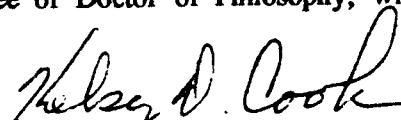


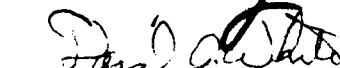
To the Graduate Council:

I am submitting herewith a dissertation written by Michael R. Troutman entitled "Studies of Ionic Keto/Enol Equilibria Utilizing Tandem Mass Spectrometry." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.



Dr. Kelsey D. Cook, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

STUDIES OF IONIC KETO/ENOL EQUILIBRIA
UTILIZING TANDEM MASS SPECTROMETRY

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Michael R. Troutman

May, 1994

Dedicated to Robert and Bess Troutman

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ABSTRACT

This research focussed upon the keto/enol tautomerization of the radical cation of 3-methyl-2-butanone (3-MB, molecular ion = m/z 86). Loss of a methyl group from opposite ends of the molecular ion yields isomeric ions at m/z 71, an acylium ion and protonated methyl vinyl ketone (MVK). Utilizing tandem mass spectrometry (MS/MS), the fragmentation characteristics of these m/z 71 ions produced from 3-MB (after introduction of neutral keto form into the mass spectrometer) was determined. Performing separate MS/MS experiments on the acylium and MVK reference ions yielded quantitative information about the m/z 71 ions produced from 3-MB itself. Furthermore, MS/MS experiments were performed on m/z 86 of 3-MB (produced from the neutral keto form) to determine the keto/enol composition of the molecular ion. By utilizing the resulting quantitative information obtained for m/z 71 and m/z 86, it was possible to partially deconvolute the mass spectrum expected for the enol ion. The mass spectrum thus obtained agreed well with the mass spectrum of the neutral enol form reported in the literature. A structurally similar compound, 2-pentanone, was also studied. These results represent an application of the linear superposition of MS/MS spectra.

For this research, a novel, custom-built hybrid mass spectrometer of QEB geometry and an Ion Trap Mass Spectrometer (ITMS) were utilized. The design and operation of the QEB are discussed, and differences in MS/MS data between the QEB and ITMS are addressed. The acylium ion (m/z 71) rearranged unexpectedly on the ITMS due to the longer time-frame for ITMS MS/MS experiments, thus complicating data interpretation. However, the keto/enol composition of the m/z 86 ions was unaffected.

TABLE OF CONTENTS

1. INTRODUCTION	1
General Information Regarding Mass Spectrometry	1
General Information on the Keto/Enol Chemical System	2
Summary of Previous Mass Spectrometric Studies on Keto/Enol Systems ...	6
Factors Influencing Reactions Of Ionic Species	8
Utilization of Tandem Mass Spectrometry for Study of Keto/Enol Tautomers	11
Characteristics of the MS/MS Experiment	14
Mass Spectrometers Utilized in These Experiments	14
The Quadrupole Mass Analyzer	18
The Magnetic Sector Analyzer	19
The Electrostatic Analyzer (ESA)	20
EB Combination For High Resolution Analyses on Ion Beams Possessing an Energy Spread	21
Trapping Instruments	22
Factors Influencing the MS/MS Experiment	25
Summary of Research Objectives	28
2. EXPERIMENTAL	30
Basic Description of the QEB hybrid mass spectrometer	30
The Vacuum System of the QEB	30
Procedure for operation of source and quadrupole electronics at high voltage.	36
Assembly of the ion optical components.	38
Placement of the entire assembly into the main vacuum chamber	46
Preliminary tests performed before attaining an ion beam	46
Sample introduction system	48
Acquisition of data	51
Tuning of the QEB Instrument	53
Acquisition of a mass spectrum.	53
Initially Attaining an Ion Beam on the QEB: Where to Start.	53
Attaining an Ion Beam with High Voltage Applied.	55
Setting of the Acceleration Lens Elements	56
Ion Beam Characteristics Monitored After the ESA.	57
Obtaining an Ion Beam Through the Magnet and Magnet Position Adjustment.	61
Quadrupole Mass Filter Operation in RF/DC Mode	62
Validation of the Acceleration Lens Settings	65
Performance of low energy MS/MS experiments on the QEB ...	66
Performance of high energy MS/MS experiments on the QEB ...	68

Performance of MS/MS/MS on the QEB	70
Ion Trap Mass Spectrometer	71
Chemicals Utilized	74
 3. INSTRUMENTATION: QEB HYBRID MASS SPECTROMETER	 75
Initial Behavior of the Ion Optical System Following the Quadrupole	76
Focussing of the ion beam into the low energy collision cell	80
Design of the acceleration lens system	82
Response of Acceleration Lens System to Product Ion Energy Spread	89
 4. RESULTS AND DISCUSSION	 96
3-METHYL-2-BUTANONE	96
QEB Results for m/z 71 ions of 3-methyl-2-Butanone	96
Application of the Linear Superposition Principle	109
QEB High Energy CID Results for m/z 71 ions of 3-MB	116
QEB MS/MS Results on M/Z 86 of 3-Methyl-2-Butanone	120
Ion-Molecule Reaction for Keto/Enol Determination on QEB	130
Partial Determination of Enolic Ion Mass Spectrum From QEB MS/MS Results	132
ITMS MS/MS Results for M/Z 71 Ions of 3-methyl-2-Butanone	136
ITMS MS/MS Results on the M/Z 86 Ion of 3-MB	145
Proposed Model for the Keto/Enol System of 3-Methyl-2-Butanone	146
2-PENTANONE	159
Approach	161
QEB Results for m/z 71 ions from 2-P	162
Behavior of the 2-Pentanone m/z 86 and m/z 71 ions on the ITMS	164
 5. CONCLUSIONS AND FUTURE WORK	 168
Comparison between the beam instrument and the trapping instrument ..	168
Future Work	171
Instrumentation	171
Keto/enol studies	173
Linear Superposition Principle	174
 REFERENCES	 176
 VITA	 183

LIST OF TABLES

TABLE 2.1

Components connected to the various numbered feedthroughs on the main mounting flange shown in Figure 2.3.

.....35

TABLE 2.2

List of materials and vendors utilized for construction of the QEB EI source shown in Figure 2.5.

.....40

TABLE 2.3

Components utilized for the construction of the QEB sample inlet system shown in Figure 2.8.

.....50

TABLE 3.1

Potentials applied to the acceleration lens elements as a function of precursor ion kinetic energy (collision energy).

.....85

TABLE 3.2

Typical potentials applied to acceleration lenses listed with respect to the collision cell potential.

.....86

TABLE 3.3

Voltage ratios which determined focussing properties of the QEB acceleration lens system.

.....87

TABLE 3.4

Variation of acceleration lenses with kinetic energy of product ions. 90

TABLE 3.5

Calculation of kinetic energies of m/z 86 precursor ion from 2-pentanone and its m/z 43 product ion resulting from 50 eV collision energy. 93

TABLE 4.1

Quantitation of 3-MB m/z 71 ions as a function of low energy collision energy on the QEB. 115

TABLE 4.2

Behavior of m/z 71 reference ions (A and MVK) and 3-MB m/z 71 ions under high energy CID conditions on the QEB. 119

TABLE 4.3

Determination of the enol content of the molecular ion of 3-methyl-2-butanone (3-MB) from QEB low energy CID data on m/z 86. 128

TABLE 4.4

MS/MS dissociation efficiencies and m/z 43 product ion ratios experimentally measured on the ITMS for the 3-MB m/z 71 ions and the m/z 71 reference ions A and MVK. 141

TABLE 4.5

Determination of $\% \Sigma$ (defined in text) for the keto and enol molecular ions (m/z 86) of 3-MB. 148

TABLE 4.6

Calculation of keto:enol composition at the source temperature (423 K) and standard temperature (298 K). 151

TABLE 4.7

Quantitation of 2-P m/z 71 ions as a function of low energy collision energy on the QEB.	
.....	163

TABLE 4.8

MS/MS dissociation efficiencies and m/z 43 product ion ratios experimentally measured on the ITMS for the 2-P m/z 71 ions and the m/z 71 reference ions A1 and MVK.	
.....	165

LIST OF FIGURES

Figure 1.1. General example of keto/enol tautomers showing the transfer of a proton (circled) from a carbon atom to the oxygen atom.	3
Figure 1.2. Structures of the keto/enol systems studied.	5
Figure 1.3. Fragmentation scheme for 3-methyl-2-butanone showing loss of C(1) methyl occurring from keto form to yield the acylium ion.	13
Figure 1.4. Schematic diagram of the QEB hybrid mass spectrometer utilized in this thesis.	15
Figure 1.5. Cross sectional view of the Ion Trap Mass Spectrometer (ITMS) employed in this thesis (cross sectional view taken from reference 48).	17
Figure 2.1. Schematic diagram of the QEB hybrid mass spectrometer showing the basic ion optical components located before the source slit.	31
Figure 2.2. Schematic diagram of the vacuum system employed on the QEB hybrid mass spectrometer.	32
Figure 2.3. Schematic diagram of the main mounting flange upon which ion optical components are mounted.	34
Figure 2.4. A typical potential diagram for the QEB mass spectrometer employed in these experiments	37
Figure 2.5. Cross sectional view of the EI source used for the QEB.	39
Figure 2.6. Construction of filaments made for the EI source on the QEB mass spectrometer.	43
Figure 2.7. Cross sectional view of the assembled lens stack used on the QEB.	45
Figure 2.8. Schematic diagram of the inlet system used on the QEB.	49
Figure 2.9. Schematic diagram of the acceleration lens elements utilized for the QEB.	58
Figure 2.10. Plots of ion current obtained at the monitor plate located after the ESA.	59

- Figure 2.11.** Schematic diagrams (not to scale) of the monitor plate mounted after the ESA on the QEB. 60
- Figure 2.12.** Schematic diagram indicating behavior of ion beam (ion beam intensity vs. accelerating voltage). 63
- Figure 2.13.** Setting of acceleration lens elements for low energy CID experiments performed on the QEB. 67
- Figure 2.14.** Diagram of needle collision cell used on the QEB for high energy MS/MS experiments. 69
- Figure 2.15.** Schematic diagram indicating the method of determination of isobaric species at m/z 43 is performed on the ITMS. 73
- Figure 3.1.** Schematic diagram of the QEB EI source showing spacing of the various lenses and typical voltages applied to these lenses during operation of the instrument. 77
- Figure 3.2.** Initial version of the lens system utilized on the QEB between the quadrupole mass analyzer and the source slit. 78
- Figure 3.3.** Diagram of lens system (not to scale) utilized between the quadrupole mass filter and the low energy collision cell on the QEB. 81
- Figure 3.4.** Schematic diagram of modified acceleration lens region utilized on the QEB. 83
- Figure 3.5.** MS/MS spectrum of m/z 86 from 2-pentanone obtained on the QEB at 50 eV (50% attenuation of the precursor ion beam with argon collision gas, sample pressure = 1×10^{-6} torr). 94
- Figure 4.1.** Fragmentation scheme for 3-methyl-2-butanone (3-MB), previously shown in Figure 1.3, showing formation of acylium ion (A) at m/z 71 from loss of the C(1) methyl and formation of protonated methyl vinyl ketone (MVK) at m/z 71 by loss of the C(4) methyl. 97
- Figure 4.2.** Behavior of the 3-MB m/z 71 ions under low energy CID conditions on the QEB. 99
- Figure 4.3.** Fragmentation of MVK and A to yield 43L and 43H, respectively. 101
- Figure 4.4.** Generation of the A and MVK reference ions. 102

- Figure 4.5.** Typical peak profiles obtained for MS/MS of m/z 71 reference ions and 3-MB m/z 71 ions yielding m/z 43 product ion(s) under low energy MS/MS conditions on the QEB. 104
- Figure 4.6.** 43L:43H product ion ratio generated by CID of the MVK m/z 71 reference ion over the collision energy range of 5-50 eV on the QEB. 105
- Figure 4.7.** Acylium reference ion (A) MS/MS dissociation probability ($\epsilon_{A, 43H}$) measured on the QEB under low energy CID conditions. 113
- Figure 4.8.** Protonated methyl vinyl ketone (MVK) reference ion MS/MS dissociation probability ($\epsilon_{MVK, 43H + 43L}$) measured on the QEB under low energy CID conditions. 114
- Figure 4.9.** Behavior of m/z 71 ions for the 3-MB system under high energy CID conditions on the QEB. 118
- Figure 4.10.** A typical MS/MS spectrum obtained for m/z 86 from 3-methyl-2-butanone (3-MB) on the QEB under low energy CID conditions. 122
- Figure 4.11.** Ratio of total m/z 71 ion signal intensity : 43H signal intensity obtained from low energy CID of m/z 86 from 3-MB on the QEB. 123
- Figure 4.12.** Schematic diagram indicating how MS/MS/MS is performed on the QEB. 125
- Figure 4.13.** Characterization of m/z 71 ions produced from low energy CID (5-50 eV) of m/z 86 from 3-MB by MS/MS/MS on the QEB. 126
- Figure 4.14.** 70 eV mass spectrum of 3-methyl-2-butanone (3-MB) obtained on the QEB. 134
- Figure 4.15.** Demonstration of deconvolution of m/z 71 and m/z 86 data to experimentally determine the expected (calculated) enol ion mass spectrum. 135
- Figure 4.16.** Comparison of raw data (i.e., uncorrected for reference ion behavior) obtained from QEB low energy CID and ITMS MS/MS of the 3-MB m/z 71 ions. 137
- Figure 4.17.** Comparison of MS/MS behavior of the MVK reference ion on the QEB and the ITMS. 139

- Figure 4.18.** Comparison of MS/MS behavior of the acylium (A) reference ion on the QEB and the ITMS. 140
- Figure 4.19.** EI mass spectrum of 3-MB obtained on the ITMS (sample pressure = 1×10^{-6} torr). 143
- Figure 4.20.** Calculation of entropy differences for structurally related compounds. 153
- Figure 4.21.** The two neutral enol structures possible for 3-methyl-2-butanone (3-MB). 155
- Figure 4.22.** The possible fates of enol ion 2. 157
- Figure 4.23.** Fragmentation scheme of 2-pentanone (2-P) showing formation of the acylium ion, A1 (by loss of the C1 methyl), from the keto form and MVK from the enol form (by loss of the C5 methyl) of the molecular ion. 160
- Figure 4.24.** Comparison of MS/MS spectra of m/z 86 from 2-pentanone (2-P) obtained on the QEB (top spectrum) and the ITMS (bottom spectrum). 166

CHAPTER 1

INTRODUCTION

General Information Regarding Mass Spectrometry

Mass spectrometry is an important analytical technique because of its high sensitivity and the wealth of information obtainable from a mass spectrum. A mass spectrum consists of a plot of relative abundance of the ions produced by ionization of the original precursor molecule, vs. the mass-to-charge ratio (m/z) of those ions. The initially formed molecular ion is the ionic analogue of the original neutral precursor in that the mass of this ion is the same as that of the original neutral. Fragment ions arising from the molecular ion can enable the mass spectrometrists to elucidate the structure of the original neutral precursor. Mass spectrometry, therefore, can provide molecular weight and structural information.

Traditional ionization techniques such as electron ionization (EI) and chemical ionization (CI) see heavy use in mass spectrometry. The EI technique produces a radical cation from the original neutral precursor by bombarding the sample with energetic electrons, typically of 70-100 eV kinetic energy (produced from a heated filament) for standard operating conditions. Chemical ionization typically produces a protonated molecular ion from the neutral precursor by means of an ion-molecule reaction with a reagent ion. This reagent ion is produced by EI to create species which can serve as proton donors for the subsequent analyte. Since EI and CI require that the neutral

precursor be present in the gas-phase for the ionization process to occur, these techniques are most suitable for those compounds with sufficient volatility to be introduced into the mass spectrometer via a chromatographic inlet or direct insertion probe with little or no thermal degradation.

Many volatile compounds have been the subject of mass spectrometric studies due to their importance in environmental and biological applications. Such applications have generated the need for a fundamental understanding of gas-phase ionic reactions. Organic ketones are one example of a compound class subjected to mass spectrometric studies because of the need to analyze them at trace levels in environmental and biological samples; furthermore, a unique structural characteristic of these compounds has also generated interest in fundamental studies of the mass spectrometric behavior of these compounds.

General Information on the Keto/Enol Chemical System

This unique property is the possibility of an intramolecular proton transfer known as tautomerism or prototropic exchange. Due to the electron withdrawing ability of the carbonyl group, the hydrogens on the α -carbons are slightly acidic. Consequently, these protons can migrate to and bond with the oxygen atom to form the isomeric enol structure, as shown in Figure 1.1. Mass spectrometry may be useful in characterizing the two structures present since they are chemically distinct species which could show different fragmentation patterns provided that they do not interconvert rapidly on the μ sec

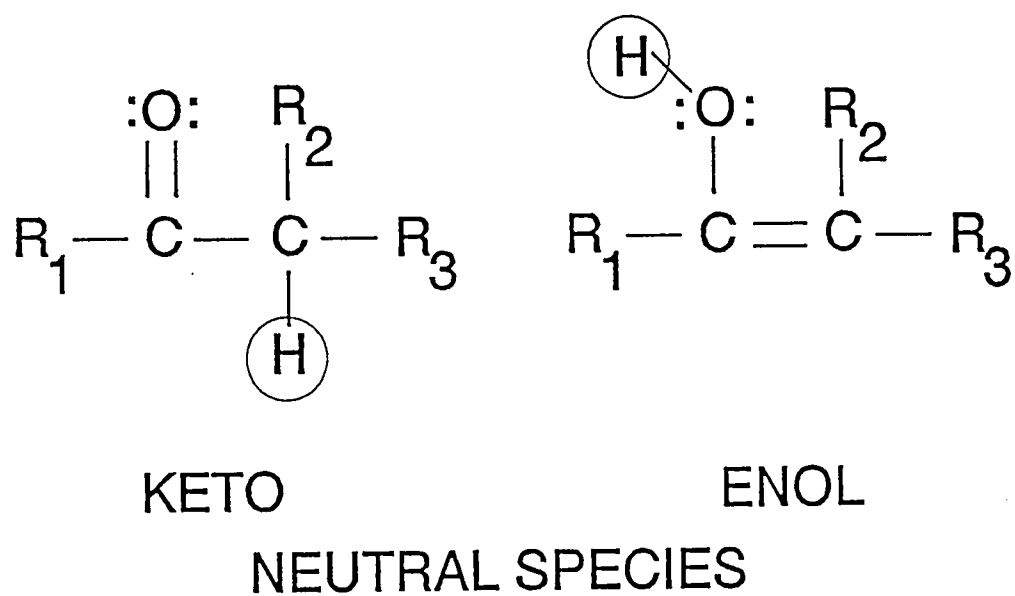


Figure 1.1. General example of keto/enol tautomers showing the transfer of a proton (circled) from a carbon atom to the oxygen atom. Alkyl, phenyl and other possible hydrogen atoms are denoted by the various R groups.

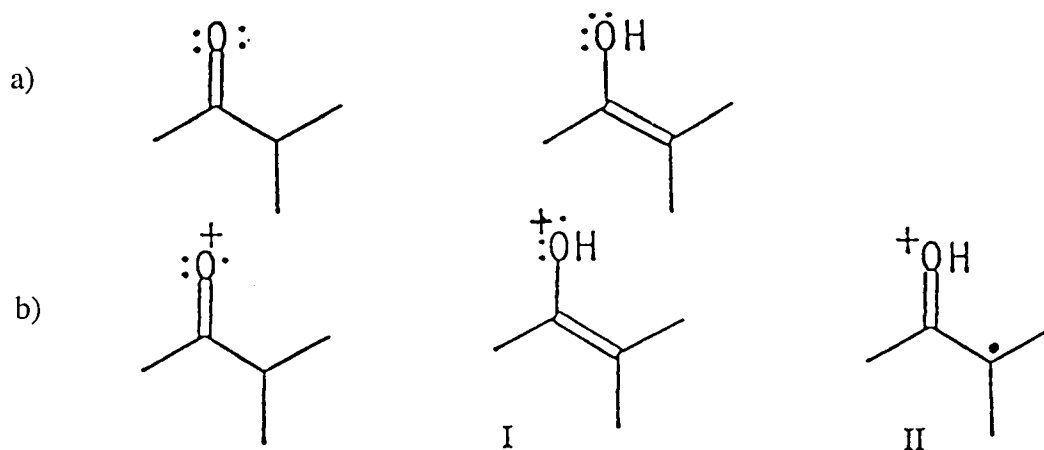
to msec time-scale of the mass spectrometric experiment.

Since mass spectrometry involves ions generated from the original neutral precursor, it is of interest to examine the thermodynamic properties of the neutral keto and enol forms to determine what proportion of each form should be present at the instant of ionization. The neutral enol form is less stable than the neutral keto form as determined by solution phase measurements (1-4), gas phase measurements (2,4), and theoretical computational chemistry (5,6). Consequently, the neutral keto form predominates at equilibrium to an extent greater than 99%. The neutral keto form being more stable is plausible due to the greater strength of the C=O bond as compared to the C=C bond; this is expected since a stronger bond generally forms between atoms of different electronegativity.

Following the ionization of a neutral ketone within the mass spectrometer under EI conditions, it is expected that the molecular ion should initially reflect the structure of the keto form since it is present to an extent of > 99%. Once the ions are formed, the possibility of keto/enol isomerization must also be considered for the ionic system. It has been shown that the enol isomer of a molecular ion is **more stable** than the keto isomer of the molecular ion (7). This is plausible since the enol structure has more resonance forms than the keto form of the molecular ion as shown in Figure 1.2.

Because of the greater stability of the enol molecular ion, the originally formed keto molecular may rearrange to the enol structure. This tautomerism question has intrigued mass spectrometrists for many years; in fact, these keto-enol systems have been the subject of many previous mass spectrometric studies (7-39).

3-ME-2-BUTANONE



2-PENTANONE

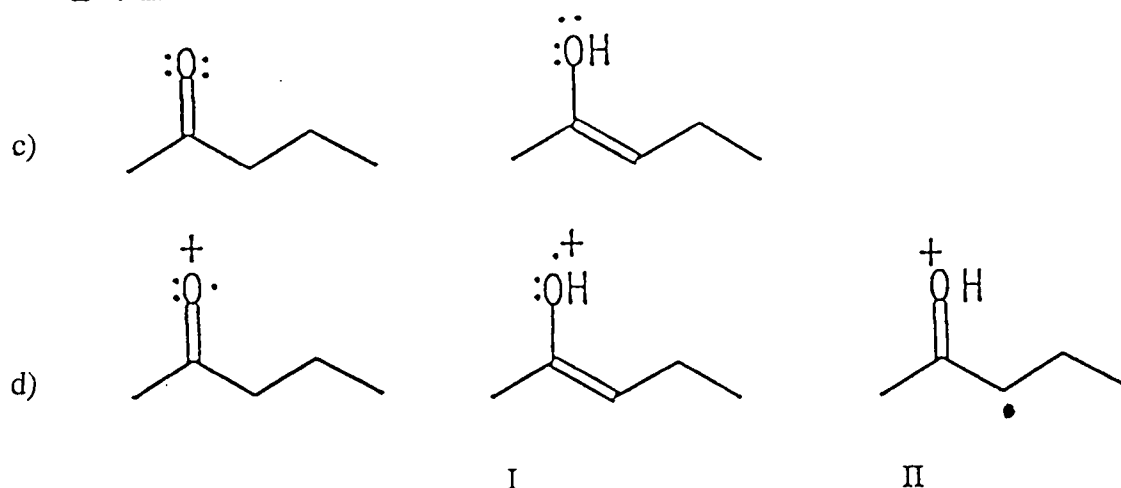


Figure 1.2. Structures of the keto/enol systems studied.

a) shows the structures for the neutral keto form and the thermodynamically most stable neutral enol form for 3-methyl-2-butanone.

b) shows the structures for the keto and enol radical cations of 3-methyl-2-butanone. I and II are resonance structures of the enol ion.

c) shows the structures of the neutral keto and the thermodynamically most stable neutral enol form of 2-pentanone.

d) shows the structures for the keto and enol radical cations of 2-pentanone, where I and II are the resonance forms of the enol ion.

Summary of Previous Mass Spectrometric Studies on Keto/Enol Systems

The goal of these previous mass spectrometric studies has been to determine a variety of information regarding the ionic keto and enol forms (7-39). Such information has included thermodynamic information (heats of formation, appearance potentials, etc.) for the neutral and ionic species and structural characterization of the various fragment ions arising from the keto and enol forms. This has been attempted by performing separate experiments in which the appropriate ionic form is formed in the mass spectrometer ion source. The keto molecular ion is formed from the neutral keto form, while the enol ion is generally formed by a McLafferty rearrangement of a suitable ionized precursor (7,15,17,23,26,31).

Studies performed in this manner (7,15,17,23,26) show that the fragmentation patterns of the two forms are very similar in many cases, leading to the conclusion that the ionic keto and enol forms interconvert extensively. However, significant questions remain regarding such experiments. First of all, what effect does the internal energy of the initially formed keto molecular ion have upon the results? Are the fragmentation patterns seen for the ionic keto form a function of the internal energy of the keto molecular ion, with isomerization to the enolic form being negligible? Alternatively, are the fragmentation patterns seen a result of partial isomerization of the keto form to the enol form with dissociation coming from a mixture of the two forms? The same questions can be raised for the enol molecular ion since formation of this ion via fragmentation from another ionic precursor does not allow for very precise control over

the internal energy of the resulting enol molecular ion.

If the keto and enol forms of the molecular ion do interconvert extensively, the mass spectrum resulting from introduction of either pure neutral into the mass spectrometer ion source should be the same. With the neutral enol form being the less stable form, it is difficult, yet possible, to generate the neutral enol form in-situ within the vacuum system via appropriate chemical reactions. Turecek has been most successful at this procedure and has demonstrated that the mass spectra obtained for the enol forms under standard EI conditions (electron energy = 70-80 eV) are significantly different than those of the isomeric keto form (18-21, 32). Turecek's procedure involves a retro Diels-Alder reaction in which the neutral enol is generated by heating an appropriate precursor to high temperatures (500 - 600 ° C) sufficient for a retro Diels-Alder reaction to occur in which the neutral enol is generated.

Turecek's results suggest that the keto and enol forms may be distinguishable on the mass spectrometric time-scale since distinct mass spectra are generated from the two neutral forms. If this is the case, the question of why previous results indicated interconversion to the extent that the ionic keto and enol forms exhibit, in many cases, nearly identical fragmentation patterns must be examined much more closely. An important factor to consider is the type of mass spectrometric experiment performed which lead to the results indicating extensive interconversion.

Those experiments involved analysis of metastable ions (7,15,17,23,26). These metastable ions are of an internal energy such that they neither fragment in the ion source nor survive to be detected as normal ions; they possess just enough internal energy to

fragment in a relatively well-defined time frame (40,41). These constraints indicate that metastable ions are of a relatively narrow internal energy distribution. Consequently, metastable ions undergo reactions, either fragmentations or rearrangements, by the lowest energy pathways available to them. Turecek obtained distinct mass spectra for enol forms since he observed *stable ions* which survived long enough after their initial formation in the source to be detected in the normal mass spectrum. These stable ions are of an internal energy different than the metastable ions which indicate that the keto and enol forms interconvert extensively. The reactions which an ion can undergo are internal energy dependent and the factors affecting such reactions are outlined below.

Factors Influencing Reactions Of Ionic Species

A mass spectrometric experiment is actually a kinetics experiment in which a series of competitive and consecutive reactions yields the ions detected. To fully interpret spectra, it is important that the dependence of the rate constant on energy be known and understood. The generally accepted theory relating the rate of fragmentation to internal energy is the Rice-Ramsperger-Kassel-Marcus (RRKM) theory which states that the rate constant for a particular reaction will be a function of the energy state population of its activated complex relative to the population of all other energy states of the decomposing ion. Furthermore, it is assumed that after the initial ionization event, the ion will redistribute its internal energy among all its various degrees of freedom (including electronic, vibrational, and rotational modes) on a time-scale faster than dissociation

processes (40-42). The minimum internal energy that an ion must possess to undergo a particular reaction is denoted as E_0 , the critical energy or threshold energy (40-43). After attaining this critical energy, the rate constant will increase with increasing internal energy until it reaches a limiting value determined by the magnitude of the frequency factor of the reaction in question. A reaction may also show a kinetic shift; that is, internal energy in excess of the critical energy may be needed to make a reaction occur on the time-scale of the mass spectrometric experiment.

The model described above for dependence of the rate constant upon internal energy dictates at what values of internal energy the rearrangement reactions open to an ion can compete effectively with direct cleavage reactions. For a direct cleavage reaction, the bond is stretched in the transition state which allows for more freedom of rotation about the bond and also decreases the vibrational frequency of the bond. Increased freedom of rotation about a bond increases the density of rotational states, resulting in a rapid rise of reaction rate with increasing internal energy. For a rearrangement process, the nature of the transition state demands that atoms be held in certain positions in the transition state meaning that rotation about bonds must then be restricted for the activated complex to form (41). This results in the loss of rotational degrees of freedom. Since rotational energy levels are more closely spaced than vibrational energy levels, the density of rotational states is greater than the density of vibrational states. The resulting effect on the rate constant for the rearrangement reaction is that the rate will increase more slowly with increases in internal energy than will that of a direct cleavage reaction. These generalities are in agreement with the predictions of theories relating rate

coefficients to internal energy (42). These generalities can explain why a rearrangement process is more competitive with direct cleavage processes at lower values of internal energies; at lower internal energies, the rates of reaction are comparable. As the rate of the direct cleavage reaction rises more rapidly with increasing internal energy, the rearrangement process cannot compete effectively at higher internal energies. Of course, the reaction (whether it be a rearrangement or direct cleavage reaction) with the lower critical energy will dominate at lower internal energies.

Not only is the rate of reaction as a function of internal energy important, but the internal energy distribution imparted to an ion in the ionization step is also important. That internal energy distribution, convoluted with the internal energy-dependent rate constants, determines the reactions open to the ion. The distribution of internal energy deposited into an ion during electron impact ionization is quite broad and extends to very high internal energies. By reducing the electron energy, the average internal energy can be lowered and the distribution of internal energies in the molecular ion is narrowed (43). This is a convenient method for producing radical cations of lower internal energy by EI.

Due to the internal energy dependence of ionic reactions, the ionic keto/enol isomerization should be competitive with the cleavage reactions of the individual forms at lower internal energies. Metastable ion studies are consistent with this fact. Turecek's studies show that the cleavage reactions of the individual keto or enol form predominate at the higher internal energies utilized for acquiring standard EI spectra. The neutral keto forms are commercially available and thus easily obtained. Ionization of this form at standard electron energies (70 eV) imparts large internal energies to the initially formed

keto molecular ion. At such high internal energies, the ionic keto form and any ionic enol form resulting either from direct ionization of neutral enol or from rearrangement of the ionic keto form should undergo their respective cleavage reactions within the ion source. From the above discussion, the rearrangement process may not compete at these higher internal energies. Nevertheless, the ions corresponding to the two ionic forms should both be present in the ion source and can be characterized to yield quantitative information about the amount of keto and enol forms present.

Utilization of Tandem Mass Spectrometry for Study of Keto/Enol Tautomers

Characterization of the relevant ions produced in the source from the keto and enol forms can be achieved if the initially formed ions are mass selected, activated to yield fragment ions characteristic of the mass selected ion, followed by mass analysis of the fragment ions. This experiment is known as tandem mass spectrometry (MS/MS). The fragmentation pattern of the selected ion can then be determined. The determination of fragmentation patterns for the relevant ions in the keto/enol system as determined by MS/MS could be extremely useful.

The utilization of MS/MS for the study of the tautomeric keto-enol ionic species is best understood by outlining the approach for the two compounds studied for this thesis, 3-methyl-2-butanone and 2-pentanone. Their structures are shown in Figure 1.2. These compounds are interesting and intriguing because methyl groups from the opposite ends of the molecular ion are lost upon ionization of the neutral keto or enol form by EI

(7). It has been shown that the loss of the C(1) methyl results in the production of an acylium ion and the loss of the terminal methyl (C(4) for 3-methyl-2-butanone and C(5) for 2-pentanone) results in the formation of protonated methyl vinyl ketone, as outlined in Figure 1.3 for 3-methyl-2-butanone. As a result, two isomeric ions form at m/z 71 in the normal mass spectrum. The formation of the acylium ion occurs from the keto form, and formation of protonated methyl vinyl ketone occurs from the enolic form (7). Therefore, these ions can be utilized as diagnostic ions for the keto and enol forms of the molecular ion. One could use isotopically labelled compounds for this purpose so that the loss of the two methyl groups yields ions of different mass, but isotope effects on the formation of these ions may influence the results. A further consideration is that the two m/z 71 ions may undergo decomposition in the source but not to the same degree. In the normal mass spectrum, it is not usually possible to associate a fragment ion with a specific precursor so the quantitation of the keto and enol ionic forms by examination of their diagnostic m/z 71 ions from the mass spectrum may be ambiguous. MS/MS would prove to be quite useful since the diagnostic m/z 71 ions produced in the source could be isolated and their fragmentation patterns determined. Such information should allow the quantitation of the two m/z 71 ions present in the source. This information, coupled with MS/MS results on m/z 86 (the molecular ion), may allow for the quantitative determination of the proportion of the keto and enol ionic forms of the molecular ion present since fragmentation pathways can be assigned by performing MS/MS experiments (44).

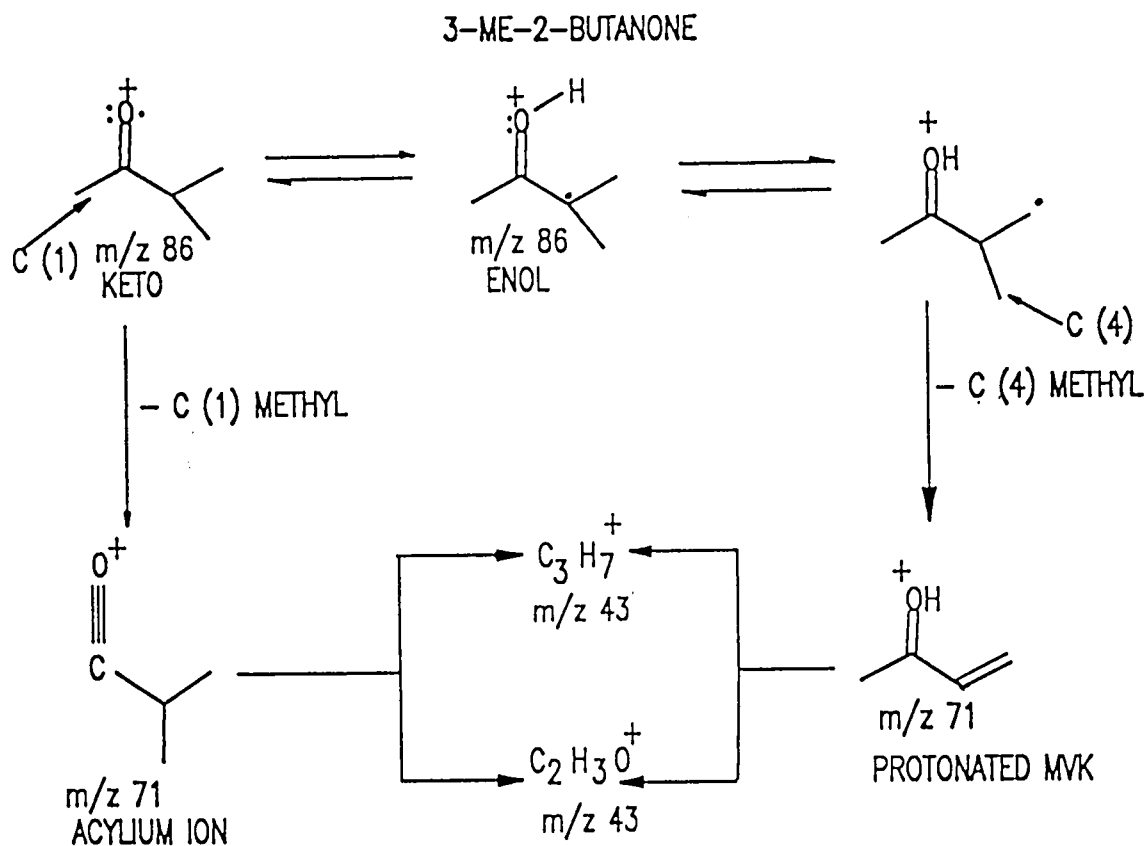


Figure 1.3. Fragmentation scheme for 3-methyl-2-butanone showing loss of C(1) methyl occurring from keto form to yield the acylium ion. Loss of C(4) methyl occurs from the enol form to form protonated methyl vinyl ketone. The two m/z 71 ions, in turn, can form $C_3H_7^+$ (43H) and/or $C_2H_3O^+$ (43L).

Characteristics of the MS/MS Experiment

The simplest way of performing an MS/MS experiment is to alter the kinetic energy of the selected ion and have it undergo collisions with a target gas in order to activate the ion. The variables involved with this type of experiment are the nature and pressure of the target gas and the collision energy (44-46). A parameter often overlooked is the time-scale of the MS/MS experiment; that is, the duration of time periods before, during, and after MS/MS activation. For a rearrangement process such as the keto-enol isomerization, it may be possible that the more stable enol ionic form may be formed if sufficient time is allowed after the initial ionization event for the isomerization to proceed. An increasingly long activation step itself may allow slower rearrangement processes to be observed which must be taken into account when the data is interpreted. Variation of the time period after MS/MS activation may allow a more thermodynamically favored product to be observed provided the time period after activation is long enough. The study of this time-scale effect is best carried out by utilizing mass spectrometers operating on widely differing time-scales.

Mass Spectrometers Utilized in These Experiments

The mass spectrometers utilized in this thesis are a custom-built QEB hybrid mass spectrometer (46) and a commercially available ion trap mass spectrometer (ITMS) (48, 49). The QEB is a beam instrument and is shown in block diagram form in Figure 1.4. Beam instruments are the traditional type of mass spectrometer in which an ion beam is

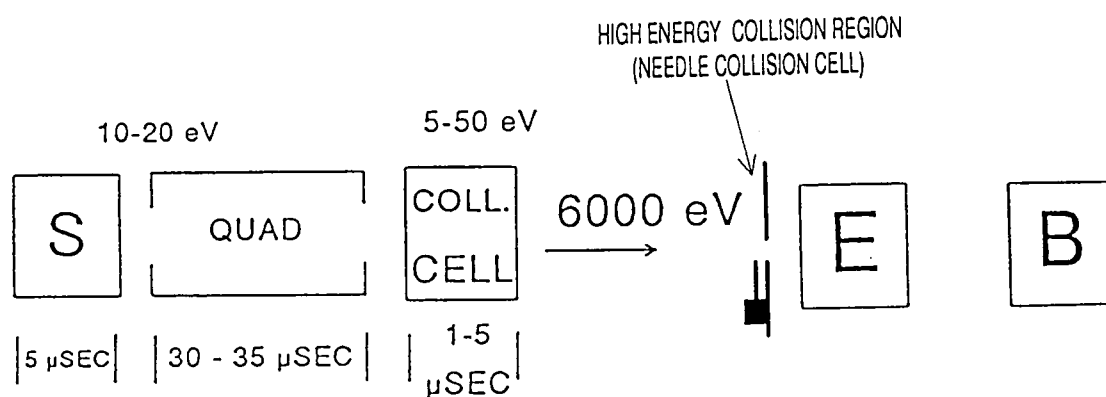


Figure 1.4. Schematic diagram of the QEB hybrid mass spectrometer utilized in this thesis. S denotes the ion source, QUAD is a quadrupole mass filter, COLL. CELL is a low energy collision cell, E is an electrostatic sector, and B is a magnetic sector. Shown below the diagram are the approximate times ions reside within each indicated portion of the instrument.

transported from the point of its formation to the detector by application of electric and/or magnetic fields. In such instruments, the ionization and mass analysis are performed in discrete portions of the instrument requiring the transmission of the ions from one portion of the instrument to another. From time of ionization to time of detection typically requires μsec , based on the kinetic energy of the ions and the physical size of the instrument. On the other hand, trapping instruments such as the ITMS utilize electric and/or magnetic fields to store ions in a defined volume in space; subsequent manipulations of the ions for MS/MS purposes are carried out within this volume. The time-frame for experiments performed on this instrument, as shown in Figure 1.5, is of millisecond duration. Beam instruments and trapping instruments clearly operate on different time-scales.

For the time-scale studies to be performed, it is essential to know the time duration of the periods before, during and after MS/MS activation. These time periods are of certain lengths depending on the type of mass spectrometry instrumentation employed. For the QEB and the ITMS, the relevant factors regarding the instrumentation are discussed below.

