11-1990

Part I: Oxidation of Heavy Metal Sulfides in Relation to the Environment; Part II: Fundamental Theory & Experiments Concerning Gas Chromatography & Mass Spectrometry

Judith Morgan
Western Kentucky University

Morgan,

Judith R.

1990

Part I: OXIDATION OF HEAVY METAL SULFIDES

IN RELATION TO THE ENVIRONMENT

Part II: FUNDAMENTAL THEORY AND EXPERIMENTS CONCERNING

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

A Thesis

Presented to

the Faculty of the Department of Chemistry

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Judith R. Morgan

November 1990

AUTHORIZATION FOR USE OF THESIS

Permission is hereby

Signed: _Judith R Morgan_

Date: ___12-5-90___

Please place an "X" in the appropriate box.

This form will be filed with the original of the thesis and will control future use of the thesis.

Part I: OXIDATION OF HEAVY METAL SULFIDES

IN RELATION TO THE ENVIRONMENT

Part II: FUNDAMENTAL THEORY AND EXPERIMENTS CONCERNING

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Recommended   October 25, 1990
                                    (Date)

Director of Thesis

Approved   *December 13, 1990*
                    (Date)

Dean of the Graduate College

To Brittany, Erica, and Holly

Acknowledgements

I would like to express my gratitude to the following:

My husband, Rodney Morgan, who always found time to support me in this venture.

My three children, Brittany, Erica, and Holly who enlightened me when I needed it the most.

Dr. Robert W. Holman for the many hours he devoted to this project and for the positive outlook that he helped me to maintain.

Dr. David J. Wilson for welcoming non-traditional research between Vanderbilt and WKU, and for his guidance in the course of this project.

Dr. Lowell Shank for all of the help he has given me in structuring my masters program.

Judy Campbell for all the love and patience she has with the students who need her the most, such as myself.

Hewlett Packard for original drawings and diagrams.

Dr. Ann Clark for without whom I would not have had the pleasure of knowing Dr. Wilson.

TABLE OF CONTENTS

Chapter One

Chapter Two

LIST OF TABLES

LIST OF FIGURES

ix

Part I: OXIDATION OF HEAVY METAL SULFIDES

IN RELATION TO THE ENVIRONMENT

Part II: FUNDAMENTAL THEORY AND EXPERIMENTS CONCERNING

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Judith R. Morgan   November 1990   113 pages

Directed by: Robert W. Holman, David J. Wilson, Lowell Shank

Department of Chemistry   Western Kentucky University

Oxidation of heavy metal sulfides is a thermodynamically spontaneous process. Because of this, metal sulfides in the presence of oxygen are not stable. Currently there are over 100 streams and rivers, within the U.S., contaminated with heavy metal mine drainage; therefore an approved method of restoration is necessary. Precipitation of heavy metals as sulfides using H_2S as a reductant has been favorably reviewed as a restorative technique to clean up mining areas. However, using this technique on the laboratory scale does not prove to be a viable answer and shows strong pH dependence.

In the past three years the ILI (Instrumentation and Laboratory Improvement) has awarded funding to over 50 institutions, in the U.S., for the purchase of GC/MS. Therefore, there is a great need for laboratory experiments to properly train students in this field in American universities. A strong theoretical treatment for sophomore-level

students is presented within this thesis and from this, three experiments have been developed in order to educate young professionals seeking a career in the field of chemistry.

INTRODUCTION

A. Statement of Problem

The motivation for this research centers on the problem of heavy metal contamination of soil and groundwater in the vicinity of mining areas. Problems associated with heavy metal deposits at and in the vicinity of mine sites are two-fold. First, due to the toxicity of the heavy metals serious health problems exist. Second, the clean up of such sites can potentially cause serious economic impact.

The effects of mining have greatly increased the amount of metals present in water supplies and because biological adaptation to trace metals occurs slowly or not at all, plant and animal life have suffered greatly.[1] Trace metals, in small quantities, are essential to life, except in the case of metals such as arsenic and cadmium which have no known biological function. However, in high concentrations most essential elements can be toxic. Toxicity can mean anything from acute discomfort to death. Most heavy metals are cumulative in the system and are deposited in many areas of the body, such as the bones or liver, which can cause gradual or even immediate malfunction.

Aquatic life and vegetation have suffered the most from the effects of mine drainage.[2] Fish have been studied in their natural environment and have shown that lead compounds

cause suffocation due its reaction with organics found in the mucous membranes.[3] Cadmium has also been shown to be a lethal substance, especially in combination with other metals such as zinc which, when present, actually increase the effects.[4] Although water quality standards do not regard copper to be a highly toxic substance to man, the lethal dose in trout may be as low as 0.08 micrograms/liter,[5] which is quite small compared to the 1000 micrograms/liter allowable limit for water supplies. Human consumption of lead (Pb) can affect the central nervous system and cause a variety of other disturbances such as paralysis, mental confusion, anemia, and convulsions. Cadmium (Cd) exposure can cause choking, vomiting, and gastrointestinal disturbances. Exposure to high amounts of these metals may lead to problems such as chronic anorexia, paralysis, convulsions and ultimately death.[6]

In the mining of metal ores either by traditional or solution mining techniques, groundwater is frequently contaminated with transition and heavy metals.[7] The remediation techniques utilized vary depending upon the mine location and the groundwater characteristics. Remediation procedures result in substantial costs the magnitude of which varies depending on the type of remediation necessary.

