An Interface with a Linear Quadrupole Ion Guide
for an Electrospray-Ion Trap Mass Spectrometer System

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Abstract

A new interface for an electrospray-3D ion trap mass spectrometer system has been developed and characterized. The interface allows ion accumulation, resonant ejection and ion isolation through dipole excitation. Linear quadrupoles have an intrinsic ion storage capability that can improve the duty cycle of the combined system. Also, dipole excitation with high resolution is possible in a quadrupole field in contrast to other linear multipole fields. Thus, selective resonant ejection allows the interface to achieve precise mass selection of ions trapped in the linear quadrupoles. The resolution of resonant ejection $(m/\Delta m)_{FWHM}$ was found to be up to 308 with the molecular ion of reserpine. One useful way of utilizing the high resolution of resonant ejection is isolating ions by applying composite waveform excitation. A composite waveform is generated from a computer by superimposing a range of frequencies. Within a band of frequencies a notch is placed around the secular frequency of an analyte ion, so that the waveform can excite all the ions except the ion with the secular frequency matching the notch. Ion isolation is shown to improve the sensitivity and resolution of the ion trap dramatically compared to the electrospray-ion trap system without ion isolation. A demonstration with a PPG (polypropylene glycol) solution with a trace amount of reserpine proves that some trace analysis can benefit greatly by eliminating background ions and thus reducing space charge problems in the ion trap.

Quadrupole resonances are an alternative to dipole excitation for excitation of ions in the quadrupole trapping field. Although both dipole and quadrupole resonance excitation share similar characteristics, quadrupole excitation has a unique resonant frequency scheme. This complicated excitation frequency scheme for higher order resonances ($n \geq 1$) was first investigated experimentally in this thesis work. The results show that the maximum mass resolution in quadrupole excitation was less than in dipole

excitation. Many interesting aspects of quadrupole excitation remain to be further investigated.

Table of Contents

Abstract	ii
Table of Contents	iv
List of Tables	vii
List of Figures	viii
List of Symbols	xvi
List of Abbreviations	xviii
Acknowledgement	xx
1. Introduction	1
2. A Review: Instrumentation for Interfacing Electrospray Ionization and 3D Ion Trap Mass Spectrometers	4
2.1 Quadrupole RF Mass Spectrometry	4
2.2 Linear Quadrupole Mass Spectrometry	7
2.3 3D Ion Trap Mass Spectrometry	14
2.4 Electrospray Ionization	25
2.5 Electrospray-3D Ion Trap Instrumentation	30
3. Experimental	33
3.1 Electrospray Ionization Source	33
3.2 Linear Quadrupoles	34
3.3 The interface	36
3.4 3D Ion Trap Mass Spectrometer	42

3.5 Analytes	43
4. The Linear Quadrupole Ion Guide: Storage Capacity	48
4.1 The Theory of the RF Linear Quadrupole Ion Guide	48
4.2 Characteristics of Linear Quadrupole Ion Guides	58
4.3 Summary and Conclusion	66
5. Resonant Ejection (I) : Dipole Excitation	67
5.1 Introduction	67
5.2 The Theory of Dipole Resonant Excitation	69
5.3 Experiments with Dipole Excitation	70
5.4 Resolution with Dipole Excitation	73
5.5 Summary and Conclusion	85
6. Application of Dipole Excitation: Ion Isolation	86
6.1 Introduction	87
6.2 Experimental	88
6.3 Results and Discussion: Ion Isolation	93
6.4 Summary and Conclusion	110
7. Resonant Excitation (II) : Quadrupole Excitation	112
7.1 Introduction	112
7.2 The Theory of Quadrupole Resonant Excitation	113
7.3 Experimental	118
7.4 Results and Discussion (I) : Higher Order Resonances	120
7.5 Results and Discussion (II) : Resolution	133
7.6 Summary and Conclusion	139

8. Summary and Conclusion	140
---------------------------	-----

Bibliography	142
--------------	-----

List of Tables

Table 5.1. Resolutions at various trapping q and excitation periods.

The amplitude to achieve 90% attenuation is in volts, shown in parentheses.

82

Table 7.1. High order quadrupole resonances at different trapping β .

Frequencies are in kHz. The amplitude $V_{\text{threshold}}$ (volts, peak-to-peak, pole-to-ground) was measured when 90% of the original intensity was attenuated. Resolutions are found from the FWHM of the frequency response curve.

121

List of Figures

Figure 2.1. A schematic representation of a linear quadrupole. The field radius is r_0 . Pole pairs are labeled either "A" or "B" on the rod ends. 9

Figure 2.2. Top: The Mathieu stability diagram for motion in two dimensions (x , y). Regions of simultaneous overlap of the x and y stability regions are labeled A, B, C and D. Bottom: The first stability diagram (A). Iso- β lines are drawn inside of the stability zone. 13

Figure 2.3. The first Mathieu stability region of the 3D quadrupole trapping field. Two possible scan lines are shown. 16

Figure 2.4. (A): Schematic of a commercial 3D ion trap mass spectrometer. The output of a GC is injected into the quadrupole trapping field. Electron impact ionization generates ions inside the trap. (B): Operation of the 3D ion trap in the mass-selective instability scan. The sequence contains electron impact ionization. 18

Figure 2.5. Operation of the 3D ion trap in the MS/MS/MS mode with electron impact ionization. Labels on RF voltage on the ion trap are A: ionization, B,C: precursor ion selection, D,E: fragmentation and precursor ion selection, F: mass scanning. 21

Figure 2.6. A schematic of a homebuilt electrospray ionization (ESI) source (similar to the SCIEX design). Compressed air forms a

nebulizer flow around the inner SS tube. A voltage of +3000-+5000 V is usually applied to the inner tube. 26

Figure 2.7. Schematic of major processes occurring in electrospray ionization. Penetration of an imposed electric field into a liquid leads to formation of a cone and jet, emitting droplets with excesses of positive ions. Charged droplets shrink by evaporation and split into smaller droplets and finally produce gas-phase ions. 27

Figure 3.1. Schematic diagram of the electrospray-3D ion trap interface. Q_1 , L_1 , L_2 and the 3D ion trap are contained in the same manifold. Helium can be added to this manifold through a variable leak valve. 38

Figure 3.2. An example of the timing diagram for a trapping experiment. Ions are drained from Q_0 for 0.1 ms and injected into the ion trap for 5 ms. Ions are stored in Q_1 for 20 ms. 41

Figure 3.3. The structures of (A) reserpine, (B) Polypropylene glycol (PPG). 45

Figure 3.4. Mass spectrum of 1 μ M reserpine in pure ethanol. The inset is a scale expansion near m/z 609. The isotopic structure of reserpine is shown. 46

Figure 3.5. Mass spectrum of 50 μ M PPG solution in ethanol. No buffer was used. 47

Figure 4.1. Results of trajectory calculation of ions with different m/z stored in the linear quadrupole field, in amplitude vs. time plane. $r_0 = 2.85$ mm, $\Omega = 768$ kHz, pressure: 0.75 mTorr (He), $\beta = 0.306$. The y scale is in mm. 52

Figure 4.2. Simulation of dipole excitation. Reserpine m/z 609 is trapped in the linear quadrupole field with $r_0 = 2.85$ mm, $\Omega = 768$ kHz, $\beta = 0.306$, pressure: 0.75 mTorr He; Excitation is in the y-direction: $\omega = 117.5$ kHz, amplitude = $0.30 V_{pp}$. The abscissa is in mm. 53

Figure 4.3. The timing chart for the storage experiment in Q_0 . A different delay time is applied to change the accumulation time in Q_0 . 57

Figure 4.4. Ion transmission through the linear RF quadrupole ESI-3D ion trap interface. The ion signal intensity of reserpine in arbitrary unit ($I(S, \text{arb.unit})$) is plotted against the RF level on Q_1 . 60

Figure 4.5. Ion storage in Q_1 . The reserpine signal (m/z 609) is plotted against different storage times in Q_1 . 63

Figure 4.6. Reserpine ion signal as a function of acquisition time in Q_0 . The ion intensity of reserpine (m/z 609) is in arbitrary units. The acquisition time is the sum of ion storage time in Q_1 , injection time, mass scan time and delay time in seconds. 65

Figure 5.1 Schematic diagram of the circuitry used for dipolar resonant excitation. A-A' is the direction of the dipole excitation. Pole pair B is connected in the normal fashion. The output A of the quadrupole power supply is connected to the center tap of the secondary of the transformer. 71

Figure 5.2. A timing chart for the resonant excitation experiments. Ions are stored in Q_1 for 500 ms with 0.1 ms for the IQ gate width and 5 ms for the L_1 gate width. There is no delay after the mass scan ramp. 72

Figure 5.3. A response curve with dipole excitation. The signal from reserpine ions (m/z 609) is monitored while the excitation frequency is scanned near the resonant frequency (206 kHz, amplitude = 0.170 V_{pp}). Excitation is applied for 100 ms. The FWHM is 0.810 kHz, which corresponds to a mass resolution of 254.

74

Figure 5.4. Response curves at different q parameters. Other experimental parameters are identical to those of figure 5.3. Refer to table 5.1 for the amplitudes of excitation and the frequency resolution.

78

Figure 5.5. Frequency resolution vs. the trapping q parameter with a 100 ms excitation period. The maximum resolution of 254 is at $q = 0.70$.

83

Figure 5.6. Response curves at excitation periods other than 20, 50 or 100 ms. The excitation amplitudes were 0.400 V_{pp} for (A), 0.150 V_{pp} for (B) and 0.140 V_{pp} for (C), and other experimental parameters were identical to the experiments in figure 5.3. R = frequency resolution. The trapping q was 0.60.

84

Figure 6.1. An example of a composite waveform with a notch at 180 kHz. Top: the waveform in the time domain; Bottom: the spectrum of the waveform in the frequency domain. The width of the notch window is 2 kHz.

91

Figure 6.2. The close-up view of the spectrum in figure 6.1. The “comb structure” is clearly shown with frequencies spaced by 0.25 kHz. A 2 kHz window is placed at 180 kHz.

92

Figure 6.3. Ion isolation with a composite waveform. The sample was 350 μ M PPG and 0.70 μ M reserpine with 500 μ M ammonium acetate

in pure ethanol. (A): No isolation waveform. (B): The waveform in figure 6.1 was applied to Q_1 for 500 ms. 95

Figure 6.4. Close-up views of figure 6.3 near m/z 610. Note that (A) and (B) have the same vertical scale maximum. 96

Figure 6.5. Calibration curves for reserpine. Concentrations of reserpine are between 0.2 and 5 μM . Each test solution contains 350 μM PPG and 500 μM ammonium acetate in pure ethanol. Other experimental conditions are identical except for the ion isolation waveform. 97

Figure 6.6. The timing chart for ion isolation in the 3D ion trap. Note that ion storage in Q_1 is 3.0 s and the L_1 gate is 500 ms. 101

Figure 6.7. Ion isolation in the 3D ion trap. The ion isolation waveform was applied to the ion trap for 3.0 s. Other experimental conditions were identical. (A): No ion isolation waveform applied: (B): with ion isolation. 102

Figure 6.8. Close-up views of figure 6.7 near m/z 610. (A) and (B) have the same vertical scale. 103

Figure 6.9. Ion fragmentation/ isolation of reserpine in the 3D ion trap and in the linear quadrupole. The fragment at m/z 448 still needs to be isolated in (B). In (C) m/z 448 is collected by applying a composite waveform to the linear quadrupole for 500 ms. 105

Figure 6.10. Separation of +15 state of cytochrome c by ion isolation. The ion intensity increases 21 fold in (B). Excitation is applied for 500

ms, notched at 255 kHz with a 20 kHz width. Other experimental parameters are identical except ion isolation.

107

Figure 6.11. Comparison of collection efficiency of the m/z 812 fragment of mellitin. (A) is the mass spectrum of mellitin with no fragmentation. The ion of m/z 812 is produced from m/z 712 which must be isolated in the ion trap to perform MS/MS. The intensity is increased 8 fold in (C) compared to (B).

109

Figure 7.1. Trajectory calculations for reserpine ion (m/z 609) with quadrupole excitation. Trapping $\beta = 0.306$; $r_0 = 2.85$ mm; trapping RF frequency = 768 kHz; 0.75 mTorr of helium background gas; excitation: $\omega = 234.2$ kHz, amplitude = $0.70 V_{pp}$ (pole-to-ground); Trajectories are shown in the (amplitude, time) plane. This particular example is for the ($n=0$, $K=1$) resonance. The y scale is in mm.

116

Figure 7.2. Trajectory simulation results of quadrupole excitation in the x-y plane. (A): ($n=0$, $K=1$) resonance; (B): ($n=+1$, $K=1$) resonance. Simulation parameters: $r_0 = 2.85$ mm; trapping RF $\omega = 768$ kHz, $\beta = 0.306$; 0.75 mTorr of helium with linear damping model. Ions start from the origin and disappear when they reach the field boundary. The x and y scales are in the units of r_0 (2.85 mm/unit). Amplitudes are in volts, peak-to-peak, pole-to-ground.

117

Figure 7.3. The circuitry for quadrupole excitation. T_1 and T_2 are identical. The variable capacitors (C_1 , $C_2 = 10 - 50$ pF) are for balancing out any RF feedback to the primary of the transformers. Q_0 is capacitively coupled to Q_1 through high voltage capacitors (200 pF).

119

Figure 7.4. $\log V_{\text{threshold}}$ vs. $1/K$ for different (n, K) resonances. The amplitude $V_{\text{threshold}}$ (peak-to-peak, pole-to-ground) was measured in volts. The trapping β is 0.521. 122

Figure 7.5. $\log V_{\text{threshold}}$ vs. $1/K$ at different trapping β . 123

Figure 7.6. The slopes of $\log V_{\text{threshold}}$ vs. $1/K$ plots at different values of β . Slopes are found by least square fitting. 126

Figure 7.7. Response curves of $(n=0, K=1)$ excitation with different amplitudes. Resonance points shift toward lower frequency with higher amplitude. The trapping β is 0.306. 128

Figure 7.8. The $(n=+1, K=1)$ resonance at different trapping β . (A): $\beta = 0.150$, $m/\Delta m = 54$, amp = 2.20 V; (B): $\beta = 0.225$, $m/\Delta m = 68$, amp = 3.01 V; (C): $\beta = 0.306$, $m/\Delta m = 66$, amp = 3.65 V. Amplitudes are in volts, peak-to-peak, pole-to-ground. 131

Figure 7.9. The $(n=-1, K=1)$ resonance at different trapping β . (A): $\beta = 0.150$, $m/\Delta m = 54$, amp = 1.690 V; (B): $\beta = 0.225$, $m/\Delta m = 53$, amp = 2.05 V; (C): $\beta = 0.306$, $m/\Delta m = 71$, amp = 2.11 V. Amplitudes are in volts, peak-to-peak, pole-to-ground. 132

Figure 7.10. Response curves of quadrupole resonances at $\beta = 0.306$. (A): $(n=0, K=1)$ resonance, $m/\Delta m = 85.9$, amp = 0.591 V; (B): $(n=0, K=2)$ resonance, $m/\Delta m = 93.7$, amp = 6.96 V; (C): $(n=0, K=3)$ resonance, $m/\Delta m = 101$, amp = 8.90 V; (D): $(n=+2, K=1)$ resonance, $m/\Delta m = 92.1$, amp = 53.6 V; (E): $(n=+2, K=2)$ resonance, $m/\Delta m = 128.3$, amp = 36.8 V; (F): $(n=-2, K=1)$ resonance, $m/\Delta m = 68.0$, amp = 21.6 V; (G): $(n=-2, K=2)$ resonance, $m/\Delta m = 82.8$, amp = 32.0 V. Amplitudes are in volts, peak-to-peak, pole-to-ground. 134

Figure 7.11. Mass resolutions of (n, K) resonances vs. trapping β . (A) :

$(n=0, K=1)$, (B): $(n=0, K=2)$, (C): $(n=+1, K=1)$, (D): $(n=-1, K=1)$

137

List of Symbols

Symbol	Description
E	Electric field
E_0	Time-invariant position-independent factor for electric field
x, y, z	Spatial coordinates
λ, σ, γ	Weighting constants for coordinates
ϕ	Electric potential
E_x	Electric field in the x-direction
r_0	Field radius
F_x, F_y, F_z	Force in the x, y and z-direction
m	Ion mass
e	Ion charge
$m/e, m/z$	Mass to charge ratio of an ion
U	DC potential
V	Amplitude of RF potential
Ω	Angular frequency of driving RF
ϕ_0	Potential applied to electrodes
r_o	Quadrupole field radius
u	Arbitrary coordinate
a_u, q_u	Mathieu parameters for motion in the u-direction
$u(\xi)$	Mathematical expression for the solution to Mathieu equation
C_{2n}	Coefficients of $u(\xi)$
A, B	Amplitudes of oscillation in the expression for $u(\xi)$
n, β	Parameters of the harmonic part of $u(\xi)$

ω_n	Angular resonant frequency at a given n
V_{eff}	Effective potential
k	Force constant
V_D	Potential well depth
Q	Total charge
ω	Resonant frequency, fundamental
G	Effusive Flow
L	Length of the quadrupole
ρ	Charge density per unit length
I(S, arb.unit)	Ion signal intensity in arbitrary units
F_0, F'_0	Amplitude of external excitation
$\omega(n, K)$	Quadrupole resonance excitation frequency with (n, K)
h	Amplitude of parametric oscillation
λ	Damping constant
$\Delta\omega$	FWHM in frequency from the frequency response curve
Δm	FWHM in mass from the mass response curve

List of Abbreviations

MS	Mass Spectrometer
RF	Radio Frequency
3D	Three-Dimensional
FTICR	Fourier Transform Ion Cyclotron Resonance
ESI	Electrospray Ionization
MALDI	Matrix Assisted Laser Desorption Ionization
TOF	Time-of-Flight
LC	Liquid Chromatography
GC	Gas Chromatography
MS/MS	Mass Spectrometry / Mass Spectrometry
MS/MS.MS	Mass Spectrometry / Mass Spectrometry / Mass Spectrometry
MS ⁿ	n Steps of Mass Spectrometry
V ₀	Initial RF
SS	Stainless Steel
CID	Collision Induced Dissociation
FFT	Fast Fourier Transform
FT	Fourier Transform
DC	Direct Current
i.d.	Inner Diameter
API	Atmospheric Pressure Ionization
Q ₀	The First Quadrupole in the Interface
Q ₁	The Second Quadrupole in the Interface
P ₁	Pressure between the Sampling Orifice and Skimmer
P ₂	Pressure in Q ₀
P ₃	Pressure in Q ₁ and the 3D Ion Trap.

IQ	Inter-Quad
L_1	The First Lens Element
L_2	The Second Lens Element
PPG	Polypropylene glycol
V_{pp}	Peak-to-peak Voltage
SWIFT	Stored-Waveform Inverse Fourier Transform
FNF	Filtered Noise Field
V_{0-p}	Zero-to-peak Voltage
DSP	Digital Signal Processing
FWHM	Full Width at Half Maximum
R_f	Frequency Resolution
R_m	Mass Resolution
$(m/\Delta m)_{FWHM}$	Mass Resolution from FWHM

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Psalm 107:1

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Chapter 1

Introduction

Mass spectrometry first appeared in the early 20th century. Thomson's parabola instrument of 1910 and Aston's velocity focusing device in 1919 first separated charged particles such as ions by their mass to charge ratio. Although the principle of separation by ion mass to charge ratio has not changed, the field of mass spectrometry has experienced immense growth especially in the number of commercial mass spectrometers used in analysis. Five different types of mass spectrometers have been developed and established, and are commercially available; magnetic/electric sector mass spectrometers, RF linear quadrupole mass spectrometers, 3D ion trap mass spectrometers, Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometers and Time of Flight (TOF) mass spectrometers. Considering the huge success of mass spectrometry in the commercial market, their current and future outlook is promising with growing demands from the bio-analytical field.

The development of ionization sources is the main reason for the successful applications of mass spectrometry. Electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) are recently developed ionization techniques for mass spectrometry. ESI has the advantage of producing multiply charged ions from large molecules, which can expand the analytical mass range of certain mass spectrometers. The popularity of ESI also derives from its ease of construction, stable and convenient operation at atmospheric pressure, and easy sample introduction. Some popular commercial mass spectrometers, such as linear RF quadrupoles and ion traps, regularly

use ESI as their ionization source. Electrospray is particularly important ionization source to interface liquid chromatography (LC) to mass spectrometers.

The RF quadrupole mass spectrometers were originally invented as mass analyzers and storage devices by W. Paul ¹. The RF linear quadrupole and 3D ion trap are closely related in their operational characteristics since ion separation is based on ion stability in quadrupole fields. The success of quadrupole mass analyzers in commercial use is due to their versatile operation and relatively low cost. RF quadrupole mass spectrometers are widely used as chromatographic detectors. Electrospray is a good match with linear quadrupole mass spectrometers since ESI is a continuous ionization source. The combination of ESI and a linear quadrupole mass spectrometer provides a detection system with potentially wide applications in the analysis of bio-molecules.

