azimuthal motion Eq. 2-12 may be further simplified by introduction of two new "V-vector" complex variables, $V^+$ and $V^-$

$$V^+ = V_x^+ + i V_y^- = \rho^+ - i \omega^- \rho$$  \hspace{1cm} (2-14a)

$$V^- = V_x^- + i V_y^- = \rho^- - i \omega^+ \rho$$  \hspace{1cm} (2-14b)

In which

$$\omega_\pm = \frac{1}{2} \left( \omega_c \pm \sqrt{\omega_c^2 - 2\omega_x^2} \right)$$  \hspace{1cm} (1-17)

Here $\omega_c$ is the unperturbed cyclotron frequency, $\omega_+$ is the reduced cyclotron frequency, and $\omega_-$ is the magnetron frequency. $V^+$ and $V^-$ represent the cyclotron and magnetron velocities, including both the orbital motion in the absence of power absorption and the time rate of change of cyclotron and magnetron radius due to power absorption. $V^+$ and $V^-$ may also be thought of as coordinates for cyclotron and magnetron motions treated as independent "normal modes".

The position vector, $\rho$, and its derivative ($\dot{\rho}$) may be expressed as functions of $V^+$ and $V^-$

$$\rho = -\frac{i(V^+ - V^-)}{\omega_+ - \omega_-}$$  \hspace{1cm} (2-15a)
and

\[ \dot{\rho} = \frac{\omega_+ V^+ - \omega_- V^-}{\omega_+ - \omega_-} \]  \hspace{1cm} (2-15b)

(The quantity, \( \omega_+ - \omega_- \), is called the "parametric" frequency.) The azimuthal motion equations may then be rewritten as

\[ \frac{dV^+}{dt} + i\omega_+ V^+ + \eta^+ V^+ + i \frac{\Omega(t)}{2} (V^{+*} - V^+) = 0 \]  \hspace{1cm} (2-16a)

\[ \frac{dV^-}{dt} + i\omega_- V^- - \eta^- V^- + i \frac{\Omega(t)}{2} (V^{-*} - V^-) = 0 \]  \hspace{1cm} (2-16b)

in which

\[ \eta^+ = \frac{\gamma \omega_+}{\omega_+ - \omega_-} \]  \hspace{1cm} (2-17a)

\[ \eta^- = \frac{\gamma \omega_-}{\omega_+ - \omega_-} \]  \hspace{1cm} (2-17b)

and

\[ \Omega(t) = \frac{2\phi_{xy}(t)}{\omega_+ - \omega_-} = \frac{6 q \alpha V_{xy}(t)}{m a^2 (\omega_+ - \omega_-)} \]  \hspace{1cm} (2-18)

and

\[ V^{+*} = V^+_x - i V^+_y \]  \hspace{1cm} (2-19a)

\[ V^{-*} = V^-_x - i V^-_y \]  \hspace{1cm} (2-19b)
(We shall later see that $\Omega(t)/2$ is the frequency of interconversion between magnetron and cyclotron modes during azimuthal quadrupolar excitation in the absence of collisional damping.) If only the interconversion between magnetron and cyclotron motion is considered, $\nu^+\nu$ and $\nu^-\nu$ may be dropped from Eqs. 2-16a and 2-16b, since they rotate twice as fast as $\nu^\nu$ and $\nu^\nu$, leaving

$$\frac{d\nu^+}{dt} + i \omega_+ \nu^+ + \eta^+ \nu^+ - i \frac{\Omega(t)}{2} \nu^-\nu = 0$$ \hspace{1cm} (2-20a)

$$\frac{d\nu^-}{dt} + i \omega_- \nu^- - \eta^- \nu^- + i \frac{\Omega(t)}{2} \nu^+\nu = 0$$ \hspace{1cm} (2-20b)

**RESONANT AZIMUTHAL QUADRUPOLAR EXCITATION**

Solution of the azimuthal ion motion Eqs. 2-20 is much simplified if the applied excitation voltage, $V_{xy}(t)$, oscillates at the resonant frequency of the conversion process, $\omega_c (= \omega_+ + \omega_-)

$$V_{xy}(t) = V_{xy0} \sin \omega_c t$$ \hspace{1cm} (2-21)

$\Omega(t)$ may then be rewritten as

$$\Omega(t) = 2k_0(e^{i\omega_c t} - e^{-i\omega_c t})$$ \hspace{1cm} (2-22)

in which

$$k_0 = \frac{3 q \alpha V_{xy0}}{2 i m \alpha^2 (\omega_+ - \omega_-)}$$ \hspace{1cm} (2-23)
As suggested by Bollen et al., we use the following transformation to further simplify Eqs. 2-22.

\[ V^+(t) = A^+(t) e^{-i \omega_+ t} \quad (2-24a) \]
\[ V^-(t) = A^-(t) e^{-i \omega_- t} \quad (2-24b) \]

Eqs. 2-24a and 2-24b may be interpreted as transformations to coordinate frames rotating at the cyclotron and magnetron frequencies, respectively. Substituting Eqs. 2-24 into Eqs. 2-16, we obtain equations describing the (much slower) motions in the cyclotron and magnetron rotating frames.

\[ \frac{dA^+}{dt} = -\eta^+ A^+ - ik_0 A^- \quad (2-25a) \]
\[ \frac{dA^-}{dt} = \eta^- A^- + ik_0 A^+ \quad (2-25b) \]

or, in x- and y-component equations,

\[ \frac{dA^+_x}{dt} = -\eta^+ A^+_x - k_0 A^-_y \quad (2-25c) \]
\[ \frac{dA^+_y}{dt} = -\eta^+ A^+_y - k_0 A^-_x \quad (2-25d) \]
\[ \frac{dA^-_x}{dt} = \eta^- A^-_x + k_0 A^+_y \quad (2-25e) \]
\[ \frac{dA^-_y}{dt} = \eta^- A^-_y + k_0 A^+_x \quad (2-25f) \]
Solution of the four equations is equivalent to a standard eigenvalue problem whose eigenvalues may be obtained from the roots of the characteristic equation

\[
\begin{pmatrix}
-\eta^+ - \lambda & 0 & 0 & -k_0 \\
0 & -\eta^+ - \lambda & -k_0 & 0 \\
0 & k_0 & \eta^- - \lambda & 0 \\
k_0 & 0 & 0 & \eta^- - \lambda
\end{pmatrix} = 0 \quad (2-26)
\]

The equation has two degenerate eigenvalues

\[
\lambda^\pm = \frac{(-\eta^+ + \eta^-) \pm \left( (\eta^+ + \eta^-)^2 - 4 |k_0|^2 \right)^{1/2}}{2} \quad (2-27)
\]

in which \( \eta^+ \), \( \eta^- \), and \( k_0 \) are defined by Eqs. 2-17 and 2-23 respectively. The solutions to Eqs. 2-25 may now be expressed as linear combinations of the eigenfunctions, \( e^{\lambda^+_+ t} \) and \( e^{\lambda^-_- t} \)

\[
A^+(t) = C_1 e^{\lambda^+_+ t} + C_2 e^{\lambda^-_- t} \quad (2-28a)
\]
\[
A^-(t) = D_1 e^{\lambda^+_+ t} + D_2 e^{\lambda^-_- t} \quad (2-28b)
\]

in which \( C_1 \), \( C_2 \), \( D_1 \), and \( D_2 \) are constants which depend on the initial conditions for \( A^+(t) \) and \( A^-(t) \) (or \( V^+(t) \) and \( V^-(t) \)).

\[
C_1 = (\lambda^- - \eta^-) \left( \frac{-(-\eta^+ + \lambda^+)A^+(0) + k_0 A^-(0)}{(\eta^+ + \lambda^+)(\eta^- - \lambda^-) - |k_0|^2} \right) \quad (2-29a)
\]
The final solution for \( V(t) \) and \( V'(t) \) may then be expressed in the form

\[
V(t) = C_1 e^{(\lambda^+ - i\omega_+)t} + C_2 e^{(\lambda^- - i\omega_-)t}
\]

(2-30a)

\[
V'(t) = D_1 e^{(\lambda^+ - i\omega_+)t} + D_2 e^{(\lambda^- - i\omega_-)t}
\]

(2-30b)

**Relation between \( V \)-vectors and cyclotron and magnetron radius vectors**

Up to this point, the \( V \)-vector formulation have extensively been used to derive the equations of ion motion. Although the \( V \)-vectors defined in Eqs. 2-14 completely describe the ion azimuthal position and velocity at any given instant, it is useful to relate them to the more familiar cyclotron and magnetron radius. In the absence of excitation, the position (in complex notation, as usual), \( \rho \), of an ion has been derived as the sum of the two independent cyclotron and magnetron components

\[
\rho = x + iy = \rho_+ e^{-i\omega_+ t} + \rho_- e^{-i\omega_- t}
\]

(1-16)
in which \( p_+ \) and \( p_- \) are the cyclotron and magnetron radii. The relation between the rotating-frame A-vectors (Eqs. 2-28) and the cyclotron and magnetron radii may be found by substituting Eqs. 2-24 and Eqs. 2-15a into Eq. 1-16 to give

\[
\rho_+ e^{-i\omega_+ t} + \rho_- e^{-i\omega_- t} = -\frac{i(A^+ e^{-i\omega_+ t} - A^- e^{-i\omega_- t})}{\omega_+ - \omega_-}
\]  
(2-31)

By comparing the coefficients preceding \( e^{-i\omega_+ t} \) and \( e^{-i\omega_- t} \), we find

\[
\rho_+ = -\frac{1 A^+}{\omega_+ - \omega_-}
\]  
(2-32a)

\[
\rho_- = \frac{1 A^-}{\omega_+ - \omega_-}
\]  
(2-32b)

Finally, from the relation between the rotating-frame A-vectors and the lab-frame V-vectors (Eqs. 2-24), we find the relation between the V-vectors and magnetron and cyclotron radius

\[
\rho_+ = -\frac{i V^+_e e^{i \omega_+ t}}{\omega_+ - \omega_-}
\]  
(2-33a)

\[
\rho_- = \frac{i V^-_e e^{i \omega_- t}}{\omega_+ - \omega_-}
\]  
(2-33b)

With Eqs. 2-33 and 2-30, we can describe the trajectory of ion during resonant azimuthal quadrupolar excitation and/or collisional damping.

Fig. 2.3 illustrates the trajectory of a singly charged ion \((m/z = 10000\) e/u) trapped by 1 V trapping voltage and 3 T magnetic field in a 5 cm
cubic trap. We set conditions in which the damping constant is equal to 500 s\(^{-1}\) (~5 × 10\(^{-7}\) torr) and rf excitation amplitude (V\(_{pp}/2\)). Initial cyclotron radius, magnetron radius are 0.3 V, 5 mm, 2 cm. The final radius of each motion was 0.6 mm and 0.4 mm for cyclotron and magnetron motion due to 50 ms quadrupolar resonant (at \(\omega_c\)) excitation in the presence of collision gas. At every 0.01 sec, the accumulated ion trajectory in the xy-plane perpendicular to the magnetic field is displayed with continuous radial trajectory. We easily find the decreasing radial distribution. The initial z-direction oscillation motion, 1 cm, shows fast damping rate and final amplitude in z-direction is ~5 × 10\(^{-6}\) cm. Next we simplify the initial conditions with reasonable assumptions and systematically analyze the ion motion during axialization.

**Ion trajectory during resonant azimuthal quadrupolar excitation and/or collisional damping**

Ion trajectories for resonant azimuthal quadrupolar excitation and/or collisional damping are completely described by the solution (Eqs. 2-20) of the azimuthal equation of ion motion. The various qualitatively different ion behaviors will be considered corresponding to different experimental conditions.
Radial Trajectory

Axial Trajectory

Figure 2.3 The radial trajectory of ion during the axialization by azimuthal quadrupolar excitation and collisional cooling.
COLLISIONAL DAMPING, NO XY EXCITATION \((V_{xy0} = 0)\)

In the absence of \(xy\)-excitation \((k_0 = 0\) on the ordinate axis in Fig. 2.3), the eigenvalues are real, \(\lambda^+ = -\eta^+\) and \(\lambda^- = \eta^-\). Cyclotron and axial motions behave as damped harmonic oscillators (i.e., negative damping rate constant in Eqs. 2-34a and 2-34c, respectively), whereas magnetron velocity increases exponentially with time (i.e., positive damping rate constant in Eq. 2-34b).

\[
V^+(t) = V^+(0) e^{-\eta^+ t} e^{-i\omega_+ t} \quad (2-34a)
\]
\[
V^-(t) = V^-(0) e^{\eta^- t} e^{-i\omega_- t} \quad (2-34b)
\]
\[
z(t) = z(0) e^{-\gamma t/2} \sin(\omega_z t) \quad (2-34c)
\]

In more familiar terms, we may use Eqs. 2-33 to express Eqs. 2-34a and 2-34b in terms of cyclotron and magnetron radii.

\[
\rho^+(t) = -\frac{i V^+_e^{i\omega_+ t}}{\omega_+ - \omega_-} = -\frac{i V^+_e(0)}{\omega_+ - \omega_-} e^{-\eta^+ t} \quad (2-34d)
\]
\[
\rho^-(t) = \frac{i V^-e^{i\omega_- t}}{\omega_+ - \omega_-} = \frac{i V^-e(0)}{\omega_+ - \omega_-} e^{\eta^- t} \quad (2-34e)
\]

The damping rate constants for cyclotron and axial motions are \(\eta^+ (= \gamma \omega_+/(\omega_+ - \omega_-))\) and \(\gamma/2\), respectively. The rate constant for increase in magnetron motion is \(\eta^- (= \gamma \omega_-/(\omega_+ - \omega_-))\). The trajectories of the three motions are shown in Fig. 2.1 along with a graph showing the time
evolution of cyclotron radius, magnetron radius, and trapping oscillation amplitude on a common time scale. Note that the cyclotron radius damps about twice as fast, $\gamma \omega_+/(\omega_+ - \omega_-)$ as the trapping oscillation amplitude, and that the cyclotron radius damps much faster ($\omega_+/\omega_-$) than the magnetron radius grows with time due to ion-neutral collisions. These relative amplitudes account for the efficacy of azimuthal quadrupolar excitation for axialization of ions, because such excitation converts magnetron motion to cyclotron motion, which is then collisionally damped rapidly so that ions end up with very small magnetron radius (i.e., "axialized"). Fig. 2.4 illustrates the trajectory of ion which is in the same condition as described in Fig. 2.3. for axialization, ion will escape the trap within 2 ms by the collisional magnetron expansion.

**AZIMUTHAL QUADRUPOLAR EXCITATION AT FREQUENCY $\omega_c$**

In this section, the initial axial amplitude and cyclotron radius are taken to be zero. This assumption is justified in actual experiments, because (see Figure 2.1) cyclotron and axial motion are rapidly cooled to near-zero amplitude by ion-neutral collisions, before azimuthal quadrupolar excitation is applied. Because the exponential growth in magnetron radius is much slower than damping of cyclotron and axial motions, such initial cooling does not result in ion radial diffusive loss.\textsuperscript{25}
Figure 2.4  The collisional expansion of magnetron motion without azimuthal quadrupolar excitation
No collisional damping ($\gamma = 0$), magnetron /cyclotron interconversion

In the zero-pressure limit (i.e., zero collisional damping), the eigenvalues become

$$\lambda^+ = -i \omega_{\text{beat}} \quad (2-35a)$$

$$\lambda^- = i \omega_{\text{beat}} \quad (2-35b)$$

in which

$$\omega_{\text{beat}} = |k_0| = \frac{3q_0 V_{xy0}}{2m\alpha^2(\omega_+ - \omega_-)} = |\Omega(t)|/2 \quad (2-36)$$

If the initial cyclotron radius is zero, $V^+(0) = 0$, the V-vector may be written

$$V^+(t) = V^-\sin(\omega_{\text{beat}} t) e^{-i\omega_+ t} \quad (2-37a)$$

$$V^-(t) = V^-\cos(\omega_{\text{beat}} t) e^{-i\omega_- t} \quad (2-37b)$$

or

$$\rho_+(t) = \frac{V^-}{\omega_+ - \omega_-} \sin(\omega_{\text{beat}} t) \quad (2-37c)$$

$$\rho_-(t) = \frac{1}{\omega_+ - \omega_-} \cos(\omega_{\text{beat}} t) \quad (2-37d)$$

It is clear that initially pure magnetron motion is converted into pure cyclotron motion after an irradiation period, $t = \pi/(2 \omega_{\text{beat}})$ and pure
Figure 2.5  Successive stages of ion motion during azimuthal quadrupolar excitation, in the absence of collisional damping.
cyclotron motion is converted back into pure magnetron motion after an equal additional period (see Fig. 2.5). It is of interest to note that the cyclotron motion and magnetron motions have 90° initial phase difference, even though the initial cyclotron amplitude is zero (see Eqs. 2-37c and 2-37d).

**Collisional damping and azimuthal quadrupolar excitation**

With quadrupolar excitation, the magnetron radius is also damped by collisions. From the Eqs. 2-33, we calculate the trajectory of ion excited by different azimuthal quadrupolar excitation voltages at the same conditions except 10 times lower pressure \((\sim 5 \times 10^{-8}\) torr\) than the previous trajectory calculation (Fig. 2.3). We find that higher pressure of cooling gas will decrease the volume of the radial distribution faster than lower pressure with the same axialization period \((\sim 0.04\) s\). However, the high amplitude of azimuthal quadrupolar excitation voltage will excite the cyclotron motion and thus result in a large ion radius (see Fig. 2.6(a)). If we use lower amplitude of azimuthal quadrupolar excitation voltage, the slowly increased cyclotron motion maintains the small radius of ion motion by collisional damping until complete conversion of magnetron motion to cyclotron motion, as in Fig. 2.6(d). Thus, in ion axialization experiments, it is desirable to apply quadrupolar excitation with an amplitude as low as possible. At that excitation amplitude, the final ion radius will be minimized.
Figure 2.6 The ion trajectory during azimuthal quadrupolar excitation with different amplitudes. (a) 0.1 V, (b) 0.01 V, (c) 0.001 V, (d) 0.0001 V, damping constant = 50 s\(^{-1}\)
BROADBAND AZIMUTHAL QUADRUPOlar EXCITATION

The theory described in the previous sections shows that ions of a single mass to charge (m/z) ratio (i.e., a single \( \omega_c \) value) may be axialized by azimuthal quadrupolar excitation at the unperturbed cyclotron frequency, \( \omega_c \). For analytical mass spectra, it is more desirable to axialize ions over a broad m/z range. The high-amplitude single-event azimuthal quadrupolar excitation has not been successful in producing highly selective magnetron-to-cyclotron conversion over a wide m/z range.

However, if the azimuthal quadrupolar excitation is sufficiently low in amplitude, then the corresponding magnetron-to-cyclotron conversion process is linear and we may therefore apply a train of low-amplitude successive identical stored-waveform inverse Fourier transform (SWIFT) excitations\(^{20}\) (Fig. 2.7, top), in azimuthal quadrupolar mode, to ions in the presence of a buffer gas. The stored waveform is generated by inverse Fourier transform of a desired magnitude-mode excitation spectrum with appropriate phase modulation.\(^{26,27}\) Each SWIFT irradiation converts a fraction of the magnetron motional amplitude into cyclotron motional amplitude (Fig. 2.7, middle); during the interval between SWIFT excitations; the cyclotron motion quickly damps to near-zero radius (Figure 12, bottom), and the magnetron radius expands only slightly (see the previous section). The process is then repeated, thereby successively reducing the magnetron radius stepwise to zero, and leaving the ions well-localized near the center of the trap. Because the magnetron radius
Azimuthal Quadrupolar SWIFT Irradiation

Figure 2.7 Broad-band axialization procedure. A train of azimuthal quadrupolar SWIFT excitations reduces magnetron radii of ions stepwise. Each step converts only a fraction of magnetron radius to cyclotron radius and the cyclotron radius damps nearly to zero before the next repetition. After many such stages, the large initial magnetron radius is reduced to zero. Reproduced by permission from Guan et al. [Reference 20]
is reduced by only a few percent by any one SWIFT irradiation, the azimuthal quadrupolar excitation process becomes linear. 28

APPLICATIONS OF ION AXIALIZATION

For optimal ICR detection, ions should initially possess zero kinetic energy and be located near the center of the trap. After excitation of cyclotron motion, ions have a relatively high velocity; thus, detection should be conducted at low pressure in order to avoid collisional damping of the cyclotron motion. 29 As discussed in the previous sections, ion cyclotron and axial motions can be damped effectively by ion-neutral collisions, and magnetron radius can be reduced by azimuthal quadrupolar excitation in the presence of collisional damping. Unfortunately, in order to reduce the initial ion kinetic energy and localize ions to the center of the trap, one must carry out cooling and axialization procedures at relatively high collision gas pressure, which would appear to be incompatible with high mass resolving power by conventional FT-ICR excite/detect procedures.

However, ion axialization and ion detection need not take place at the same location and/or at the same time. An obvious solution is to axialize ions at high pressure in a first ICR ion trap, and then transport them to a second spatially separated trap at lower pressure for excitation/detection. Alternatively, a buffer gas may be pulsed into the trap to provide for collisional damping during the axialization process,
and then pumped away before excitation/detection in the same trap. Both methods are in use.

For the first method, we used a dual-trap instrument\textsuperscript{15}, in which ions were axialized at an argon pressure of \( \sim 5 \times 10^{-7} \) torr in a source trap for 10-30 s and then transferred through a conductance limit to an analyzer trap for high-resolution detection. A schematic wiring diagram for producing azimuthal quadrupolar excitation in the source trap and carrying out dipolar excitation/dipolar detection in the analyzer trap is illustrated in Fig. 2.8. The second method has been demonstrated by Speir \textit{et al.} in a single-trap instrument for improvement of ion remeasurement efficiency\textsuperscript{17}. Finally, we have also developed a multiply-pulsed collision gas technique for ion axialization in a dual trap for further improving mass resolving power of laser-desorbed ions.\textsuperscript{18} Very rapid progress has been made in the application of ion axialization since it was introduced into the field of FT-ICR/MS in 1991.
Figure 2.8 Trap configuration for quadrupolar excitation (left) and conventional dipolar excitation (right). In the present experiments, ions are axialized in the presence of buffer gas in the source trap (left) and then passed to the analyzer trap (right) for conventional high-resolution dipolar excitation/detection at low pressure. [Reference 2.2]
Prolonged and efficient ion trapping at high collision gas pressure

An important feature of the ion axialization technique is its ability to trap ions at high neutral gas pressure for a long time period. Ions generated by a variety of ionization methods possess high kinetic and internal energy. However, a wide distribution in ion kinetic energy is undesirable for ion detection in any mass analyzer, including ICR.

In ion axialization experiments, ions in any region of the trap are brought back to the center of the trap by application of low amplitude azimuthal quadrupolar excitation voltage on the electrodes parallel to the magnetic field. With the axialization method, ions may be trapped at a high collision gas pressure for a prolonged period allowing a large number of ion neutral collisions. The long trapping period allows very slow ion molecule reactions to be observed. Also, it is possible to cool ion internal and external energies to thermal temperature and therefore avoid unimolecular decomposition due to high-energy deposition during ionization processes.

Trapping efficiency is clearly enhanced by quadrupolar excitation/collisional cooling because ion kinetic energy after ion formation, partitioned into cyclotron, axial, and magnetron motions upon entry into the trap, is effectively removed. Axialization should therefore prove very useful in extending the m/z range of laser-desorbed ions detectable by FT-ICR/MS. High ion kinetic energy following the
desorption event limits the highest observable \( m/z \), because ions of high kinetic energy have large cyclotron radii. With our recently introduced thin gold film-assisted laser desorption/ionization technique (see chapter 5), we have been able to trap efficiently peptide ions up to \( m/z \sim 2000 \) after axialization, even though the signal for the same ions without axialization was almost undetectable. More experimental results will be discussed in chapter 5.