B. Nature and Magnitude of Problem

Mining operations expose large quantities of previously undisturbed ore in the search for valuable minerals. In their natural crystalline form metal sulfides are relatively stable.[8] The natural environment containing these metals and

minerals is mechanically agitated during the mining process, which provides potential for many sources of pollution. The nature of the mining process involves techniques such as milling and grinding which are used to maximize metal recovery. The underground agitation loosens the material, thus exposing increased surface area to oxygen and water, resulting in weathering at a much faster pace. In an undisturbed area the weathering process is much slower. The products of weathering or natural breakdown include acids and metal ions formed by the oxidation of metal sulfides.[9]

During the course of active mining, the groundwater must be either pumped from the mine or allowed to drain freely from the work area. Many times the groundwater may be controlled and treated or even diverted around the mining site. Once the mining ceases, maintenance frequently ceases as well, resulting in increased pollution potential. A good example of this is the situation in Leadville, Colorado, where lead mining in the late 1800's was performed with pumping to keep the water out of the mine.[10] Once the demand for lead decreased it was not profitable to keep the mine in operation and subsequently the mine was allowed to fill with water. Finally, in 1943 the construction of a drainage tunnel began, to restart mining operations, and once again maintenance ceased when demand decreased. Currently the tunnel is blocked, and heavily contaminated drainage has backed up in the tunnel, posing a threat to the municipal water supply.

Due to the large number of inactive mines the potential for mine pollution in our nation is phenomenal. The Bureau of Mines cited 19,000 abandoned and inactive ore and mineral mines and a total of 90,000 mines of all types in an unpublished report.[11] Over 100 streams and rivers have been adversely affected by these mines (see map fig. 1.1). The principal contaminants are acid drainage and heavy metals. Heavy metals associated with mine drainage can be carried into streams and lakes in their soluble forms, be sorbed onto sediments, and settle to the bottom. Through biochemical or other chemical processes these metals can cycle back and forth from their insoluble to their soluble forms. The soluble forms, such as metal sulfates, can be released into the environment steadily for many years after the drainage has stopped.[12] Known inactive mines have been estimated to discharge 30,000 to 50,000 metric tons of acid and 10,000 metric tons of metal annually into the nation's surface waters.[13]