While both the linear quadrupole and 3D ion trap have advantages of their own, the 3D ion trap has some aspects more advantageous as a chromatographic detector. It has fast mass scanning, lower cost of manufacturing, and easy tandem MS capability. Nowadays many examples of ESI-3D ion trap are encountered in LC applications. In commercially available ESI-3D ion trap systems, RF multipole devices such as hexapoles or octopoles are usually employed to enhance the ion transport efficiency from electrospray ²⁻⁴. An RF quadrupole is also an efficient ion guide with the additional advantage of resonant mass selection. Resonant mass selection through dipole excitation is possible only in the quadrupole field as will be explained in Chapter 2. This mass filtering capability will allow the interface to exclude unwanted ions from entering the 3D ion trap, so the analytical characteristics of ESI-3D ion trap can be dramatically improved by using resonant ejection.

This thesis discusses new instrumentation for an interface between a commercial 3D ion trap mass spectrometer and an electrospray ion source. The focus of the thesis is the enhanced ion signal, resolution and mass accuracy of the ion trap with the new interface. A linear RF quadrupole is the key component. These capabilities have been characterized with experiments for future applications such as trace analysis. In addition, two different methods of resonance excitation, dipole and quadrupole excitation will be discussed.

Chapter 2 is a general review of the components of an electrospray-3D ion trap system. The theory of quadrupole mass spectrometry is briefly reviewed. The theory of operation of 3D ion traps and linear quadrupole ion guides is also explained. Electrospray, the ion source of the system, is also described. The advantages of each component of electrospray-3D ion trap and some anticipated disadvantages when different techniques are combined are discussed. Chapter 3 is the main experimental section. Instrumentation of the interface, timing charts, and reagents and solutions commonly used in the experiments are explained. The characteristics of linear quadrupole ion guides are discussed in Chapter 4. Their theory of operation and usage are described in detail. Simulations of ion trajectories in the linear quadrupole are shown to verify the theory. Chapter 5 demonstrates the performance of the linear quadrupole ion guide in the interface. Advantages of dynamic mass pre-filtering capability through resonant ejection are discussed with results. Optimization of the experimental parameters for dipole resonant excitation such as trapping RF amplitude, excitation amplitude and period is discussed, with a resolution study. Chapter 6 discusses an important application of dipole resonant excitation with composite waveform technique; ion isolation. A notch waveform accomplishes accumulating certain precursor ions from electrospray to improve the tandem MS capability in 3D ion traps. An alternative to dipole excitation, quadrupole excitation, is discussed in Chapter 7. A complex frequency scheme for quadrupole excitation is discussed in conjunction with resolution study and other information contained in the resonances. Chapter 8 is a summary of this thesis.

Chapter 2

A Review: Instrumentation for Interfacing Electrospray Ionization and 3D Ion Trap Mass Spectrometers

This chapter describes the basic principles of operation of the different components in an electrospray-3D ion trap mass spectrometer system. Quadrupole mass spectrometry in general, linear quadrupole mass spectrometers, 3D ion trap mass spectrometers, and electrospray ionization sources are discussed with the theory of their operation. The key component of the interface, the RF linear quadrupole ion guide and its advantage over other types of conventional ion guides will be discussed.

2.1 Quadrupole RF Mass Spectrometry

The pioneering work on the use of the quadrupole field for mass spectrometry was first published by Paul and Steinwedel in 1953¹. Different from the “static” mass spectrometry that utilizes static electric/magnetic fields to separate ions with different mass to charge ratio (m/z), mass selection involves oscillating ion motion in a “dynamic” mass spectrometry such as quadrupole mass spectrometry. Ions injected into an RF quadrupole field can have oscillating motion. If the amplitude of this oscillating motion is less than the quadrupole field radius (r_0), ions remain indefinitely stable unless they are interrupted by gas phase reactions or scattered by collisions. The stability of ion motion is determined by m/z when other operating parameters are fixed.

Depending on the geometry of the electrodes, many variations of quadrupole devices are possible ⁵. Some examples are the monopole field, the linear quadrupole field and the 3-dimensional quadrupole trapping field. The linear quadrupole and 3D quadrupole ion trap (or 3D ion trap in brief) are currently the most widely used mass spectrometry techniques. Both were first suggested and developed by Paul and Steinwedel ^{1,6}. After Paul's original publications and related patents ^{7,8}, many practical aspects of quadrupole mass spectrometry as an analytical tool were developed and improved. These include sensitivity, mass resolution, and scan speed. Practical applications of quadrupole mass spectrometry first appeared in the field of vacuum technology with growing demand for simpler partial pressure measuring devices ⁵.

Linear quadrupole devices drew attention to the technology earlier than the 3D ion trap. They made its first debut in research field in the 1960s. Linear quadrupoles quickly prevailed in atomic physics, molecular physics, medical applications, environmental science and many industrial applications. It turned out that the combination of gas chromatography (GC) with the linear quadrupole mass spectrometry was a great success in the late 1960s. Nowadays, GC-linear quadrupole MS has become a standard system for analysis. The recent development of tandem mass spectrometry gives linear quadrupoles much wider and more versatile applications in chemical analysis. A triple quadrupole mass spectrometer is an example of successful tandem mass spectrometers. In triple quadrupole systems, three separate steps of precursor ion selection, collisional fragmentation and ion detection are accomplished in three discrete linear quadrupoles, a process called MS/MS ^{9,10}. Many useful applications can be found with triple quadrupole instruments in trace analysis ^{11,12}.

Although 3D ion trap mass spectrometry was included in Paul's original patent ^{7,8}, it was developed as a technology later than the linear quadrupole. Ion trap was first recognized as an excellent storage device for charged particles ¹³, and later widely used as an optical cuvette for spectroscopic studies of ions ¹⁴⁻¹⁶. The industrial application of the 3D ion trap emerged when it was used as a residual gas analyzer. In 1982, Finnigan MAT announced a novel scanning technique for ion traps to generate a mass spectrum ¹⁷. Mass selective end-cap excitation was already available when the mass-selective scanning technique was developed ¹⁸ but the combination of both techniques in the 3D

ion trap generated many new applications ¹⁹. Ion traps are now fully recognized as a successful analytical tool with advantages such as simple tandem MS capability ¹⁶.

Ions, on average, experience restoring forces toward the center of the quadrupole field. Because of the focusing efficiency, quadrupoles are popular focusing devices although the most extensive use of quadrupole fields is mass analysis. Both linear quadrupole traps and 3D ion traps are used in the spectroscopic study of gas phase ions ^{14,15}. The focusing capability of the quadrupole field also enables quadrupole devices to be utilized as highly efficient injection platforms for other devices such as Time-of-Flight (TOF) mass spectrometers ²⁰, ion mobility measurements ²¹ and FTICR mass spectrometers ²².

When an external oscillating field is applied with its frequency tuned to the frequency of an ion motion along the same direction of excitation electric field, kinetic energy is selectively deposited in the motion and the amplitude of ion motion increases. Ions are ejected from the quadrupole field when they acquire enough kinetic energy to collide with an electrode. In the presence of background gas, however, increased ion kinetic energy induces collisions energetic enough to initiate fragmentation. Ejection and fragmentation compete with each other but the consequence of both processes is that certain ions disappear from the trapping quadrupole, and this provides a method to eliminate unwanted ions.

Selective fragmentation in the quadrupole field is an important feature of quadrupole devices. It allows tandem mass spectrometry capabilities. With certain types of mass spectrometers such as TOF, using quadrupole devices to perform MS/MS in the ion injection stages gains recognition ²³⁻²⁵.

2.2 Linear Quadrupole Mass Spectrometry

This section deals with details of the theory and operational characteristics of the linear quadrupole mass analyzer. The equation of ion motion in the quadrupole field, the Mathieu equation, and its mathematical solution are derived for use in discussing both 3D ion traps and RF linear quadrupole ion guides later in this chapter. From the solution, ion stability and common mass scanning sequences will be further explained.

i) The field equations

A linear quadrupole consists of four sets of round rods aligned in parallel as shown in figure 2.1. Hyperbolic surfaces form a perfect quadrupole field but they are often approximated by round rod surfaces for engineering convenience. The mathematical expression of the quadrupole field starts from the electric field expression in the Cartesian coordinates x , y and z .

$$E = E_0(\lambda x + \sigma y + \gamma z) \quad (2.1)$$

Here λ , σ and γ are weighting constants for the coordinate variables and E_0 is a time-invariant position-independent factor. To form a focusing field, the restoring force acting upon ions should increase according to the displacement of the ions from the origin. Assuming no space charge is formed within the field, one more constraint is given by Laplace's equation.

$$\nabla \cdot \vec{E} = 0 \quad (2.2)$$

To satisfy equation (2.2), the three constants λ , σ and γ , must satisfy the following relation.

$$\lambda + \sigma + \gamma = 0 \quad (2.3)$$

Two possible solutions to equation (2.3) are shown in equations (2.4) and (2.5), while a final solution, $E_0 = 0$, is trivial.

$$\lambda = -\sigma ; \gamma = 0 \quad (2.4)$$

$$\lambda = \sigma ; \gamma = -2\sigma \quad (2.5)$$

Now, the electric field E is related to the electric potential ϕ by equation (2.6).

$$E_x = -\left(\frac{\partial\phi}{\partial x}\right), E_y = -\left(\frac{\partial\phi}{\partial y}\right), E_z = -\left(\frac{\partial\phi}{\partial z}\right) \quad (2.6)$$

Equation (2.7) satisfies both equation (2.1) and (2.6).

$$\phi = -\frac{1}{2}E_0(\lambda x^2 + \sigma y^2 + \gamma z^2) \quad (2.7)$$

By plugging the constraint of equation (2.4) into the equation (2.7), the following expression for the electric potential is obtained.

$$\phi = -\frac{1}{2}E_0\lambda(x^2 - y^2) \quad (2.8)$$

In figure 2.1 the potentials on the two rod pairs are the same in magnitude but different in sign. Since all four rods are equivalent, poles of quadrupoles ("A" or "B") are defined by the applied potential as shown in figure 2.1. If the potential ϕ_0 is applied between a pole and ground, equation (2.8) reduces to the following.

$$\phi = \frac{\phi_0(x^2 - y^2)}{r_0^2} \quad (2.9)$$

where r_0 is half of the minimum distance between opposite electrodes. This is the mathematical representation of the linear quadrupole field in the xy-plane. A similar derivation made with the other constraint, equation (2.5), results in the mathematical representation of a 3D trapping quadrupole field in equation (2.10).

$$\phi = \frac{\phi_0(x^2 + y^2 - 2z^2)}{r_0^2} = \frac{\phi_0(r^2 - 2z^2)}{r_0^2} \quad (2.10)$$

Figure 2.1. A schematic representation of a linear quadrupole. The field radius is r_0 . Pole pairs are labeled either "A" or "B" on the rod ends.

ii) The Mathieu equation

The Mathieu equation describes periodic wave motion with elliptical boundaries²⁶, and also ion motion in a quadrupole field with a DC and oscillating potential. To derive the equation, forces acting along the x , y and z -direction are first expressed in terms of charge, e and the electric potential, ϕ :

$$F_x = ma_x = m \frac{d^2 x}{dt^2} = -e \frac{\partial \phi}{\partial x} \quad (2.11)$$

$$F_y = ma_y = m \frac{d^2 y}{dt^2} = -e \frac{\partial \phi}{\partial y} \quad (2.12)$$

$$F_z = ma_z = m \frac{d^2 z}{dt^2} = -e \frac{\partial \phi}{\partial z} \quad (2.13)$$

The electric potential on RF quadrupole devices is generally a combination of radio frequency (RF), $-V \cos \Omega t$, and direct current (DC), U , voltages (equation (2.14)).

$$\phi_0 = U - V \cos \Omega t \quad (2.14)$$

where Ω is the angular frequency of the driving RF and V is the zero-to-peak amplitude of the RF, measured pole-to-ground. Substituting equation (2.14) into (2.9) yields a new expression for the linear quadrupole potential.

$$\phi = (U - V \cos \Omega t) \frac{x^2 - y^2}{r_0^2} \quad (2.15)$$

From equation (2.11), (2.12) and (2.15) ion motion along the x or y -direction is determined by

$$m \frac{d^2 x}{dt^2} = -\frac{2xe}{r_0^2} (U - V \cos \Omega t) \quad (2.16)$$

$$m \frac{d^2 y}{dt^2} = +\frac{2ye}{r_0^2} (U - V \cos \Omega t) \quad (2.17)$$

Rearranging both equations gives two equations for ion motion.

$$\frac{d^2 x}{dt^2} + \frac{2xe}{mr_0^2} (U - V \cos \Omega t) = 0 \quad (2.18)$$

$$\frac{d^2 y}{dt^2} - \frac{2ye}{r_0^2} (U - V \cos \Omega t) = 0 \quad (2.19)$$

Equations (2.18) and (2.19) are known in the literature as the Mathieu equation ²⁶.

$$\frac{d^2 u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0 \quad (2.20)$$

where u represents a coordinate (x or y). Transforming equation (2.18) and (2.19) to the Mathieu equation requires a definition of the parameter ξ and two other Mathieu parameters (a and q).

$$\xi = \frac{\Omega t}{2} \quad (2.21)$$

$$a_x = -a_y = \frac{8eU}{mr_0^2 \Omega^2} \quad (2.22)$$

$$q_x = -q_y = \frac{4eV}{mr_0^2 \Omega^2} \quad (2.23)$$

The Mathieu parameters are important in indicating the stability of ion motion in a quadrupole field and will be encountered throughout this thesis.

iii) Stability diagram

The solution to the Mathieu equation cannot be found in closed analytical form and thus the series method is introduced ²⁶.

$$u(\xi) = A \sum_{-\infty}^{\infty} C_{2n} \cos(2n + \beta)\xi + B \sum_{-\infty}^{\infty} C_{2n} \sin(2n + \beta)\xi \quad (2.24)$$

Equation (2.24) has two new parameters, n and β . From equation (2.24), β is an important parameter that determines oscillation frequencies of the solution. The relation of β to (a , q) is given by the recursion formula ¹⁶.

$$\begin{aligned} \beta^2 = a + & \frac{q^2}{(2 + \beta)^2 - a - \frac{q^2}{(4 + \beta)^2 - a - \frac{q^2}{(6 + \beta)^2 - a - \dots etc}}} \\ & + \frac{q^2}{(\beta - 2)^2 - a - \frac{q^2}{(\beta - 4)^2 - a - \frac{q^2}{(\beta - 6)^2 - a - \dots etc}}} \end{aligned} \quad (2.25)$$

When q is small, however, equation (2.25)' is a valuable approximation, and is often quoted throughout the literature ^{5,16}.

$$\beta^2 \approx a + \frac{q^2}{2} \quad (2.25)'$$

The amplitude of motion is also determined by (a, q) and is the most important characteristic that determines the ion trajectories in the quadrupole field. If an ion has amplitude of motion smaller than the field radius (r_0), it is confined in the quadrupole field. If the amplitude is larger than the field radius, the ion will collide with an electrode. Figure 2.2 shows two stability boundaries for both the x and y -directions. The a and q parameters are determined by trapping parameters as defined in equation (2.22) and (2.23). When an (a, q) combination of a certain m/z is located inside the stability boundary, the ion has a "stable" trajectory. The overlap of both stability regions determines the stability region since x and y -stability have to be considered simultaneously. The Mathieu stability diagram in figure 2.2 shows four simultaneous overlapping regions labeled A through D. Because a_x and a_y (and equally q_x and q_y) are symmetric with respect to the origin on the a - q plane as referred to equation (2.22) and (2.23), the first quadrant is enough to represent the entire stability diagram. Figure 2.2 shows the first stability region ("A" in figure 2.2) with iso- β lines. As shown in the stability diagram, the β_x and β_y parameters vary between 0 and 1. Equation (2.25) gives a relation between a and q for a given β . When β is equal to 0 or 1, the resulting relationships of between a and q correspond to the boundaries of the first stability region.

Figure 2.2. Top: The Mathieu stability diagram for motion in two dimensions (x, y). Regions of simultaneous overlap of the x and y stability regions are labeled A, B, C and D. Bottom: The first stability diagram (A). Iso- β lines are drawn inside of the stability zone.

iv) Mass scanning

The most common use of a linear quadrupole is as a mass analyzer. A mass spectrum is obtained by monitoring the ion current at the exit of the quadrupole while U and V are simultaneously scanned along a scan line. A scan line is a sequence of U and V passing through the origin of the stability diagram that places ions of different mass to charge ratio at the tip of the stability region. Points on the scan line have a fixed ratio of a to q (or equally U to V in equation (2.14)). At any given (U, V) , only ions of one m/z fall just inside of the stability region at the “tip” and have stable motion, while ions with other mass to charge ratios have unstable motion and thus cannot pass through the quadrupole. By monitoring the ion current at the exit of the linear quadrupole continuously while scanning (U, V) along the scan line, a complete mass spectrum is obtained.

2.3 3D Ion Trap Mass Spectrometry

The theory of operation of the 3D ion trap has many similarities to that of the linear quadrupole. The Mathieu equation for the 3D quadrupole trapping field is the same with slightly different definitions of the parameters. From the solution to the Mathieu equation, the secular frequency of ion motion is derived. Resonant ejection, mass scanning and ion storage capabilities of the 3D ion trap will be discussed in this section.

i) Field equation

The expression for the 3D quadrupole trapping field was shown in equation (2.10). A process similar to that for deriving the Mathieu equation (equation (2.16) through (2.19)) can be done for the 3D quadrupole trapping field. From equation (2.10) and (2.14), the 3D quadrupole trapping field is defined as.

$$\phi = (U - V \cos \Omega t) \frac{r^2 - 2z^2}{r_0^2} \quad (2.26)$$

The equations of ion motion are

$$\frac{d^2 z}{dt^2} - \frac{4e}{mr_0^2} (U - V \cos \Omega t) z = 0 \quad (2.27)$$

$$\frac{d^2 r}{dt^2} + \frac{2e}{mr_0^2} (U - V \cos \Omega t) r = 0 \quad (2.28)$$

The definitions of the Mathieu parameters are slightly different from those for the linear quadrupole field.

$$a_z = -2a_r = \frac{-16eU}{mr_0^2 \Omega^2} \quad (2.29)$$

$$q_z = -2q_r = \frac{-8eV}{mr_0^2 \Omega^2} \quad (2.30)$$

ii) Stability diagram and mass scanning sequences

The first stability region for the 3D trapping quadrupole field is shown in figure 2.3. From the plot, z and r stability diagrams are asymmetric which derives from the relation of a_z to a_r and q_z to q_r . (equation (2.29) and (2.30)). There are three possible ways of operating the 3D ion trap depending on the potential applied to the end-caps and ring electrode ¹⁶. Biasing the ring electrode and end-caps with the same RF amplitude but 180° different in phase, will apply twice the potential applied between electrodes as between electrodes and ground ^{16,27}. Alternatively, the end-caps can be grounded while the potential is applied to the ring electrode only; this is currently the most common operating mode. RF can be applied to the ring electrode and DC only to the end-caps ²⁸. All three modes were developed by different research groups with different approaches to mass scanning/storage sequences ¹⁶.

Figure 2.3. The first stability region of the 3D quadrupole trapping field. Two possible scan lines are shown.

One way of scanning the mass is by monotonically increasing U and V along a scan line that passes through the origin and either apices of the first stability region. This process is the same as in the linear quadrupole mass analyzer except the fact that an extraction sequence (usually a DC pulse on the end-caps) is required in the 3D ion trap. Ions are unstable and ejected unless their m/z fall inside the apices of the stability region. Scanning of (U, V) allows different m/z to become stable and a mass spectrum is obtained by monitoring ion current after the selected m/z is released from the trap. Alternatively, stable ions can be stored continuously in the ion trap. Regardless of the scan modes, it is required to refill the ion trap with ions after each measurement.

Figure 2.4 shows a schematic of the 3D ion trap. Both end-caps are perforated so that they can pass ions from the external ion source. Also, the current by ions ejected through the end-cap is monitored by an electron multiplier. A mass-selective instability scan is developed by the Finnigan MAT Corporation (figure 2.4). The trapping RF is applied directly to the ring electrode with no DC component, so that all the trapped ions will have corresponding points on the q axis in the (a, q) plane. At a fixed driving RF amplitude (V_0), ions with their q parameters between 0 and 0.908 remain confined in the trapping field. Equation (2.30) shows that the q parameter of a given m/z will increase as V increases. Thus, the q of the trapped ions approach the stability boundary as the amplitude of the driving RF increases. The value of q is inversely proportional to m/z and lighter ions will reach the boundary and be ejected through the end-cap earlier than heavier ions, so that the mass spectrum is taken in the order of increasing mass to charge ratio. The mass-selective instability scan has the benefit of simplicity and high scan speed.

Figure 2.4. (A): Schematic of a commercial 3D ion trap mass spectrometer. The output of a GC is injected into the quadrupole trapping field. Electron impact ionization generates ions inside the trap. (B): Operation of the 3D ion trap in the mass-selective instability scan. The sequence contains electron impact ionization.

iii) Secular frequency and resonant excitation

Ions have unique frequencies of motion along the z and r -direction at a fixed RF level. This secular frequency, ω_n is given by

$$\omega_n = \frac{\Omega}{2}(2n + \beta) \quad (2.31)$$

where n is an integer and Ω is the frequency of the trapping RF. The parameter β is determined for low a and q values by equation (2.25) or (2.25)', so that β is related to m/z . External excitation tuned to this frequency can excite the ion in a resonant manner. In the 3D ion trap, external excitation is applied between the two end-caps to perform many useful tricks such as early β -ejection, ion isolation and collision induced dissociation (CID) ¹⁶. It is called dipole excitation since the end-caps have different polarity from each other. It is possible to excite higher-order resonances ($n \geq 1$ in equation (2.31)) ²⁹. However, the fundamental resonance ($n = 0$) has much lower threshold voltage than higher order resonances ²⁹, and thus will be used for resonant external excitation with low amplitude (Chapter 5).