**Enhanced mass resolving power**

Because ions can be cooled and axialized in the source trap and detected at a much lower pressure in the analyzer trap, high mass resolving power may be achieved. The increased mass resolving power is likely due to the collisional cooling of ion initial kinetic energy prior to excitation/detection. Because damping of cyclotron and axial motions is much faster than magnetron expansion, collisional cooling for a short period (\( \sim 0.5 \text{ s} \), without axialization) removes ion kinetic energy without causing significant ion cloud radial expansion. The collisionally cooled ions are therefore localized in a region of more homogeneous magnetic and electric field during excitation and detection; consequently, inhomogeneous spectral broadening is reduced. However, due to the high pressure in the source trap, the time-domain ICR signal damps too quickly to allow ultrahigh mass resolving power. If ions are axialized, the cooled ions may then be transferred to the analyzer trap where the
pressure is about two orders of magnitude lower—they may be detected at much higher mass resolving power.

Clearly it is desirable to maintain the analyzer trap at the lowest pressure possible to achieve high mass resolving power during detection. Axialization compresses an ion cloud to very small radial dispersion and should therefore allow for use of a conductance limit smaller than the $\sim 2$ mm diameter in our current instrument, to provide a larger pressure difference between the two ion traps. The analyzer trap pressure may be lowered even further by use of pulsed collision gas into the source trap for axialization. Finally, the frequency drift correction, which will be discussed in next chapter, may improve the resolving power further.

**Mass-selective axialization**

The ion axialization method is inherently mass-selective. For single-frequency azimuthal quadrupolar excitation, only ions resonant to the excitation (i.e., excitation frequency equal to the unperturbed cyclotron frequency) will be axialized. The illustration of the selectivity of the axialization process at high mass is shown in Fig. 2.9. Laser desorption of a gold film coated on a glass plate yields a wide distribution of gold cluster anions (Fig. 2.9, top). The gold cluster $\text{Au}_{25}^-$ may be selectively axialized from the $\text{Au}_n^-$ mixture by application of quadrupolar excitation in an Ar atmosphere ($4 \times 10^{-7}$ torr) followed by subsequent transfer to the analyzer trap for excitation and detection (Fig. 2.9, bottom).
For analytical purposes, axialization of ions over a broad m/z range is highly desirable, and may be achieved by the application of repeated low-amplitude SWIFT excitations to produce magnetron/cyclotron conversion. Fig. 2.10 illustrates the highly mass-selective isolation of ions of 1,050 ≤ m/z ≤ 1,450 from a poly(ethylene glycol) sample (mixture of PEG 1000 and PEG 5000) originally containing components ranging from ~850 ≤ m/z ≤ 2,100.

In an ICR ion trap with perfectly quadrupolar electrostatic trapping potential and spatially uniform dipolar excitation and detection fields, mass selectivity from axialization of ions of desired m/z range(s) should be comparable to that obtained by selective dipolar SWIFT radial ejection of ions of the complementary undesired m/z range(s).\textsuperscript{27,32-37} Nevertheless, we believe that axialization is preferable for several reasons. First, axialization requires relatively low amplitude excitation voltage to keep ions near the center of the trap, whereas radial ejection requires higher excitation voltage because ions must be driven to the walls of the trap to achieve radial ejection. Second, for the common case that ions of a single or very narrow range of m/z values are selected, quadrupolar excitation is narrow-band whereas radial ejection is broadband, so that axialization requires even lower amplitude. Third, the axialization mass selectivity demonstrated here was achieved with a relatively large conductance limit aperture (2 mm diameter); higher selectivity could be achieved with a smaller conductance limit aperture.
Figure 2.9 Axialization for high-resolution mass selectivity. A minor component, Au$_{25}^-$, from a mixture of gold cluster anions generated by a Nd:YAG laser pulse from a thin gold film (top) may be selectively axialized and transferred to the analyzer trap for detection (bottom). Such experiments make possible improved parent ion selectivity for MS/MS.
PEG 1000 and PEG 1500
MALDI FT-ICR/MS with 3rd Harmonic of Nd:YAG laser

Figure 2.10 Broadband mass-selective axialization of poly(ethylene glycol) ions. Top: matrix-assisted laser desorption/ionization (355 nm third harmonic of Nd:YAG laser), dihydroxybenzene matrix doped with KBr and fructose, of a mixture of PEG 1000 and PEG 1500, showing quasi-molecular (M+K)+ ions ranging from 850 ≤ m/z ≤ 2,100. Bottom: broadband mass-selective axialization of ions of 1,050 ≤ m/z < 1,450. Note the sharp selectivity without loss in signal-to-noise ratio. [Reference. 2.2]
**Ion capture efficiency from external ion injection**

Another obvious application for axialization is for improved efficiency in injection of externally formed ions into an ICR ion trap. The problem is that during injection of ions from a low-current continuous ion beam, the ions which arrive first in the trap begin to diffuse radially outward due to collision-induced magnetron orbit expansion. Thus, by the time later-arriving ions show up, the ions which arrived earlier have leaked out, so that it is impossible to fill the trap with ions even after an indefinitely long injection period. Therefore, it is preferable to fill the trap with ions from a series of pulsed injections (each accompanied by pulsed buffer gas during axialization), as recently demonstrated by Hasse et al.\(^{38}\) for Au\(_n^+\) ions, for a continuous beam of electrosprayed ions of a single \(m/z\) ratio,\(^{39}\) and for continuously injected ions of a wide range of \(m/z\) ratio.\(^{40}\)

**CONCLUSIONS**

Ion axialization by quadrupolar excitation/collisional cooling is still a very new technique. Nevertheless, it already has an assured place in the arsenal of FT-ICR techniques for improved signal-to-noise ratio, improved mass resolving power, extended ion trapping period for efficient cooling of internally excited ions and study of slow unimolecular
processes, higher mass selectivity for MS/MS, higher CID efficiency, improved ion capture efficiency, and higher ion remeasurement efficiency.

Future improvement should include the use of a smaller conductance limit aperture for improved differential pumping and higher mass resolving power. Future applications should include improved efficiency for photodissociation (since ions can be packed closer together to intercept all of the laser beam), as well as direct detection of optical absorption of trapped mass-selected ions.
REFERENCES


CHAPTER III

FREQUENCY DRIFT CORRECTION IN FOURIER TRANSFORM SPECTROSCOPY

INTRODUCTION

Apart from the resolving power improvement by axialization\textsuperscript{1-10} during the experimental event sequence, the accumulated data also can be improved to generate the spectrum with higher resolving power than that of the original spectrum. There are several different ways to enhance the information content of the accumulated data. For example, the multiplication of the time domain data with a suitable function will generate a frequency domain spectrum with either improved resolving power or higher signal-to-noise ratio. The detailed properties of these manipulation will be discussed in the following chapter. Another very practical and useful method to increase the resolving power in high resolution FT/ICR/MS is the correction of frequency drift during detection.

At sufficiently low pressure, the resolution of an FT/ICR spectrum is not dominated by collisional broadening. The inhomogeneous electric
field experienced by the ions during the detection period is the dominant factor. Guan and Marshall recently proposed the use of digital quadrature heterodyne data reduction for correcting the frequency drift, however, the program code heavily depends on the specific data station. Here we will introduce an improved algorithm for frequency drift correction with ANSI compatible C language that is easily used on any general desktop PC with a C compiler. The correction method is applied to generate an ultra-high resolution mass spectrum of insulin B chain and will be used on various data from samples discussed in later application chapters.

**THEORY**

The frequency drift is observed by Fourier transformation of successive time domain data segments. For example, 16 K time domain data points can be separated into eight equal segments of 2 K data points each. After Fourier transformation, the eight reduced cyclotron frequencies are plotted vs. time and fitted by polynomial expansion of maximum seventh order (less than the number of segments) of in time as shown in Eq. 3-1

\[
\omega_+(t) = \omega_+(0) + a_1 t + a_2 t^2 + \cdots = \omega_+(0) + \sum_{n=0}^{\infty} a_n t^n
\]
in which the sum is the time dependent deviation. It can be expanded by polynomial expansion to maximum order limited by the length and number of segments of time domain data.

The exponentially damped sinusoidal time-domain signal can be described by Eq. 3-2

\[ f(t) = A e^{-t/\tau} \cos[\phi(t)] \]  

or

\[ = A e^{-t/\tau} \frac{e^{+i\phi(t)} + e^{-i\phi(t)}}{2} \]  

in which \( A \) is the initial signal amplitude, \( \tau \) is the exponential damping constant, and \( \phi(t) \) is the instantaneous cyclotron phase. The angular velocity can be derived from the derivative of phase vs. time, Eq. 3-3.

\[ \omega_+(t) = \frac{d \phi(t)}{dt} \]  

Therefore, the instantaneous cyclotron phase can be determined from integrating the experimentally determined frequency expression, Eq. 3-1, with respect to time and thus the time domain signal can be described as

\[ f(t) = A e^{-t/\tau} \cos[\phi(t)] = A e^{-t/\tau} \cos\left(\phi_0 + \omega_+(0)t + \delta(t)\right) \]  

\[ \delta(t) = \frac{a_1}{2} t^2 + \frac{a_2}{3} t^3 + \frac{a_3}{4} t^4 + \ldots = \sum_{n=1}^{\infty} \frac{a_n}{n+1} t^{n+1} \]

in which, \( \delta(t) \) is the time-dependent frequency drift. From the fit of frequency change to time, we can calculate the coefficients, \( a_n \). Finally,
we can remove the frequency drift by the multiplication of the original data with an exponential function which has the same drift but opposite sign.

\[
f(t) = A e^{-t/\tau} \left( \frac{e^{+i(\phi_0 + \omega+(0)t + \delta(t))} + e^{-i(\phi_0 + \omega+(0)t + \delta(t))}}{2} \right) \times e^{-i\delta(t)}
\]

\[
= A e^{-t/\tau} \left( \frac{e^{+i(\phi_0 + \omega+(0)t)} + e^{-i(\phi_0 + \omega+(0)t + 2\delta(t))}}{2} \right)
\]  (3-5a)

Fig. 3.1, shows the time domain signals for a counter-clockwise rotation which produces a delta function signal in the real positive frequency spectrum (Fig. 3.1). The direction of rotation determines the sign of the frequency. The angular velocity sets the position in the
frequency domain. Finally if we assume a zero initial phase then the final spectrum will have only real components. (Even with nonzero initial phase the phase can be corrected; besides, FT-ICR data is displayed in absolute value (magnitude) mode because phase information is not usually relevant.) The ICR time-domain data is collected as a real sinusoidal signal. It can be converted to complex form, Eq. 3-2b, for Fourier transformation to obtain a frequency domain spectrum. The resulting frequency domain spectrum has both positive and negative frequencies with time dependent deviation, $\delta(t)$. If we correct the frequency drift as in Eq. 3-5b, then we find the corrected positive frequency and a negative frequency signal broadened by a factor of 2. Usually the negative and positive frequencies have exactly the same information, therefore we ignore the negative frequency. The positive frequency component is the frequency drift corrected high resolution mass spectrum. Fig. 3.2 illustrates the corrected high resolution positive frequency and the broadened negative frequency signal.

**EXPERIMENT**

The time domain data of insulin B chain mass spectrum has been acquired as discussed in chapter 8. 128 K time-domain data were collected in heterodyne mode at 1185 Hz signal bandwidth and 14226 Hz reference frequency with 0.422 ms dwell time. The data transferred from an Extrel-Odyssey® data station to a Macintosh Quadra 840AV as a
\[ f(t) = e^{i(\omega_0 t)} + e^{i(\omega_0 t + 2\delta)} = e^{i2\pi \nu_0 t} + e^{i(2\pi \nu_0 t + 2\delta)} \]

\[
\begin{align*}
\text{Real}[F(v)] & \quad \text{Imag}[F(v)] \\
\nu_0 + 2\delta/2\pi & \quad \nu_0 \\
0 & \quad 0
\end{align*}
\]

\[ F(t) = \delta(v - \nu_0) + \delta(v + (\nu_0 + 2\delta/2\pi)) \]

**Figure 3.2** Complex Fourier transformation of frequency drift corrected time domain signal. The positive frequency is corrected and negative frequency is broadened.

ASCII code by use of a program provided by Waters Extrel FTMS. Time-domain data sets were segmented into 32 equal size data sets and each transient was Fourier transformed on a Macintosh Quadra 840AV computer (Apple Computer, Cupertino, CA) with 40 MHz Motorola 68040 CPU and 64 megabyte RAM, with an algorithm compiled with THINK C Version 7.0.3 (Symantec Corporation, Cupertino, CA). A standard Cooley-Tukey FFT algorithm\(^ \text{12} \) converted the ICR time-domain data to a frequency-domain spectrum, which was converted to a mass spectrum by a standard mass calibration formula based on a pure quadrupolar trapping potential.\(^ \text{13} \) The frequency drift functions were calculated and
mass spectra were plotted by a KaleidaGraph Version 3.0.1 (Synergy Software, Reading, PA) spreadsheet program.

RESULTS AND DISCUSSION

Frequency drift during detection. Fig. 3.3 illustrates the asymmetric peak shape of the insulin B chain molecular ion signal; the higher mass side (right side) is wider than lower mass side of the molecular ion peak. The distortion been interpreted as arising from time dependent frequency change during the detection (frequency drift). Also, note the low resolving power (~3000) and low signal-to-noise ratio (~16). To apply the frequency drift correction as previously described, we cut the time domain data each into 32 segments of 4 K time domain data and we use only the first 31 segments because the signal-to-noise ratio drops below 2 for the 32nd segment. Fig. 3.4 shows a frequency drift of 35 Hz over a 55.3 second detection period. The instantaneous frequencies for each 1.7285 second period were fitted vs. time to a ninth order polynomial expansion. The frequency drift coefficients were determined by the fit.

Frequency drift correction. The calculated frequency drift was removed from the time domain data by multiplication of the exponential function, which include the time dependent phase shifting by the space charge potential as in Eqs. 3-5, with the time domain data. The corrected frequency domain data shows a wider peak on the lower
Figure 3.3  Fourier transform mass spectrum of insulin B chain without frequency drift correction.
Figure 3.4  Cyclotron frequency change of insulin B chain molecular ion during detection.
frequency side and the sharper and higher peak on the high frequency side. The negative frequency peak appears on the positive side instead of being on the negative frequency side, because we detect the signal with heterodyne detection mode. The advantage of frequency drift correction is not only the higher resolving power but also the improvement in signal-to-noise ratio. For insulin B chain, we increased the resolving power by approximately a factor of ten and improved signal-to-noise ratio as in Fig. 3.6. Additional applications of this method will be shown in chapter 8.

CONCLUSION

The peak shape of the mass spectrum was improved by correction of the frequency drift caused by the inhomogeneous electrostatic field during the detection period. After frequency drift correction, resolving power and signal-to-noise ratio are dramatically improved. Several points in drift correction should be discussed. First, the number of segments will be limited by the lower resolution of reduced number of data points in each segment, but the fitting process inherently has an averaging effect and some resolving power limitation can be relaxed by the fitting process. Second, the length of the time domain signal should be long enough to produce a spectrum with high resolving power. Finally, a higher order polynomial expansion in the fitting function calculates the drift function with higher accuracy, but the maximum order of the polynomial expansion will be limited by the number of segments.
Figure 3.5  Frequency drift corrected frequency domain spectrum of cyclotron frequency of insulin B chain molecular ion
Insulin Chain B

Resolving Power

= 35000

Figure 3.6 Frequency drift corrected mass spectrum of insulin B chain molecular ion
REFERENCES


CHAPTER IV

Magnitude-Mode Multiple-Derivative Spectra for Resolution Enhancement without Loss in Signal-to-Noise Ratio in Fourier Transform Spectroscopy

INTRODUCTION

It has long been recognized that the frequency-derivative of a spectrum aids in visual resolution of overlapped spectral peaks in (e.g.) optical\textsuperscript{1,2} and electron paramagnetic resonance\textsuperscript{3} spectroscopy, by sharpening inflection points of the original spectrum. With the advent of the fast Fourier transform (FFT) algorithm, a particularly simple method for obtaining the \textit{n}th frequency-derivative of a spectrum is simply to multiply the corresponding time-domain discrete signal by \(t^n\) before performing an FFT computation.\textsuperscript{1,4} However, two obvious and important disadvantages of the derivative FT spectrum are: (a) a decrease in spectral signal-to-noise ratio (since multiplication by \(t^n\) gives highest weight to data points at the end of the time-domain data set, where signal-to-noise ratio is poorest), and (b) an asymmetrical (for odd-integral
derivatives) and/or multiple-lobed (for odd- or even-integral derivatives) spectral line shape.

In an approach initially directed at FT-NMR spectra, Y. Balcou recently proposed the use of FFT methods for producing an $n$th derivative discrete spectrum, but with two significant improvements: (a) additional time-domain weighting with a damped-exponential function (Cameron and Moffatt had used time-domain sinc weighting) to counteract the loss in signal-to-noise ratio produced by $t^n$ weighting, and (b) magnitude-mode display to eliminate negative-lobed peaks in odd-integral derivative spectra. In this chapter a systematic analysis of Balcou's derivative method has been applied to simulated and experimental Fourier transform ion cyclotron resonance (FT-ICR) mass spectral data.

**THEORY**

Let $F(\omega)$ denote the (mathematically complex) frequency-domain spectrum of a time-domain signal, $f(t)$, which has been acquired for an infinite length of time.

$$F(\omega) = \int_{-\infty}^{\infty} e^{-i\omega t} f(t) \, dt$$  \hspace{1cm} (4-1)
It is easily shown that the $n$'th derivative of $F(\omega)$ with respect to frequency is simply the Fourier transform of the original time-domain signal, $f(t)$, multiplied by the weight ("window") function, $(-1 \cdot t)^n$.\(^4\)

$$\frac{d^nF(\omega)}{d\omega^n} = \frac{d^n}{d\omega^n} \left( \int_{-\infty}^{\infty} e^{-i\omega t} f(t) \, dt \right) \quad (4-2a)$$

$$= \int_{-\infty}^{\infty} e^{-i\omega t} \left( (-1 \cdot t)^n \cdot f(t) \right) \, dt$$

or

$$\frac{d^nF(\omega)}{d\omega^n} = FT \left( (-1)^n \cdot t^n \cdot f(t) \right) \quad (4-2b)$$

in which $FT$ denotes the Fourier transform operation. In Eq. 4-2b, the factor, $(-1)^n$, shifts the phase of the absorption and dispersion mode of Fourier transform spectrum by $3\pi n/2$, so that absorption and dispersion spectra in successive derivatives are interchanged with alternate sign. However, the magnitude-mode spectrum, $|F(\omega)|$, is phase-independent (see Eq. 4-3). Thus, the magnitude of the $n$th derivative of the complex frequency-domain spectrum is simply equal to the magnitude of the Fourier transform of the time-domain signal weighted by a $t^n$ "window" function.

$$\left| \frac{d^nF(\omega)}{d\omega^n} \right| = \left| FT \left[ t^n \cdot f(t) \right] \right| \quad (4-3)$$

We may proceed to characterize the properties of the derivative spectrum analytically by assuming a particular functional form for $f(t)$. 
For an exponentially damped sinusoidal time-domain signal of infinite duration,

\[ f(t) = A e^{-t/\tau} \cos(\omega_0 t); \quad 0 \leq t \leq \infty \]  

(4-4)

in which A is the time-domain signal initial amplitude, \( \tau \) is the exponential damping constant, and \( \omega_0 \) is the "natural" frequency of the system of interest, the (complex) Fourier transform spectrum, \( F(\omega) \), is given by Eq. 4-5.

\[ F(\omega) = FT \left[ f(t) \right] = \frac{A}{2} \int_0^\infty (e^{i \omega_0 t} + e^{-i \omega_0 t}) e^{-t/\tau} e^{i \omega t} dt \]  

(4-5)

Because \( F(\omega) \) is symmetric about \( \omega = 0 \), we henceforth consider only the positive-frequency (physically relevant) spectral range.

\[ F(\omega) = \frac{A}{2} \left( \frac{\tau}{1 + (\omega - \omega_0) \tau} \right) \]  

(4-6a)

\[ = \frac{A}{2} \left( \frac{\tau}{1 + (\omega - \omega_0)^2 \tau^2} + \frac{\omega_0 \tau^2}{1 + (\omega - \omega_0)^2 \tau^2} \right) \]  

(4-6b)

Direct differentiation \( n \) times of frequency-domain signal, Eq. 4-6a, with respect to frequency, yields the following analytical expression for the \( n \)th-derivative spectrum.

\[ \frac{d^n F(\omega)}{d\omega^n} = A \frac{(-i)^n n!}{2 \left( (1/\tau) + i\cdot(\omega - \omega_0) \right)^{n+1}} \]  

(4-7)
The magnitude, $M_n(\omega)$, of the multiple-derivative spectrum may then be obtained as the absolute value of the complex $n$'th derivative spectrum of Eq. 4-7.

$$M_n(\omega) = \frac{A \cdot n!}{2} \left( \frac{1}{\sqrt{1/\tau^2 + (\omega - \omega_0)^2}} \right)^{(n+1)/2}$$  \hspace{1cm} (4-8)

Moreover, an analytical expression for the full peak width, $\Delta \omega_{1/2}$, at half-maximum peak height of magnitude-mode multiple-derivative spectral peak height may be obtained analytically from Eq. 4-8.

$$\Delta \omega_{1/2} = 2 \left( \frac{1}{\tau} \right) \sqrt{2 \left( \frac{2}{n+1} - 1 \right)}$$ \hspace{1cm} (4-9)

**EXPERIMENTAL SECTION**

Simulated time-domain data sets of exponentially damped N-point sinusoids, $4096 \leq N \leq 32768$, were generated and processed on a Macintosh Quadra 840AV computer (Apple Computer, Cupertino, CA) with 40 MHz Motorola 68040 CPU and 64 megabyte RAM, with an algorithm compiled with THINK C Version 7.0.3 (Symantec Corporation, Cupertino, CA). When desired, the effect of noise was simulated by addition of Gaussian-distributed random noise whose rms value was 20% of the initial time-domain signal amplitude.\(^6\) A standard Cooley-Tukey FFT algorithm\(^6\) converted the ICR time-domain data to a frequency-domain spectrum, which was converted to a mass spectrum by a standard mass calibration formula based on pure quadrupolar trapping...
potential. Mass spectra were plotted by a KaleidaGraph Version 3.0.1 (Synergy Software, Reading, PA) spreadsheet program.

Experimental time-domain ion cyclotron resonance data were obtained from a synthetic mixture of crude oil components by electron impact ionization (30 eV, 5 µA, 5 ms) with an Extrel FTMS-2000 instrument (Waters Extrel FTMS, Madison, WI) operating at 3.0 T at ~1 V/inch trapping potential. Ions were excited by chirp excitation with ~140 V_{p-p} excitation amplitude and 2000 Hz/µs sweep rate for 0.2 ms, and the signal was detected in the source compartment of a dual cubic trap. 4 K time-domain data were accumulated in direct mode for 400 KHz Nyquist bandwidth and transferred from an Odyssey® data station to the Quadra 840AV as a ASCII code by use of a program provided by Waters Extrel FTMS.

RESULTS AND DISCUSSION

Peak width as a function of derivative order. Plots of $M_n(\omega)$ vs. $\omega$ (i.e., $n$'th-derivative magnitude-mode spectrum of Eq. 4-8) for an infinitely long, noiseless, exponentially damped, sinusoidal time-domain signal are shown in Fig. 4.1. The width of the magnitude of the $n$'th-derivative spectrum (Eq. 4-9) clearly decreases monotonically with increasing derivative order, as is apparent graphically in Fig. 4.1. For example, Eq. 4-9 shows that the width of the "0'th" derivative magnitude spectrum (i.e., the underivatized FT spectrum, $F(\omega)$), is $2\sqrt{3}/\tau$, whereas
Figure 4.1 Simulated magnitude-mode spectra of a noiseless exponentially damped time-domain sinusoid which has been weighted by $t^n$, $n = 0$ to 10 before Fourier transformation. The spectrum resulting from $t^n$ time-domain weighting is the $n$'th frequency-derivative of the FT spectrum corresponding to $n = 0$. Note the monotonic increase in resolving power (i.e., decrease in peak width) with increasing derivative order.
the width of the first-derivative magnitude spectra is 2/τ. [As an aside, it
is interesting to note (see Eq. 4-8) that the first-derivative magnitude
spectrum is in fact identical in shape and width to the underivatized
absorption spectrum.] Thus, one might hope to improve FT magnitude-
mode spectral resolution simply by computing the n'th derivative of the
spectrum before performing the magnitude calculation.