C. Approaches to the Problem

In undisturbed areas, weathering is a natural and con- tinuous process which takes place at a slow pace that allows for biological adaptation. Once an area is exposed to increased oxidation, the weathering process is accelerated. In-situ remediation of mining areas may be the solution to slow or stop contamination. Several in-situ methods have been used for remediation; these are discussed briefly below.
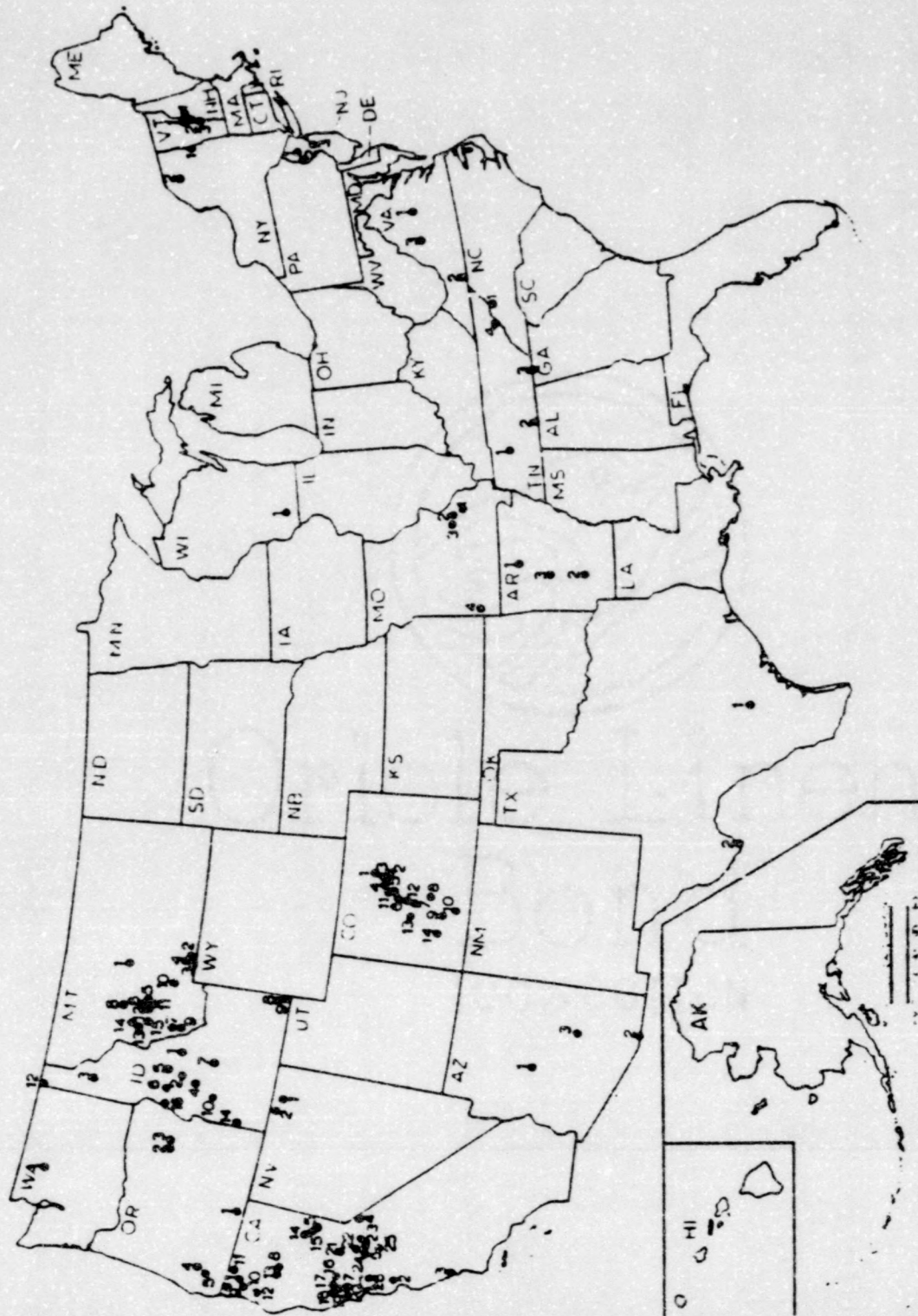
FIGURE 1.1   Map of streams and rivers contaminated by mining
             activities

Foam flotation[14] has been used in the mineral industry and has been suggested by the EPA as a method for heavy metal and acid mine drainage remediation. This technique is performed by adding an organic complexing agent to the aquifer, which reacts with the metals to form an anionic complex. The complex is then removed from the solution by flotation.

Reverse osmosis has also been shown to be a feasible method for mine drainage treatment. Moxley and Catchpole[15] used this technique for restoration of the Bison Basin uranium mine in Wyoming. The primary goal of their research was to restore the groundwater to its original state before the solution mining was done. The project required pumping of the contaminated water from the contaminated aquifer to a processing plant, then treating the water using reverse osmosis, followed by reinjection of the water back into the aquifer. This treatment was performed until the groundwater metal concentrations were at or below their original values. The water was monitored for a six-month stability period and showed upward trends, in concentration, toward the later portion of the study. This suggests a problem with this remediation technique.

Precipitation of metals using a two-step method has also been suggested by EPA.[16] In the first step lime is added to the aquifer, in order to elevate the pH, causing precipitation of iron and aluminum. Barium sulfide is added in the second step which will react with the metals to form insoluble metal sulfide precipitates. The main drawback to this procedure is

the high cost of barium sulfide. It was suggested however that research be conducted to find a more economical sulfide source.

Precipitation using H_2S as a source to form metal sulfide precipitates was developed to replace the former sulfide precipitation technique.[17] EPA suggests this to be a feasible method for transition and heavy metal removal.

D. Hypothesis for Research

The research reported in this thesis centers specifically upon the metal sulfide precipitation technique employed to restore groundwater at mining sites to its natural state. Heavy metal sulfides are generally very insoluble, with solubility products of 2.5×10^{-27}, 8×10^{-27}, and 6×10^{-36}, for PbS, CdS, and CuS respectively at 25 degrees celsius.[18] Because of this property, very little metal ion would be expected to leach into groundwater in contact with these sulfides. The metal sulfides seem to be quite stable even at rather acidic pHs. As such, sulfide precipitation should immobilize the contaminants and hence should be a viable solution to the problem.

The stability pattern exhibited by the metal sulfides at different pH values is shown in the following equations and graph.