External excitation can increase the kinetic energy of ion motion along the direction of excitation resonantly if the excitation frequency is tuned to the secular frequency of ion motion. In a linear quadrupole field, there are two independent "modes" of ion motion along the x and y -directions, which can be excited by dipolar excitation. One mode (either x or y) is excited by applying an excitation voltage between a set of poles (either A or B) in figure 2.1. The usefulness of dipolar excitation is that a prudently chosen frequency can selectively excite an ion of interest. By applying excitation with longer periods and/or higher amplitudes, ions gain more kinetic energy, which results in ion ejection when the kinetic energy is large enough to overcome the confining force of the trapping quadrupole field.

It is notable that different frequencies of external excitation work independently since the motions of ions of different mass to charge ratios are independent. This is advantageous because it allows the application of different frequencies simultaneously to excite ions of different m/z values. This also can be furthermore exploited by using

Fourier transform techniques. Many frequencies can be incorporated into one waveform to accomplish simultaneous ejection of many different ions. This composite waveform technique is utilized in isolating an ion of a single mass to charge ratio out of a mixture of ions in the quadrupole field, as will be discussed in Chapter 6.

It was previously mentioned that the 3D ion trap is an efficient ion storage device. Combining this storage capability with resonant excitation results in an important application of the ion trap, tandem mass spectrometry. With a 3D ion trap MS/MS consists of repeated steps of precursor ion selection, fragmentation and fragment ion detection. Selecting a precursor ion would be a trivial process for the ion trap. The trapping RF can be ramped until the unwanted ions are forced to pass the stability boundary, and are ejected. Although it is a commonly used method of isolating ions, ramping the RF does not eject ions with greater m/z than the analyte. As an alternative, resonant excitation and ion isolation by composite waveform techniques is widely utilized in commercial trap systems, as is explained in references ^{16,30} and also Chapter 6 of this thesis. Once the precursor ions are isolated, an excitation voltage tuned to the secular frequency of the precursor is applied to achieve fragmentation. A mass spectrum of the resulting fragments is obtained by ramping the driving RF of the trap. If required, any successive isolation/dissociation/fragment-collection sequences are repeated to complete the tandem sequence and the resulting fragments are detected. By repeating the tandem mass spectrometry sequences in the 3D ion trap, many steps of MS/MS are possible in theory. The expression MS^n is often used to describe this experiment where n represents the number of mass analysis steps. An example of timing diagram for an $MS/MS/MS$ sequence is shown in figure 2.5. In this figure, ions are generated by electron ionization in step A. Unwanted ions lighter than the ion of interest are excluded by increasing the trapping RF in step B and the remaining ions are stored in the trap (step C). In step D external excitation tuned to the secular frequency of the analyte ion is applied to achieve fragmentation while the trapping RF is maintained constant, followed by an isolation sequence of single m/z by composite waveform as will be explained in Chapter 6. Once fragments from the precursor are formed, another sequence of fragmentation through resonant excitation can be applied (step E), which yields a mass spectrum of fragments from the fragment of the precursor ion (step F). In real analysis, a finite

number of tandem mass spectrometry steps is allowed due to restrictions such as the initial number of precursor ions, the efficiency of storing precursor ions, the yield of fragmentation, and the efficiency of fragment collection ¹⁶. Yet, it is relatively easy to perform tandem mass spectrometry with a 3D ion trap since minimal hardware modifications, such as electronics for generating excitation waveforms are required.

Figure 2.5. Operation of the 3D ion trap in the MS/MS/MS mode with electron impact ionization. Labels on RF voltage on the ion trap are A: ionization, B,C: precursor ion selection, D,E: fragmentation and precursor ion selection, F: mass scanning.

iv) Storage capability

It has been mentioned that the 3D ion trap is an ion storage device. This section describes the theoretical calculation of the storage capacity of the ion trap. Later in this thesis, the storage capacity of linear quadrupoles will also be discussed and compared with that of the 3D ion trap. Linear quadrupoles serve as ion storage devices in the interface built for this thesis work. Analytical tools that measure the actual number of ions stored in both linear quadrupoles and 3D ion traps are limited. It is useful, then, to estimate the number of ions that can be stored in RF quadrupole devices. Ions in the 3D ion trap have harmonic motion and the trapping quadrupole field can be approximated as a harmonic potential well. The restoring force exerted on ions is towards the center of the trap. The fundamental frequency ($n = 0$) of ion motion as discussed previously is expressed by β and the driving RF frequency, Ω (equation (2.32))

$$\omega_0 = \frac{\beta\Omega}{2} \quad (2.32)$$

Equation (2.25), ($\beta^2 \approx \frac{q^2}{2}$) is a good approximation when $a = 0$ and the q parameter is lower than 0.4.

$$\omega_0 = \frac{q\Omega}{2\sqrt{2}} \quad (2.33)$$

For any harmonic motion along the z -axis, its effective potential, V_{eff} , and oscillation frequency is expressed in the following equations.

$$V_{eff}(z) = \frac{1}{2}kz^2 \quad (2.34)$$

$$\omega_0 = \sqrt{\frac{k}{m}} \quad (2.35)$$

where k is a force constant for the oscillator. Equating (2.33) to (2.35), gives an expression for k in terms of m , q and Ω_0 .

$$k = \frac{mq_z^2\Omega^2}{8} \quad (2.36)$$

Then k in equation (2.34) can be replaced with equation (2.36) to give a full expression of V_{eff} in terms of trap parameters.

$$V_{eff}(z) = \frac{1}{2} \frac{mq_z^2 \Omega^2}{8} z^2 = \left(\frac{z}{z_0} \right)^2 \frac{mq_z^2 \Omega^2 z_0^2}{16} = \left(\frac{z}{z_0} \right)^2 V_D \quad (2.37)$$

Here, the potential well depth (V_D) is defined as the height of the trapping potential or simply the value of V_{eff} at $z = z_0$.

$$V_D = \frac{mq_z^2 \Omega_0^2 z_0^2}{16} = \frac{-eVq_z}{4} \quad (2.38)$$

The well depth, V_D , is positive since $q_z = \frac{-4eV}{m\Omega^2 z_0^2}$. Now one can further simplify equation (2.37).

$$V_{eff}(z) = - \left(\frac{z}{z_0} \right)^2 \frac{eVq_z}{4} \quad (2.39)$$

The restoring force can be found from the definition of the effective potential.

$$force = - \frac{\partial V_{eff}(z)}{\partial z} = \frac{z}{z_0^2} \frac{eVq_z}{2} \quad (2.40)$$

At the boundary of the quadrupole trapping field ($z = z_0$), the force is

$$force = \frac{eVq_z}{2z_0} \quad (2.41)$$

Suppose ions are clustered in a sphere with its radius of z_0 and total charge Q , around the center of the trap. The electric field exerted by this charged sphere is

$$E = \frac{Q}{4\pi\epsilon_0 z_0^2} \quad (2.42)$$

The expression for the force experienced by a charge e in the field from the other charges is

$$force = eE = \frac{eQ}{4\pi\epsilon_0 z_0^2} \quad (2.43)$$

Equation (2.43) is directly equated with equation (2.41) to give an expression for the total charge that can be stored in the 3D quadrupole field.

$$Q = 2\pi\epsilon_0 Vq_z z_0 \quad (2.44)$$

The well depth (V_D) and the total charge (Q) that can be confined in the r-direction are determined in the similar way.

$$V_D = \frac{eVq_r}{4} = \frac{-eVq_z}{8} \quad (2.45)$$

$$Q = \frac{\pi\epsilon_0 Vq_z z_0}{\sqrt{2}} \quad (2.46)$$

Equation (2.45) shows that the well depth is shallower in the r-direction, expressed in terms of q_z and z_0 . As a result, the total charge in the r-direction is expressed similar to equation (2.44), assuming that the radius of the sphere of charge that can be confined is limited by z_0 , because r_0 is always greater than z_0 . By comparing equation (2.44) and (2.46), the total number of stored ions is limited by the well depth in the r-direction. From equation (2.46), it is possible to estimate the number of ions that can be stored in a 3D ion trap with 1.00 cm ring diameter (r_0) with a trapping RF frequency of 909,090.909 Hz. Assuming singly charged reserpine molecular ions (m/z 610) are trapped at $q_z = 0.300$, a trapping voltage of 777 V is require and the corresponding well depth is 4.66×10^{-18} J (29.1 eV) in the r-direction. The estimated total charge is 4.58×10^{-11} C, which corresponds to 2.87×10^8 singly charged ions. When ions are externally injected into the ion trap the driving RF is maintained very low. With the trapping $q_z = 0.100$, the trapping voltage is 259 V and the well depth in the r-direction is 5.18×10^{-20} J (0.324 eV). The total charge at $q_z = 0.100$ is 5.09×10^{-12} C or 3.18×10^7 reserpine molecular ions.

The theory is based upon the assumption that ions can fill the potential well until the strength of the interaction between ions is equal to the confining force of the potential well, so that space charge limits the total number of trapped ions. The total number calculated by equation (2.46) is a useful indicator of the storage capacity of the ion trap.

v) Limitations of the 3D ion trap

There have been many successful applications of the 3D ion trap, and these indicate the potential of the 3D ion trap for more numerous applications in the future^{2,16}. However, to take the full advantage of the 3D ion trap, the limitations of coupling to different ionization sources should be considered. Space charge, for example, can be a

significant problem. When numerous ions are confined in the 3D quadrupole field, the interactions between charges increase. Thus as the number of trapped ions increases, ion-ion interactions are no longer negligible compared to the RF trapping potential ³¹. The resulting perturbations to the trapping field deteriorate properties like mass accuracy, resolution and sensitivity. A limited duty cycle is another problem of the ion trap. It will be particularly significant when a continuous external ionization source is used. The ion trap is a pulsed device and the ions from continuous ionization sources are accepted intermittently. For example, an injection pulse width of 0.1 ms is commonly used for this thesis work. If the interval between injections is 500 ms, the duty cycle is 0.02%. Ions from the source are wasted with such a low duty cycle. This becomes even more significant when the ion trap runs time-consuming sequences such as MSⁿ. A solution to these problems is an interface with ion storage and ejection capability.

2.4 Electrospray Ionization

Electrospray ionization (ESI) has been a revolutionary technique for mass spectrometry. Along with matrix-assisted laser desorption ionization (MALDI) it introduced new possibilities for the analysis for high-molecular-weight compounds. Molecular weights of macromolecules such as proteins, oligonucleotides, and polymers can now be conveniently measured with mass spectrometers.

Some major advantages of electrospray set it apart from other ionization techniques. First, electrospray is an efficient ionization source. Ions produced by electrospray can bear multiple charges, so that mass spectrometers with limited mass to charge ranges can detect compounds with a greater range of masses. Second, liquid samples or solutions can be introduced into ESI directly. This allows important separation techniques, such as liquid chromatography or capillary electrophoresis to be easily interfaced with mass spectrometry. Third, ESI is an extremely soft ionization technique and non-covalent complexes can be generated and detected by electrospray.

Figure 2.6. A schematic of a homebuilt electrospray ionization (ESI) source (similar to the SCIEX design). Compressed air forms a nebulizer flow around the inner SS tube. A voltage of +3000-+5000 V is usually applied to the inner tube.

Figure 2.7. Schematic of major processes occurring in electro spray ionization. Penetration of an imposed electric field into a liquid leads to formation of a cone and jet, emitting droplets with excesses of positive ions. Charged droplets shrink by evaporation and split into smaller droplets and finally produce gas-phase ions.

Electrospray is also a mechanically simple source. A schematic view of the electrospray source (figure 2.6) shows that sample solution is supplied through a capillary into the spray tip that is another thin metal capillary usually made of stainless steel (SS). A high voltage DC bias is applied to the metal capillary to form a strong electric field between the spray tip and an adjacent plate. In this design, an air supply forms a nebulizing flow around the sprayed ion flow to facilitate solvent evaporation and to stabilize the spray. Other features such as sheath liquid or ultrasonic transducer assistance can be added depending on the applications ².

Ion generation in electrospray consists of three major steps (figure 2.7): charged droplet generation, solvent evaporation and fission, and gas-phase ion generation. The high DC voltage on the electrospray tip forms a strong electric field gradient between the tip and an adjacent counter-electrode with a low potential. This forces ions to migrate toward the spray tip and generates a region concentrated with positive or negative charge depending on the polarity of the spray voltage. Electrospray generates ions at atmospheric pressure. The migration force exerted on ions by the electric field and the surface tension of the sample solution causes the solution surface to be dragged out of the spray tip to form a cone, called the Taylor cone. If the field strength is sufficient, the tip of the Taylor cone emits small charged droplets. As the charged droplets pass through the space between the ESI tip and counter-electrode, solvent evaporates. The result is that the droplets bear more concentrated charge as they shrink. Because of intensive Coulomb interactions between charges, fission takes place and produces multitudes of smaller droplets. Gas-phase ions are formed from the smaller droplets either by successive fission ^{2,32} or the ion ejection process ^{33,34}.

Any interface between electrospray and mass spectrometers requires a series of differentially pumped pressure stages. Most interface designs adapt a free-jet expansion for sampling ions from the source ³⁵. It is desirable that ions pass through the various stages with high efficiency because this determines the sensitivity of the system. Ion guides based in RF multipoles are often used because of their focusing and storage capability. The most common RF multipole ion guides in commercial electrospray systems are RF quadrupoles ^{25, 30}, hexapoles ³ and octopoles ³⁶. Multipole fields with RF

potentials applied between poles (a set of electrodes with the same potential), form a potential well that can confine ions. Because of the alternating potential on the poles, ions in RF multipole fields can have oscillating stable trajectories depending on their mass to charge ratios. Secular motion of ions in a linear RF quadrupole or 3D ion trap is an example of oscillating ion motion in a multipole field. The restoring force toward the center of the multipole field is efficient in focusing ions since ions scattered by collision with background gas, can still be trapped. Ions especially with low mass are easily ejected by collisions with background gases in electrostatic lens systems, so their focusing ability is dependent on the background pressure. The focusing forces of RF multipole fields are strong enough to reduce ion losses by the scattering through a region of high pressure. When a stopping potential is applied to the entrance and exit of a multipole, ions are confined within the field provided that they have stable ion trajectories. Since operation of the RF multipoles is not as sensitive to the quality of vacuum or ion kinetic energy as conventional ion focusing devices, they are often preferred in building electrospray-3D ion trap systems. An electrospray-3D ion trap system (LCQTM) developed by Finnigan Corporation uses RF octopoles³⁶.

Electrospray can cause space charge problems when it is combined with 3D ion traps. Since electrospray is a soft ionization method, it generates unwanted ions with high efficiency. These include water molecule adducts of any charged species (including organic and metallic cations), single/multi-protonated ions, and cation-bound complexes². Ideally unwanted ions should be removed before analyte ions are injected into the ion trap for analysis. One apparent solution to this problem is filtering unwanted ions from the ion current within the interface. Linear quadrupole ion guides provide a solution to these potential problems, as will be discussed in the next section

2.5 Electrospray-3D Ion Trap Instrumentation

A challenge to interfacing electrospray to the 3D ion trap mass spectrometer is coupling two methods which operate in very different pressure regions, atmospheric pressure for electrospray and high vacuum for the ion trap. Several different but successful approaches have been described. Van Berkel et al. first succeeded in interfacing electrospray to the 3D ion trap with differential pumping stages containing skimmers and electrostatic lens stacks to focus ions ³⁷. The first instrumentation was immediately followed by Schwartz and Jardine at Finnigan Corporation. They used a capillary tube between the source and the skimmer to interface electrospray to an ion trap that had been modified for high mass range ³⁸. The first successful modification of a commercial ion trap (Saturn IITM, Varian Instruments, Palo Alto, CA) was reported by Mordehai and co-workers ³⁹. Mordehai's design was intended for use of atmospheric pressure chemical ionization (APCI) for LC and CE studies. Skimmers and conventional electrostatic lenses were used in the differential pumping stages.

Although conventional electrostatic devices are easy to manufacture, RF multipoles have gained popularity because of their advantages. Ion scattering can cause significant ion losses in an einzel lens depending on the pressure of background gas and the initial kinetic energy of ions. Linear RF multipole fields, on the other hand allow scattered ions to maintain stable trajectories by exerting restoring forces toward the center of the ion guides ². Ion loss by scattering is not as significant as in conventional electrostatic devices. This characteristic allows RF multipoles to be very efficient in transporting ions between stages ⁴⁰. Different RF multipoles are similar in focusing capabilities. If necessary, background gas is introduced to the multipole field to provide collisions that reduce the kinetic energy of injected ions in the axial and radial directions. In interfaces for electrospray-3D ion trap systems, the storage ability of RF multipoles ^{3,30} can be used to improve the duty cycle of the electrospray-3D ion trap system.

Different RF multipoles induce distinctive ion trajectories. In linear quadrupoles, ions have uncoupled motions in the x and y-directions. When dipolar excitation is employed in the quadrupole field, an external oscillation can selectively excite motion in

one direction (either x or y) in a resonant manner. If there is no coupling between modes, any gain or loss in kinetic energy of motion in one direction does not change the energy in the other direction. Motion in the x-direction is independent of the motion in the y-direction and vice versa. In hexapoles and octopoles, however, the x and y-motions are coupled and kinetic energy in different modes can be distributed between modes. Selectively exciting ion motion in the x-direction is impossible without exciting motion in the y-direction. As a result, the frequencies of ion motion depend on the amplitude and initial conditions of the ions and no resonant excitation of ion motion is possible when dipolar excitation is applied in the hexapole or octopole fields ⁴⁰. Resonant excitation with high resolution is only possible in the RF-only 3D quadrupole ion trap or linear quadrupoles. For linear quadrupoles, β in equation (2.31) is equal for x and y motion and determined by (a_x, q_x) or (a_y, q_y) in equation (2.25).

Possible space charge and duty cycle problems of the ion trap with electrospray ionization were discussed in section (2.4). The intrinsic storage capability of RF multipoles can improve the duty cycle but reducing space charge problems requires a mass filtering process. The composite waveform technique used with linear quadrupoles and 3D ion traps, which will be discussed in detail in Chapter 6, can provide a solution to the space charge problem. However, many advantages are found by isolating ions before they are injected into the 3D ion trap. The feasibility of using a hexapole excitation scheme to isolate ions from the hexapole field has been discussed ⁴¹, but the resolution of resonant ejection was very low compared to that of quadrupole resonant excitation. Although hexapoles and octopoles are effective ion guides, only RF quadrupoles are capable of providing solutions to both space charge and duty cycle problems in electrospray-ion trap combination.

Examples of using linear quadrupoles as ion guides for electrospray mass spectrometry are found with TOF ²⁵, 3D ion trap ³⁰ and FTICR ⁴². Using linear quadrupole mass analyzers as ion storage devices for FTICR was first shown by Senko et al. Campbell et al. demonstrated the use of resonant excitation in linear quadrupoles for mass selection and fragmentation with mass analysis in a TOF ²⁵. The possibility of enhancement in resolution, mass accuracy and signal sensitivity of a 3D ion trap was first

suggested by Douglas ⁴³. The first linear quadrupole interface fully equipped with ion storage and resonance excitation capability is discussed in this thesis.

Chapter 3

Experimental

This chapter describes the instrumentation used for the work of this thesis. The electrospray ionization source and quadrupole rod set were constructed in the workshop at UBC. The bias voltage settings for plates, orifices, quadrupoles and lenses are explained. The reagents used to prepare sample solutions for electrospray are also discussed. Specific additional details are described in the relevant chapters.

3.1. Electrospray Ionization Source

i) Schematic of electrospray

A schematic diagram of the electrospray source is provided in figure 2.6. The design was based on a SCIEX design. The sprayer has two SS tubes. The tip of the inner capillary is where the spray is formed. The outer SS tube forms a channel for a nebulizer gas, compressed air. Higher nebulizer flow is required for a stable spray with higher solution flow rates.

A high voltage DC bias was applied through a ballast resistor ($1\text{ M}\Omega$, $\frac{1}{4}\text{ W}$) placed in series with a high voltage power supply (PS350, Stanford Research Systems, Sunnyvale, CA).

Sample solutions were placed in a 1 ml gas-tight syringe (Model 1001, Hamilton Co., Reno, NV), and pumped through a silica capillary (TSP075150, 75 μm i.d., Polymicro Technologies Inc., Phoenix, AZ) to the tip of the inner SS capillary using a syringe pump (model 55-2222, Harvard Apparatus Canada, Saint-Laurent, PQ, Canada). Flow rates of 1.0 to 5.0 $\mu\text{l}/\text{min}$ were used. A sprayer voltage between 1500 and 10,000 V was used depending on the solution characteristics and the solution flow rate. A higher voltage is usually required at higher sample flow rates and for less volatile solvents, but the spray voltage is also affected by the nebulizer gas flow ².