The QEB hybrid mass spectrometer consists of a quadrupole mass filter (Q) to allow mass selection, at unit mass resolution, of a precursor ion which will be subjected to low energy MS/MS. Following low energy MS/MS, the ions are accelerated to kinetic energies appropriate for analysis by the EB portion of the instrument. E is an electrostatic sector analyzer (ESA) and B is a magnetic sector analyzer; proper configuration of these two sectors provides what is known as a double focussing sector instrument. Use of such

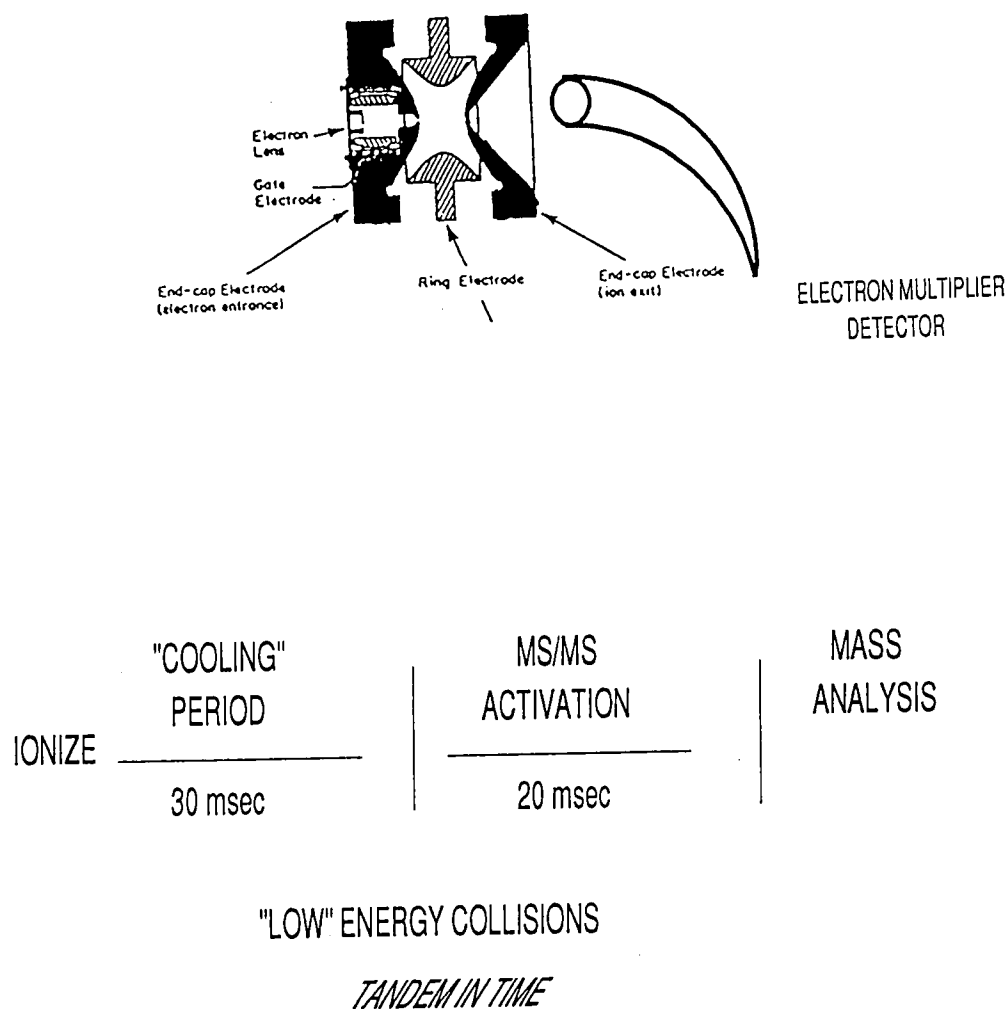


Figure 1.5. Cross sectional view of the Ion Trap Mass Spectrometer (ITMS) employed in this thesis (cross sectional view taken from reference 48). Shown below the ITMS is a timing diagram relevant to the operation of this instrument. It should be noted that a "delay period" of variable length (up to several hundred milliseconds) can be implemented after the MS/MS activation step.

a double focussing instrument provides high mass resolution on an ion beam possessing an energy spread.

The Quadrupole Mass Analyzer

Quadrupole mass filters are devices constructed from 4 parallel rods to which are applied RF (radiofrequency) and DC voltages (49-53). Ions are injected on-axis into the center of the array, and upon application of the proper voltages, it is possible to selectively pass an ion of a particular m/z value through the quadrupole mass filter. If no resolving DC is applied to the rod structure, the quadrupole acts as a high pass filter with a low mass cutoff determined by the value of the applied RF voltage. A range of masses is then passed by the quadrupole in such an RF-only mode of operation; the sensitivity of the quadrupole is also increased in the RF-only mode. The popularity of these devices has resulted from their ease of use and their adaptability to computer control.

In operation, the RF/DC ratio is maintained in a manner that yields unit resolution over the mass range of interest. The mass resolution is easily varied on a quadrupole by electronic means as opposed to sector analyzers where the resolution is determined by the appropriate slit settings. The mass resolution of the quadrupole is generally limited to unit mass resolution in contrast to the high resolving power of a double focussing mass spectrometer. A mass spectrum can be obtained from the quadrupole by scanning the RF and DC potentials to sequentially pass each m/z value over the mass range of interest.

Proper mass resolution from a quadrupole also requires attention to the kinetic energy of the ion as it passes through the quadrupole mass filter and the input conditions for the ions as they enter the mass filter (50-53). For proper mass resolution, ions must spend enough time in the RF field to experience several RF cycles. Given the typical operating frequency of 1.1-2 MHz and a length of 10-20 cm for the quadrupole, kinetic energies of the ions are required to be very low (5-40 eV). For an ion of m/z 100 possessing 10 eV kinetic energy, the time for the ion to traverse through the quadrupole is about 40 μ sec; this is a parameter to keep in mind for the time-scale studies to be conducted here.

The second mass analyzer, MS 2, for the QEB hybrid mass spectrometer is the EB portion of the instrument. The factors controlling the operation of these sectors are detailed below.

The Magnetic Sector Analyzer

Historically, the separation of ions of different mass has long been performed by passing the ions through a magnetic field (49, 54-59). If ions are subjected to a magnetic field whose direction is perpendicular to the direction of their motion, the ions will execute a circular trajectory through this field. The radius of this trajectory can be determined by equating the forces resulting from magnetic deflection and centrifugal force to yield the equation:

$$r = (mv) / (Bz) \quad \text{Eq. 1.1}$$

where r is the radius of the trajectory, m is the mass of the ion, B is the magnetic field strength, v is the velocity of the ion, and z is the number of charges on the ion. This equation clearly shows that a magnetic field separates ions based on their momentum. By adjusting the magnetic field strength appropriately, ions of different mass are allowed to pass through the magnetic field. Ions of high kinetic energy (> 1 keV) are utilized so that the relative energy spread of ions passing through the magnet is greatly reduced which effectively increases mass resolving power of the magnetic sector. Greater mass dispersion can also be achieved with the higher kinetic energy ions since they will undergo a greater trajectory radius at a given field strength resulting in greater differences in trajectories for ions very similar in mass.

A limitation to the use of a single magnetic sector is the limit in the ultimate mass resolution obtainable due to an energy spread in the ion beam (49, 55, 56). An additional component is needed which will account for the energy spread of the beam by dispersing the ion beam according to energy in conjunction with the mass dispersing properties of the magnetic sector. The component needed for the energy dispersion is known as an electrostatic analyzer (ESA) or simply an electric sector (54, 56, 58).

The Electrostatic Analyzer (ESA)

The simplest and most common type of electric sector consists of two parallel cylindrical plates held at equal and opposite potentials, $+V$ and $-V$. Under these conditions, ions of appropriate kinetic energy will travel along the central axis of the

electric sector if the electrostatic force on them is balanced by the centrifugal force acting on them according to the equation:

$$mv^2/r_e = zF \quad \text{Eq. 1.2}$$

where m is the mass of the ion, v is the velocity of the ion, r_e is the radius of the electric sector, z is the number of charges on the ion, and F is the field strength existing between the two plates. Since the kinetic energy of an ion can be expressed as $1/2 mv^2 = zV$ (where V is the accelerating voltage) equation 1.2 can be rewritten as:

$$r_e = 2V/F = 2(\text{kinetic energy})/F \quad \text{Eq. 1.3}$$

This equation clearly shows that the radius of trajectory of an ion through the electric sector depends on the kinetic energy of the ion (at a fixed value of F) and is independent of the mass of the ion. Ions with the kinetic energy zV will travel along the central radius, r_e , given a certain value of F ; ions of greater or smaller energies will describe greater or smaller radii. The radial electric field thus produces an energy dispersion in the ion beam in much the same way a prism disperses white light into its component colors. If a slit is placed behind the electric sector, it can act as an energy filter.

EB Combination For High Resolution Analyses on Ion Beams Possessing an Energy Spread

The combination of this ESA with a magnetic sector can be utilized advantageously since the energy dispersion properties of the ESA will compensate for the lack of ability of the magnetic sector to focus ions of widely varying energy by itself.

By appropriate arrangement of the ESA and the magnetic sector (B), ions of the same exact mass possessing differing kinetic energy first undergo energy dispersion in the ESA. Following this energy dispersion, these ions then enter the magnetic field at an appropriate position and angle to ensure that they are refocused at the final slit located after the magnet. In this manner, high resolution analyses can be performed on ion beams possessing an energy spread. This arrangement of the sectors is known as the Nier-Johnson geometry, one of several popular options for the coupling of sectors.

Trapping Instruments

In addition to beam instruments, trapping instruments have become increasingly popular over the last few years. Two common types of trapping instruments are the Fourier Transform mass spectrometer (FTMS) and the quadrupole ion trap mass spectrometer (ITMS) (49, 53). Since only the ITMS was used for studies in this thesis, it will be exclusively described below. In contrast to the beam instrument, the ions, once confined within the trapping device, are not transmitted through space until they are purposely ejected from the trap. The sensitivity of the trapping devices may then be expected to be higher than that of the beam instruments since trapping instruments do not suffer from the transmission losses which beam instruments do. Such an advantage has sparked a large amount of interest in the development of trapping instruments and the understanding of how ions behave in such instruments.

The ITMS (Figure 1.5) is a device which in which ions are trapped in a potential

well created by the presence of a three dimensional electric field (48, 53). In normal operation, the ions within the trap are stored by means of an RF voltage applied to the ring electrode. Furthermore, it has been discovered that the utilization of helium bath gas (usually at 1 mtorr) is helpful in greatly increasing the trapping efficiency of this device (59, 60). This presumably results from the fact that the ions undergo collisions with the helium bath gas which results in loss of excess kinetic energy with consequent damping of their motion to the center of the trap. The formation of an ion cloud at the center of the ITMS seems to be important to the proper operation of this device; it has been postulated that efficient detection of the ions occurs when this ion cloud is about 2 mm in diameter (61). By comparison, the total available trapping volume is 2 cm (60, 61).

Once the ions are trapped within the ITMS, the mass spectrum can be obtained by ramping the RF voltage applied to the ring electrode in a linear fashion. As the RF voltage is steadily increased, the amplitude of the RF causes the trajectory of an ion with a particular m/z value to become unstable resulting in the ejection of that m/z value from the trap. This ejection occurs in the axial (z) direction and the ions therefore can be detected if an electron multiplier is placed behind an endcap. The efficiency of this ejection is increased if an auxiliary AC signal is applied across the two endcaps during this RF ramp; this procedure is known as axial modulation (61). This results in increased sensitivity and better mass resolution since the ions can be ejected as a packet at the appropriate time during the mass scan.

The initial storage of ions in the ITMS occurs for a time period of tens of milliseconds; lower values for this initial time period cause a great reduction in the

observed ion intensity (61). During this initial storage period, the ions undergo many collisions with the helium bath gas which effectively damps their motion so that the ions are more efficiently trapped within the center of the field. Further manipulation of the ions for MS/MS purposes can be performed at this point. The millisecond time-frame of the ion trap is drastically different from the μ sec time-frame of the beam instrument.

Collisions with the helium buffer gas can also have an effect on the internal energy of the trapped ions since they lose some of their initial internal energy in these collisions. The ions residing in the trap will then have a different internal energy content than those encountered in a beam instrument since the beam instrument operates under conditions to limit the number of collisions within the vacuum system to prevent ion losses due to scattering. In fact, the "cooling" process for loss of internal energy under the beam instrument collision-free conditions can require several milliseconds (61).

Recall that the ITMS operates with a pressure of 1 millitorr of helium as buffer gas. If the precursor ion can be kinetically excited in some way, the resulting increase in kinetic energy can be converted to internal energy upon collision with the helium buffer gas to induce fragmentation. This is the basis for the MS/MS experiment on the ITMS. Ions stored in the ion trap can be excited by placing a supplementary AC voltage across the endcaps of the device at a frequency corresponding to the fundamental frequency of ion motion, resulting in an increase in kinetic energy of the excited ion (48, 49, 61-65). This voltage is called the tickle voltage; though the tickle voltage can be varied in a systematic manner, the actual kinetic energy attained by this excitation process is not truly known. The MS/MS experiment on the ITMS lasts for several milliseconds

(10-20 msec) since the ion, at thermal kinetic energy after the cooling period, undergoes many collisions with the helium bath gas as the tickle voltage is applied. The ion simply cannot accelerate greatly between each collision in the ITMS during the activation process (61); the rate of increase of internal energy of an ion during the activation step on the ITMS is slow. This phenomenon therefore requires that the activation period lasts for several milliseconds to ensure adequate internal energy deposition into the ion for efficient fragmentation. Furthermore, because the duration of this period in the ion trap is on the order of milliseconds, the MS/MS experiment is conducted under multiple collision conditions. Consequently, the resulting MS/MS activation process may be fundamentally different than that on a beam instrument. On a beam instrument, the ions accelerate to the desired collision energy before encountering the collision gas, whereas the ions must be activated from thermal energies within the ion trap and undergo many collisions while attempting to accelerate them to higher kinetic energies. Again the different time-scales for the ITMS and the QEB experiments may influence the results obtained.

Factors Influencing the MS/MS Experiment

Since the goal of this study is to examine effects of these different time-scale factors on the MS/MS data resulting from a beam instrument and a trapping instrument, it is appropriate to examine the factors which influence the MS/MS process itself. As

stated above, MS/MS involves the activation of a selected ion. The characteristics of the internal energy dependent rate constants for fragmentation and rearrangement described above should apply. The activation step typically involves the collision of the precursor ion with a target gas (usually an inert gas such as helium or argon) to increase the internal energy of the precursor ion and thereby induce its fragmentation. The maximum amount of internal energy deposited into the precursor ion, E_{cm} (center of mass collision energy), is given by the following equation:

$$E_{\text{cm}} = [M_t/(M_t + M_p)] \text{ K.E.} \quad \text{Eq. 1.4}$$

where M_t is the mass of the target gas, M_p is the mass of the parent (precursor) ion and K.E. is the kinetic energy of the ion as it undergoes collision (also called the collision energy). The collision energy regimes are roughly classified into two categories: high energy collisions are those where the collision energy is 1 keV or greater and low energy collisions are those occurring at collision energies less than 1 keV.

The variables adjustable in the MS/MS experiment include the mass of the target gas and the kinetic energy; greater internal energy deposition is achieved with heavier target gases and/or high collision energies. The pressure of the collision gas can also be increased to create the situation known as multiple collision conditions. Under such conditions, the ion undergoes many activating collisions within the collision cell; each collision increases the internal energy of the ion. For analytical purposes, this is desirable since the yield of fragment ions is increased with multiple collision conditions. Such conditions can be a disadvantage if enough internal energy is deposited into the ion to induce isomerization before fragmentation; under such conditions, isomeric compounds

can yield virtually identical MS/MS spectra. In this situation, the collision gas pressure can be lowered sufficiently to provide single collision conditions where the ion undergoes only a single activating collision. The experimental determination of single vs. multiple collision conditions for MS/MS experiments has been described (46).

Other methods of ion activation can also be utilized to increase the internal energy of a precursor including surface-induced dissociation, electron dissociation, and photodissociation (44, 46). The important factor to consider is the shape of the $P(E)$ vs. E curve for each type of activation method; that is, the probability of depositing various internal energies into the precursor ion. The shape of this curve is important since it will determine the types of reactions which an ion can undergo. It has been demonstrated that high energy MS/MS can deposit much higher internal energies into a precursor ion than low energy MS/MS can (45, 47). This is important for cases where increased internal energy is needed to "drive" a fragmentation reaction. Such a situation occurs for highly endothermic reactions or for large ions which have a large number of degrees of freedom over which to distribute the internal energy. This high internal energy input characteristic of high energy CID can overcome kinetic shift problems to some degree. Low energy CID, on the other hand, allows more control over the internal energy input with this input being quite sensitive to changes in collision energy. If the relative abundances of product ions are plotted versus the collision energy for low energy MS/MS, the resulting "breakdown curve" resembles those obtained from photoionization data (where internal energy of the precursor ion is varied in a known manner). Low energy MS/MS conditions are also suitable for ion-molecule reactions in which an intermediate complex

forms between the reactants to yield the desired product. Such ion-molecule reactions can be quite useful for the differentiation of isomers (46). Clearly, MS/MS is versatile in its ability to yield structural information about ions.

Summary of Research Objectives

Because of the different operational characteristics of the QEB and the ITMS, the MS/MS results may differ due to the different time-scales upon which the two instruments operate. Since the keto-enol isomerization question is intriguing, the differing time-scales may provide insight into the problem of whether the enol ion can form from the keto ion on the longer time-scale of the ion trap.

For the proposed approach to the chemical problem, adequate resolution of isobaric species from low energy MS/MS experiments is desirable in order to avoid any complications due to isotope effects on the fragmentations studied (refer to Figure 1.3). The scheme outlined in Figure 1.3 demonstrates that isobaric ions are produced at m/z 43 from the diagnostic m/z 71 ions. These isobaric m/z 43 ions, having different chemical formulas and hence different exact masses, can be resolved by mass spectrometry. However, the m/z 71 ions are isomeric and therefore have identical formulas and exact masses; they are not directly resolvable by mass spectrometry. Nevertheless, if it can be shown that each m/z 71 ion shown in Figure 1.3 produces a unique ratio of m/z 43 ions under given MS/MS conditions, the ability to directly resolve the m/z 43 ions should, in turn, allow quantitation of the m/z 71 ions. This information can then be utilized in

conjunction with low energy CID results on m/z 86 to provide quantitative information regarding the keto-enol problem. Low energy MS/MS may prove advantageous for the m/z 71 and m/z 86 studies since the internal energy deposition in the low energy MS/MS experiment is quite sensitive to the collision energy, as stated above.

The QEB is an ideal hybrid instrument for such an experiment since resolution of the m/z 43 isobars is possible with this instrument from both high and low energy MS/MS experiments. The ITMS is also capable of high resolution analysis of ions produced in the MS/MS process utilizing a variety of experimental approaches (63-66). However, ITMS experiments conducted for this thesis involved an unusual approach for determination of isobaric species based on natural isotopic abundances; the approach is described further below in the experimental chapter of this thesis. The power of MS/MS utilizing the different time-scales of the two instruments along with their capability to perform high resolution MS/MS analyses should allow unique insight into the keto/enol isomerization question.

The ultimate goal is to determine the extent to which slow rearrangements may contribute to differences between beam and trap instrument MS/MS spectra. A further goal is to quantitate any time-scale effect on MS/MS spectra seen for the two classes of instruments.

CHAPTER 2

EXPERIMENTAL

Basic Description of the QEB hybrid mass spectrometer

The basic layout of this instrument is shown in Figure 2.1 (67). The instrument consists of an ion source, a quadrupole mass filter (model C50, Extrel Corp., Pittsburgh, PA), an einzel lens which focusses ions into a low energy collision cell and an acceleration lens system to provide ions of kinetic energies appropriate for analysis by the sector (EB) portion of the instrument. The sector portion of the instrument is an MS50 mass spectrometer (Kratos Analytical, Inc., Manchester, UK) (68). High energy collisional activation can be performed following acceleration. A more detailed description of the instrument and the experimental details regarding its operation are outlined below.

The Vacuum System of the QEB

The vacuum system utilized on the QEB, shown in Figure 2.2, was similar to that utilized on the initial version of this instrument (67). The main vacuum housing consisted of a 4-way cross having 6 inch Conflat flanges (MDC Vacuum Products Corp, Hayward, CA, Part No. 405006). Flange number 1 mated with the mounting flange upon which are mounted the source, quadrupole, and other ion optical components before the source slit.

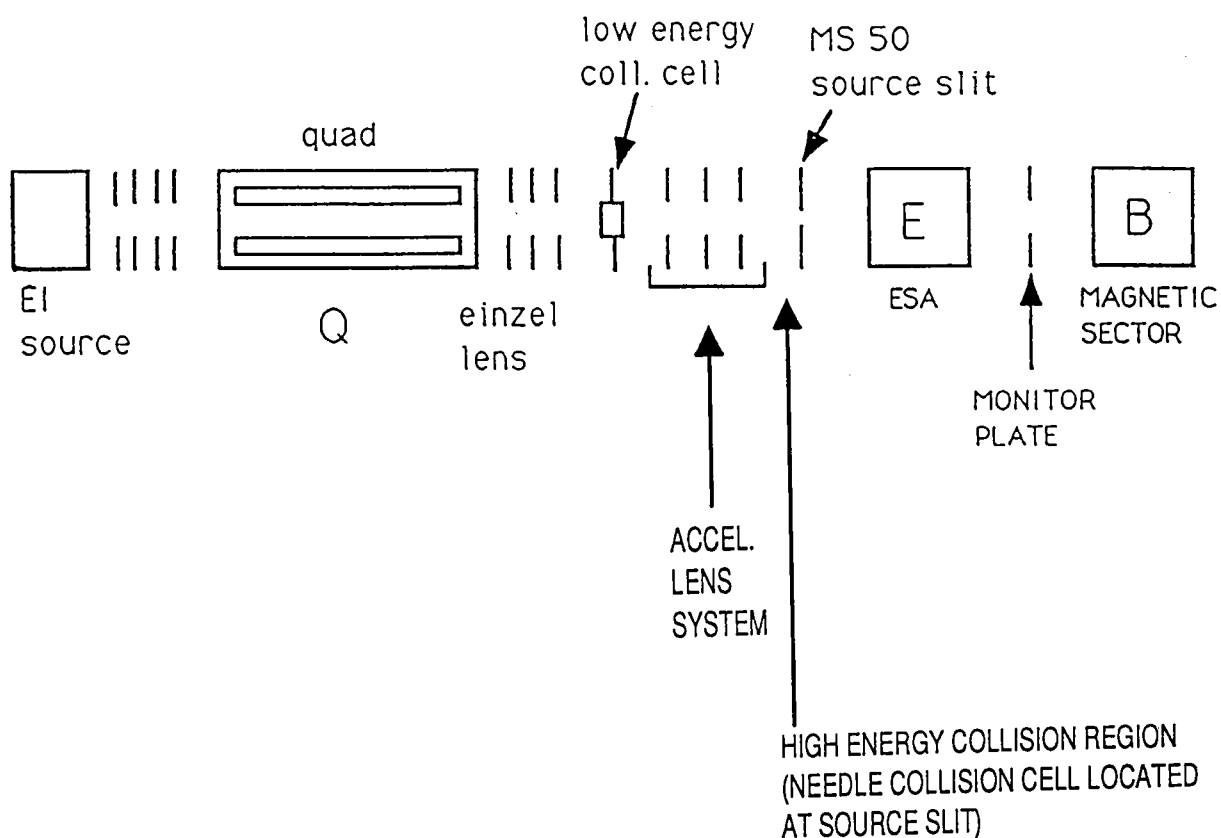


Figure 2.1. Schematic diagram of the QEB hybrid mass spectrometer showing the basic ion optical components located before the source slit. The quadrupole (Q) is an Extrel C50 unit; the sector portion (EB) is an AEI (Kratos) MS50. The high energy collision cell region is shown in greater detail in figure 2.14.

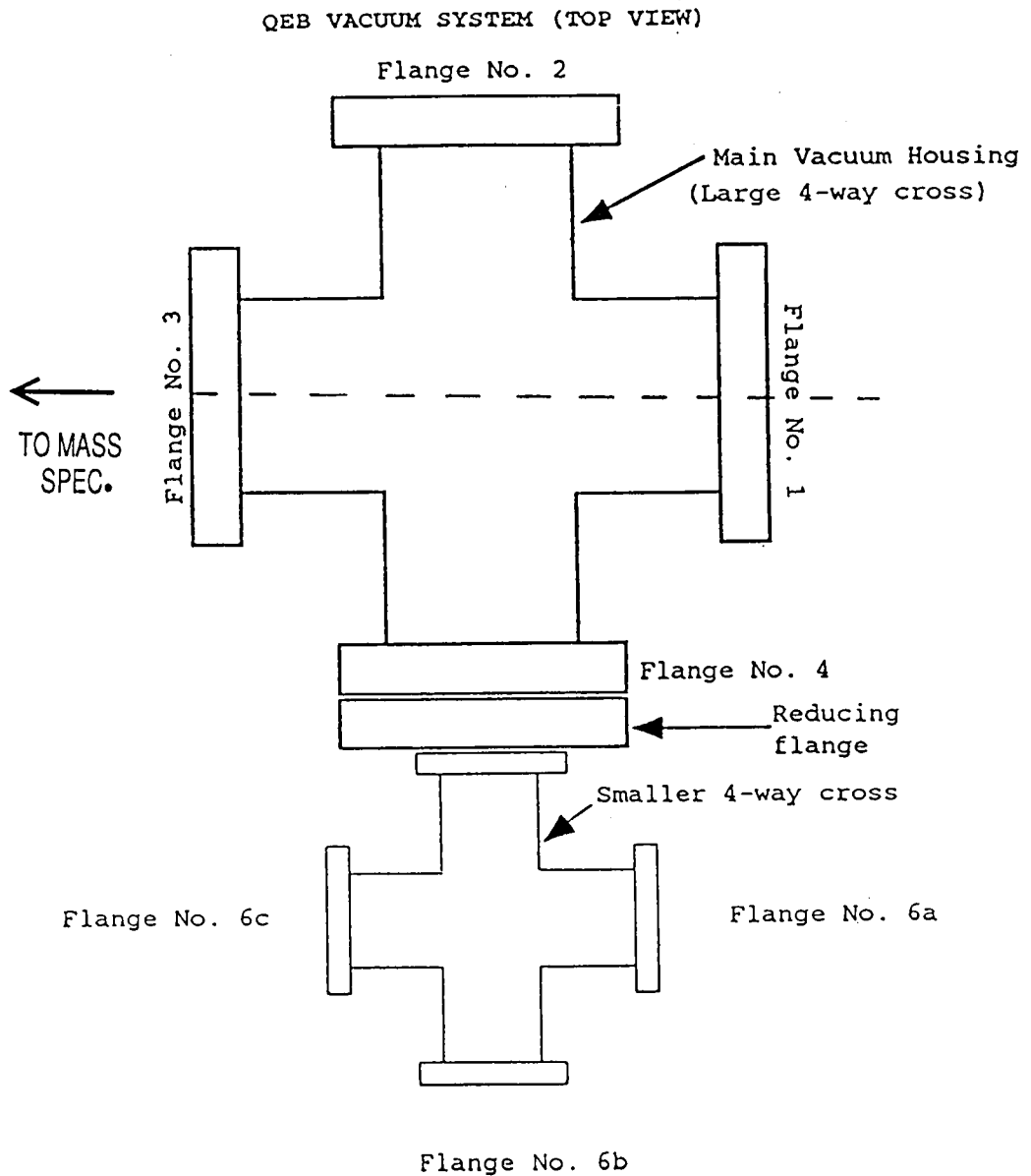


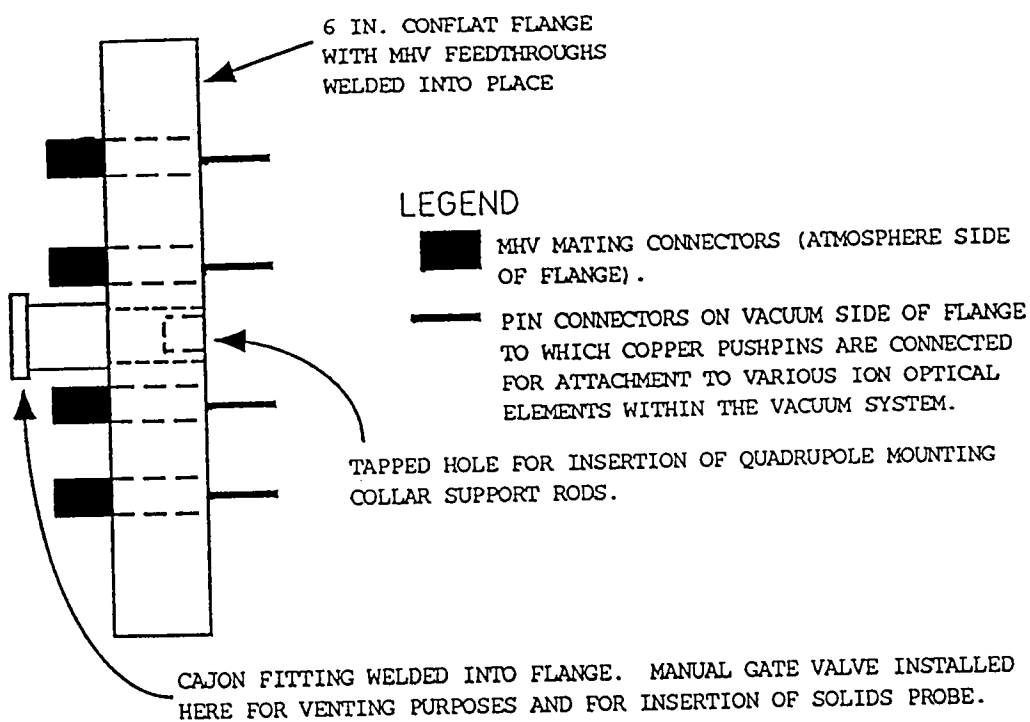
Figure 2.2. Schematic diagram of the vacuum system employed on the QEB hybrid mass spectrometer.

A high vacuum turbomolecular pump (Balzers TPU 270/330, Hudson, NY) was attached to flange number 2. Flange number 3 was utilized for the attachment of the main vacuum housing to the sector instrument. Attached to flange number 4 was an adaptor (reducing) flange which allowed attachment of a smaller 4 way cross consisting of 2.75 inch Conflat flanges.

Attached to this smaller 4 way cross were other 2.75 in. flanges containing feedthroughs for electrical leads to the acceleration lenses and quadrupole rods. Operation of this instrument required that the quadrupole RF/DC voltages be floated at the accelerating potential of the sector instrument which is typically 6-10 kV. This requirement imposed constraints on the electrical feedthroughs to avoid electrical discharge and resulting damage to the power supplies, cables, and associated electronics. Consequently, an "electrical break" (MDC Vacuum Products Corp., Hayward, CA, Part No. = 464001) was used to ensure isolation of the RF voltage feedthroughs from the grounded main vacuum housing.

The main mounting flange upon which the source, quadrupole, and other ion optical components were mounted also contained many of the feedthroughs necessary for application of voltages to these components. Figure 2.3 shows a schematic diagram of this flange. The feedthroughs shown were MHV connectors having a voltage rating of 5000 volts DC and a current rating of 3 amperes. The connections from each feedthrough to its corresponding component within the vacuum system are noted in table 2.1. Each lead within the vacuum system, except for the filament leads, was insulated with teflon tubing insulation (part no. = TS-250/18, Dixie Electronics, Oak Ridge, TN.); connections

SIDE VIEW



VIEW FROM VACUUM SIDE

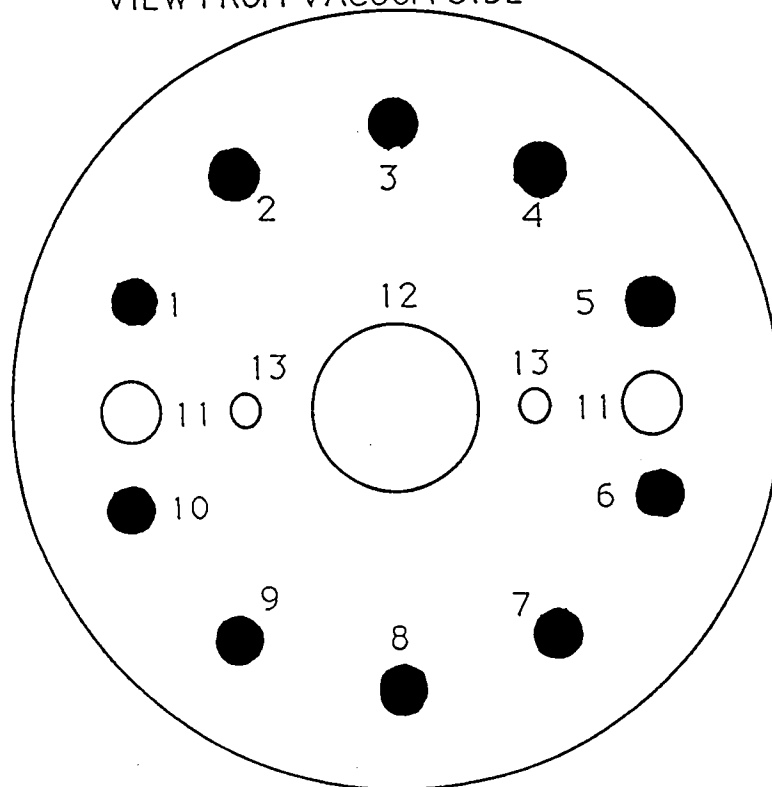


Figure 2.3. Schematic diagram of the main mounting flange upon which ion optical components are mounted. The function of each numbered component on this flange is listed in table 2.1 on the next page.

TABLE 2.1

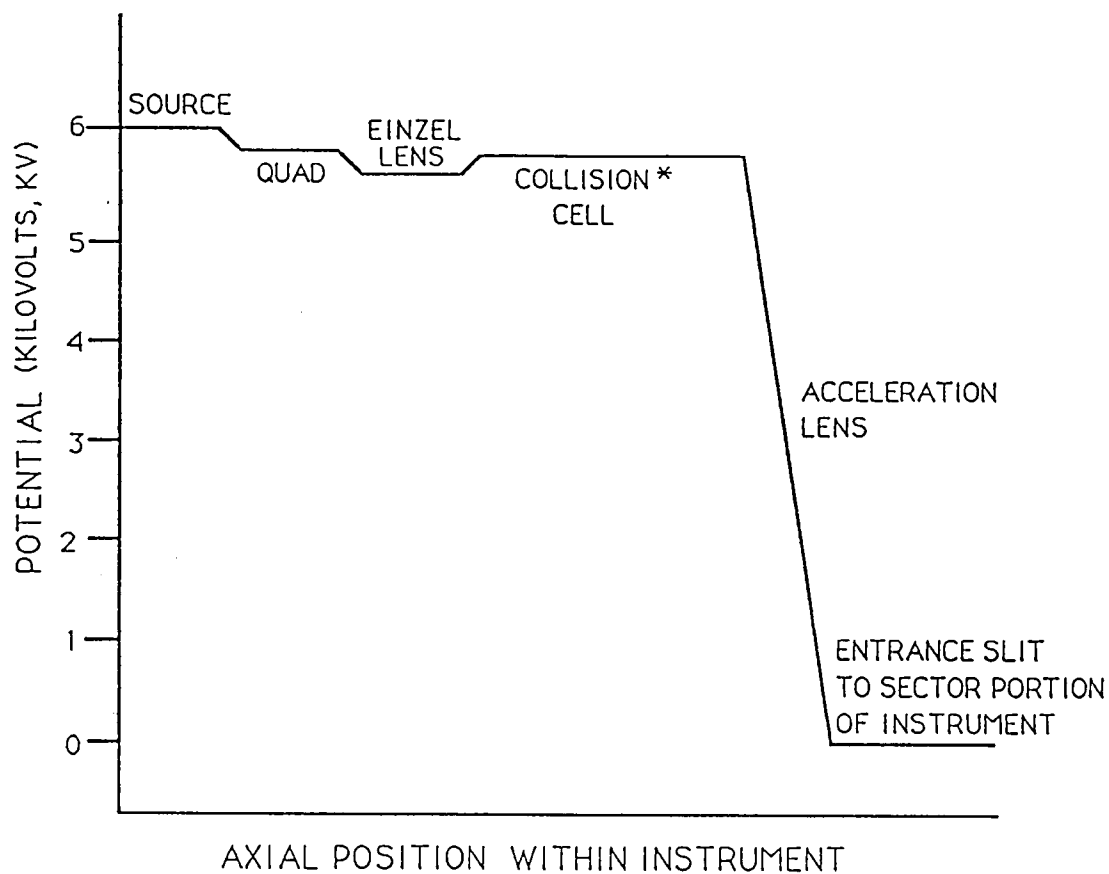
Components connected to the various numbered feedthroughs on the main mounting flange shown in Figure 2.3.

CONNECTOR NUMBER	COMPONENT
1	FILAMENT
2	FILAMENT
3	SOURCE LENS L2
4	SOURCE BLOCK
5	QUADRUPOLE ENTRANCE LENS
6	SOURCE LENS L1
7	COLLISION CELL
8	ACCELERATION LENS 1
9	SPARE CONNECTOR
10	EINZEL LENS
11	QUADRUPOLE COLLAR MOUNTING RODS
12	CAJON FITTING FOR MANUAL GATE VALVE
13	CAJON FITTINGS FOR INSERTION OF 1/8 IN. DIA. PLASTIC TUBING FOR INTRODUCTION OF SAMPLE AND COLLISION GAS

from the lead to the MHV connector were made with push-on copper electrical connectors (part no. = 9924003, Insulator Seal, Inc., Hayward, CA). Since the teflon insulation could not tolerate the higher current (2-2.5 amperes) flowing through the filament leads, portions of ceramic tubing (part no. = R19, Scientific Instrument Services, Inc., Ringoes, NJ) were placed over these leads.

Procedure for operation of source and quadrupole electronics at high voltage.

The quadrupole electronics and other power supplies were designed to be operated at 120 volts AC (RMS); however, the sector portion of the mass spectrometer required ions of 1-10 keV kinetic energy. Since the entrance slit of the sector portion of the mass spectrometer is at ground potential, the source and the quadrupole and associated components were operated in such a way that the ions attained the required kinetic energy upon entering the grounded entrance slit of the sector mass spectrometer. The potential diagram in Figure 2.4 demonstrates how this was accomplished. It is seen that the source and quadrupole were held at potentials near the accelerating potential, but that in this region of the instrument the potential differences between the various ion optical elements were low so that the kinetic energy of the ions (determined by these potential differences) remains at the values of 5-50 eV typically required for the proper operation of the quadrupole mass filter. The electronics for all the ion optical elements before the acceleration lens were "floated" at the acceleration potential. Floating is the process whereby the reference potential of electronic power supplies are altered from true ground



* POTENTIAL ON THE COLLISION CELL IS VARIABLE

Figure 2.4. A typical potential diagram for the QEB mass spectrometer employed in these experiments. Since the entrance slit to the sector portion of the mass spectrometer is held at ground potential it is necessary to operate (float) the portion of the instrument where the kinetic energy of the ions is low so that upon acceleration the ion beam remains focussed and of the proper kinetic energy for analysis by the sector portion of the instrument.

to some other known potential; in this way, a known and precise potential difference was maintained between the various "floating" elements. Attention was paid to certain details when floating the electronics (69). These details include the setup of the power supplies to be floated and the use of an isolation transformer. The isolation transformer utilized here was rated for use to 20 kV with a current rating of 5 amperes (DEL Electronics Corp., Mount Vernon, NY, model no. AD 1647).

Furthermore, any surfaces at high voltage must be insulated from ground potential so that high voltage arcing can be prevented. Typically, the power supplies to be floated were isolated from grounded surfaces by means of plexiglass sheets. In the configuration utilized for this QEB instrument, the entire instrument rack in which the Extrel power supplies were located was held at the accelerating potential. The instrument rack was then isolated from grounded surfaces by enclosing it in plexiglass sheets.

Assembly of the ion optical components.