H+ ion concentration:

$H_2S(aq) \rightleftharpoons HS^-(aq) + H^+(aq) \quad K_1 = 8.9 \times 10^{-8}$

$HS^-(aq) \rightleftharpoons S^{2-}(aq) + H^+(aq) \quad K_2 = 1.2 \times 10^{-13}$ [19]

$H_2S(aq) \rightleftharpoons 2H^+(aq) + S^{2-}(aq)$
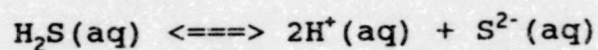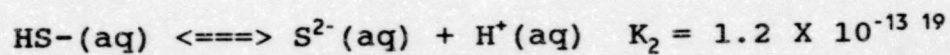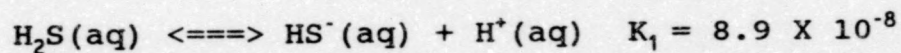
$$K_1 K_2 = \frac{[H+]^2 [S^{2-}]}{[H_2S]} \qquad (1.1)$$

$$[S^{2-}] = \frac{[H_2S] K_1 K_2}{[H^+]^2} \qquad (1.2)$$

Metal sulfide:

$$MS(s) <===> M^{2+}(aq) + S^{2-}(aq) \qquad K_{sp}$$
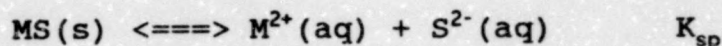
$$K_{sp} = [M^{2+}] [S^{2-}] \qquad (1.3)$$

Substitution of equation 1.2 into 1.3 and solving for metal ion concentration gives:

$$M^{2+} = \frac{K_{sp} [H+]^2}{[H_2S] K_1 K_2} \qquad (1.4)$$

K_{sp} values:[20]

$$Cd = 8.0 \times 10^{-27}$$
$$Cu = 6.0 \times 10^{-36}$$
$$Pb = 2.5 \times 10^{-27}$$

See graph in fig. 1.2.

An important reaction of these sulfides, however, must be considered. A reaction that might be able to convert water insoluble metal sulfides into water soluble metal sulfates is as follows:

$$MS(s) + 2O_2(g) <====> M^{+2}(aq) + SO_4^{-2} (aq)$$
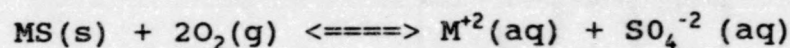
where MS = metal sulfide

Since metal sulfates are soluble, the metal is now mobile and the potential environmental hazard increases. The above reaction is the oxidation process that takes place when the metal sulfides are exposed to oxygen. Kelley[21] has documented that infiltration water from rain and snow promotes the formation of oxidation zones in mine areas. Kelley reported that the oxidation zone expands as the oxidative process continues.
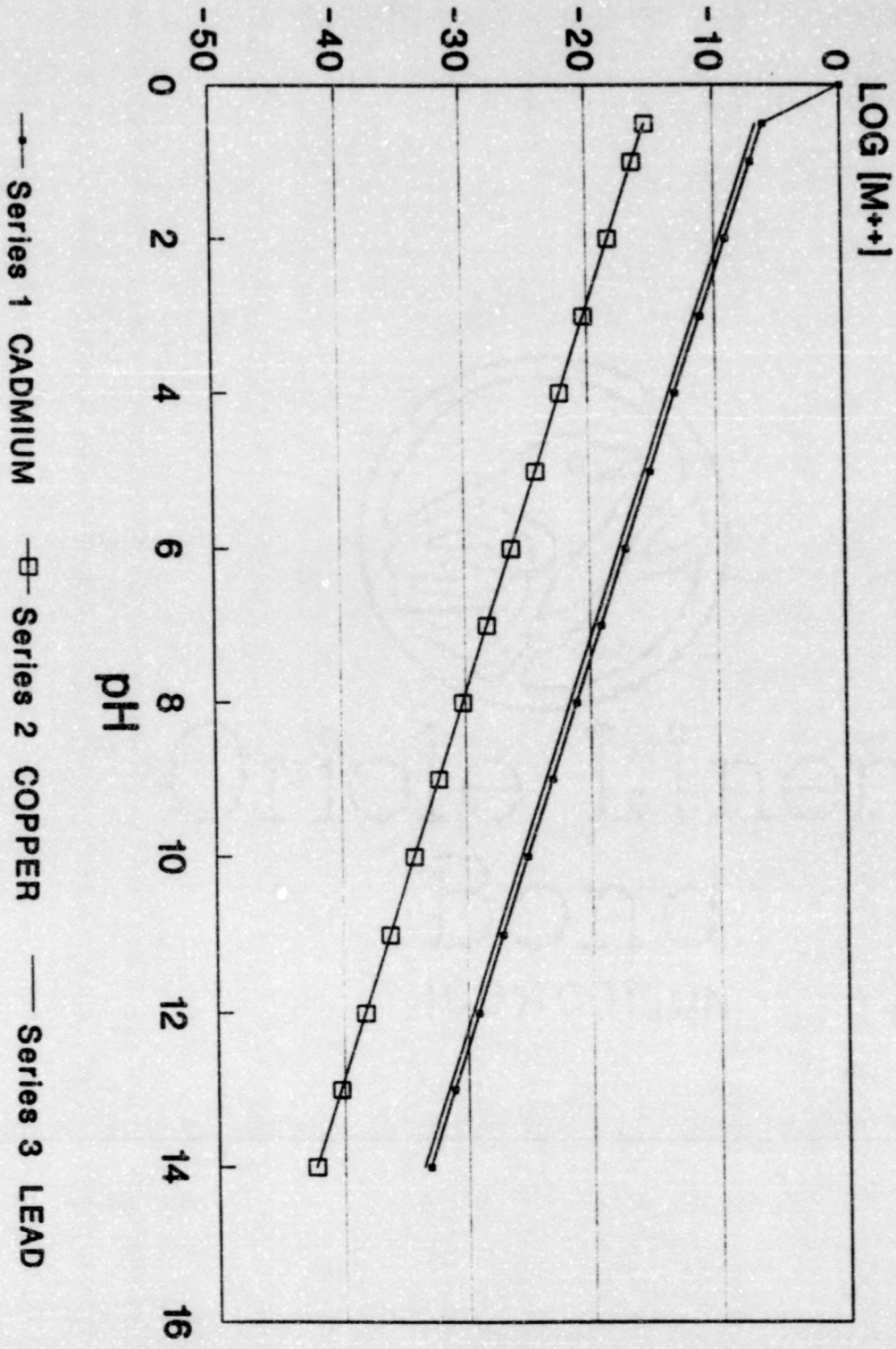
FIGURE 1.2   Metals: Log of Concentration vs pH