**Signal-to-noise ratio as a function of derivative order.**
Unfortunately, experimental data are not noiseless, and the signal-to-
noise ratio of the derivative spectrum decreases monotonically with
increasing derivative order, as is easily seen from Eq. 4-3. Since one way
to generate an n'th-derivative spectrum is to multiply the time-domain
data by a weight function, t^n, it is clear that such weighting will
emphasize the later portion of the time-domain signal (where the signal-
to-noise (S/N) ratio is lower) relative to the initial portion of the time-
domain signal (where S/N ratio is higher). The reduction in S/N ratio
for a first-derivative spectrum is evident graphically in Fig. 4.2 (compare
panels (d) and (b)) for a noisy time-domain signal,

\[ f(t) = A e^{-t/\tau} \cos(\omega_0 t) + 0.2 \ A \ G(t); \quad 0 \leq t \leq \infty \] (4-10)

in which G(t) represents normalized Gaussian-distributed random noise\(^8\)
whose root-mean-square displacement is 20% of the initial time-domain
signal amplitude.
Figure 4.2 Various means for generation of magnitude-mode FT spectra, by FT followed by magnitude calculation. Fourier transformation of an unapodized noisy exponentially-damped truncated sinusoidal time-domain discrete (4K) signal (a) leads to the magnitude-mode spectrum (b). FT of the product of (a) and the linear weight function, (c), produces the first-derivative magnitude-mode spectrum (d), with enhanced resolving power but reduced S/N ratio compared to (b). FT of the product of (a) and an exponentially decreasing time-domain weight function (e), yields a magnitude-mode spectrum (f) with increased S/N ratio but lower resolving power than (b). Finally, FT of the product of (a) and (g), namely, the original data windowed by successive multiplication by (c) and (e), yields a magnitude-mode spectrum (h) with enhanced mass resolving power without loss in S/N ratio compared to (b).
Figure 4.2
Restoration of S/N ratio for derivative spectra. If the effect of time-domain \( t^n \) weighting is to emphasize the later portion of the time-domain data, then an obvious compensating procedure would be to apply an additional time-domain weight function, \( W(t) \), which decreases monotonically with time. Of the many possible time-domain windows used to smooth and/or increase the S/N ratio of an FT spectrum, we choose the simple decreasing exponential weight function, \( \exp(-t/\tau_c) \).

The well-known effect of an exponential weight function by itself is seen by comparing panels (f) and (b) of Fig. 4.2: exponential time-domain weighting increases signal-to-noise ratio, but also increases magnitude-mode line width (by \( 2\sqrt{3}/\tau_c \) s\(^{-1} \), or \( \sqrt{3}/\pi \) Hz) for the time-domain signal of Eq. 4-10.

The combined effect of the differentiating weight function, \((-i)^n t^n\), and the applied exponential weight function is provided by the "power-exponential" window function, \( W(t) \).

\[
W(t) = t^n \exp(-t/\tau_c)
\]  

(4-11)

Fig. 4.2h shows that the power-exponential window achieves higher resolving power (i.e., narrower peak width) without reducing signal-to-noise ratio. In the presently assumed limit that the data acquisition period, \( T \), is much longer than the time-domain signal exponential damping constant, \( \tau \) (i.e., \( T >> \tau \)), we can derive expressions for resolving power, \( \omega_0/\Delta \omega_{1/2} \), and S/N ratio for magnitude \( n' \)th-derivative frequency-domain spectrum obtained by Fourier transformation of the time-domain
signal of Eq. 4-10 weighted by the window function of Eq. 4-11. The peak width will be given by Eq. 4-9, with \( \tau \) replaced by an effective time constant, \( \tau_{\text{eff}} \).

\[
\frac{1}{\tau_{\text{eff}}} = \frac{1}{1/\tau + 1/\tau_c}
\]

(4-12)

so that the resolving power (relative to that for an underivatized magnitude-mode spectrum) is given by Eq. 4-12.

\[
\frac{\omega}{\Delta \omega} \frac{\text{(mag-mode } n'\text{'th derivative, exponentially apodized)}}{\text{(underivatized magnitude-mode)}} = \frac{\tau}{\tau + \tau_c} \sqrt{\frac{3}{2^{n+1} - 1}}
\]

(4-13)

Similarly, we may characterize the signal-to-noise ratio (relative to that for an underivatized magnitude-mode spectrum) for an exponentially apodized magnitude-mode \( n'\text{'th-derivative spectrum.}^{13} \)

\[
\frac{S/N \text{ (mag-mode } n'\text{'th derivative, exponentially apodized)}}{S/N \text{ (underivatized magnitude-mode)}}
\]

\[
= \frac{\int_{0}^{T} t^n e^{-t/\tau_{\text{eff}}} \, dt}{\int_{0}^{T} t^n e^{-t/\tau_c} \, dt} = \left( \frac{\tau_{\text{eff}}}{\tau_c} \right)^{n+1}
\]

(4-14)
Thus, the additional damped exponential window helps to reduce the amplification of noise produced by the derivative operation, at the cost of some loss in resolving power. We may now proceed to determine the optimal derivative order for given time-domain exponential damping constant, $\tau$, and additional time constant, $\tau_e$, for time-domain exponential weighting.

**Effect of finite truncation of time-domain data.** Simulated 32 K time-domain data were generated from Eq. 4-10 ($\nu_0 = \omega_0/2\pi = 630$ kHz) except that data were acquired for $T = 3\tau$ sec. The time-domain data were multiplied by $t^n$, followed by Fourier transformation at magnitude calculation to yield the magnitude-mode spectra shown in Fig. 4.3. (For comparison to experimental FT-ICR mass spectra, we converted the frequency scale to a mass-to-charge ratio, $m/z$, scale, based on an assumed magnetic field induction of 3.0 tesla.) Fig. 4.3 shows, as expected, that the width of a magnitude-mode first-derivative spectrum is narrower and the noise level is higher than that of the underivatized spectrum, but (surprisingly) the peak width for higher-order derivatives increases until it is actually wider than that of the underivatized spectrum. We shall now address the noise and width problems.

As for a time-domain signal of infinite duration (Fig. 4.2), additional time-domain exponential weighting of a truncated time-domain signal by the window function, $\exp(-t/\tau_e)$, improves the S/N ratio for magnitude-mode higher-order derivative spectra (see Fig. 4.4), at the cost of some
Figure 4.3 Magnitude-mode of a simulated FT-ICR mass spectrum (bottom front) and its 1st-, 3rd-, 5th-, and 7th-order frequency derivatives for the exponentially damped noisy sinusoidal time-domain discrete (32 K) signal of Eq. 10, acquired for $T = 3\tau$ seconds, in which $\tau$ is the time-domain exponential damping constant. The $n$'th-derivative spectrum was generated by multiplication of the corresponding time-domain discrete data by $t^n$ before FFT. In contrast to the spectra obtained from an infinitely long time-domain signal (Fig. 4.1), the spectral peak first narrows with increasing derivative order and then broadens to become wider than the underivatized spectrum, due to time-domain truncation (see text).
Figure 4.3
Figure 4.4 Magnitude-mode of a simulated FT-ICR mass spectrum and its 1st-, 3rd-, 5th-, and 7th-order frequency derivatives as in Figure 4.3, except that the time-domain data has been additionally multiplied by a decreasing exponential with time constant, $\tau_e = T/9$. The additional exponential weighting reduces the amplification in noise by the derivatization process. Also, resolving power now increases monotonically with increasing derivative order, as for the infinitely acquired time-domain signal of Figure 4.1.
Figure 4.4
broadening of the peak width, $\Delta \omega_{1/2}$, at half-maximum peak height. Even so, Fig. 4.4 shows a net gain in resolving power, $\omega_0/\Delta \omega_{1/2}$, (relative to an underivatized unapodized magnitude-mode spectrum) for derivatives of order, $n \geq 4$. More important, the exponential weighting results in much narrower peak width (relative to an unapodized underivatized spectrum) for the magnitude-mode $n$'th derivative spectrum of a truncated time-domain signal at (say) 10% of peak height.

The increased peak broadening for magnitude-mode higher-order derivative spectra seen in Fig. 4.3 is a direct consequence of truncation of the time-domain signal, as shown by the results in Fig. 4.5 for simulated magnitude-mode $n$'th-derivative spectra obtained by FT of a noiseless exponentially damped sinusoid time-domain signal truncated after acquisition periods, $T = 3\tau$, $6\tau$, $12\tau$, and $120\tau$, as well as for the corresponding untruncated (infinitely extended) time-domain signal. The desired monotonic increase in resolving power with increasing derivative order (for a noiseless time-domain signal) is attained only for an inconveniently long acquisition period, $T >> 10\tau$.

**Optimal apodization for magnitude-mode $n$'th derivative spectra.** Fig. 4.4 showed that time-domain windowing with a decreasing exponential weight function reduces noise in the magnitude-mode $n$'th-derivative spectrum, and also increases resolving power for magnitude higher-order derivative spectra. An exponential damping window thus effectively compensates for time-domain data truncation, as far as magnitude-mode higher-derivative spectra are concerned. Therefore, an
Figure 4.5 Theoretical resolving power for magnitude-mode $n$th derivative FT spectra as a function of derivative order, $n$, for each of several time-domain data acquisition periods, $T = 3\tau, 6\tau, 12\tau, 120\tau$, and $\tau \to \infty$, in which $\tau$ is the time-domain signal exponential damping constant. For an experimentally typical case, $T = 3\tau$, resolving power is improved only up to second-order derivatization.
Figure 4.5

- $T \to \infty$
- $T = 3\tau$
- $T = 6\tau$
- $T = 12\tau$
- $T = 120\tau$
optimum strategy for use of n'th-derivative spectra is to select, for a given acquisition period (say, \( T = 3t \)), the time-domain damped exponential windowing time constant, \( \tau_e \), which yields the desired combination of resolving power and S/N ratio for a given derivative order, \( n \). It will then be clear which derivative order is best.

Such an optimization is illustrated in Fig 4.6, for magnitude-mode n'th-derivative spectrum obtained by FT of a power-exponential \( (\tau_e = T/9) \) windowed time-domain noisy exponentially damped sinusoid truncated at \( T = 3t \). Resolving power increases monotonically (relative to an unapodized underivatized magnitude-mode spectrum), and S/N ratio decreases monotonically with increasing derivative order, up to \( n = 7 \). Moreover, the product of resolving power and S/N ratio is maximal for the first-derivative spectrum, with about a 30% improvement relative to the unapodized underivatized magnitude spectrum.

**Experimental FT-ICR mass spectral examples.** Fig. 4.7 shows the effect of each of three different time-domain windows on an experimental magnitude-mode spectrum of an electron-ionized mixture of three components of crude oil. Fig. 4.7a, the spectrum obtained without windowing, exhibits peaks which are very broad at the base, making it difficult (even for a computer) to determine the position and height of overlapped spectral peaks. In contrast, the magnitude-mode second-derivative spectrum (Fig. 4.7b) obtained by prior multiplication of the time-domain data by the power-exponential window function, \( t^2 \exp(-t/\tau_e) \), in which \( \tau_e = T/2 \) (optimized as in Fig. 4.6), shows a slight
Figure 4.6 Plots of each of three figures of merit (S/N ratio, resolving power, and their product) for magnitude-mode n'th derivative FT spectra as a function of derivative order, n. In this theoretical comparison, the time-domain signal of Eq. 10 has been acquired for $T = 3\tau$ seconds, in which $\tau$ is the time-domain exponential damping constant, and additional time-domain decreasing exponential windowing with exponential time constant, $\tau_c = T/9$, has been applied as in Fig. 4.4.
Relative S/N
Relative Resolving Power
Relative Resolving Power*S/N

Figure 4.6

Derivative Order
widening of the peaks at half-maximum height, but significant narrowing
of the bases of the peaks, for much-improved peak separation without
significant loss in signal-to-noise ratio. Note that the peaks at integral
$m/z$ spacings above and below each of the $m/z$ values for the five
principal ionic species are baseline-resolved in each case. The peak
width in each case is well-approximated by Eq. 4-13, with $T = 7 \tau_{\text{eff}}$, and
the optimization in choice of $\tau_{\text{e}}$ is quite similar to Fig. 4.5 for $T = 6\tau$.

Alternatively, Fig. 4.7c shows resolution-enhancement of the
spectrum by prior exponential weighting of the time-domain signal with
an increasing exponential designed to overcome (partially) the time-
domain damping of the ICR signal itself before FFT. Although resolving
power is significantly improved (note the now-resolved splitting of the
peaks at $m/z$ 165 and 166), the noise level is correspondingly higher as
well. Thus, the peak at $m/z$ 158, clearly identified in Fig. 4.7b, has
disappeared into the noise in Fig. 4.7c.

Finally, Fig. 4.7d shows the effect of exponential-Gaussian
weighting. The resolving power is comparable and the S/N level higher
than for resolution-enhancement by a time-domain increasingly
exponential alone (Fig. 4.7c), but the noise level is substantially higher
than for the magnitude-mode second-derivative spectrum (Fig. 4.7b)
obtained by FT of a power-exponential windowed time-domain signal.
Figure 4.7 Experimental FT-ICR magnitude mass spectra of a 3-component mixture of crude oil components, all obtained from the same time-domain data acquired for $T = 5.12$ ms. (a) conventional FT-ICR magnitude mass spectrum; (b) the magnitude-mode second-derivative mass spectrum obtained by FFT of time-domain data multiplied by an optimized (with respect to $\tau_e$ for best resolving power) power-exponential weight function, $t^2 \exp(-t/\tau_e)$, $\tau_e \sim T/5$ (c) magnitude-mode FT-ICR mass spectrum in which the time-dependent exponential damping has been compensated by multiplication by the weight function, $\exp(+t/\tau_e)$, $\tau_e \sim T/2$. (d) magnitude-mode second-derivative mass spectrum in which the time-dependent exponential damping component has been partly compensated by multiplication by the weight function, $\exp(+t/\tau)$, $\tau = T/5$ and then further windowed by multiplication by the power function, $t^2$, and then a Gaussian weight function, $\exp[-t^2/(2\sigma^2)]$, $\sigma \sim T/7$. Note that the power-exponential windowing (b) produces the best improvement in mass resolving power without sacrificing signal-to-noise ratio (see text).
Figure 4.7
Figure 4.7 continued.

(B)

m/z
CONCLUSIONS

Based on both theoretical and experimental characterization of time-domain power-windowing to provide magnitude-mode multiple-derivative FT spectra, we find that, with appropriate (and readily chosen) parameters, derivative FT analysis can improve the product of resolving power and S/N ratio. Under typical experimental conditions ($T = 3\tau$, in which $T$ is time-domain acquisition period and $\tau$ is the time constant for exponential damping of the time-domain signal), resolving power (especially near the base of the spectral peaks) may be significantly enhanced without loss in S/N ratio by use of first or second derivative display produced by power-exponential weighting with the function, $t^n \exp(-t/\tau_e)$, $\tau_e \sim T/9$, $n = 1$ or $2$. The method should prove especially useful for resolving and identifying peaks in dense magnitude-mode FT spectra, such as those arising from FT-ICR mass analysis of complex mixtures (e.g., crude oil). The derivatization method thus appears to offer an attractive alternative to standard windowing methods for producing apodized FT spectra.
REFERENCES


CHAPTER V

THIN GOLD FILM-ASSISTED LASER DESORPTION/IONIZATION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY OF BIOMOLECULES

INTRODUCTION

High instantaneous power deposition achieved by illuminating a solid sample with a short pulse of intense laser light can produce molecular or quasimolecular ions from picomole amounts of thermally labile organic samples with minimal fragmentation\(^1\)\(^,\)\(^2\) making laser desorption/ionization (LDI) a very attractive technique for mass spectrometric analysis of biomolecules.\(^3\) LDI performance is affected by laser power density and wavelength, sample absorption characteristics, incident angle of laser irradiation, sample thickness, and nature of the substrate on which the sample is placed.\(^4\)\(^,\)\(^5\) The most commonly used laser wavelengths are in the far infrared (e.g. 10.6 μm from a CO\(_2\) laser) or near-ultraviolet (e.g. 266 nm from a frequency-quadrupled Nd:YAG laser).\(^6\) In the absence of a matrix (see below), LDI is most effective for molecules with chromophoric groups in the chosen wavelength range,
although LDI has also been applied to samples which are transparent at the laser wavelength.\textsuperscript{3,7}

The introduction of a UV-absorbing matrix (with which dilute sample molecules are mixed) vastly extended the applicability of LDI. Proteins with molecular weights above $10^5$ Da\textsuperscript{8} may now be analyzed by matrix-assisted LDI (MALDI).\textsuperscript{9} In a less commonly practiced and less sensitive variant of MALDI, a liquid matrix combined with fine metal powder is used.\textsuperscript{10} LDI and MALDI have been demonstrated on a wide variety of mass analyzers for most classes of biomolecules. Because laser desorption/ionization is inherently a pulsed ion source, it is optimally matched to a pulsed mass analyzer;\textsuperscript{11} time-of-flight (TOF), as in the above-cited examples, or Fourier transform ion cyclotron resonance (FT/ICR). LDI and MALDI FT/ICR mass spectrometry have been demonstrated for a wide range of biological molecules,\textsuperscript{12} including peptides,\textsuperscript{13-16} oligo- and polysaccharides,\textsuperscript{17-20} nucleotides,\textsuperscript{21} nucleosides and glycosides,\textsuperscript{18} as well as porphyrins.\textsuperscript{22,23} Phospholipids, although more commonly analyzed by liquid secondary ion mass spectrometry (liquid SIMS), have also been successfully desorbed and ionized by UV-LDI with TOF detection\textsuperscript{24-26} (for review see Jensen \& Gross.\textsuperscript{27}) More recently Amster \textit{et al.} introduced a technique called substrate-assisted LDI (SALDI),\textsuperscript{28} in which a UV-absorbing matrix is sandwiched as a separate layer between the analyte layer and the probe tip, so as to reduce the number of detected interfering matrix ions. In other experiments,\textsuperscript{29} ammonium bromide mixed with the analyte provided for chemical ionization of the analyte molecules.
Employing MALDI with TOF mass analysis, Beavls and Chait\textsuperscript{30} demonstrated accurate molecular weight determinations for proteins of up to about 27 kDa. High mass accuracy requires resolution of different quasimolecular ion species. TOF mass resolving power can be limited by the range in ion kinetic energy resulting from laser irradiation in LDI experiments. LDI has recently been interfaced to a quadrupole (Paul) ion trap mass spectrometer (ITMS) and used for mass analysis of biologically interesting molecules.\textsuperscript{31} An advantage claimed for ITMS is the use of a high pressure inert buffer gas in the analyzer for cooling the potentially high ion kinetic energy following laser desorption. Similarly, we have recently demonstrated high-pressure collisional ion cooling to reduce ion kinetic energy in FT/ICR trapped-ion experiments.\textsuperscript{32,33} LDI-FT/ICR mass resolving power, sensitivity, mass selectivity,\textsuperscript{34} and ion remeasurement efficiency\textsuperscript{35} are also enhanced by prior quadrupolar excitation with collisional cooling to axialize ions prior to mass analysis (see below).

Despite the enormous impact of MALDI on mass spectrometric analysis of nonvolatile biomolecules in general (and of peptides and proteins in particular), the method suffers from some limitations. Although desorption and ionization processes for some matrix systems have been investigated,\textsuperscript{36} our understanding of the fundamental role of the matrix in MALDI processes is incomplete. For example, matrix compounds of highly similar structure and/or optical absorption spectra may yield large differences in desorption/ionization efficiency. In general, a good matrix should: (a) have strong optical absorbance at the
laser wavelength and should also be inert toward chemical reactions with
the analyte; (b) be soluble in the same solvent as the analyte; and (c) be
sufficiently volatile and photostable. Thus, among more than 300
tested matrices, only seven proved acceptable for MALDI. Because of
the lack of a detailed understanding of the desorption/ionization
mechanism, considerable time and resources are being spent to discover
the appropriate "magic potion" of analyte and matrix for optimal
performance at each new laser wavelength.

In this chapter, we describe LDI-FT/ICR experiments performed at
the fundamental (near-IR, 1064 nm) and first harmonic (visible, 532 nm)
wavelengths of a Nd:YAG laser. Prior attempts at LDI of biomolecules at
these wavelengths has met with limited success. Nelson et al. obtained
mass spectra of nucleic acids by laser ablation of frozen aqueous
solution films at 581 nm. Bovine insulin, albumin, and horse heart
cytochrome c were mass analyzed by MALDI at 532 nm from a two
component matrix consisting of rhodamine 6G and 3-nitrobenzyl
alcohol. Demirev et al. have obtained bovine insulin MALDI spectra at
496 nm.

In this chapter, we introduce a qualitatively new method which has
proved successful in laser desorption/ionization of biomolecules at 532
and 1064 nm. This technique resembles the abovementioned SALDI in
that we coat a glass plate with thin gold film to assist desorption and
ionization of analyte molecules deposited onto the gold-coated glass
surface. Importantly, the thickness of the gold film can be easily
adjusted to match its maximum absorption wavelength to the laser wavelength. We term the new technique thin gold film assisted/laser desorption ionization (TGFA/LDI). All samples tested in this experiments were prepared in the same manner with addition of only potassium bromide to the analyte solution.

Other research groups also have employed gold (and other metal) substrates in laser desorption experiments. In the SALDI experiments conducted by Speir and Amster, peptide samples were deposited on a sinapinic acid substrate which in turn was layered on a gold/palladium coated Macor probe tip. However, the laser wavelength (248 nm) was tuned to the absorption of the sinapinic acid and not to that of the metal film which was of unidentified thickness. Alternatively, Wilkins and co-workers produced a gold film by application of several drops of a commercial gold solution to a probe tip and heating to 500 - 600 °C; they then characterized the reactivity of laser-desorbed metal ions toward alcohols, hydrocarbons, and alkyl halides deposited on the surface. The same researchers also performed similar experiments with gold oxide surfaces and gold surfaces covered with layers of other metal oxides. Desorption was achieved by use of a CO$_2$ laser (10.6 μm). Those authors do not indicate the thickness of their gold films (presumably several orders of magnitude thicker than the gold films used for the experiments described in this manuscript) or their optical absorption behavior. Kahr and Wilkins performed LDI experiments in the far IR (10.6 μm) and UV (355 nm) range with polybutadiene and polystyrene in which a 0.1 mm thick commercial gold foil served as a substrate. The
role of the gold substrates in the desorption/ionization processes in abovementioned experiments is unclear. In related studies by Llenes and O'Malley\textsuperscript{44} and Dean and O'Malley,\textsuperscript{45} bulk aluminum, chromium, iron, copper, silver, and gold probe tips covered with polystyrene, polyethylene glycol, or polybutadiene samples produced LDI-FT/ICR mass spectra showing metal ion adducts to polymer molecules. 1064 nm laser light with similar irradiance as in the present experiments ($\sim 10^8$ W/cm$^2$) was used in those investigations. In contrast, in our experiments the gold film serves merely as a vehicle for desorbing the analyte into the gas phase, whereas ionization is achieved by attachment of alkali ions which are present as contaminants or as an added matrix component.