Many adjustable parameters such as solution flow rate, spray voltage, and nebulizer gas flow, can affect the stability of electrospray. The sample solutions were usually prepared with excess buffer (up to 500 fold higher concentration than the analyte). Such a high ion concentration helps to minimize the temporal fluctuations of electrospray ². Organic solvents and organic acids are common additives as an alternative to buffers. In addition, acids play an essential role by protonating molecules. Acetic acid is commonly used for protein samples.

3.2. Linear Quadrupoles

i) Quadrupole rod sets

Two quadrupoles, Q_0 and Q_1 , were used for the electrospray-3D ion trap interface. For both quadrupoles the field radii (r_0) were 2.85 mm and the rod diameters were 6.50 mm. Quadrupole Q_0 was 12.0 cm long and the tips of its quadrupole rods were sharpened at a 30° angle, so that Q_0 could be placed 4.0 mm downstream of the skimmer orifice. Quadrupole Q_1 was 8.0 cm long with flat ends. The collars of both quadrupoles were machined from Macor[®] to provide rigid supports. All quadrupole rods were made from SS.

ii) Quadrupole RF power supply

Drive RF was supplied by an RF quadrupole power supply (API 3, maximum voltage = 10,000 V_{pp,pole-to-ground}, SCIEX[®], Concord, Ontario) at a frequency of 768,000 Hz. This frequency was chosen to minimize the interference between the drive RF of the linear quadrupole and the trapping RF of the 3D ion trap (909090.909 Hz). For both quadrupoles, the capacitance of the pole A (or pole B) was approximately 70 pF to ground when no RF cables were attached.

The difference in capacitance between poles was adjusted by changing the length of RF cables (RG58/U, capacitance = 93.5 pF/m, Amphenol Canada, Scarborough, Ontario, Canada). The cable capacitance, which is directly proportional to the length of the cable, is parallel to the pole capacitance to ground, and the total capacitance of each pole is the sum of both capacitances. A pair of short cables (12 cm) were attached to the A poles where external excitation is applied while a long cable (35 cm) is used to connect the B pole. The capacitance difference between poles was within the range (30 pF) that the RF quadrupole supply can compensate.

Quadrupole Q₁ was connected to the output of the RF supply and Q₀ was operated in a passive mode, coupled to Q₁ through high voltage capacitors (200 pF, in figure 7.3). This arrangement allowed 74% of the RF level of Q₁ to be applied to Q₀. The pole offsets of the two quadrupoles were separately provided through resistors (10 M Ω) by two DC outputs of a DC power supply (Model LXQ 60-1, 0-15 V variable DC output, Xantrex, Vancouver, BC, Canada) as shown in figure 5.1. The high resistance of 10 M Ω was necessary to prevent RF leakage from the RF quadrupole supply to the DC power supply.

3.3. The Interface

i) Differential pumping stages

Figure 3.1 is a schematic diagram showing the main components of the linear quadrupole interface. Ions, generated from the electrospray source, pass through the differential pumping stages, and into the 3D ion trap under vacuum. In a differentially pumped state the relation between the gas flow (Q), pressure (P) and the pump speeds (S) is given by

$$Q = P \cdot S \quad (3.1)$$

In most cases the pump speed is a constant and a different orifice size gives different pressure from the equation.

The curtain plate orifice was 5 mm in diameter. Dry nitrogen (1400 sccm) was introduced between the curtain plate and the sampling orifice to generate a counter flow toward the spray tip. This flow excludes large droplets from entering into the ion sampling orifice. The spacing between the curtain plate and sampling orifice is 5.0 mm. The diameter of the sampling orifice is 0.25 mm and the diameter of the skimmer orifice is 0.75 mm. The sampling orifice and skimmer are 3.0 mm apart. The region between the sampling orifice and skimmer was kept at 1.2 Torr (P_1) by a rotary pump (6 L/s, E1M18, Edwards High Vacuum, West Sussex, U.K.). A free jet expansion was formed by the pressure difference between atmosphere and the 1.2 Torr region. The skimmer (60° angle) samples a part of the gas in the jet expansion into the first quadrupole (Q_0) region. The Q_0 region was pumped to a pressure (P_2) of 7.0 mTorr using a turbo molecular pump (50 L/s, Turbovac 50, Leibold Vacuum Products Inc., Woodbridge, ON, Canada). The pressure in Q_0 is an important parameter that affects the ion accumulation characteristics of Q_0 , especially the efficiency of ion trapping. Through collisions with background gas in Q_0 , the initial kinetic energy of ions injected into Q_0 can be dissipated so that ions reflected back towards the entrance of Q_0 by the high potential on the Q_0 exit aperture, do not escape from the quadrupole. Collisions can also decrease the amplitude of the radial motion of ions, focusing ions toward the center of the Q_0 , a process termed collisional

cooling. Reduced ion motion in the radial direction contributes to an increase in the injection efficiency into the second quadrupole, Q_1 , by shrinking the size of the ion cloud in Q_0 . The pressure in Q_0 (P_2) was fixed at 7.0 mTorr for all the experiments reported in this thesis.

An orifice plate, 2.0 mm apart from Q_0 and Q_1 is placed between the two quadrupoles. The orifice diameter is 1.6 mm and the plate is machined from stainless steel. A gate pulse is applied to this Inter-Quad (IQ) orifice to control the ion current from Q_0 to Q_1 .

The 3D ion trap and Q_1 are contained in the same manifold with a pressure P_3 . The pressure in Q_1 is a critical property of the system. The injection efficiency of ions into Q_1 and the 3D ion trap is directly affected by the background pressure. Collisions with background gas should occur to dissipate the initial ion kinetic energy, so it is important to maintain a high pressure in the manifold to enhance injection efficiency. The resolution of resonant excitation and the resolution of the mass scan in the 3D ion trap are also affected by collisions¹⁶. Helium is the most popular buffer gas because of its low mass⁴⁴. The pressure in Q_1 and the ion trap (P_3) is maintained at 0.75×10^{-3} Torr with a turbo pump (50 L/s, THP062, Pfeiffer Vacuum Inc., Milpitas, CA) and a regulated helium leak through a leak valve (Variable Leak, Granville Philips Co., Boulder, CO) connected to a purified helium supply. Adjusting the helium leak can increase P_3 above 0.75×10^{-3} Torr when it is required.

Two lens elements are located between Q_1 and the 3D ion trap. An aperture lens (L_1) is 2.0 mm from the end of Q_1 and its aperture diameter is 2.0 mm. A cylinder lens (L_2) is 16.5 mm long and 8.5 mm in internal diameter, and located 2.0 mm from L_1 and from the end-cap of the 3D ion trap. Both lens elements are machined from stainless steel and biased with DC to focus the ion current from Q_1 to the 3D ion trap. A gate pulse is applied to L_1 to control the ion current at the exit of Q_1 . The cylinder lens is located 4.0 mm from the 2.0 mm diameter entrance aperture of the end-cap of the 3D ion trap.

Figure 3.1. Schematic diagram of the electrospray-3D ion trap interface. Q_1 , L_1 , L_2 and the 3D ion trap are contained in the same manifold. Helium can be added to this manifold through a variable leak valve.

ii) Potentials

A positive or negative voltage between 1500 and 10,000 V is applied to generate electrospray for the applications in general. For the experiments in this thesis, a positive ion production mode was used with the spray voltage between +3000 to +5000 V. The biases on other plates were chosen to be lower than the spray voltage, so that ions can be extracted by the potential difference. Voltages applied were: +100 V to the curtain plate, +50 V to the sampling orifice and +20 V to the skimmer orifice. Bias voltages on the quadrupoles (pole offset voltages) were typically +17 V for Q_0 and +10 V for Q_1 .

For trapping and resonant excitation experiments in Q_1 , a series of synchronized pulse sequences were applied to the IQ (inter-quad) orifice and L_1 . Lens element L_2 could be used for gating ion current between Q_1 and the ion trap although it is mainly used for focusing the ion output of Q_1 into the 3D ion trap. The L_2 bias was set to +10 V for all the experiments in this thesis.

DC voltages were generated by a custom-built DC power supply at the electronic service shop of the UBC Chemistry Department. The four outputs of the DC power supply could be adjusted between 0 and +150 V. Pulse generation for the IQ orifice and L_1 is discussed in the next section.

iii) Timing chart (figure 3.2)

Gate pulses were generated by a pulse generator (BNC model 500A, Berkeley Nucleonic Corp., Richmond, CA), synchronized to the trigger signal from the 3D ion trap. The height, duration and delay of the pulses with respect to the trigger were controlled by the pulse generator. The gate pulse voltages varied between 0 (low) and +20 V (high). The interface (figure 3.1) allows two methods of gating the ion current, at the IQ orifice and L_1 . Both IQ gating and L_1 gating are equally effective in controlling the ion injection into the 3D ion trap. When IQ and L_1 were gated synchronously, ions could be stored in Q_1 as shown in figure 3.2, an example of 20 ms ion storage time.

Quadrupole Q_1 has more versatile control of ion trapping. The synchronized IQ orifice gate controls ion entrance to Q_1 , and the L_1 gate controls the exit of Q_1 , so that the number of ions injected as well as the ion storage time in the quadrupole can be conveniently manipulated.

With the timing sequence in figure 3.2, ions accumulate in Q_0 . When the IQ gate is high (+20), a blocking potential is applied to the exit of Q_0 , and thus prevents ions from escaping from Q_0 . In Q_0 ions have many collisions with background gas, so that the ions lose their initial kinetic energy and become trapped. Lowering the IQ gate for a brief moment, typically 0.1 ms, injects ions from Q_0 into Q_1 . At the same time, the L_1 gate is high and a blocking potential is applied to the exit of Q_1 . After the desired period, the L_1 gate is lowered to ground for 5 ms and most of the ions in Q_1 are injected into the 3D ion trap. The time difference between the two drain gates determines the trapping time in Q_1 .

Figure 3.2. An example of the timing diagram for a trapping experiment. Ions are drained from Q_0 for 0.1 ms and injected into the ion trap for 5 ms. Ions are stored in Q_1 for 20 ms.

3.4 3D Ion Trap Mass Spectrometer

The 3D ion trap (Teledyne 3DQ, Teledyne Electronic Technologies, Mountain View, CA), originally a part of a GC-MS system with an electron impact ionization source, was modified to accept the linear quadrupole interface. The SS trap has a field radius (r_0) of 1.0 cm. The driving RF (909,090.909 Hz) is applied between the ring electrode and ground. The maximum mass range of the trap is $m/z = 650$ with the mass selective instability ejection at $q_z = 0.908$ ¹⁸.

Ions can be ejected using a supplemental AC voltage applied between the two end-caps, tuned to a frequency corresponding to q_z lower than 0.9 (or equally, β_z lower than 1). If ions are ejected before they reach the stability boundary, heavier ions can be ejected for a given maximum amplitude of the drive RF power supply. For example, when this resonance ejection is placed at $\beta_z = 0.5$, the largest m/z value ejected from the ion trap will be twice as large as the maximum m/z with the mass instability ejection at $\beta_z = 1$. The maximum mass range of the Teledyne 3DQ with early ejection is $m/z = 2000$.

With resonance ejection at $\beta = 0.5$, however, the ion signal decreases by 50 % and the resolution degrades. Thus, a maximum mass range of 900 amu was typically used for this thesis. A scan speed of 2,000 amu/sec was typically used although the maximum scan speed can be 10,000 amu/sec. The 3DQ system is operated with Teledyne Discovery control software and the Sequel data acquisition program, installed in an Intel 80486 CPU (33 MHz) based PC.

The Teledyne 3DQ system uses analogue detection with an electron multiplier (Model 7707mh3, K&M Electronics Inc., West Springfield, MA). The current from the multiplier is amplified and processed by the Teledyne circuitry. The vertical scales of the mass spectra are in arbitrary units, and can be changed with different electron multiplier gain by applying different high DC voltages, between 800 and 2500 V, to the electron multiplier. When quantification of the ion signal was required, such as comparing ion intensities from different mass spectra, a bias voltage of 1200 V was typically used.

3.5. Analytes

i) Reserpine

The chemical structure of reserpine is shown in figure 3.3 (A). It generates a singly protonated molecular ion in ESI (figure 3.4). The inset is an expanded view near m/z 609, which shows the isotopic peaks. A 1.0 μM solution of reserpine (M.W. 608.7, Sigma Chemical Co., St. Louis, MO) was prepared in absolute ethanol (Commercial Ethanol, Vancouver, Canada) with 500 μM ammonium acetate buffer (Fisher Scientific, Nepean, ON, Canada). This sample solution was used for most of the trapping and resonant excitation experiments. A 100 μM stock solution was prepared in pure ethanol and diluted as necessary.

ii) Polypropylene Glycol (PPG)

Polypropylene glycol (PPG) (average $M_n \approx 425$, Aldrich Chemical Co., Milwaukee, WI) is commonly used as an MS mass calibrant ⁴⁵. Its chemical structure is shown in figure 3.3(B). A mass spectrum taken with a 50 μM PPG solution prepared in pure methanol with no added buffer is shown in figure 3.5. Sample solutions of PPG were prepared from a 1.0 mM stock solution of PPG in acetonitrile (Fisher Scientific) with no buffer. For the ion isolation experiments described in Chapter 7, a series of 350 μM PPG solutions were prepared in 500 μM ammonium acetate with varying amounts of trace reserpine (0.2 to 5 μM).

iii) Mellitin

Mellitin (Sigma Chemical Co.) is a toxin found in wild bees. It is a small peptide with a molecular weight of 2845.6, and has the following sequence: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-

amide. A 100 μM stock solution of mellitin in pure ethanol with no buffer was prepared. A 5 μM mellitin sample solution with 5% acetic acid (Fisher Scientific) was prepared in order to demonstrate the ion isolation/CID capability of the composite waveform technique in the linear quadrupole interface (described in Chapter 7).

iv) Cytochrome *c*

Cytochrome *c* is a protein with a molecular weight of 12,384 amu (horse heart cytochrome *c*, Sigma). A 3.0 μM solution of cytochrome *c* was prepared in pure ethanol with 40 mM acetic acid and 0.20 mM ammonium acetate buffer for the ion isolation experiments of Chapter 7. Because of the high alcohol content in the sample solution, the protein is completely denatured and ions with high charge states are generated from electrospray.

(A) Reserpine (C₃₃H₄₀N₂O₉, m.w.=608.7)

(B) Polypropylene glycol (PPG)

Figure 3.3. The structures of (A) reserpine, (B) Polypropylene glycol (PPG).

Figure 3.4. Mass spectrum of 1 μ M reserpine in pure ethanol. The inset is a scale expansion near m/z 609. The isotopic structure of reserpine is shown.

Figure 3.5. Mass spectrum of 50 μ M PPG solution in ethanol. No buffer was used.

Chapter 4

The Linear Quadrupole Ion Guide: Storage Capacity

4.1 The Theory of the RF Linear Quadrupole Ion Guide

This section describes quadrupole ion guide theory and ion motion in the linear quadrupole field. The result of a simulation is given to verify the theory, followed by estimation of the storage capacity of quadrupoles.

i) Ion motion in the linear quadrupole field

The mathematical representation of a quadrupole field was described in Chapter 2. From the field equation the Mathieu equation was already shown as in equation (2.20).

$$\frac{d^2 u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0 \quad (2.20)$$

If an RF potential only is applied, equation (2.20) reduces to a simplified form (equation (4.1)).

$$\frac{d^2 x}{d\xi^2} + (-2q_x \cos 2\xi)x = 0 \quad (4.1)$$

Ion stability is determined by the Mathieu parameter q_x and q_y in equation (2.23).

$$q_x = -q_y = \frac{4eV}{mr_0^2 \Omega^2} \quad (2.23)$$

From equation (4.1), the Mathieu stability reduces to one dimension, on the q axis, in (a, q) plane and the criteria for stable ion trajectory reduces to inequality.

$$q \leq 0.908 \quad (4.2)$$

Ions in the quadrupole field undergo oscillating motion in the x and y -directions, independently, with the frequency of motion is determined by equation (2.31).

$$\omega_n = \frac{\Omega}{2}(2n + \beta) \quad (2.31)$$

This equation suggests that there could be many possible frequencies, at a given β (or equally at a given q). The fundamental resonance ($n = 0$) is more significant than the higher order resonances ($n \geq 1$) using dipolar excitation because higher amplitude and frequency are required to excite higher order resonances²⁹. Considering only the fundamental frequency, the expression reduces to the simple relation given in equation (4.3).

$$\omega = \frac{\beta\Omega}{2} \approx \frac{q\Omega}{2\sqrt{2}} \quad (4.3)$$

Here, β is approximated using the q parameter ($\beta^2 \approx \frac{q^2}{2}$, equation (2.25)') although this approximation is not accurate enough to predict secular frequencies when parameter q is higher than 0.4. A more accurate expression of β for higher values of q will be discussed in Chapter 5.

The secular frequency (ω) of ion motion, defined in equation (4.3), is a unique property of ions and is only dependant on ion mass to charge ratio when the driving RF amplitude is constant. It was also mentioned in Chapter 2 that ω 's for ions with different m/z are independent and will maintain their own secular frequency regardless of the presence of other m/z ions in the linear quadrupole field.

ii) Trajectory calculations

The Mathieu equation allows the calculation of the ion trajectory in a quadrupole field. The actual calculation is carried out in the phase space using a matrix method as is described in reference ¹⁶. A program to calculate trajectories was written by Dr. M. Sudakov, Department of Physics, Ryazan State Pedagogical University, Ryazan, Russia.

Three stable trajectories, calculated for ions with different m/z , are shown in figure 4.1. The value of β used in the calculation was 0.306 for the m/z 609 ions. The corresponding amplitude of the trapping RF (768 kHz) was 130 V_{zero-to-peak}, as measured between a pole and ground (pole-to-ground). The calculation used a helium background pressure of 0.75 mTorr and the collision cross section of 2.80×10^{-18} m² between helium and reserpine (m/z 609). Also, a linear damping model was used for simulating collisions. The trajectories in figure 4.1 plot time-amplitude. Axial motion is not shown. The y-motion is plotted in microsecond time scale.

Very light ions will have unstable trajectories (not shown) because their trapping q will be higher than 0.908. Figure 4.1 shows stable trajectories in the y-direction for ions with 300 m/z (A), 609 m/z (B) and 1000 m/z (C). The β parameters corresponding to the mass to charge ratios of ions, at a given RF amplitude of 130 V (zero-to-peak on pole-to-ground), are 0.620, 0.306 and 0.186 respectively. For the stable trajectories, their secular frequencies are inversely proportional to m/z , consistent with equation (2.31). In addition, collisional damping along the radial direction is shown in the simulation result. The amplitude of radial motion decreases, as the ion remains in the trapping quadrupole field due to collisions with the background gas longer. The efficiency of collisional damping of the radial ion motion is dependent on the mass of the collision gas and the ion mass ¹⁰. Heavier ions will require more number of collisions to lose the same amount of kinetic energy in the radial direction than the lighter ones, which is also depicted in the result (figure 4.1).

Sudakov's software is also capable of simulating resonant excitation, both dipole (Chapter 5) and quadrupole excitations (Chapter 7). The trajectories of m/z 609 in the x and y-direction are plotted in figure 4.2 without excitation (A) and with external

excitation applied in the x-direction (B), and in the y-direction (C)). Dipole resonance excitation (frequency = 117.5 kHz, amplitude = 0.30 V_{pp}) is applied between the two rods of pole A (figure 2.1). The vertical scale in figure 4.2 has been changed from figure 4.1 because of the increased amplitude in the presence of dipole excitation.

Without excitation (figure 4.2 (A)), m/z 609 ion has a stable trajectory and the amplitude in the radial direction decreases through collisions with the buffer gas. With external excitation applied in the y-direction, the amplitude in the excitation direction (y) increases until the ion is lost by colliding with the rod surface. However, ion motion in the x-direction, perpendicular to the excitation direction (y), is not affected by the external excitation and remains the same as the ion motion without excitation. This is a demonstration of how an ion gains kinetic energy through external excitation. Since the x and y-motion are independent from each other, only the ion motion in the excitation direction will acquire energy in a resonant manner.

Figure 4.1. Results of trajectory calculation of ions with different m/z stored in the linear quadrupole field, in amplitude vs. time plane. $r_0 = 2.85 \text{ mm}$, $\Omega = 768 \text{ kHz}$, pressure: 0.75 mTorr (He), $\beta = 0.306$. The y scale is in mm.

Figure 4.2 Simulation of dipole excitation. Reserpine m/z 609 is trapped in the linear quadrupole field with $r_0 = 2.85 \text{ mm}$, $\Omega = 768 \text{ kHz}$, $\beta = 0.306$, pressure: 0.75 mTorr He ; Excitation is in the y -direction: $\omega = 117.5 \text{ kHz}$, amplitude $= 0.30 \text{ V}_{pp}$. The abscissa is in μs .

iii) The storage capability of linear quadrupoles

The storage capacity of a 3D ion trap was estimated using the space charge calculation described in Chapter 2. A similar approach can be used to calculate the maximum number of ions that can be stored in a linear quadrupole. Ions undergo harmonic motion in the quadrupole field, so that the harmonic potential well approximation is valid. Instead forming an ion sphere in a 3D quadrupole trapping field, ions are distributed along the center of the linear quadrupole trap, in the z -direction in figure 2.1 and form a “line of charge”. The restoring force that an ion experiences is toward the central axis of the quadrupole field in the z -direction. The force acting against this restoring force is the Coulombic repulsion between “the line of charge” and the ion.