The rate of this reaction changes with pH, being fastest at a low pH and slowest at a high pH. Therefore, in acidic soil or water, the problem is intensified, and in basic media it is less acute.

The natural weathering process of iron sulfide results in the formation of sulfuric acid:

$$FeS_2(s) + {}^{15}/_4O_2 + {}^7/_2H_2O ==> Fe(OH)_3(s) + 2H_2SO_4$$
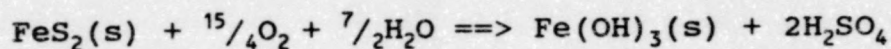
This reaction will provide a low pH medium which will enhance the formation of soluble metals. Bacteria present in the soil also aid in increasing sulfide oxidation rates.[22,23] Areas containing limestone slags exhibit natural buffering properties. This natural buffer increases the pH and thus neutralizes the acid being formed. The most common natural buffers in streams are bicarbonate and carbonate from limestone sources. In areas where natural buffers are not present, the pH of mine water has been found to be as low as 1.4 as reported for a drainage pond in the Rocky Mountains.[24]

The possibility of oxidation of the sulfides to the more soluble sulfates is thermodynamically quite favored. Also, lead sulfate has a calculated molar solubility of 1.3×10^{-4}, and cadmium and copper sulfates are quite soluble in water.[25] We examined the reaction of the three sulfides with dissolved oxygen to form the more soluble sulfates:

$$MS(s) + 2O_2(g) ==> M^{+2} + SO_4^{-2}(aq)$$
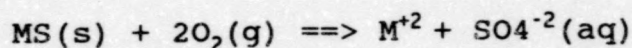
where M = Cu, Cd, and Pb

The standard free energy changes (shown below) for these reactions are all negative and quite large, so these reactions

are thermodynamically spontaneous. The standard free energy changes can be calculated using the above reaction and the relationship shown below:

$$\Delta G = \Delta G^{\circ}_f(SO_4^{-2}) + \Delta G^{\circ}_f(M^{+2}) - \Delta G^{\circ}_f(MS)$$

where

Species	ΔG°_f, kJ/mol [26]
SO4-2(aq)	-741.99
PbS	-96.7
Pb+2(aq)	-24.3
Cu+2(aq)	64.98
Cd+2(aq)	-77.74
CdS	-141.0
CuS	-53.6

CuS $\Delta G^{\circ} = -741.99 + 64.98 - (-53.6) = -623.4$ kj

PbS $\Delta G^{\circ} = -741.99 - 24.3 - (-96.7) = -669.6$ kj

CuS $\Delta G^{\circ} = -741.99 - 77.74 - (-141.0) = -629.0$ kj

Since $\Delta G^{\circ} = -RT(\ln K)$ and $K = \exp(-\Delta G^{\circ}/RT)$ hence, the equilibrium constants for the oxidation of the sulfides are as follows:

CuS, $K(298) = \exp(250.8) = 10^{+109}$

PbS, $K(298) = \exp(269.4) = 10^{+117}$

CdS, $K(298) = \exp(273.1) = 10^{+119}$

Our hypothesis is that if the groundwater contains dissolved oxygen, this will permit the oxidation of metal sulfides to metal sulfates, so that sulfides which were initially immobile will be released as the mobile metal sulfates. This can cause a contamination release for an extended period of time (potentially years).   If this is the case then sulfide precipitation is not a viable restoration method.