The electron ionization (EI) source designed for these experiments is shown in Figure 2.5. The source block consisted of a parabolic ionization region with a 1.5 mm diameter hole used for the electron entrance aperture for electrons emitted from a rhenium filament. A 2.5 cm diameter hole was located at the rear of the source block to prevent high sample pressures within the ionization region and to allow for insertion of a probe by which solid samples may be analyzed. An ion exit plate with a 3 mm circular aperture was attached to the EI source with 4 0-80 thread type screws. Four source lens elements

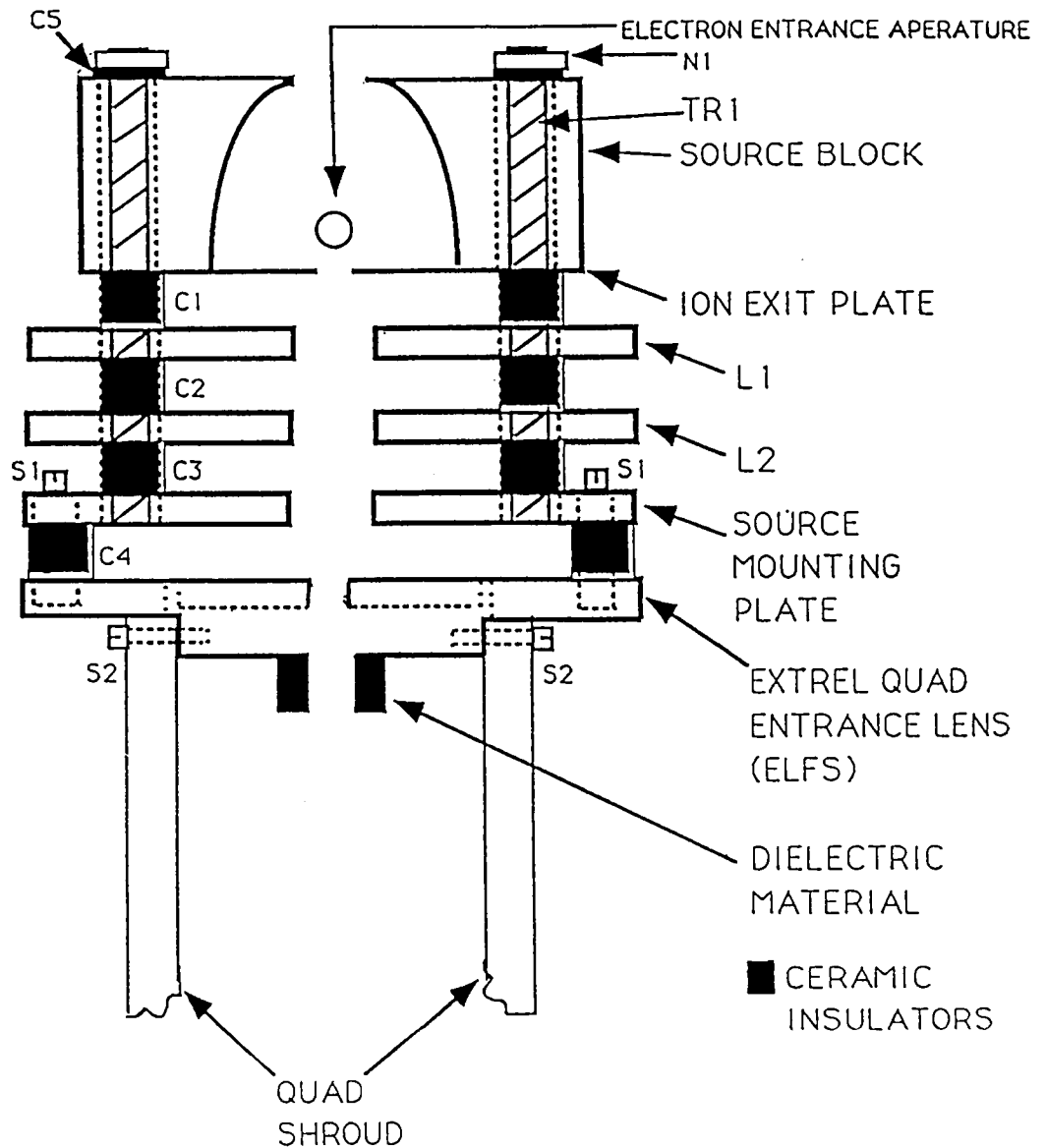


Figure 2.5. Cross sectional view of the EI source used for the QEB. Table 2.2 on the next page identifies the various components referred to in the above figure. The filament and the ceramic tubing surrounding TR1 are not shown for clarity.

TABLE 2.2

List of materials and vendors utilized for construction of the QEB EI source shown in Figure 2.5.

C1	TWO VGI-2 2 mm spacers (SIS*)
C2	ONE VGI-2 and ONE VGI-1 (1 mm spacer) for spacing of 3 mm (SIS*)
C3	SAME AS C2.
C4	ONE VGI-2 spacer (SIS*)
C5	ONE VGI-1 spacer (SIS*)
TR1	THREADED ROD (0-80 thread) for securing source lenses L1 and L2 and source block to source mounting plate (ORNL lab stock)
N1	0-80 nut; attaches to TR1 (ORNL lab stock)
L1 and L2	Source lenses with 6 mm dia. aperture (eV Parts**)
S1 and S2	Assembly screws (ORNL lab stock)

Notes:

The ion exit plate (3 mm dia. aperture and 1.5 mm thick) and the source mounting plate (3 mm dia. aperture and 3 mm thick) were retained from the original QEB instrument (see reference 60).

*SIS = Scientific Instrument Services, Inc. (Ringoos, NJ).

**eV Parts = Kimball Physics, Inc. (Wilton, NH).

(L1, L2, source mounting plate, quad entrance lens) followed the source block assembly and focussed ions into the quadrupole mass filter. The thickness of the alumina spacers was matched to 0.002 in. to ensure that the source was aligned correctly once it was assembled.

The assembly of the EI source was carried out in the following manner. The four main assembly threaded rods (TR 1) were screwed into the source mounting plate and ceramic tubing of the correct dimensions was slipped over each threaded rod. Ceramic spacers, C3, were then slid over the ceramic tubing. Lens element 2 (L2) was then placed in position. Ceramic spacers (C2) were then slid over the ceramic tubing and placed on top of L2; lens element 1 (L1), identical in geometry to L2, was then placed over these spacers. Ceramic spacers (C1) were then placed over L1. The source block, with the ion exit plate attached, was then placed over C1. The source block was then secured with assembly nuts, N1; these nuts were isolated from the source block itself by ceramic spacers, C5. The source block itself had a threaded hole in it for attachment of an electrical lead to allow a potential to be applied to the source block. The lead also served as a means of monitoring the total electron emission current from the filament, thus providing a means by which the current passed through the filament can be regulated. The source mounting plate was held at high voltage common (typically 6 kV), while the voltages applied to the other three source lenses were variable from +200 to -200 volts relative to this common.

Once the EI source was assembled and attached to the source mounting plate, it was attached to the Extrel quadrupole entrance lens using screws S1 as shown in Figure

2.5. This entrance lens was then secured to the quadrupole shroud by four allen screws, S2, (one every 90°) which mated with four threaded holes located along the edge of this quadrupole entrance lens. The central portion of this lens was electrically isolated from the outer portion of the lens by 4 ruby balls. This allowed a potential of ± 185 volts relative to high voltage common to be applied to this lens to aid in focussing of ions into the quadrupole. This procedure allowed the source to be securely mounted to and aligned with the quadrupole mass filter.

This source mounting procedure allowed for easy maintenance of the EI source; removal of the source was easily achieved by removing the four allen screws, S2. Consequently, the quadrupole shroud and the ion optics following the quadrupole assembly were not disturbed if the EI source was removed. Typically, the EI source was removed for cleaning purposes or for filament replacement. The filaments constructed for this source (Figure 2.6) were home-built in the following manner. The filament was assembled by securing the two posts between the ceramics with the assembly screws. A length of rhenium wire was drawn out and one end of it was spotwelded to one of the filament posts by utilizing a current setting of 3 or 4 on the spotwelder (model no. SW 110-B, Scientific Instrument Services, Inc., Ringoes, NJ). The entire assembly was then rotated 90° and a second weld was made in the same manner for increased strength. The procedure was then repeated with the other post. In practice, it was found that merely laying the wire across the posts and spotwelding both ends of the wire in place resulted in high currents being passed through the filament during operation (3-4 amperes) which greatly reduced filament lifetime. Using a longer portion of wire was found to decrease

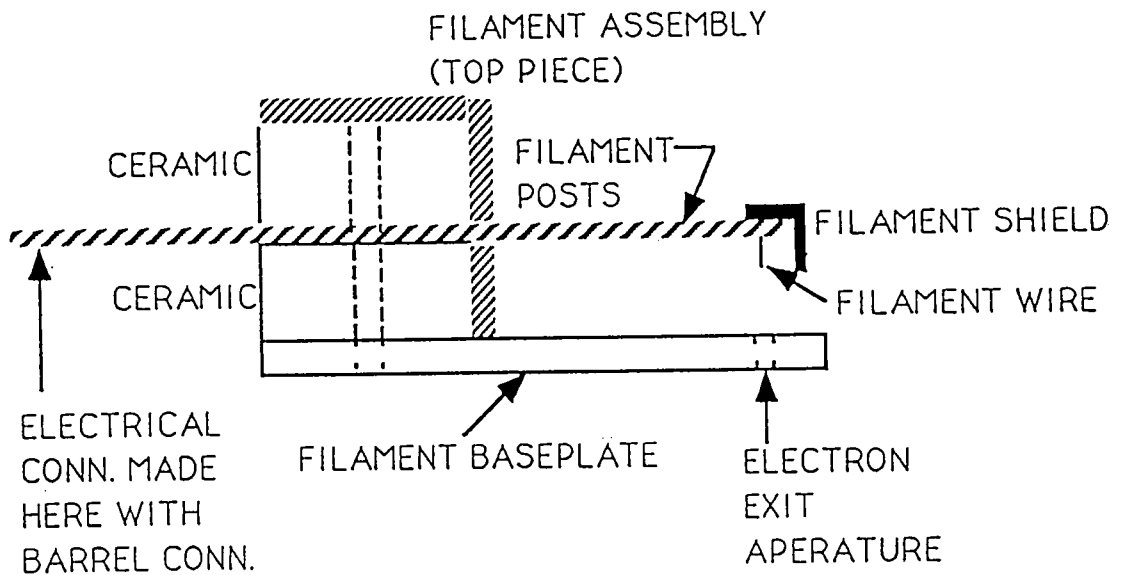


Figure 2.6. Construction of filaments made for the EI source on the QEB mass spectrometer. All components, except for the filament wire, were purchased from Kratos Analytical, Inc. (Ramsey, NJ) as part of the MS50 filament assembly package. The filament wire, part no. W6, was purchased from Scientific Instrument Services, Inc. (Ringoes, NJ).

this current by a factor of 2. Therefore, after spot welding the wire in place on one post the wire was brought toward the exit aperture plate by 2-3 mm, bent 90°, drawn straight across the length corresponding to the distance between the filament posts, followed by another 90° bend to bring the other end of the wire into a position for spotwelding to the opposite post. After making the filament in this manner, the filament shield was then spotwelded to one of the filament posts. After assembly, the filament was secured to the source block with 2 0-80 screws.

With the EI source attached to the quadrupole shroud, the shroud was then mounted inside of a machined mounting collar, as shown in Figure 2.7. The quadrupole shroud was securely fastened in place with two screws to assure correct alignment with the main flange.

The ion optical components following the quadrupole mass filter were mounted on four lexan support rods. These lexan support rods, in turn, were secured to the mounting collar described above. The lens elements were separated by one or two 1 cm thick lexan spacers, shown in Figure 2.7. To assure proper alignment of these lens elements, the thickness of each lexan spacer was checked to a tolerance of 0.002 in. After stacking the lens elements on the lexan support rods separated with the appropriate spacing, the lens elements were secured by use of 4-40 thread screws at the end of each lexan support rod. In this manner, the lens elements were held securely in place on the lexan support rod assembly. The entire ion optical assembly utilized is depicted in Figure 2.7. The low energy collision cell shown in Figure 2.7 consisted of a stainless steel cylindrical (1.5 cm diameter, 1 cm length). Removable aperture plates, located at each

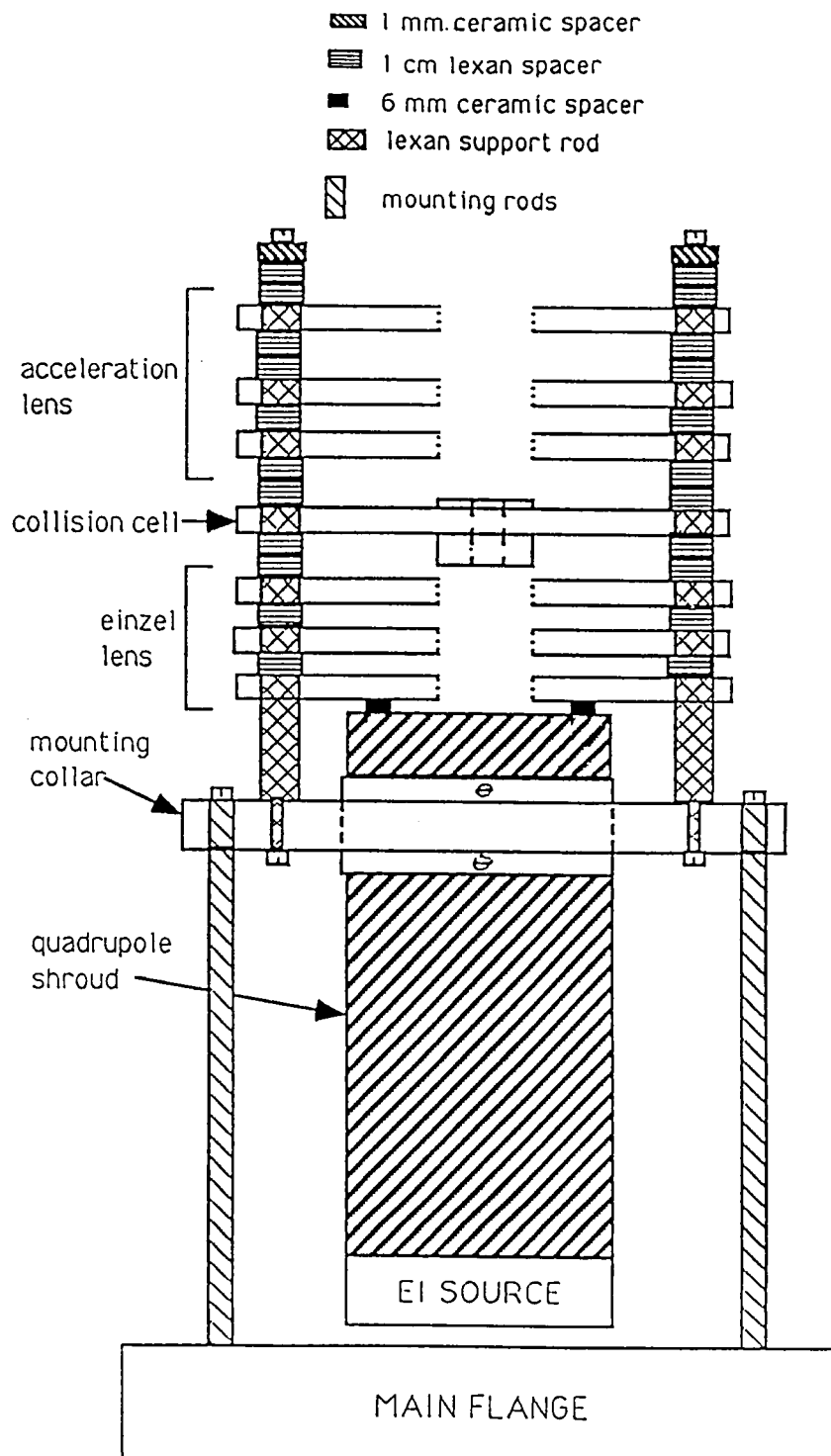


Figure 2.7. Cross sectional view of the assembled lens stack used on the QEB. The version shown is the final and most successful version of the various lens configurations tested.

end of the collision cell, allowed pressurization of the cell. These aperture plates were 1.5 cm diameter with 3 mm diameter holes. The design of the einzel and acceleration lens elements is described below in chapter three of this thesis.

Placement of the entire assembly into the main vacuum chamber

After the ion optical assembly was inserted into the main vacuum housing, the main mounting flange was secured to the vacuum housing with nylon bolts. Nylon bolts were used since the main flange was floated as described below. Floating of the main flange required the use of a lexan insulating flange to isolate the main flange from the grounded vacuum housing. The 2.75 in. feedthrough flanges containing the RF and acceleration leads were then sealed to the small 4 way cross by means of viton o-rings suitable for use with Conflat flanges (part no. = 191005, MDC Vacuum Products Corp., Hayward, CA).

Preliminary tests performed before attaining an ion beam

The preparation of the RF cables required special attention because of the floating arrangement used. It was necessary to remove the final 2 inches of the outer copper shield, held at the accelerating potential, from the spectrometer end of the cable. This prevented RF discharge between the central conductor of the cable and the outer copper shield. After achieving an initial vacuum (10^{-5} torr or lower as indicated on the source

ionization gauge), it was necessary to "tune" the quadrupole mass filter for proper resonance conditions and proper balancing of voltages between the two pairs of quadrupole rods following the manufacturer's procedure.

Another test of the system performed once the pressure was below 5×10^{-6} torr was degassing of the EI source filament. This degassing procedure involved operation of the filament at 70 eV electron energy with a total emission current of 100 μ A for 15 - 20 minutes; the source pressure would increase slightly (especially when a new filament was being degassed) for the first 5 minutes of this procedure and would then decrease steadily thereafter. This would also allow the source block to attain a temperature of 125-150 ° C during this 15 minute period before sample introduction. No separate source heater was used.

As stated earlier, the main flange was floated at the acceleration potential. In order to insulate this flange from the remainder of the grounded high vacuum system, a special lexan flange was used, as in the initial version of the instrument (67). Alternatively, high voltage feedthroughs could have been used, but they are expensive and bulky. Because all the elements before the acceleration lens are within 100-1000 volts of the accelerating voltage, floating allowed the use of cheaper MHV connectors on the main flange. Use of this type of connector allowed utilization of coaxial cables rated at 500-1000 volts between the outer shield and the central conductor. With the main flange floated at the accelerating potential, the potential difference between the central conductor and the outer shield of these cables was then maintained at values within the insulation rating of the cables. It was the use of these types of cables and connectors which

required floating the main flange at the accelerating voltage.

Before making any electrical connections to this main flange, the insulation properties of the lexan flange were tested with a home-built high voltage insulation tester. Insulation was tested between the floated flange and the grounded vacuum housing; insulation was excellent to at least 10 kV as evidenced by leakage currents of less than 1 μ A at 10 kV. The insulation between the grounded vacuum housing and other feedthroughs for introduction of the quadrupole RF/DC and acceleration lens voltages was also excellent up to 10 kV.

Sample introduction system

The sample inlet system is depicted schematically in Figure 2.8 where the various connections to the roughing pump, the high vacuum system and associated control components are shown. The connections were made with Cajon fittings (Oak Ridge National Laboratory stock items, Oak Ridge, TN) which are fingertight fittings utilizing viton o-rings as the vacuum seals. Liquid samples were placed in small glass test tubes of an appropriate diameter such that they fit snugly into the appropriate Cajon fitting. These liquid samples were degassed by repeated freeze-pump-thaw cycles until the rapid bubbling of the sample under vacuum conditions ceased. All the tubing utilized here was of polyethylene or teflon construction. No problems with memory effects were encountered due to adsorption of organic material onto the surface of the tubing. In order to prevent condensation of liquid sample within the leak valves, they were gently heated

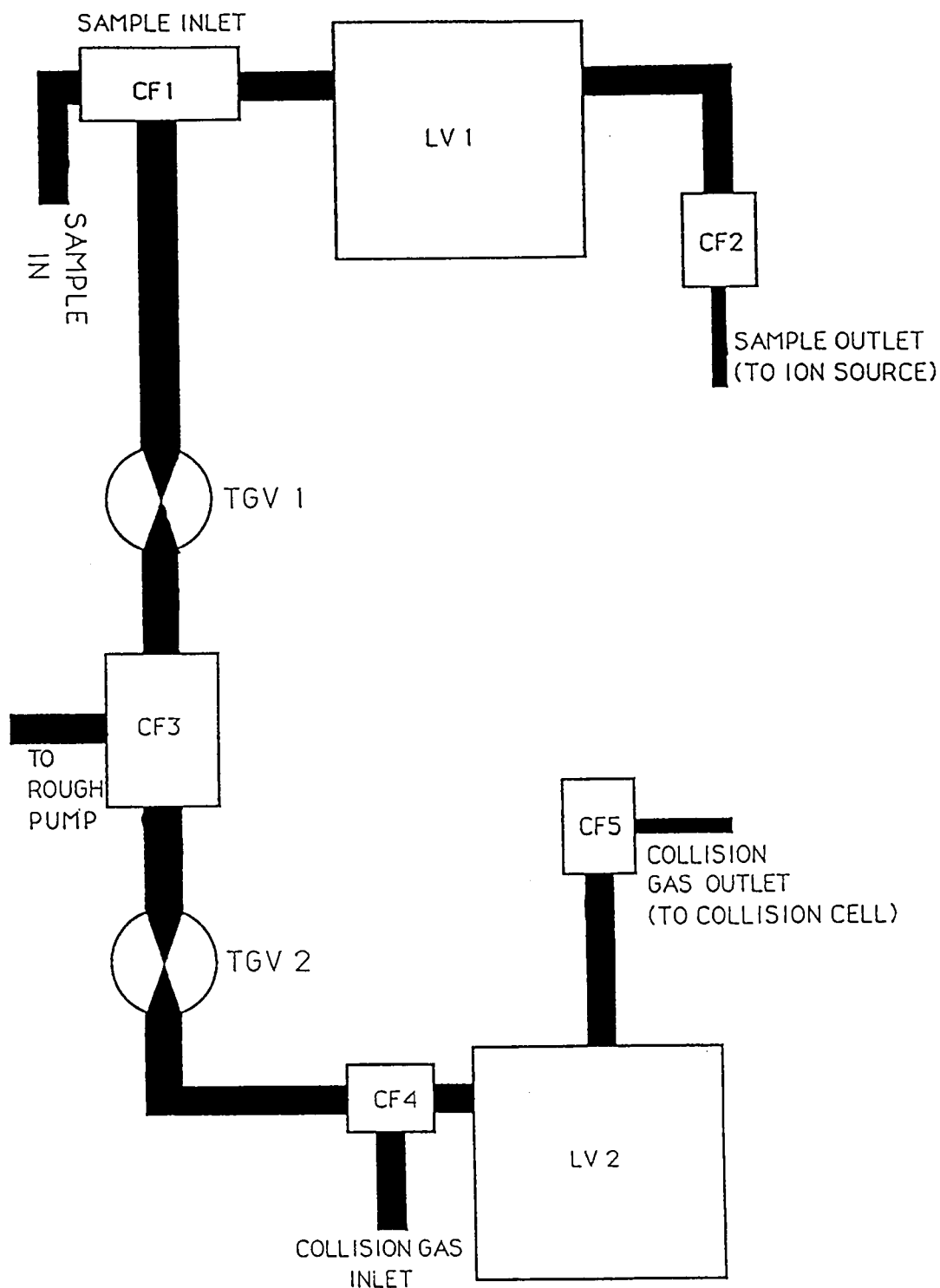


Figure 2.8. Schematic diagram of the inlet system used on the QEB. The components used in the construction of this inlet system are listed in table 2.3 on the next page.

TABLE 2.3

Components utilized for the construction of the QEB sample inlet system shown in Figure 2.8.

COMPONENT	DESCRIPTION
CF1,CF3,CF4	CAJON FITTING, TEE (1/4 IN. O.D. TUBING)
CF2,CF5	CAJON FITTING, REDUCER (1/4 IN. O.D. TUBING TO 1/8 IN. O.D. TUBING)
TGV1, TGV2	TOGGLE VALVES (1/4 IN. O.D. TUBING)
LV1, LV2	GRANVILLE-PHILLIPS VARIABLE LEAK VALVES*

Notes:

*Vendor = Granville-Phillips Co., Boulder, CO, Model #203.

All other items utilized above were purchased as ORNL laboratory stock items.

to a temperature of 70° C by means of heating tape; several of the metal Cajon fittings were also gently heated. Sample was introduced to the ion source through the leak valve until the indicated pressure on the source ionization gauge read 8×10^{-7} torr to 2×10^{-6} torr. Collision gas for low energy MS/MS experiments was admitted via a leak valve into the low energy collision cell. Performance of MS/MS experiments generally resulted in a maximum total pressure of 1×10^{-5} torr in the region before the source slit. The sector analyzer (EB) remained at pressures of 2×10^{-7} torr or lower during all experiments.

Acquisition of data

Mass spectra were collected by means of a digital oscilloscope (model N150C, Nicolet Corp., Madison, WI). Reproducible triggering was essential to allow for optional signal averaging. Mass spectra and low energy MS/MS spectra reported were averages of at least 10 scans. An appropriate trigger signal was the so-called bright-up signal which activated the analog oscilloscope employed on the MS50. This signal rose from a value of 0.2 volts to 4.5-4.6 volts when a scan was initiated; the signal remained at a level of 4.5-4.6 volts for the duration of the scan and then decreased to 0.2 volts at the end of the scan. This sequence was repeated as long as scans were enabled. The trigger level was set therefore to a level of 3.5 volts on the rising portion of the signal to avoid false triggering.

It was then necessary to convert this time domain signal to one which is a function of mass. No data system was utilized for these experiments, and no software was

available to directly convert the time domain signal into mass information. Since ions were being observed across a narrow mass range (typically 30-90 amu) a manual procedure for identification of peaks was performed. This was conveniently done by observing the intensity of ions of known mass. Initially, the ions were detected by manually adjusting the magnetic field to pass a given ion. It should be noted that a mass marker display unit (Kratos Analytical, Inc, Manchester, UK) was utilized with this instrument which would indicate what mass was being passed by the magnet based on the magnetic field strength and the value of the accelerating potential. The mass marker display unit was calibrated for correct readings by following the manufacturer's instructions. Values of signal intensity as a function of mass were tabulated. Following this manual procedure, the magnet could then be scanned over the range of 100-10 amu at a rate of 30 seconds/decade. The intensities of the peaks obtained from scanning of the magnet could then be compared to those obtained by the manual tuning method and the peak assignments for the magnet scan spectrum could then be achieved. When performing a magnet scan, it should be noted that the filter bandwidth in the signal processing circuitry needed to be set to a value sufficient to allow detection of peaks which are of narrow width in time; the time width of these peaks depends on the rate of magnet scanning and the mass resolution utilized. As stated above, the scan rate was 30 sec/decade and a mass resolution of 2000 was generally employed. For the experiments here, bandwidth settings of 300-1000 Hz were sufficient to avoid "clipping" of peaks and great reductions in peak intensities. This type of procedure was followed since the manual tuning procedure was a daily check of instrument operation.

The peaks utilized for this calibration procedure were m/z 87 (^{13}C isotope of the molecular ion of the ketones 3-methyl-2-butanone and 2-pentanone), m/z 86 (the molecular ion of these ketones), m/z 71 (molecular ion - methyl), and m/z 43 fragment ions produced from these compounds. The peaks from reference compounds such as perfluorokerosene (PFK) or perfluorotributylamine (PFTBA) were also utilized in some cases.

Tuning of the QEB Instrument

Acquisition of a mass spectrum.

Initially Attaining an Ion Beam on the QEB: Where to Start.

The experimental details regarding the actual attainment of the ion beam through the entire instrument will now be considered. This instrument had only one electron multiplier, located after the magnetic sector, requiring that the ion beam be transmitted through the entire instrument for subsequent signal processing and data acquisition. The ion beam intensity could be monitored at three convenient points prior to the detector: the low energy collision cell, the top ESA plate, and a monitor plate located after the ESA. The low energy collision cell is a convenient point to monitor the ion current before the source slit since it is located after the quadrupole mass filter and can therefore serve as a means of checking for ion transmission through the quadrupole mass filter. This test does not require that high voltage be applied since the kinetic energy of the ions in this

portion of the instrument is low (< 50 eV) whether the high voltage is applied or not. Although tuning of lens elements will not necessarily be exactly the same as those utilized for transmission of ions through the entire instrument, 10-20 nanoamperes could be measured at the collision cell utilizing 1×10^{-6} torr of 3-methyl-2-butanone. The quadrupole was generally operated in the RF-only mode (no resolving DC applied to the quadrupole rods) to ensure maximum transmission of ions to the low energy collision cell. The source and einzel lenses, shown in Figure 2.7, were adjusted to yield the optimal current.

When performing this test, it was important to determine that the ion current measured was not merely an artifact due to any noise pickup by the electrometer system and its measuring cables. True ion current correlated with 1) sample pressure, 2) filament current, and 3) individual lens voltages, when each was varied independently of the others. It should also be noted that the ion current could be measured at other lens elements before the quadrupole if trouble was encountered in obtaining a beam at the low energy collision cell. Furthermore, measuring the ion current at various lens elements also proved helpful in verifying the integrity of the electrical connection to the lens. In general, this procedure was utilized at the initial set-up, then repeated only when trouble was encountered in obtaining an ion beam through the sector portion of the instrument or when ion optical changes were made.

Attaining an Ion Beam with High Voltage Applied.

Once an ion current was achieved at the collision cell, the accelerating potential could then be applied to attempt ion beam transmission through the sector portion of the instrument. The acceleration potential initially applied was 1 kV, the lowest value supported by the MS50 control console. The high voltage level was maintained at this level for five minutes to ensure that high voltage insulation was maintained. The acceleration lens potentials were initially set at values of 1 kV for this same reason. After this initial five minute period, both the accelerating potential and the acceleration lens potentials were increased steadily from 1 kV to 6 kV (in increments of 1-1.5 kV). At each stage, the voltages were monitored for 1-2 minutes to ensure that high voltage insulation was being maintained. This procedure was performed routinely. Normal operation of this instrument used a nominal accelerating potential of 6 kV (actual measured potential = 6.18-6.20 kV).

After attaining the required high voltage, the instrument could then be tuned for maximum transmission of ions through the source slit. An attempt to find an ion current at the monitor plate could be made at this point; in many cases, it was easier to search for the beam transmitted through the source slit by measuring an ion current to the top ESA plate with no voltage applied to either plate. In this manner, the ion current could then be measured and checked in the manner described above to determine if this was indeed a true ion current being measured. In order to obtain an initial ion beam (though not necessarily one of greatest sensitivity), it was found easier if at least a few of the

many ion optical variables were held constant at the values determined by optimizing ion current at the collision cell in the absence of high voltage. In this manner, obtaining an ion beam through the source slit and into the ESA was made much easier.

Setting of the Acceleration Lens Elements

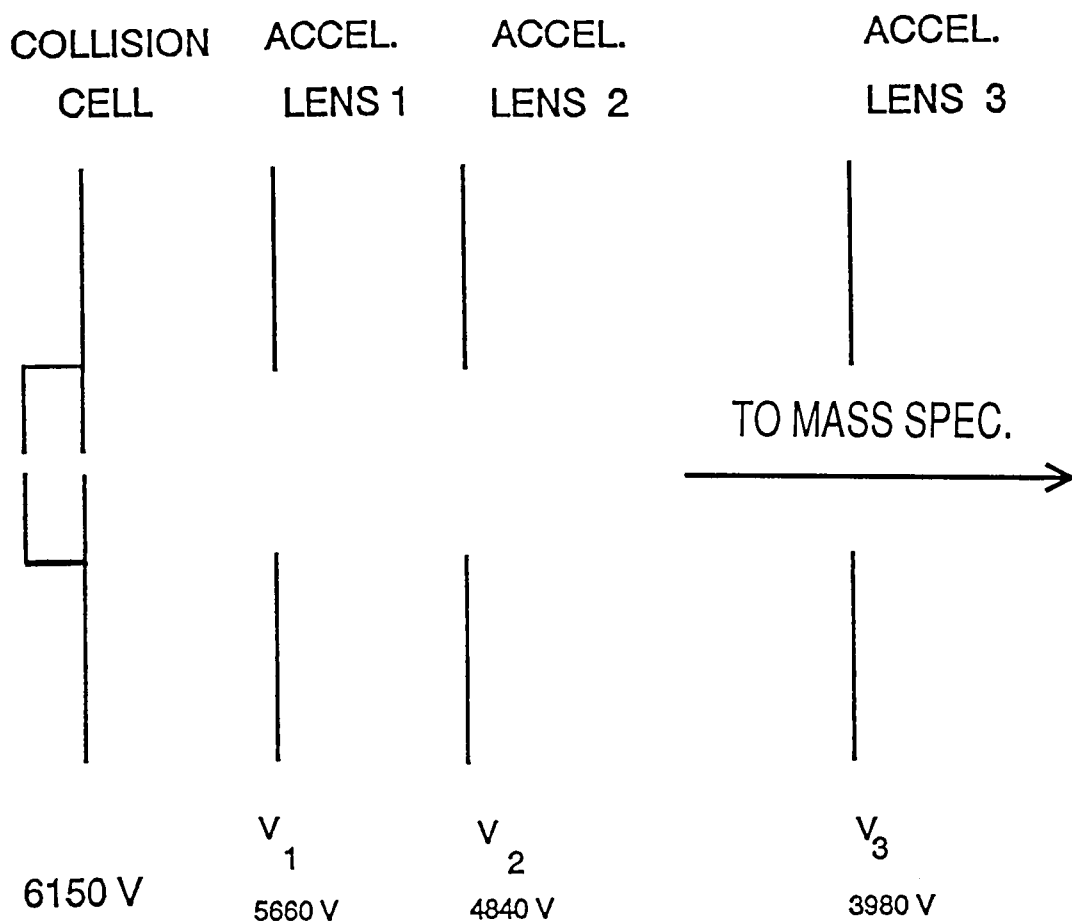
The most critical lens settings were found to be those for the acceleration lens system. In initial tuning of these lenses, it was critical to avoid setting up a potential barrier of sufficient height to prevent ion passage to the source slit. Generally, an initial ion beam of some intensity, though perhaps far from that which could be achieved with optimization of the lenses, was attained by maintaining the first acceleration lens at 5700 V and varying the following two acceleration lenses in such a way that the ions are gradually accelerated. Typically, this would yield a value of 4000-4800 V for the second acceleration lens, and 3000-3500 V for the third acceleration lens. In this manner, an initial ion current was measured at the top ESA plate. The measured current was quite small if the voltages applied to the acceleration lens elements were not optimal; currents as small as 1×10^{-13} amperes would be measured, but the criteria for a true ion current discussed above were met.

After reattaching the electrical leads to the ESA plates, the ion beam was then detected by observing the ion current at the monitor plate located after the ESA. The acceleration lens elements were then tuned to yield the maximum current obtainable at this monitor plate. This was achieved by maintaining the voltage ratio $(V_3)/(V_1)$ at

various values between 3 and 15; the use of this terminology is explained in Figure 2.9. These particular voltage ratios were used since they are typical values used for such a three element lens (69). At each value of $(V_3)/(V_1)$, the potential applied to acceleration lens element 2 (accel. 2) was adjusted to yield the maximum ion current at the monitor plate. These experiments were performed by holding the same ion optical parameters constant as was done for obtaining an ion beam at the top ESA plate. Further optimization of the acceleration lens system is discussed below. The voltages applied to the various acceleration lens elements (Figure 2.9) were typical optimum values, giving a monitor plate ion current of 0.5 - 1.0 μA .

Ion Beam Characteristics Monitored After the ESA.

Another crucial experiment to perform while monitoring the current at the monitor plate was the "alignment" of the ion beam as it exits the ESA. This was determined by making a plot of the ion current at the monitor plate vs. the accelerating potential. The radius of an ion's trajectory through the ESA varied directly with this change in the acceleration potential. Therefore, the ions were essentially swept across the opening in the monitor plate. As shown in Figures 2.10 and 2.11, this process allowed determination of the accelerating potential corresponding to the central ion optical axis. The central ion optical axis was the point where ions were traveling along the central radius of the ESA. This placed the ions in an optimal position for analysis by the magnetic sector portion of the instrument.



ACCEL. LENS 1 POTENTIAL IS 5660 V. WITH RESPECT TO COLLISION CELL, POTENTIAL IS $-490 \text{ V} = V_1$

LIKEWISE, $V_2 = -1310 \text{ V}$.

$V_3 = -2170 \text{ V}$.

$$(V_3) / (V_1) = 4.43$$

$$(V_2) / (V_1) = 2.67$$

Figure 2.9. Schematic diagram of the acceleration lens elements utilized for the QEB. An example demonstrating calculation of relevant voltage ratios for these acceleration lens elements is shown.

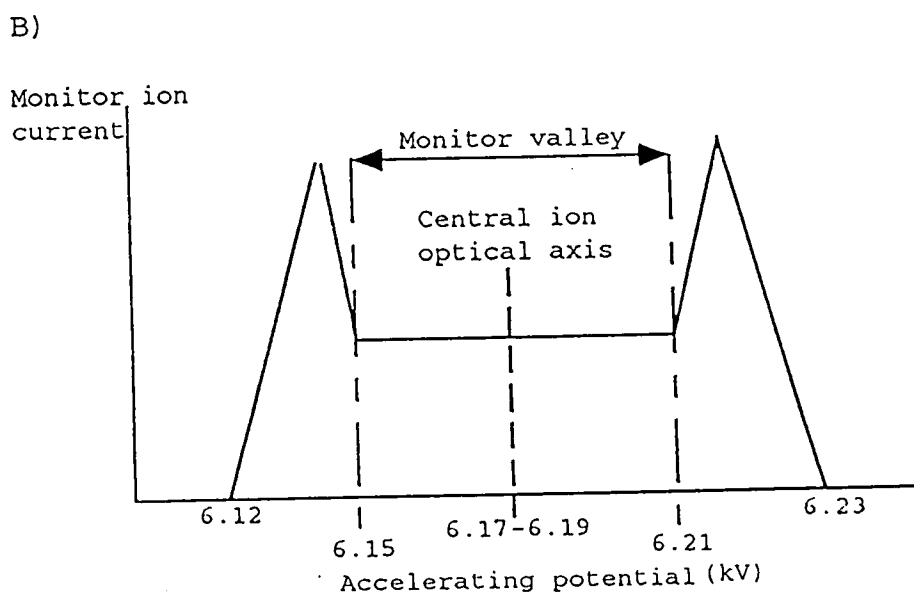
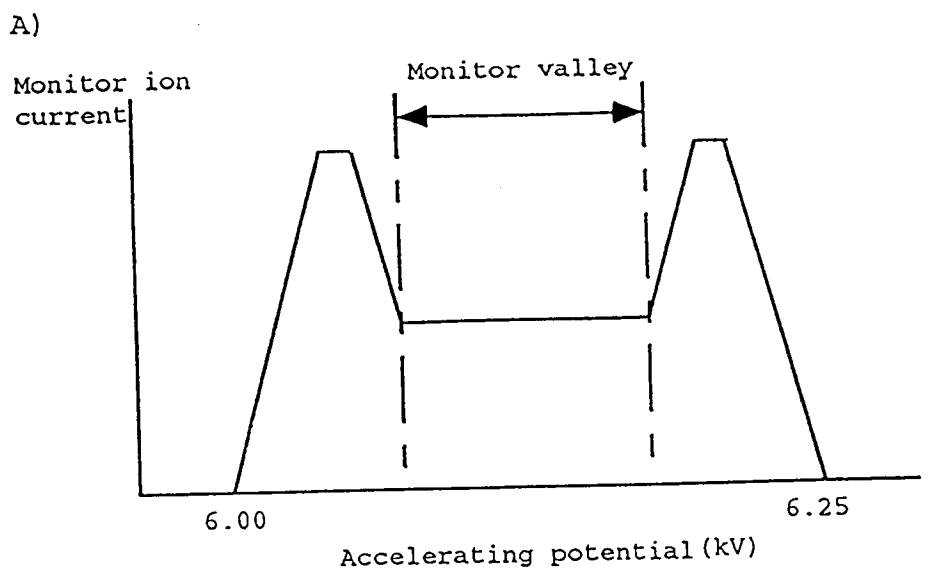


Figure 2.10. Plots of ion current obtained at the monitor plate located after the ESA. A) shows an expected, ideal profile (based on information from the Kratos MS50 operating manual, Kratos Analytical, Inc., Manchester, UK). B) represents a typical observed profile obtained using the QEB under normal operating conditions.

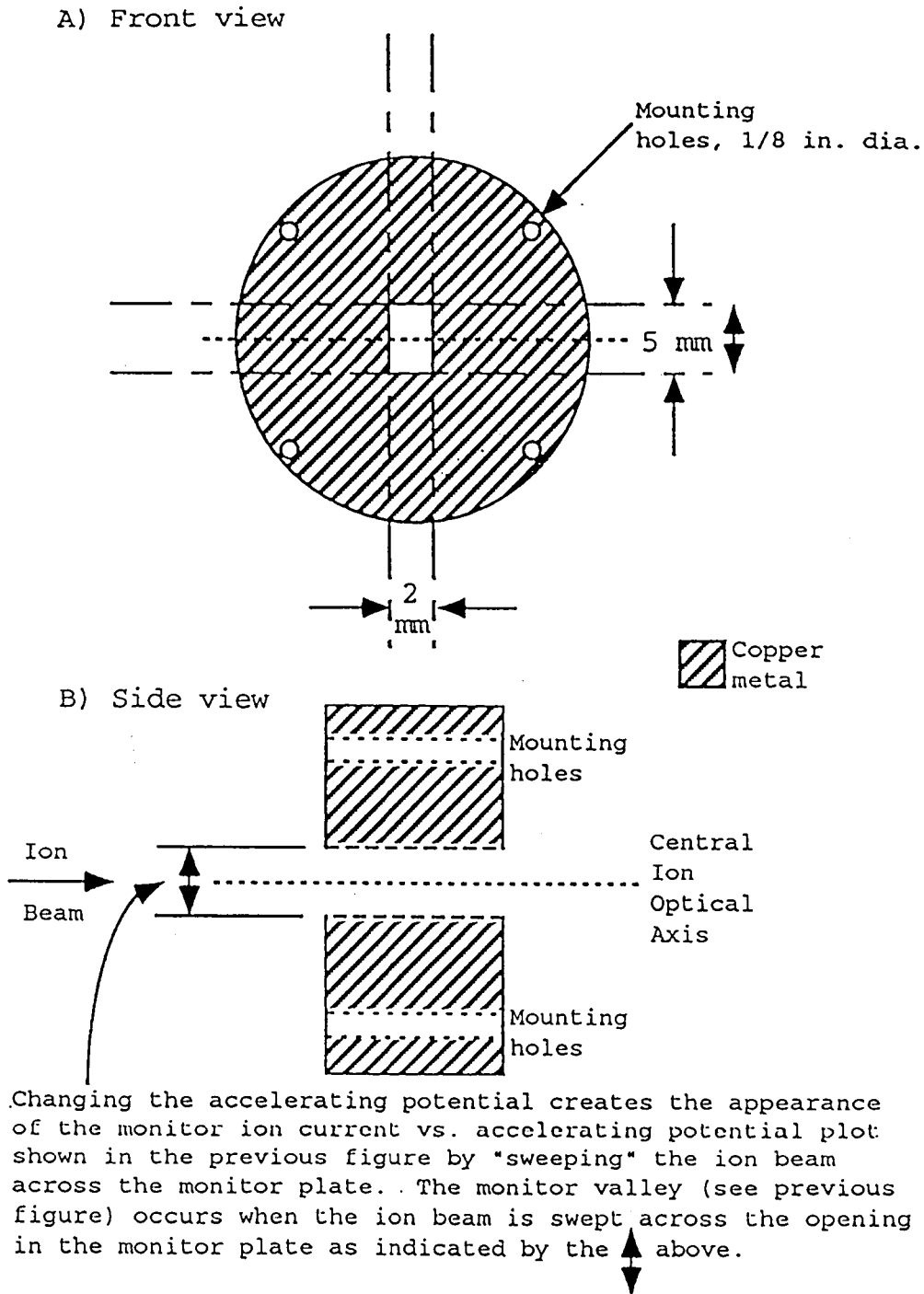


Figure 2.11. Schematic diagrams (not to scale) of the monitor plate mounted after the ESA on the QEB. A) shows a front view in which the ion beam is moving into the plane of the paper. B) shows a cross sectional view of the monitor plate with the direction of the ion beam as shown.