**EXPERIMENTAL**

**Gold films.** 99.95\% purity gold (Johnson Matthey, Ward Hill, MA) was deposited on a microscope cover glass plate (1.8 cm diameter, No. 48380046, VWR Scientific, Media, PA) by vapor deposition to a final thickness of 10 nm at a typical pressure of $<10^{-6}$ torr. Deposition rate (1 nm/s) and layer thickness were monitored with a quartz crystal device (R.D. Mathis Co., Long Beach, CA) and optical absorption was measured with a UV/VIS/NIR Lambda 19 spectrometer (Perkin Elmer, Überlingen, Germany). The visible/near IR absorption spectrum (Figure 5.1) of the thin layer gold film was obtained after baseline correction relative to a clean blank glass plate; for a 10 nm thick gold film, the wavelength at which the (broad) absorbance is maximum ($\sim 950$ nm) nicely matches the
Figure 5.1 VIS/NIR absorption spectrum of thin layer gold film. Gold is deposited by resistively heated evaporation. Pure glass plate is used as a reference of base correction. [Note the broad maximum near the fundamental wavelength (1064 nm of a Nd:YAG laser)]
fundamental wavelength (1064 nm) of Nd:YAG laser. The purity and stability of the gold film were verified from the LDI-FT/ICR mass spectrum of the pure gold film along: in contrast to similar films of other metals, no impurities or oxidized compounds were found even after several weeks of air exposure. The gold-plated glass plate was affixed to a stainless steel probe with double-sided adhesive tape. Gold covered glass plates may be reused with care. It is best to detach the plate from the sample holder before cleaning the surface with methanol. Therefore, the double-sided adhesive tape used to attach the plates should not be too strong if reuse is attempted. Reuse of the probe tips is of course possible only as long as gold film is left on the surface (about 0.3 mm² surface material is ablated by each laser shot).

**Samples.** All samples were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Almost all samples tested in this work were prepared in the same manner with addition of only potassium bromide in the analyte solution. Unless otherwise stated, analyte was dissolved or dispersed in methanol to a final concentration of 10⁻³ M and mixed with an equal volume of 10⁻² M potassium bromide in methanol. 10 – 20 μl of the mixture (5-10 nmole of analyte) was applied to the gold film and air dried. For sensitivity and detection limit determinations, samples were prepared similarly but from analyte stock solutions of lower concentration. As control experiments, we prepared samples similarly but with a matrix solution of 10⁻² M KBr and a varying concentration ratio of rhodamine 6G (molar ratio 100:1 -
Figure 5.2 FT/ICR mass spectra obtained by thin gold film-assisted laser desorption/ionization (TGFA-LDI) of gramicidin S deposited at a concentration at which 10 pmol of peptide is desorbed per laser shot. (A) Peptide dissolved in rhodamine/KBr methanol solution and deposited on a stainless steel probe tip. (B) Peptide dissolved in KBr methanol solution and deposited on a glass plate. (C) Peptide in pure methanol deposited on a thin gold film. (D) Peptide dissolved in KBr methanol solution and deposited on a thin gold film. Vertical scale is expanded by a factor of 20, 100, and 10 in (A), (B), and (C) relative to (D). Note that at this peptide concentration, peptide quasimolecular ions, (M+K)+, are observed only by laser desorption/ionization from the gold surface.
A) Matrix Ions

Gramicidin S + Rhodamine 6G/KBr
(Stainless Steel)

B) Gramicidin S + KBr
(Glass)

C) Gramicidin S
(Thin Gold Film on Glass)

D) Gramicidin S + KBr
(Thin Gold Film on Glass)

Figure 5.2
500:1 rhodamine 6G:analyte, Fig. 5.2a), a matrix solution of 10^{-2} M KBr only and subsequent deposition on an uncoated glass plate (Fig. 5.2b), or no matrix solution and deposition on a gold-covered glass plate (Fig. 5.2c). For comparison with UV-SALDI employing sinapinic acid and a gold/platinum covered probe tip, 28 samples were prepared similarly by premixing methanolic analyte (10^{-3} M) and matrix solutions to yield final matrix:analyte mole ratios of 100:1 - 500:1.

**Choice of solvent.** Methanol or other organic solvent with low surface contact angle may be used to disperse the sample over the smooth surface of the sample holder. Even samples of limited methanol solubility could be successfully dispersed uniformly over the gold surface, e.g., methanol suspensions of sugars. We are currently investigating deposition of a gold film on a rougher substrate (e.g., a ceramic or etched glass plate) to enhance surface wettability by aqueous and other more polar solvents. In any case, a major advantage of the gold film is its stability with respect to any of a broad spectrum of solvents.

**Laser desorption/ionization.** In all experiments, we used a frequency-doubled Nd:YAG laser (YG-660A, Continuum, Santa Clara, CA) with a mixed 1064/532 nm output (95/5 ratio). For best results, the laser was operated at highest power density, corresponding to 10^8 - 10^9 W/cm^2 (power meter, Diamond Ophir Optics, Inc., Wilmington, MA) over a spot size of ~0.3 mm^2 and a pulse width of ~10 ns. The laser light impinged on the probe tip at an incident angle of ~30°. The laser-mass spectrometer interface has been described elsewhere. Taking into
account the surface area of the sample holder (254.5 mm²) and assuming uniform coverage of analyte, we estimate that each laser shot typically desorbs about 10 pmol of analyte under standard conditions. Ablation of the gold film in spots where the laser hit indicated that all sample deposited in that area was removed. For the detection limit determination, similar assumptions and calculations were used. Analyte solutions were carefully adjusted in concentration by serial dilution of a 1 mM stock solution. Unless otherwise stated, each FT/ICR mass spectrum resulted from a single laser shot.

**Fourier transform ion cyclotron resonance mass spectrometry.**
All FT/ICR experiments were performed at 3 tesla on an FTMS-2000 spectrometer (Extrel FTMS, Madison, WI) whose source and analyzer vacuum chambers are equipped with Helix Technology CryoTorr-8 2000 L/s cryopumps (Waltham, MA). The standard dual-trap design provides for high pressure (~2-3 x 10⁻⁸ Torr) in the source and low pressure (~1 x 10⁻⁹ Torr) in the analyzer 4.76-cm cubic traps separated by a 2 mm diameter conductance limit. Trapping and probe bias voltages were set to 2 V in all experiments. Time domain ICR signals were produced by frequency-sweep excitation from 1-500 kHz at 500 Hz/μs sweep rate, followed by broadband detection at 500 kHz bandwidth to yield 8 - 16 K time-domain data, which were zero-filled once and Fourier transformed without apodization. High-resolution mass spectra were acquired in heterodyne mode at a 2 - 3 kHz detection bandwidth to give 8 K time-domain data.
**Quadrupolar excitation and collisional cooling for ion axialization.** For collisional cooling and axialization experiments, argon or nitrogen buffer gas was admitted into the source compartment through a leak valve (Model 951-5100, Varian, Palo Alto, CA) connected to the batch inlet system of the instrument to a partial pressure of $3 \times 10^{-7}$ Torr, thereby increasing pressure in the analyzer to $2 \times 10^{-9}$ Torr. Wiring diagrams and experimental details on quadrupolar axialization have been reported previously.$^{32,33}$

**RESULTS AND DISCUSSION**

**Mass spectral features due to the gold substrate.** When detected directly on the source side of the dual trap, the LDI-FT/ICR mass spectra in general exhibited gold cluster ions, $\text{Au}_n^+$ and $\text{Au}_n^-$, in varying numbers, but generally not gold adducts, $(\text{M}+\text{Au})^+$ or $(\text{M}+\text{Au})^-$. Pure gold cluster anions, $\text{Au}_n^-$, are particularly abundant, as will be reported separately.$^{46}$ Among the gold cluster cations, $\text{Au}_n^+$, clusters up to $n = 5$ could be observed, but disappeared for a delay period between laser shot and ICR excitation of more than ~0.5 s. As expected, $\text{Au}_n^+$ species were absent from LDI-FT/ICR mass spectra acquired after source-side axialization and transfer to the analyzer trap, due to the high mass selectivity of the axialization.$^{33}$ All $\text{Au}_n^+$ mass spectral peaks were easily identified, and under high-resolution conditions served as internal mass calibrants for $m/z \leq 1,000$ or so.
**Essential role of the thin gold film.** We have been able to obtain TGFA/LDI-FT/ICR mass spectra for peptides up to m/z 2000 (e.g., potassiated gramicidin D). It is possible that higher mass ions are desorbed but not easily ionized or trapped by our instrument (see below). We attempted to detect higher mass peptides [insulin (FW 5733.5) and insulin A and B chains (oxidized, FW 2532 and 3496, respectively)] without success.

Although potassiated gramicidin S (m/z 1180) is easily detected by TGFA/LDI-FT/ICR in the source trap at high abundance, LDI of the same peptide deposited on a stainless steel probe surface failed to yield molecular or quasimolecular ions at practically useful sensitivity, either alone or in combination with any of several matrices (e.g., rhodamine 6G/KBr), as shown in Figure 5.2. In both cases, gramicidin S concentration was adjusted to yield ~10 pmol per laser shot. For the steel probe surface, LDI-FT/ICR mass spectra exhibited only matrix ions unless gramicidin S was premixed with KBr and deposited as a thick slurry (i.e., unacceptably high concentration). Alkali metal ions, presumably present in the glass and as a contaminant on the gold surface, yielded sodiated and potassiated gramicidin S in good abundance even when the peptide was deposited alone on the gold film (i.e., without KBr) (Fig. 5.2c). The sodiated molecular ion is about twice as abundant as the potassiated molecular ion. Addition of potassium bromide yields the potassiated molecule as the most abundant species, with signal-to-noise ratio enhanced about tenfold (Fig. 5.2c,d).
Potassium bromide gave slightly larger quasimolecular ion abundance than sodium bromide applied at the same concentration; thus, KBr was used in all further experiments. Gold films are absolutely required for observation of the molecular ion, as indicated by absence of analyte-derived ions in the LDI-FT/ICR mass spectrum (Fig. 5.2b) obtained from gramicidin S deposited on a pure glass plate together with potassium bromide.

**Laser wavelength selectivity.** A mixed output from a Nd:YAG laser (532 and 1064 nm) was used in the experiments described herein. In order to determine the influence of each component, we also used isolated IR (1064 nm) or visible (532 nm) beams for laser desorption (see Fig. 5.3). At the fundamental (1064 nm) laser output (which accounts for ~95% of the total power output, namely, ~2.5 x 10^8 W/cm^2), the resultant mass spectra are virtually identical to those obtained with the mixed output (Figs. 5.3a,b). On the other hand, the visible (532 nm) wavelength by itself is incapable of desorbing any ions throughout the whole m/z range (Fig. 5.3c). This finding underlines the importance of tuning the absorption of the gold film (by adjustment of its thickness) to the laser wavelength used for desorption/ionization. Of course, higher-intensity irradiation at 532 nm might produce some desorption/ionization. The green light was maintained in the output for the following experiments because it is a convenient marker for the otherwise invisible (and therefore difficult to focus) laser beam.
Figure 5.3 FT/ICR mass spectra of gramicidin S/KBr deposited on a thin gold film. The spectra compare the desorption/ionization efficiency at the two wavelengths contained in the mixed output of the Nd:YAG laser. 1064 nm-only illumination (b) achieves virtually the same result as irradiation at the combined Nd:YAG fundamental and first harmonic (a), showing abundant (M+K)$^+$ ions. On the other hand, no desorption/ionization was observed by use of only the first harmonic (c) at 532 nm.
Gramicidin S
Thin Gold Film on Glass

A) 1064 nm + 532 nm
~ 2.5 x 10^8 W/cm^2

B) 1064 nm
~ 2.1 x 10^8 W/cm^2

C) 532 nm
~ 1.0 x 10^7 W/cm^2

Figure 5.3
**Peptides.** To increase trapping efficiency and transfer to the analyzer compartment of the dual trap as well as to stabilize internally "hot" molecules which may undergo unimolecular decomposition on the FT/ICR-MS time scale (about 1 s for standard source side detection), we axialized potassiated quasimolecular ions for 10-60 s at 3x10^{-7} Torr of argon in the source trap before transfer to the analyzer. LDI-FT/ICR mass spectra with resolving power (m/Δm, with Δm measured as the full magnitude-mode peak width at half-maximum peak height) up to 113,000 for gramicidin S could be obtained (Fig. 5.4, middle spectrum). During the detection period, the detected cyclotron frequency drifts downward due to rearrangement of the ion packet and the peak broadens. Such frequency drift can be eliminated to improve the resolving power by another factor of two to 248,000 for the gramicidin S data.

The detection limit of the method was tested on gramicidin S (see Figure 5.5). As little as 100 fmol of desorbed protein per laser shot yielded an FT/ICR mass spectrum with signal-to-noise ratio of ~5 (Figure 5.5d), in close agreement with the detection limit obtained by Amster et al. for SIMS-FT/ICR of peptides coated on solid gold surfaces. Excellent signal-to-noise ratio was obtained for 1 pmol per laser shot on either side of the dual trap (Figure 5.5b), whereas 100 fmol per laser shot was sufficient to generate high resolution (m/Δm ~ 10,000) FT/ICR mass spectra of high signal-to-noise ratio after prior collisional
Figure 5.4 FT/ICR mass spectra of three peptides obtained by TGFA-LDI followed by axialization in the source side of a dual-trap and transfer to the analyzer of the dual-trap. The reported mass resolving power may be further improved dramatically (e.g., to >1,000,000 for leucine enkephalin, and to ~250,000 for gramicidin S) by correcting for cyclotron frequency drift during data acquisition.
Figure 5.5 FT/ICR mass spectra of gramicidin S at various concentrations, obtained by TGFA-LDI with source-side detection. No prior axialization was used. The direct detection limit of ~100 fmol per laser shot for a signal-to-noise ratio of ~5 may be improved by prior axialization (see next figure) and/or ion remeasurement (see text). The following quasimolecular ions are resolved and assigned: \((M+K-CO)^+\) at m/z 1152, \((M+Na)^+\) at m/z 1163, and \((M+K)^+\) at m/z 1180.
Gramicidin S

A) 10 pmol/shot

B) 1 pmol/shot

C) 360 fmol/shot

D) 100 fmol/shot

Figure 5.5
Figure 5.6  Relative FT/ICR mass spectral peak magnitude for potassiated gramicidin S detected on the source side of a dual trap as a function of amount of sample desorbed per laser shot. The inset shows the 10-fold improvement in signal magnitude following prior axialization.
axialization. For example, Figure 5.6 (see insert) shows an improvement in signal-to-noise ratio of about a factor of 10 by the addition of a prior axialization event. The leveling off of the detected signal at higher sample concentration (Fig. 5.6) may be due to saturation either in access to or capacity of the source ion trap. The assumption of uniform surface coverage by the analyte (see Experimental section) which was used to calculate the average amount of analyte desorbed per laser shot is obviously approximate. Uniform surface coverage was judged by visual inspection of the surface before mass spectral analysis. A series of laser shots gave standard deviations ranging from ~5% (high concentration) to ~27% (low concentration) in measured mass spectral signal magnitude. However, every shot hitting the target plate yielded a detectable signal.

Unfortunately it is not possible for us to compare directly the results described above to UV-MALDI or SALDI experiments on our instrument because we lack the capability to produce laser output in the UV. Moreover, we do not have the capability to electrospray-deposit the sample on the probe tip, as done by Speir and Amster for SALDI-FT/ICR/MS. Our use of nicotinic acid instead of potassium bromide as the matrix (in molar ratios of 100:1 - 500:1, matrix:analyte) in TGFA-LDI yielded matrix-related species, but not protonated molecules or other pseudomolecular ions. Sinapinic acid at the same concentration ratio gave an LDI-FT/ICR mass spectrum similar to that obtained in the absence of any type of matrix: i.e., sodiated and potassiated molecules in
about a 2:1 ratio (compare to Fig. 5.2c). Protonated molecules were again absent. Presumably these standard UV-MALDI matrices require irradiation at or close to their absorption maxima to achieve protonation of the analyte, which may explain the success of SALDI analysis of peptide samples on a sinapinic acid substrate deposited on a gold/palladium coated probe tip.28

Axialization prior to FT/ICR detection also proved very effective for mass-selective isolation33 of quasimolecular ions. By comparison, typical detection limits for MALDI-TOF fall in the fmol and sometimes sub-fmol range.49,50 TOF detection sensitivity, based on direct ion counting with an electron multiplier detector, is intrinsically higher than FT/ICR sensitivity, which is based on detection of the voltage derived from charge induced on the detector electrodes by a coherently orbiting ion packet.51,52 We hope to be able approach those values with FT/ICR detection, by use of the ion remeasurement technique originally introduced by Williams et al.53 and recently improved by combination with prior axialization by Speir et al.35 When operated in broadband mode, our FT/ICR mass spectrometer is capable of detecting as few as ~100 charges in a single detection event; with remeasurement, it should be possible to reduce the detection limit by at least an order of magnitude.

Gramicidin S is a cyclic peptide. Linear peptides may also be laser-desorbed/ionized efficiently from the gold film. Although the signal-to-noise ratio for potassiated molecular ions (m/z 594) of leucine
enkephalin is low for detection in the source trap, the TGFA-LDI process nevertheless must have produced a huge number of ions from the gold film, because axialization (10 - 20 s) and transfer of those ions to the analyzer trap yielded a much higher signal-to-noise ratio and high mass resolving power, as shown in Fig. 5.4 (top). Correction for the large downward drift in cyclotron frequency during detection can increase the FT/ICR mass resolving power to > 1,000,000\textsuperscript{34}

The largest peptide ion we detected by TGFA-LDI was potassiated gramicidin D (m/z 1919). Detected directly in the source trap, the quasimolecular ions did not yield a signal significantly higher than the noise. However, axialization and subsequent transfer of ions to the analyzer resulted in remarkable improvements in signal-to-noise ratio and mass resolving power (m/△m ≈ 31,000), as shown in Fig. 5.4 (bottom). These observations suggest that high-mass ions may retain considerable kinetic energy after the TGFA-LDI process (see below).

We also tested some smaller peptides (Gly-Phe, Met-Leu-Cys, Thr-Tyr-Ser) and obtained high mass resolving power and high signal-to-noise ratio TGFA/LDI-FT/ICR mass spectra (not shown). All molecules tested were desorbed as the potassiated molecular species and they could be easily axialized. In the analyzer trap, mass resolving power for these peptides on the order of 10,000 was routinely achieved.

**Nucleosides and nucleotides.** (Oligo-) nucleotides and nucleosides are difficult to ionize and mass analyze because of their high polarity
and instability. LDI provides an avenue for mass spectrometric analysis of these molecules. In the absence of a matrix, LDI of a nucleotides typically cleaves off the phosphate to give a mass spectrum similar to that of the corresponding nucleoside. Both positive\textsuperscript{18,54} and negative\textsuperscript{55} ions have been investigated for these classes of molecules by use of LDI with FT/ICR mass analysis. Nucleosides usually yield positive quasimolecular ions such as \((M+K)^+\) when a salt is added.\textsuperscript{6} Negative ions are usually observed as the deprotonated molecular ion, \((M-H)^-\). Matrix assistance allows observation of intact cationized or protonated nucleotides and oligonucleotides. Mass spectrometric investigations of these simple biological compounds provide a simple and fast method for characterization of modified residues in DNA and RNA.\textsuperscript{56} More than 70 modified nucleosides are known in RNA, whereas the number in DNA approaches 20\textsuperscript{6} and new discoveries can be anticipated in the future.

Our results with TGFA/LDI-FT/ICR/MS accord with the above direct LDI observations. Nucleosides typically exhibit abundant \((M+K)^+\) ions, usually as the base peak of the spectrum, and nucleotides are dephosphorylated to yield spectra similar to the corresponding nucleoside. For example, Figure 5.7 (top) shows a TGFA/LDI-FT/ICR mass spectrum of guanosine, acquired in the source trap without prior axialization. Following collisional axialization and transfer of ions to the analyzer trap, mass resolving power of \(\geq 20,000\) was routinely achieved, as shown in Figure 5.7, bottom, for guanosine.
Figure 5.7 TGFA/LDI-FT/ICR mass spectra of guanosine. Top: Broadband source-side detection without prior axialization. Bottom: Narrowband analyzer-side detection after prior axialization. Fragments are readily observed by broadband detection, whereas narrowband detection of axialized ions yields much higher mass resolving power and signal-to-noise ratio.
**Saccharides.** To date we have been able to desorb intact only mono- and disaccharide quasimolecular ions, as shown in Figure 5.8 for cellobiose. Saccharides exhibit limited solubility in the methanol solvent used throughout this study. A more uniform solution may be needed to facilitate spatially uniform precipitation of the analyte on the sample probe tip. Insoluble samples in our experiments were applied as a fine suspension (sonication for ~10 min) at the same concentration as other biomolecules examined in this experiment. Limited solubility in the solvent (methanol) is not the sole limitation, because longer-chain oligosaccharides exhibit generally higher methanol solubility compared to mono- or disaccharides, but yet gave no TGFA/LDI-FT/ICR mass spectra. We speculate that inefficient ionization was the reason; if so, then an additional chemical ionization (CI) step after the desorption event should help. However, neither sodium- nor ammonium salts proved any more effective than potassium bromide. As noted in the Experimental section, perhaps a roughened surface on which the gold film is deposited might assist wettability of the sample holder by more polar solvents (e.g., water), as well as provide increased surface area for desorption/ionization.

**Phospholipids.** Phospholipids represent the major structural lipid class of biological membranes. Accurate mass measurement in combination with high mass resolving power (both provided by FT/ICR/MS) could greatly facilitate mass spectral analysis of membrane phospholipid composition. However, we were able to achieve only limited
Figure 5.8 TGFA/LDI-FT/ICR mass spectrum of cellobiose. Potassiated and sodiated quasimolecular ions dominate the spectrum, which is virtually devoid of fragmentation.
success with TGFA/LDI of phospholipids. Five phospholipid samples were investigated and quasimolecular ions were observed, but at low ion abundance and low signal-to-noise ratio. Figure 5.9 shows a representative single-shot TGFA/LDI-FT/ICR mass spectrum of dipalmitoyl phosphatidic acid (DPPA) taken in the source trap.

Attempts to transfer axialized phospholipid quasimolecular ions to the analyzer trap were unsuccessful. Perhaps the ions are unstable and fragment during the (lengthy) axialization process. Ion abundances remain approximately constant for ~10 ms to 1 s after desorption, but decrease thereafter. Abundances are not improved by a higher background pressure of argon or nitrogen which could result in collisional cooling and therefore stabilization of the ions. Onset of the quadrupolar excitation may actually produce collisional fragmentation of the ions in this case. If so, then use of a less massive buffer gas (say, helium) at lower temperature should increase the number of ions that survive the collisional damping during quadrupolar irradiation. In our instrument, in which both trap compartments are pumped by separate cryopumps, the use of helium as a collision gas is difficult, because helium is removed only very slowly by cryopumping. We therefore refrained from using helium as a collision gas in quadrupolar axialization experiments although it has been successfully employed by Amster et al.\textsuperscript{35} Nitrogen represents a slight decrease in mass relative to argon but did not result in axializing the phospholipid quasimolecular ions. It is worth noting that the quadrupolar axialization/collisional cooling for ion axialization is a new technique whose various parameters
Dipalmitoyl Phosphatidic Acid

Figure 5.9  TGFA/LDI-FT/ICR mass spectrum of dipalmitoylphosphatidic acid showing the quasimolecular ion region.
(quadrupolar excitation amplitude, frequency, and duration; buffer gas pressure; trapping potential; subsequent ion transfer period; etc.) are not well-characterized. Of course, it is possible that the ions are simply not stable over the several seconds time scale of the present experiments. Collisions of ions with much smaller neutrals may not induce fragmentation but can remove kinetic energy from an ion, until the ion eventually becomes thermalized. If the cooling process is faster than the redistribution of energy (leading to bond rupture) after the desorption process, fragmentation may be avoided. Relative to this issue, we recently observed a very slow unimolecular decay of gramicidin S after desorption from the gold plates. Because a circular molecule such as gramicidin S requires breakage of two bonds to produce fragment ions, observation of unimolecular decay of gramicidin S suggests the possibility of more rapid decay for linear molecules (for which only one bond need be broken) under similar conditions. We are presently investigating this possibility.