In support of our hypothesis, several field studies have been conducted that suggest that the rates of metal sulfide

oxidation may be appreciable. The U.S. Geological Survey reports[27] that several areas have been studied in which conclusive creek and stream contamination was observed. Tar Creek, Kansas, has shown a low pH, with high concentrations of dissolved lead, zinc, and cadmium. This contamination resulted from open drill holes that penetrated shale which confined a shallow underlying mined aquifer. This is only one of several mine-related problems in the Kansas area. A study performed at the Front Range mineral belt in Colorado showed that sulfide ores do indeed yield significant amounts of toxic trace metal quantities in groundwater.[28] An Italian study showed that concentrations of heavy metals and sulfate varied significantly in groundwater in the vicinity of sulfide ore mines.[29] In Georgia a study was conducted on stream water in the vicinity of a Cu-Zn-Pb sulfide ore deposit. The result showed heavy metals deposited on ceramic plates immersed in the stream-water.[30] Ion exchange was shown to be an important consideration in controlling the movement of these metals.[31]

While the above studies suggest that sulfide oxidation may take place under field conditions, a thorough literature search uncovered no controlled laboratory investigation on this to date. Archer et al.[32] conducted a study in Papua, New Guinea, on mine tailings contained in lysimeters. Archer's study showed 60% sulfide oxidation and pH decrease over a period of 5 years with predicted complete oxidation in 7 years. Our investigation stems from the hypothesis that the metal sulfide remediation process supported by the EPA is not

a satisfactory remediation process because dissolved oxygen oxidation of the metal sulfides to metal sulfates may take place. This thesis centers upon the laboratory testing of that hypothesis.

E. Related Studies

We next provide an overview of a related precipitation-based restoration process. While several mine remediation techniques have been employed in the field, there is not a single agreed upon general technique available that is suitable for all sites.

Schmidt and co-workers[33] combined several groundwater restoration techniques on site at the Ruth orebody uranium mine of the Powder River Basin in Wyoming. In the past, the requirement to restore the groundwater of leaching areas to pre-operational quality has posed quite a problem due to the fact that the host rock is heavily damaged by certain leaching techniques. This restoration must be completed since an unrestored area poses major contamination problems.

In order to satisfactorily restore the Ruth orebody several restoration techniques were performed in combination. The orebody itself is composed of sandstone and was mined using a solution type leaching technique. The lixiviant (leach solution) used was sodium bicarbonate. Monitor wells were strategically placed in and around the leach wells, firstly to determine pre-operational baseline concentrations and secondly to monitor the area to ensure that the lixiviant was not escaping the leach zone during mining. Leaching began in

February of 1983 and continued for 12 months. The amount of uranium collected during that time period was 35,000 pounds with an average headgrade (concentration) of 84 ppm.

Restoration of pre-operational groundwater chemistry necessitates the removal of all lixiviant remains, mobilized compounds, and metals (U, V, Se, As, Fe, Mn, Mo, Ra), as well as restoration of the initial pH. The first step for restoration was a groundwater sweep. This is performed by pumping contaminated groundwater to the surface and replacing the water with clean uncontaminated water. This is only effective for pumping out the heavily contaminated water, and disposal of this liquid must be addressed. Since groundwater is not found abundantly in the western states and water quality must be preserved, discharge into streams or rivers is not usually permitted. In the second step of restoration the recovered solution is purified for reinjection. During purification the contaminants are removed from the groundwater and disposed of in the form of sludge. By treating the water the volume of toxic material which is disposed of is greatly reduced. In the final step, a reagent used to precipitate or inactivate the contaminants is injected.

The reagent used in the precipitation process was H_2S, which is not only a precipitant but a strong reductant as well. While all three steps contributed to restoration, this final step was considered to be the most important.

By September 1984, upon completion of the groundwater sweep and the purification/reinjection treatments (which were

performed over a nine month period), a total of 7.2 million gallons of water had been removed and treated. Approximately 8.6 million gallons of water were injected or reinjected after purification. At this time elevated metal concentration levels were still present and had shown an upward trend since August. The authors rationalized this increase by stating: "Most likely, ore remainders still reacted with dissolved atmospheric oxygen in the reinjected solutions. Atmospheric oxygen had already demonstrated its capability in uranium mobilization during groundwater circulation in January 1983, prior to injecting any leach chemicals." In order to remedy this increase a strong reductant was deemed necessary to facilitate oxygen reduction in the injection stream while at the same time reducing the heavy metals and fixing them underground as insoluble metal sulfides.

The injection of hydrogen sulfide began in November 1984 and continued until December 1984. The treatment lasted for a total of six weeks. Upon completion of reductant injection a stabilization period began in January 1985 and lasted 12 months. During this time concentration levels of the metals remained below or very close to target restoration levels. It was concluded that the H_2S precipitation-based restoration process worked well and that it will be used in the restoration of future commercial operations. The process has been approved by both the DEQ and the NRC as a suitable demonstration for a permit to be granted to perform this type of restoration on a commercial level.