Obtaining an Ion Beam Through the Magnet and Magnet Position Adjustment.

Once the central ion optical axis was determined as described above, the ion beam was passed through the magnet by proper selection of the magnetic field. Generally, the total ion current measured up to this point consisted of several masses; proper selection of the magnetic field allowed only one of these masses to pass through to the detector. For initial experiments, the collector slit located after the magnet was fully opened, the electron multiplier set to its normal voltage of -1000 to -1200 V, and the magnetic field was varied until the collector reading gave the most intense reading for a particular ion derived from one of the compounds utilized above for mass assignment.

If the horizontal magnet position was correctly optimized according to Barber's rule (49), the signal on the collector meter remained constant and the mass resolution increased as the magnet exit (collector) slit was closed. Upon optimizing the horizontal magnet position, closing the collector slit changed the peak shape from flat-topped to gaussian before attenuating the peak signal recorded on the collector meter. The slit setting for which a gaussian shape was first realized is conventionally described as 100% T, and narrower collector slit settings are described by comparison with this peak transmission. The horizontal position of the magnet was adjusted empirically by means of a small stepper motor provided with the original sector instrument.

The vertical position of the magnet was also important since it influenced the focussing at the collector slit of ions having the same exact mass but varying kinetic energy. While measuring the signal for ion current vs. accelerating potential at the

monitor plate, the signal at the electron multiplier located after the magnet was measured simultaneously in order to observe the energy spread of the ion beam passable through the magnet as shown in Figure 2.12. The vertical position of the magnet was then altered to maximize the energy spread passable by the magnet by adjusting four large jacking screws on the magnet body to raise or lower the magnet. As the vertical position of the magnet was adjusted, it was necessary to keep the body of the magnet parallel with the ESA vacuum housing to ensure that the magnet is not tilted during vertical adjustment, which could result in unwanted focussing effects from the nonnormal entry of ions into the magnetic field. After vertical optimization, the horizontal position of the magnet was readjusted, if necessary, to adhere to Barber's rule as outlined above. Once the magnet position (both horizontal and vertical) was found to be satisfactory, the maximum energy spread passed through the magnet at 100% T was found to be 30 eV; the energy focussing properties of the EB portion of the instrument should prove adequate for ion beams possessing an energy spread, such as those resulting from low energy MS/MS experiments on the QEB.

Quadrupole Mass Filter Operation in RF/DC Mode

In order to obtain maximum transmission of ions through the quadrupole mass filter during initial construction of the instrument, the quadrupole was operated in the RF-only mode. However, the precursor ion for an MS/MS experiment was selected by operating the quadrupole mass filter in the RF/DC mode. With the quadrupole set in the

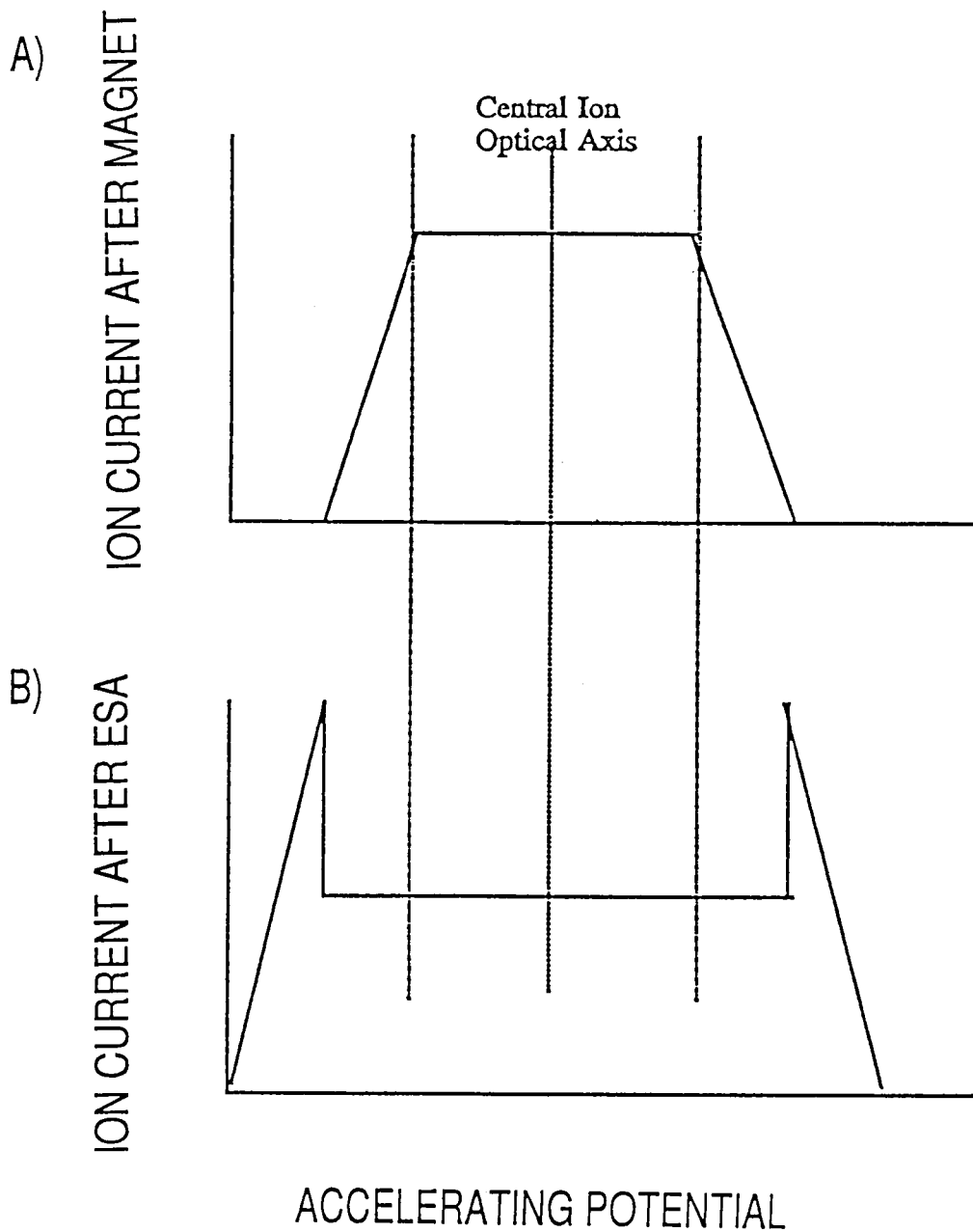


Figure 2.12. Schematic diagram indicating behavior of ion beam (ion beam intensity vs. accelerating voltage). A) shows the plateau in ion current observed after the magnet when the ion current emerging from the ESA is at its minimum (monitor valley, as shown in B). The dotted lines indicate that, for a 6 kV accelerating potential, the maximum energy spread passable by the magnet is 30 eV (15 eV either side of the central ion optical axis).

RF-only mode to allow for maximum sensitivity, the magnet was set to pass the precursor ion to be selected by the quadrupole. After obtaining an ion beam through the magnet with the quadrupole operated in the RF-only mode, the quadrupole was set to the RF/DC mode and the ion beam was again detected after the magnet. Since the transmission of the mass filter decreases in the RF/DC mode as compared to the RF-only mode, it was necessary to optimize source lens potentials and the quadrupole resolution controls in order to achieve the maximum transmission of the selected ion. Proper tuning of the source lenses and the quadrupole yielded ion transmission for the RF/DC mode which was 25-35% of that in the RF-only mode; other ion optical parameters were not adjusted. Improper setting of ion optical parameters resulted in 5% or less relative transmission.

After attaining a beam through the entire instrument in the RF/DC mode operation of the instrument, it was then necessary to ensure that the mass selected ion was the only species being passed by the quadrupole mass filter. First of all, separation of the mass of interest from its ^{13}C isotope peak was ensured by adjusting the resolution (RF/DC ratio) potentiometer on the quadrupole power supply while performing an accelerating voltage scan to pass a narrow range of masses through the magnet (set at constant field). A magnet scan was then performed to ensure that other peaks were not present over the mass range of 10-100 amu. Generally, improper setting of the pole bias on the quadrupole power supply resulted in the kinetic energy of the ions being too high as they traversed through the quadrupole resulting in the ions not spending enough cycles in the RF field to ensure efficient separation in the quadrupole. Optimization of these parameters yielded adequate operation of the quadrupole in the RF/DC mode.

Validation of the Acceleration Lens Settings

With the double focussing characteristics of the sector portion of the QEB confirmed and an ion selected for MS/MS analyses with the quadrupole, the next problem to attack was how to adjust the lenses before and after the low energy collision cell in order to focus at the source slit an ion beam which possessed a variable kinetic energy *before acceleration*. This type of situation occurred following the low energy CID experiment on the QEB. This situation was simulated by varying the collision energy systematically for a chosen parent ion so that the kinetic energy of this ion was precisely known before acceleration. Furthermore, if no collision gas was initially present in the collision cell to cause scattering or dissociation of the parent ion, the intensity of the selected ion should have remained constant with collision energy variations.

For this simulation, the selected ion was m/z 86 or m/z 71 derived from the ketones studied. Upon changing the collision cell potential, the optimum potential of the central element of the einzel lens in Figure 2.7 was found to vary with collision energy such that its potential was twenty times the kinetic energy of the ion in the collision cell. Since the kinetic energy of the ions in the collision cell is known (defined as the potential difference between the source block and the collision cell), the potential on the central element of the einzel lens can then be set for each value of collision energy.

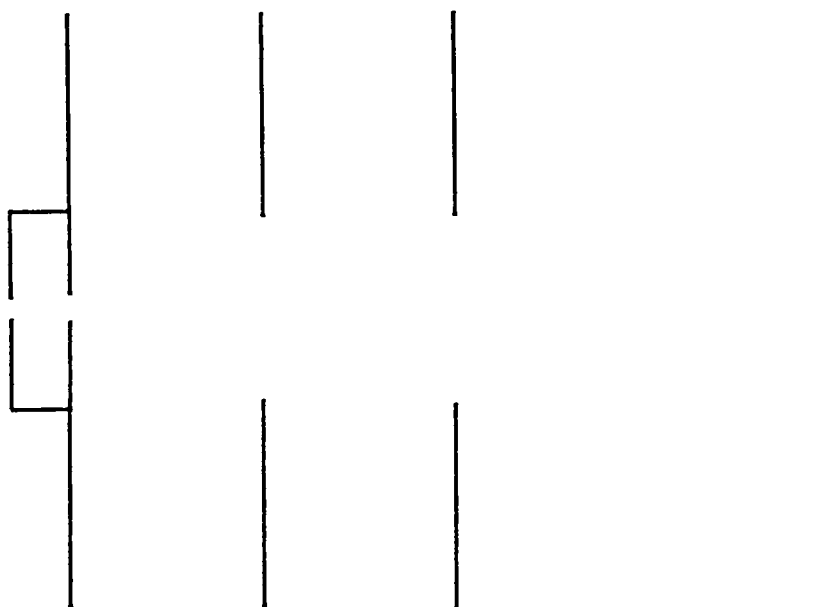
The acceleration lens elements also had to be altered in a systematic fashion with such collision energy variations to maintain constant focussing conditions at the source slit. Best sensitivity was achieved by operating the acceleration lens elements such that

$(V_3)/(V_1)$ was 4-8 and $(V_2)/(V_1)$ was 1.5-2.5 (refer to terminology in Figure 2.9) at each collision energy while maintaining the einzel lens/collision cell potential settings described above.

Performance of low energy MS/MS experiments on the QEB

Once the appropriate precursor ion was selected by the quadrupole, it was then possible to perform low energy MS/MS experiments. After selection of an initial collision energy (for example, 30-40 eV) a collision gas (generally argon) could then be introduced into the collision cell to achieve a desired percentage attenuation of the precursor ion beam. At this point, the magnetic field was decreased manually and the acceleration lenses were adjusted to yield maximum intensity for each product ion. It was found experimentally that only the potential applied to the third acceleration lens element needed to be varied with the mass of the product ion. Furthermore, at collision energies up to 60 eV, the "average" value for this potential could be utilized for detection of all MS/MS product ions; this "average" potential is defined in Figure 2.13. Once this average potential was applied, the magnet could be scanned in the normal manner with the resulting MS/MS spectrum being acquired on the Nicolet digital oscilloscope as described above. Provided that the ratio V_3/V_1 was less than 8 and the einzel lens potential was set according to the criteria outlined above, little or no sensitivity for product ion detection was lost by tuning the acceleration lenses in this manner.

COLLISION	ACCEL.	ACCEL.	ACCEL.
CELL	LENS 1	LENS 2	LENS 3



VOLTAGES
SET AS
SHOWN IN
TABLE
BELOW.

SOURCE POTENTIAL = 6180 V; COLLISION ENERGY = 6180 V - 6150 V = 30 V.

EXAMPLE SHOWN BELOW IS FOR 30 eV COLLISION ENERGY WHEN M/Z 86 OF 2-PENTANONE SUBJECTED TO MS/MS.

POTENTIALS ON ACCEL. 1 AND ACCEL. 2 WERE HELD CONSTANT.

UNDER SUCH CONDITIONS, POTENTIAL ON ACCEL. 3 WAS SET AS SHOWN BELOW:

PRECURSOR ION	M/Z	POTENTIAL ON ACCEL. 3
	86	3980 V
PRODUCT IONS	71	3870 V
	58	3820 V
	43	3750 V

POTENTIAL APPLIED TO ACCEL. 3 WAS AVERAGE OF ACCEL. 3 LENS SETTINGS SHOWN ABOVE FOR PRODUCT IONS. IN THIS CASE, 3810 V WAS THEN APPLIED TO ACCEL. 3.

Figure 2.13. Setting of acceleration lens elements for low energy CID experiments performed on the QEB.

Performance of high energy MS/MS experiments on the QEB

After acceleration of ions to the proper kinetic energy necessary for analysis by the sector portion of the instrument, it was also possible to perform high energy MS/MS experiments. The setup employed for this experiment was a "needle collision cell" shown in Figure 2.14 (67). The needle consisted of a luer lock fitting which was welded onto the source slit shield ground plate. A gas connection was made to this needle by means of a Cajon fitting welded into place on an existing vacuum port within the original Kratos source vacuum housing. A piece of teflon tubing was then passed from this cajon fitting into the vacuum housing and attached to the needle. Argon collision gas was admitted via a Granville-Phillips leak valve. Before admitting the collision gas into this region, a diffusion pump located directly across from the needle valve assembly was put into operation by opening the isolation valve of this diffusion pump. After opening the isolation valve, collision gas was admitted into the needle to achieve the desired attenuation of the precursor ion beam. This needle was situated at a focal point in the system, the entrance slit to the sector portion of the sector mass spectrometer. This setup approximated a jet of gas at this focal point with the result that the product ions were formed in a well-defined region leading to a greater collection efficiency of these product ions.

Formation of the product ions at high energy CID required that the ESA potential be decreased to allow passage of product ions with lower kinetic energy; likewise, the magnetic field must be decreased to allow these product ions of lower energy to pass the

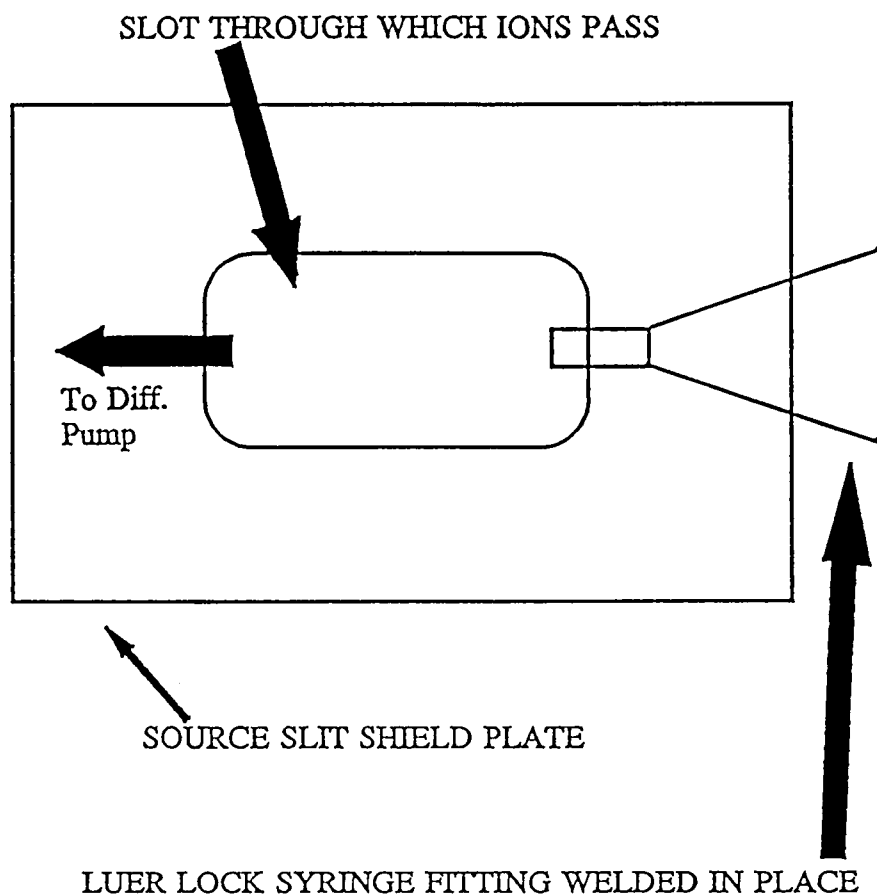


Figure 2.14. Diagram of needle collision cell used on the QEB for high energy MS/MS experiments. Since the shield plate is held at ground potential, the high energy MS/MS collision energy is fixed at the accelerating potential. The source slit (not shown) is 2 mm behind the shield plate. This shield plate was a part of the original MS50 and was not modified in any way except for the attachment of the needle (as described in the text). In this figure, the ion beam is heading into the plane of the paper.

magnet. This process is known as a linked scan at constant B/E ratio (37). With this particular instrument, it was found easiest to manually perform this experiment. First of all, the main beam (parent ion beam) was found in the normal manner as described above and the accelerating potential and the ESA voltage were noted. After introduction of collision gas into the high energy collision region, the ESA voltage was decreased to a value appropriate to pass an expected product ion as indicated by a response at the monitor plate located after the ESA. After obtaining a product ion beam through the ESA, the magnetic field was adjusted and a signal recorded at the electron multiplier. For obtaining a peak profile in this manner, the ESA was changed in 0.1 volt increments and the magnetic field was varied at each ESA increment by an amount determined by the constant B/E ratio needed to pass the parent ion and the various product ions. This required recording the Hall probe reading to the fifth decimal place with a highly accurate voltmeter (Kiethly model 501, Kiethley Corp., Morristown, NJ). The intensities obtained in this manner could then be plotted with software offline.

Performance of MS/MS/MS on the QEB

For characterization of a fragment ion produced by low energy MS/MS on the QEB, it is possible to subject that ion to high energy MS/MS. This experiment involved procedures incorporating both the low and high energy MS/MS experiments described above. An initial precursor ion was selected by the quadrupole and subjected to low energy MS/MS; utilizing the characteristics of the B/E linked scan, a chosen fragment ion

was then subjected to high energy MS/MS and relevant fragments from this ion were then detected utilizing the procedure described above for high energy MS/MS. The characteristics of the linked scan at constant B/E ratio ensure that the fragments are derived from a selected ion produced in the low energy collision cell since the ESA and magnet are set by the B/E scan law to pass ions of certain energies and momenta, respectively, based on the precursor from which they are derived. In this case, the precursor is produced by low energy MS/MS instead of being produced in the ion source.

Ion Trap Mass Spectrometer

The ion trap mass spectrometer (ITMS) utilized for these experiments was not modified in any way; the standard instrument available from Finnigan Corporation (San Jose, CA) was utilized. The ion trap instrument operates by utilizing various time periods for ionization, ion isolation, and MS/MS activation. Ionization times were 1-5 milliseconds (msec) in duration. The cooling period was typically 10-500 msec in length. MS/MS activation occurred for 10-20 msec utilizing a tickle voltage of 300-400 mV (peak-to-peak). These values for MS/MS activation were utilized since they provided nearly 100% dissociation efficiency of the chosen ion. MS/MS dissociation efficiency was defined as the ratio of (sum of product ion intensities from MS/MS) : (precursor ion signal strength before activation). Higher values of tickle voltages resulted in increasingly greater losses of precursor ion from the trap; longer activation times produced a similar result. The time period following MS/MS activation was varied from 0-1 second. All

of these time periods were under data system control and were varied independently of one another throughout these studies. The basic experimental procedure to be followed for the ion trap MS and MS/MS experiments can be obtained from the instruction manual issued with the instrument.

Samples introduced into the ITMS were thoroughly degassed using the same type of freeze-pump-thaw cycle as outlined above for the QEB sample introduction system. Samples were introduced into the ITMS by means of a leak valve to attain a sample pressure of 5×10^{-7} torr to 1×10^{-6} torr (uncorrected ion gauge readings). The leak valve inlet system was interfaced directly to the standard Finnigan inlet system for the ITMS by means of standard Swagelok and Cajon fittings. For all experiments, the helium bath gas pressure was maintained at 1 millitorr. The temperature of the ion trap was maintained at 100°C throughout these experiments.

To obtain the ratio of m/z 43 isobars from the the m/z 71 ions on the ITMS, MS/MS experiments involving the naturally occurring ^{13}C isotope peak of a precursor ion were performed. The experimental procedure followed for this process, developed at Oak Ridge National Laboratory, is described in detail in the literature (70). For m/z 71, MS/MS experiments are performed on m/z 72 and the product ion ratio at m/z 43 and m/z 44 are noted. The measured ratio is compared to a calculated ratio. The calculated ratio is based upon the probability of losing the ^{13}C (present in natural abundance) in the neutral fragment. This is outlined in Figure 2.15. For example, loss of ^{13}CO from m/z 72 yields a product ion at m/z 43, while loss of ^{12}CO from m/z 72 yields a product ion at m/z 44. Since the ^{13}C is present in natural abundance, it can be found at any of the

OBTAINING MASS RESOLUTION ON THE ITMS

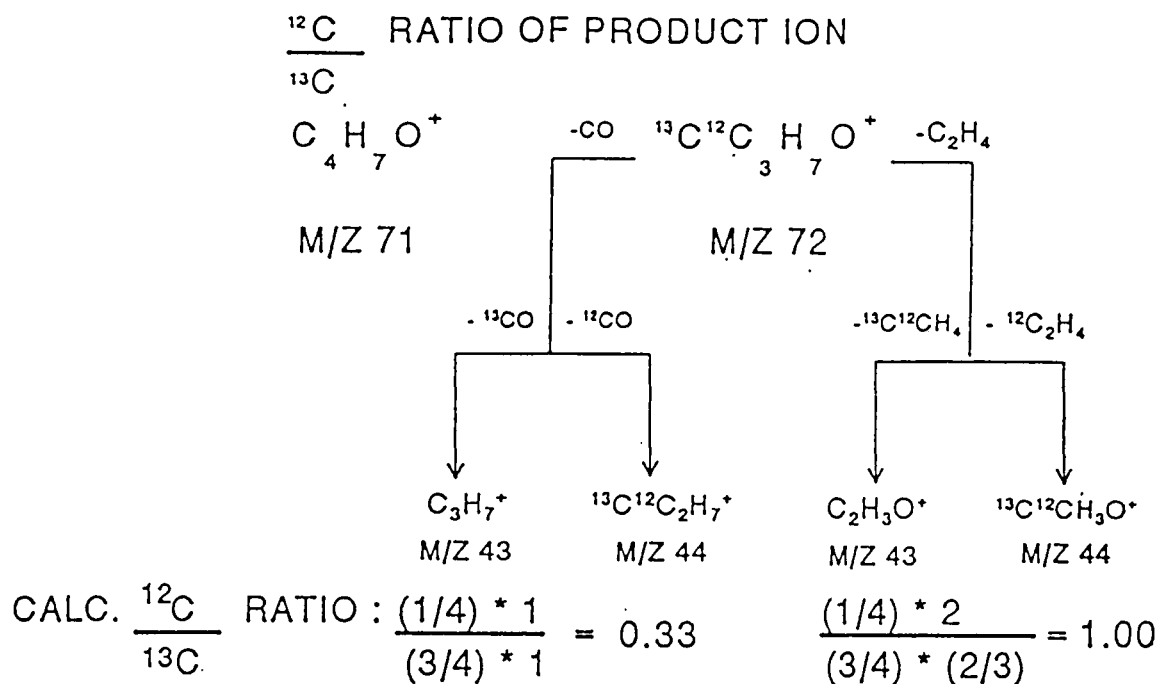


Figure 2.15. Schematic diagram indicating the method of determination of isobaric species at m/z 43 is performed on the ITMS. If one desires to know the proportion of isobaric species at m/z 43 produced from MS/MS of m/z 71 from 3-methyl-2-butanone or 2-pentanone on the ITMS, the MS/MS experiment is performed on m/z 72 (the ^{13}C isotope of m/z 71). The resulting product ion ratio at m/z 43 and m/z 44 is then observed. This experimentally determined ratio is then compared to the theoretical values calculated above so that the proportion of isobaric m/z 43 ions can then be accurately determined (see text for further details).

four carbons in the m/z 72 ion. The calculated ratio shown in Figure 2.15 is the ratio of ^{12}C product ion (m/z 43) : ^{13}C (m/z 44) product ion. If the experimentally measured ratio is 0.33, only CO is lost from m/z 72, but if the measured ratio is 1.00 only C_2H_4 is lost. If an intermediate ratio is measured, both losses have occurred. The ratio of precursor ions yielding these product ions can be determined by solving an equation which states that the experimentally measured $^{12}\text{C}:^{13}\text{C}$ ratio is a weighted average of the theoretically expected $^{12}\text{C}:^{13}\text{C}$ ratio which can be generated from each individual precursor ion (70).

Chemicals Utilized

All the chemicals utilized for these experiments were purchased commercially and used without further purification. The chemicals utilized included 3-methyl-2-butanone (Eastman Kodak Co., Rochester, NY), 2-pentanone, isobutyryl chloride, butyryl chloride, methyl vinyl ketone, and mesityl oxide (Aldrich Chemical Co., Milwaukee, WI).

CHAPTER 3

INSTRUMENTATION: QEB HYBRID MASS SPECTROMETER

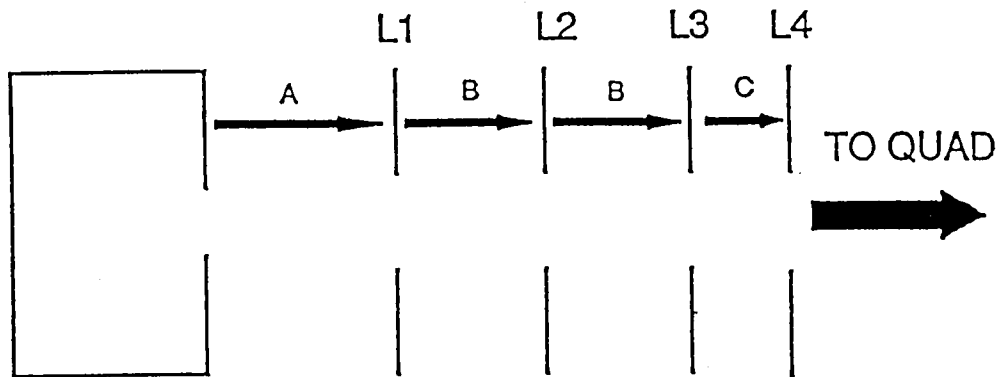
The QEB hybrid mass spectrometer is a novel piece of instrumentation having the capability of performing high resolution product ion analyses from both high and low energy CID experiments. The capability to perform high resolution analyses on product ions resulting from low energy CID experiments is perhaps the most unusual aspect of the QEB since such experiments allowed control over the internal energy deposition in a known and precise manner since collision energies are known. This makes ion structural analysis easier. Certain factors had to be considered while designing the QEB to ensure adequate sensitivity from the low energy CID experiments. First of all, the ions exiting from the quadrupole must be focussed into the low energy collision cell so that product ion formation will occur from a relatively well-defined region within the low energy collision cell. Such a situation approximated a point source of ions, making focussing of the ion beam by subsequent ion optical elements following the collision cell easier. An acceleration lens system follows the collision cell to provide the ions with the kinetic energy necessary for analysis by the sector portion of the instrument. The acceleration lens must be capable of focussing product ions of variable kinetic energies at the source slit; the product ions may also have a relatively large angular divergence resulting from scattering processes within the low energy collision cell. Studies outlined below concentrated on focussing the ion beam exiting from the quadrupole into the low energy collision cell and design of an acceleration lens system. The ion source and the

quadrupole were not altered during these studies. The typical voltages applied to the source lenses are listed in Figure 3.1.

Initial Behavior of the Ion Optical System Following the Quadrupole

The lens system used initially for focussing the beam exiting the quadrupole at the source slit is shown in Figure 3.2. Spacings of the various elements and voltages applied to those elements were determined using SIMION (71), an ion optical computer modeling program. Unfortunately, it was found that the lens system performed poorly in practice, even with many different combinations of voltages applied to the various lens elements. For example, in order to obtain a 1 volt signal for m/z 86 (molecular ion) from 2-pentanone utilizing a multiplier voltage of -1300 volts, a sample pressure (uncorrected, indicated on the source ion gauge) of $2 - 3 \times 10^{-5}$ torr had to be utilized. Furthermore, total electron emission from the filament was generally 500-1000 μA under these conditions. These criteria were somewhat arbitrary, but they provided a benchmark for comparing different lens systems on the QEB. With this initial lens system, the high sample pressure and the relatively high electron emission required indicated poor sensitivity based on the author's previous experience with other mass spectrometers.

Another poor feature of this initial system was the "lens" effect of the low energy collision cell. This "lens" effect referred to the fact that the signal obtained for m/z 86 of 2-pentanone, after being optimized at a single value of collision energy, decreased by over 90 % at other values of collision energy. The signal was not recovered by adjusting



EI SOURCE
BLOCK

DISTANCES:

A = 4 mm

B = 3 mm

C = 2 mm

TYPICAL VOLTAGES USED IN OPERATION

SOURCE BLOCK : 6180 V

L1: 6075 V

L2: 6170 V

L3: 6160 v (HELD AT ACCEL. VOLTAGE)

L4: 6030 V

Figure 3.1. Schematic diagram of the QEB EI source showing spacing of the various lenses and typical voltages applied to these lenses during operation of the instrument. Drawing not to scale.

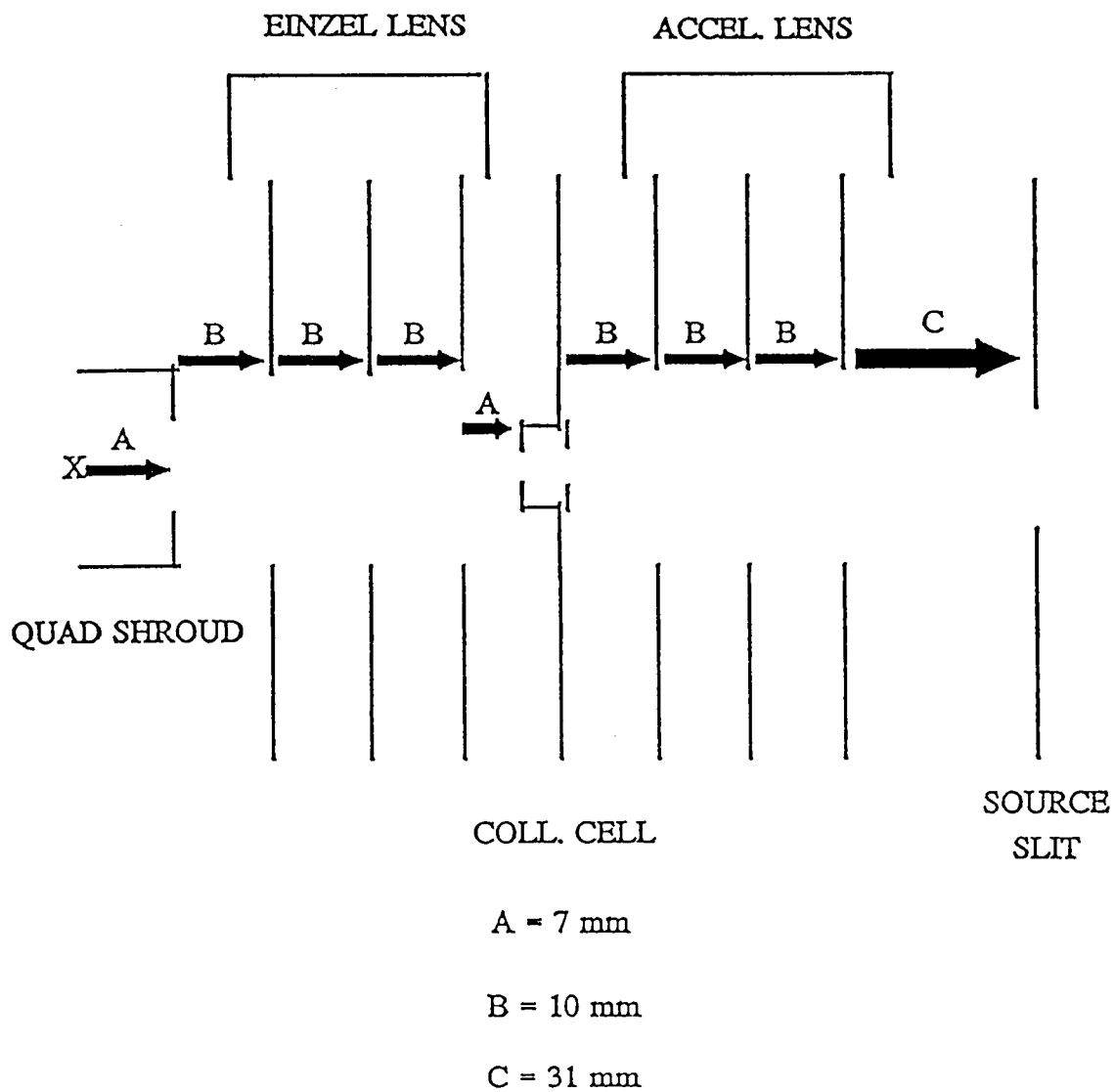


Figure 3.2. Initial version of the lens system utilized on the QEB between the quadrupole mass analyzer and the source slit. "X" indicates the position of the ion beam as it exits the quadrupole mass filter.

voltages on the einzel lens or the acceleration lenses. Since the unattenuated precursor ion (m/z 86 in the presence of no collision gas) showed undesirable sensitivity to collision energy variations, it was expected that the product ion spectra would also be influenced. The sensitivity of the low energy MS/MS experiments was indeed low since less than 1% of the original unattenuated precursor ion signal appeared as product ions after low energy CID was performed. Optimizing lens potentials on either the precursor ion or various product ions did not improve system performance. This 1% value was obtained for low energy CID of m/z 86 from 2-pentanone utilizing sufficient argon collision gas to attenuate the intensity of the precursor ion beam by 50%. With high sample pressures and high electron emission yielding abundant precursor ion, the product ion yield was expected to be higher. Therefore, the extraction and focussing characteristics of the acceleration lens system were suspected to be deficient. The focussing characteristics of lens elements before and after the collision cell were examined to overcome these problems.

The goal was to develop a lens system which would not only overcome this collision cell "lens" effect, but also improve MS and MS/MS sensitivity while utilizing the same available amount of space in the vacuum system as for the initial lens system. This was an economical approach, in terms of time and money, since a new vacuum system did not have to be built.

Focussing of the ion beam into the low energy collision cell

The approach taken, though empirical, was systematic. In order to keep the number of variations of this lens system manageable, only the distance B in Figure 3.3 was varied, thereby keeping the remaining distances within the lens system (A and C in Figure 3.3) constant. Three distances, dictated by available spacers, were tried; 10 mm (the original distance shown in Figure 3.2), 6 mm, and 3 mm. The final lens system utilized for focussing the ion beam into the low energy collision cell is shown in Figure 3.3, showing that a distance of 6 mm for B was optimal for removal of the collision cell "lens" effect described above since the dramatic variations in unattenuated precursor ion intensity with collision energy changes were removed.

It is difficult to say exactly why this distance worked best since the lens system may have been performing as a four element lens consisting of the einzel lens elements and the collision cell (see Figure 3.3). Such four element lenses are quite complicated and are not easily treated (72). However, it was interesting to note that the following relationship did hold for the final lens system used in Figure 3.3: $D_1/D_2 = 3.3 \text{ cm}/0.7 \text{ cm} = 4.71$. The significance of this relationship is noteworthy and is accounted for as follows. For this lens system, the collision cell and central einzel lens element voltages were adjustable. The experimentally measured optimum voltage ratio of central einzel lens element : collision cell fell within the range of 20-24. This range corresponds well with the relationship $(D_1/D_2)^2 = (4.71)^2$. Significantly, such a relationship between the geometry of a lens and the voltage ratio applied to various elements in that lens has been

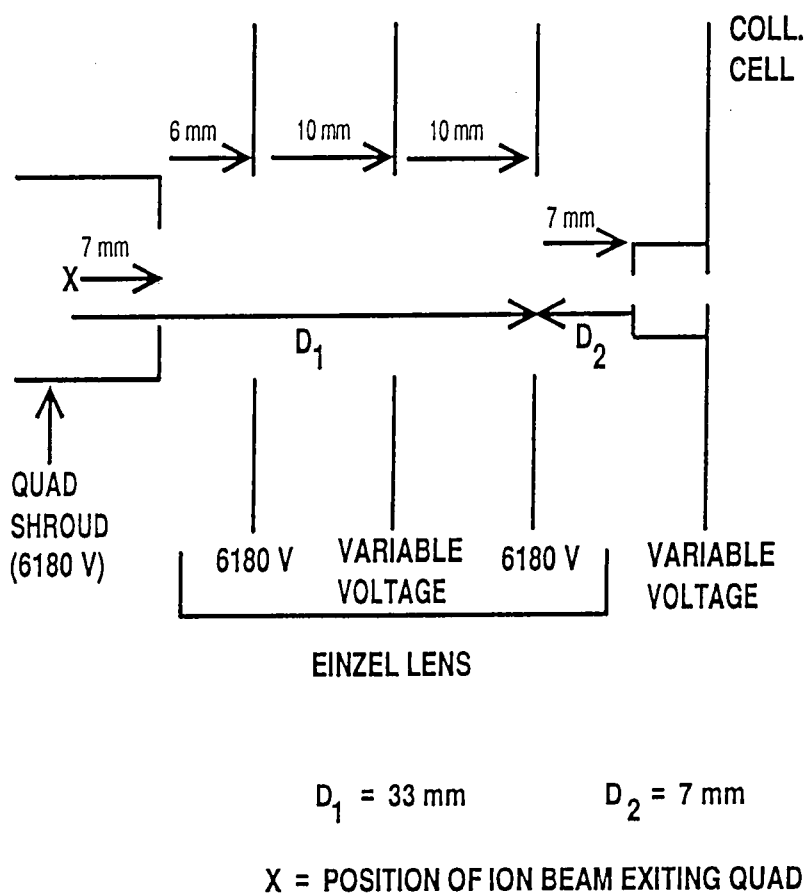


Figure 3.3. Diagram of lens system (not to scale) utilized between the quadrupole mass filter and the low energy collision cell on the QEB. Aperture sizes for the einzel lens elements are 2 cm diameter, while the aperture size for the collision cell is 3 mm. Spacing between various lens elements are as indicated above.

previously established in the literature for three and four element lenses (72,73).

Though the collision cell "lens" effect was removed by this procedure, it was noted that the MS and MS/MS sensitivity were not greatly improved, according to the protocol discussed above, by modifying only this portion of the lens system. Attention was then focussed on the acceleration lens system for further improvements in sensitivity.

Design of the acceleration lens system

The acceleration lens system utilized is shown in Figure 3.4. Large apertures (2 cm) were used; these would prove advantageous in this case since the ion beam exiting the collision cell may be angularly divergent after the collisional activation event due to scattering within the cell. Use of large apertures also avoided aberration problems since only the central portion of the lens would be utilized; utilizing only the central 40-50% of the available aperture diameter is recommended for ion lenses to avoid aberrations (68,69). Abberations are simply the inability of a lens to focus all ions to a point. With the 2 cm diameter apertures for the acceleration lenses, the largest diameter beam which entered the acceleration lens system was limited by the aperture size of the collision cell (3 mm). Only 15% of the available lens diameter was utilized, indicating that abberations should be reduced with this type of lens system.