Equation (2.32) through (2.38) are valid for a linear quadrupole field. The definition of trapping potential after harmonic treatment of ionic motion is

$$V_{eff}(x) = \left(\frac{x}{r_0} \right)^2 V_D \quad (4.4)$$

where r_0 is the linear quadrupole field radius. The well depth is defined as following.

$$V_D = \frac{eVq_x}{4} \quad (4.5)$$

Equation (4.5) is derived from the relation $q_x = \frac{4eV}{m\Omega^2 r_0^2}$ and equation (4.4). Equation (4.4) is expressed in terms of x , r_0 , e , V and q_x as following.

$$V_{eff}(x) = \left(\frac{x}{r_0} \right)^2 \frac{eVq_x}{4} \quad (4.6)$$

The confining force is determined from the effective trapping potential.

$$force = -\frac{\partial V_{eff}(x)}{\partial x} = -\frac{x}{r_0^2} \frac{eVq_x}{2} \quad (4.7)$$

The force at the trapping boundary ($x = r_0$) is expressed in terms of ion charge, the amplitude of RF potential, q and the quadrupole field radius (r_0).

$$|force| = \frac{eVq_x}{2r_0} \quad (4.8)$$

Assuming that the ions are clustered along the center axis of the quadrupole field, the line of charge has a charge density of ρ (C/m) per unit length. The electric field exerted by this line of charge is found by using Gauss' law ($\epsilon_0 \int \vec{E} \cdot \vec{S} = \rho$),

$$2\pi r_0 LE = Q / \epsilon_0 = \rho L / \epsilon_0 \quad (4.9)$$

where L is the length of the quadrupole. The expression for the electric field in equation (4.9) is

$$E = \frac{\rho}{2\pi\epsilon_0 r_0} \quad (4.9)'$$

The force is,

$$force = eE = \frac{e\rho}{2\pi\epsilon_0 r_0} \quad (4.10)$$

Equating this directly to equation (4.8) will result in the expression for the charge density,

$$\rho = \pi\epsilon_0 V q_x \quad (4.11)$$

where V is zero-to-peak amplitude of the driving RF, pole-to-ground. From the theory, the total number of ions stored is directly proportional to the length of a linear quadrupole (L), since the number of charges stored in the linear quadrupole is a product of ρ and L . An example of calculating the number of ions trapped in a 3D quadrupole trapping is given in Chapter 2. Trapping of singly charged reserpine molecular ion (609 amu) at $q = 0.300$ in a linear quadrupole with a field radius of 2.85 mm, requires trapping voltage of 94.60 V. For this estimation, the driving RF frequency is set at 768,000 Hz. The maximum charge density is calculated to be 7.89×10^{-10} C/m. The length of Q_0 is 12.0 cm and the maximum number of ions is 9.47×10^{-11} C which corresponds to 5.92×10^8 ions. This number is not necessarily an accurate count of the number of ions that can be stored in a linear quadrupole because other factors can affect the trapping. However, it provides valuable information on the storage capacity of linear quadrupoles. Longer linear quadrupoles can store more ions than the shorter quadrupoles. One can attempt to compare the total ion capacity of linear quadrupoles and 3D ion traps (Chapter 2), but the acceptance of different quadrupole devices must also be considered prior to the direct comparison. Although the ion capacity of the linear quadrupoles calculated in this chapter is one order of magnitude greater than the capacity of the 3D ion trap in Chapter 2, linear

quadrupole devices ⁵ have larger acceptance and the injection efficiency is greater than 3D ion traps ¹⁶. Because of the ion capacity and injection efficiency, it may be advantageous to use linear quadrupoles not only the ion guides but also the ion storage devices when constructing an ESI-3D ion trap interface.

To examine the storage capability of the quadrupoles, a timing sequence different from figure 3.2 was introduced. Figure 4.3 has a new timing chart with a variable delay after the mass scanning ramp in the 3D ion trap. By changing the length of delay, the ion accumulation time in Q_0 can be changed. The width of the extraction pulse is 0.10 ms. Each extraction empties the ion content in Q_0 , so that the number of ions accumulated in Q_0 is determined by the time difference between the ion extractions. The length of exposure time of Q_0 to electrospray, a continuous ion source, determines the total number of ions retained during the accumulation process when the IQ gate is high. To examine this, the ion signal was monitored as a function of acquisition time. A similar result of the ion acquisition with RF hexapoles was demonstrated by Voyksner and Lee ³.

Figure 4.3. The timing chart for the storage experiment in Q_0 . A different delay time is applied to change the accumulation time in Q_0 .

4.2 Characteristics of Linear Quadrupole Ion Guides

i) Ion transmission through linear quadrupole ion guides

Theory predicts the low mass cutoff at high q (equation (4.2)) and changes in acceptance as a function of parameter q ¹⁶. The combination of both gives a response curve of transmittance for a linear quadrupole with the maximum existing between q of 0 and 0.908 ⁵. Experiments show that the transmission is also dependent on the RF field strength. Figure 4.4 shows the response of ion signal as a function of the change in the trapping RF level. In this experiment, electrosprayed reserpine molecular ions (3,000 V spray voltage) were injected from Q_0 into Q_1 for 0.1 ms, and stored in Q_1 for 500 ms. After the storage time, ions were injected into the 3D ion trap by opening the L_1 gate for 5 ms, stored in the ion trap for 5 ms, and the mass spectrum was taken. The ion signal of the reserpine molecular ion was monitored as a function of the RF level on Q_1 . The helium background pressure was 1.6×10^{-3} Torr for this experiment. The DC biases on plates, orifices, quadrupoles and lenses were at the values described in Chapter 3. A sharp increase of ion signal was observed at low RF after which a flat response was reached at parameter q greater than 0.3. The ion guide becomes more transmitting as q parameter becomes higher than 0.3 because of the increase in ion acceptance of the ion guide ⁵. The quadrupole is 24 % more transmitting at maximum than it is at $q = 0.3$. The maximum is located when q is close to mass cut-off point of 0.908. When the trapping q was increased above 0.908, the transmittance drops to zero.

A minor attenuation in ion signal was observed at $q = 0.8$. This is probably from multipole components those are incorporated into the pure quadrupole field in mechanically imperfect quadrupoles ⁴⁶. The attenuation becomes severer at this q parameter when background pressure in Q_0 (P_3) was 0.75×10^{-3} Torr. At certain trapping q 's, non-linear resonance of multipole field higher than quadrupole can occur. In the

presence of a hexapole or octopole field, the x and y-motion are no longer independent and the motions in the x and y-direction start to interact with each other. This coupling between ion motions can induce ejection of ions before the stability boundary ($q = 0.908$). Attenuation of ion signal becomes more significant as the trapping q approaches the multipole resonant points such as the hexapole ($4\beta_r = 2$) and octopole ($2\beta_z + 2\beta_r = 2$) resonant points, close to $q = 0.80$ ⁴⁶. The attenuation is more severe because sufficient collisions cannot be provided at low pressure.

At higher background pressure, however, the collisions provided by the background gas can effectively dampen the radial motion and the coupling is diminished. The result in figure 4.4 was obtained with a higher background helium pressure of 1.6×10^{-3} Torr, showing 17% attenuation of the original ion signal intensity ($I(S)$ in the figures). It can be concluded from this result, that a relatively high background pressure is necessary to assure a linear response of ion transmission of linear quadrupole ion guides at different trapping RF level. The background pressure becomes a more significant factor to affect the efficiency of transporting ions with linear quadrupole ion guides throughout wide range of RF, especially when quadrupoles with low mechanical precision are used as ion guides.

Figure 4.4. Ion transmission through the linear RF quadrupole ESI- 3D ion trap interface. The ion signal intensity of reserpine in arbitrary unit ($I(S, \text{arb.unit})$) is plotted against the RF level on Q_1 .

ii) Storage capacity of Q_1 : experimental results

The storage capacity of Q_1 can be assessed by accumulating different number of ions while monitoring the increase in the ion signal. Quadrupole Q_1 is contained in the manifold of the ion trap, which normally operates in the sub-mTorr pressure range. The background pressure in Q_1 is a critical operation parameter. Although quadrupole Q_1 is not intended for accumulating ions, it is also desirable to have a substantial storage capacity for Q_1 because resonant excitation, taking from few tens of milliseconds up to few seconds, is implemented in Q_1 .

Figure 4.5 is the response curve for the reserpine molecular ion signal as a function of trapping time in Q_1 . For this curve, a sample solution containing 1.0 μM reserpine was electrosprayed at 3,000 V with bias voltages on plates, orifices, quadrupoles and lenses at DC voltages described in Chapter 3. Trapping q in Q_1 was 0.50. Ions were gated into Q_1 for 0.1 ms, stored in Q_1 for a given time, and injected into the ion trap to obtain mass spectra. This result suggests that ions can be stored in Q_1 up to 800 ms without significant loss when the background helium pressure is 0.75×10^{-3} Torr. The existence of a maximum in the response curve is possibly from a thermal equilibrium process taking place in Q_1 . Ions, initially accelerated into Q_1 by voltage difference between Q_0 and Q_1 ($\Delta V = 7\text{V}$), experience collisions with the background gas when they are trapped in Q_1 . Collisions with the background gas not only concentrate ions toward the middle of the quadrupole but also induce ions to reach a thermal equilibrium with the background gas at a given temperature. A narrow ion profile in the radial direction can improve the injection efficiency into the 3D ion trap¹⁶. With a trapping time longer than 500 ms, however, the thermalization may be further driven until the advantage of diminished radial profile does not contribute to enhance the ion signal any more. Ion density is higher near the exit of Q_1 because of the initial ion kinetic energy and also penetration of DC voltage provided by pole offset of Q_0 into the quadrupole field in Q_1 . The drain gate width of 5 ms is long enough to extract nearly 99 % of ions concentrated near the exit of Q_1 . For ions with wide profile in the axial direction as the result of thermalization process, a gate width longer than 5 ms would be required for complete extraction. This may be the result of attenuation in ion signal with storage times longer

than 500 ms. Furthermore, fragmentation may be easily facilitated with longer storage time in Q_1 , which also attenuates the reserpine ion signal. This could be verified with a detection method suitable for monitoring m/z 609 and its fragments simultaneously. However, possible fragments were not efficiently detected in the 3D ion trap used in this thesis work because of the high q values associated with possible fragments of reserpine. Otherwise, further verification of fragmentation would have been possible.

Nevertheless, an optimized storage time of 500 ms in Q_1 was chosen from the result in figure 4.5, and used for most of the resonant excitation experiments.

Figure 4.5. Ion storage in Q_1 . The reserpine signal (m/z 609) is plotted against different storage times in Q_1 .

iii) Duty cycle improvement

A limited duty cycle is a problem when electrospray is combined with a 3D ion trap. Because of this low duty cycle, most of the sample is wasted if the interface does not have any ion storage ability. The duty cycle is lowest when the 3D trap is running lengthy ion manipulation sequences such as MS^n .

Figure 4.6 is the experimental result obtained using the timing chart depicted in figure 4.3. The reserpine sample solution contained 1.0 μM reserpine and 5000 μM ammonium acetate in pure ethanol and the infusion rate was 1.0 $\mu l/min$. The spray voltage was 3,000 V. The IQ gate width was 0.1 ms and L_1 gate wide was 5.0 ms while delay times were applied to measure the ion signal at the exit of the 3D ion trap. The delay time was converted to the total acquisition time in seconds, by adding the ion storage time in Q_1 (50 ms), ion injection time (20 ms) and the mass scanning time of the 3D ion trap (250 ms). Ion accumulation is interrupted only by the IQ gate that drains ions from Q_0 . The acquisition time was varied between 200 ms and 3.0 s.

In figure 4.6, the solid line is from a linear least square fit of data points. The ion signal increases monotonically as the acquisition time in Q_0 increases. It is deducible from this result that more ions can be accumulated in Q_0 for an ion acquisition period longer than 3.0 s. Since the result suggests that ions can be stored in the linear quadrupole for a long acquisition period, a duty cycle improvement should be possible. Enhanced duty cycle is a particularly important characteristic of an ESI-3D ion trap system, considering the possibility of running a lengthy process such as MS^n in the ion trap. As the ion trap remains closed until the next ion injection, ions from the sample solution will be wasted because of low duty cycle.

When a trace analyte is electrosprayed and analyzed using a 3D ion trap, ion storage in the interface becomes important since the ion signal can increase by accumulating more ions in the quadrupole. A similar result of enhanced ion signal with RF hexapoles was demonstrated by Voyksner and Lee ³. However, trace analysis with ESI-3D ion trap is not entirely benefited, since ion storage in the interface gives rise to not only an increase in the number of analyte ions but also a large increase in the number of background ions, yielding a space charge problem.

Figure 4.6. Reserpine ion signal as a function of acquisition time in Q_0 . The ion intensity of reserpine (m/z 609) is in arbitrary units. The acquisition time is the sum of ion storage time in Q_1 , injection time, mass scan time and delay time in seconds.

4.3 Summary and Conclusion

In the new electrospray-3D ion trap system, ions can be stored in linear quadrupole ion guides while the 3D ion trap is not able to accept sprayed ions during mass scan analysis. The ion signal is increased by the stored ions otherwise wasted without storage capability in the interface. This chapter discussed the results of the ion storage experiment in the first quadrupole (Q_0) in which different delay times were applied to the IQ orifice drain gate, so that the storage capability of Q_0 could be examined. Through these storage experiments, it was demonstrated that the linear quadrupole can store ions from the source for up to 3.0 s without significant ion loss. By using ion acquisition in the linear quadrupoles, it has also been shown that the sampling efficiency of an electrospray-3D ion trap system can be dramatically improved. For trace analysis, the ion signal can be increased by accumulating electrosprayed ions for longer periods in the interface. Also, the storage capability of Q_1 was examined. A storage time of 500 ms in Q_1 yielded an optimum ion signal.

Chapter 5

Resonant Excitation (I) : Dipole Excitation

The storage capability of linear RF multipole ion guides was discussed in the previous chapter. When ion accumulation is used in an RF multipole interface, too many ions can be collected and injected in the ion trap, and the performance of the ion trap can be degraded. Using the RF linear quadrupoles, ions of unwanted mass to charge ratio can be pre-filtered using resonant excitation. This chapter discusses the theory and experiments of dipole resonant excitation.

5.1 Introduction

In comparison to other multipole fields, the quadrupole field can provide relatively high resolution for ion ejection by resonant excitation. This technique has been used extensively in 3D quadrupole ion traps for mass range expansion, ion isolation, ion fragmentation and MS^n ¹⁶. Most commercial ion traps are equipped with these capabilities. The same techniques are equally applicable to linear quadrupoles, however, linear quadrupoles have not been used as extensively as ion storage devices. In the new interface described in this thesis, the quadrupoles are not only used as ion guides but also as storage and ion isolation devices.

The resonant excitation process in linear quadrupoles is similar to the 3D ion trap. In this work, linear quadrupoles were mostly used as ion guides. Ions are introduced in

the axial direction (z-direction in figure 2.1), while excitation is applied along the radial directions (x and y-direction). Likewise, a linear quadrupole can have a higher ion capacity with lower space charge problems (Chapter 2 and Chapter 4). Just as in the 3D ion trap, groups of ions can be manipulated for isolation and/or fragmentation.

Two methods of exciting ion motion in a quadrupole field are currently in use: dipole and quadrupole excitation. The term dipole or quadrupole describes the geometry of the excitation field. One can choose one set of poles (either “pole A” or “B” in figure 2.1) and form a dipole electric field between two electrodes (dipole excitation). Alternatively, a quadrupole field can be established between two sets of poles. The excitation field overlaps with the trapping quadrupole field (quadrupole excitation). Both techniques can be implemented into existing linear quadrupoles with simple electric circuitry. This chapter discusses dipole excitation and its resolution and dependence on trapping parameters such as q values, the excitation period and the excitation amplitude. The optimum experimental results on resolution study will be discussed later in this chapter.

5.2 The Theory of Dipole Resonant Excitation

Motion of trapped ions in the RF linear quadrupole field is dependent on the q parameter in equation (4.1), the simplified Mathieu equation.

$$\frac{d^2x}{d\xi^2} + (-2q_x \cos 2\xi)x = 0 \quad (4.1)$$

Leaving out detail of the mathematical procedure, the secular frequency expression is given as a function of driving RF frequency (Ω) and parameter β (equation (2.31)).

$$\omega_n = \frac{\Omega}{2}(2n + \beta) \quad n = 0, \pm 1, \pm 2, \pm 3, \dots \quad (2.31)$$

Equation (5.1) is a good approximation to β when q is close to 0.

$$\beta = \frac{q}{\sqrt{2}} \quad (5.1)$$

When β becomes close to 1, however, this approximation is no longer valid and β is given by ⁴⁷.

$$\beta = 1 - \sqrt{\frac{0.908 - q}{1.14}} \quad (5.2)$$

External excitation is included in equation (5.3), which is the expression for dipole excitation with a forced oscillation term on the right-hand side

$$\frac{d^2x}{d\xi^2} + (-2q_x \cos 2\xi)x = F'_0 \cos(\omega t + \phi) \quad (5.3)$$

where ω is the frequency of the external excitation and ϕ is its phase. A more physically meaningful expression is obtained after replacing ξ with Ω and t ($\xi = \frac{\Omega t}{2}$).

$$\frac{d^2x}{dt^2} + \frac{\Omega^2}{4}(-2q_x \cos \Omega t)x = F'_0 \cos(\omega t + \phi) \quad (5.4)$$

Also a new definition of the amplitude of forced oscillation is $F_0 = \frac{\Omega^2}{4} F'_0$.

The interaction between a free oscillator system and external excitation is the strongest when the two systems have the same frequency ⁴⁸. The resolution of the resonance is affected by damping characteristics of the entire system ⁴⁹. From equation (5.4), a

similar analysis is possible for the free Mathieu oscillators and the external excitation at frequency ω . Strong resonance excitation occurs when ω is equal to the frequency of the free Mathieu oscillator.

From the definition of the resonant frequencies in equation (2.31), n is a parameter included in a series of frequencies as a solution to the Mathieu equation. An infinite number of resonant frequencies are expected to exist for a single β . For resonances with higher n parameters ($n \geq 1$), higher amplitude is required to achieve the same degree of excitation as for the fundamental resonance ($n = 0$)²⁹. The fundamental resonance frequency will be significantly used in experiments with low amplitude of excitations.

5.3 Experiments with Dipole Excitation

A 1.0 μM reserpine sample solution containing 500 μM ammonium acetate buffer was prepared in pure ethanol. The spray voltage was 3,000 V and DC biases on plates, orifices, quadrupoles and lenses were as shown in Chapter 3. The pressure in Q_1 was 0.75×10^{-3} Torr of helium.

i) The circuitry

Figure 5.1 shows the circuitry that used to apply the dipole excitation. Normally pole pair A is connected to output A and pole pair B is connected to output B of the RF quadrupole supply. To apply dipole excitation, however, the electric connection to the two electrodes in pole pair A is split and connected to separate ends of the secondary of a toroid transformer. The center tap of the secondary is then connected to output A of the quadrupole supply. External excitation is introduced through the primary of the toroid. If the center tap of the secondary is balanced, no RF from the quadrupole supply will be induced on the toroid, and no RF will be picked up by the external excitation source.

To facilitate the experiments and to protect the function generator, a variable gain homebuilt amplifier was inserted between the function generator and toroid. The function generator (SR347 arbitrary waveform generator, Stanford Research Systems, Sunnyvale, CA) was used exclusively for single frequency experiments. The maximum output is 10.0 V_{pp}. This can be increased up to 30.0 V_{pp} with an RF amplifier.

Figure 5.1. Schematic diagram of the circuitry used for dipolar resonant excitation. A-A' is the direction of the dipole excitation. Pole pair B is connected in the normal fashion. The output A of the quadrupole power supply is connected to the center tap of the secondary of the transformer.

ii) Timing chart

The timing chart for all of the resonant excitation experiments is shown in figure 5.2. Ions are drained from Q_0 for 0.1 ms and stored in Q_1 for a total of 500 ms. While ions are stored in Q_1 , external excitation is applied. Programming the function generator can vary the length, amplitude and frequency of the excitation.

Figure 5.2. A timing chart for the resonant excitation experiments. Ions are stored in Q_1 for 500 ms with 0.1 ms for the IQ gate width and 5 ms for the L_1 gate width. There is no delay after the mass scan ramp.

5.4 Resolution with Dipole Excitation

Theory predicts that resonance excitation only takes place when the frequency of external excitation matches that of the motion of trapped ions, so there is very sharp response to frequency change when no collisional damping is present ⁴⁹. Broadened resonances can result from damping collisions with the background gas, interactions between ions ⁴⁸ and imperfections in the trapping quadrupole field ⁴⁷, so it is necessary to optimize parameters to achieve the highest resolution.

An example of the response curve with resonant excitation is shown in figure 5.3. The ion signal from the reserpine sample solution (1.0 μM) was monitored while the excitation frequency was scanned near the resonant frequency of reserpine ions (206 kHz, amplitude = 0.170 V_{pp}). The trapping q was 0.70 in Q_1 and the excitation period was 100 ms.