Based on the study performed by Schmidt, the approval of the restoration of sites contaminated by heavy metals using hydrogen sulfide precipitation as a immobilization method suggests that it is a permanent fixation method. The fact that transition and heavy metal sulfides possess extremely low solubility products also suggests that sulfide precipitation as an immobilization technique is a feasible method for removing these metals from wastewater.[34] This would allow in-situ treatment and also allow burial of sulfide sludges or solidification followed by appropriate disposal (burial).

There have been several studies done that have shown that sulfide sludge reacts much in the same manner as mine tailings and spoil. That is, sulfide oxidation does take place and metal concentrations in groundwater increase over time. The fact that researchers suggest that reoxidation happens so slowly that the metal ions would be diluted sufficiently by the groundwater does not solve the problem of what happens to the soluble metals as they accumulate in the ecosystem. Cataldo Mission Flats, Idaho[35] is a large area of tailing deposits. In this area groundwater quality has suffered due to a low pH and high metal concentrations. It has been shown that fish and vegetation suffer dramatically when exposed to only small amounts of soluble metals. The release rates must be comparable to those resulting from normal weathering of undisturbed natural minerals to be biologically acceptable.

Mine tailings and sludge generated from precipitation of metals from wastewater have been disposed of in a variety of

ways. Many times they were buried at dump sites. In the situation reported by Kelley,[36] the oxidation of the dumped material was expected to occur in the same way as in the mines. Kelley reported that in areas where dumping has occurred oxygen can enter the soil via diffusion, convection, and advection. The presence of oxygen thus triggers the process of sulfate formation and hence a release of solubilized metals.

It has been shown by Ernst[37] that plants can become contaminated from the metals in dump areas. Agriculturally, crops have shown unacceptable levels of heavy metals in the areas of disposal. This information is provided to inform readers that this metal mobilization problem is not limited to improperly restored mines but rather to all aspects of the metal industry where waste water and metal by-product disposal is involved.

EXPERIMENTAL

A. <u>Equipment</u>

Below is a complete list of chemicals and equipment necessary to reproduce the chemistry that is described in Chapter 1 of this thesis.

1. Chemicals

Thioacetamide - Fisher Scientific Co.

Lead Nitrate - Fisher Scientific Co.

Cadmium Nitrate - Fisher Scientific Co.

Copper Nitrate - Baker Scientific Co.

Hydrochloric Acid - Fisher Scientific Co.

Sodium Hydroxide - Fisher Scientific Co.

2. Miscellaneous

Whatman 42 filter paper

18 250mL Erlenmeyer flasks

Millipore 0.45 micron filter paper

compressed nitrogen

4 rubber self-sealing septums

2 small gauge needles (1 long, 1 short)

Buchner funnel

filtration flask

micro-filtration funnel

vacuum pump

compressed air

magnetic stirrer and stirring bars

pH meter

Buffers: pH of 4, 7, 10 - to calibrate pH meter

Items to construct aeration system: 10 polyethylene T's

1 glass Y

1 polyethylene quick disconnect

8 ft. tygon tubing

B. Metal Solution Preparation

Dissolve 1.61g $Cu(NO_3)_2 \cdot 3H_2O$, 2.75g $Cd(NO_3)_2 \cdot 4H_2O$, and 1.60g $Pb(NO_3)_2$ in 1 Liter of 0.1 M HCl. This solution will contain 1.0 g of each metal.

C. Formation of Metal Sulfides

1. Dilute 50 mL of metal solution to 150 mL in an Erlenmeyer flask. Repeat for a total of eight flasks. Fill one extra flask with 150 mL water as a blank.

2. Acidify each sample to pH = 3. Heat all flasks to boiling and add thioacetamide using a scoopula until the reaction is complete. Check reaction for completeness by taking an aliquot from each flask and testing for the presence of metal using atomic absorption. At this point the solutions are brownish-black with a substantial amount of precipitate. If metal is still present, while the solution is still hot, add more thioacetamide until all metal has reacted and is no longer detectable in solution.

D. <u>Preparation of Metal Sulfides for Oxidation Aeration</u>

Two methods were used:

Method 1:

Leave metal sulfides in reaction solution and adjust pH in the flasks to 5,6,7,7,8,9 using 0.1M HCl and 0.1M NaOH. Two samples will be controls, adjust control-1 to pH=5 and control-2 to pH=9. These control samples will be stored under nitrogen using a nitrogen tank, flasks with self-sealing septums, gas inlet needle, and a needle for oxygen escape. Purge samples for 5-7 minutes while allowing air to escape, turn nitrogen flow down to a gentle bubble then pull outlet needle out and allow nitrogen pressure to build slightly in the flask. With nitrogen still flowing pull inlet needle out.