After collisional activation within the collision cell, the different product ions possessed various kinetic energies due to the partitioning of kinetic energy of the original precursor ion with the mass of the product ion according to the following relationship:

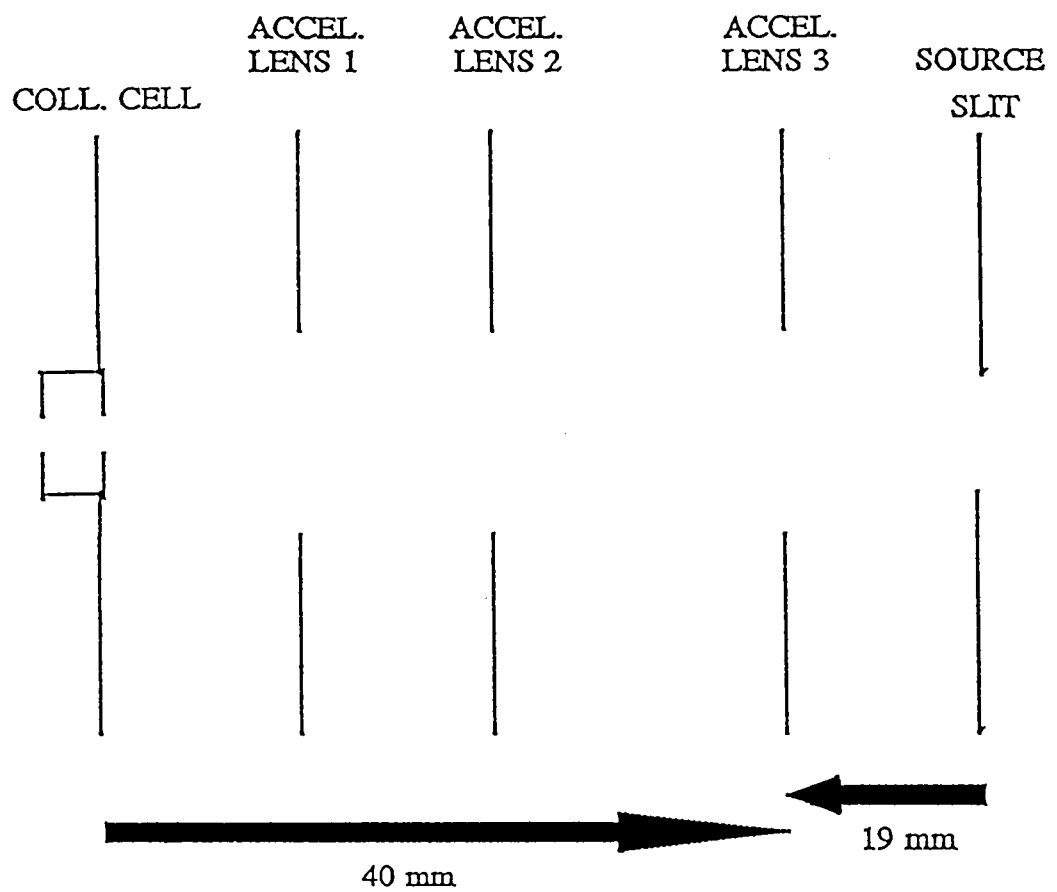


Figure 3.4. Schematic diagram of modified acceleration lens region utilized on the QEB.

kinetic energy of product ion = $[(\text{mass}_{\text{product ion}})/(\text{mass}_{\text{precursor ion}})] * \text{collision energy}$. The acceleration lens system must be able to deal with this energy spread. The key to making the acceleration lens system work reliably was to accelerate the ions gradually from the collision cell to the source slit. If too great a change in kinetic energy occurred within the lens system, the ions achieved a focus at a point before the source slit since the lens was too "strong". This problem was handled with the lens system described below.

The first three elements in the acceleration lens system were treated as a three element lens in which the voltage ratio V_3/V_1 determines the kinetic energy change through the lens and V_2/V_1 determines the focal properties of this lens (72-82). These properties should be useful in handling an ion beam possessing an energy spread of the type outlined above since a kinetic energy spread can be handled with the ratio V_3/V_1 and the focussing abilities of the lens can be maintained constant by adjusting the ratio V_2/V_1 . Three element lenses have seen much use because of their simplicity and flexibility (72-82). The same relationship noted above for the einzel lens/collision cell system relating geometrical distances and voltage ratios was used for the acceleration lens. Using distances from Figure 3.4, the ratio $40 \text{ mm}/19 \text{ mm} = 2.10$ and $(2.10)^2 = 4.43$. The voltage ratio V_3/V_1 should then be about 4.43 for optimal performance of the acceleration lens. The acceleration lens performance was checked by observing the focussing characteristics of a precursor ion with no collision gas present as a function of collision energy. In this way, the kinetic energy of the ions was changed in a known manner before acceleration. The optimum voltages used in such experiments for the acceleration lens are listed in tables 3.1 and 3.2; relevant voltage ratios are listed in table 3.3.

TABLE 3.1

Potentials applied to the acceleration lens elements as a function of precursor ion kinetic energy (collision energy).

COLLISION ENERGY	ACCEL. 1 VOLTS	ACCEL. 2 VOLTS	ACCEL. 3 VOLTS
5 eV	5660 V	5430 V	3440 V
10 eV	5660 V	4960 V	3750 V
15 eV	5660 V	4830 V	3860 V
20 eV	5660 V	4810 V	3990 V
25 eV	5660 V	4730 V	3990 V
30 eV	5660 V	4790 V	4020 V
35 eV	5660 V	4810 V	4030 V
40 eV	5660 V	4790 V	3980 V
45 eV	5660 V	4850 V	4140 V
50 eV	5660 V	4830 V	4200 V

Note:

Potentials applied to Accel. 2 and Accel. 3 varied by $\pm 10\%$ day-to-day from the values listed. Source potential = 6180 V typically.

TABLE 3.2

Typical potentials applied to acceleration lenses listed with respect to the collision cell potential.

COLLISION ENERGY	VOLTS COLL. CELL	VOLTS, ACCEL.1	VOLTS, ACCEL.2	VOLTS, ACCEL.3
5 eV	6175 V	-515 V	-745 V	-2735 V
10 eV	6170 V	-510 V	-1210 V	-2420 V
15 eV	6165 V	-505 V	-1335 V	-2305 V
20 eV	6160 V	-500 V	-1350 V	-2170 V
25 eV	6155 V	-495 V	-1425 V	-2165 V
30 eV	6150 V	-490 V	-1360 V	-2130 V
35 eV	6145 V	-485 V	-1335 V	-2115 V
40 eV	6140 V	-480 V	-1350 V	-2160 V
45 eV	6135 V	-475 V	-1285 V	-1995 V
50 eV	6130 V	-470 V	-1300 V	-1930 V

Notes:

Accel. 1 potential was held constant. Other lens elements were variable. Source potential was held at 6180 V, generally. Values for Accel. 2 and Accel. 3 varied from listed values by $\pm 10\%$ on a day-to-day basis.

Potentials utilized for above calculations are listed in table 3.1.

TABLE 3.3

Voltage ratios which determined focussing properties of the QEB acceleration lens system.

COLLISION ENERGY	VOLTS, COLL. CELL	V_2/V_1	V_3/V_1
5 eV	6175 V	1.45	5.31
10 eV	6170 V	2.37	4.75
15 eV	6165 V	2.64	4.56
20 eV	6160 V	2.70	4.34
25 eV	6155 V	2.88	4.37
30 eV	6150 V	2.78	4.35
35 eV	6145 V	2.75	4.36
40 eV	6140 V	2.81	4.50
45 eV	6135 V	2.71	4.20
50 eV	6130 V	2.77	4.11

Notes:

The ratios are based on voltages shown in the table 3.2.

To determine the above ratios, define voltages as follows:

V_2 is the voltage applied to Accel. lens 2 with respect to (w.r.t.) the collision cell (see table 3.2).

V_1 is the voltage applied to Accel. lens 1 w.r.t. to collision cell (see table 3.2).

V_3 is the voltage applied to Accel. lens 3 w.r.t. to collision cell (see table 3.2).

When utilizing the voltage ratio of 20 for the central einzel lens/collision cell and the voltage ratios shown in table 3.3, the MS sensitivity was found to improve dramatically according to the criteria listed above (the MS/MS sensitivity is discussed below). To obtain a 1 volt signal for the m/z 86 molecular ion of 2-pentanone in the absence of collision gas at a multiplier voltage of -1300 volts, a sample pressure (uncorrected, indicated on source ion gauge) of 8×10^{-7} torr and 100 μ A total electron emission were used. This 1 volt signal was maintained across the desired collision energy range of 5-50 eV. As expected, the voltage ratios for the acceleration lens system remained constant with collision energy variations in order to keep focussing conditions at the source slit constant. The value of V_3/V_1 was very close to the expected value of 4.43 at all but the lowest collision energy (5 eV), as shown in table 3.3. The behavior of the acceleration lens system at 5 eV collision energy appeared not to agree well with the behavior at higher collision energies. This anomaly will be discussed further in the future work section of this thesis (chapter five).

The ion beam emerging from the quadrupole was focussed into the collision cell by altering the central einzel lens element potential in conjunction with the collision energy as described above. Since the collision cell was, by definition, a field-free region, the acceleration lenses would not influence ion extraction and focussing until the ions exited from the collision cell. The exit of the collision cell would then be the actual "ion source" for subsequent lens elements. The voltages applied to the acceleration lens varied with the collision energy since kinetic energy before acceleration was being varied. However, the voltage ratios applied to the acceleration lenses should remain essentially

constant in order to maintain constant focussing conditions at the source slit. The ability to maintain constant signal values for a precursor ion (with no collision gas present) with collision energy variations was an important first step for increasing MS/MS sensitivity. Focussing of product ions from low energy MS/MS experiments also involved focussing of ions possessing variable kinetic energies before acceleration.

Product ions from low energy CID, however, did not exist until the precursor ion was activated in the low energy collision cell. The apparent "ion source" for product ions was then the exit of the collision cell since the collision cell was a field-free region. As described above for the precursor ion exiting the collision cell, the acceleration lens elements would not influence product ion extraction and focussing until the ions exited from the collision cell. The exit of the collision cell would also be the actual position of the "ion source" for the product ions. The behavior of the acceleration lens with regard to the expected energy spread of the product ions was examined to determine if the lens system, shown to increase MS sensitivity, also increased MS/MS sensitivity.

Response of Acceleration Lens System to Product Ion Energy Spread

Table 3.4 shows the potentials applied to the various acceleration lens elements to obtain the maximum product ion signals from low energy CID of m/z 86 of 2-pentanone. The potential on acceleration lens 3 was reduced to compensate for the lower kinetic energy of the product ion upon its formation within the collision cell. The following relationship between the ratio V_3/V_1 for the precursor (denoted as prec.) and

TABLE 3.4

Variation of acceleration lenses with kinetic energy of product ions. Specific example is for MS/MS of m/z 86 of 2-pentanone at 30 eV collision energy.

M/Z	K.E.	VOLTS, ACCEL.1	VOLTS, ACCEL.2	VOLTS, ACCEL.3	V_3/V_1
86	30 eV	5660 V	4840 V	3980 V	4.34
71	25 eV	5660 V	4840 V	3870 V	4.56
58	20 eV	5660 V	4840 V	3820 V	4.66
43	15 eV	5660 V	4840 V	3720 V	4.86

Notes:

K.E. is the kinetic energy of the ion in the low energy collision cell.

V_3/V_1 was calculated in the same manner as in table 3.3. Acceleration lens potentials are referenced to the collision cell potential to calculate this ratio.

For 30 eV collision energy, the collision cell potential is 6150 V.

product ions (denoted as prod.) was empirically derived as shown below:

$$V_3/V_{1\text{prod.}} = (V_3/V_{1\text{prec.}})*[(K.E._{c.c.\text{prec.}})/(K.E._{c.c.\text{prod.}})]^{1/4} \quad \text{Eq. 3.1}$$

In this equation, the K.E. terms refer to the kinetic energies of the ions within the collision cell. The kinetic energy of the precursor ion was known and was equal to the collision energy. The expected kinetic energy of the product ions was taken to be that from partitioning of kinetic energy according to the mass of the product ion and the mass of the precursor ion. The above relationship was derived empirically through experimentation, but it could prove useful to others utilizing similar lens systems.

A further step was taken in the analysis of the accelerating lens system. Since the first three elements in the acceleration lens system were treated as a three element lens, the properties of that type of lens were investigated in hopes of gaining further insight into the operation of this acceleration lens system. Data from Read indicated that the focussing properties of three element lenses possess a relatively broad maximum for values of V_3/V_1 between 2-10 and V_2/V_1 values of 2-3 (72, 74). Interestingly, voltage ratios within these ranges were utilized for the acceleration lens employed on the QEB. Furthermore, since the maximum did not shift to other values of V_2/V_1 with changes in V_3/V_1 , the observed behavior of keeping the first two acceleration lens elements at constant voltages (i.e., V_2/V_1 constant) as V_3/V_1 was varied for the product ions at a given collision energy was justified and understandable (see table 3.4).

The maxima in Read's curves were also used advantageously to simplify operation of the acceleration lens system. His tabulated data were used as a guide to predict that if all of the acceleration lens elements were held at constant potentials, the lens system

would yield approximately the same type of performance as described above where V_3/V_1 was varied with product ion mass. The following case example was studied. Over the collision energy range examined (5-50 eV), the largest energy spread resulting from low energy CID covered 25 eV, since a factor of two in mass range was observed (m/z 43-86) in the product ion spectra. If this extreme case worked well, other cases at lower collision energies should be valid also since the energy spread was lower in the less extreme cases.

This problem was approached as follows. Since the kinetic energy (K.E.) of an ion at a point within the acceleration lens was related to the value of the potential within the lens, it was appropriate to examine the kinetic energy ratios for ions of varying kinetic energy at specified points within the lens. Therefore, the ratio $(K.E._{accel. 2} / K.E._{accel. 1})$ was utilized in place of V_2/V_1 and the ratio $(K.E._{accel. 3} / K.E._{accel. 1})$ was utilized in place of V_3/V_1 . The appropriate calculations are shown in table 3.5. Clearly, the values for the above ratios fell within the ranges corresponding to those on the broad maximum of the curves determined by Read (72, 74).

This calculation indicated that the low energy MS/MS spectrum for m/z 86 of 2-pentanone could be acquired at 50 eV collision energy without scanning of the acceleration lenses. Such a spectrum is shown in Figure 3.5; the relative intensities of the product ions corresponded well with low energy MS/MS spectra obtained on other types of tandem mass spectrometers at the same collision energy (31). Furthermore, the MS/MS sensitivity indicated that 12-20% of the unattenuated precursor ion (precursor ion present before addition of collision gas) could be collected as product ions. The MS/MS

TABLE 3.5

Calculation of kinetic energies of m/z 86 precursor ion from 2-pentanone and its m/z 43 product ion resulting from 50 eV collision energy.

M/Z	K.E. _{CC}	K.E. ₁	K.E. ₂	K.E. ₃	$\frac{K.E._2}{K.E._1}$	$\frac{K.E._3}{K.E._1}$
86	50 eV	530 eV	1350 eV	1980 eV	2.55	3.74
43	25 eV	495 eV	1325 eV	1955 eV	2.68	3.95

Notes:

K.E._{CC} is the kinetic energy of the ion in the collision cell.

K.E.₁ is the kinetic energy of the ion at accel. lens 1 as defined above.

K.E.₂ is the kinetic energy of the ion at accel. lens 2 as defined above.

K.E.₃ is the kinetic energy of the ion at accel. lens 3 as defined above.

Kinetic energy at each lens is assumed to be equal to (Collision cell potential - Potential applied to acceleration lens element) + (kinetic energy of ion in collision cell).

Potential applied to each lens element is listed below.

Collision cell potential = 6130 V

Accel. lens 1 potential = 5660 V

Accel. lens 2 potential = 4830 V

Accel. lens 3 potential = 4200 V

The kinetic energy ratios relevant to the operation of the acceleration lens are listed in the last two columns.

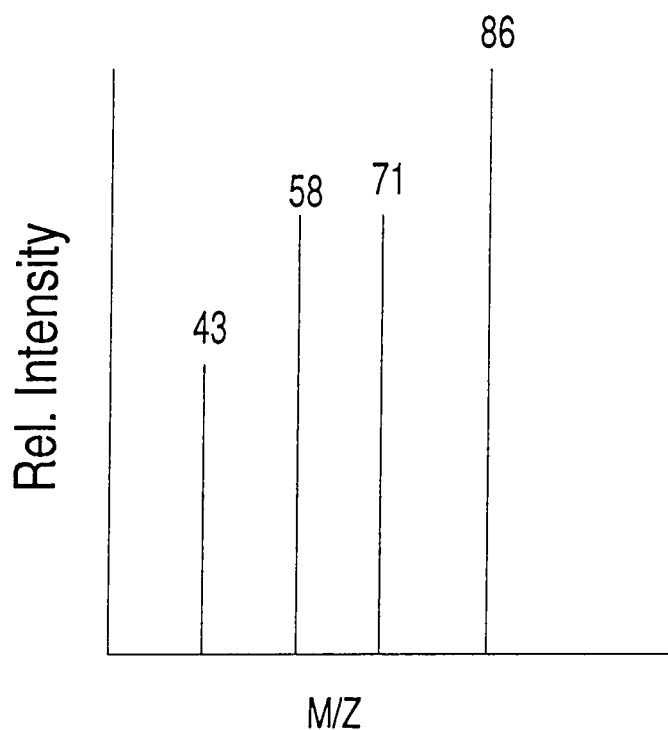


Figure 3.5. MS/MS spectrum of m/z 86 from 2-pentanone obtained on the QEB at 50 eV (50% attenuation of the precursor ion beam with argon collision gas, sample pressure = 1×10^{-6} torr). This MS/MS spectrum was acquired using the final lens system shown in Figures 3.3 and 3.4.

sensitivity is defined as (sum of product ion intensities/intensity of unattenuated precursor ion) and is also referred to as the dissociation efficiency of the precursor ion. Of course, this value depended on the collision energy utilized due to internal energy deposition considerations. Nevertheless, these values were much better than the values of 1% or less obtained for MS/MS sensitivity with the initial version of the lens system shown in Figure 3.2. Furthermore, proper design and operation of the entire lens system between the quadrupole and the source slit yielded much better MS/MS sensitivity while using less sample (8×10^{-7} torr vs. 3×10^{-5} torr) and lower electron emission from the filament (100 μ A vs. 500-1000 μ A). This design thus allowed the QEB to be operated under EI conditions which were comparable to those of other mass spectrometers. The successful lens system designs shown in Figures 3.3 and 3.4 were therefore utilized for collection of the QEB data discussed in this thesis.

CHAPTER 4

RESULTS AND DISCUSSION

3-METHYL-2-BUTANONE

QEB Results for m/z 71 ions of 3-methyl-2-Butanone

The fragmentation scheme shown in Figure 4.1 predicts that the keto isomer of the molecular ion of 3-methyl-2-butanone preferentially loses the C (1) methyl to form an acylium ion (A), while the enol form of the molecular ion preferentially loses the C(4) methyl group from the isopropyl side-chain to form protonated methyl vinyl ketone (MVK). This fragmentation scheme is based upon literature studies on this compound confirming the identification of the two m/z 71 ions by MS/MS techniques (7,26). Therefore, initial steps in differentiating between and quantifying the keto and enolic forms of the molecular ion of 3-methyl-2-butanone (3-MB) involved the investigation of the MS/MS dissociation characteristics of diagnostic m/z 71 fragment ions formed from the keto and enol forms. Previous MS/MS studies indicated that these isomeric m/z 71 ions lose C₂H₄ and/or CO to form the possible isobaric product ions of m/z 43, C₃H₇⁺ (43H, exact mass = 43.0546 amu) and/or C₂H₃O⁺ (43L, exact mass = 43.0183 amu) (7, 23, 26, 29, 83). These previous studies indicated exclusive formation of 43H from A, while MVK formed both 43H (minor) and 43L (major).

Nevertheless, the behavior of A and MVK must be studied under the experimental conditions employed for this thesis. On the QEB, source-generated m/z 71 ions can be

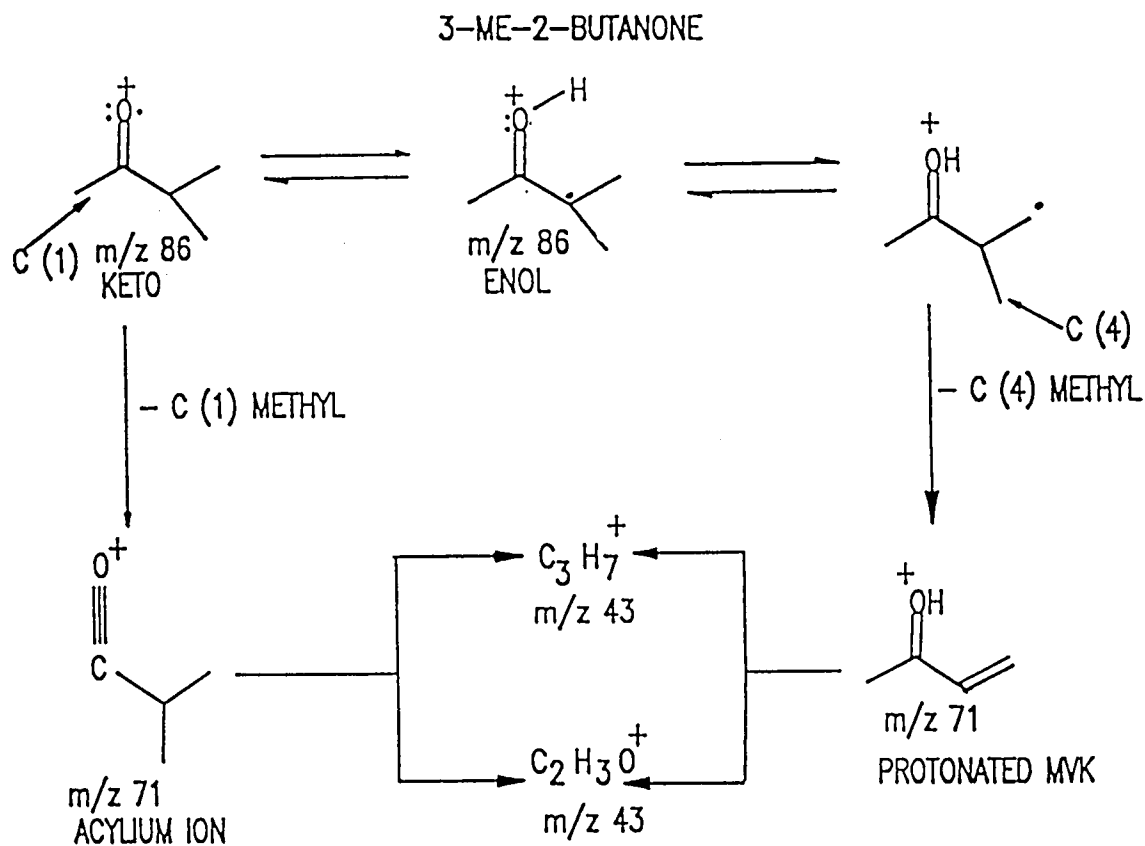


Figure 4.1. Fragmentation scheme for 3-methyl-2-butanone (3-MB), previously shown in Figure 1.3, showing formation of acylium ion (A) at m/z 71 from loss of the C(1) methyl and formation of protonated methyl vinyl ketone (MVK) at m/z 71 by loss of the C(4) methyl. The m/z 71 ions, in turn, can be characterized by observing the production of $C_3H_7^+$ (43H; exact mass = 43.0546) and/or $C_2H_3O^+$ (43L; exact mass = 43.0183) in MS/MS experiments. Based on the difference in exact mass of 43H and 43L (0.0363 amu), a mass resolution of 1200 is needed to resolve these isobars.

selected with the quadrupole (Q), subjected to low or high energy CID, followed by high resolution analyses of the resulting product ions. The m/z 71 ions generated from 3-MB (hereafter referred to as 3-MB m/z 71 ions) can be characterized by the relative proportions of 43H and 43L generated from MS/MS of the 3-MB m/z 71 ions. Based on the previous MS/MS results discussed above, it may be postulated that if only 43H forms from QEB MS/MS experiments of 3-MB m/z 71 ions, the composition of the m/z 71 ion would be entirely A. On the other hand, if both 43H and 43L form from 3-MB m/z 71 ions as a result of QEB MS/MS experiments, the composition of the 3-MB m/z 71 ions may be a mixture of A and MVK. With these ideas in mind, MS/MS experiments were performed on the QEB to determine the behavior of the m/z 71 ions.

The low energy CID results obtained for the 3-MB m/z 71 ions fragmenting to m/z 43 over the collision energy range 5-50 eV are shown in Figure 4.2. Over this collision energy range, the product ion ratio at m/z 43 (43H:43L) remained constant, within experimental error, at 4.27 ± 0.214 (81% 43H : 19% 43L, $\pm 4.8\%$). Since the ratio remained constant, the two m/z 71 ions were not isomerizing as a result of collisional activation under the low energy MS/MS conditions utilized on the QEB. This does not preclude, however, the possibility that the m/z 71 ions may undergo rapid isomerization within the source before they are subjected to low energy CID. However, it has been demonstrated that A and MVK do not equilibrate on the μsec time-scale since distinct MS/MS spectra are obtained from the individual A and MVK m/z 71 ions (7, 13, 29, 83).

The 43H:43L ratios stated above were the raw data values obtained from the low energy MS/MS of 3-MB m/z 71 source-generated ions. In order to accurately quantitate

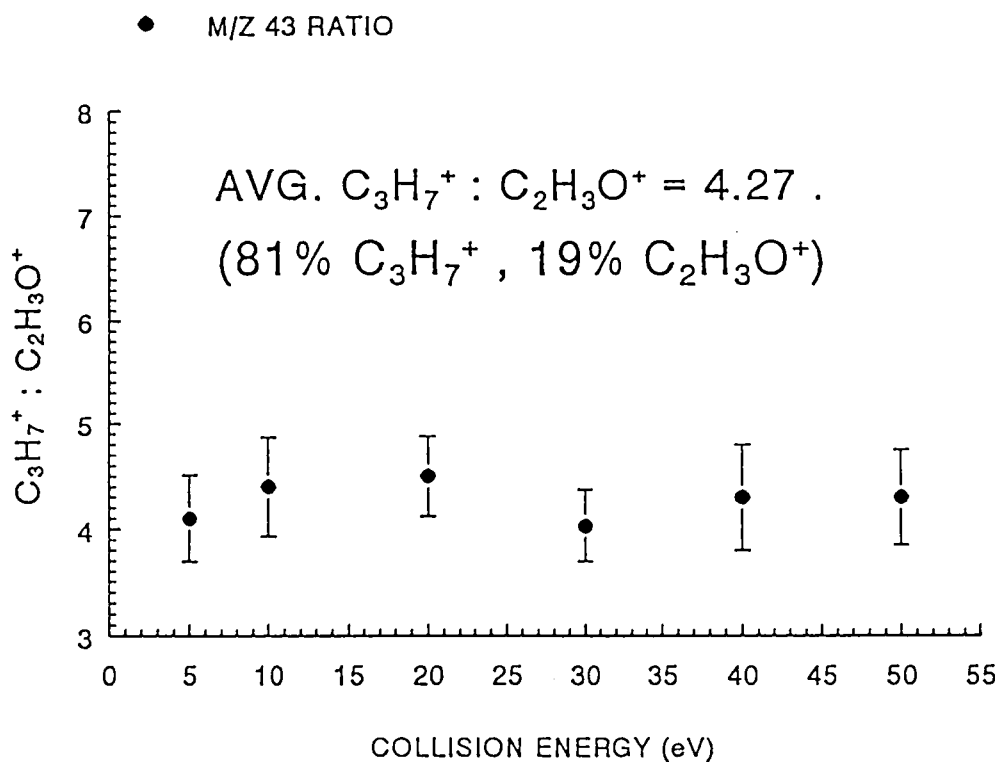


Figure 4.2. Behavior of the 3-MB m/z 71 ions under low energy CID conditions on the QEB. Argon collision gas was utilized to attenuate the precursor ion intensity by 50-70%. The m/z 43 product ions were resolved by fixing the magnet to pass m/z 43 and scanning the accelerating voltage over a narrow range. Ionization conditions for 3-MB: 70 eV electron energy, sample pressure of 8×10^{-7} torr.

the two m/z 71 ions present, it is necessary to develop an approach which weights the relative dissociation efficiency of the two m/z 71 ions under the MS/MS conditions employed for the 3-MB m/z 71 ions. The measured (raw data) value stated above would be equal to the actual proportion of the two 3-MB m/z 71 ions at the collision cell if, and only if, *the two ions dissociated to the same extent under the MS/MS conditions employed for collection of the raw data*. The characteristics of the MVK and A reactions provided insight into this problem. As indicated in Figure 4.3, the reaction of A to produce 43H is a direct cleavage reaction, while the MVK reaction involves a rearrangement for formation of 43L and rearrangement (probably to the acylium ion) to form 43H. Furthermore, the reaction of A is of lower endothermicity than the MVK reactions, indicating that with its lower critical energy (83), it will be favored at lower internal energies. Since the rate of the direct cleavage reaction (acylium ion reaction) would increase faster with increases in internal energy, it would be continue to be favored with increasing internal energies. These factors indicated that the raw data may not accurately reflect the true composition of the m/z 71 ions reaching the collision cell since A may dissociate more efficiently than MVK under the MS/MS conditions employed.

For the 3-MB m/z 71 ions, attempts to determine if the reaction of A was more favorable than the MVK reaction within the low energy collision cell were complicated by the fact that a *mixture of A and MVK* reached the collision cell when produced from 3-MB. In order to determine if A indeed reacts preferentially, the characteristics of the *pure, individual* A and MVK reference ions were examined. In Figure 4.4, typical methods used for generation of the A and MVK reference ions is shown (7, 26, 29, 83).

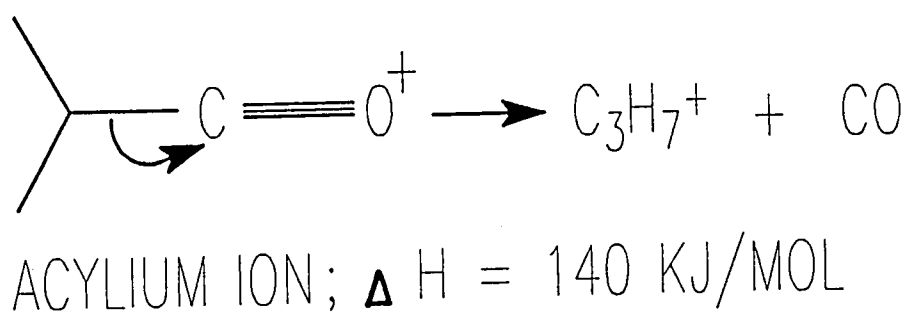
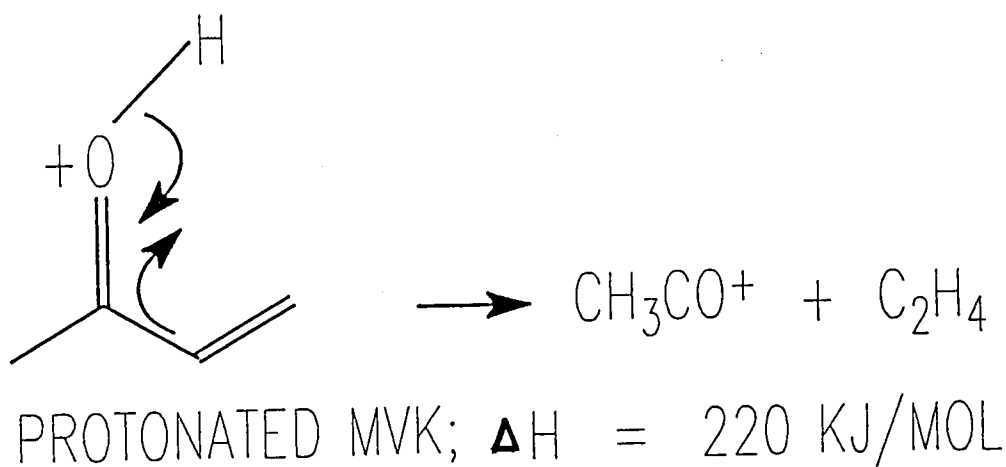
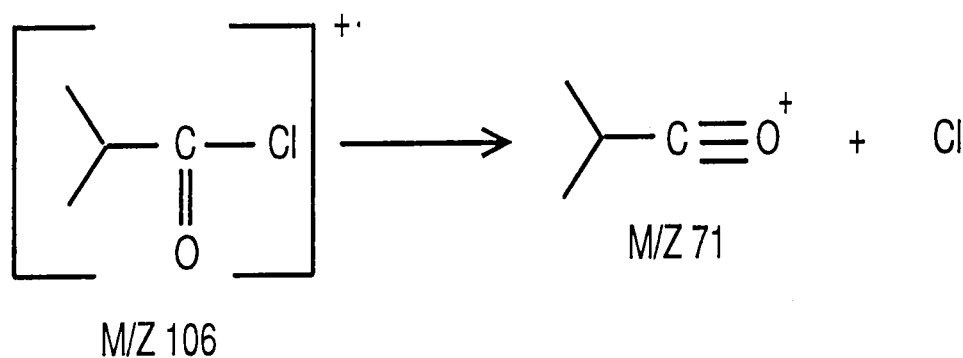


Figure 4.3. Fragmentation of MVK and A to yield 43L and 43H, respectively. The predominant fragmentation pathway for MVK, based on literature studies, is shown. The figure indicates that the MVK reaction, in addition to being a rearrangement reaction, is more endothermic than the reaction of A. The reaction of A would be expected to be more favorable.

REFERENCE ION GENERATION

1. ACYLIUM ION (A)



2. PROTONATED METHYL VINYL KETONE (MVK)

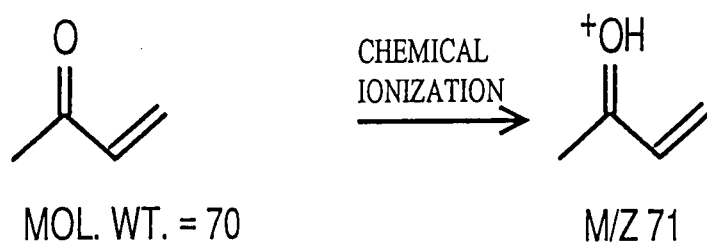


Figure 4.4. Generation of the A and MVK reference ions. A is generated by 70 eV dissociative EI of isobutyryl chloride. MVK is generated by "self-CI" of the neutral ketone. For "self-CI", the neutral pressure is 1×10^{-5} torr; 70 eV electron energy is used for this process. This "self-CI" process is exothermic by 5-6 kJ/mol (9, 10, 29).

Following formation of each reference ion in its pure form in the ion source, the appropriate reference ion, mass selected by the quadrupole, was subjected to low energy CID over the range of 5-50 eV.

As shown in Figure 4.5, the relative proportions of the isobaric product ions at m/z 43 is very different for the A and MVK reference ions. Over the collision energy range employed (5-50 eV), A produced solely 43H. MVK, on the other hand, produced 43H and 43L over this collision energy range. The behavior of MVK is shown in Figure 4.6. The characteristic behavior of the A and MVK reference ions indicates that they are not equilibrating with one another as a result of collisional activation under the MS/MS conditions employed on the QEB; the same conclusion was reached earlier for the 3-MB m/z 71 ions. Furthermore, the data in Figure 4.2 indicates that the balance between production of 43H and 43L from 3-MB m/z 71 ions is not affected by collision energy variations. The same is true for the A and MVK reference ions. The characteristic behavior of the A and MVK reference ions and 3-MB m/z 71 ions was used advantageously to analyze the mixture of the 3-MB m/z 71 ions. Since the m/z 71 reference ions produce unique ratios of 43H:43L, the displayed ratio for the 3-MB m/z 71 ions is a convolution of the behavior of these reference ions. The system discussed here resembled the spectroscopic method of mixture analysis by Beer's law in that the total signal present was a weighted sum of the contributions resulting from the components comprising the mixture. In the Beer's law experiment, the weighting factor arises from experiments performed on standards which allow determination of the probability of absorption (ϵ) for a given species. An analogous procedure should also be

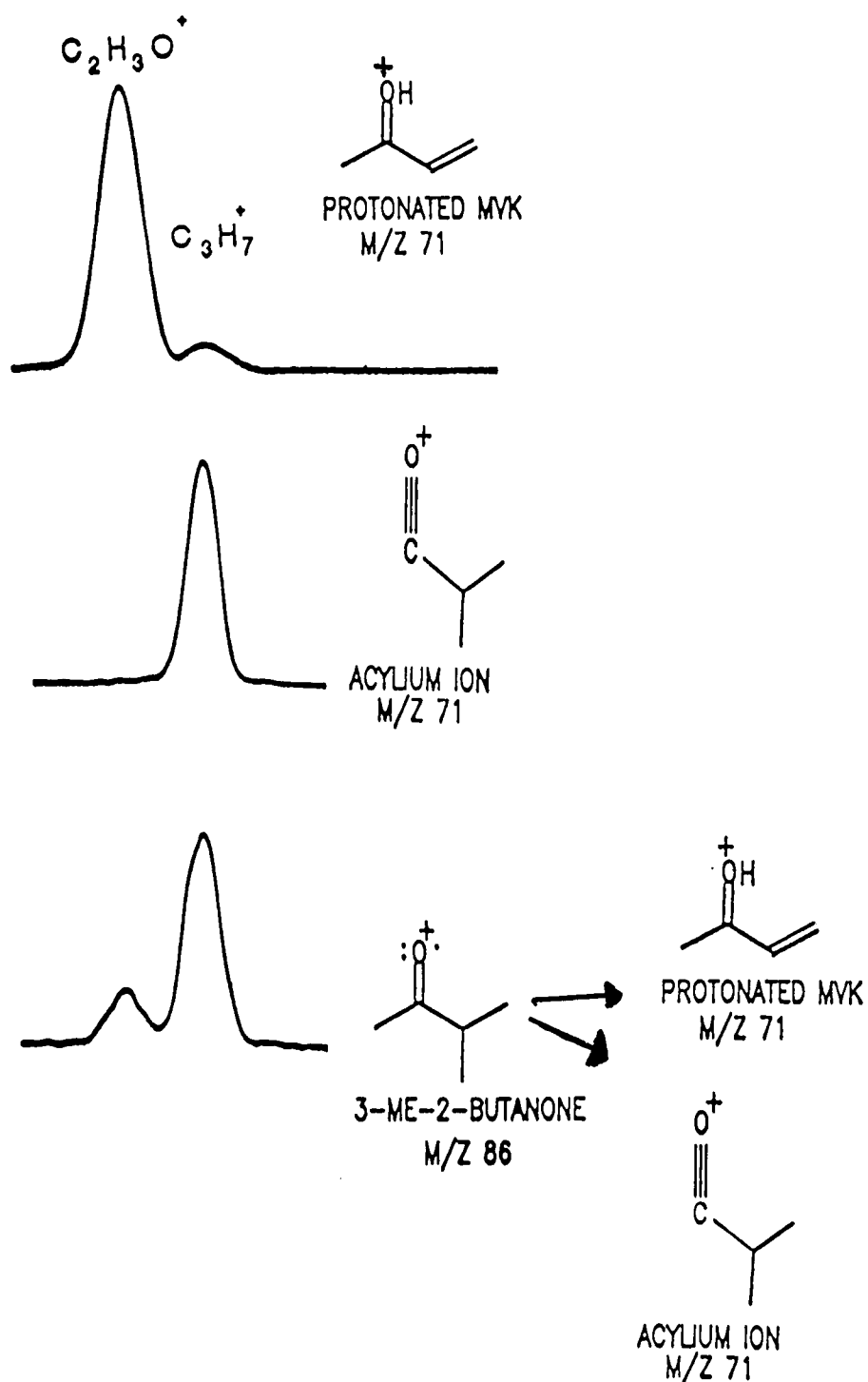


Figure 4.5. Typical peak profiles obtained for MS/MS of m/z 71 reference ions and 3-MB m/z 71 ions yielding m/z 43 product ion(s) under low energy MS/MS conditions on the QEB. Argon collision gas was used to attenuate each m/z 71 precursor ion by 50-70%. The peak profiles shown were generated at 30 eV collision energy. Reference ions were generated as shown in figure 4.4.