Approximate kinetic energy distribution of TGFA-laser desorbed ions. Desorption with our laser presumably yields an ion population with a high absolute number (e.g., leucine enkephalin) and a large range of kinetic energy (which translates into large preexcitation magnetron and cyclotron radii as well as z-axial amplitudes, resulting in a potential loss of ions before or during excitation).57 A wide kinetic energy distribution and large magnetron radii also prevent the transfer of laser-desorbed ions to the analyzer of the dual trap. In support of this analysis, we find that mass resolving power increases following
collisional cooling of ions in the source trap, even in the absence of quadrupolar excitation. Wilkins et al. have suggested that adding a sugar co-matrix acts to reduce ion kinetic energy during the desorption process. Spengler et al. have reported kinetic energy distributions for TOF-analyzed laser desorbed ions. Heavy ions (m/z ~ 5000) carry as much as 10 eV of kinetic energy. Similar results were obtained by Hogan et al. based on IR laser irradiation. Even for a relatively low-mass MALDI-desorbed nucleotide dimer (m/z 530) observed by FT/ICR/MS, Hettich and Buchanan found a kinetic energy distribution spanning several eV. High ion kinetic energy obviously makes it difficult to trap heavy ions for FT/ICR mass analysis: if the ion kinetic energy is higher than the axial trapping potential, then ions will escape axially from the trap.

The assumption that ions produced by our laser setup have a wide kinetic energy distribution is confirmed by the observation that analytes (especially leucine enkephalin and gramicidin D) often exhibit very weak signals when detected in the source trap but give excellent spectra after axialization and collisional cooling (see Fig. 5.10). Furthermore, because neutrals do not respond to quadrupolar excitation, the detected ions must have been formed before onset of the cooling process. Also, ions required for the cationization process (e.g., K+, K2Br+) diffuse radially outward and are lost from the trap during single-frequency collisional axialization of the quasimolecular ions, so that (M+K)+ ions must have been formed prior to the axialization step. We therefore infer that
Figure 5.10 Comparison of source trap (no axialization /cooling) and analyzer trap (after 10 s of axialization/cooling) detection of gramicidin D. Possibly a high absolute amount of kinetic energy retained by the ions after desorption/ionization limits the trapping capability of the instrument.
quasimolecular ions are formed in high abundance, even at high m/z, but are not necessarily detected due to inefficient trapping and/or insufficient cooling before excitation/detection.

**Mechanism of desorption and ionization.** The detailed mechanism of production of a plasma useful for mass analysis by TGFA-LDI as well as by other MALDI or SALDI techniques remains an open question. However, we may make some inferences from Tanaka’s MALDI experiments,\(^1\) in which a finely dispersed metal powder in a liquid serves as the matrix; a proposed initial step in desorption is the heating of the metal particles by laser light of a wavelength longer than the particle diameter. The spatially coherent rapidly varying electric field of the irradiation induces electric current in the particles, resulting in resistive heating of the metal particles and the surrounding solution. In the present TGFA-LDI experiments, the gold film thickness is about two orders of magnitude smaller (~10 nm) than the laser fundamental wavelength (1064 nm). Therefore, a similar rapid heating event after laser irradiation could be operating here. Low-power laser pulses could provide sufficiently high energy to vaporize the thin gold film and bring single or clustered atoms of metal into the gas phase along with the analyte, but without appreciable multiphoton absorption leading to extensive fragmentation of sample molecules. In contrast, atomization from bulk metal near the surface requires high laser irradiation power due to the high electrical and thermal conductivity of gold. In that case, analyte molecules may readily undergo multi-stage fragmentation and/or
ion-molecule reactions, so that the resulting mass spectrum fails to show abundant molecular or quasimolecular ions.

For the present experiments, the thickness of the gold film was adjusted to have an absorption maximum at 1064 nm (measured by an UV/VIS/NIR spectrometer), to match the fundamental Nd:YAG laser light. However, the absorbance of the thin gold film measured with a low-power continuous light source could differ from that from a high-power pulsed laser source. The relation between film thickness, optical absorbance, and LDI performance will be a subject for future investigation.

Regarding the ionization mechanism in TGFA/LDI, we presume that \((M+K)^+\) ions are produced by reaction of neutral molecule with \(K_2Br^+\), as has previously been demonstrated for LDI. We are currently investigating postdesorption chemical ionization techniques to widen the range of samples which can be analyzed and to increase the sensitivity of TGFA/LDI.

CONCLUSIONS

A new thin gold film-assisted laser desorption/ionization technique has been applied to each of a variety of chemically different molecular classes of biological interest. All analyte-containing samples were prepared by premixing equal amounts of methanolic solutions of analyte \((10^{-3} \text{ M})\) and \(KBr (10^{-2} \text{ M})\) and abundant quasimolecular ions \((M+K)^+\)
were detected. A detection limit of ~100 femol per laser shot was attained for potassiated gramicidin S. In general, analyte deposited on a stainless steel probe tip or a glass surface at the same concentration as for TGFA did not yield detectable molecular ions by LDI. Even gramicidin S, a standard molecule for demonstration of intermediate molecular weight-LDI, did not yield molecular ions by direct LDI unless deposited as a thick slurry in methanol/KBr solution.

The work presented here clearly demonstrates that laser desorption/ionization of analyte deposited on a thin gold film substrate on a glass surface profoundly increases the applicability to biological molecules of LDI in the near IR/visible range. Laser desorption/ionization efficiency can be vastly higher than in the absence of the gold film. In general, it appears that the thin gold film extends the applicability of Nd:YAG laser desorption to organic molecules previously LDI-detected much more efficiently with a CO\textsubscript{2} laser (10.6 \mu m). We are exploring the use of chemical ionization to form quasimolecular ions to further lower the detection limit and open the way to analysis of more fragile molecules. Finally, because the gold film optical absorption spectrum can be tuned by varying the film thickness, we predict that it should be possible to change the laser wavelength as necessary to avoid resonant laser excitation of a chromophore that would otherwise absorb laser light directly, without any other change in the experimental protocol.
REFERENCES


INTRODUCTION

The first reports of large carbon clusters by the Exxon 1 and Rice 2 groups with supersonic laser vaporization sources have stimulated greatly increased interest in the structure and reactivity of all sorts of atomic clusters, including those from noble metals, particularly gold. Methods for producing charged clusters of gold atoms include: secondary ion mass spectrometry (SIMS) 3-8, liquid-metal ion sources 8,9, electrohydrodynamic sources 10, field emission followed by laser desorption 11, gas aggregation followed by electron impact 12,13, flowing afterglow 14, and most commonly laser desorption/vaporization (including supersonic sources) 15-21. These clusters are usually detected and identified by mass spectrometry 3-19, although photoelectron spectrometry has been used to observe negatively-charged gold clusters and to measure their electron affinities (EA's) 14,20,21. Mass
spectrometry ion abundance variations show that metal ion clusters with odd-numbers of atoms tend to be more abundant than even-numbered clusters \(^{4,6-8,12,22}\); moreover, collision-induced dissociation (CID) experiments indicate that odd-numbered cluster ions tend to be more stable than even-numbered cluster ions \(^{18,19}\). These mass spectrometric observations are supported by corresponding photoelectron spectroscopy showing that odd-numbered clusters have higher EA’s than even-numbered clusters \(^{14,20,21}\), consistent with theoretical stability predictions based on the electronic shell model of clusters, with magic numbers for neutral gold cluster stability at 8, 18, 20, 34, 40, and 58 corresponding to shell closings \(^{4,8,20,23-25}\).

With a few exceptions \(^{4,7,13,19,20,23,24}\), previously reported gold clusters, \(\text{Au}_n^{+/-}\), have been relatively small (\(n < 10\)). In fact, until now it has been impossible to detect large gold clusters produced by laser vaporization except by use of supersonic-jet expansion (cooling) techniques \(^{19,20}\). In this chapter, a simple method for producing large gold cluster ions will be discussed based on a technique which has been used previously in chapter 5 for desorption of biomolecules from a gold film which absorbs strongly at the fundamental wavelength of a Nd:YAG laser. Interestingly, when certain peptides are deposited onto a thin gold film coated on a glass substrate, large gold cluster ions (\(\text{Au}_n^+\) to \(n = 21\) and \(\text{Au}_n^-\) to \(n = 46\)) are generated and resolved by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometric detection. These large clusters are not produced in the absence of the peptide on the gold layer, and gold adducts with peptides (\(M + \text{Au})^{+/-}\) are not observed. Initial
results using this technique are described and related to predicted gold cluster ion stabilities.

EXPERIMENTAL

Sample Preparation. The pure gold films were prepared by the same evaporator and conditions which have been discussed in Chapter 5. The gold film plates were then coated with 40 µl of a 1 x 10^{-3} M peptide (gramicidin S for negative ions or gramicidin D for positive ions) solution in methanol to enhance the production of large gold cluster ions (see Results and Discussion). Peptides were purchased from Sigma Chemical Co. (St. Louis, MO), and used without further purification.

FT-ICR Mass Spectrometry. Laser desorption FT-ICR experiments were performed on a Waters Extrel (Madison, WI) FTMS-2000 instrument operated at 3.0 tesla, and equipped with differentially pumped 4.76 cm dual cubic traps separated by a 2 mm diameter conductance limit. (The data for Fig. 6.4 were collected with a Waters Extrel Odyssey Data Station). Two Helix Technology CryoTorr-8 cryopumps (Waltham, MA) were used to evacuate both source and analyzer vacuum chambers. Laser desorption was performed with a YG-660A Continuum Nd:YAG laser (Santa Clara, CA) at 1064 nm with 10 ns pulses at 75 mJ/pulse, for which 5% of the power was included from the 532 nm green line to facilitate laser alignment.
Ions were trapped promptly after laser desorption in the source-side cubic trap (-2 V for negative ions in Figs. 6.1, 2, 3, +2 V for positive ions in Fig. 6.4), and the probe bias voltage was adjusted to match the trapping voltage. After a specified delay period (10 ms to 5 s), the gold cluster ions were then excited by means of a frequency sweep (60 V(p-p) from 1-250 kHz at a rate of 600 Hz/μs). Direct-mode detection (500 kHz Nyquist bandwidth, single 32 K time-domain transient padded with 32 K zeroes) was followed by fast Fourier transformation (without prior apodization) and magnitude-mode calculation. The pressure ranged from 6-12 x 10⁻⁸ torr in all experiments.

RESULTS AND DISCUSSION

**Conditions for production of small or large Auₙ⁻ cluster ions.**

Fig. 6.1 shows the laser desorption/ionization FT-ICR negative-ion mass spectrum of a pure gold film. As in prior studies from other laboratories (see Introduction), abundant small Auₙ⁻ clusters (1 ≤ n ≤ 5) are observed. The absence of signals corresponding to oxides and other impurities testifies to the purity and stability of gold film surface. A striking increase in the production of larger cluster anions, Auₙ⁻, occurs if 40 μl of 1 x 10⁻³ M gramicidin S in methanol is deposited onto the thin gold film before laser desorption/ionization: Fig. 6.2 shows gold negative ion clusters mass-resolved up to at least Au₄₆⁻, and clusters with more than 50 Au atoms (m/z ≥ 10,000) are probably present. Similar clustering has been observed with laser desorption (in the absence of supersonic jet
**Figure 6.1** Negative-ion Nd:YAG laser desorption/ionization FT/ICR mass spectrum of a pure thin gold film.
Figure 6.2  Negative-ion Nd:YAG laser desorption/ionization FT ICR mass spectrum of a thin gold film overcoated with gramicidin S. The even/odd Au\textsubscript{n}\textsuperscript{−} abundance variations are consistent with prior SIMS and supersonic jet expansion observations of Au\textsubscript{n}\textsuperscript{−}.
cooling) for both aluminum anions \(^{26}\) and fullerenes to form giant carbon clusters \(^{27}\). Odd-numbered \(\text{Au}_{n}^-\) clusters tend to be more abundant than even-numbered clusters, but higher relative abundances for closed-shell clusters (\(\text{Au}_{n}^-, n = 7, 17, 19, \text{and } 33\)), are less pronounced for \(\text{Au}_{n}^{+/−}\) than for other (e.g., sodium) atomic cluster ions \(^{23,24}\).

**Time evolution of the \(\text{Au}_{n}^-\) cluster size distribution.** The negatively-charged gold clusters appear to be unstable with respect to evaporation due to high temperature and due to multiple collisions with background neutrals. Initially, it appears likely that highly coagulated hot gold clusters of random sizes are produced in the hot plasma of ions (both gold and peptide) and neutrals formed by the laser pulse. Changes in relative abundances of \(\text{Au}_{n}^{+/−}\) cluster ions of different size (i.e., different \(n\)-value) thereafter result from the interplay of several effects: decrease in temperature of the background neutrals as the laser-desorbed plume cools, electron transfer from species of lower electron affinity (e.g., organics and small gold clusters) to species of higher electron affinity (e.g., large gold clusters), evaporation of atoms from large clusters (with consequent cooling of the cluster left behind), collision-Induced dissociation (resulting in loss of pairs of gold atoms), and (in the ICR ion trap) continuous expansion of the ion magnetron radius resulting in eventual radial diffusional loss of the gold cluster ions.

Fig. 6.3 shows the time evolution of the absolute and relative abundances of \(\text{Au}_{n}^-\) cluster ions following a laser desorption/ionization
pulse. Generally, the large clusters appear to break up, presumably due to a combination of evaporation and collision-induced dissociation (CID). The odd/even abundance alternation is maintained, however, suggesting that collisions between cluster ions and neutrals tend to displace even number (probably pairs) of gold atoms, consistent with prior CID observations \(^{18,19}\). As a result, the absolute abundance of smaller \(\text{Au}_{n^-}\) ions increases with time at first, but eventually even the small cluster ions are lost (presumably by magnetron radial diffusion). Since background neutral pressure decreases monotonically with time following the laser pulse, and since the overall size distribution of the \(\text{Au}_{n^-}\) clusters appears to change mainly from collision-induced dissociation, it appears that the \(\text{Au}_{n^-}\) ions, once thermalized, are stable. Thus, axialization of such cluster ions by quadrupolar excitation in the presence of a thermal buffer gas \(^{28-30}\) should make them amenable to trapping for extended periods.

**Effect of added peptides on \(\text{Au}^{+/−}\) cluster formation and stability.** Laser desorption from electrochemically or laser-roughened surfaces has previously proved useful for producing small metal cluster ions \(^{18}\). Here, we too are able to produce highly abundant small gold clusters from a thin gold film produced by depositing gold atoms under high vacuum. The formation of large gold clusters may be explained by (a) the higher electron affinities of large gold clusters, (b) the inhomogeneity of aggregated metal atoms on the glass surface, and (c) the peptide additive (see below). We suggest that gold clusters on the glass plate absorb the laser light and excite the surface plasmon mode of
Figure 6.3  Negative-ion Nd:YAG laser desorption/ionization FT/ICR mass spectra obtained of thin gold films coated with gramicidin S, following each of various periods (between ion formation and excitation/detection) of exposure to collisions with background gas at ~10^-7 Torr.
Figure 6.3

LD-FT/ICR/MS

100 ms delay between formation and detection

500 ms delay between formation and detection

1 s delay between formation and detection

5 s delay between formation and detection

$\text{Au}_n$
electrons in the cluster. The excited plasmon mode (see Fig. 5.1) loses energy by transfer to surface clusters and peptide molecules.

The intriguing new feature is that addition of a small amount of peptide makes possible the generation and detection of giant gold clusters detectable by FT/ICR mass spectrometry. To date, we have only been able to produce large negatively-charged gold clusters by coating the cyclic peptide, gramicidin S, on the thin gold film. Gramicidin S and its fragments may transfer electrons to the neutral gold clusters during the laser desorption process, because the electron affinity of a large gold cluster is much higher than that of a typical organic molecule. Molecular cations of gramicidin S are in fact observed in the positive-ion laser desorption/ionization FT-ICR mass spectrum. However, other (linear) peptides (e.g., gramicidin D, insulin A chain) of comparable electron affinity are ineffective.

At this stage, we have not separated the factors leading to peptide enhancement of large gold cluster ion formation: e.g., the cyclic nature of gramicidin S, possible shielding effect of the gold substrate by the peptide, and/or charge transfer between gramicidin S and gold clusters. Possibly electrons produced in the desorption step are thermalized by collisions with the gramicidin S so that they (the electrons) more readily attach to the gold clusters. However, such an explanation does not account for the failure of the other linear peptides to form massive negative clusters.
Finally, gold cluster positive ions are in fact observed by addition of the linear peptide, gramicidin D, under otherwise identical experimental conditions (Fig. 6.4). Although the positive cluster ions are smaller than the negative cluster ions, the shell-closing number for neutral stability at 8, 18, 20 (corresponding to n= 9, 19, 21 for positive Au_{n}^{+} ions) and odd number preference in clustering are similar to those for negative cluster ions.

**Relevance to surface-enhanced Raman scattering (SERS).** It is well established that a metal surface can enhance the Raman scattered intensity of molecules adsorbed on the metal surface. Moreover, in the sol state, metal atoms must aggregate to generate a large enhancement in Raman scattered intensity. Thus, clustering is essential to the SERS phenomenon. SERS experiments with thin metal films show that the large enhancement in Raman scattering appears to correlate with the presence of large cluster structures on the substrate (cover glass). For example, a great enhancement in Raman scattering has been observed for thiocyanate ion on a thin silver film. Unfortunately, a silver surface is not stable in air; moreover, the presence of multiple silver isotopes complicates the interpretation of the mass spectra of highly aggregated silver clusters. In the present experiments, we therefore chose to examine high-mass metal clusters from a thin gold film, which shows almost the same SERS effect as a silver film. The presence of large gold cluster ions, Au_{n}^{+/−}, (Figs 6.2,3,4,) in the present experiments thus offers additional evidence for a
Figure 6.4 Positive-ion Nd:YAG laser desorption/ionization FT/ICR mass spectrum of a thin gold film coated with gramicidin D. The even/odd Au$_n^{+}$ abundance variations are consistent with prior SIMS and supersonic jet expansion observations of Au$_n^{+}$.
clustered (inhomogeneously aggregated) atomic structure of the gold films used for SERS experiments.

CONCLUSIONS

Now that gold cluster ions may be easily and consistently produced, it should be possible to mass-isolate and axialize particular clusters by quadrupolar excitation in the presence of collisional cooling \(^{28-30}\) for subsequent investigation of cluster ion structure, reactivity, photodissociation, collisional dissociation, and determination of ionization energy and electron affinity by FT-ICR mass spectrometry.
REFERENCES


CHAPTER VII

ANALYSIS OF ATMOSPHERIC PARTICULATES, SRM-1648, BY FT/ICR MASS SPECTROMETRY

INTRODUCTION

Urban atmospheric particulates are an exceptionally complex mixture of compounds. These particulates consist of inorganic compounds onto which various organic compounds are adsorbed at levels from ultra-trace to ppm (part-per-million) concentrations. One major source of atmospheric particulates is the thermal conversion of municipal solid waste. More than 600 organic compounds have been identified in municipal incinerator fly ash.¹ There are many other sources of atmospheric particulates besides fly ash and the large number of compounds will cause difficulty in reliably identifying the components of the mixture.

Fourier transform ion cyclotron resonance (FT/ICR) mass spectrometry offers the advantages of simultaneous high mass resolving power and sensitivity² as well as MS/MS with high resolution in both stages.³ Laser desorption/ionization (LDI)⁴ is a convenient means for
generating ions from insoluble and/or involatile particulate organic components. Previously these methods have been successfully used for the analysis of crude oil, another complex environmental mixture. In this chapter, we discuss the analysis of Standard Reference Material (SRM) 1648, "Urban Particulate Matter" and trial methods to detect the toxic material which may be included in this material.

**EXPERIMENTAL**

**Sample Preparation.** The SRM 1648 and 1649 samples were purchased from the National Institute of Standard and Technology, Gaithersburg, MD. The SRM 1648 sample was prepared from urban particulate matter collected in the St. Louis, Missouri area over a period in excess of 12 months. Fig. 7.1 shows the methods used to generate the ions from the SRM 1648 material. For direct laser desorption ionization FT/ICR/MS, the SRM 1648 powder was directly applied to double sided sticky tape. Higher sensitivity was obtained for the chlorinated organic materials, by use of toluene extract of the SRM 1648, prepared by one day extraction of 20 mg SRM 1648 sample in 5 ml toluene. The 5 µl toluene extract was either directly applied to the stainless steel probe or on the thin gold film. The thin gold film surfaces were produced in the same way as in chapter 5 and 6. The gold film plates were then coated with 5 µl of a 0.245 x 10⁻³ M heptachloro-dibenzofuran (HpCDF) solution in a toluene (10%) and iso-octane mixture. The methane gas
Figure 7.1 Experimental Procedures for Identification of Organic Compounds in Urban Atmospheric Particulates
for chemical ionization of HpCDF was purchased from Air Products and Chemicals, Allentown, PA.

**FT-ICR Mass Spectrometry.** Laser desorption FT-ICR experiments were performed on an Extrel Waters (Madison, WI) FTMS-2000 instrument operated at 3.0 tesla, and equipped with differentially pumped 4.76 cm dual cubic traps separated by a 2 mm diameter conductance limit. The data were collected with an Extrel Waters Odyssey Data Station. Two Helix Technology CryoTorr-8 cryopumps (Waltham, MA) were used to evacuate both source and analyzer vacuum chambers. Laser desorption was performed with a Surelite I Continuum Nd:YAG laser (Santa Clara, CA) at 1064 nm with 10 ns pulses at ~100 mJ/pulse, for which 20% of the power was included from the 532 nm green line to facilitate laser alignment.

Ions were trapped promptly after laser desorption in the source-side cubic trap and the probe bias voltage was adjusted to match the trapping voltage. After a specified delay period (10 ms to 5 s), the ions were then excited by means of a frequency sweep. Direct-mode detection was followed by fast Fourier transformation (without prior apodization) and magnitude-mode calculation. The pressure ranged from 6-12 × 10⁻⁸ torr in all experiments. For the methane chemical ionization, a pressure of 10⁻⁷ torr was maintained during the experiment.
RESULTS AND DISCUSSION

DIRECT LASER DESORPTION IONIZATION FT/ICR/MS OF SRM 1648.

Figure 7.2 illustrates the positive ion LDI FT/ICR mass spectrum of SRM 1648 obtained by applying the particulate matter directly to the probe tip and desorbing the ions using a Nd:YAG laser. Of the polynuclear aromatic hydrocarbons in this mixture, the spectrum shows molecular ions for benzopyrene/perylene (m/z 252.0958) chrysene (m/z 228.0913) fluranthene/pyrene (m/z 202.0801) and anthracene/phenanthrene (m/z 178.0764). The predicted structures are shown in Figure 7.3. The mass calibration was accomplished by use of perfluorotri-n-butyl amine. The average error for these mass measurements was 10 ppm. Positive ion LDI FT/ICR spectra of these particulates always contained a prominent ion at m/z 149.0238, protonated phthalic anhydride, a fragment ion from the alkyl phthalate esters in the mixture.

Negative ion LDI FT/ICR mass spectra from SRM 1648 were always dominated by an ion at m/z 266.9984 (Figure 7.4). The measured mass generates the elemental composition $C_{13}H_{935}Cl_{2}O_{2}$. This ion has been shown by gc/ms using a quadrupole mass spectrometer to arise from 2,2'-methylenebis[4-chlorophenol], "dichlorophene", a compound that has been used as a fungicide and anthelmintic. The presence of this compound was not mentioned in previous reports on the composition of SRM's 1648 and 1649.
Figure 7.2 Positive ion LDI FT mass spectrum of Standard Reference Material 1648.
Figure 7.3  Predicted polynuclear aromatic hydrocarbons in SRM 1648 material from the positive ion mass spectrum generated by LDI/FT/MS.
Figure 7.4 Negative ion LDI FT mass spectrum of Standard Reference Material 1648.
Figure 7.5 Negative LDI FT mass spectrum of a toluene extract of Standard Reference Material 1648 on a thin gold film substrate (magnitude-mode of the first derivative mass spectrum)
Thin gold film assisted laser desorption ionization FT/ICR/MS of SRM 1648. Negative ion LDI FT/ICR spectra are more susceptible to thin gold film matrix effects than their positive ion counterparts, as Figure 7.5 illustrates. Figure 7.5 shows the negative ion LDI FT/ICR spectrum obtained by placing 5 µl of a toluene extract of SRM 1648 on thin gold film followed by laser desorption. In addition to the m/z 267 ion the spectrum shows the presence of numerous polychlorinated materials. The ion at m/z 400 may correspond to octachloronaphthalene. The cluster at m/z 361 may represent a C_{14}H_{6}C_{13}O isomer, possibly a mixture of trichloro-(phenanthrene/anthracene)ols.