As the excitation frequency approaches resonance, a dramatic decrease in ion signal is observed. At the resonance point, the signal reaches its minimum. It recovers quickly as the frequency moves away from resonance. The excitation amplitude was adjusted so that the minimum signal was less than 10 % of the original signal. The resolution of resonant ejection ($R_{1/2}$) is determined from the full width at the half maximum (FWHM).

$$R_{1/2} = \frac{\omega}{\Delta\omega_{1/2}} \quad (5.5)$$

For the data in figure 5.3, a resolution of 254 is calculated from the resonance frequency of 206 kHz and FWHM of 0.810 kHz.

Figure 5.3. A response curve with dipole excitation. The signal from reserpine ions (m/z 609) is monitored while the excitation frequency is scanned near the resonant frequency (206 kHz, amplitude = 0.170 V_{pp}). Excitation is applied for 100 ms. The FWHM is 0.810 kHz, which corresponds to a mass resolution of 254.

The shape of the response curve will differ at different excitation amplitudes (Chapter 7). At lower amplitudes with the same excitation period, the response curve will have a narrower FWHM and less attenuation of signal. The resolution will increase as a result, but the excitation is not sufficiently energetic to achieve substantial ejection of the reserpine ions. With an amplitude higher than the optimum, on the other hand, the excitation will deposit excessive energy not only on resonance but also near-resonance. Since ions are ejected at near-resonant frequencies, the response curve will have a wider FWHM and a flat minimum.

Changes in the trapping parameter, q , will result in different response curves. Excitation parameters such as the amplitude and the excitation period also directly affect the resolution. Some examples of this are shown in figure 5.4. The trapping q directly affects the secular frequencies of ions (equation (2.31)) and the well depth of the trapping field (equation (4.5))¹⁶. The excitation period also affects resolution. As the excitation is applied for a longer period, more energy is deposited in the ion motion. To keep the same total energy deposited in the ion motion, the amplitude of excitation should be lower. In a similar context, a shorter excitation period requires a higher amplitude to achieve the same degree of attenuation. These trends are shown in table 5.1 where the required amplitudes to achieve 90% attenuation of the original signal are shown in the numbers in parentheses, in volts peak-to-peak, pole-to-ground.

Each column represents a different excitation period and each row represents a different trapping RF level expressed in terms of the q value. The response curves in figure 5.4 were obtained with an excitation period of 100 ms (curves with 20 and 50 ms excitation period are not shown).

A frequency resolution ($\omega/\Delta\omega$) of 254 was observed at a trapping q of 0.70, with an excitation period of 100 ms and excitation amplitude of 0.170 V_{pp}. The corresponding mass resolution ($m/\Delta m$) using equation (5.2), was 297.4.

As the experimental parameters change from the optimum point, systematic trends are apparent. For example, the highest resolution for a excitation period always occurs when the trapping q is equal to 0.70, regardless of the excitation period. When a series of resolutions were observed at the same q parameter but with different excitation

periods, better resolution was observed as the excitation period became longer. Amongst 20, 50 and 100 ms excitation periods, periods of 100 ms show the highest resolution regardless of trapping q . The variation of frequency resolution with q is plotted in figure 5.5.

When excitation was applied for periods longer than 100 ms, the resolution was further improved. Figure 5.6 shows three response curves when excitation was applied for 10, 200 and 500 ms at $q = 0.6$. The resolution ($\bar{R}_{1/2}$) was 50 with the excitation period of 10 ms. When excitation was applied for 200 ms, the frequency resolution was 308, which corresponds to a 33% increment compared to the resolution of 231 when excitation was applied for 100 ms. Also, the resolution was decreased from 308 by 14 % when the excitation was applied for 500 ms. The peak-to-peak amplitudes of excitation are shown in the caption of figure 5.6. The origin of the second peak in figure 5.6 (A) is unknown.

Most response curves in figure 5.4 are asymmetric. This may result from the non-linear resonance due to the imperfect quadrupole field ⁴⁹. A non-linear resonance can cause energy coupling between ion motions and thus it is no longer possible to deposit kinetic energy in the direction of excitation by applying an external oscillation. The deviation from a pure quadrupole field is worse near the surface of mechanically imperfect quadrupole rods than at the center of the quadrupole field. The ion motion has beats with an excitation frequency tuned near resonance. This beat motion superimposed on the secular motion causes the ion to travel close to the rod surfaces, where the non-linear components (hexapole or octopole field) are greater. With excitation frequencies higher than the resonance frequency, ions are forced to travel more frequently and closer toward the rod surface than with the lower frequencies. Thus, the degree of energy coupling is not symmetric near resonance and this results in asymmetric response curves. At high q values, the strength of non-linear resonance becomes significant and splitting in the response curve results in some extreme cases ⁴⁶.

Resonant excitation is also possible in the 3D ion trap. Commercial ion trap systems extensively use resonant excitation to achieve mass selection and MS^n . Although the resolution of resonant ejection with a 3D ion trap will be comparable to the resolution of linear quadrupoles observed in the experiments, developing an interface for the 3D ion trap is the focus of this thesis, so a comparison of the resolution of resonant ejection in

RF linear quadrupole and 3D ion traps was not carried out. A 3D ion trap might be used to build an interface for an electrospray-ion trap system as there are many examples of an ion trap used as an injection platform for other detection techniques ²⁰⁻²². With an ion trap-ion trap system, it would be of interest to compare the dipole excitation resolution of the linear quadrupole and 3D ion trap interface. However, injection efficiency for 3D ion traps is not as high as for linear quadrupoles, so the ion trap would not be adequate for guiding ions from electrospray.

Ion-ion interactions were briefly discussed in the ion trap section in Chapter 2. The magnitude of the interaction is significant when ions are stored at high concentration. Even when the space charge problem is not as significant, however, ion-ion interactions still exist in the quadrupole field. Most of the experiments in the thesis were undertaken with many ions stored in the linear quadrupole because efficient ion transport was the goal in developing a new interface. Thus, the dipole excitation experiments of this thesis are subject to ion-ion interactions, although it remains to quantify the degree of perturbation caused by interactions between ions during the resonant ejection process.

(A) $q = 0.30$

$R = 68$

(B) $q = 0.35$

$R = 86$

(C) $q = 0.40$

$R = 108$

Figure 5.4. Response curves at different q parameters. Other experimental parameters are identical to those of figure 5.3. Refer to table 5.1 for the amplitudes of excitation and the frequency resolution.

(D) $q = 0.45$

$R = 163$

(E) $q = 0.50$

$R = 200$

(F) $q = 0.55$

$R = 210$

Figure 5.4 Continued.

Figure 5.4 Continued.

(J) $q = 0.75$

$R = 205$

(K) $q = 0.80$

$R = 125$

(F) $q = 0.85$

$R = 150$

Figure 5.4 Continued.

Table 5.1. Resolutions at various trapping q and excitation periods. The amplitude to achieve 90% attenuation is in volts, shown in parentheses.

q / excitation	20ms	50ms	100ms
0.30	52 (0.160)	61 (0.130)	68 (0.120)
0.35	53 (0.200)	65 (0.160)	86 (0.140)
0.40	62 (0.220)	88 (0.170)	108 (0.160)
0.45	66 (0.240)	134 (0.170)	163 (0.170)
0.50	65 (0.260)	151 (0.190)	200 (0.170)
0.55	76 (0.260)	148 (0.200)	210 (0.170)
0.60	78 (0.260)	142 (0.210)	231 (0.170)
0.65	91 (0.260)	148 (0.210)	238 (0.170)
0.70	130 (0.260)	163 (0.210)	254 (0.170)
0.75	126 (0.260)	159 (0.210)	205 (0.180)
0.80	75 (0.260)	103 (0.210)	125 (0.180)
0.85	95 (0.260)	127 (0.210)	150 (0.180)

Figure 5.5. Frequency resolution vs. the trapping q parameter with a 100 ms excitation period. The maximum resolution of 254 is at $q = 0.70$.

Figure 5.6. Response curves at excitation periods other than 20, 50 or 100 ms. The excitation amplitudes were $0.400 V_{pp}$ for (A), $0.150 V_{pp}$ for (B) and $0.140 V_{pp}$ for (C), and other experimental parameters were identical to the experiments in figure 5.3. R = frequency resolution. The trapping q was 0.60.

5.5 Summary and Conclusion

It has been demonstrated experimentally that the resolution with dipole resonant excitation is affected by the trapping parameter q , the excitation period, and the amplitude of excitation. A correlation trend has been found between excitation period and resolution as well as between trapping RF and resolution. A lower amplitude of excitation is required to produce the same attenuation of ion signal when excitation is applied for a longer period. The optimum resolution was found at $q = 0.7$, regardless of the length of the excitation period. This trend makes it possible to predict optimum operational parameters for high resolution resonant ejection. Under optimized experimental conditions, the highest frequency resolution was observed to be 254 when the excitation was applied for 100 ms. With a longer excitation period (200 ms), a frequency resolution of 308 (corresponding mass resolution of 306) was observed at $q = 0.60$. These conditions were applied to perform ion isolation using a composite waveform, discussed in the following chapter.

Chapter 6

Application of Dipole Excitation: Ion Isolation

This chapter discusses ion isolation, one of the most useful applications of dipole resonant excitation. Using the independence of secular frequencies of ions of different m/z , a broadband waveform is synthesized with a narrow notch window in the frequency domain. Using this composite waveform, ions with a single m/z value can be separated from a mixture of ions trapped in the linear quadrupole field. Applying ion isolation in the 3D ion trap is also advantageous because it can reduce any interference caused by coexisting ions, such as space charge. However, implementation of ion isolation in the linear quadrupole interface will impact analysis with dramatic improvement of signal intensity and mass resolution. Further demonstrations will prove the advantages of isolating analyte ions before injected into the 3D trap.

6.1 Introduction

It was demonstrated in Chapter 5 that resonant ejection is possible in a linear quadrupole field. Considering that a resonant frequency is uniquely related to the mass to charge ratio of an ion, one can treat a mixture of ions as a collection of independent oscillators. Thus, one can generate a waveform that contains the frequencies corresponding to the secular frequencies of all ions and eject all ions from the trapping quadrupole field. One also can retain ions of a single mass to charge ratio by intentionally omitting the frequency that matches the secular frequency of the ion of interest. This assumes that the interactions between ions are negligible compared to the interaction with the trapping field.

Constructing a waveform that contains carefully chosen frequencies is a key part of the ion isolation process. This is usually done using computer-assisted waveform generation. Experimental details on production of the waveform and how the experiments are performed, are given in the following sections. However, the approach is essentially based upon Fourier Transform (FT) in which the spectrum in the frequency domain is converted to an amplitude waveform in the time domain. One can construct the waveform in the frequency domain with a notch window around the frequency of interest, and the transform generates a waveform in the time domain. A simpler method of composing a waveform is to simply add the amplitudes of chosen frequencies in the time domain. However, this process is only possible for discrete frequencies rather than a continuous band of frequencies. In Chapter 5, it was shown that the resolution with resonant dipole excitation is finite. A set of discrete frequencies will have the same effect as a band of continuous frequencies if the spacing between frequencies is less than the resolution limit. This process is fast (relative to the FT method) and does not sacrifice resolution (discussed in the composite waveform generation section). The process is called a "comb" wave because of its appearance with many discrete frequencies equally spaced over a range of frequencies.

Ion isolation can be used in many applications and it will be demonstrated that this method can be used to improve the performance of the electrospray-3D ion trap system. In particular, the mass accuracy of the ion trap has been a key issue with ion trap technology because space charge problems are believed to produce mass shifts ³¹. The notched waveform technique applied in linear quadrupoles can reduce the space charge problems in the 3D ion trap by isolating ions of a single m/z .

Ejection and fragmentation processes compete with each other and both processes contribute to attenuating the ion signal ²⁵. A composite waveform with properly chosen excitation amplitude and period allows fragmentation, ejection, or a combination of both processes to take place concurrently in the linear quadrupoles. This can shorten the length of analysis, by replacing MS^n in the 3D ion trap with one step of mass selection / fragmentation in the linear quadrupoles. Some distinguishing results using this approach are discussed later in this chapter.

6.2 Experimental

A 350 μM PPG sample solution was prepared in pure ethanol from a 1 mM stock solution (Chapter 3) with 0.7 μM reserpine as trace. To produce a concentration calibration curve, a series of 350 μM PPG solutions with 500 μM ammonium acetate were made in pure ethanol with reserpine concentrations between 0.2 and 5 μM . The spray voltage was 3,000 V and DC biases on plates, orifices, quadrupoles and lenses were at the values shown in Chapter 3. The pressure in Q_1 was 0.75×10^{-3} Torr of helium.

i) The circuitry and timing chart for ion isolation

The circuitry used in ion isolation experiments was similar to the one used for dipole excitation (figure 5.1). Instead of the function generator, however, a computer-installed arbitrary waveform generator board (PCI-312 arbitrary function generator, PC Instruments Inc., Akron, OH) was the excitation source. The output level could be adjusted by PCI control software (Benchtop[®] for Windows[®], PCI Instruments Inc., Akron, OH).

The timing chart for ion isolation was the same as shown in figure 5.2 except that a composite waveform was used for excitation. The drain pulse width from Q_0 was 0.1 ms and the gate width from Q_1 was 5.0 ms. Ions extracted from Q_0 were stored in Q_1 for 500 ms while a composite waveform was applied for 500 ms.

ii) Composite waveform generation

The composition is started by choosing a proper frequency window. Software from SCIEX (PCI3WAVE, SCIEX[®], Concord, Ontario, Canada) was used to generate the composite waveform. It allows setting the start and end frequencies of the window anywhere between 10 kHz to 500 kHz. The software also controls the PCI arbitrary waveform generator board with variable duration of the excitation waveform. The excitation period should be kept longer than 100 ms to ensure the maximum resolution of dipole excitation as was discussed in Chapter 5. A 500 ms excitation period was used for all the ion isolation experiments in this thesis. The step size determines the difference between neighboring frequencies. A finer step size can better approximate a continuous frequency band. A step size of 0.25 kHz was used. This is well within the resolution limits of dipole excitation. (At 250 kHz, which is the middle of the frequency band, it corresponds to a frequency resolution ($\omega/\Delta\omega$) of 1,000.)

The frequency of the notch should be determined first. The frequency is chosen so that ions of one m/z have a secular frequency matching the notch. The software starts by

calculating the amplitude at $t = 0$ by adding the amplitudes of all sine waves 0.25 kHz apart between 10 and 500 kHz. The amplitudes of sine waves with different frequencies are the same and their phases are randomly chosen. When the frequency reaches the notch, the program omits frequencies until the notch window is passed. After the amplitude of the first point in time is calculated, the value of the amplitude is stored into a text file. The program automatically repeats the same process with the next point at $t = 0.040 \mu\text{s}$, $0.080 \mu\text{s}$, etc. The sampling rate of the PCI function generator is 25 MHz. This determines that the separation between points is $0.040 \mu\text{s}$. The entire process is repeated until the amplitudes between $t = 0$ and 500 ms are completely calculated. After completing the calculation, the software initiates programming of the PCI board so that it can generate the waveform beginning with an external trigger. An example of a waveform both in the time and frequency domain is shown in figure 6.1. The waveform appears to be noise in the time domain but it has a well-defined structure in the frequency domain. The close-up view of the frequency domain spectrum in figure 6.2 clearly shows the comb structure of the spectrum with frequencies 0.25 kHz apart, and a window at 180 kHz with a width of 2 kHz in this example.

The ion isolation is affected by the same experimental parameters that influence dipole excitation: the amplitude of excitation, period of excitation and the trapping RF level. Obtaining an optimum isolation requires adjustment of these parameters and this is discussed in section 6.3. The amplitude of the waveform can be easily changed, either by software manipulation (SCIEX software) or by the gain of the amplifier, until the desired degree of isolation is obtained. The trapping RF was adjusted at $q = 0.63$ for the analyte ion (reserpine ion) and its resonant frequency was 180kHz.

Figure 6.1. An example of a composite waveform with a notch at 180 kHz. Top: the waveform in the time domain; Bottom: the spectrum of the waveform in the frequency domain. The width of the notch window is 2 kHz.

Figure 6.2. The close-up view of the spectrum in figure 6.1. The “comb structure” is clearly shown with frequencies spaced by 0.25 kHz. A 2 kHz window is placed at 180 kHz.

6.3 Results and Discussion: Ion Isolation

i) Ion isolation

Many applications are possible with the waveform shown in figure 6.1. By applying this waveform, all ions in the quadrupole field can be ejected except ions with a secular frequency of 180 kHz. Since the frequency notch window has a width of 2 kHz, some ions will be retained if their secular frequency is within the window. However, higher amplitude of excitation may decrease the resolution of dipole excitation. Thus frequencies with high amplitude near the resonance can eject ions with frequencies within the notch, as explained in Chapter 5. When a properly chosen isolation sequence is applied, however, the result is dramatic. An example is shown in figure 6.3. A solution with 350 μ M PPG and 0.70 μ M reserpine, a 500 fold excess PPG, was run. Ammonium acetate (500 μ M) was added to the pure ethanol solution to facilitate the electrospray. Because of the excess of PPG, the mass spectrum (figure 6.3 (A)) appears different from figure 3.5 of PPG solution in low concentration with no buffer. Singly protonated reserpine should appear at m/z 609.3, but it is not obvious that reserpine is present, even in the scale expanded view (figure 6.4 (A)).

When the excitation waveform is applied to Q_1 for 500 ms, however, the result is very different as shown in figure 6.3 (B). The reserpine molecular ion becomes the most intense ion in the spectrum instead of the peak from PPG near m/z 615. Some other ions near m/z 610 survive, but their intensities are dramatically reduced. The close-up view of two spectra in figure 6.4 clearly shows that the base peak has become the reserpine molecular ion at m/z 609.

Figure 6.4 (A) demonstrates an extreme case of space charge exerted by the intense PPG peaks near the reserpine molecular ion. Comparing figure 6.4 (A) with figure 6.4 (B) shows the benefits of isolating an analyte ion from the ion mixture. First, the mass resolution improves substantially. No isotopic structure of the reserpine

molecular ion is apparent in figure 6.4 (A) while figure 6.4 (B) shows well-resolved isotope structure. The resolution of dipole resonance ejection allows isolation of the reserpine ion which is only 7 amu apart from the adjacent intense PPG peak. Second, the signal of the analyte ion increases 15.6-fold (peak height measurement) when ion isolation is used. This directly lowers the detection limit for trace analysis with an ESI-3D ion trap system. Also, a 15.6 fold enhancement in the ion signal will allow for more MS/MS steps in the ion trap, if the analyte ion is to be further dissociated. Third, the mass accuracy of the ion trap was dramatically improved. The usual effect of space charge is a shift of apparent mass to charge ratio toward higher mass (figure 6.4 (A)), although the low resolution of the spectrum does not allow an accurate mass assignment. Low mass accuracy can be a serious disadvantage in automation of an ion trap system. To improve the mass accuracy of the 3D ion trap, automatic gain control has been incorporated with most of the commercial ion trap systems. However, this linear quadrupole interface can improve the mass accuracy and resolution simultaneously without reducing the signal.

Figure 6.3. Ion isolation with a composite waveform. The sample was 350 μM PPG and 0.70 μM reserpine with 500 μM ammonium acetate in pure ethanol. (A): No isolation waveform. (B): The waveform in figure 6.1 was applied to Q_1 for 500 ms.

Figure 6.4. Close-up views of figure 6.3 near m/z 610. Note that (A) and (B) have the same vertical scale maximum

Figure 6.5. Calibration curves for reserpine. Concentrations of reserpine are between 0.2 and 5 μM . Each test solution contains 350 μM PPG and 500 μM ammonium acetate in pure ethanol. Other experimental conditions are identical except for the ion isolation waveform.

ii) Calibration curve

To demonstrate another practical aspect of ion isolation, variable concentrations of reserpine analyte were tested and a calibration curve was constructed. A series of solutions were made with their reserpine content ranging from 0.20 μM to 5.0 μM while the concentrations of the other components, PPG (350 μM) and ammonium acetate (500 μM), were kept the same. The timing of the gate pulses for ion storage in Q_1 was the same as that used for figure 6.4.

A comparison of signal intensities with and without ion isolation is shown in figure 6.5. In the plot, all the points were taken from the peak height of the reserpine molecular ion. Solid lines are from a linear least square fit of the data points. With no ion isolation, the reserpine ion peak height increases slightly as the concentration of reserpine increases. The calibration curve is nearly flat. With the same experimental conditions, however, with use of the composite waveform, the signal increased and a linear calibration curve ($R^2 = 0.9823$) was produced.

iii) Comparison of ion isolation in the linear quadrupole and the 3D ion trap

The similarities of the 3D ion trap and linear quadrupole traps, also allow 3D ion traps to reject matrix ions. Instead of injecting a pre-filtered portion of the ions with ion isolation in the linear quadrupole, all ions can be injected into the 3D ion trap. This injection process can be accompanied by the application of a composite waveform (Stored-Waveform Inverse Fourier Transform (SWIFT), Filtered Noise Field (FNF) or arbitrary waveform generation ⁴⁶) on both end-caps to eject unwanted ions. The SWIFT technique uses an inverse Fourier Transform to generate a waveform containing a frequency notch. For FNF, rapid digital filtering enables the generation of a composite waveform in much faster fashion than SWIFT, so that FNF is widely utilized in ion trap systems for chromatography. An example of an arbitrary waveform generation has been already shown in section 6.2.