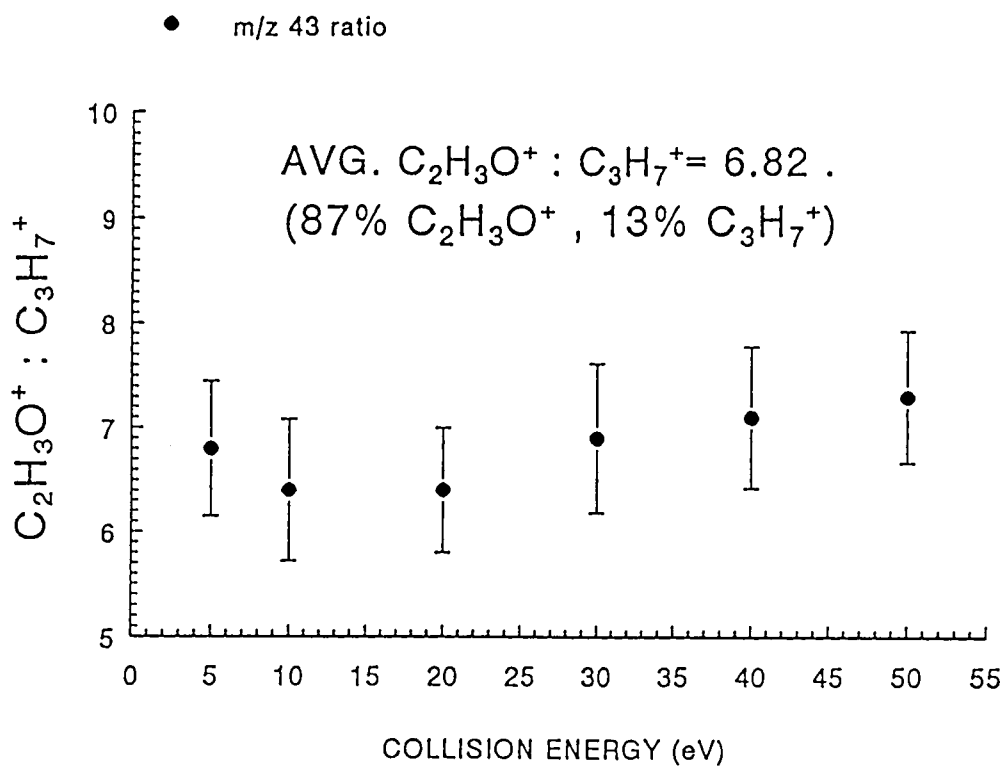


Figure 4.6. 43L:43H product ion ratio generated by CID of the MVK m/z 71 reference ion over the collision energy range of 5-50 eV on the QEB. Argon collision gas was utilized to attenuate the precursor ion intensity by 50-70%.

applicable to the ionic system here if the probability of dissociation of the A and MVK reference ions can be ascertained.

The dissociation probability (efficiency) of the two m/z 71 ions was determined by observing the dissociation characteristics of the m/z 71 reference ions. The dissociation efficiency was defined as:

$$\epsilon = \frac{\text{(intensity of product ion from CID of ref. ion)}}{\text{(unattenuated precursor ion intensity)}} \quad \text{Eq. 4.1}$$

The unattenuated precursor ion intensity refers to the precursor ion intensity in the absence of collision gas. Experiments described above indicated that the collision energy did not influence the ratios of 43H and 43L formed from the reference ions or the 3-MB generated m/z 71 ions. However, the probability of dissociation as defined above will be sensitive to collision gas pressure since the cross section for production of product ions is pressure dependent (45-47). In experiments conducted here, the initial precursor ion intensity was attenuated by 50-70% with argon collision gas to yield the product ion of interest. Therefore, to use equation 4.1 for the different precursors, one would have to assume that the two m/z 71 reference ions (A and MVK) and the 3-MB m/z 71 ions undergo 50% attenuation at the same pressure. For this to be the case, the scattering cross-section of the m/z 71 ions should be similar since scattering is the major cause of loss of ions in CID. For isomeric ions such as these m/z 71 ions, the scattering cross sections are generally quite similar (46, 83, 84). Using the ratio as defined in equation

4.1 is preferable since it is difficult to exactly reproduce analyte pressures, electron current and energy and collision gas pressures from sample to sample. Using an alternative ratio of (product ion intensity)/(residual precursor ion remaining after attenuation) would make the results much more uncertain since the residual precursor ion remaining would be quite sensitive to the above mentioned variables. The definition proposed above is preferable since it would not be as sensitive to such variables when constant percentage attenuation is utilized from sample to sample and this attenuation is reproducible. Since this was rather easily achieved experimentally, it was felt that the definition proposed above was valid.

For MS/MS experiments, collision gas pressure is certainly important since each individual collision which a precursor ion experiences increases its internal energy. An equally important consideration is the effect of the internal energy of the precursor ion before MS/MS activation. This may be of importance here since the A and MVK reference ions are prepared as pure standards in one case and are generated as a mixture from 3-MB in another case. These different methods of preparation may not allow very precise control over the internal energy content of the precursor ion before activation. An estimate of the precursor ion internal energy before activation was obtained by observing formation of 43H and 43L from A, MVK, and 3-MB m/z 71 ions in the absence of collision gas. These experiments demonstrated very low ion yields; signal intensities of 43H and 43L were only 4-4.7 times greater than background noise, in contrast to the signal to noise ratio of 10-100 : 1 observed in Figure 4.5. for production of 43H and 43L by CID of the various m/z 71 ions. At equal collision pressures (ca. 2

mtorr), the percentage attenuation (70-80%) for the m/z 71 isomers is the same within $\pm 10\%$, as is expected for isomeric ions like MVK and A (46, 83, 84). The formation of 43H and 43L do indeed result from internal energy deposition via collisional activation of A, MVK, or 3-MB m/z 71 ions and show little dependence on the method of preparation or the internal energy of the precursor ion. This behavior should improve the precision and accuracy of determination of the dissociation probability since internal energy of the precursor ion, perhaps not easily controlled, does not affect product ion yield significantly. This insensitivity of 43H and 43L formation to precursor ion internal energy may also contribute to the nonvariant 43H:43L ratios obtained from A, MVK, and 3-MB m/z 71 ions.

After initial observation of the characteristic low energy CID behavior of A, MVK, and the 3-MB m/z 71 ions, it seemed entirely plausible that the MS/MS spectrum of the 3-MB m/z 71 ions, being a mixture of unknown composition of A and MVK, was simply a linear superposition of the MS/MS spectra of A and MVK. As with Beer's law, the total signal of interest from the mixture is the weighted sum of the individual components which comprise that signal of interest. The weighting factor used in this case is the probability of dissociation (ϵ), defined in equation 4.1, as derived from the A and MVK reference ions. The theory of linear superposition of MS/MS spectra has been discussed by McLafferty and coworkers (84, 87, 88), Bass and Bowers (89), and Kim and coworkers (85, 86) and is currently of great interest in mass spectrometry for the analysis of mixtures of isomeric ions.

Application of the Linear Superposition Principle

The application of the linear superposition principle has several criteria or assumptions which must be met if it is to yield reliable results (84-89). For a mixture of two isomers C and D, the following assumptions have been made by previous investigators to derive expressions for the isomeric composition: (i) each of the isomers C and D can be generated in pure form in the ion source; (ii) the relative peak heights in a given CID spectrum correspond directly to the relative numbers of fragment ions produced at each m/z value; (iii) the CID spectrum for a given isomer is independent of the internal energy content of the isomer; (iv) no isomerization occurs between the time of formation of a given ion and the time at which CID occurs; (v) relevant peak heights in the CID spectrum of each isomer can be measured accurately; (vi) the number of ions of each structure which undergoes CID is representative of the number of ions of each structure which is present in the collision cell. The previous discussion has demonstrated that all of these assumptions are valid for the system studied here, but assumption (vi) must be addressed carefully. Assumption (vi) dictates that the CID cross-sections for the two isomers be the same; McLafferty and coworkers have pointed out that this cannot be assumed but must be tested experimentally. A method for testing this assumption experimentally is outlined below.

Many equations have been proposed and utilized for determining the composition of an ionic mixture using the linear superposition principle (84-89). A series of simple equations were developed and used for determining the composition of the 3-MB m/z 71

ions. The steps involved are shown below.

The intensity of a particular product ion is, according to the linear superposition principle, the weighted sum of the individual components which comprise that signal of interest. For the system under study, this can be expressed by the following equations:

$$I_{43H} = [A]\epsilon_{A,43H} + [MVK]\epsilon_{MVK,43H} \quad \text{Eq. 4.2}$$

$$I_{43L} = [A]\epsilon_{A,43L} + [MVK]\epsilon_{MVK,43L} \quad \text{Eq. 4.3}$$

I_{43H} = Intensity of 43H generated from MS/MS of 3-MB m/z 71 ions.

$\epsilon_{A,43H}$ = Probability of dissociation of A to form 43H.

$\epsilon_{MVK,43H}$ = Probability of dissociation of MVK to form 43H.

I_{43L} = Intensity of 43L generated from MS/MS of 3-MB m/z 71 ions.

$\epsilon_{A,43L}$ = Probability of dissociation of A to form 43L.

$\epsilon_{MVK,43L}$ = Probability of dissociation of MVK to form 43L.

[A] = Unknown concentration of A in the mixture of m/z 71 ions from 3-MB.

[MVK] = Unknown concentration of MVK in the mixture of m/z 71 ions from 3-MB.

These equations can be simplified for the system studied by noting the behavior of the two reference ions on the QEB. Figure 4.5 shows that the acylium ion (A) produces no 43L under low energy CID conditions; therefore, $\epsilon_{A,43L} = 0$. Equation 4.3 then reduces to:

$$I_{43L} = [MVK]\epsilon_{MVK,43L} \quad \text{Eq. 4.4}$$

The relevant equations for quantitation of the 3-MB m/z 71 ions are:

$$I_{43H} = [A]\epsilon_{A,43H} + [MVK]\epsilon_{MVK,43H} \quad \text{Eq. 4.2}$$

$$I_{43L} = [MVK]\epsilon_{MVK,43L} \quad \text{Eq. 4.4}$$

Dividing Eq. 4.2 by Eq. 4.4 yields the following result:

$$\frac{I_{43H}}{I_{43L}} = \frac{[A]\epsilon_{A,43H} + [MVK]\epsilon_{MVK,43H}}{[MVK]\epsilon_{MVK,43L}} \quad \text{Eq. 4.5}$$

Rearrangement of Eq. 4.5 yields an expression for $[A]/[MVK]$; quantitation of the 3-MB m/z 71 ions is then possible utilizing Eq. 4.6:

$$\frac{(I_{43H}/I_{43L})(\epsilon_{MVK,43L}) - \epsilon_{MVK,43H}}{\epsilon_{A,43H}} = [A]/[MVK] \quad \text{Eq. 4.6}$$

The quantities on the left hand side of Eq. 4.6 can be measured experimentally. The term I_{43H}/I_{43L} is the experimentally determined ratio of product ions produced from CID of the ionic mixture having unknown composition (the 3-MB m/z 71 ions in this case). The ratio I_{43H}/I_{43L} was presented earlier in Figure 4.2 as a function of the QEB collision energy over the range of 5-50 eV. The ϵ values in Eq. 4.6 are determined in

separate CID experiments utilizing reference ions (ions known to possess the A or MVK structure). Figure 4.7 indicates the values of $\epsilon_{A,43H}$ obtained over the low energy collision range studied on the QEB. Since $\epsilon_{A,43L} = 0$, the total probability for A forming an m/z 43 product ion upon CID (i.e., $\epsilon_{A,43L + 43H}$) is then $\epsilon_{A,43H}$. Clearly, $\epsilon_{A,43L + 43H} = \epsilon_{A,43L} + \epsilon_{A,43H}$; the total probability is the sum of the individual probabilities which can give rise to the event in question. Realizing this relationship aids in dealing with cases where one of the individual probabilities is nonzero, as is the case for MVK. Figure 4.8 shows that the $\epsilon_{MVK,43L + 43H}$ (probability for total production of m/z 43 product ions from MVK) is only about 2%; Figure 4.6 further shows that MVK produces a relative proportion of only about 13% 43H under low energy CID conditions, so $\epsilon_{MVK,43H}$ is then $(0.02)(0.13) = 0.0026 = 0.26\%$. Furthermore, since MVK produces 87% 43L, $\epsilon_{MVK,43L} = (0.87)(0.02) = 0.0174 = 1.74\%$. Note that equation 4.6 demands determination of both $\epsilon_{MVK,43L}$ and $\epsilon_{MVK,43H}$. Based on data presented earlier in this chapter, this is a convenient and easy way of finding these ϵ_{MVK} values. Further calculations regarding quantitation of the 3-MB m/z 71 ions are presented in table 4.1.

Table 4.1 indicates the composition of the 3-MB m/z 71 ions as a function of QEB low energy collision energy. The average composition of these ions is 48.5% A, 51.5% MVK ($\pm 0.74\%$); the composition shows no great systematic dependence on the collision energy. Therefore, no equilibration of the m/z 71 ions appears to be occurring as a result of the collisional activation event, thus making the linear superposition principle easier to apply. The data in table 4.1 also demonstrates that the total CID efficiency of MVK (denoted as $\epsilon_{MVK,43T}$ in table 4.1) is less than that of A even at 50 eV collision energy.

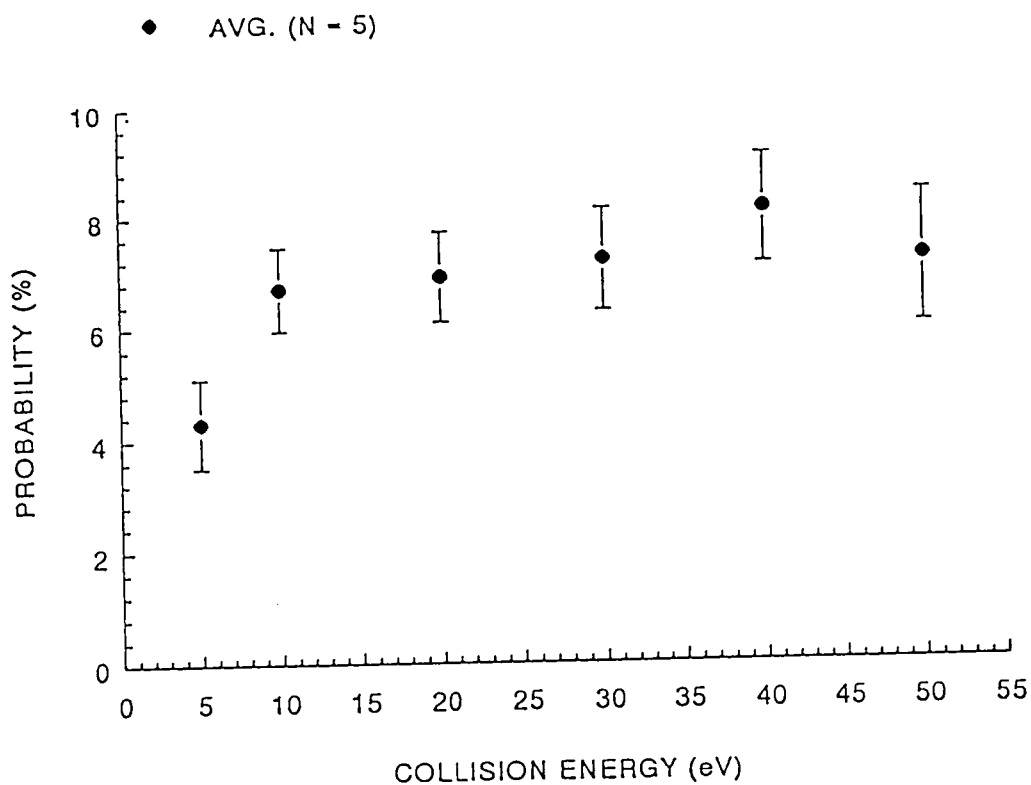


Figure 4.7. Acylium reference ion (A) MS/MS dissociation probability ($\epsilon_{A,43H}$) measured on the QEB under low energy CID conditions. MS/MS conditions included 50-70% attenuation of the precursor ion with argon collision gas. The experimental determination of $\epsilon_{A,43H}$ is outlined in the text and is defined in equation 4.1. The acylium reference ion was generated as shown in Figure 4.4.

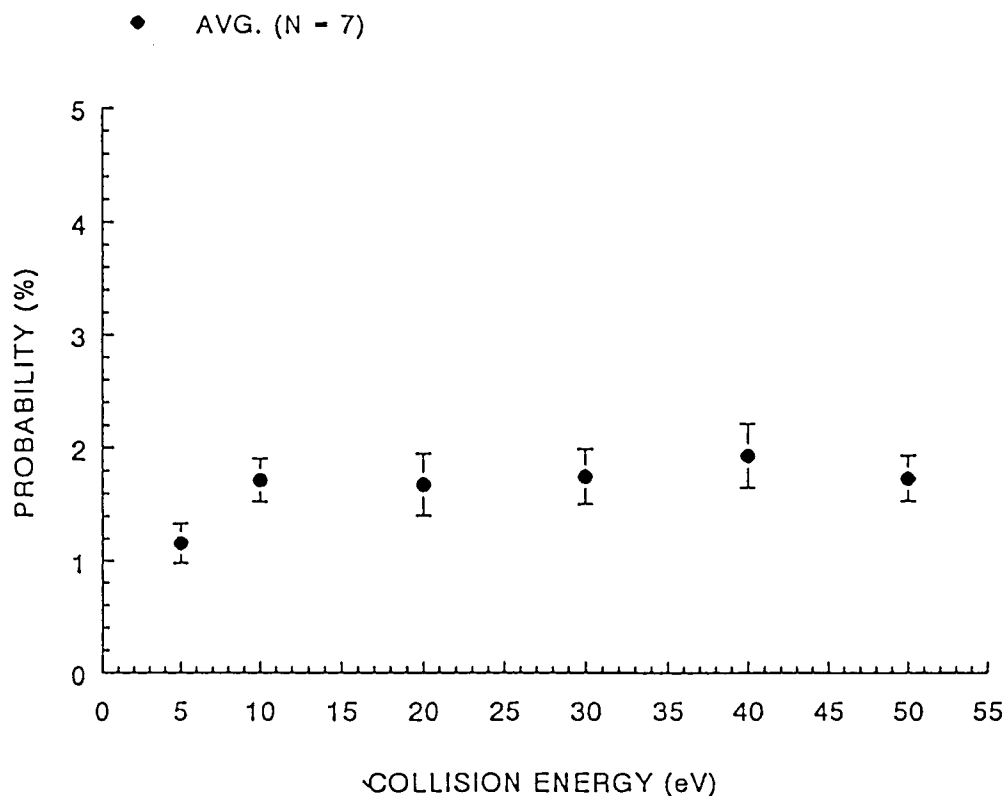


Figure 4.8. Protonated methyl vinyl ketone (MVK) reference ion MS/MS dissociation probability ($\epsilon_{\text{MVK}, 43\text{H} + 43\text{L}}$) measured on the QEB under low energy CID conditions. This definition accounts for the total production of m/z 43 product ions from MVK. MS/MS conditions included 50-70% attenuation of the precursor ion with argon collision gas. The experimental determination of $\epsilon_{\text{MVK}, 43\text{H} + 43\text{L}}$ is outlined in the text. The MVK reference ion was generated as shown in Figure 4.4.

TABLE 4.1

Quantitation of 3-MB m/z 71 ions as a function of low energy collision energy on the QEB.

C.E. ^A	$\epsilon_{A, 43H}$	$\epsilon_{MVK, 43T}$ ^B	43R ^C	$\epsilon_{MVK, 43H}$	$\epsilon_{MVK, 43L}$	I_{43H}/I_{43L}	[A]/[MVK]
5 eV	4.37%	1.20%	6.82	0.154%	1.05%	4.10	0.949
10 eV	6.67%	1.72%	6.38	0.234%	1.49%	4.37	0.941
20 eV	6.63%	1.64%	6.39	0.221%	1.42%	4.51	0.933
30 eV	6.78%	1.80%	6.85	0.229%	1.57%	4.02	0.897
40 eV	7.81%	2.06%	7.03	0.258%	1.80%	4.25	0.946
50 eV	7.17%	1.97%	7.21	0.240%	1.73%	4.22	0.985

^Acollision energy

^BTotal production of m/z 43 product ions from MVK; also referred to as $\epsilon_{MVK, 43H + 43L}$.

^C43L : 43H ratio obtained from CID of MVK.

Notes:

The values listed for the various ϵ terms, 43R, and I_{43H}/I_{43L} are averages of 5-7 determinations.

Based on above results, $([A]/[MVK])_{AVG.} = 0.942$ corresponding to 48.5% A, 51.5% MVK ($\pm 0.74\%$).

The suspected disparity between the MS/MS raw data and the true proportion of the m/z 71 ions at the low energy collision cell due to factors pointed out above (refer to Figure 4.3) controlling relative rates of reactions proved to be true.

It was suspected that the higher barrier to dissociation of the MVK reaction coupled with the fact that it was also a rearrangement process may have resulted in a kinetic shift for the MVK reaction. That is, internal energy in excess of the minimum required for reaction would be needed for the reaction to occur within the time-frame of the mass spectrometric experiment (41, 45). At the lower internal energy depositions encountered in low energy CID and given the μsec time-scale upon which the QEB operates, this kinetic shift phenomenon may prevent the MVK reaction from being competitive with the dissociation of A. Although the direct cleavage of A should be faster at all collision energies, the disparity between A and MVK CID efficiency should diminish at very high collision energies. Thus, if the composition of the 3-MB m/z 71 ions is truly about a 1:1 proportion of A:MVK, the probability of dissociation (ϵ) of A and MVK should be similar at high energy CID. Consequently, the *uncorrected* ratio 43H:43L should be near 1 for high energy CID of the 3-MB m/z 71 ions.

QEB High Energy CID Results for m/z 71 ions of 3-MB

Application of the linear superposition of MS/MS spectra to the high energy MS/MS results may also confirm the proportion of m/z 71 ions determined above by low energy MS/MS. The m/z 71 reference ions were prepared in the same manner as for the

low energy MS/MS experiments (Figure 4.4). The observed behavior of the m/z 71 reference ions and the 3-MB m/z 71 ions from high energy MS/MS is shown in Figure 4.9. Their dissociation efficiencies were measured and defined as above and are shown in table 4.2. Application of the linear superposition is again performed using equations 4.2 and 4.3:

$$I_{43H} = [A]\epsilon_{A,43H} + [MVK]\epsilon_{MVK,43H} \quad \text{Eq. 4.2}$$

$$I_{43L} = [A]\epsilon_{A,43L} + [MVK]\epsilon_{MVK,43L} \quad \text{Eq. 4.3}$$

As done previously for the low energy CID data analysis, division of Eq. 4.2 by Eq. 4.3, followed by rearrangement of the resulting equation along with subsequent separation of variables leads to the following expression $[A]/[MVK]$:

$$\frac{(I_{43H}/I_{43L})(\epsilon_{MVK,43L}) - \epsilon_{MVK,43H}}{\epsilon_{A,43H} - (I_{43H}/I_{43L})(\epsilon_{A,43L})} = [A]/[MVK] \quad \text{Eq. 4.7}$$

Note that Eq. 4.7 reduces to Eq. 4.6 in cases where $\epsilon_{A,43L} = 0$; this situation occurred for the QEB low energy CID results discussed above. However, the acylium ion, A, produces a small, yet measurable, amount of 43L under high energy CID conditions so Eq. 4.7 is used for analyzing these data. The results of this analysis are shown in table 4.2.

The agreement between the QEB high energy and low energy CID results is quite

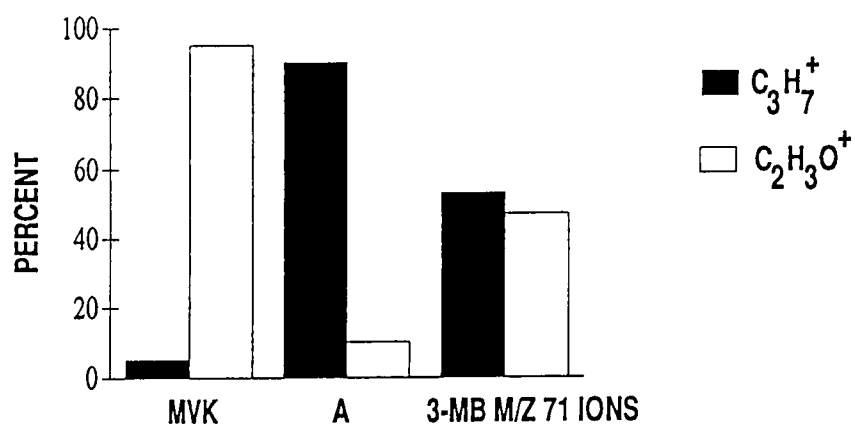


Figure 4.9. Behavior of m/z 71 ions for the 3-MB system under high energy CID conditions on the QEB. From left to right, results are shown for protonated MVK reference ion (MVK), the acylium reference ion (A), and the 3-MB m/z 71 ions. The collision energy was 6.2 keV and argon collision gas was used to attenuate the precursor ion beam by 70%.

TABLE 4.2

Behavior of m/z 71 reference ions (A and MVK) and 3-MB m/z 71 ions under high energy CID conditions on the QEB. The collision energy was nominally 6 keV utilizing argon collision gas to attenuate the precursor ion by 70%. The 43H : 43L ratios stated below vary by 5-7%.

COMPOUND	$\epsilon_{X, 43H + 43L}^*$	43H : 43L
A	1.2% ($\pm 0.11\%$)	95 : 5
MVK	1.1% ($\pm 0.091\%$)	10 : 90
3-MB	Not applicable	53 : 47

*Probability for total production of m/z 43 product ions from X, where X is either MVK or A.

Notes:

$$\epsilon_{A, 43L} = (1.2\%)(0.05) = 0.06\%$$

$$\epsilon_{A, 43H} = (1.2\%)(0.95) = 1.14\%$$

$$\epsilon_{MVK, 43L} = (1.1\%)(0.90) = 0.99\%$$

$$\epsilon_{MVK, 43H} = (1.1\%)(0.10) = 0.11\%$$

$$I_{43H}/I_{43L} = 1.13$$

Substitution of these values into equation 4.7 (see text) yields:

$$[A]/[MVK] = 0.94$$

$$= 48.5\% \text{ A, } 51.5\% \text{ MVK } (\pm 6.2\%)$$

remarkable after the linear superposition is applied to both sets of data; identical results are obtained for the composition of the 3-MB m/z 71 ions. The low energy CID results have thus been confirmed by the high energy MS/MS results which, due to its higher internal energy deposition, allowed the more endothermic MVK rearrangement reaction to attain a CID efficiency (ϵ) nearly equal to that of the acylium ion (A) direct cleavage reaction.

QEB MS/MS Results on M/Z 86 of 3-Methyl-2-Butanone

Even assuming that each m/z 71 ion has a unique precursor (i.e., MVK from enol and A from keto), the proportion of the m/z 71 ions determined above does not necessarily represent the proportion of keto and enol molecular ions at m/z 86. The composition of the 3-MB m/z 71 ions is 51.5% MVK, 48.5% A after accounting for the probability of dissociation for MVK and A under the various CID conditions employed. MVK and A decompose equally only at the very high internal energy depositions characteristic of high energy CID. Since the 3-MB m/z 71 ions are fragment ions generated from the original molecular ions, their internal energy content, immediately after formation in the ion source, is not expected to be as great as those of the originally formed molecular ions (41). The low energy CID results, where internal energy of the fragmenting ions is lower, indicate preferential fragmentation of A. Therefore, A formed from 3-MB itself would decompose more facilely than MVK formed from 3-MB, leading to a measured ratio of m/z 71 ions which does not accurately reflect the composition of

the ionic precursors from which A and MVK arose (i.e., keto and enol, respectively).

In order to determine the correct composition of the molecular ion, MS/MS experiments were performed on the molecular ion of 3-MB (m/z 86). Due to the finer control of internal energy deposition available in low energy MS/MS experiments, the behavior of m/z 86 from 3-MB was investigated over the collision energy range of 5-50 eV. Information will allow ions to be assigned as arising from either the keto or enol form of the molecular ion to allow quantitation of the two forms.

A typical MS/MS spectrum for m/z 86 of 3-MB is shown in Figure 4.10. Over the collision energy range of 5-50 eV, fragment ions of m/z 71, m/z 58, and m/z 43 (43H and 43L) were produced. In order to assess the composition of m/z 86, it is necessary to determine whether the fragment ions above resulted from the keto or enol form of the molecular ion. The formation of m/z 58 occurs via a McLafferty rearrangement of the molecular ion and can be attributed to the keto form of the molecular ion (7, 15, 23, 26). In order to assign the m/z 71 and m/z 43 peaks as arising from either the keto or enol form the behavior of these ions across the collision energy range was examined.

Figure 4.11 shows how the ratio of 71:43H varied with collision energy. Above 20 eV collision energy, it is noted that this ratio attains a value of 1.10. Since a possible source of 43H is from decomposition of the acylium ion, it was of interest to compare the 71:43H ratio in Figure 4.11 (above 20 eV) to the value of MVK:A found for the 3-MB m/z 71 ions determined above. Upon making this comparison, it was noted that the two ratios were nearly identical. This may be consistent with the fact that the acylium ion, after its formation from the keto molecular ion within the low energy collision cell,

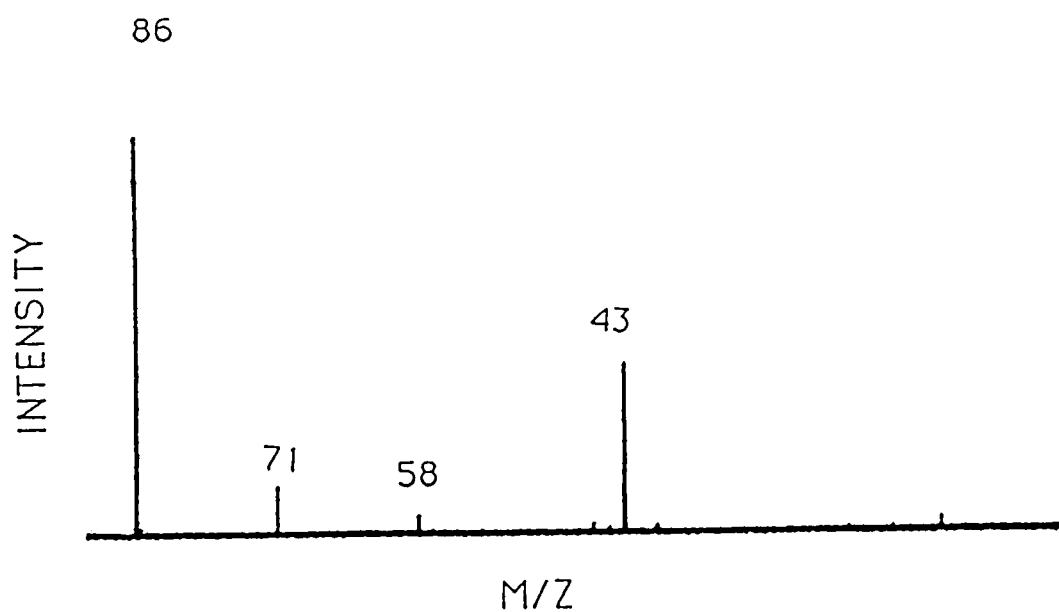


Figure 4.10. A typical MS/MS spectrum obtained for m/z 86 from 3-methyl-2-butanone (3-MB) on the QEB under low energy CID conditions. CID conditions for this spectrum include 60% attenuation of the precursor ion with argon collision gas at 30 eV collision energy. For 3-MB, sample pressure = 1×10^{-6} torr, while an electron energy of 70 eV was employed.

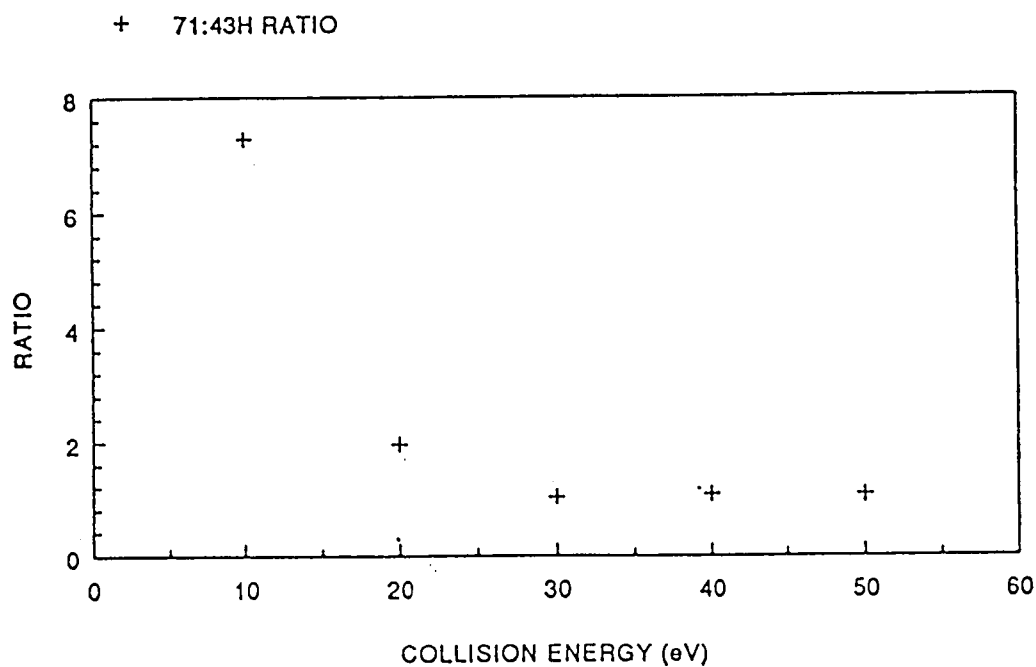


Figure 4.11. Ratio of total m/z 71 ion signal intensity : 43H signal intensity obtained from low energy CID of m/z 86 from 3-MB on the QEB. CID conditions: 50-70% attenuation of precursor ion with argon collision gas. For 3-MB, sample pressure = 1×10^{-6} torr, while an electron energy of 70 eV was employed.

undergoes further decomposition to form 43H at collision energies above 20 eV.

Alternatively, 43H can form by direct cleavage of the keto form. This pathway is the highest energy pathway for direct cleavage of the keto form ($E_0 = 142$ kJ/mol) (7). This direct cleavage pathway for 43H production would require higher internal energy deposition and may also contribute to the 43H signal in the MS/MS spectrum of m/z 86. Regardless of the pathway, 43H can be attributed as predominantly arising from the keto form of the molecular ion.

The proportion of MVK and A produced at m/z 71 from MS/MS on m/z 86 must also be determined if quantification of the keto and enol forms of the molecular ion is desired. The structure of the m/z 71 ions formed by CID of m/z 86 can be ascertained by performing a further step of MS/MS. This is known as an MS/MS/MS experiment and is described in Figure 4.12. After forming m/z 71 in the low energy collision cell by dissociation of m/z 86, that m/z 71 fragment ion was accelerated, then subjected to high energy MS/MS. The overall fragmentation pattern observed was $86 \rightarrow 71 \rightarrow 43$. It is noted that at collision energies below 20 eV, both 43H and 43L are produced in the high energy CID step of the MS/MS/MS experiment. At collision energies between 20 and 50 eV, production of 43L predominates in the high energy CID of m/z 71 in the MS/MS/MS QEB experiment, as shown in Figure 4.13. In fact, between 20 and 50 eV, the 43H:43L ratio is virtually identical to that obtained for the MVK reference ion subjected to high energy CID (Figure 4.9). Below 20 eV, a mixture of MVK and A is produced, but between 20 and 50 eV, only MVK survives at m/z 71.

At this point, 43H and m/z 58 are attributed as arising from the keto molecular

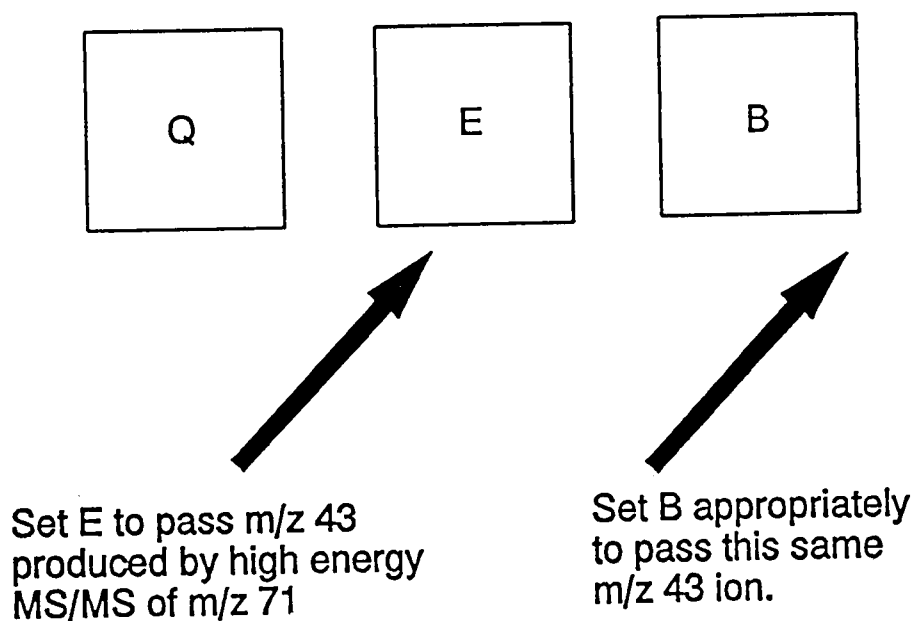


Figure 4.12. Schematic diagram indicating how MS/MS/MS is performed on the QEB. After selection of a particular ion produced in the ion source with the quadrupole (Q), fragments are formed from this ion by low energy CID. All ions surviving and all those produced by the collisional activation event enter the high energy MS/MS collision region. Appropriate setting of E and B allows only product ions arising from a unique precursor in the high energy collision cell to be observed at the final detector (located after B).

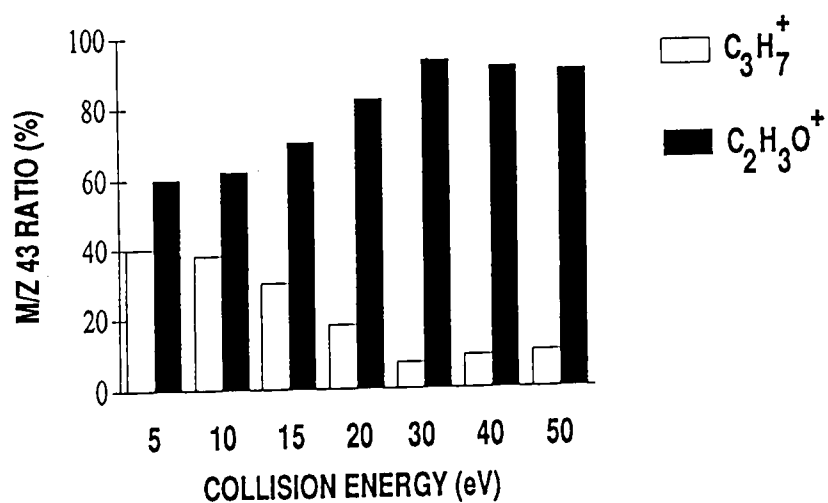


Figure 4.13. Characterization of m/z 71 ions produced from low energy CID (5-50 eV) of m/z 86 from 3-MB by MS/MS/MS on the QEB. The m/z 43 ratio is that produced from the high energy CID of the m/z 71 ions produced in the low energy collision cell.

ion; above 20 eV m/z 71 is comprised of MVK, according to the MS³ results, and can therefore be attributed to arising from the enol molecular ion. In the MS/MS spectrum of m/z 86, the most abundant product ion is 43L. It can result from a direct α -cleavage reaction of the keto form or from decomposition of MVK formed at m/z 71. Since MVK survives at m/z 71 above 20 eV, the ratio of 43L:71 can be observed above 20 eV. If a significant amount of MVK decomposes after its initial formation in the low energy collision cell, this ratio should change with collision energy. Table 4.3 summarizes the collision energy dependence of the MS/MS spectrum of m/z 86 above 20 eV. Above 20 eV, the ratio 43L:71 remains essentially constant at an average value of 3.5 ($\pm 4.3\%$). If MVK were decomposing to 43L, this ratio would be expected to increase since MVK would be decreasing. Though some of the MVK may indeed decompose to 43L, the above data are consistent with the fact that such a pathway is not the predominant source of 43L in the MS/MS spectra for m/z 86 from 3-MB. The direct α -cleavage of the keto molecular ion must be the major contribution to formation of 43L. From the data, it is not possible to determine the exact contribution of the two pathways. Nevertheless, 43L can be attributed to arising primarily from the keto molecular ion.

The presence of MVK in the low energy MS/MS spectrum of m/z 86 indicates that a portion of the molecular ion exists in the enol form. It would therefore be tempting to apply the linear superposition of MS/MS spectra in this case for quantitation of the two forms. However, preparation of the pure, individual ionic keto and enol forms of the molecular ion is difficult due to their propensity to isomerize upon ionization and the general difficulty of preparing the pure, neutral enol isomer (32). However, the structural

TABLE 4.3

Determination of the enol content of the molecular ion of 3-methyl-2-butanone (3-MB) from QEB low energy CID data on m/z 86.

C.E.	M/Z 71	M/Z 58	M/Z 43H	M/Z 43L	% E
20 eV	9.30%	3.19%	6.60%	33.0%	21.7%
30 eV	10.0%	3.37%	8.83%	42.7%	18.2%
40 eV	11.9%	4.44%	11.2%	41.3%	20.9%
50 eV	9.03%	1.92%	8.38%	29.4%	22.7%

Notes:

C.E. = COLLISION ENERGY

For each m/z value, the percentage listed is the relative intensity of that ion in the MS/MS spectrum of m/z 86 from 3-MB, normalized to the m/z 86 intensity (the base peak in the MS/MS spectrum).