FT/ICR/MS of heptachlorodibenzo-furan. We have independently demonstrated that the LD FT/ICR system is sufficiently sensitive to detect nanogram quantities of molecules like heptachlorodibenzo-furan by use of the thin gold film, Figure 7.6(a), and methane-chemical ionization, Figure 7.6(a). The fact that we have seen no evidence for the presence of octachlorodioxin and the other polychloro dioxins and dibenzofurans that are known to be present in this sample shows that competition for the ionization in negative ion LD FT/ICR will require some fractionation of the mixture prior to analysis for these compounds.
Figure 7.6  Negative ion Spectrum of heptachlorodibenzofuran by methane chemical ionization and thin gold film assisted LDI FT/ICR/MS.
CONCLUSION

The power of LDI FT/ICR for the analysis of complex mixtures is illustrated by the detection of several compounds in the mixture that were not mentioned in the literature. We first found the 2,2'-methylenebis[4-chlorophenol] in SRM 1648 and 1649. For the negative ion mass spectrum of the polychlorinated organic mixture, the thin gold film assisted LDI/FT/MS shows high sensitivity. To simplify the spectrum and increase the dynamic range, we may need to fractionate the SRM samples.
REFERENCES


INTRODUCTION

The development of matrix assisted laser desorption (MALDI) has been one of the most striking advances in mass spectrometry for the last decade. Since its introduction by Hillenkamp and Karas in 1987, MALDI has become the method of choice for biochemical applications of mass spectrometry. Electrospray ionization (ESI), or a MALDI source coupled with a time of flight (TOF), FT/ICR, quadrupole ion trap or double focusing sector mass spectrometer has become an essential tool in many biochemical laboratories, allowing rapid (few seconds), sensitive (subpicomole levels) and accurate (far more than traditional) method for determining molecular weights of biologically active molecules. A particularly interesting potential capability is the protein and oligonucleotide sequencing. Although this is still far away from
practical applications regarding current mass range limit and sensitivity, tandem mass spectrometry (MS/MS) is nowadays recognized as a very useful complement to standard oligopeptide sequencing methods because of its high sample throughput, applicability to complex mixture analysis and versatility for sequencing of modified peptides and proteins with unusual amino acids.\textsuperscript{17} Moreover, there is an ample motivation to tackle these issues: routine MS analysis of proteins or unconventional MS sequencing of oligonucleotides can turn hours of tedious traditional chemical or ultrafast electrophoretic work into seconds. Recently Chait et al. demonstrated the feasibility of development of a fully automated protein sequencer with mass spectrum detection, based on so called ladder sequencing, consisting of modified stepwise Edman degradation followed by readout of sequence from MALDI mass spectrum.\textsuperscript{18,19}

MALDI, as one of the dominant ionization techniques with respect to biochemical applications, has shown rapid progress during the past decade. Yet, development in this field continues to be predominantly an empirical search for new matrices which reflects our poor understanding of the underlying principles. However, it is widely accepted that the common matrix properties are the resonant absorption frequency at laser fundamental wavelength, coupling between the vibrationally and/or electronically excited states and crystal lattice, and electronic structure and properties of excited states.\textsuperscript{20,21}

By far the most common mass analyzer used for MALDI generated ions is the time-of-flight (TOF) mass spectrometer. Although
MALDI/TOF combination holds the record in the upper mass limit, i.e., singly-charged proteins and protein clusters with m/z ≥ 200,000 \(^{11}\) have been detected, MALDI/TOF mass spectra have poor resolving power, i.e., m/Δm is typically 200-500 at full width at half maximum (fwhm), that even decreases to ≈ 50 for m/z > 100,000, and peaks are typically ill shaped (broad and asymmetrical). One of analytical impacts of the limited resolving power in mass spectrometric protein analysis is ambiguous differentiation between some residues, i.e., Asp (aspartate: 133 u) or Asn (asparagine: 132 u) and Glu (glutamate: 147 u) or Gln (glutamine: 146 u), at higher ion masses, i.e., m/z > 3,000.\(^{18,19}\) The main reasons for poor performance with respect to resolving power are the wide distribution of kinetic energies of "injected" ions\(^{22,23}\) and unimolecular decay\(^{24}\) of these high-mass biomolecules. As intrinsic characteristics of the LD process, these problems limit the performance of any mass analyzer combined with MALDI. Thus, the highest mass resolving power obtained with double focusing sector instruments equipped with an array detector is about 1000 for bovine ubiquitin (m/z ~ 8500 u), based on fwhm, at picomole sensitivity.\(^{25}\) A quadrupole ion trap also shows a drop in resolution and sensitivity for m/z ≥ 3,000 due to the inefficient trapping.\(^{8}\) However, very large spatial and kinetic energy distribution of desorbed ions should particularly affect FT/ICR analyzer because of the low voltage generally used to trap and store ions.

Although FT/ICR/MS appears to have more limited mass range than that demonstrated for TOF MS, it has a unique advantage of ultrahigh resolving power and detection sensitivity potential as well as ion
selection/storage ability for mixture analysis and sequencing which can be crucial for many biological applications. Mass range has recently been extended to ~ 157,000 u, corresponding to transferrin dimer ion, by use of a wire ion guide inside the ion trap. However, the resolving power, as a major strength of the technique, is limited by the nature of MALDI process itself. Ion trapping is heavily disturbed due to the high energy content (well above thermal) of desorbed ions as has been demonstrated by spectacular improvement in trapping efficiency and consequently in upper mass limit as well as ICR mass resolving power after ion cooling. Russell et al. reported that a so-called "waiting room" device mounted in front of analyzer cell provides collisional relaxation prior to MS analysis, thus allowing trapping of higher mass ions, although without significant improvement in resolution. Castro et al. obtained mass resolution in excess of 100,000 for several peptides and ~ 30,000 for bovine insulin by using carefully timed gated trapping/deceleration, and use of a sugar co-matrix, thus demonstrating the versatility of cooling for overcoming poor resolving power associated with non optimal ion trapping and metastable decay of high-mass biomolecules.

The highest mass resolving power ever demonstrated for MALDI generated ions has been obtained with an external ion source and r.f.-only quadrupole ion guide. Using gated trapping and pulsed collisional cooling, McIver et al. obtained mass resolution of 1,100,000 and 90,000 for sodiated gramicidin S and bovine insulin, respectively. Consequently, according to current results it seems that the trapping
and excitation/detection efficiency for MALDI generated ions is somewhat higher for external ion source method than for conventional internal ionization with standard dual cell configuration. This could be rationalized as a direct consequence of quadrupole ion guide which focuses the ions on the z-axis and serves as a bandpass filter reducing the total number of trapped ions and consequently reduces space charge effects. Similarly, high mass resolving power obtained by ESI, as an intrinsically external ion source, could be explained on the same basis, although a relatively wide distribution in kinetic energies still has to be reduced in order to obtain high-resolution ICR detection. Results obtained by McLafferty's group nicely illustrated this point: $m/\Delta m \approx 500,000$ has been achieved for undissociated carbonic anhydrase ($m/q = 29,025$), while >100 isotopic clusters are assigned in the mass spectrum obtained after nozzle/skimmer dissociation of the same peptide. 42

Collisonal damping is not only the most common, but also by the far the most efficient way of cooling of heavy ions. However, during this process ions could be easily lost from the trap due to the magnetron expansion associated with lowering of trapping potential energy. Thus, it is necessary to shrink an ion cloud back to the center of the trap prior to ICR excitation/detection event. The ion axlallzatlon method (see chapter 2), consisting of collisional damping and simultaneous azimuthal quadrupolar excitation of desired ions has been proved to be extremely efficient method for obtaining ultahigh mass resolving power and for trapping the high-mass ions generated by MALDI or ESI. The mass
resolving power of MALDI generated ions may be further improved by the frequency drift correction procedure as discussed in chapter 3.

Due to the resolution enhancement by frequency drift correction we found the Coulomb coupling between the ions with slightly different masses. According to some recent experimental data,\textsuperscript{43,44} the Coulomb interaction between ions with slightly different $\omega_c$ (m/q) values induces the merged ion motion and thus result single cyclotron frequency of ion mixture of different masses.\textsuperscript{52} Thus, we change the distance between the ions by decreasing the trapping voltage and we can avoid the Coulomb coupling in low density of ions in trap.

Finally, we should point the dependence of ICR mass resolving power, upper mass limit and nonlinear effects associated with space charge on the magnetic field strength. MALDI or ESI FT/ICR/MS high resolution and/or high mass work, reported so far, has been mostly done at 7 Tesla. In this chapter, we use a lower magnetic field (3 T), however, the resolving power of spectra shown in Figures of this chapter is compatible with the results from a higher magnetic field. Therefore the application of axialization and frequency correction method in MALDI experiment with higher magnetic field will dramatically improve both obtainable upper mass limit and mass resolving power.
**Experimental**

**Sample Preparation.** 2,5-Dihydroxybenzoic acid (DHB) and D-fructose (Aldrich Chem. Co, Milwaukee, WI) were used as the matrix and co-matrix, respectively, for all reported MALDI experiments. All oligopeptides, except nonanpeptide Arg-Leu-Cys-Ile-Phe-Ser-Cys-Phe-Arg, were purchased from Sigma Chemical Co, St Louis, MO, and were used without further purification as 1 mM solutions in 0.1% aqueous trifluoroacetic acid (TFA). Oligopeptide solutions were mixed with an appropriate quantity of 1 M matrix and 1 M co-matrix solution in order to obtain molar ratio of 1000/500/1 for DHB/D-fructose/oligopeptide. Deuterated samples were prepared by using H$_2$O/D$_2$O mixture as a solvent (D$_2$O, Aldrich Chem. Co, Milwaukee, WI). Approximately 10 µl of mixture (1 - 10 nmol of analyte) was applied to the solids insertion probe tip and allowed to dry in air before insertion in the mass spectrometer.

**Laser desorption/ionization.** All experiments were performed at 3 T on an Waters Extrel FTMS 2000 (Madison, WI, USA) instrument equipped with dual cubic trap (4.76 cm plate to plate distance), separated by a 2 mm diameter conductance limit differentially pumped by Helix Technology CryoTorr-8 cryopumps and an Odyssey data system. The frequency-tripled output (355 nm) of a Nd:YAG laser (Continuum, Surlite 1-10; 7 ns pulse) was interfaced to mass spectrometer as shown in Fig.1.2. Near UV photons were separated from IR (1064 nm) and VIS (532 nm) by a UV reflective mirror; variable attenuation of the laser power has been achieved by changing the Q-switch delay. The highly
attenuated laser pulse was focused through the conductance limit separating the two halves of the dual trap on the stainless steel probe tip, located behind the source trap plate. The focusing was accomplished by using two lenses as a telescope (2:1) assembly. The spot size was estimated to \( \sim 1 \text{ mm}^2 \), while the typical laser pulse energy was 1-2 mJ (powermeter, Diamond, Ophir Optics Inc., Wilmington, MA). Therefore, final power density at the probe tip was approximately \( 10^7 \text{ W cm}^{-2} \) in accordance with accepted optimal values.

**Fourier transform ion cyclotron resonance mass spectrometry.** The detailed experimental conditions for laser desorption and ion axialization have been described in previous papers.\(^{45,46}\) Briefly, after laser desorption/ionization event, ion z-axis translational energy was minimized using gated deceleration, i.e., raising the potential on the conductance limit plate to 9.75 V for short period of time (20 - 200 ms) and grounding probe tip and source trap plate; a 1 s delay provided additional "relaxation" of the initial cyclotron and axial motion. The axialization was provided by the train of low amplitude successive identical SWIFT excitations, in azimuthal quadrupolar mode, at the cyclotron frequency of the ion(s) of interest, in the presence of the collisional gas; amplitude and duration of quadrupolar excitation have been optimized for each sample. Ions were than transferred to the low-pressure analyzer trap. After the ion transfer event, static trapping was been smoothly lowered from 2 - 3 V to 0.2 - 0.6 V. Conventional frequency - sweep excitation from 1 - 500 kHz at 500 Hz/\( \mu \text{s} \) sweep rate or broad-band SWIFT excitation was followed by heterodyne detection at 1 -
5 kHz detection bandwidth to yield 64K - 256K time-domain data points. Collision gas, namely N\textsubscript{2} or Ar, was admitted into the source trap through a leak valve (Model 951-5100, Varian, Palo Alto, CA) to a partial pressure of $0.2 - 2.0 \times 10^{-7}$ torr in the source trap, corresponding to $0.1 - 0.5 \times 10^{-9}$ torr in the analyzer trap. Each FT/ICR mass spectrum resulted from a single laser shot.

**Frequency drift correction.** The frequency change during detection has been corrected by calculating the frequency drift from Fourier transformation of segmented small transients of time domain data as discussed in chapter 3. 256 K time-domain data from a bradykinin sample were accumulated in heterodyne mode for 1.7007 kHz signal bandwidth and 45,674 Hz reference frequency with 294 μs dwell time and transferred from an Odyssey\textsuperscript{®} data station to the Quadra 840AV as a ASCII code by use of a program provided by Waters Extrel FTMS. The 256 K data were separated into 64 segments of 4 K data points and Fourier transformed on a Macintosh Quadra 840AV computer (Apple Computer, Cupertino, CA) with 40 MHz Motorola 68040 CPU and 64 megabyte RAM, with an algorithm compiled with THINK C Version 7.0.3 (Symantec Corporation, Cupertino, CA). A standard Cooley-Tukey FFT algorithm\textsuperscript{47} converted the ICR time-domain data to a frequency-domain spectrum, which was converted to a mass spectrum by a standard mass calibration formula based on pure quadrupolar trapping potential.\textsuperscript{48} The frequency drift functions were calculated by the 9th order polynomial expansion and mass spectra were plotted by a KaleidaGraph Version 3.0.1 (Synergy Software, Reading, PA) spreadsheet program. For the
Insulin B chain 126 K time domain data were collected in heterodyne mode at 1.1848 kHz signal bandwidth and 14.226 MHz reference frequency with 422 µs dwell time and separated into 32 segments of 4 K data points. The frequencies of the segments were fitted to time with 5th order polynomial expansion, followed by the same procedure as in bradykinin data.

RESULTS AND DISCUSSION

Cooling and axialization of ion. The high resolution mass spectra of several oligopeptides (leucine enkephalin, oxytocin, bradykinin, nonapeptide Arg-Leu-Cys-Ile-Phe-Ser-Cys-Phe-Arg, angiotensin, and somatostatin), small peptides (bovine insulin chain B, bovine insulin) and some of its deuterated derivatives are detected by MALDI FT/ICR/MS. Long-lasting transient signal (typically 5 - 60 s) is generated by the optimization of experimental parameters (deceleration times, collisional gas pressure, amplitude and duration of quadrupolar excitation; and trapping voltage) and consequently high mass resolving power is obtained. FT/ICR mass resolving power is linearly proportional to the data acquisition time in the absence of signal decay and inhomogeneous line broadening during detection which is determined by the stability of ions, i.e., metastable decay, the rate of the collisions of the ions with the background neutrals, and inhomogeneous electric field within the trap. In our experiments ions have been generated with minimum laser power which can generate the ions, and then decelerated
Arg-Leu-Cys-Ile-Phe-Ser-Cys-Phe-Arg

(b₈+H+OH)⁺

(M+H)⁺

Figure 8.1 The MALDI FT/ICR magnitude-mode mass spectra of nonapeptide Arg-Leu-Cys-Ile-Phe-Ser-Cys-Phe-Arg detected a) in the source trap at the pressure of ~ 1.5 x 10⁻⁷ torr, and b) in the analyzer trap, after 20 s of quadrupolar excitation in the presence of 1.5 x 10⁻⁷ torr Ar in the source trap, at the pressure of ~ 0.2 x 10⁻⁷ torr.
by gated trapping. The ions are then cooled by collisions with neutrals and simultaneously shrunk to the center of the trap by applying quadrupolar excitation. Thus, one can safely assume minimal excess internal energy. Also, collisional relaxation at sufficiently low pressure (~0.1 - 0.2 x 10^{-8} torr) can be practically neglected. Consequently, fast unimolecular decay of higher mass ions (high number of vibrational degrees of freedom) and non-ideal electric field in the trap remain as main mass resolving power limiting factors.

Fig. 8.1 and Fig 8.2. show high resolution MALDI FT/ICR spectra of several oligopeptides obtained using axialization scheme described above. Detection of nonapeptide ions in the source trap, at a base pressure of ~1 x 10^{-7} torr, results in poor mass resolving power, m/Dm ~ 2000 (Fig 8.1a.). After 20 s of axialization and transfer to the analyzer trap, where the pressure is about two orders of magnitude lower, m/Dm ~ 120,000 has been obtained (Fig 8.1b.). Although m/Δm in the source trap can be increased if the ions are collisionally cooled, the transient damps too quickly to allow ultrahigh mass resolving power. Consequently, usage of the axialization technique may improve the resolution by an order of magnitude. Once desorption and axialization parameters have been optimized for ion(s) in a certain m/z range, the same experimental sequence could be used for any sample within that mass range. For example, the spectra presented in the Fig. 8.2 have been obtained by using the same train of low amplitude (~12.5 V <sub>p-p</sub>) successive identical SWIFT excitations, in azimuthal quadrupolar mode.
Figure 8.2  High resolution MALDI FT/ICR magnitude-mode mass spectra of: a) angiotensin, b) neurotensin and c) somatostatin.
Differences in obtained mass resolving power and signal to noise ratio are presumably due to different amounts of collisional gas present in the source trap. Since the efficiency of axialization depends on azimuthal quadrupolar excitation amplitude and collisional damping, while low-pressure is essential during detection, it is necessary to simultaneously optimize these two parameters in order to obtain the best results. Nevertheless, relatively high performance FT/ICR MS analysis of desorbed ions (not necessarily oligopeptides) could be easily routinized.

**Frequency drift correction.** Bradykinin molecular ions (Fig. 8.3.) have been acquired for more than a minute, which theoretically should correspond to m/Δm ~ 2,000,000 in the low-pressure limit at 3 T. The experimental value, obtained after frequency drift correction, is m/Δm ~ 1,500,000. It has been shown that ion frequency may (and usually does) drift during detection period and thus significantly broaden the observed ICR signals. The effect has been interpreted as arising from time variation of the space charge potential and can be eliminated by the frequency drift correction procedure explained in chapter 3.

**Axialized Ion cyclotron resonance mass spectrum at high ion density.** However, an even more serious mass resolving power limiting factor seems to be the loss of individual ICR signals for ions of slightly different m/z values (Fig 8.4. and 8.5.). Signal merging is a direct consequence of ion-ion interaction associated with moderate to high ion densities in the trap. The MALDI FT/ICR mass spectrum of a deuterated
Figure 8.3 MALDI mass FT/ICR magnitude-mode spectra of bradykinin: a) FFT of raw time domain data and b) frequency drift correction yields an ultrahigh resolution mass spectrum; this spectrum represents the highest mass resolving power obtained to date for any laser desorbed biomolecule.
Figure 8.4 MALDI FT/ICR magnitude-mode mass spectra of a) deuterated leucine enkephalin and b) deuterated bradykinin.
Figure 8.5 Bovine insulin chain B at high ion density (unresolved after frequency drift correction)
Figure 8.6 MALDI/FT/MS of Bovine insulin chain B at low ion density (resolved).
leucine enkephalin sample, shown in Fig 8.4, was obtained using a standard pulsed axialization scheme. Based on spectrum simulated for Lorenzian lines with m/Δm > 700,000 for m/q ~ 558, one would expect to see separate signals corresponding to $^{12}\text{C}_2\text{H}_3\text{N}_5\text{O}_7+$ and $^{13}\text{C}_2\text{H}_3\text{N}_5\text{O}_7+$. However, only a single peak appears. Similarly, in the case of deuterated bradykinin sample (Fig 4b), a measured m/Δm of approximately 200,000 should be enough to at least indicate the presence of $^{13}\text{C}_1\text{H}$ and $^{12}\text{C}_2\text{H}$ signals, but again only a single peak is obtained. This effect is even more pronounced in the spectrum of bovine insulin chain B (Figs 8.5, and 8.6). Since the resolution, after frequency drift correction, for the spectrum shown in Fig 8.5 is about 0.004 u one would expected to see characteristic isotope pattern in the range 3493-3499 u. However, only one mass peak appears, the mass being the weighted average of all species. Actually, the average frequency is shifted to the lower value due to the high ion density in the trap (i.e., space charge).

**Axialized Ion cyclotron resonance mass spectrum in low ion density.** Spectra obtained with different ion densities clearly show Coulomb-induced coupling of the ion motions. Ion densities were varied by means of changing i) the number of desorbed ions (i.e., laser power), ii) axialization efficiency (i.e., time of quadrupolar excitation and/or collision gas pressure), iii) ion transfer efficiency (i.e., transfer time), and/or iv) the number of trapped ions (i.e., trapping voltage). In all cases, the isotope pattern is partially or completely lost as the ICR signal
Increases. Similar behavior has been reported for ions stored in a quadrupole ion trap, and recently for a CO/N₂ mixture in an ICR trap.\textsuperscript{43} Thus, at relatively high ion densities in an ion trap, cyclotron motions of the ions with a small ICR frequency difference are strongly coupled. Instead of independent motions, collective oscillations, arising from the ion-ion interactions, are observed.\textsuperscript{43} Thus, high ion density in an ICR ion trap results not only in coulomb-induced frequency shift and peak broadening, but also in cross-coupling among the cyclotron modes of the ion cloud.

**Collective oscillations of stored ions.** Since this phenomenon strongly resembles non-neutral plasma physics, quantitative description is quite complex, mostly because of the normal modes dependence on the unknown ion cloud geometry. There are few reports on collective oscillations of stored ions in the recent literature. Ion dynamics in Paul quadrupolar trap is reasonably described by the high-temperature model of ion clouds.\textsuperscript{44} A recent report by Huang et al is an experimental study of ion coupling in an ICR trap, with emphasis on resulting effects on mass spectra and an explanation of two-particle system based on a simple harmonic oscillator approximation.\textsuperscript{43} Unfortunately, there is no simple analytical treatment of the ion dynamics associated with multiple-ion coulomb interactions. The appropriate model is an equation of motion:

\[
F = \frac{m_i q_i \mathbf{r}_i}{dt^2} = q_i \mathbf{E}(r_i, t) + \mathbf{v}_i \times \mathbf{B}(r_i, t) - m_i \gamma \mathbf{v}_i + \sum_{j>1} \frac{q_i q_j}{4\pi \varepsilon_0 r_{ij}^2} \tag{8-1}
\]
where \( m_i, q_i, \mathbf{r}_i = x_{i1} \hat{i} + y_{i1} \hat{j} + z_{i1} \hat{k} \), \( \mathbf{v}_i = \frac{dx_i}{dt} \hat{i} + \frac{dy_i}{dt} \hat{j} + \frac{dz_i}{dt} \hat{k} \) are \( i \)th ion mass, charge, position vector and velocity, respectively, in a magnetic field \( \mathbf{B} \) and electric field \( \mathbf{E} \); third term represents collisions modeled as frictional damping, while the last term describes Coulombic interaction between the \( i \)th ion and all the other ions present in the trap. The Coulomb potential is determined by the ion distribution which in turn depends on the total potential. Thus, even in an idealized case of quadrupolar trapping potential and uniform excitation field, analytical solution will require an iterative procedure to obtain self-consistency.