Ion selection incorporated with the 3D ion trap, however, has to allow more abundant ions, primarily from the matrix, to be introduced into the 3D ion trap. Since the 3D ion trap can only accommodate a finite number of ions, the majority of ions contained by the ion trap are from an unwanted source, the matrix. Thus the population of analyte ions will be relatively low compared to the matrix ions since the analyte ions must compete with matrix ions to be trapped by the 3D ion trap. The result is that the injection efficiency of analyte ions may be relatively low compared to the efficiency of injecting pre-selected ions from the interface.

To increase the number of trapped analyte ions, ion isolation should be applied during the injection process in the 3D ion trap, so that only analyte ions can be selectively stored. A notch waveform similar to figure 6.1 was applied between the two end-caps of the ion trap. However, a different timing sequence, shown in figure 6.6, was introduced. It was found that the same ion signal in the 3D ion trap could not be obtained with the timing sequence of figure 5.2. The new timing sequence employs a 3 second ion acquisition period in Q_0 , which allows the interface to accumulate enough ions to produce an intense reserpine ion peak.

The unusually long injection period in this timing diagram (500 ms, compared to 5.0 ms in figure 5.2) is intended to produce an ion signal sufficiently intense to be detected. The q parameter of the ion trap was maintained at 0.100 during injection. Because the RF level is low, a noise waveform, however, interferes with the trapping RF field and hinders efficient trapping of injected ions. Thus the long injection period has to be associated with low amplitude of waveform to ensure the minimum interference of the composite waveform with the trapping RF. In the linear quadrupole trap, the composite waveform is applied 10 ms after the ions are injected, so that the external excitation does not interrupt the injection process.

The two mass spectra in figure 6.7 were obtained with this new timing chart. Close-up views of the region near $m/z = 609$ are shown in figure 6.8. The timing sequence, figure 6.6, is designed with a 3.0 s ion accumulation period, and a 500 ms ion isolation and ejection period in the 3D ion trap. The width of the L_1 gate is 500 ms, compared to the 5 ms gate width in figure 5.2. The total length of the timing sequence for

ion isolation in the 3D ion trap (figure 6.6) is 7 times longer than the sequence used with ion isolation in the linear quadrupole.

The results in figure 6.7 and 6.8 suggests that the benefits of isolating an analyte in 3D ion traps may be similar to the benefit of ion isolation in the linear quadrupole traps. The resolution, sensitivity and mass accuracy have been improved simultaneously by ion isolation in the 3D ion trap. However, the improvement is not as great as with ion isolation in the linear quadrupole. For example, the signal increases from 200 in figure 6.8 (A) to 530 in figure 6.8 (B), a factor of 2.7. The improvement was from 164 in figure 6.4 (A) to 2600 in figure 6.4 (B) with ion isolation in the linear quadrupole, a factor of 15.6. In both cases the resolution has also been enhanced, but the remains of resonant ejection from the PPG base peak are shown near m/z 617 in figure 6.8 (B). Some other ions also survived with ion isolation in the linear quadrupole, but they are less abundant compared to ion isolation in the 3D ion trap.

The relatively low resolution of ion isolation in the 3D ion trap can derive from various sources. First, the resolution of ion isolation will be low because the trapping q_z was 0.100, as discussed in Chapter 5. Because of the low resolution of ion selection, the notch window should be maintained wide to prevent any attenuation of analyte ion signal, which results in less attenuated neighboring ions (7 amu from m/z 609). Second, space charge effects in 3D ion traps can also affect the resolution of resonant ejection. Many more ions were simultaneously injected to the 3D ion trap in this experiment, while only pre-selected analyte ions (reserpine) were injected in figure 6.4. In Chapter 4, it was shown that the number of ions detected with the 3D ion trap is directly proportional to the length of the acquisition time in Q_0 , which suggests that approximately 6 times more ions are injected to the ion trap with the timing chart in figure 6.6, compared to that of figure 5.2. Consequently the matrix ions are not ejected instantly after being injected into the ion trap.

In contrast, ion isolation in the linear traps is less subject to the problems discussed above. First, the ion storage capacity of the linear quadrupole trap is higher than the capacity of the 3D ion trap, so that more ions can be stored without reaching the space charge limit (chapters 2 and 4). Second, a high trapping q can be used with injection of ions into the linear quadrupole trap because injection is not critically

determined by q in the linear traps. Higher trapping q produces an even higher ion capacity (Chapter 4). Because of the high ion capacity, efficient ion injection and ion isolation with high resolution are concurrently applicable.

Figure 6.6. The timing chart for ion isolation in the 3D ion trap. Note that ion storage in Q_1 is 3.0 s and the L_1 gate is 500 ms.

Figure 6.7. Ion isolation in the 3D ion trap. The ion isolation waveform was applied to the ion trap for 3.0 s. Other experimental conditions were identical. (A): No ion isolation waveform applied; (B): with ion isolation.

Figure 6.8. Close-up views of figure 6.7 near m/z 610. (A) and (B) have the same vertical scale.

iv) Other applications of ion isolation

1) Fragment collection

Resonant ejection competes with collisional dissociation in the quadrupole field when external excitation is applied to the quadrupole. When a notched waveform is applied, it not only ejects a wide range of masses but also fragments ions. Thus, applying a notched waveform can be considered as collecting fragments of one m/z while the waveform ejects and fragments all other ions. Figure 6.9 shows an example of isolating the m/z 448 fragment ions formed by dissociating reserpine molecular ions.

A 1.0 μM reserpine solution with 500 μM ammonium acetate buffer was electrosprayed with a 3,000 V spray voltage. DC biases on plates, orifices, quadrupoles and lenses were at the values shown in Chapter 3. To obtain the mass spectrum of reserpine with no ion isolation or fragmentation in figure 6.9 (A), the timing sequence in figure (5.2) was used.

Figure 6.9 (B) is an MS/MS spectrum of reserpine with isolation / fragmentation done in the 3D ion trap. Since lower background pressure in the trap is used, the mass spectrum appears to be different from what is normally obtained with some commercial ion trap systems. After injecting ions into the 3D ion trap for 5.0 ms, the molecular reserpine ions were selected by ion isolation. Excitation time of 500 ms is an optimized parameter for ion isolation in the 3D trap, which is unusually long. Isolated reserpine ions were stored for 1.0 s while fragmentation was achieved by excitation with single frequency at a trapping q_z of 0.50. The fragment mass scan required 100 ms, so that this MS/MS spectrum required a total time of 2.1 s. The mass spectrum in figure 6.9(B) shows three major fragments of reserpine, which still require a further isolation sequence to isolate the m/z 448 fragments.

Figure 6.9 (C) is the spectrum obtained with ion isolation done in the linear quadrupole. Ions were stored in the linear quadrupole (Q_1) for 500 ms while the broadband ion isolation waveform was applied. The notch frequency of the waveform was at 190 kHz, which corresponds to the secular frequency of m/z 448, and the width of the notch was 10 kHz. After the isolation sequence was applied, the resulting ions were

injected to the 3D ion trap for 5.0 ms and the mass spectrum was obtained. The total length of this MS/MS sequence was 620 ms. In the spectrum, the m/z 448 fragments were isolated from other fragments. If figure 6.9 (C) is with compared figure 6.9 (B), the signal of the m/z 448 fragments increased 8 fold, while the total analysis time is one third.

Figure 6.9. Ion fragmentation/ isolation of reserpine in the 3D ion trap and in the linear quadrupole. The fragment at m/z 448 still needs to be isolated in (B). In (C) m/z 448 is collected by applying a composite waveform to the linear quadrupole for 500 ms.

2) Cytochrome *c*

A composite waveform can not only be used to isolate ions of a certain charge state from a protein solution but can also be used to increase the ion signal. Figure 6.10 (A) is a mass spectrum obtained with a 3 μ M cytochrome *c* sample solution prepared as described in Chapter 3 (M.W. = 12,384 amu). The infusion rate for the solution was 5 μ L/min and the spray voltage was 3,700 V. DC biases on plates, orifices, quadrupoles and lenses were at the values shown in Chapter 3. The timing sequence in figure 5.2 was used to obtain the mass spectra in figure 6.10 (A) with +13 to +16 states of cytochrome *c* ions. Previous studies of cytochrome *c* show that high charge states of up to +20 are detected from a sample solution denatured by alcohol or acid content^{50,51}, but states higher than +17 were not significantly detected with the electrospray-3D ion trap system used in this thesis. This is probably because of the 500 ms trapping time in the second quadrupole (Q_1). Through collisions with the background gas, high charge states of cytochrome *c* may lose charges and converge toward lower charge states.

A notch waveform with a notch frequency at 255 kHz, the secular frequency of +15 cytochrome *c* ions, and with a notch width of 20 kHz was applied. The only surviving charge states after ion isolation are + 15 ions and some +14 ions in figure 6.10 (B). Comparison of figure 6.10 (A) and 6.10 (B) shows that the intensity of the +15 state near m/z 830 increased 21 fold.

This enhancement is possibly from space charge problems caused by high charge states of cytochrome *c*. By ion isolation, the extent of the ion-ion interactions is reduced and the efficiency of trapping the +15 ions in Q_1 improves, a further benefit of ion isolation.

Figure 6.10. Separation of +15 state of cytochrome c by ion isolation. The ion intensity increases 21 fold in (B). Excitation is applied for 500 ms, notched at 255 kHz with a 20 kHz width. Other experimental parameters are identical except ion isolation.

3) Mellitin

Acquiring an intense signal for a certain charge state of a protein sample could be useful for analytical purposes because one can overcome the lower limit of concentration for detection of a trace. In addition, isolation of singly charged ions would be of great interest in gas-phase study of macromolecules such as spectroscopy of gas-phase proteins. The two examples above show fragmentation or charge-transfer process produced by application of a composite waveform. The isolation of mellitin fragments described below is an example of both.

A 5 μ M mellitin (M.W. = 2845.6 amu) solution was prepared in absolute ethanol with 5% acetic acid (Chapter 3). The spray voltage was 3,400 V and the DC biases on plates, orifices, quadrupoles and lenses were at the values shown in Chapter 3. The timing sequence in figure 5.2 was used to obtain the mass spectra in figure 6.11. Figure 6.11 shows three mass spectra taken with the mellitin solution. With no isolation or fragmentation, the base peak is a molecular ion with 4 protons (+4 charges, figure 6.11(A)).

In the 3D ion trap, +4 ions were collected by ion isolation with a composite waveform applied for 500 ms. Isolated ions (m/z 712) from the previous step were fragmented with a single frequency applied for 1.0 s in the 3D ion trap and the mass spectrum of the resulting fragments was obtained. The m/z 812 fragments are the only fragments recorded with MS/MS in the 3D ion trap (figure 6.11(B)).

A waveform was composed with a notch frequency at 115 kHz, window width of 10 kHz for the m/z 812 ion and applied to Q_1 while ions from the mellitin solution were stored for 500 ms. The result, shown in figure 6.11 (C), suggests that the isolation / fragmentation steps in the 3D ion trap can be replaced with an ion isolation sequence in Q_1 . This particular example has the ion isolation and fragmentation occurring simultaneously in the linear quadrupole with the composite waveform technique. The signal of m/z 712 ions was increased 8.1 fold by replacing MS/MS in the ion trap with ion fragmentation and isolation in the linear quadrupole.

Figure 6.11. Comparison of collection efficiency of the m/z 812 fragment of mellitin. (A) is the mass spectrum of mellitin with no fragmentation. The ion of m/z 812 is produced from m/z 712 which must be isolated in the ion trap to perform MS/MS. The intensity is increased 8 fold in (C) compared to (B).

6.4 Summary and Conclusion

Resonant excitation in quadrupole devices can be applied for ion isolation using composite waveform techniques. Isolating ions with a composite waveform in a linear quadrupole trap improves the signal from a trace analyte dramatically. The mass accuracy and resolution of the 3D ion trap was also increased with this method. The calibration curve of trace reserpine with ion isolation shows increased sensitivity over a 0.2 – 5 μM range of reserpine concentration.

With ion isolation through resonant excitation, some beneficial results of an enhanced signal of certain charge state ions or fragment ions were obtained through a process not clearly understood at this stage. The processes responsible could include fragmentation, ion isolation and charge transfer reactions. Lengthy and less efficient MS^n sequences in the 3D trap can be replaced with ion isolation sequences in the linear quadrupoles. The results also suggest that the analytical performance of an electrospray-3D ion trap system can be dramatically improved especially for trace analysis.

Although it was not tested with this thesis work, the performance using a noise waveform can be further improved by using digital signal processing (DSP). Slow process speed is a disadvantage of using Fourier transform in generating a composite waveform, so that it is not adequate for a 3D ion trap system combined with chromatography. Also, unwanted modulation is encountered when a finite number of data points are used in Fourier transforms. Windowing⁵², a common DSP technique, applies a filter function to the initial data set in the frequency domain before performing the transform. By doing this, the resolution of the waveform in the time domain improves without increasing the number of points in the original data set. Thus, generating a noise waveform can be faster and also better characteristics of waveform can be obtained in the time domain⁵³. For example, unwanted modulations in time domain waveform can be reduced, so that the resulting waveform through digital filtering gives better resolution in isolating ions. In composing a waveform from a comb structure, the phase of each sine function is at least randomized to give a wider dynamic range of isolation. In phase over

modulation, a quadratic function of frequency is used as phase for each sine function, which can prevent missing frequencies in the time domain waveform ⁵³.

Chapter 7

Resonant Excitation (II) : Quadrupole Excitation

Quadrupole excitation has been studied as an alternative to dipole excitation. It shares some features with dipole excitation but has many different characteristics especially in the complexity of the resonance spectrum. Instead of single strong fundamental resonance, many high order resonances and their sub-resonances are observed at a given trapping β . This chapter discusses new observations of these resonances in the linear quadrupole trap.

7.1 Introduction

In Chapter 5 and 6, the basics and applications of dipole excitation in linear quadrupoles were discussed. So far, the focus of the study on dipole excitation has been the resolution of resonant ejection. The term “quadrupole” describes the geometry of the excitation electric field. Although the setup for quadrupole excitation is similar, this technique distinguishes itself from the dipole excitation in several aspects. In dipole excitation, only one pair of poles (either pole pair A or pole pair B) is used to apply the

excitation, so that its geometry with respect to the trapping quadrupole field is asymmetric. Quadrupole excitation uses both poles since excitation is applied between pole pairs, so it has exactly the same geometry as the trapping field. As a result, the excitation circuitry is symmetric between poles and can be installed in the quadrupole RF power supply easily. The external quadrupole potential exerts forces in both the x and y-directions and simultaneously excites ion motion in both directions, while dipole excitation utilizes only one direction.

Unlike dipole excitation where the fundamental resonance is most often used, the fundamental and higher order resonances can be used for quadrupole excitation. Studies on higher order quadrupole resonances in the 3D trap can be found in the literature ^{54,55}. Also, the theory of quadrupole excitation with linear quadrupoles was published recently ^{56,57}. This chapter describes experimental results with quadrupole excitation in linear quadrupoles, including ion trajectory simulations, the characteristics of higher order resonances especially their resolution, the thresholds of different resonances as well as any correlation to the trapping/excitation parameters. The main purpose of this study is to investigate the potential of quadrupole excitation as an alternative to dipole excitation for resonant ejection and excitation.

7.2 The Theory of Quadrupole Resonant Excitation

i) Mathieu equation and resonant frequencies

The mathematical description of quadrupole excitation is related to that of dipole excitation. Assuming no external excitation is applied, any ion behaves approximately as a free Mathieu oscillator, which is the solution to the simplified Mathieu equation (equation (4.1)).

$$\frac{d^2x}{d\xi^2} + (-2q_x \cos 2\xi)x = 0 \quad (4.1)$$

One can place the forced oscillation term on the right-hand side of the equation.

$$\frac{d^2x}{d\xi^2} + (-2q_x \cos 2\xi)x = F_0' \cos(\omega t + \phi)x \quad (7.1)$$

This equation is similar to equation (5.3) but the excitation is quadrupole and has the same functional form as the potential on the left-hand side. Replacing ξ with Ω and t gives a physically more intuitive expression.

$$\frac{d^2x}{dt^2} + \frac{\Omega^2}{4}(-2q_x \cos \Omega t)x = F_0 \cos(\omega t + \phi)x \quad (7.2)$$

Finding a solution to equation (5.4) is not trivial. A perturbation treatment led to a formula for the resonant frequencies ^{49,56}. The order of the perturbation calculation (K) is introduced as a new parameter in the definition of resonant frequencies. Now the resonant frequencies $\omega(n, K)$ are expressed in terms of β , n and K at given β .

$$\omega(n, K) = \frac{|n + \beta|\Omega}{K} \quad (7.3)$$

where n is any integer ($n = 0, \pm 1, \pm 2, \pm 3, \dots$), K is any positive integer greater than zero ($K = 1, 2, 3, \dots$) and Ω is the frequency of the trapping RF.

Since an infinite number of (n, K) combinations are possible, the resonances will appear throughout a wide range of frequencies, although not all the resonances are resolved in experiments. This is because quadrupole resonances have a sharp threshold of excitation if ion motion is damped ⁵⁷. Dipole excitation can continuously deposit energy into the ion motion, so the amplitude of ion motion increases gradually regardless of the amplitude of excitation. With quadrupole excitation, however, the amplitude of ion motion can decrease if the excitation is below the threshold and the desired degree of excitation will not be achieved. A distinguishing characteristic of the quadrupole resonance is that the threshold amplitude for different (n, K) resonances can vary considerably. Even ions of a single m/z ions trapped at a fixed β , low threshold resonant frequencies can frequently overlap with higher threshold resonances. Ions will be predominantly ejected by the low threshold resonances when the excitation amplitude is high, even though the excitation frequency is off-resonance. To observe any low threshold (n, K) resonances, the resonant frequencies should be located far from any low-threshold resonances. Experiments have revealed that certain (n, K) resonances were only observable by adjusting the trapping β .

ii) Simulation of ion trajectories

Sudakov's software was used for simulating ion trajectories in a linear quadrupole field in Chapter 4. This software calculates ion trajectory by a matrix method ^{16,46}. The geometry of quadrupoles, trapping RF frequency, m/z of ions, DC and RF voltages (U , V) are given as variables for simulations. The linear collision damping model is used, in which energy loss is proportional to the kinetic energy of ions. The trajectories of ions as well as kinetic energies are displayed in the choice of the (x , z), (y , z) or (x , y) planes, or in phase space with position (in the x or y -direction) and the corresponding momentum. During the calculation, dipole and quadrupole excitations can be incorporated, so that characteristics of resonant excitation can be predicted.

To obtain figures 7.1 and 7.2, the trapping β of 0.306 for m/z 609 ions (reserpine) was used with 0.75 mTorr of helium background pressure. The frequency of the trapping RF was 768,000 Hz. The collisional cross section of the reserpine-helium pair was from $2.80 \times 10^{-18} \text{ m}^2$ ^{10,48}.

The trajectories with dipole excitation, shown in Chapter 4, differ from the trajectories with quadrupole excitation (figure 7.1). In figure 4.2, the amplitude in the y direction increases when the excitation is applied in the y direction. With quadrupole excitation, ion motion in both x and y directions is excited. Figure 7.1 shows the increase of amplitude of ion motion in both the x and y -directions. Figure 7.2 shows trajectories of a reserpine ion drawn on the xy -plane. The elapsed time of each point is not shown in this simulation, but it is proportional to the distance from origin. Figure 7.2 (A) is the trajectory with excitation of the ($n=0$, $K=1$) resonance and figure 7.2 (B) is that of the ($n=+1$, $K=1$) resonance. This is a demonstration that trajectories for different (n , K) resonances can be drastically different. There is a circular trajectory centered to the middle of the trapping field figure 7.2 (A) or a trajectory aligned along a line passing the center of the trap figure 7.2 (B).

Figure 7.1. Trajectory calculations for reserpine ions (m/z 609) with quadrupole excitation. Trapping $\beta = 0.306$; $r_0 = 2.85$ mm; trapping RF frequency = 768 kHz; 0.75 mTorr of helium background gas; excitation: $\omega = 234.2$ kHz, amplitude = $0.70 V_{pp}$ (pole-to-ground); Trajectories are shown in the (amplitude, time) plane. This particular example is for the ($n=0$, $K=1$) resonance. The y scale is in mm.

(A)

($n=0$, $K=1$)
 $\omega = 235$ kHz
Amp = 0.60 V

(B)

($n=+1$, $K=1$)
 $\omega = 1003$ kHz
Amp = 3.5 V

Figure 7.2. Trajectory simulation results of quadrupole excitation in the x-y plane. (A): ($n=0$, $K=1$) resonance; (B): ($n=+1$, $K=1$) resonance. Simulation parameters: $r_0 = 2.85$ mm; trapping RF $\omega = 768$ kHz, $\beta = 0.306$; 0.75 mTorr of helium with linear damping model. Ions start from the origin and disappear when they reach the field boundary. The x and y scales are in the units of r_0 (2.85 mm/unit). Amplitudes are in volts, peak-to-peak, pole-to-ground.