Average % E (enol) = 20.9% ($\pm$ 2.83%).

assignments made above for the various product ions should allow an estimation of the proportion of keto and enol forms to be made based on the intensities of those peaks in the MS/MS spectrum of the molecular ion. This method assumes that the cross-sections of the various dissociation processes are nearly equal. In the collision energy range of 20-50 eV, all possible combinations of ratios of intensities among the various product ions remained constant. At these higher internal energy depositions, the assumptions regarding equal cross-sections for the various processes appears to be valid. However, production of MVK from the enol form of the molecular ion involves a rearrangement process in contrast to the direct cleavage processes of the keto form yielding 43H and 43L. The amount of enol present may be underestimated unless the higher internal energy deposition above 20 eV makes the rearrangement process for MVK formation competitive with the direct cleavage processes of the keto form. By noting the intensity of the fragment ions the approximate keto/enol composition of the molecular ion was approximated by the following equation (R.I. denotes the relative intensity of the various product ions in the MS/MS spectrum, where m/z 86 is the base peak):

$$\begin{aligned} \% \text{Enol} &= (\text{m/z } 71 \text{ R.I.}) / (\text{m/z } 58 \text{ R.I.} + 43\text{H R.I.} + 43\text{L R.I.} + \text{m/z } 71 \text{ R.I.}) \times 100 \\ &= ([\text{enol}] / ([\text{enol}] + [\text{keto}])) \times 100 \quad \text{Eq. 4.8} \end{aligned}$$

The results obtained from this equation are listed in table 4.3. This procedure indicated that the relative proportion of keto : enol ions was 79.1% : 20.9% ($\pm 4.3\%$, 95% confidence interval).

Ion-Molecule Reaction for Keto/Enol Determination on QEB

An ion-molecule reaction was performed to confirm the proportion of keto : enol determined by MS/MS of m/z 86. If an organic ketone is placed into the low energy collision cell, the keto form of the molecular ion of 3-MB will undergo charge exchange with this reagent to form M^+ (radical cation) of the neutral reagent. This will occur if the ionization potential of the neutral reactant is lower than that of 3-MB, making the charge exchange reaction exothermic and therefore favorable. The enol form of the molecular ion, on the other hand, will undergo proton transfer with the neutral reagent gas to form the $(M + H)^+$ ion from the neutral, provided that the neutral has a higher proton affinity than the enol ion. This also makes the proton transfer reaction exothermic and favorable. After careful deliberation (discussed further below), mesityl oxide (4-methyl-3-penten-2-one, $C_6H_{10}O$) was chosen as the neutral reagent species. Its ionization potential is lower than that of 3-MB (9.08 eV vs. 9.30 eV) (90) and its proton affinity is 202 kcal/mol (91). It has been demonstrated that compounds of this structure accept a proton from enol ions, but do not abstract protons from the keto form (92, 93). Furthermore, the enol form, in contrast to the keto form, will not charge exchange with such reagents since such a reaction is endothermic and unfavorable. A measured $(M + H)^+ : M^+$ ratio produced from the neutral reagent gas should provide a reasonable measure of the proportion of keto:enol molecular ion composition. Since the molecular weight of mesityl oxide is 98 amu, the ratio $(M + H)^+ : M^+$ corresponds to measuring the intensities of m/z 99 and m/z 98. The experimentally measured intensity of m/z 99 was corrected for ^{13}C

isotope contributions from m/z 98 by subtracting the quantity $(0.066)(\text{measured signal intensity of } m/z \text{ 98})$ from the m/z 99 measured intensity. The factor 0.066 takes into account the 1.1% natural abundance of ^{13}C and the six carbons present in mesityl oxide.

If the above reaction scheme is to be used for quantitation of the keto and enol forms of the molecular ion, it is important that the two reactions (charge exchange and proton transfer) have similar rate constants. Experimental measurements have verified that the ratio of the rate constants $k_{\text{charge transfer}} : k_{\text{proton transfer}}$ for the keto and enol ion, respectively, with an α,β -unsaturated ketone such as mesityl oxide are in the ratio of 1.1 (92, 93). It may seem surprising that these two different types of reactions would possess similar rate constants, but ion-molecule reactions are affected by strong ion-dipole and ion-induced dipole interactions which influence the formation of an intermediate collision complex (94, 95). Formation of such complexes results in a lowering of potential energy of the reactants which is, in turn, counterbalanced by an increase in internal energy of the reacting species (94, 95). Furthermore, kinetic energy of the precursor reactant ion may be converted to internal energy upon collision. Both of these factors help in overcoming barriers to reaction and enable the two types of reactions studied here to proceed at comparable rates.

The use of a neutral compound such as mesityl oxide also helped with the observation of the desired ion-molecule reaction. This reasoning can be understood by examining the Langevin rate constant equation: $k = 2\pi z(\alpha/\mu)^{1/2}$ (94, 95). In this equation, z = the charge on the ion, α = the polarizability of the neutral molecule, and μ = the reduced mass of the ion-molecule pair. Mesityl oxide, being an α,β -unsaturated

ketone, possesses highly polarizable π electrons and therefore yields a greater rate constant for the reactions of interest with the keto and enol ions. This consideration is especially of importance on beam instruments where the reaction time is limited to several microseconds. In fact, mesityl oxide yielded 2-3 times more ion-molecule reaction products than a simple aliphatic ketone such as acetone. Acetone, with its lower polarizability, simply does not react as quickly on the beam instrument time-scale.

With the above concepts in mind, the experiment was performed on the QEB. The precursor ion, m/z 86, was mass selected with the quadrupole so that a specific ion was subjected to reaction. Upon performing the ion-molecule reaction at a collision energy of 1-2 eV (80% attenuation of precursor ion) and correcting for the ^{13}C isotope contribution at m/z 99, the $(\text{M} + \text{H})^+ : \text{M}^+$ ratio of m/z 99 : m/z 98 is 14% : 86% ($\pm$ 3.7%, 95% confidence interval). The agreement between the MS/MS results on m/z 86 and the ion-molecule reaction is quite good. It appears that the composition of the molecular ions which survive the ionization process in the source are of an approximate composition of 80-85% keto form and 15-20% enol form according to the QEB results.

Partial Determination of Enolic Ion Mass Spectrum From QEB MS/MS Results

The composition of the m/z 86 and m/z 71 ions was determined above by MS/MS data. The contributions of each ionic form (keto and enol) to these measured ion intensities was estimated, based on this compositional data, as a means of predicting the relative abundance of each ion in a hypothetical mass spectrum of a pure enol or keto

precursor. First of all, the normal, 70 eV mass spectrum obtained when the keto form is introduced into the ion source must be obtained. This mass spectrum is shown in Figure 4.14. By utilizing the compositional data determined above for m/z 86 and m/z 71, the contributions to each peak resulting from the keto and enol ions are determined. As shown in Figure 4.15, these "enol ion" contributions were normalized to 100% to yield the expected relative intensities of these peaks in the normal mass spectrum of the enol ion. The QEB MS/MS data did not allow for **direct** determination of the contributions of the keto and enol forms to 43H and 43L in the simple mass spectrum since multiple pathways are possible for formation of these ions. For example, MVK and the keto form can both yield 43L, while the keto form and A yield 43H. The expected intensity of 43H and 43L in the enol spectrum cannot therefore be determined directly as for m/z 86 and m/z 71.

The results obtained for the enol ion mass spectrum via the QEB results are in excellent agreement with those obtained by Turecek for the mass spectrum of the neutral enol form (32). Turecek's data showed other ions (10% or less relative intensity) which resulted from higher energy processes or stepwise decompositions which were not studied with the QEB. Nevertheless, the good agreement found places confidence in the QEB MS/MS results and the subsequent data analysis.

The time between ionization and collisional activation on the QEB is 30-40 μsec . In order to determine if a longer time period would favor enol ion formation, experiments were conducted on the ion trap mass spectrometer (ITMS) since the time between ionization and collisional activation on the ITMS is several *milliseconds*.

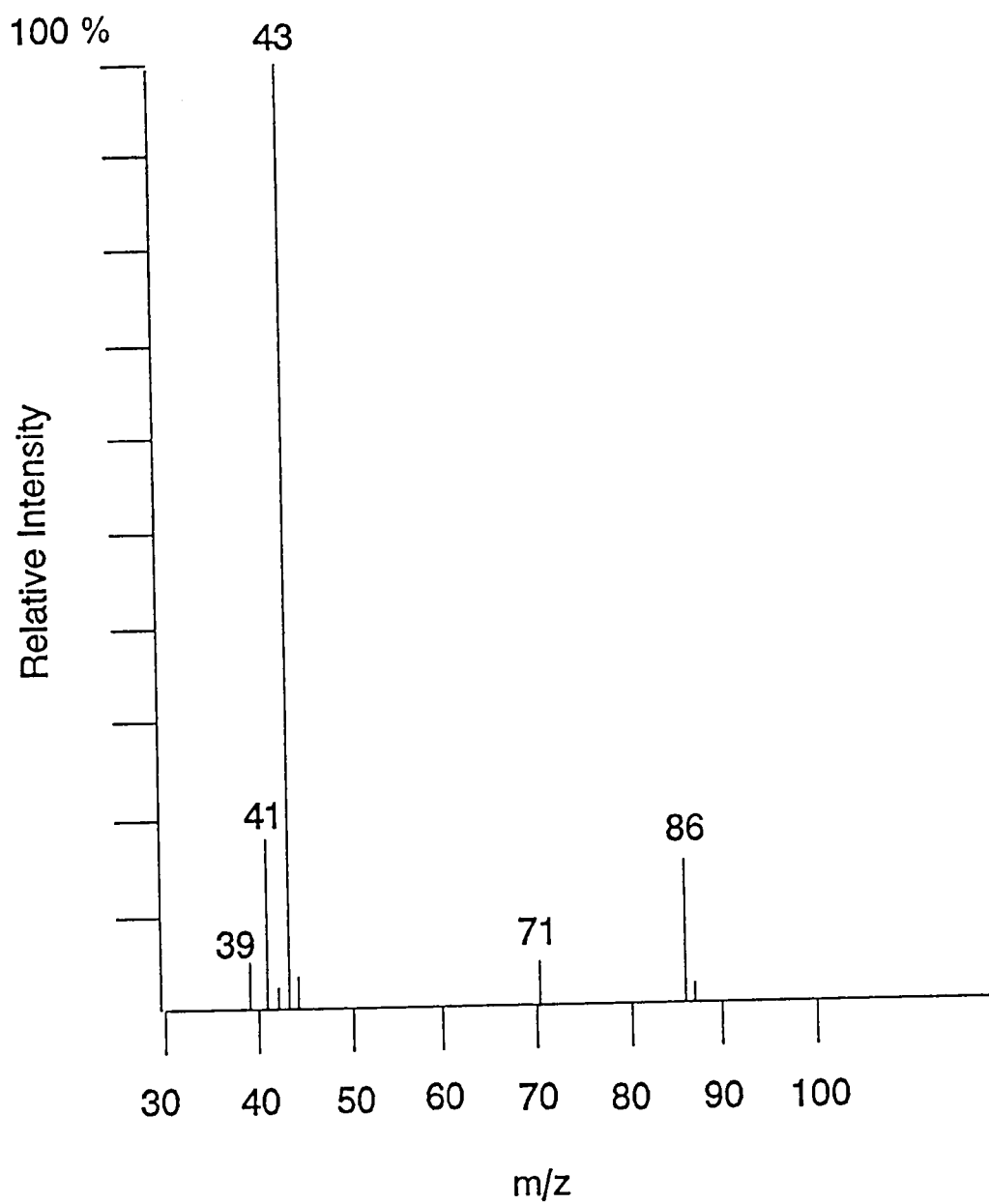
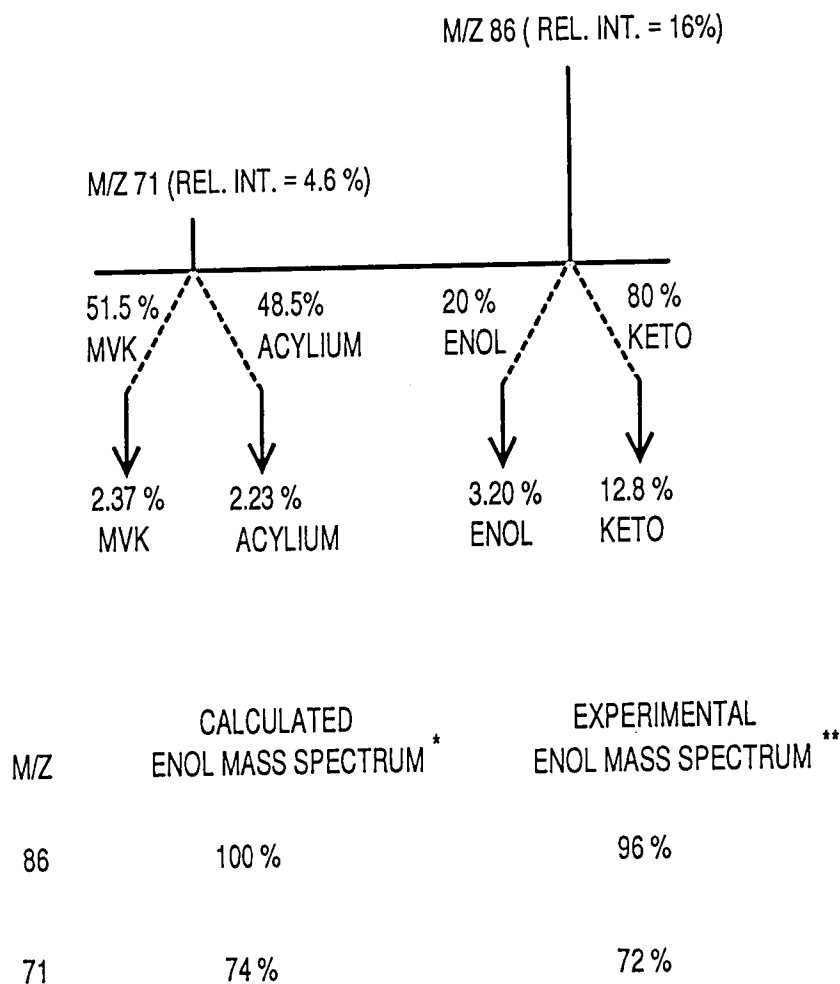


Figure 4.14. 70 eV mass spectrum of 3-methyl-2-butanone (3-MB) obtained on the QEB.



*BASED ON EXPERIMENTALLY DETERMINED VALUES SHOWN ABOVE.

**BASED ON DATA FROM REFERENCE 32.

Figure 4.15. Demonstration of deconvolution of m/z 71 and m/z 86 data to experimentally determine the expected (calculated) enol ion mass spectrum. The relative intensities are those from the 70 eV mass spectrum of the 3-MB keto form obtained on the QEB at a sample pressure of 1×10^{-6} torr (see figure 4.14 for the full mass spectrum). The composition of the m/z 71 and m/z 86 ions were determined by low energy CID experiments on the QEB.

ITMS MS/MS Results for M/Z 71 Ions of 3-methyl-2-Butanone

The procedure for determining the proportion of the m/z 43 product ions from MS/MS of various ions was described in the experimental section of this thesis. The initial raw data results obtained for MS/MS of 3-MB m/z 71 ions on the QEB and the ITMS are shown in Figure 4.16. The relative proportions of 43H and 43L were essentially inverted, leading one to believe that the enol ion predominates on the ITMS since 43L is associated with the enol form via MVK.

As a means of checking this possibility, the ion-molecule reaction involving mesityl oxide, performed on the QEB for determination of keto/enol content of the molecular ion, was performed on the ITMS. To ensure that m/z 86 was the only ion reacting with mesityl oxide (pressure = 5×10^{-7} torr), m/z 86 was isolated from other ions after the cooling period by application of appropriate RF and DC voltages to the ring electrode. After isolation, a variable reaction period of up to 1 second was employed and the $(M + H)^+ : M^+$ ratio (m/z 99 : m/z 98) obtained from reaction with mesityl oxide was observed as a function of this reaction time. *Utilizing this method and correcting for ^{13}C isotope contributions to m/z 99, the same proportion of keto and enol forms of the molecular ion were found on the ITMS and the QEB. The proportion was found to be independent of the time duration of the cooling period before and the reaction period after RF/DC isolation of m/z 86 on the ITMS. This applied to cooling and reaction periods as long as 1 second, indicating that no time dependence was associated with the keto/enol isomerization in question. The increased 43L production cannot, therefore, be*

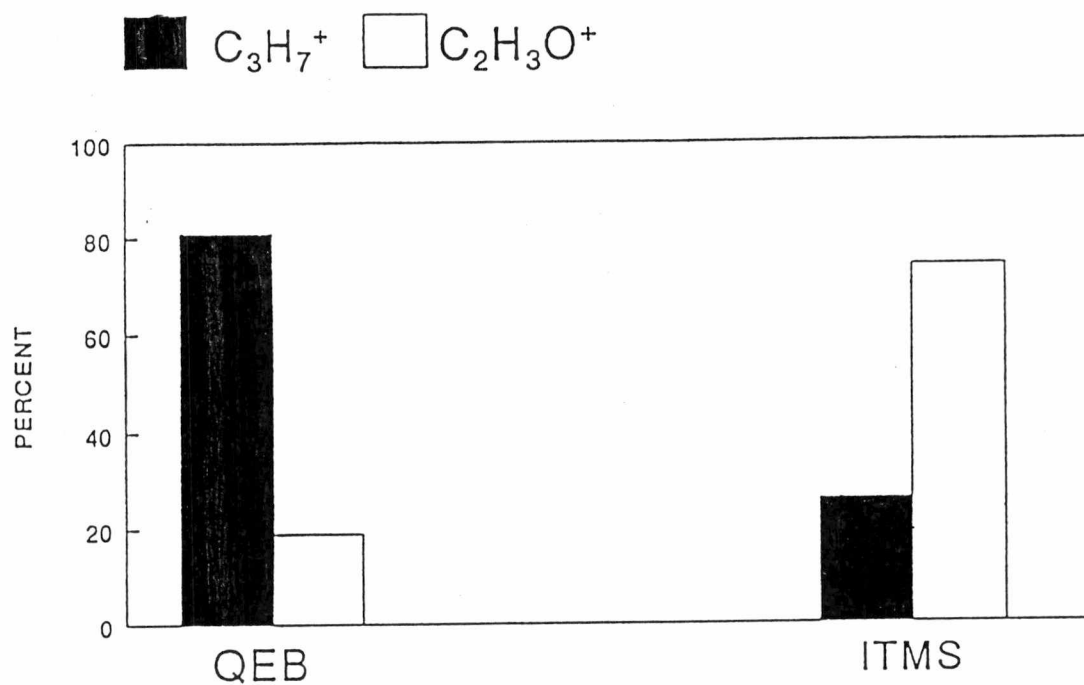


Figure 4.16. Comparison of raw data (i.e., uncorrected for reference ion behavior) obtained from QEB low energy CID and ITMS MS/MS of the 3-MB m/z 71 ions.

attributable to increased enol content; another possibility is different behavior of the MVK and A ions under the longer MS/MS time-frame of the ITMS.

The MS/MS behavior of the m/z 71 reference ions on the ITMS was then examined. The reference ions were generated in the same manner as on the QEB (Figure 4.4). Figure 4.17 shows the MS/MS dissociation of the MVK reference ion on the two instruments; the behavior was quite similar. Figure 4.18 shows the drastically different MS/MS behavior of the acylium reference ion (A) on the two instruments. The acylium ion apparently undergoes a rearrangement process on the ITMS time-scale in order to yield a significant amount of 43L.

Taking into account the fragmentation behavior and dissociation efficiencies (ϵ) of the reference ions, the linear superposition principle can be applied to the ITMS data to determine the composition of the 3-MB m/z 71 ions. Equations 4.2 and 4.3, pertaining to the linear superposition principle, are reproduced below. The 43H:43L ratio and ϵ values for MVK, A, and 3-MB m/z 71 ions pertaining to the ITMS MS/MS data are shown in table 4.4.

$$I_{43H} = [A]\epsilon_{A,43H} + [MVK]\epsilon_{MVK,43H} \quad \text{Eq. 4.2}$$

$$I_{43L} = [A]\epsilon_{A,43L} + [MVK]\epsilon_{MVK,43L} \quad \text{Eq. 4.3}$$

Table 4.4 indicates that the measured 43H:43L ratio is 28:72 which equals 0.39. The appropriate values may then be inserted into Eq. 4.7 (see high energy MS/MS section of this chapter) and $[A]/[MVK]$ may then be determined. The composition of the 3-MB m/z 71 ions is determined to be 40% A, 60% MVK ($\pm 2.2\%$, 95% confidence interval).

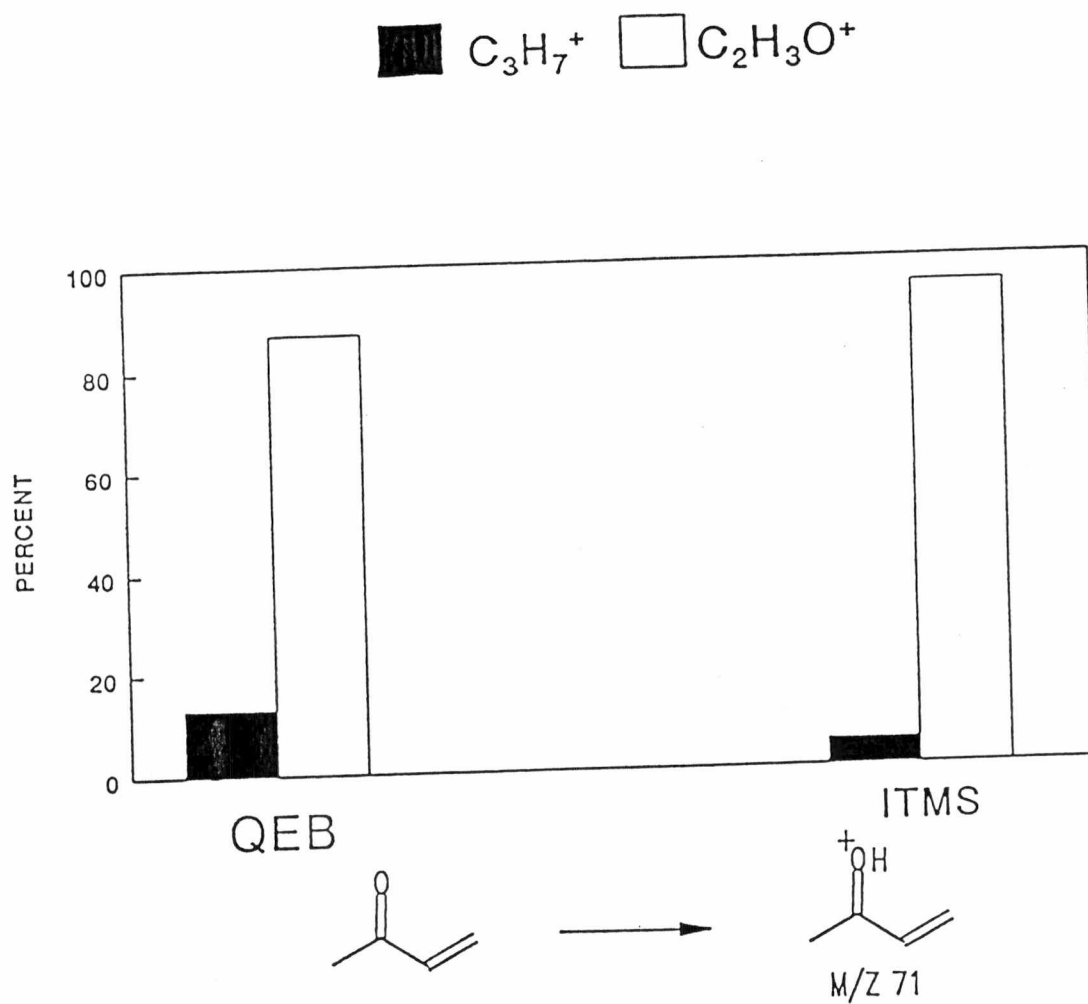


Figure 4.17. Comparison of MS/MS behavior of the MVK reference ion on the QEB and the ITMS.

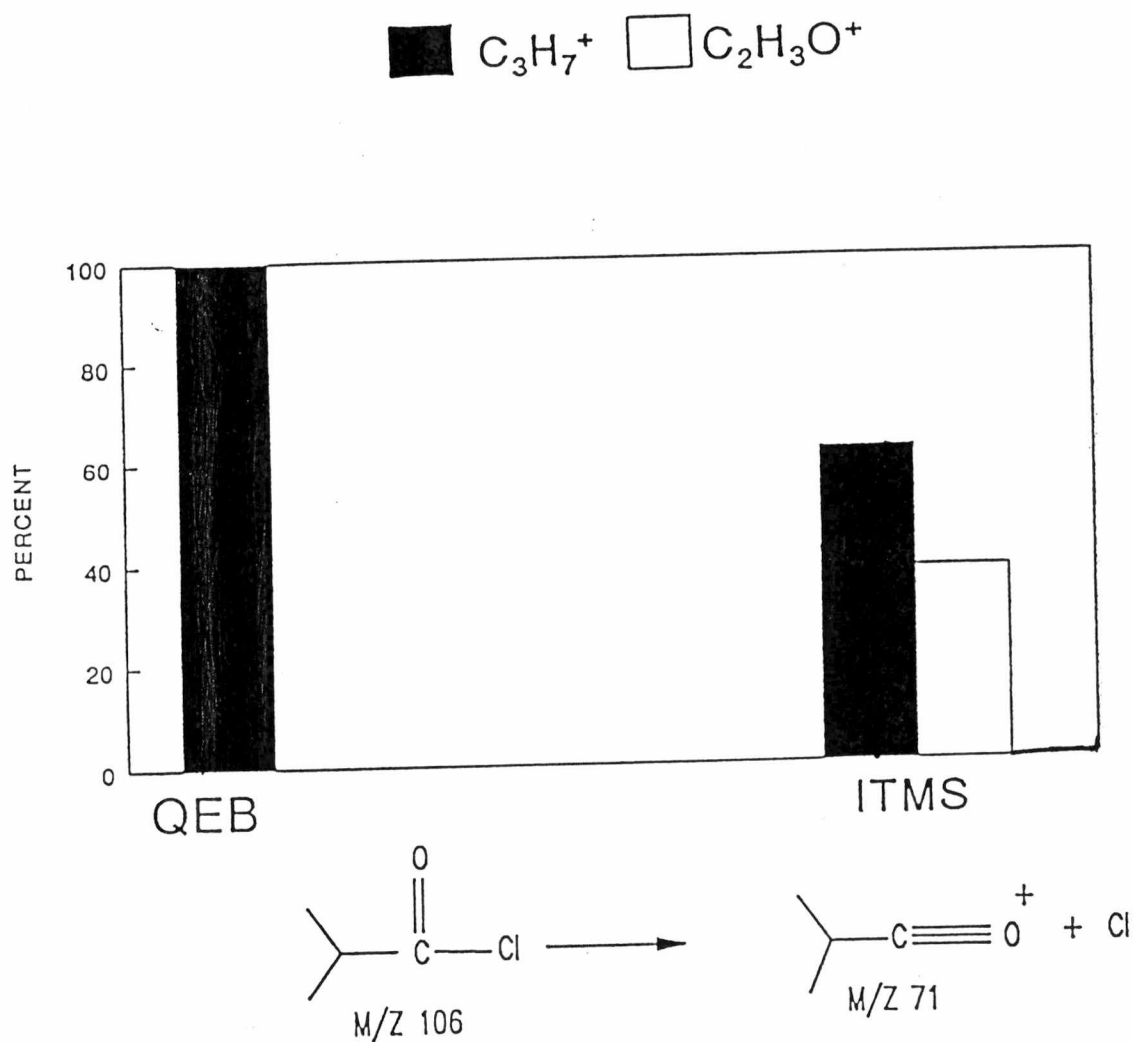


Figure 4.18. Comparison of MS/MS behavior of the acylium (A) reference ion on the QEB and the ITMS.

TABLE 4.4

MS/MS dissociation efficiencies and m/z 43 product ion ratios experimentally measured on the ITMS for the 3-MB m/z 71 ions and the m/z 71 reference ions A and MVK.

COMPOUND	ϵ^A	43H:43L ^B
A	30% ($\pm$ 2.3%)	62:38 ($\pm$ 2.7%)
MVK	28% ($\pm$ 3.5%)	5:95 ($\pm$ 1.8%)
3-MB	31% ($\pm$ 2.4%)	28:72 ($\pm$ 2.0%)

^A ϵ defined as (total m/z 43 product ion intensity)/(m/z 71 ion intensity before MS/MS activation). Also referred to as $\epsilon_{X, 43H + 43L}$, where X is either A or MVK.

^B43H:43L measured using the ¹²C/¹³C ratio technique described in the text (see chapter 2).

Notes:

$$\epsilon_{A,43H} = (0.62)(0.30) = 0.19$$

$$\epsilon_{MVK,43H} = (0.05)(0.28) = 0.014$$

$$\epsilon_{A,43L} = (0.30)(0.38) = 0.11$$

$$\epsilon_{MVK,43L} = (0.95)(0.28) = 0.27$$

$$I_{43H}/I_{43L} = 0.39$$

Substitution of these values into equation 4.7 (see text) yields:

$$[A]/[MVK] = 0.62$$

$$= 39\% \text{ A, } 61\% \text{ MVK } (\pm 2.2\%)$$

The composition of the 3-MB m/z 71 ions determined by ITMS data is quite similar to that found on the QEB, where a composition of roughly 50% MVK, 50% A ($\pm 5\%$) was determined. This is not surprising since the ITMS mass spectrum, shown in Figure 4.19, is virtually identical to that obtained on the QEB. The mass spectrum on the ITMS is obtained after a suitable cooling period during which ions are "collected" at the center of the trap before being ejected from the trap by an RF ramp. The rearrangement of the acylium ion on the ITMS lead to an inflated proportion of 43L upon MS/MS activation. If the behavior of the reference ions is not taken into account and the linear superposition not applied to the raw data, erroneous conclusions can be reached regarding the behavior of the keto/enol ionic system on the ITMS.

Since the acylium ion rearranged in the ion trap, experiments were conducted in which time periods before and after MS/MS activation were varied. If the 43H:43L peak ratios varied with time, kinetics information may possibly be obtained. No dependence of the MS/MS results for the A reference ion or for the 3-MB m/z 71 ions was noted when the cooling period duration before MS/MS activation was varied over the range 30 msec - 1 sec. This suggested that the no rearrangement of the acylium ion was occurring during the cooling period and the original composition of the 3-MB m/z 71 ions was retained before MS/MS activation. This is supported by the fact that the linear superposition principle determined similar compositions of the 3-MB m/z 71 ions on the ITMS and QEB. The acylium ion rearrangement may have occurred during the MS/MS activation step; sufficient internal energy must be added to the acylium ion to allow the rearrangement to occur. The activation step must also be of an adequate time-duration

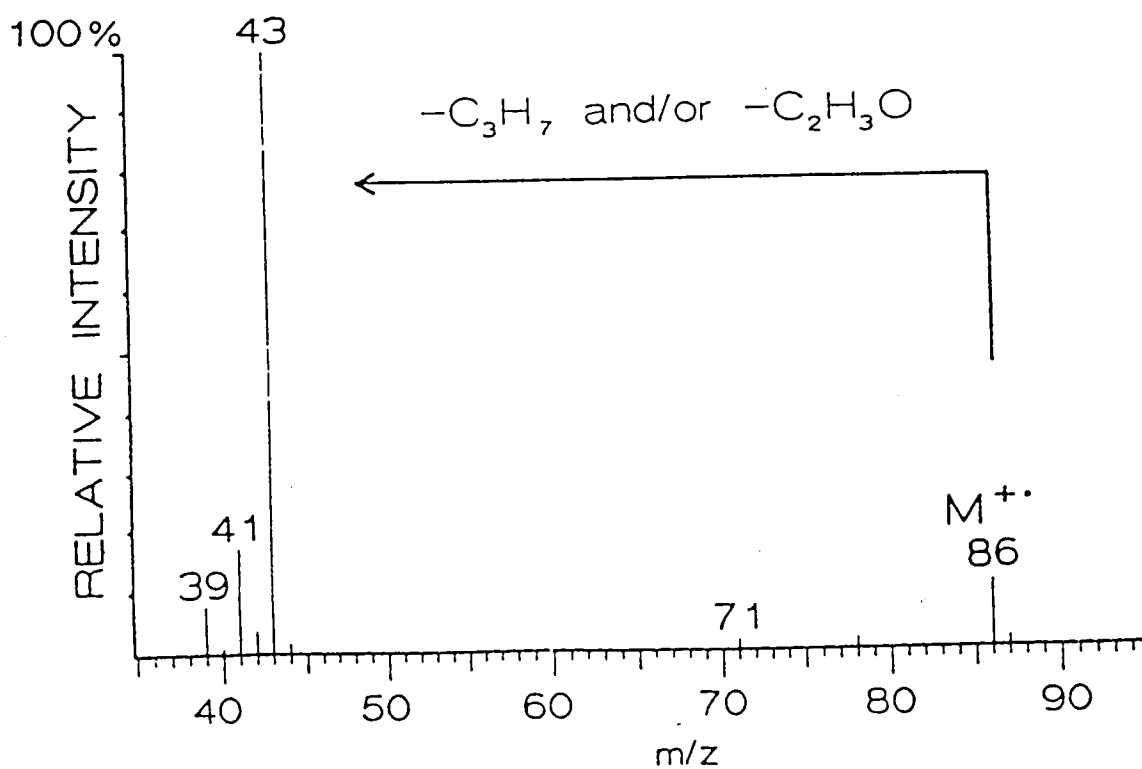


Figure 4.19. EI mass spectrum of 3-MB obtained on the ITMS (sample pressure = 1×10^{-6} torr).

to allow the rearrangement process to occur. These requirements are met with the ITMS MS/MS activation conditions.

The behavior of the A reference ion and the 3-MB m/z 71 ions can also be observed as a function of a delay time after MS/MS activation to determine if product ion ratios vary after the internal energy deposition during MS/MS activation. Given the variable time period after MS/MS activation, a more thermodynamically stable product ion may become more favored if sufficient time is available for attainment of equilibrium. Experiments performed in which a delay period was introduced after MS/MS activation did not show conversion of the acylium ion to the MVK ion since the 43H : 43L ratio from A and 3-MB m/z 71 ions did not vary for delay periods from 0 - 1 second. This indicated that the acylium ion underwent simultaneous direct cleavage and rearrangement processes during the MS/MS activation period on the ITMS. Because of these competitive processes, the acylium ion could not rearrange completely to MVK and then fragment solely from this rearranged form. Dissociation from a mixture of the two m/z 71 ions would thus occur on the millisecond time-frame of the ITMS for both the A reference ion and the 3-MB m/z 71 ions. It appears that the difference in the MS/MS data for the 3-MB m/z 71 ions can be attributed to the different behavior of the acylium ion in the ITMS. The ITMS data had to be carefully interpreted to avoid the erroneous conclusion that the enol form was predominant.

ITMS MS/MS Results on the M/Z 86 Ion of 3-MB

MS/MS experiments were performed on m/z 86 from 3-MB to confirm the hypothesis that the enol ion did not predominate after the cooling period. It should be noted that the appearance of the ITMS MS/MS spectrum is greatly affected by the RF level applied to the ring electrode during MS/MS activation. If too high an RF level is employed, the relative intensity of the m/z 43 peak is greatly reduced while the relative intensities of the m/z 71 and m/z 58 ions are increased. Being under data system control, the RF level used for storage of ions is simply set by typing in a mass level. The actual magnitude of the RF potential is proportional to this mass level. At "low" RF levels (29-31 amu mass level), the ITMS MS/MS spectra are very similar to those obtained from low energy QEB CID spectra. However, at "high" RF levels, the spectrum looks much different. The m/z 71 and m/z 58 ions are greatly enhanced in intensity, while the m/z 43 ion is greatly reduced in intensity. Two possible factors can account for this variation: 1) discrimination against storage of low mass ions at the higher RF level; 2) greater internal energy of the m/z 86 molecular ion at the higher RF level leading to a different fragmentation pattern. For either the low or high RF level, the MS/MS spectrum is not affected by variations in the "tickle" voltage over a range of 100 mV - 1 V. The difference in spectra is then likely attributed to a mass discrimination at higher RF levels.

Proposed Model for the Keto/Enol System of 3-Methyl-2-Butanone

The same proportion of ionic keto and enol forms existed on the QEB and the ITMS after ionization, even though the time-scale of experiments are vastly different on the two instruments. This indicates that formation of the enol ion occurs within the first several microseconds after the initial ionization event and the measured composition of the molecular ion (approximately 80% keto, 20% enol) does not change over the course of several milliseconds. However, the approximate composition of the neutral species, based on computational chemistry results, is 99.7% keto, 0.3% enol at 25°C according to heats of formation of the respective neutral species (6). This calculation assumes that $\Delta H = \Delta G$ (i.e., entropy effects are negligible). The composition of the molecular ion *immediately after the ionization event* should correspond to the composition of the original neutral. Clearly, the measured composition of the molecular ion differs greatly from the calculated composition of the neutrals.

One plausible explanation for this discrepancy may be the preferential fragmentation of the ionic keto form since its barriers to decomposition are lower than those of the enol ion (7, 32, 34-39). Furthermore, the enol ion is also thermodynamically more stable than its keto ion counterpart, thus allowing a greater proportion of the enol ion to survive the ionization process (7, 32). The question of quantifying the extent of this preferential fragmentation becomes of interest. By calculating the relative percentage of the total ion current which the m/z 86 ion carries in the mass spectrum of the keto or enol ion, it is possible to obtain the desired quantitative information. This relative

percentage is called $\% \Sigma$ and is defined as $(\text{rel. int. } m/z \ 86)/(\Sigma \text{rel. int., all ions})$.

Table 4.5 shows the $\% \Sigma$ values for the keto and enol molecular ions. The relative intensities of $m/z \ 71$ and $m/z \ 43$ ions are listed since these are the dominant fragment ions in the mass spectrum of the keto and enol ions. The relative intensities for $m/z \ 86$ and $m/z \ 71$ are those determined after deconvolution of data based on the QEB MS/MS results. The $\% \Sigma$ for the enol ion is 3.17 times greater than the $\% \Sigma$ for the keto ion, indicating that the keto ion fragments 3.17 times more readily than the enol ion.

Knowing this preferential fragmentation factor (PFF), the compositional data determined above by MS/MS experiments can be manipulated using this PFF to determine the initial keto : enol composition using the procedure outlined below.

First of all, multiply the molecular ion composition determined via MS/MS experiments by PFF. Since these initial MS/MS results showed no dependence on time-scale of the MS/MS experiments, the "new" composition determined using PFF can be taken as the equilibrium composition for the ionic keto : enol mixture. If the ionization cross-section for the keto and enol neutral species are very similar at 70 eV electron energy (i.e., no preferential ionization) and the isomerization of keto molecular ion to enol molecular ion after the initial ionization event is negligible, the composition of the neutral species will be the same as that for the ionic species (corrected by PFF). At this point of the data analysis, the extent of isomerization of the ionic species following ionization cannot be ascertained, so the above assumption regarding such isomerization is made. This point will be addressed further once the data analysis is completed at the end of this section.

TABLE 4.5

Determination of $\% \Sigma$ (defined in text) for the keto and enol molecular ions (m/z 86) of 3-MB.

IONIC FORM	REL. INT M/Z 86	REL. INT M/Z 71	REL. INT. M/Z 43	$\% \Sigma_{M/Z\ 86}$
KETO	12.8%	2.23%	96.7%	11.4%
ENOL	3.20%	2.37%	3.30%	36.1%

Notes:

The values listed for m/z 86 and m/z 71 are those following deconvolution of data according to Figure 4.15 (p. 135)

The relative intensities for m/z 43 for each form were calculated as follows:

From Tureck's data (ref. 32), the ratio of m/z 43: m/z 86 in the 70 eV mass spectrum of the enol ion is $(100\%/96\%) = 1.04$. Therefore, the value for m/z 43 in the table above = $(3.20\%) \times 1.04$ since the relative intensity of the enol ion at m/z 86 was determined to be 3.2%. Since the relative intensity of m/z 43 in the 70 eV mass spectrum resulting from introduction of the neutral keto form is 100%, the relative intensity attributable to the keto form is $100\% - 3.30\% = 96.7\%$.

Secondly, knowing the equilibrium composition of the neutral species, ΔG for the equilibrium keto $\rightleftharpoons$ enol can be calculated from the expression $\Delta G = -RT \ln K$, where R is the gas constant, T is the absolute temperature in kelvins, and K is the equilibrium constant. It should be noted that the temperature at this stage is the ion source temperature (423 K = 150°C) since the neutral species would be expected to undergo many collisions with the walls of the ion source before being ionized; any equilibrium would then be attained at this source temperature.

Finally, the value of ΔG obtained from step 2 is then used in calculating ΔG at 25°C for the neutral species. In this manner, the results obtained here with can be compared to other results for the neutral species obtained at the standard temperature of 25°C. Utilizing the following equation for the temperature dependence of ΔG (available from any standard physical chemistry text), the calculation of ΔG at 25°C can be calculated based upon the value derived in step two above.

$$d(\Delta G/T) = -\Delta H/T^2 dT \quad \text{Eq. 4.9}$$

Upon integrating equation 4.9, the following result is obtained:

$$(\Delta G_{298}/298) - (\Delta G_{423}/423) = \Delta H (1/298 - 1/423) \quad \text{Eq. 4.10}$$

$$\Delta G_{298} = \Delta G \text{ at } 298 \text{ K}$$

$$\Delta G_{423} = \Delta G \text{ at } 423 \text{ K (source temp.); calculated in step 2 above.}$$

$$\Delta H = 14.4 \text{ kJ/mol (taken from ref. 6); assumed to be independent of temperature.}$$

Utilizing equation 4.10, the value of ΔG_{298} can be calculated. Once this value is known, the equilibrium constant can be calculated at 298 K.