**Empirical threshold of ion motion coupling.** According to experimental results, we can estimate that the minimal frequency difference (\( \delta v \)) that can be detected separately in FT/ICR/MS with 3 T magnetic field is about 0.45 ~ 3.75 Hz. Bovine insulin chain B is detected with a frequency difference, \( \delta v \sim 3.75 \) Hz, however, deuterated leucine enkephaline cannot be detected as a unresolved peak even though the expected frequency difference is \( \delta v \sim 0.45 \) Hz. The \( \text{C}_{21}\text{H}_{22}^+ \) and \( \text{C}_{18}\text{H}_{26}\text{S}^+ \) signals in crude oil sample\(^{51}\) were partly separated and shows the reasonable intermediate frequency difference value, \( \delta v \sim 2.0 \) Hz.

**Advantage of High magnetic field.** Since frequency drift can be eliminated from a spectrum by deconvolution, this effect cannot be viewed as an limiting factor. Quite the contrary, ion-ion coupling between ions with slightly different \( \omega_C \) values, cannot be easily eliminated and represents the most serious limitation for high-
performance ICR measurements. Notice, however, that ICR frequency difference, as well as upper mass limit, mass resolving power, mass accuracy and signal-to-noise ratio (i.e., maximum number of trapped ions) strongly depend on magnetic field strength, \( B_0 \). Because the high magnetic field magnet is becoming more popular and inexpensive, therefore strong magnetic field may solve most of present problems. In this chapter, we have explored what can be done at 3 T, and showing that a significant increase in performance can be achieved at higher magnetic field.

**CONCLUSIONS**

The combination of MALDI, cooling-axialization techniques, and frequency drift correction methods provide ultrahigh resolution mass spectra using a relatively inexpensive 3 T magnet. Ultrahigh mass resolving power is obtained for protonated oligopeptide molecular ions, e.g., \( m/\Delta m \approx 1,500,000 \) and 100,000 for bradykinin after frequency drift correction and for insulin chain B, respectively. These results present the highest mass resolving power demonstrated for internally MALDI generated ions analyzed at 3 T, and are comparable to results obtained with higher-field magnets. The ion-ion Coulombic interactions appears as a FT/ICR/MS resolving power limiting factor in practical problems.
REFERENCES


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CHAPTER IX

QUADRUPOLARIZED ICR CUBIC TRAP

INTRODUCTION

The basic structure of ICR ion traps has been discussed in chapter 1. In this chapter we will introduce an improved trap design for future instrument development. The standard ICR trap has two trapping electrodes along the z-axis and two pairs of excitation and detection plates along the x and y-axis. Excitation electrodes should excite ions in the x-direction and then ion cyclotron radius will increase linearly with the amplitude of excitation electric field at a given duration or linearly with the excitation duration at a given amplitude. Also, the detecting plates receive the differentially induced charge (or voltage) generated by the post-excitation ion motion and the differentially induced charge is linearly proportional to the post-excitation ion radius. The ICR experiment is carried out within the electrostatic field generated by the voltage applied to the trap plates. The ion motion and detected signal are strongly influenced by the trapping electrostatic field.
There have been many attempts many trial to improve the trapping electric field.\textsuperscript{1,2} For higher dynamic range (greater ion storage capacity) and higher mass accuracy, the elongated trap has been design\textsuperscript{3} and improved.\textsuperscript{4} For electrostatic field improvement of the cylindrical trap, the addition of voltage adjustable trapping rings increases the accuracy, resolving power and sensitivity.\textsuperscript{5} In addition to segmenting the electrodes, modification of the physical shape of electrodes also can improve the trapping field. For example, a hyperbolic ICR ion trap was developed\textsuperscript{6} and systematically tested\textsuperscript{7} with observation of improved peak shape and resolving power. However, the finite structure of the trap does not allow one to produce a pure quadrupolar electrostatic potential.

In this chapter, we will design and test a quadrupolarized ICR cubic trap which has a near quadrupolar electrostatic trapping field while maintaining the advantages of the cubic trap (i.e., higher dynamic range). Two previous trials\textsuperscript{5-7} were combined to design our new trap. We kept the large cubic trap volume as in Naito's design for improvement in cylindrical trapping field, but we segmented the excitation and detection plate instead of the trapping electrodes. To improve the the electric field, we applied the required voltage to satisfy the boundary conditions of a quadrupolarized trap to each segment.
The importance of a quadrupolar electrostatic trapping potential can be explained by solving the equations of ion motion, in static electric and magnetic fields. As we can see in Eqs. 1-13a, 1-13b, 1-16, and 1-17, if we assume a quadrupolar trapping field, Eq. 1-11, the (reduced) cyclotron frequency, $\omega_+$, is purely independent of radius although the cyclotron frequency is no longer simply $qB/m$ as shown in chapter I.

The highly desirable independence of ion cyclotron frequency on radius requires an electrostatic field whose radial component is proportional to the radius, $r$. Also Laplace's equation, Eq. 1-7, requires that the potential must vary with $z^2$. Unfortunately, for a cubic ion trap, and essentially all tetragonal and cylindrical traps, the trapping electric field is not purely quadrupolar. Thus the cyclotron frequency is not constant during detection. Fortunately, near the center, every ICR trap has approximately a pure quadrupolar trapping field. The electrostatic potential inside a cubic, tetragonal, or cylindrical trap deviates from the quadrupolar form with increasing distance from the center of the trap. As a result, the ion cyclotron frequency varies with $r$ and $z$. Therefore, the quadrupolarization of the trapping potential inside an ion trap is one of the main goals of ion trap designs.
EXPERIMENTAL

We propose a new way to improve the trapping potential by showing that the electrostatic potential field in a conventional ICR cubic ion trap may be shimmed to near-perfect quadrupolar form without change in overall trap shape (cubic in this case) by segmenting the excitation and detection electrodes and coupling them by an appropriate RC network (see Figure 9.1). Four of the six plates (other than the two trapping plates) of the cubic quadrupolarized trap (4.15 cm) are cut into 20 segments along the z-axis, which are connected together by an RC network. In the conventional trapping mode, terminals 1 and 2 are grounded. In the modified trapping mode, terminal 1 is shorted with 3, and so is 2 with 4. This trap is called a quadrupolarized trap because it generates a more nearly quadrupolar trapping potential over a larger space than in a conventional cubic trap.

The quadrupolar trapping field is established by fitting the electrostatic trapping potentials of every segment to a quadratic function of the distance from the center plane. The trapping field is separated from the rf excitation/detection fields by capacitors of carefully chosen values. The trap design has been refined theoretically by use of SIMION\textsuperscript{10} (see Figure 9.2). Although the simulated trapping potential well in a conventional trap is deeper than that of a quadrupolarized trap, the overall trapping potential profile in the quadrupolarized trap approaches a perfect quadrupolar field much more closely than does that of a conventional trap, especially at large ion cyclotron radius.
Figure 9.1 Configuration of a quadrupolarized cubic ion trap and its resistor-capacitor network.
Figure 9.2  Electrostatic trapping potential (thick grey line) in a conventional cubic trap (top) and in a quadrupolarized cubic trap (bottom) with a pure quadrupolar potential (thin balck contour lines) for comparison.
A three-dimensional view of the trapping potentials in a conventional cubic trap and a quadrupolarized trap are presented in Figure 9.3. When ions are excited to a large radius, the trapping potential well in a conventional trap is not quadratic any more. In fact, it looks very close to a rectangular potential (Fig. 9.3, top). In the quadrupolarized trap, the shape of the trapping potential well is basically quadrupolar except at the edge of the trap where the trapping well becomes a step function because of the finite number of segments (Fig. 9.3, bottom).

All spectra of benzene were obtained on an Extrel Waters FTMS-1000 mass spectrometer at a field strength of 3.0 Tesla and a pressure of $5 \times 10^{-8}$ torr. The cyclotron motion of benzene molecular ions was excited by single frequency on-resonance dipolar resonant excitation at 1 Vp-p trapping potential. Carbon film resistors and ceramic capacitors were used for the RC network after baking for 48 hours at 150°C.

RESULTS AND DISCUSSION

A striking advantage of the quadrupolarized trap is evident from a comparison of benzene molecular ion FT/ICR mass spectral peak shapes for quadrupolarized and cubic ICR ion traps at different ICR orbital radii (see Figures 9.4 & 5). The elimination of peak splitting in the quadrupolarized trap shows that peak splitting previously observed for conventional ICR ion traps arises from non-quadrupolar electrostatic trapping potential. Moreover, mass resolving power is enhanced by ~10% in the quadrupolarized trap.
Figure 9.3 Three dimensional plot of trapping field in a conventional cubic trap (top) and in a quadrupolarized cubic trap (bottom).
Figure 9.4  Peak splitting at different excitation amplitudes (or radii of ion cyclotron motion) in a conventional cubic trap.
Figure 9.5 Peak splitting at different radii of cyclotron motion in the quadrupolarized cubic trap.
However the quadrupolarized cubic ICR ion trap still has peak position shifts with ion cyclotron radius (excitation amplitude), which shows the trapping electric field is still far away from the ideal quadrupolar electrostatic field. We are attempting to add additional boundary conditions to improve the electrostatic field by segmenting the trapping electrodes. In this first attempt of quadrupolarized design, we only put the z-direction trapping field boundary conditions. The radial boundary condition on the trapping plates was totally ignored for design simplification. The frequency shift (peak position shifts with the ion cyclotron radius) in Figure 9.5 implies that there is still a possibility to improve the trapping field by applying the radial boundary condition. Currently we are trying to simplify the electric circuit which can control the segmented electrodes. The radial and axial segmentation of the ICR trap electrodes will improve the trapping field without a loses in dynamic range and will produce a more accurate and high resolving power mass spectrum.
REFERENCES


CHAPTER X

FAST NEUTRAL BEAM FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

INTRODUCTION

Advantages of FT/ICR/MS include extremely high mass resolving power, high mass accuracy, MS^n experiments, and nondestructive ion detection. However, high mass resolving power (10^5 - 10^6 at m/z $\leq$ 1,000) requires low (10^-8 - 10^-9 torr) pressure, whereas several of the currently popular ionization sources (e.g., fast atom bombardment (FAB) and electrospray) operate optimally at several orders of magnitude higher pressure.

The general solution to the problem of differential pressure between the ion source and mass analyzer is to separate the two functions in spatially distinct regions. In a "dual trap", the "source" and "analyzer" regions are separated by a conductance limit wall through whose small central aperture ions may transit from "source" to "analyzer". The dual trap can maintain a pressure ratio of about a factor of up to 100-500 between the source and analyzer regions. A greater pressure differential
requires more than two states of differential pumping, which is typically achieved by moving the ion source outside the solenoidal magnet. Ions may then be injected into the ion trap inside the solenoidal magnet by: (a) passage through rf-only quadrupole or octupole rods;\textsuperscript{2-6} (b) acceleration and focusing with electrostatic lenses;\textsuperscript{7-10} or (c) entrainment in a supersonic jet (of, e.g., helium)\textsuperscript{11}. Although each of these methods succeeds in forcing or focusing ions to trajectories close to the magnetic field symmetry axis (z-axis) to circumvent the "magnetic mirror" effect \textsuperscript{12,13}, some fraction of the ions is always lost in transit, and (in pulsed injection) quadrupole-stability and time-of-flight effects\textsuperscript{8} can lead to mass discrimination in attempts to inject ions of a wide mass-to-charge ratio into the ion trap.

Ionization methods in which ions are formed in or near the ion trap (e.g., electron ionization, chemical ionization, laser desorption/ionization \textsuperscript{14}, and Cs\textsuperscript{+} "static" secondary ionization \textsuperscript{15,16}) avoid these problems and are therefore in general preferable to external formation of ions, provided that the desired ions can be generated by those methods. Moreover, ions formed near the ion trap are already confined laterally by the strong magnetic field, and are therefore more easily directed and transmitted to the ion trap, as in a recent electrospray ion source operating inside a high-field solenoidal magnet. We try to combine the advantages of external and internal ionization: the ion source is removed from the high vacuum chamber of the mass analyzer in order to optimize production of the primary ionizing beam; the beam is focussed, differentially pumped, and directed into the bore of
the solenoidal magnet; and ions are desorbed near the ion trap to facilitate efficient capture of secondary ions at low pressure for optimal FT/ICR mass analysis.

Delmore and coworkers\textsuperscript{17-20} at the Idaho National Engineering Laboratory originally developed a fast neutral beam gun that generates a high-velocity SF$_6$\textsuperscript{−} ion beam. SF$_6$\textsuperscript{−} is chosen, because a significant fraction of the accelerated (to several keV translational energy) SF$_6$\textsuperscript{−} ions autodissociates (to SF$_6$ and an electron) during the time it takes for those ions to travel \(\sim 2\) m from the source to the sample. Thus, autodissociation of SF$_6$\textsuperscript{−} ions which have been formed, accelerated, focused, and rastered (laterally) leaves a neutral SF$_6$ beam which retains the focus of the original ion beam (because of the much greater mass of SF$_6$ compared to an electron). The neutral beam exhibits the same ionization characteristics as an ion beam, with respect to sputtering of a sample surface and generation of secondary ions for subsequent mass analysis. In that spirit, the neutral beam flux may be stated in units of the "equivalent current" of a similar number of singly-charged primary ions.

Based on a series of experiments in which a fast neutral SF$_6$ beam was interfaced to a quadrupole mass analyzer, Delmore \textit{et al.} were quick to point out the special advantage of a neutral beam source for analysis of electrically insulating samples, due to reduced electrical charging at the sample surface. In experiments with a fast neutral beam (FNB) source interfaced to an FT/ICR mass analyzer, we noted the additional
advantage for FT/ICR/MS that the neutral beam passes unaffected through the fringe field of a solenoidal magnet, thereby allowing the ion source to be moved outside the magnet without any magnetic mirror reflection of primary beam particles. Yet another advantage of a polyatomic SF$_6$ beam is that the molecular beam produces a greater secondary ion yield than an atomic (e.g., Cs$^+$) beam of the same momentum.$^{21}$

**EXPERIMENTAL**

**Ionization method** A schematic diagram of a fast neutral beam generator and its interface to an FT/ICR mass spectrometer is shown in Fig. 10.1. The 5-15 keV SF$_6^-$ beam source is formed by electron attachment to neutral SF$_6$ gas molecules; the ion beam is then focused and rastered to strike a solids insertion probe positioned adjacent to the ion trap. The source housing is ~2 meters from the ion trap and is separated from the mass spectrometer by two 0.04" conductance limits that allow the source housing to be operated at 10$^{-5}$ torr while the mass spectrometer pressure is maintained at ~1-2 x 10$^{-8}$ torr. The key feature here is the formation of the SF$_6^-$ beam at high pressure outside the magnet; after the beam is focused and accelerated toward the bore of the solenoidal magnet, the SF$_6^-$ beam partially autoneutralizes to leave a neutral beam of SF$_6$ molecules whose trajectories are unaffected by the magnetic field. Because the electron mass is only 10$^{-4}$ of the SF$_6$ mass, autoneutralization does not affect the trajectory of the original SF$_6^-$ ion,
Figure 10.1 Schematic diagram of a fast neutral primary SF6 beam source and its interface to an FT/ICR mass spectrometer. Primary beam formation is external to the magnet. The neutral beam is focused and accelerated to impact on a solid-sample insertion probe located on the far side of the ICR ion trap. Typical working pressure is ~1 x 10^-5 torr in the source housing and 1-2 x 10^-8 torr in the ICR ion trap.
and the neutral beam retains the focus of the original SF$_6^-$ beam. A primary ion current of hundreds of picoamperes can be focused to a spot diameter of a few mm, to produce a primary beam current density of $\sim$10 nA/cm$^2$.

**Fourier transform ion cyclotron resonance mass spectrometer**
The FT/ICR mass spectrometer is comprised of Nicolet FTMS-1000 data station to control signal generation, data acquisition, and data processing; a custom built vacuum system; and an Oxford 3-tesla magnet (Oxford Instruments Limited, Osney Mead, England). A manual insertion solid sample probe is mounted at the opposite end of the vacuum chamber from the beam entrance. The vacuum chamber was roughed with a Balzers (Hudson, NH) 270 L/s turbomechanical pump. A CTI Cryogenics (Waltham, MA) CryoTorr-8 cryopump produced a base pressure of $3 \times 10^{-10}$ torr before sample analysis. A 2" (o. d.) elongated cylindrical open ended ion trap$^{22,23}$ centered at the highest-field point within the solenoidal magnet was used to trap, excite and detect the secondary ions.

**Mass spectra** SF$_6$ (99.99 %) was obtained from Matheson Gas Products (Secaucus, NJ); methyl stearate (>99%) and gramicidin S from Sigma Chemical Co. (St. Louis, MO); Teflon$^\text{®}$ was obtained in tape form. Fullerene-containing toluene extracts of soot prepared by the KH method were a gift from R. Zioło (Xerox Corp.). Each sample was dissolved in a suitable solvent (if necessary) and placed onto the probe tip (occasionally
with a cellulose matrix (Kim-Wipe® fastened to the probe tip) before insertion into the vacuum chamber. The probe was then inserted to an initial position ~1 cm from end of the rear trap electrodes. The neutral beam ion optical element voltages were adjusted appropriately, and the beam focused so that it would pass through the trap and impact upon the probe tip. Generally, focusing of the beam was determined by maximizing the observed FT/ICR mass spectral magnitude-mode peak height from K⁺ ions in the sample or by maximizing the signal from the sample of interest. The observed signal was further optimized by moving the probe closer or farther from the rear trap electrodes, typically ~1-2 cm for most of the work reported here. The desorbed ions were then captured in the ion trap where they were subjected to the usual FT/ICR/MS experimental event sequence: quench period to remove any unwanted ions; delay period to allow a new population of ions to accumulate in the trap; and then excitation followed by detection. The event sequence was repeated to accumulate a sufficient number of time-domain transients to yield a frequency-domain spectrum of acceptably high signal-to-noise ratio. Chirp excitation (2 MHz bandwidth, sweep rate varying from 1200 to 800 Hz/μs over a period of 1.67 - 2.5 ms) was used, except for methyl stearate, for which single-frequency resonant dipolar excitation (500 μs at 4.2 V). Direct-mode detection (64K time-domain data points, padded with an additional 64K zeroes, exponentially apodized (to enhance spectral peak height-to-noise ratio) and then Blackman-Harris apodized (to reduce peak overlap resulting from magnitude-mode Lorentzian line shape), was followed by fast Fourier
transform, magnitude calculation, and frequency-to-mass conversion to yield a mass spectrum.

RESULTS AND DISCUSSION

Primary beam intensity We implemented a number of improvements on the previously described prototype FNB FT/ICR mass spectrometer. For example, the previous instrument configuration limited the primary beam equivalent ion current to ~10 pA. Because secondary ion yield is in general proportional to the primary ion beam current, it is desirable to increase the primary ion current while retaining low pressure in the mass spectrometer. We therefore chose to increase the conductance limit aperture at the exit of the SF$_6$ ion source by a factor of four (from 0.02" to 0.04" i.d.); the primary ion current (as measured on a Faraday cup placed in the beam path) thereby increased approximately tenfold. Unfortunately, the pressure in the mass spectrometer also rose to a level (~1 x 10$^{-7}$ torr) unsuitable for ultrahigh-resolution mass analysis. We therefore added another stage of differential pumping to the beam line; the pressure in the mass analyzer was thereby reduced to 1-2 x 10$^{-8}$ torr.

Pulsed vs. continuous primary beam Another improvement included changing the beam from a continuous to a pulsed source in order to take advantage of the inherently pulsed nature of the FT/ICR
Figure 10.2 Comparison of experimental FNB FT/ICR magnitude-mode mass spectra of SF$_6^-$ for continuous (top) and pulsed (bottom) primary beam. In the lower spectrum, the primary beam is deflected away from the ion trap during excitation and detection, resulting in significantly enhanced mass resolving power. Primary beam conditions: beam energy, 7.5 keV; beam duration, 5 s.
Continuous
SF$_6$ Beam

$m/\Delta m = 268,000$

Pulsed
SF$_6$ Beam

$m/\Delta m = 445,000$

Figure 10.2
mass spectrometer. A small switching circuit was triggered from the Nicolet 1280 computer. The final lens element (y-raster) was pulsed from 0 to 500 V in order to allow the beam to enter the mass spectrometer during ion accumulation and subsequently be deflected away during the excitation, detection and quench events. Fig. 10.2 shows the marked (66%) improvement in mass resolving power for pulsed vs. continuous beam operation: mass resolving power for SF₈⁻ increases from 268,000 to 445,000.

Open-ended cylindrical ion trap Beu and Laude recently reintroduced the open-ended trap previously used in single-ion measurements for use in FT/ICR/MS, for improved efficiency of capture of externally injected ions. Such a trap may particularly improve trapping retention of high-mass ions. We therefore constructed an elongated cylindrical open ended ion trap for the present experiments. Moreover, the increased detection sensitivity of an elongated ion trap and (slight) additional increase in detection sensitivity for a cylindrical trap over a tetragonal trap of the same aspect ratio are well documented. Experimentally, we observed a very substantial improvement in detected secondary ion signal for an elongated, open-ended, cylindrical trap compared to our prior cubic trap. First, the open-ended trap provides a completely unobstructed path for primary ions to strike the solids probe surface. Second, the open-ended trap increases the acceptance angle (measured relative to the z-axis) of secondary ions which can be captured in the ion trap; thus, ions which are initially formed off-axis may still be trapped. Finally, with the open-ended ion
trap, the probe bias voltage and position may be varied in order to increase the detected ion signal. For example, the probe could be placed inside the outer trap electrode cylinder to increase the number of ions reaching the central cylinder for excitation and detection; alternatively, moving the probe away from the outer trap cylinder (i.e., outside of the main trapping field) exposes the secondary ions to a decelerating force (due to the static trapping field) whose magnitude depends on the probe position. Based on these experiments, the open-ended ion trap proves to be the trap of choice for the present FNB FT/ICR/MS analysis.

**Fullerenes** The effectiveness of the various above-listed instrumental improvements is nicely illustrated by FNB FT/ICR mass spectral analysis of fullerene-containing soot obtained by toluene extraction from arc-welding of graphite rods. Without the above-listed modifications, no detectable fullerene signal could be obtained, presumably due to too-low primary ion current and/or inefficient capture of desorbed ions. Following the above-listed modifications, Fig. 10.3 shows a strong signal from C\textsubscript{60}\textsuperscript{+} (m/z 720) and a somewhat weaker signal from C\textsubscript{70}\textsuperscript{+} (m/z 840) from a benzene solution of soot evaporated onto the solids insertion probe tip. The isotope relative abundances for \textsuperscript{12}C\textsubscript{60}\textsuperscript{+} and \textsuperscript{13}C\textsubscript{12}C\textsubscript{59}\textsuperscript{+} are as expected, as are the relative abundances of C\textsubscript{60}\textsuperscript{+} and C\textsubscript{70}\textsuperscript{+}.
Figure 10.3 FT/ICR positive-ion mass spectrum of a fullerene sample obtained from toluene-extracted soot from arc-welded graphite. Primary beam conditions: beam energy, 8 keV; beam duration, 2s. The sample in benzene solution was evaporated onto an aluminum probe tip. C_{60}^+ and C_{70}^+ are clearly evident.
Figure 10.3
Figure 10.4 FT/ICR broadband mass spectrum of methyl stearate ionized without a matrix. The sample was dissolved in methylene chloride and evaporated onto an aluminum probe tip. Primary beam conditions: beam energy, 10 keV; beam duration 3 s. The main peak in the mass spectrum is from protonated molecular ions, (M + H)^+.
**Methyl stearate** Methyl stearate was examined because it produces abundant molecular ions by any of several other ionization methods, and is often used as a sensitivity test for mass spectrometers. Fig. 10.4 shows the FNB FT/ICR mass spectrum of methyl stearate dissolved in methylene chloride and evaporated onto the aluminum probe tip with no added matrix. The predominant signal is from the protonated molecular ion, \((M+H)^+\) at \(m/z\) 299. Although single-frequency resonant excitation was used to generate this particular spectrum, broadband excitation (not shown) showed a virtual lack of fragmentation of this sample. Variation of the beam accelerating voltage (5-10 keV) did not affect the fragmentation pattern.