7.3 Experimental

Figure 7.3 shows the circuit used to apply quadrupole excitation to the linear quadrupole. It requires two toroidal transformers. The A poles of Q_1 are connected to one end of the secondary of a toroidal transformer (T_1). The other end of the secondary is connected to ground through a variable capacitor (5 - 50 pF). The connection for the B poles to the second transformer (T_2) is the same as the A poles except the order of pole and ground are inverted. Thus, 180°-inverted AC will be applied between poles if the connections to the primaries of both transformers are identical. The variable capacitors are for matching half of the impedance on the primary to eliminate any RF pickup on the primary from the driving RF. Center taps of the secondaries are connected to the outputs of the RF quadrupole supply. The auxiliary excitation is from a function generator (SR 347, Stanford Research Systems) through a high voltage RF amplifier (ENI model 240L, ENI, Rochester, N.Y.), so that the maximum amplitude of the excitation ranges up to 200 V_{pp} (peak-to-peak, pole-to-ground). The RF amplifier was used only when a high RF amplitude was required. The timing chart in figure 5.2 was used. The drain time from Q_0 was 0.1 ms and the storage time in Q_1 was 500 ms. External excitation was applied for 500 ms. The frequency, period and amplitude of excitation were adjustable.

A 1.0 μ M solution of reserpine, prepared as described in Chapter 3, was used for the experiments. The spray bias was 3,000 V with a 1.0 μ l/min syringe pump speed. The plate biases were as described in Chapter 3.

Figure 7.3. The circuitry for quadrupole excitation. T_1 and T_2 are identical. The variable capacitors ($C_1, C_2 = 10 - 50$ pF) are for balancing out any RF feedback to the primary of the transformers. Q_0 is capacitively coupled to Q_1 through high voltage capacitors (200 pF).

7.4 Results and Discussion (I) : Higher Order Resonances

i) (0,K) resonances and their damping characteristics

Even the fundamental resonance ($n=0$) can have many different sub-resonances with different values of K . Table 7.1 shows the resonant frequencies from both theory and experiment. The threshold voltage which produces 90% attenuation of the signal is also listed in the table. The general trend is that higher sub-resonances (higher K) require higher amplitudes of excitation to attain the same attenuation. This trend is consistent with the theory of parametric resonance. Landau and Lifshitz ⁴⁹ state without proof that

$$h \propto \lambda^{1/K} \quad (7.4)$$

where h is the threshold, λ is a damping constant and K is the order of the resonance. From equation (7.4), the logarithm of the threshold voltage of each resonance is inversely proportional to its integer parameter K . Such a plot of the thresholds from the table 7.1 with $\beta = 0.521$ is consistent with equation (7.4) (figure 7.4).

When β is 0.521 as in figure 7.4, the slope is -2.44 (for the $n=0$, K resonances). Similar plots were obtained at different values of β ranging from 0.133 up to 0.439 for the $n=0$ resonance, shown in figure 7.5. Figure 7.6 is a plot of the slopes of ($n=0$, K) resonances at different trapping β . Some resonances are missing because they overlap in frequency with low threshold resonances at a given β . The variations of slopes from this plot are less than 10% of the average (average = -1.76 , standard deviation = 0.354). It can be concluded that the slope of a plot of $\log V_{\text{threshold}}$ vs. $1/K$ is proportional to a damping constant regardless of β .

Table 7.1. High order quadrupole resonances at different trapping β . Frequencies are in kHz. The amplitude $V_{\text{threshold}}$ (volts, peak-to-peak, pole-to-ground) was measured when 90% of the original intensity was attenuated. Resolutions are found from the FWHM of the frequency response curve.

β	(n, K)	ω_{theory}	$\omega_{\text{experiment}}$	$\Delta\omega_{\text{FWHM}}$	$V_{\text{threshold}}$	R_f	R_m
0.155	(0,1)	119.04	113.00	2.17	0.230	52.190	52.19
0.155	(0,2)	59.52	58.00	0.63	1.210	92.800	92.80
0.155	(+1,1)	887.04	884.50	2.16	2.200	409.398	53.84
0.155	(-1,1)	648.96	651.50	2.14	1.690	304.818	54.40
0.178	(0,1)	136.70	136.90	1.81	1.900	75.780	75.78
0.225	(0,1)	172.80	173.20	2.70	0.484	64.250	64.25
0.225	(0,2)	86.40	87.40	0.80	3.260	109.800	109.80
0.225	(+1,1)	864.00	943.50	2.58	3.010	365.478	67.92
0.225	(-1,1)	595.20	593.00	3.32	2.050	178.511	52.95
0.266	(0,1)	203.98	205.80	3.19	0.616	64.440	64.44
0.306	(0,1)	235.01	237.00	3.23	0.696	73.470	73.47
0.306	(0,2)	117.50	118.80	1.27	6.960	93.690	93.69
0.306	(0,3)	67.99	78.10	0.78	8.900	100.800	100.80
0.306	(+1,1)	1003.01	1005.00	3.65	3.650	280.648	66.18
0.306	(-1,1)	532.99	531.00	3.32	2.110	160.036	71.43
0.306	(+2,1)	1771.01	1772.50	2.57	53.600	690.495	92.13
0.306	(+2,2)	885.50	886.90	0.93	36.800	956.949	128.29
0.306	(-2,1)	1300.99	1300.00	3.47	21.600	374.640	68.01
0.306	(-2,2)	650.50	649.30	1.43	32.000	452.789	82.78
0.350	(0,1)	268.80	269.90	3.14	0.688	85.880	85.88
0.395	(0,1)	303.36	303.90	3.40	0.808	89.480	89.48
0.395	(0,2)	151.68	151.10	1.80	9.970	88.860	88.86
0.395	(+1,1)	1071.36	1070.00	4.24	4.330	252.240	71.19
0.395	(-1,1)	464.64	465.50	4.48	2.030	103.929	67.54
0.440	(0,1)	337.92	338.30	3.65	0.920	92.691	92.69
0.521	(0,1)	400.13	400.50	5.07	0.768	79.010	79.01
0.521	(0,2)	200.06	198.90	4.10	10.400	48.520	48.52
0.521	(+1,1)	1168.13	1167.00	5.86	3.490	199.147	68.09
0.521	(-1,1)	367.87	369.00	5.44	0.961	67.856	73.37

Figure 7.4. $\log V_{\text{threshold}}$ vs. $1/K$ for different (n, K) resonances. The amplitude $V_{\text{threshold}}$ (peak-to-peak, pole-to-ground) was measured in volts. The trapping β is 0.521.

Figure 7.5. $\log V_{\text{threshold}}$ vs. $1/K$ at different trapping β .

Figure 7.5. Continued.

Figure 7.5. Continued.

Figure 7.6. The slopes of $\log V_{\text{threshold}}$ vs. $1/K$ plots at different values of β and $n = 0$. Slopes are found by least square fitting.

ii) High order resonances

The theory implies the possibility of detecting higher order resonances ($n \geq 1$ or $K \geq 2$). Some higher order resonances ($n=0$, K resonances) were reported in the linear quadrupole field with a TOF mass spectrometer⁵⁷. Overlaps between frequencies were mentioned as a problem in the previous section. With dipole excitation the fundamental frequency and higher order resonances are remotely located in the frequency domain and any overlap between resonances scarcely occurs (equation 2.31).

It is especially difficult to observe a high order resonance with quadrupole excitation if the resonant frequency is near that of a low threshold resonance such as ($n=0$, K), ($n=\pm 1$, K) and ($n=\pm 2$, K) resonances. To identify weak resonances, thus, the trapping β value should be carefully chosen. Even with no overlap with strong resonances, some higher order resonances cannot be observed because they require very high excitation amplitudes. Such high amplitudes may affect trapping of ions in the quadrupole field. However, several higher order resonances ($n \geq 1$, $K \geq 2$) were observed as listed in table 7.1. An example is the ($n=+2$, $K=1$) resonance at $\beta = 0.306$. It has a threshold voltage of 26.8 V (zero-to-peak, pole-to-ground). Since the trapping β of 0.306 for m/z 609 (reserpine) corresponds to the trapping RF amplitude of 130.3 V (zero-to-peak, pole-to-ground), the excitation amplitude is 20.6% of the driving RF in amplitude.

The frequency predicted by theory agreed with experiments within 10%. In table 7.1, certain (n , K) resonances are only available at certain trapping β for the reason mentioned above. Also, with the trapping β higher than 0.5, many resonances could not be detected because of high thresholds. Relatively fewer resonances appear in the table at higher β .

Figure 7.7. Response curves of ($n=0$, $K=1$) excitation with different amplitudes. Resonance points shift toward lower frequency with higher amplitude. The trapping β is 0.306.

iii) $\log V_{th}$ vs. $1/K$ plots for higher order resonances

Since high order resonances can be observed, it is possible to draw $\log V_{th}$ vs. $1/K$ plots. Figure 7.5 shows $\log V_{th}$ vs. $1/K$ plots of (n, K) resonances at different trapping β . The slopes of the $(n=\pm 1, K)$ resonances are similar to the slope of the $(n=0, K)$ resonances when β is lower than 0.4 (figure 7.5(A) through 7.5(H)), but become greater at higher values of β (figure 7.5 (I) and 7.4).

The slopes of the $(n=\pm 2, K)$ resonances are quite different from the slopes of both the $(n=0, K)$ and $(n=\pm 1, K)$ resonances at every β . In figure 7.4 ($\beta = 0.521$), the slope of $(n=\pm 2, K)$ resonances is -0.4 and that of $(n=0, K)$ resonances are -2.4 . If equation (7.4) applies to higher order resonances, there is possibility of a different damping process or at least the need for different damping parameters. This requires additional theoretical work.

iv) Frequency shifts

Small perturbation theory is used to find solutions of equation (7.2) ⁵⁶. If the magnitude of the perturbation changes, it also affects the solution. The experiments showed that resonant frequencies shift depending upon the amplitude of the excitation. Frequency shifts similar to those observed in this thesis work have been reported and a theoretical model that has shown reasonably good correlation to the results of experiments and trajectory calculations ⁵⁸. Omitting the details of the theory, the higher amplitude of the excitation shifts the resonance to lower frequency for $(n=0, K)$ resonances.

Figure 7.7 shows response curves of the $(n=0, K=1)$ resonance with $\beta = 0.306$. Reserpine ions from a $1.0 \mu\text{M}$ solution were stored in Q_1 for 500 ms and excitation was applied for 500 ms. Instead of changing the pressure of background gas to adjust the ejection threshold ⁵⁸, different number of ions were stored in the linear quadrupole trap to give different threshold. The width of the IQ gate was adjusted to 1.2 ms, 0.5 ms and 0.3 ms so that the signal of the reserpine ions detected from the 3D ion trap was 1000, 500 and 250 in arbitrary units, respectively. A higher amplitude is required to obtain the same

degree of attenuation (90%) as the ion signal without excitation increases. With excitation applied in the linear quadrupole trap, the excitation frequency was scanned near 235 kHz while recording the signal of the reserpine molecular ion.

An amplitude of 0.990 V_{pp} was required to attenuate the maximum ion signal of 1000, 0.591 V_{pp} for 500 and 0.493 V_{pp} for 250. As higher amplitude is applied the resonance shifts toward lower frequency regardless of the amount of attenuation. In this example, a 40% increase in amplitude will shift the frequency by 1.3 kHz. All the amplitudes were measured in volts, peak-to-peak, pole-to-ground.

v) Abnormal splitting

During a series of resolution measurements, splitting of the response curve was occasionally found. As shown in figure 7.8 (A), the ($n=+1$, $K=1$) resonance at $\beta = 0.150$ (883 kHz) shows two minimum points split by 3.5 kHz. At higher β s, the splitting decreases until the two peaks eventually overlap. The ($n=-1$, $K=1$) resonances in figure 7.8 (B) and figure 7.8 (C) shows the same trend as shown in figure 7.9.

This splitting was seen only with the ($n=\pm 1$, $K=1$) resonances possibly because of field imperfections. Based on the trajectory calculation of the quadrupole excitation, these resonances allow ions to travel deeply along the asymptotes of the quadrupole field (figure 7.2 (B)) unlike any other resonances. It was also observed that the splitting decreases at higher β parameters (from 3.5 kHz (figure 7.8 (A)) to 2.1 kHz (figure 7.8 (B)); from 3.3 kHz (figure 7.9 (A)) to 2.5 kHz (figure 7.9 (B))). With trapping β higher than 0.3 splitting was not observed. This could be from non-linear components of the quadrupoles as discussed in Chapter 5, but the detailed process is not clearly understood.

Figure 7.8. The $(n=+1, K=1)$ resonance at different trapping β . (A): $\beta = 0.150$, $m/\Delta m = 54$, $\text{amp} = 2.20 \text{ V}$; (B): $\beta = 0.225$, $m/\Delta m = 68$, $\text{amp} = 3.01 \text{ V}$; (C): $\beta = 0.306$, $m/\Delta m = 66$, $\text{amp} = 3.65 \text{ V}$. Amplitudes are in volts, peak-to-peak, pole-to-ground.

Figure 7.9. The $(n=-1, K=1)$ resonance at different trapping β . (A): $\beta = 0.150$, $m/\Delta m = 54$, $\text{amp} = 1.690$ V; (B): $\beta = 0.225$, $m/\Delta m = 53$, $\text{amp} = 2.05$ V; (C): $\beta = 0.306$, $m/\Delta m = 71$, $\text{amp} = 2.11$ V. Amplitudes are in volts, peak-to-peak, pole-to-ground.

7.5 Results and Discussion (II) : Resolution

i) Frequency resolution vs. mass resolution

The resolution of resonances from table 7.1 can be found by plotting their response curves as in figure 7.10. These particular examples of response curves are for different (n, K) resonances at $\beta = 0.306$. The FWHM of each plot at different (n, K) and different β is listed in table 7.1.

The frequency resolution is found simply dividing the resonant frequency, ω , by the full width at half maximum, $\Delta\omega$.

$$R_f = \frac{\omega}{\Delta\omega} \quad (7.5)$$

The mass resolution can be found only by converting the FWHM into m/z units for the different (n, K) resonances. In the response curve, both ends of full width at half maximum correspond to $(\omega - \Delta\omega/2)$ and $(\omega + \Delta\omega/2)$. These frequencies are converted to the corresponding m/z values using equation (7.3). Also, the different relations between q and β should be used for low values of β (equation (5.1)) and high values of β (equation (5.2)). Then the resolution $((m/\Delta m))$ is calculated.

$$R_m = \frac{m}{\Delta m} \quad (7.6)$$

As listed in the table, the frequency resolution varies widely as the resonant frequency changes. It is equal to the mass resolution for all (n=0, K) resonances. For the (n, K) resonances where n is different from 0, the frequency resolution can be higher than the corresponding mass resolution. For example, the (n=+2, K=2) resonance at $\beta = 0.306$ has a frequency resolution of 957, but the mass resolution is 128. In every case, the mass resolution is of more interest than the frequency resolution.

Figure 7.10. Response curves of quadrupole resonances at $\beta = 0.306$. (A): ($n=0, K=1$), $m/\Delta m = 85.9$, amp = 0.591 V; (B): ($n=0, K=2$), $m/\Delta m = 93.7$, amp = 6.96 V; (C): ($n=0, K=3$), $m/\Delta m = 101$, amp = 8.90 V. Amplitudes are in volts, peak-to-peak, pole-to-ground.

(D) ($n=+2, K=1$)

(E) ($n=+2, K=2$)

(F) ($n=-2, K=1$)

(G) ($n=-2, K=2$)

Figure 7.10. Continued. (D): ($n=+2, K=1$), $m/\Delta m = 92.1$, amp = 53.6 V; (E): ($n=+2, K=2$), $m/\Delta m = 128.3$, amp = 36.8 V; (F): ($n=-2, K=1$), $m/\Delta m = 68.0$, amp = 21.6 V; (G): ($n=-2, K=2$), $m/\Delta m = 82.8$, amp = 32.0 V. Amplitudes are in volts, peak-to-peak, pole-to-ground.

ii) β -dependence of the mass resolution

The q -dependence of the mass resolution of a dipole excitation was discussed in Chapter 5. Similarly the resolution with quadrupole excitation shows a dependence on the trapping RF. The resolution of the ($n=0$, $K=1$) resonances at a different RF levels is plotted with respect to its β (figure 7.11) since this resonance is observed all the trapping β . The plot reaches its maximum when β is 0.440 for ($n=0$, $K=1$) (figure 7.11 (A)). The resolution increases slowly toward the maximum and decreases rapidly at higher β in this plot. For the ($n=0$, $K=2$) resonances, the maximum resolution appears at lower β . The mass resolution increases at higher β values in the ($n=\pm 1$, $K=1$) resonances.

iii) K -dependence of the resolution

Table 7.1 shows another coherent trend of the resolution. Within a series of resonances with the same n , it is always the case that higher K resonances have higher mass resolution. The only exception observed from the experiment was when the β values were higher than 0.395 and the resolution decreased with higher K values.

Figure 7.11. Mass resolutions of the (n, K) resonances vs. trapping β .
 (A): $(n=0, K=1)$, (B): $(n=0, K=2)$

Figure 7.11. Continued. (C): ($n=+1, K=1$), (D): ($n=-1, K=1$)

7.5 Summary and Conclusion

Quadrupole excitation has been studied as an alternative to dipole excitation in the linear quadrupole field. A complex resonance frequency scheme is predicted by theory. Some of the higher order resonances were found experimentally. The $(n=\pm 1, K)$ and $(n=\pm 2, K)$ resonances were observed for the first time in the linear quadrupole. Different (n, K) resonances showed different ejection threshold voltages and mass resolution. It was found that the parameter K affects the threshold voltages significantly and their correlation is affected by the order of resonance (n) . Also, the mass resolution of each (n, K) resonance was determined. When the resolution of $(n=0, K=1)$ resonances is plotted against the trapping RF level, it has a maximum at a trapping β of 0.40. Resonances of different order (n) and parameter K were found to have different resolutions, while the maximum resolution found in the study was 128.

Chapter 8

Summary and Conclusion

The goal of this thesis work was to investigate the enhanced performance possible with a new interface for an electrospray-3D ion trap system. Linear quadrupoles were used as ion guides and traps in the interface. Chapter 4 through Chapter 6 discussed the enhanced performance of the system made possible by resonant excitation techniques, especially with dipole excitation.

In Chapter 4, the ion accumulation capability of the linear quadrupole was demonstrated. A number of ions of different m/z ratio were stored in the quadrupole field. This is especially beneficial when adapting a continuous ion source like electrospray to 3D quadrupole ion traps. A 3D ion trap requires an interface that can store ions from electrospray in order to have the improved duty cycle. Linear quadrupoles are storage devices if blocking potentials are applied to the ends. The experiments with ion storage in a linear quadrupole show that the duty cycle can be improved by acquiring ions before injection into the 3D ion trap.

Details of dipole excitation in a linear quadrupole were discussed in Chapter 5. Stable ion motion with a given secular frequency can be excited along either of two perpendicular directions. The secular frequency is determined by the m/z ratio of ions when the frequency and amplitude of the driving RF are fixed. Dipole excitation was applied to the trapping quadrupole field through a toroid transformer. Parameters such as the trapping RF amplitude and the period and amplitude of excitation affect the resolving power $(m/\Delta m)_{FWHM}$ with dipole excitation. A mass resolution of 254 was observed at $q =$

0.70 when excitation was applied for 100 ms with an amplitude of 0.170 V_{pp}. At different trapping periods, the best resolution was always observed at $q = 0.700$. The resolution was improved when longer excitation was applied up to 100 ms. The highest frequency resolution ($\omega/\Delta\omega$) from experiments was 308 (a mass resolution of 306) when trapping q was 0.60, excitation amplitude was 0.150 V(peak-to-peak, pole-to-ground), and the excitation period was 200 ms.

Chapter 6 discussed the use of composite waveforms. A waveform was generated using a computer by superimposing a number of frequencies. Within a band of frequencies, a notch is placed around the frequency of ions that were to be isolated. If the resonant excitation amplitude is sufficient to eject ions, all the ions are ejected from the field except the ions with a secular frequency matching the notch. Experiments with ion isolation showed that the signal and resolution of the 3D ion trap can be dramatically improved. A demonstration with PPG solutions with trace reserpine showed that trace analysis can benefit from the elimination of background ions, thus reducing space charge problems in the ion trap. A signal improvement of 15.6 fold and dramatic enhancement in resolution were observed. Other applications of composite waveforms such as selective storage of ion fragments, or collecting a certain m/z ratio from a mixture of multiply charged ions were shown. The resulting enhancement in sensitivity contributes to lowering the detection limit of analysis and allows further steps of MS^n in the 3D ion trap.

Chapter 7 discussed quadrupole resonant excitation as an alternative to dipole excitation. The quadrupole excitation field has the same symmetry as the trapping field. As a preliminary study, higher order resonances ($n \geq 1$) and their sub-resonances in a linear quadrupole field were first observed and their mass resolution was reported. The maximum mass resolution with quadrupole excitation was lower than with dipole excitation. Significant frequency shifts appear as the amplitude of excitation increases. Peak splitting was observed with ($n=\pm 1, K$) resonances for values of β less than 0.306.

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