The method outlined above is a general description of the approach to the problem of determining the initial keto : enol composition of the molecular ion. The initial starting point is with the ionic composition of m/z 86 as determined by MS/MS experiments. The methods used to determine this composition were low energy CID of m/z 86 (QEB) and ion-molecule reactions (QEB and ITMS). The average value for each of these determinations is listed in table 4.6. The appropriate values found by applying the method outlined above are also listed in table 4.6.

The results listed in table 4.6 reveal that the composition of the neutral species is 1.2 % enol, 98.8% keto at 25°C. The value of 0.3% enol, 99.7% keto mentioned at the outset of this discussion is based on ΔH only; entropy effects were assumed negligible. It is the value of ΔG which determines the equilibrium composition, so entropy must be taken into account. Due to the similarity in the chemical structures of the two neutrals, it may be expected that the entropy difference between the two species may be quite small. The compounds differ primarily in the placement of the double bond (C=C vs. C=O). Since the C=C bond is weaker than the C=O bond, the fundamental vibrational frequency of the C=C bond is lower (91). This translates into greater vibrational "freedom" or disorder for the neutral enol. Since a species possessing the weaker bonding generally has greater entropy (96), the neutral enol is therefore expected to possess a greater entropy than the neutral ketone. Unfortunately, the magnitude of this entropy difference is not known from either computational or experimental data. However, the

TABLE 4.6

Calculation of keto:enol composition at the source temperature (423 K) and standard temperature (298 K).

METHOD	PFF	K:E _{423 K}	$\Delta G_{423 K}$	$\Delta G_{298 K}$	K:E _{298 K}
IMR: 86.0% K, 14.0% E	3.17	95.1% K, 4.90% E	10.44 kJ/mol	11.61 kJ/mol	99.08%K, 0.920% E
CID: 79.1% K, 20.9% E	3.17	92.3% K, 7.70% E	8.730 kJ/mol	10.41 kJ/mol	98.51%K, 1.490% E

Notes:

IMR = Ion-molecule reaction for quantitation of keto and enol ions. Average result used.

CID = Quantitation based on QEB low energy CID results obtained for m/z 86 of 3-MB. Average result used.

PFF = Preferential fragmentation factor determined in table 4.5.

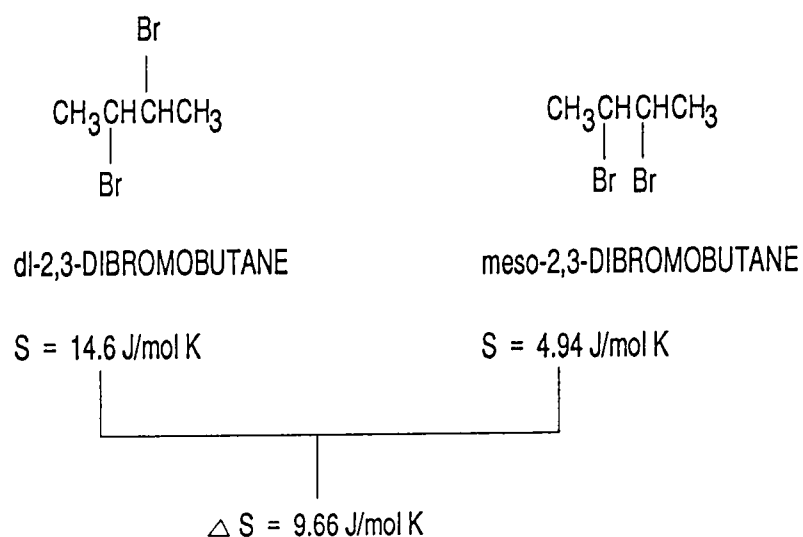
Values of ΔG are for the equilibrium keto $\rightleftharpoons$ enol.

equation $\Delta G = \Delta H - T\Delta S$ can be solved for ΔS at 298 K utilizing $\Delta G = 11$ kJ/mol (average of the two values in table 4.6) and $\Delta H = 14.4$ kJ/mol (value utilized in table 4.6). Such a calculation yields a value of 10.1 J/mol K for ΔS at 298 K.

It is constructive to compare this value to values of ΔS for other sets of structurally similar compounds, for which entropy values are known, to determine if the value calculated above is reasonable. Two such sets of compounds are shown in Figure 4.20. The diastereomers dl- and meso-2,3-dibromobutane differ in the way atoms are arranged in three dimensional space and possess different entropy values due to varying interactions for different conformers of the dl and meso compounds. This example demonstrates the small value of ΔS (9.69 J/mol K) expected from structurally similar compounds. Diastereomers are utilized in this example, whereas tautomers are discussed in the previous paragraph. The second example makes use of the "weaker bond-greater entropy" principle described earlier. Ethyl chloride and vinyl chloride are used in this case since they have similar molecular weights and chemical structures, but the C=C of vinyl chloride is a stronger bond than the C-C of ethyl chloride. As expected, ethyl chloride has the greater entropy; ΔS , favoring ethyl chloride, is 12 J/mol K. The magnitude of ΔS in both examples compares well with that calculated above for the neutral keto/enol equilibrium. Furthermore, the enol would be expected to have a slightly greater entropy since it has the weaker C=C bond. Entropy effects on the keto/enol equilibrium, though rather small, should not be ignored when calculating equilibrium compositions since the value of ΔG determines the position of an equilibrium.

The results obtained indicate the following scenario. The original vapor consisting

A)



B)

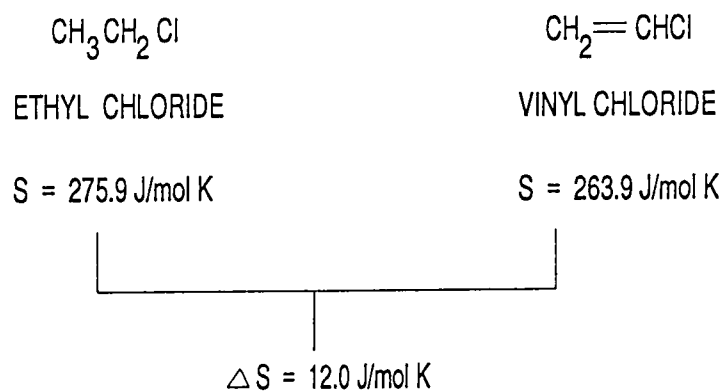
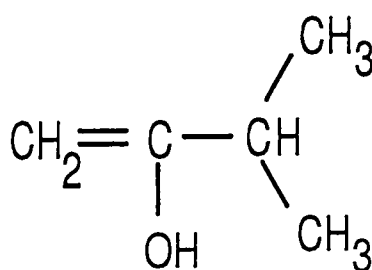


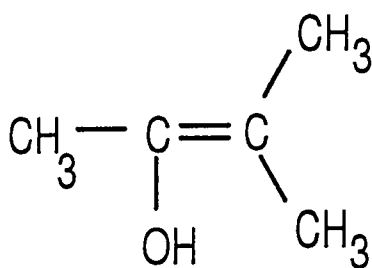
Figure 4.20. Calculation of entropy differences for structurally related compounds. In A) the diastereomers of 2,3-dibromobutane are utilized (data from ref. 97), while in B) ethyl and vinyl chloride are utilized (data from ref. 90).

of 99% keto, 1% enol at 298 K enters the heated ion source block (423 K) causing a shift in the equilibrium composition in accordance with the Le Chatelier principle. Since the isomerization of neutral keto to neutral enol is endothermic and entropy favors the enol, this temperature increase results in increased neutral enol in the equilibrium mixture. It is this equilibrium mixture which undergoes ionization. Following ionization, the keto molecular ion fragments preferentially to yield the MS/MS results obtained for the surviving m/z 86 and m/z 71 ions. The data show no indication of preferential ionization of one neutral form over the other or isomerization of the keto ion to the enol ion following ionization. For the high internal energy depositions encountered in 70 eV EI, the frequency factor governing the ionic keto/enol rearrangement dictates that this process would simply not be competitive with the fragmentation reactions which each individual ionic species may undergo (36). Consequently, the composition of the ionic species immediately following ionization reflects the composition of the neutral species at the source temperature and is merely altered by preferential fragmentation of the keto form.

The structure of neutral 3-MB allows possible formation of two neutral enol species, as shown in Figure 4.21. The above discussion assumed that enol 2 was the predominant neutral enol in the equilibrium mixture since its internal C=C would make it more stable than enol 1. As stated above, ΔH for conversion of keto to enol 2 is 14.4 kJ/mol; ΔH for conversion of keto to enol 1 is 24 kJ/mol (6). Using the same ΔS as above (ca. 10 J/mol K) for calculating ΔG at 298 K for the production of enol 1 from the neutral keto, only 0.02% enol 1 is present. The amount of neutral enol 2 (about 1% at 298 K) simply outweighs the amount of enol 1 by a factor of 50. Furthermore, even at



ENOL 1



ENOL 2

Figure 4.21. The two neutral enol structures possible for 3-methyl-2-butanone (3-MB). Neutral enol 2 is thermodynamically more stable than neutral enol 1.

an elevated source temperature of 423 K, enol I comprises only 0.35% of the equilibrium mixture. Under the same conditions, enol 2 is present at 6.4%, outweighing enol 1 by a factor of 18. The likelihood of these two neutral enols rearranging with one another under mass spectrometric conditions (high vacuum) is small since there is no external source of acid or base to catalyze such a rearrangement process. The contribution of neutral enol I therefore does not contribute greatly, if at all, to the results obtained above.

Of course, the possibility that the ionic forms enol 1 and enol 2 interconvert cannot be overlooked. The initial ionization event should produce a mixture of the two enols having a composition the same as that of the original neutral mixture (i.e., 6.4% enol 2, 0.35% enol 1). Since enol ion 2 is by far the major enol ion formed after the ionization event, it is of interest to consider its fate as shown in Figure 4.22. Enol ion 2 may: a) rearrange to enol ion 1 via a 1,3 H shift; b) undergo a 1,2 H shift via the resonance structure shown and subsequently fragment to form protonated MVK at m/z 71; c) fragment from the resonance structure shown to form an acylium ion at m/z 43. It has been established that the 1,3 H shift has a higher activation barrier (30 kJ/mol greater than the 1,2 H shift; therefore, the 1,2 H shift will be favored for enol ion 2 (7, 23, 26). The 1,2 H shift will also be facilitated by the partial overlap of the methyl C-H bonds with the p-orbital of the α -carbon radical center (hyperconjugation). Furthermore, the fragmentation of the resonance structure to form m/z 43 is driven by the stability of the acylium ion formed and the formation of a stable neutral radical. This gives enol ion 2 another favorable pathway to follow other than rearrangement to enol ion 1. It should be noted that this fragmentation pathway, along with any decomposition of MVK to form

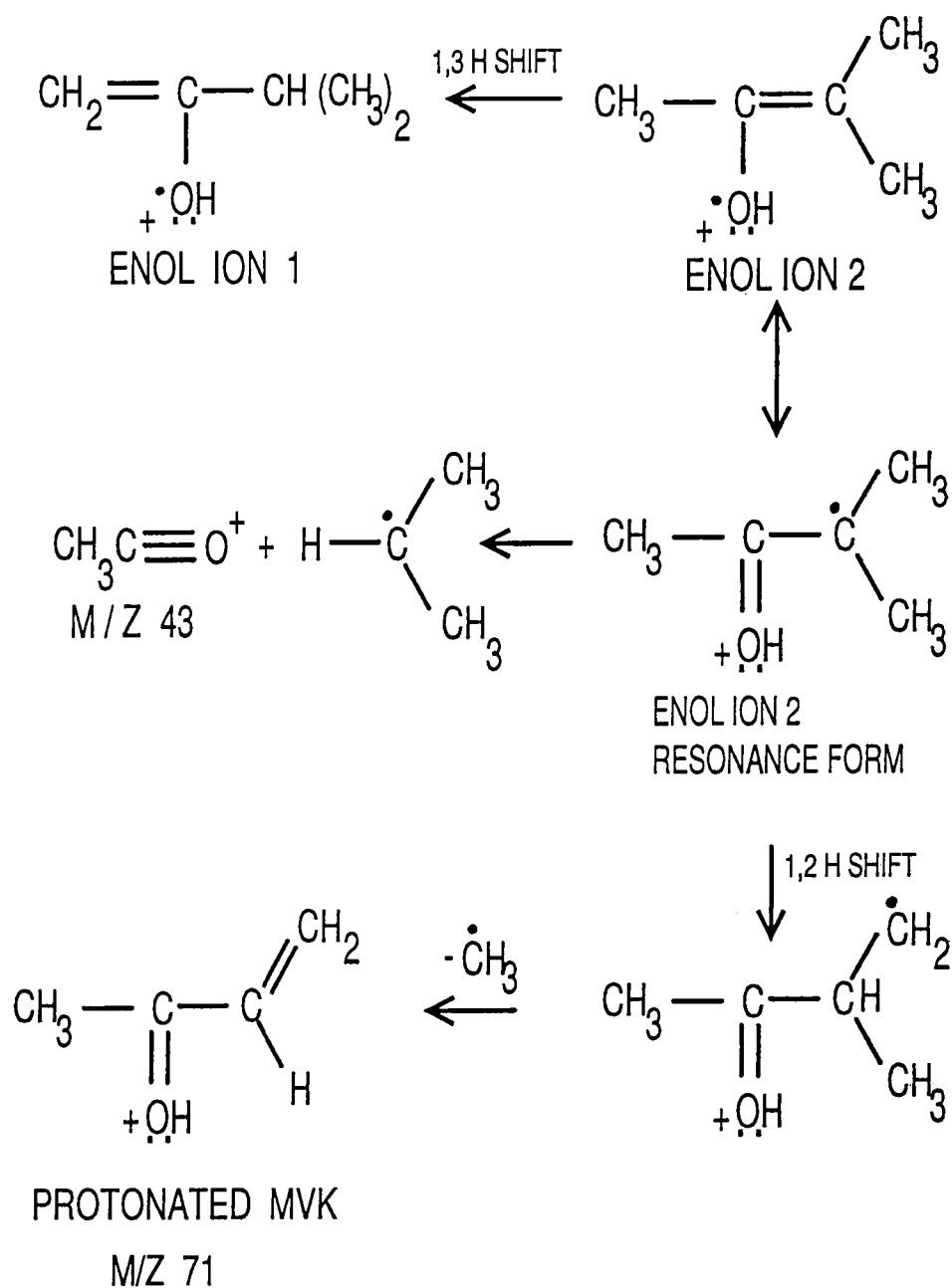


Figure 4.22. The possible fates of enol ion 2. Enol ion 2 would be initially formed from neutral enol 2 following the ionization of the neutral keto/enol mixture present at the source temperature.

m/z 43, helps account for the presence of m/z 43 in the 70 eV mass spectrum of enol ion 2. Since enol ion 1 comprises such a small amount of the original mixture and its formation from enol ion 2 after ionization would be negligible in light of the fact that enol ion 2 has other, more favorable pathways available to it, the contribution of enol ion 1 to the results obtained for the ionic system of 3-MB should be vanishingly small. The preferential fragmentation of the keto form would also tend to overwhelm any contribution from enol 1. The results obtained thus reflect the composition of the original neutral mixture, where one neutral enol (enol 2) makes, by far, the greatest contribution to this composition because of its greater stability in comparison with neutral enol 1.

2-PENTANONE

As a further test of the experiments and hypotheses outlined above for 3-MB, a related compound, 2-pentanone (2-P), was also examined. Upon ionization of the neutral keto form, C(1) and C(5) methyls are lost, as outlined in Figure 4.23. The C(5) methyl loss is believed to occur from the enol ion to produce protonated methyl vinyl ketone (MVK), while the C(1) methyl loss yields an acylium ion (A1) (29). The 2-P ionic system is very similar to that for 3-MB. The difference in stability of the keto and enol ionic forms of 3-MB and 2-P is similar (85 kJ/mol for 2-P, 91 kJ/mol for 3-MB) (7). Furthermore, the keto/enol composition of the neutral species is nearly the same for 3-MB and 2-P (2, 3, 6). Structural similarities between the 2-P and 3-MB systems should make entropy contributions about the same for the two systems. Thus, one may expect that the measured composition of the molecular ion should be very similar to that found above for 3-MB. The behavior of the 2-P m/z 71 ions, under MS/MS conditions, may be different than the 3-MB m/z 71 ions since the barrier to dissociation of MVK and A1 are very similar (220 kJ/mol) (7, 29, 76); preferential fragmentation of A1 with respect to MVK may therefore not occur as was seen above for the 3-MB m/z 71 ions. These hypotheses must be tested experimentally.

2-PENTANONE

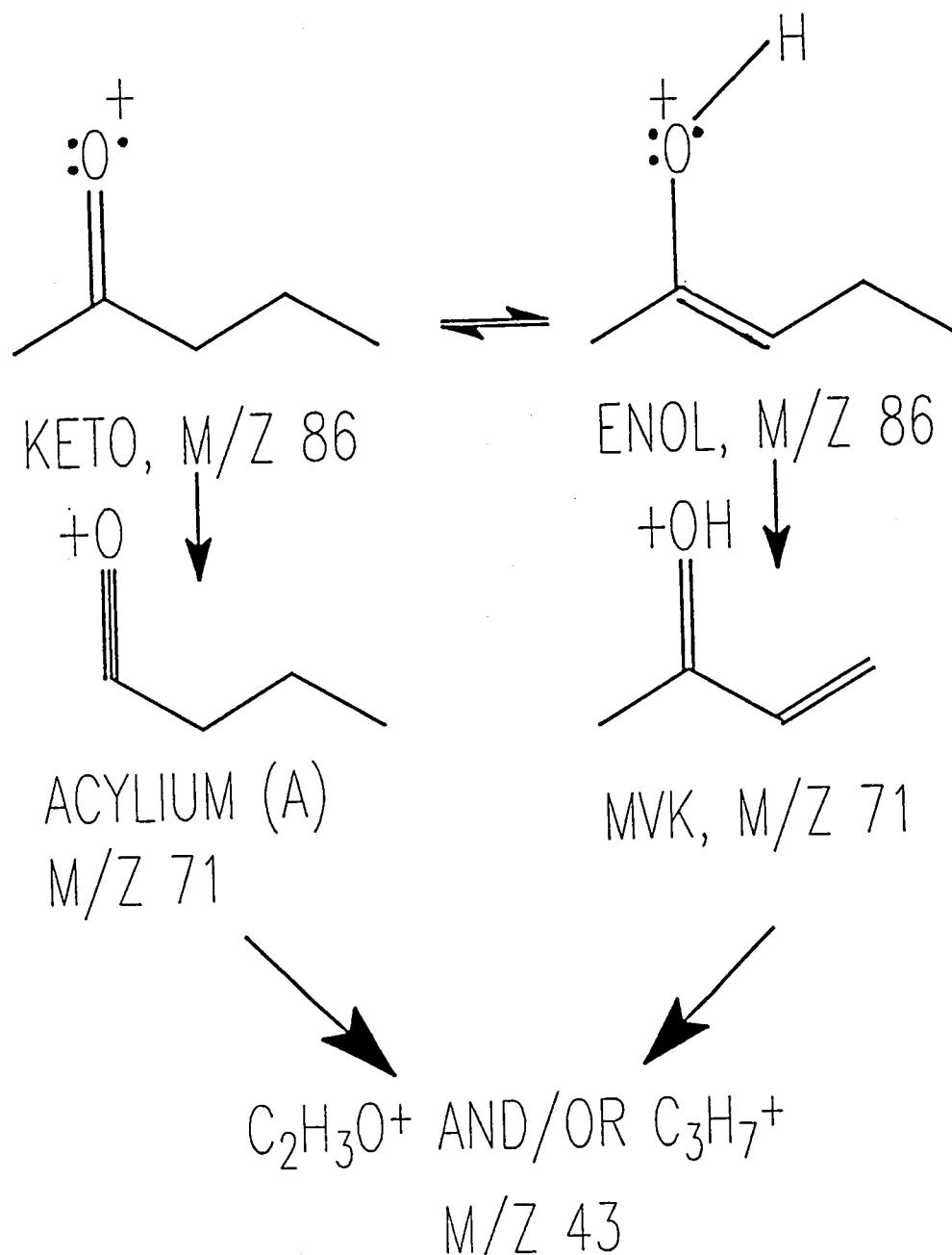


Figure 4.23. Fragmentation scheme of 2-pentanone (2-P) showing formation of the acylium ion, A1 (by loss of the C1 methyl), from the keto form and MVK from the enol form (by loss of the C5 methyl) of the molecular ion. These two m/z 71 ions can, in turn, fragment to yield 43H and 43L in a manner similar to the 3-MB m/z 71 ions.

Approach

For determination of the keto:enol composition of the molecular ion, the series of experiments most useful to perform can be determined by carefully considering which experiments provided the greatest amount of information for 3-MB. Determination of keto/enol composition of the molecular ion is easiest to achieve by utilizing an ion-molecule reaction. This directly yields the desired compositional data which can be more difficult to determine from MS/MS dissociation patterns. The MS/MS dissociation pattern was utilized for 3-MB composition, assuming nearly equivalent cross-sections for the keto and enol dissociations. For 3-MB, that proved to be the case, but the method may not always be generally applicable. The possibility also exists for ambiguous or incorrect assignment of product ions as arising from the keto or enol molecular ion.

The MS/MS of the m/z 71 ions (MVK and A1 in the case of 2-P) can be performed for compositional determination. The linear superposition principle can be applied to these m/z 71 ions for quantification purposes. The m/z 71 and m/z 86 ions of 2-P are also examined by ITMS MS/MS to examine time-scale effects for this ionic system. This will determine if the linear superposition principle holds for ITMS MS/MS of 2-P as it did for 3-MB, ensuring that the ITMS MS/MS data treatment is not unique to 3-MB.

QEB Results for m/z 71 ions from 2-P

The m/z 71 ions, MVK and A1, produced from 2-pentanone (2-P) were mass selected by the quadrupole and subjected to low energy CID over the collision energy range of 5-50 eV. As with the 3-MB m/z 71 ions, the 43H:43L ratio remained constant. For the 2-P ions, the ratio is 3.35 (77% 43H:23% 43L $\pm$ 4.3%).

In order to quantitate the 2-P m/z 71 ions, the reference ions for A1 and MVK must run under the same conditions as those used for the 2-P ions. MVK has already been studied for the 3-MB system, so its behavior is established (see Figures 4.6 and 4.8). A1 can be prepared in similar manner to A (see Figure 4.4); A1 is generated by 70 eV dissociative EI of n-butyryl chloride. Low energy CID of A1 produces only 43H. The dissociation efficiency ($\epsilon_{A1,43H}$) of A1 across the collision energy range 5-50 eV (70% attenuation using argon collision gas) is listed in table 4.7. Relevant ϵ values for MVK are also listed in table 4.7. Since A1 produces only 43H, equation 4.6 can be utilized for accurate quantitative analysis. The composition of the 2-P m/z 71 ions is then 73.8% A1, 26.2% MVK $\pm$ 2.31%. Since the two ions have nearly identical dissociation efficiencies, the measured (uncorrected for reference ion behavior) and corrected values for the composition of the 2-P m/z 71 ions are very similar. Such behavior is in direct contrast to that of the 3-MB m/z 71 ions, so preferential fragmentation of one m/z 71 ion with respect to the other should not be a problem for the 2-P system. Therefore, if the cross-sections for the fragmentations Keto $\rightarrow$ A1 and Enol $\rightarrow$ MVK are approximately equal, the directly measured (uncorrected) or corrected ratio of A1 and MVK m/z 71 ions that was

TABLE 4.7

Quantitation of 2-P m/z 71 ions as a function of low energy collision energy on the QEB.

C.E. ^A	$\epsilon_{A1, 43H}$	$\epsilon_{MVK, 43T}^B$	43R ^C	$\epsilon_{MVK, 43H}$	$\epsilon_{MVK, 43L}$	I_{43H}/I_{43L}	$\frac{[A1]}{[MVK]}$
5 eV	1.22%	1.20%	6.82	0.154%	1.05%	3.35	2.76
10 eV	1.81%	1.72%	6.38	0.234%	1.49%	3.50	2.75
20 eV	1.69%	1.64%	6.39	0.221%	1.42%	3.68	2.96
30 eV	1.88%	1.80%	6.85	0.229%	1.57%	3.44	2.75
40 eV	2.11%	2.06%	7.03	0.258%	1.80%	3.53	2.89
50 eV	2.05%	1.97%	7.21	0.240%	1.73%	3.47	2.81

^Acollision energy

^BTotal production of m/z 43 product ions from MVK; also referred to as $\epsilon_{MVK, 43H + 43L}$.

^C43L : 43H ratio obtained from CID of MVK.

Notes:

The values listed for the various ϵ terms, 43R, and I_{43H}/I_{43L} are averages of 5-7 determinations.

Based on above results, $([A1]/[MVK])_{AVG.} = 2.82$ corresponding to 73.8% A1, 26.2% MVK ($\pm 2.31\%$).

determined above will accurately reflect the composition of the molecular ion of 2-P.

The composition of the 2-P molecular ion can be determined by the ion-molecule reaction with mesityl oxide in the collision cell performed with 3-MB above. Upon performing this experiment, the keto : enol composition was determined to be 84% keto : 16% enol ($\pm 6.3\%$). The m/z 71 results and ion-molecule reaction results for 2-P were in good agreement. As hypothesized above, the proportion of keto and enol ionic forms of the molecular ion are quite similar to that measured for 3-MB.

Behavior of the 2-Pentanone m/z 86 and m/z 71 ions on the ITMS

The 43H:43L measured ratio resulting from MS/MS of 2-P m/z 71 ions is 52% 43H : 48% 43L ($\pm 2.1\%$). Table 4.8 lists the relevant information needed to determine the composition of the m/z 71 ions based upon this MS/MS data. Applying the linear superposition of MS/MS spectra to the ITMS data for m/z 71 ions in the same manner as for the QEB 3-MB m/z 71 ions, the composition of the 2-P m/z 71 ions is 78% A1, 22% MVK. As for 3-MB, the same composition of the m/z 71 ions is found on both the QEB and the ITMS; the rearrangement of the acylium ion again yields the different results between the QEB and the ITMS.

The ion-molecule reaction, performed as described above for 3-MB, with mesityl oxide on the ITMS indicated a composition of the molecular ion of 86% keto, 14% enol ($\pm 3.8\%$). The MS/MS spectrum on the ITMS for m/z 86 from 2-P is essentially identical to that obtained on the QEB (low energy CID) as shown in Figure 4.24. Use of a higher

TABLE 4.8

MS/MS dissociation efficiencies and m/z 43 product ion ratios experimentally measured on the ITMS for the 2-P m/z 71 ions and the m/z 71 reference ions A1 and MVK.

COMPOUND	ϵ^A	43H:43L ^B
A1	30% ($\pm 2.7\%$)	65:35 ($\pm 2.7\%$)
MVK	28% ($\pm 3.5\%$)	5:95 ($\pm 1.8\%$)
2-P	29% ($\pm 3.1\%$)	52:48 ($\pm 2.7\%$)

^A ϵ defined as (total m/z 43 product ion intensity)/(m/z 71 ion intensity before MS/MS activation). Also referred to as $\epsilon_{X, 43H + 43L}$, where X is either A1 or MVK.

^B43H:43L measured using the ¹²C/¹³C ratio technique described in the text (see chapter 2).

Notes:

$$\epsilon_{A1,43H} = (0.65)(0.30) = 0.195$$

$$\epsilon_{MVK,43H} = (0.05)(0.28) = 0.014$$

$$\epsilon_{A1,43L} = (0.30)(0.35) = 0.105$$

$$\epsilon_{MVK,43L} = (0.95)(0.28) = 0.27$$

$$I_{43H}/I_{43L} = 1.08$$

Substitution of these values into equation 4.7 (see text) yields:

$$[A1]/[MVK] = 3.40$$

$$= 77.5\% \text{ A, } 22.5\% \text{ MVK } (\pm 2.7\%)$$

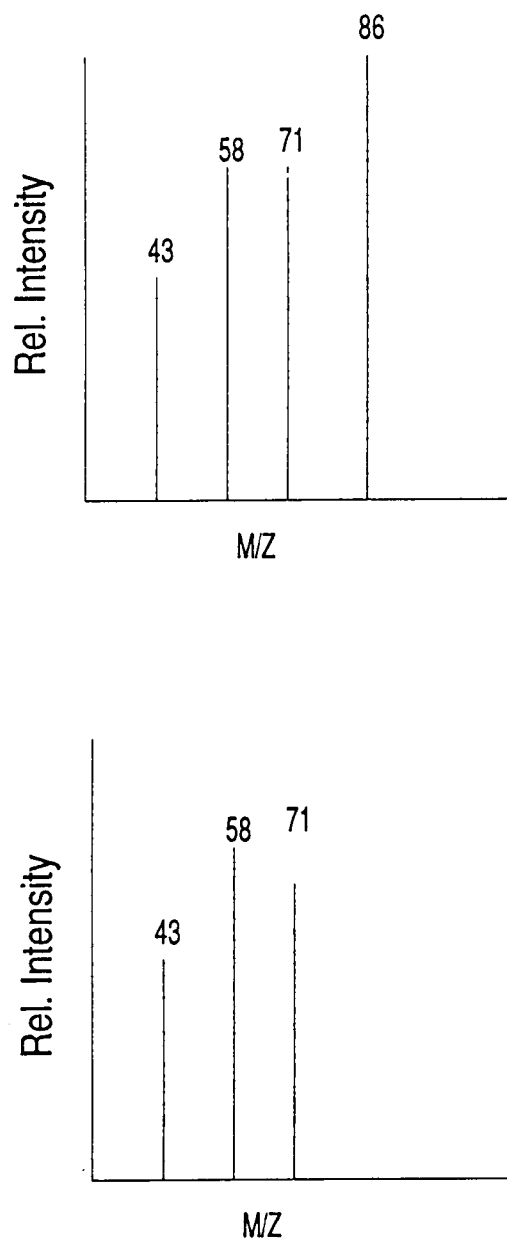


Figure 4.24. Comparison of MS/MS spectra of m/z 86 from 2-pentanone (2-P) obtained on the QEB (top spectrum) and the ITMS (bottom spectrum). Conditions for the QEB included 30 eV collision energy with 50% attenuation of the m/z 86 precursor ion with argon. An electron energy of 70 eV was used. For the ITMS, a tickle voltage of 350 mV was used and applied for 10 msec. The RF trapping level was set, with the data system, to a value of 29 amu ("low" RF level). The helium bath gas pressure on the ITMS was 1×10^{-3} torr. For both instruments, the sample pressure was 1×10^{-6} torr.

RF trapping level showed the same type of behavior as for 3-MB. No changes for m/z 86 ITMS MS/MS spectra were noted for a variable delay time after MS/MS activation (30 msec - 1 second). The thermodynamically more stable enol ion does not predominate for 2-P, as was found for 3-MB.

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

A method for quantification of isomers by tandem mass spectrometry (MS/MS) has been presented in this thesis. Utilizing tandem mass spectrometry for quantification of aliphatic keto and enol isomers is preferable since these isomers cannot be separated chromatographically. The neutral enol isomer simply does not exist in large enough quantity at normal temperatures and pressures to permit reliable chromatographic separation. After quantitation of the keto and enol isomers using MS/MS data, the expected mass spectrum of the enol isomer can be deconvoluted from the mass spectrum obtained when the pure, neutral keto form is introduced into the ion source. This is a novel discovery which has not been presented previously. The keto and enol ionic isomers, therefore, react independently under the high internal energy conditions present with standard EI conditions (70 eV electron energy) since the mass spectrum of the neutral enol can be deconvoluted from that of the keto form.

Comparison between the beam instrument and the trapping instrument

The beam instrument and trapping instrument MS/MS data were seen to differ. The longer time-scale of the ITMS allowed a rearrangement process for a diagnostic fragment ion (the acylium ion, in this case) to occur; such rearrangement processes can lead one to reach false conclusions regarding the composition of the molecular ion from

which the diagnostic fragment ions arise. The original composition of the diagnostic fragment ions in the ITMS was determined by applying the linear superposition principle. It was found that the composition of the diagnostic fragment ions was the same in the two instruments when reference ion behavior was taken into account. Ion-molecule reactions were performed on the molecular ion to confirm that its composition was also the same on the two instruments.

The beam instrument (QEB) experiment did not suffer from complications due to rearrangement processes because of the much shorter time-scale of the MS/MS experiments on the QEB. Not only was the QEB data easier to interpret, but the mass resolution capabilities of the instrument for both high and low energy CID experiments were quite helpful since isotopic labelling did not have to be performed. This avoids the possible complication of having to isotopically label compounds and account for isotope effects on fragmentation reactions during data analysis. The beam instrument also allows for greater control of internal energy deposition in low energy CID experiments which was quite useful for the ion chemistry problem studied here.

The fragmentation efficiency (denoted as ϵ in chapter 4 of the thesis) of the diagnostic m/z fragment ions studied on the QEB showed interesting behavior. For the 3-MB m/z 71 ions, the acylium ion (A) fragmented about four times as efficiently as the protonated methyl vinyl ketone (MVK) under low energy CID conditions on the QEB. Based on this difference in dissociation, the acylium ion underwent preferential fragmentation within the ion source resulting in a composition of the 3-MB m/z 71 ions not truly reflecting the keto/ enol composition of the molecular ion. QEB low energy

CID results and ion-molecule reactions on the molecular ion confirmed this suspicion. In fact, the composition of the 3-MB m/z 71 ions (48% A : 52% MVK = 0.923) multiplied by a factor of four to account for the preferential fragmentation of the acylium ion yields the experimentally measured ratio of keto:enol composition of the molecular ion (80% keto, 20% enol). The experimentally determined preference of fragmentation of the acylium ion, determined by low energy CID, seems to indicate that after initial formation from the molecular ion the composition of the 3-MB m/z 71 ions would be about 80% A, 20% MVK. This ratio is then altered by the preferential fragmentation of the acylium ion.

This hypothesis is confirmed by observing the behavior of the 2-P m/z 71 ions. For the 2-P ions, the dissociation efficiencies of the two m/z 71 ions A1 and MVK are nearly identical. In this case, the measured composition of the m/z 71 ions is 79% A1, 21% MVK which is nearly identical to the keto/enol composition of the molecular ion. For 2-P, the originally formed m/z 71 ions, if they undergo decomposition within the source, do so to the same extent. Their composition is not altered due to preferential fragmentation as was seen for the 3-MB m/z 71 ions. This behavior (preferential fragmentation) is most likely a reflection of the greater stability of the secondary carbocation formed at m/z 43 from the 3-MB m/z 71 acylium ion in contrast with the primary carbocation formed at m/z 43 from the 2-P m/z 71 acylium ion. Due to this difference in product ion stability, the 3-MB m/z 71 acylium ion would then be more likely to fragment than the 2-P m/z 71 acylium ion.

Future Work

Instrumentation

The QEB hybrid mass spectrometer can be further improved by utilizing an RF-only quadrupole collision cell instead of the simple DC collision cell used here. This would further improve sensitivity for the low energy CID experiments since the RF-only quadrupole collision cell would have a greater pathlength than the one used for this thesis (10-20 cm vs. 1 cm). This longer pathlength allows a greater number of collisions to occur within the collision cell. The nature of the field within the RF-only quadrupole would also help confine the ions in the radial direction, thus reducing scattering losses to some extent.

The acceleration lens system used on the QEB can also be modified to remove the anomalous behavior of the lens system at collision energies below 5 eV. Recall from chapter three of this thesis that the acceleration lens system showed the best performance above 5 eV with voltage ratios of $V_3/V_1 = 4.43$ and $V_2/V_1 = 2.70$ (see table 3.3) with the potential on acceleration lens element 1 held constant in such a way that the kinetic energy of ions at that lens element was 520 eV. Upon exiting the collision cell with 5 eV, the ions undergo a factor of 104 increase in kinetic energy when they reach acceleration lens element 1 (i.e., 520 eV/5 eV). This may simply be too great of a change in kinetic energy for ions exiting a field-free region such as the low energy collision cell of the QEB. Under such conditions, the lens is said to be too "strong" at

the initial stages of the lens. If the lens is too strong at an early portion of the lens, it seems logical that the lens would be correspondingly weaker in a different portion of the lens system. Referring to table 3.3, it is seen that at 5 eV, the voltage ratio V_2/V_1 is indeed lower than that utilized for other collision energies. In fact, it is lower by an amount $(104/30)^{1/2}$. The value of 104 is the increase of kinetic energy between the collision cell and acceleration lens element 1 and 30 appears to be an approximate upper limit to this kinetic energy increase necessary for proper operation of the acceleration lens system. The square root of this ratio is used since the trajectories of ions respond to voltage changes with such a relationship. This is analogous to the square root relationship between light rays and the refractive indices of the materials through which the light rays pass (69). In order to obtain a more uniform performance (i.e., constant voltage ratios between various lens elements) of the acceleration lens system across the collision energy range on the QEB, it would be necessary to add an additional lens element, between the collision cell and acceleration lens element 1, to the acceleration lens system. This element would be held at a fairly low voltage relative to the collision cell (perhaps -50 to -150 volts) to remove this "strong" lens effect seen at very low collision energies here. The computer modelling program SIMION could prove quite useful in updating the lens system while maintaining the desirable properties of the lens system discussed in chapter 3. SIMION seems to be most useful in those situations where the user has gained enough experience with ion optics to know the basic type of lens system to utilize for a given application (69, 71).

Keto/enol studies

The same type of procedure employed above for 3-methyl-2-butanone and 2-pentanone could be applied to other ketones. Knowing the diagnostic ions for the two forms of the molecular ion, analogous to the m/z 71 ions for the compounds studied here, it should be possible to utilize the linear superposition principle of MS/MS spectra if the diagnostic ions are isomeric or to directly determine the ratios of these ions in the ion source if they are not isomeric. MS/MS experiments could then be conducted on the molecular ion to determine its composition, as described for 3-methyl-2-butanone and 2-pentanone in this thesis. In order to determine the accuracy of the deconvolution method applied to 3-methyl-2-butanone for determination of the enol ion mass spectrum, the keto form of the compounds for which Turecek has generated the mass spectrum of the pure enol could be studied. In this way, the easily obtained keto forms can be used in a study involving the compilation of a data base containing keto and enol mass spectra, where enol mass spectra are determined by the deconvolution as shown in this thesis. The data base could be created initially from compounds involved with Turecek's study (e.g., 2-butanone) (32). If the approach appears successful, Turecek's method for in-situ generation of the thermodynamically less stable enol could be used to continue the compilation of the data base. However, since the method described in this thesis gives accurate results, it may prove to be the simpler and more economical approach to the study of keto/enol isomerization.

Linear Superposition Principle

The linear superposition of MS/MS spectra could prove to be a useful technique for isomer quantification. For example, if isomeric species show distinct MS/MS spectra, this principle could be applied for quantification of the isomeric species, as shown in this thesis. Furthermore, if the pure, neutral isomeric species are available, the composition of mixtures could be confirmed by chromatography. Standard mixtures of known composition could also be subjected to MS/MS experiments to determine experimental conditions most suitable to yielding reliable quantitative results by the linear superposition principle.

A further application of the linear superposition principle is the quantitative analysis of isobaric ions by MS/MS when utilizing instrumentation not capable of directly resolving the isobaric mixture. Such a situation occurs with a tandem mass spectrometer known as a triple quadrupole mass spectrometer; such an application has recently been demonstrated in the literature in which quantitation of isobaric species and deconvolution of the individual MS/MS spectra of those isobars was performed (98). The approach was similar to that used in this thesis. This may be an important future application of the linear superposition of MS/MS spectra.

The biggest advantage of utilizing MS/MS techniques for isomer analysis is the high throughput of MS/MS techniques, in direct contrast to chromatographic methods. The precursor ion(s) of interest can be mass selected from a mixture within the ion source and subjected to CID. Based on the behavior of the reference ions for these precursor

ions, the linear superposition can hopefully be applied for quantitation. Such a throughput advantage would probably be most useful to those in an industrial laboratory setting looking for ways to greatly increase the efficiency of their operation.

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VITA

Michael Ray Troutman was born in Rochelle, Illinois on September 10, 1961. He attended elementary schools in the Sycamore, Illinois School District and graduated from Sycamore High School in June, 1980. In September, 1980, he enrolled at Western Illinois University (Macomb, IL) and received a Bachelor of Science degree in Chemistry with a minor in Mathematics in May, 1984. He enrolled at Indiana University (Bloomington, IN) in September, 1986 and pursued graduate studies in Analytical Chemistry under the direction of Dr. Ron Hites. The research involved studies of liquid chromatography/mass spectrometry (LC/MS) as applied to environmental analyses. He received the Master of Science degree in Analytical Chemistry in May, 1989 from Indiana University. In September, 1989, he enrolled at the University of Tennessee, Knoxville while performing graduate research at Oak Ridge National Laboratory with Dr. Gary Glish and Dr. Scott McLuckey. His doctorate research involved studies of radical cation keto/enol tautomers utilizing tandem mass spectrometry. Included in his doctoral studies were the design, construction and characterization of a novel hybrid mass spectrometer of QEB configuration. He received his Ph.D. in Analytical Chemistry in May, 1994 from Dr. Kelsey Cook at the University of Tennessee, Knoxville.