**Gramicidin S** Gramicidin S is a cyclic peptide which has become a standard sample for demonstrating peptide analytical performance in mass spectrometry. Fig. 10.5 is the FNB FT/ICR mass spectrum of gramicidin S dissolved in methanol and evaporated onto a cellulose matrix (Kim-Wipe® tissue paper) previously attached to the probe tip by wrapping the probe tip and securing with an encircling metal strip. Abundant protonated molecular ions, \((M+H)^+\), and several ionic fragments are readily observed. The cellulose matrix was essential for generation and detection of ions of \(m/z\) > 300. The most abundant fragment ions are quite similar to those observed by FT/ICR/MS by Cs⁺ ionization ⁱ⁶, fast atom bombardment ³², and laser desorption ³³. The presently observed fragmentation pattern suggests that FNB FT/ICR/MS could be useful for peptide sequencing, since most of the fragment ions are N-terminal fragments corresponding to B-type ions ³⁴. There was no
Figure 10.5  FT/ICR broadband positive-ion mass spectrum of the cyclic peptide, gramicidin S. The sample was dissolved in methanol before evaporation onto a cellulose matrix. Primary beam conditions: beam energy, 7.2 keV; beam duration, 2.5 s. Abundant molecular ions are evident, along with several N-terminus-type fragment ions: $B_{xy}$ denotes a B-type fragment extending from amino acid residues $x$ to $y$. 
significant overlap between the relatively low-abundance cellulose matrix ions and the gramicidin fragment ions. Finally, it is noteworthy that the protonated molecular ion was readily observed by FNB FT/ICR/MS without the addition of glutathione or salts to the sample solution, as for some other prior ionization methods.16

Teflon® The fast neutral beam is uniquely suited for analysis of electrically insulating materials. Because the beam autoneutralizes on its way from the source to the sample target, a neutrals-only SF6 primary beam is available for secondary ion generation. The obvious advantage of a neutrals-only beam, as previously noted by Delmore et al. 18.19 is that surface charging from the primary ion beam can be reduced without the need for external neutralization devices.26 In this work, the primary beam consisted of an approximately 50/50 mixture of neutrals and ions. Even with the resulting large negative-ion current, Teflon®, [CxFy], with y = 2x, was easily analyzed, as shown by the FNB FT/ICR mass spectrum of a Teflon® film (Fig. 10.6). At low trapping voltage (e.g., 2 V in Fig.10.6), oligomeric species up to m/z 581 are observed; at higher trapping voltage (8V, not shown), the FNB FT/ICR mass spectrum shows oligomers up to m/z 1531.

The upward extension in mass range of detectable positive ions on increase in trapping voltage suggests that there is some beam-induced charging at the sample surface: if the heavier ions are ejected with higher kinetic energy, then a higher trapping potential is required to retain them in the trap. Preliminary measurements suggest that the upper
Figure 10.6 FT/ICR mass spectra of Teflon®. Top: broadband positive-ion mass spectrum of a Teflon® film. Teflon tape was attached directly to the probe tip and bombarded with a 10 keV beam for 5 s per scan. Bottom: same mass spectrum showing easily detectable fluorocarbon fragment ions up to m/z 581. The mass range may be extended either by increasing the trapping voltage or by reducing the ion content in the primary beam.
Figure 10.6
mass limit may be further extended by reducing the number of negative ions in the primary beam; however, under those conditions, contaminants in the vacuum chamber were also readily detected, thereby limiting the available dynamic range.

Previous FNB FT/ICR mass spectra of Teflon® obtained with a prototype instrument were limited to relatively low-mass polymer fragments, because attempts to lower the beam current (to reduce sample surface charging) led to long beam period (to generate a detectable signal), and the resultant ion-molecule reactions of the trapped ions produced CxFy+ clusters that were no longer representative of the original polymer structure. The higher primary beam current of the present instrument allows for shorter beam period, and a more readily interpretable fragmentation pattern. Future FT/ICR analysis of other insulating materials (e.g., polyethylene terephthalates, with similarly severe surface charging effect) with a neutrals-only beam appears especially attractive.

Additional considerations For a neutrals-only primary beam, sample charging of electrically insulating samples is greatly reduced, thereby largely eliminating the need for additional compensation devices (e.g., electrons flooding the sample surface 35, pulsed extraction devices 36) to neutralize the sample surface charging. Even if some sample charging occurs, the voltages applied to the probe and to the rear trapping electrodes may easily be adjusted to lower the additional translational energy acquired by the secondary ions as they are repelled
from the (positively) charged surface. Moreover, because the secondary ions are initially formed in a strong magnetic field, the cyclotron motion of the secondary ions will largely prevent them from escaping in directions perpendicular to the magnetic field direction. In addition, the open-ended ion trap facilitates entry of ions formed initially off-axis into the ion trap, thereby increasing the population of detectable ions. Finally, even if ions enter the trap off-axis, they may be driven ("shrink-wrapped") back to the center of the trap by the application of resonant or stored-waveform \(^{37}\) based rf electric quadrupolar excitation of \(xy^{38,39}\) symmetry.

Pulsing the primary beam source (as demonstrated here) to reduce ion formation during the (potentially) long FT/ICR excitation and detection periods and/or use of a "storage" trap \(^{40,41}\) to increase the duty cycle of the experiment should also improve FNB FT/ICR/MS performance. Here, we showed that the addition of an involatile cellulose matrix neither degrades the mass spectral resolving power nor produces significant "chemical noise" from ions derived from the matrix itself. If another more reactive matrix were needed, its "chemical noise" could be removed by SWIFT \(^{42}\) multiple selective simultaneous ejection of unwanted matrix peaks.
CONCLUSIONS

Secondary ion mass spectra of high signal-to-noise ratio and ultrahigh mass resolving power have been obtained from a fast neutral beam ionization source FT/ICR mass spectrometer. The signal-to-noise ratio and molecular weight range of analysis have been extended by a factor of at least 5 over those of a prior prototype instrument 25. Routine analysis of thermally fragile materials is now feasible. FNB FT/ICR/MS is uniquely advantageous for high-resolution mass spectral analysis of electrically insulating materials. General considerations for optimizing routine FNB FT/ICR secondary ion mass analysis have been discussed.
REFERENCES


CHAPTER XI

Instrumental Configuration for Direct Measurement of Optical Absorption of Ion Cyclotron Resonance Mass-Selected Trapped Ions

INTRODUCTION

Mass spectrometry is one of the most widely used analytical methods, providing for identification of single or a few ionic species, although typically hundreds to thousands of ions are required for routine experiments. Its ultrahigh sensitivity (as low as attomoles of samples which may be ionized with high efficiency) has made mass spectrometry one of the most powerful analytical tools for physics, chemistry, and biology. Until recently, mass spectrometry has been largely used to analyze pure (or chromatographically on-line separated) neutral species which are vaporized and ionized prior to mass analysis. The mass spectrum provides mass-to-charge ratios of the ionic species. Two generic advances in mass spectrometry have greatly extended its mass range and versatility of the technique. First, the introduction of "soft" ionization methods, such as $^{252}$Cf plasma desorption, fast atom
bombardment \(^2\) or liquid secondary ion mass spectrometry, matrix-assisted laser desorption/ionization \(^3,4\), and electrospray ionization mass spectrometry \(^5,6\), allow ionic species of large, thermally labile biopolymers such as proteins and DNA to be brought into the gas phase. Second, Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry, introduced by Comisarow and Marshall in 1974 \(^7,8\) is capable of: ultrahigh mass resolving power and correspondingly accurate mass measurement \(^9\), multistage tandem mass spectrometry (MS\(^n\)), high mass range, ion remeasurement \(^10\), simultaneous detection of all ions, and long ion storage time. The principles, applications, and current developments of analytical FT-ICR mass spectrometry have been repeatedly and recently reviewed \(^11-32\).

Precise mass measurement by high-resolution mass spectrometry can yield the chemical formula (e.g., C\(_x\)H\(_y\)O\(_z\)) of molecules and their fragments. However, ion structure may be inferred only indirectly by mass spectrometry alone, most generally by tandem mass spectrometry of two (MS/MS or MS\(^2\)) or more (MS\(^n\)) stages \(^33\). In the first MS stage, "parent" ions of a particular mass-to-charge (m/z) ratio are selected. Those parent ions are then induced to dissociate or react by collisions with neutrals, and the "product" ions are detected and mass-analyzed in a second MS stage. Tandem mass spectrometry has proved especially useful for identifying functional groups in small organic molecules and for peptide sequencing in the gas phase \(^34\). FT-ICR mass spectrometry offers ultrahigh mass resolving power, and moreover makes possible MS\(^n\) experiments in a single mass analyzer. However, because gas-phase ion
structures may rearrange between ion formation and ion detection, it is not always easy to determine a molecular structure from the chemical formulas of its ions and fragments.

Optical spectroscopy of ions, on the other hand, offers a direct route to ion structure, by means of correlations between spectral peaks or bands and molecular structure or (for small or sufficiently symmetric molecules) direct assignment of electronic/vibrational lines. In this chapter, we will describe the method to detect optical absorption spectra of mass-selected ions trapped in an ICR ion trap. The difficulty of interfacing an FT-ICR instrument to an optical absorption spectrometer is the wide gap in sensitivity between the two methods. FT-ICR experiments are usually conducted with ~10^3 - 10^6 ions confined to a volume of ~ 1 cm^3, whereas optical detection typically requires >10^{10} absorbers/cm^3. We therefore will discussed how to narrow the gap from both sides: namely, by increasing the number of trapped ions and by increasing the optical absorption sensitivity.

Spectroscopy of molecular ions dates back to the 1920's when Wien observed the so called "negative nitrogen band" emitted by N$_2^+$ 35. Most molecular ion spectra have been recorded in the electronic emission (rather than absorption) mode, due to its higher sensitivity, as noted in a recent review 36. The electronic absorption spectrum of N$_2^+$ in a flash discharge was recorded (much later) by Herzberg in 1968 37. The discharge method is still the dominant method for generating a large number of molecular ions for optical spectroscopy. For example, Oka
has reported the infrared absorption spectrum of $H_3^+$\textsuperscript{38}. However, direct absorption spectral studies of other molecular ions in a discharge is difficult because of background interference from neutrals. Discrimination against background neutrals was vastly improved by Saykally's introduction of velocity modulation \textsuperscript{39}.

In this paper, we propose to combine FT-ICR mass spectrometry with optical absorption spectroscopy for characterization of ionic species. The use of ICR to accumulate and trap ions of selected mass-to-charge ratio(s) offers several important potential advantages. First, ions may be generated from virtually any type of ion source. The high ion concentration provided by a discharge is not needed, because ions may be generated continuously (or repeatedly, for pulsed ion sources) and accumulated in an ICR ion trap to attain the required high concentration. Second, one gains access to a host of ions not necessarily available by direct ionization (e.g., fragment ions formed by ion-neutral collisions, and ions formed by ion-molecule reactions). Third, because ICR is optimally conducted at low pressure ($\leq 10^{-8}$ torr, or about $10^8$ neutrals/cm$^3$), background interference from neutrals is negligible. Direct measurement of optical absorption requires a relatively large number of ions ($\sim 10^9$) in the optical path, and a highly sensitive absorbance detector. In a preliminary experiment, we have successfully detected $2 \times 10^7$ methylacetylene cations in a two-inch cubic trap with conventional ICR dipolar excitation and dipolar detection at 3.0 tesla. We propose to increase the number of trapped ions by a factor of $\sim 50$ by increasing the magnetic field to 7.0 tesla and by use of
quadrupolar excitation and collisional cooling to axialize the ions and eliminate radial diffusive loss of ions from the ICR ion trap. The most sensitive optical absorption method currently available is O'Keefe and Deacon's cavity ringdown technique whose implementation will be discussed below.

**FT-ICR Techniques**

**Multi-section open-ended ion traps.** The spatially homogeneous (to within 20 ppm) region near the center of a solenoidal superconducting magnet for FT-ICR mass spectrometry is typically cylindrical with a long aspect ratio (2 x 2 x 8 cm). To make maximal use of that homogeneous region, we propose the multi-section open-ended trap shown in Fig. 11.1. Each rf section is bounded by narrow rings which are biased with a high d.c. voltage to produce a nearly quadrupolar trapping potential in each rf section. Use of trapping rings rather than cylinders reduces the total length of the trap compared to a conventional open-ended trap design. Moreover, the ringed cylindrical trap provides dipolar or quadrupolar rf excitation potentials in the trap without the need for capacitive coupling because the non-excitation electrodes occupy only a small fraction of the total electrode surface area. Three sections (each 5 cm long) thus almost completely fill the magnetically homogeneous volume near the center of the magnet.
Figure 11.1 Schematic representations of a multi-section trap and cavity ringdown layout for mass-selected ion optical absorption experiments. The three-section trap fills most of the spatially homogeneous region of the magnet. The electrodes of the trap may be connected so that dipolar excitation/detection as well as quadrupolar excitation for ion axialization can be carried out.
Ion Axialization. A strong quadrupolar electrostatic trapping potential overcomes the Coulomb repulsions ("space charge") which would otherwise induce ions to escape axially along (or opposed to) the magnetic field direction. However, the same quadrupolar trapping potential which produces a deep potential well in the axial direction also produces a steep radial potential hill at the center of the trap. Moreover, space charge adds to the height of the radial potential hill. Thus, the resulting radially outward-directed electric field combines with the axial static magnetic field to produce a drift velocity, $\mathbf{E} \times \mathbf{B}/B^2$ that drives ions slowly around the hill in a circular so-called "magnetron" motion. If ions are able to lose (potential) energy (e.g., by ion-neutral or ion-ion collisions), then ions can "roll" down the radial potential hill until they strike the side electrodes and are lost.

Such radial diffusive loss of ions can be overcome by use of azimuthal quadrupolar excitation (i.e., $x^2 - y^2$ symmetry) at the unperturbed ion cyclotron frequency, $\omega_c = qB/m$, in which $q$ and $m$ are ion charge and mass, and $B$ is magnetic field induction, to convert magnetron motion (i.e., off-axis cyclotron orbit center) into cyclotron motion centered on the z-axis of the trap; collisions then shrink the cyclotron orbit, leaving ions "axialized" on or near the central z-axis of the trap. Fig. 11.1 shows the electrical connections of the multi-section trap required to produce an azimuthal quadrupolar excitation potential as well as conventional dipolar excitation/detection.
By restricting ion axial motion with a strong axial quadrupolar electrostatic trapping potential \( (x^2 + y^2 - 2z^2) \) symmetry, whose x-z component is shown in Fig. 11.2, and compressing the ion radial distribution by continuous azimuthal quadrupolar excitation, we expect to achieve a much higher ion number density. The purpose of the multiple-section trap shown in Figs. 11.1 and 11.2 is to extend the z-dimension of the trap (and hence the number of trapped ions and optical path length) while maintaining the xy-component (i.e., \( x^2 + y^2 \) symmetry) of the quadrupolar electrostatic potential needed to perform axialization. Moreover, the axialization process is also mass-selective, so that ions of all other mass-to-charge ratios will be lost by passive radial diffusion. Initially, we shall generate ions by electron impact or internal chemical ionization, by passing a beam of 10-70 eV electrons axially through the ion trap. In a strong magnetic field, electrons closely follow the magnetic field lines. We can therefore inject electrons off-axis (thereby avoiding obstruction of the laser beam by the electron gun), because ions formed off-axis will be axialized by azimuthal quadrupolar excitation anyway.

**ABSORPTION SPECTROSCOPY**

Following a long history of ion electronic spectroscopy \(^{35,51-53}\), direct IR absorption spectroscopy of ions became feasible in the early 1980's by use of various continuous-wave lasers in multipass ion absorption spectroscopic experiments \(^{38,54}\). Velocity modulation and other techniques improved signal-to-noise ratio and sensitivity \(^{54}\), with
Figure 11.2 Electrostatic trapping isopotential contours. Top: Perfect axial quadrupolar electrostatic trapping potential, for optimal ion axialization (see text). Bottom: Electrostatic trapping potential in a three-section ICR ion trap (see Fig. 11.1) to which d.c. voltages of 7.2, 10, 10, and 7.2 V have been applied to the four "ring" electrodes which bound the three cylindrical rf segments of the trap. Note the close agreement between the isopotential contours near the center of the three-section trap and the desired perfect quadrupolar potential.
Figure 11.2  Three section ICR ion trap trapping potential.
sensitivity approaching 1 ppm difference between incident and transmitted beam intensity. Further improvement appears to be limited by path length: diffraction limits require that the reflective mirror of the absorption cell be as large as possible to achieve longer path length, but ions of interest are then distributed over a larger volume with resultant decrease in ion density.

Cavity ringdown detection (CRD) is a new absorption technique developed in 1988 by A. O'Keefe and coworkers. In conventional absorption spectroscopy, one measures the ratio of incident to transmitted light intensity. In the limit of very weak absorption, the signal increases in direct proportion to the optical path length through the sample; thus, a very long path are almost universally used for low density measurements. In the conceptually different CRD experiment, a beam of incident light is directed into a cavity bounded by highly reflective mirrors, so that photons traverse the cavity up to $10^5$ or more times (the actual number of passes depends on the reflectivity of the cavity mirrors), thereby greatly increasing the effective absorption path length in a relatively short absorption cell. Absorbance is then determined from the logarithm of the ratio of the exponential decay time constants in the presence and absence of sample inside the cavity (see below). Cavity ringdown spectroscopy has recently been successful applied to high-resolution molecular spectroscopy, metal clusters formed in a molecular beam, and kinetic studies. The technique has also been extended to the near UV.
A cavity ringdown apparatus consists of a pair of highly reflective mirrors whose radii of curvature, $R_1$ and $R_2$, and separation, $L$, satisfy the stable cavity condition,

$$0 < \left(1 - \frac{L}{R_1}\right)\left(1 - \frac{L}{R_2}\right) < 1$$ \hspace{1cm} (11-1)

Photons injected into such a cavity from a pulsed dye laser will bounce back and forth between the mirrors many times before escaping from either end. Photons leaking out of the cavity may be detected by a photomultiplier tube placed at the far (from the pulsed dye laser) end of the cavity. Immediately following the laser pulse, the exponential time constant, $\tau$, for decrease in number of photons in the cavity is determined by photon escape through the mirrors and absorption by species inside the cavity.

The sensitivity of detection of absorbance may be estimated as follows. From the Beer-Lambert law, one can show that

$$\frac{I_{\text{absorber + cavity}}}{I_{\text{cavity}}} = \exp(-\sigma \rho l) = \frac{\tau_{\text{cavity}}}{\tau_{\text{absorber + cavity}}}$$ \hspace{1cm} (11-2)

in which $\sigma$ is absorption cross section of the absorbing species (in cm$^2$), $\rho$ is number density (molecules or ions per cm$^3$) of absorbers, and $l$ is the total path length (in cm). Suppose that the smallest detectable change in integrated (over the ringdown detection period) intensity, $I$, of photon leakage is $\sim$2%. A typical ion visible absorption cross section is $\sigma = 10^{-16}$ cm$^2$; thus, if photons bounce back and forth $10^5$ times (i.e., decrease in number of photons in the cavity by $10^{-5}$ per round trip in the cavity), and
the Ions are spread out evenly over a 10 cm axial path per trip in the ICR ion trap, the predicted detection limit is $-2 \times 10^8$ ions cm$^{-3}$.

**EXPERIMENTAL PROCEDURES**

A proposed experimental layout is shown in Fig. 11.3. The FT-ICR mass spectrometer is based on a 6” bore horizontal superconducting 7.0 tesla magnet. The vacuum chamber pumped by a 270 L s$^{-1}$ turbomolecular pump is mechanically isolated from the pump by a 4”-diameter 6”-long bellows. Two cavity mirrors are mounted on the chambers with gimbal assemblies. The mirror reflectivity is 99.999% at 514 nm. The cavity length is about 150 cm.

A dye laser (DL14, Laser Photonics, Orlando, FL) is pumped by a N$_2$ laser (UV24, 10 mJ/pulse, Laser Photonics, Orlando, FL) triggered at a rate of 2 Hz. Wavelength scanning is accomplished by rotation of the wavelength dial with a stepping motor. 4 TTL signals from a spectral acquisition PC are converted into a signal (12 V, 2A) to control the stepping motor. Frequency scanning resolution of the homebuilt spectrometer is $\sim 0.5$ cm$^{-1}$, which is comparable to the specified resolution of the dye laser. Laser pulses from the dye laser are collimated and split into two beams. The minor beam ($\sim 5\%$ of the total intensity) is sent directly to a photodiode whose output is amplified and digitized by one of the channels of a digital oscilloscope card installed in the spectral acquisition PC. The laser pulse intensity may be monitored
FT-ICR mass-selected ion optical absorption Spectrometer

Figure 11.3 Instrumental layout for FT-ICR mass-selected ion optical absorption experiments.
by means of the photodiode signal. The major beam (~95% of the total intensity) is sent to the ringdown cavity. (Of course, only ~10^{-5} of that beam passes through the mirror into the cavity.) The cavity ringdown signal is monitored by a photomultiplier tube (Hamamatsu R928) operated at 500-1000 V. The signal is amplified by 2 stages of 20 dB broadband amplifiers from Stanford Research Systems (SR445, Sunnyvale, CA). The digitization is performed by a 8-bit 100 Msample/s digital oscilloscope card from Gage Applied Sciences Inc., (CS250, Montreal, Canada) operated at 10 Msample/s. Fig. 11.4 shows the overall instrumental layout. 1024 data points are collected following each 10 ns laser pulse, and typically 50-100 scans are averaged to achieve a signal-to-noise ratio of >100. Programs written in ANSI C provide hardware control, computer interface, and data processing of ringdown signals. The raw ringdown signal is baseline-corrected, and a two-parameter linear least-squares fit is performed to extract the ringdown exponential decay time constant from a plot of the logarithm of detected intensity vs. time.

A proposed event sequence for the mass-selected ion spectroscopy experiment is shown in Fig. 11.5. After a short-duration electron beam ionizes the sample molecules, ions are selectively axialized to a tight packet near the central axis of the trap. A laser pulse is directed through the ion cloud, and the corresponding cavity ringdown signal is collected. Axialization is maintained throughout the optical detection period to keep the ions from diffusing radially out of the ICR ion trap. Ions may be detected (to determine their number^{59}) by conventional FT-ICR
techniques. The process can be repeated many times to achieve higher signal-to-noise ratio at a given laser wavelength. The integrated area of the cavity ringdown intensity decay curve provides a measure of the "transmitted" intensity, $I_{\text{absorber + cavity}}$. The experiment is repeated for an empty cavity to obtain the "incident" intensity, $I_{\text{cavity}}$, and the absorbance, $A = \sigma \rho l$, at that wavelength is then evaluated as $\ln(I_{\text{cavity}}/I_{\text{absorber + cavity}})$. 

**Figure 11.4** Schematic relations between various components of an FT-ICR mass-selected ion optical absorption spectrometer.
Figure 11.5 Experimental event sequence for FT-ICR mass-selected ion optical absorption experiments. The ICR event sequence (top) is identical to that for a conventional ion axialization experiment. The optical event sequence (bottom) shows that cavity ringdown measurements are conducted during the ICR axialization period.

The laser wavelength is then incremented (or decremented), and the above event sequence is repeated to generate the next spectral absorbance value. Wavelength calibration is carried out in two steps. First, a small monochrometer (SDMC1-03, Optometrics USA, Inc., Ayer, MA) provides approximate calibration (to ~ 1 nm). For high-resolution spectroscopic calibration, an optogalvanic technique is used: the photodiode in Fig. 11.4 is replaced by a hollow cathode lamp filled with Ne (Model L233-26NU, Hamamatsu, Bridgewater, NJ), and the
resonance-enhanced absorption spectrum of Ne is recorded to yield a very accurate wavelength calibration.
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LIST OF REFERENCES


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