The purpose of the control samples is to show that metal sulfides that are unexposed to air will not oxidize.

Method 2:

After metal sulfides are formed, filter them onto Whatman 42 filter paper, using vacuum filtration, and place in a 250 mL Erlenmeyer flask. Dilute each sample to 150 mL and adjust pH as stated in part 1. Prepare two controls as instructed in part 1.

E. <u>Aeration Apparatus</u>

See fig. 1.3
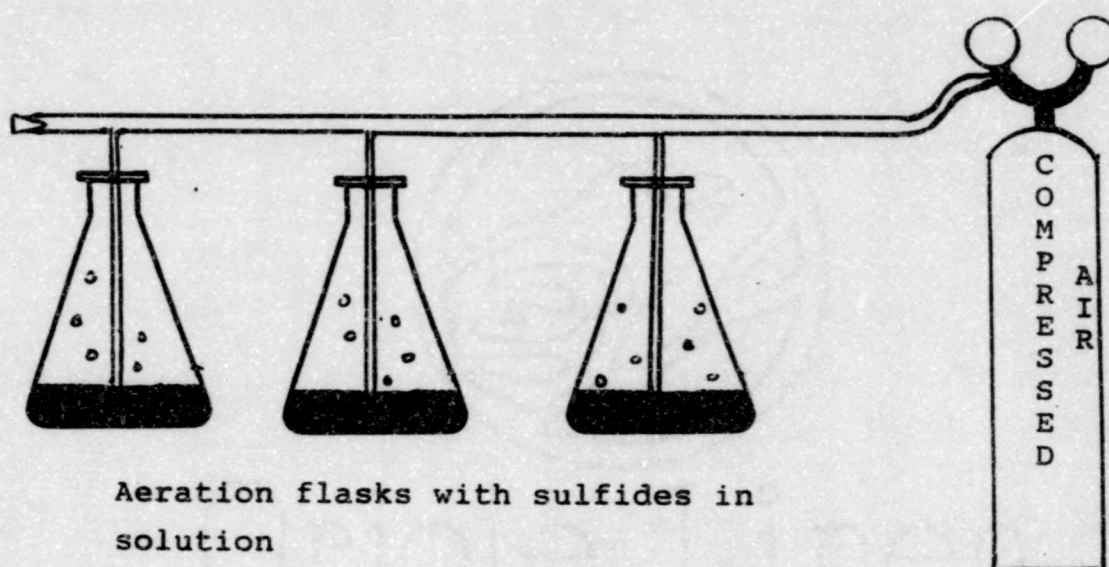
Aeration flasks with sulfides in
solution

FIGURE 1.3   Aeration apparatus

F. Quantitative analysis

1. Instrumentation

Perkin Elmer Atomic Absorption - Model 305 B

2. Calibration and Analysis

Using standards for each metal a standard curve was
prepared for each metal. The average correlation for each

standard curve was .9980. The appropriate wavelength was selected for each metal as listed:

$$Cu = 324.7 \text{ nm}$$

$$Cd = 228.8 \text{ nm}$$

$$Pb = 217.0 \text{ nm}$$

Each sample was analyzed and the linear regression program on a TI55-III calculator was used to calculate the concentrations.

RESULTS

A. Oxidation rate

Each sample flask was monitored regularly every 3-4 days in order to see the effect of time on the reaction rate. Since the flasks were always saturated with oxygen at atmospheric conditions (0.2 atm partial pressure) the actual amount of oxygen was not considered an issue. This bench study was designed to show that in the presence of oxygen metal sulfides will oxidize to release dissolved metal sulfate. The results show that, once the reaction was initiated, it progressed rather quickly. Forty percent of the original concentration oxidized in 1 month. As expected, our control samples which were kept under a nitrogen atmosphere, showed no change from the beginning of the study to the end (approx. 1.5 months). These control samples were prepared at the same time as the others and were monitored for a longer time and no detectable amount was present in the final analysis.

B. pH Dependence

As expected the metals were more soluble at the lower pH values. As the pH approached alkaline conditions the dissolution rate decreased by an average factor of four. The acid production that occurred due to the oxidation of the metals posed a problem with pH control and at times the pH dropped to

a level of 3.0 pH units. Under these conditions the pH fluctuated from the initial pH quite frequently. However, the pH was monitored regularly and was always brought to initial conditions upon notice of deviation.

In the following data it is shown how each metal reacted under laboratory conditions. Copper has shown great stability throughout the study. The solubility product for copper sulfide is 10 orders of magnitude less than those of cadmium and lead sulfides; hence it was expected that CuS may not oxidize during the course of this study, which was what was observed.

DATA

Cadmium Aeration Concentration
Aeration Started 2/12/90

DATE	fil- tration	S-1 pH = 5	S-2 pH = 6	S-3 pH = 7	S-4 pH = 7	S-5 pH = 8	S-6 pH = 9	C-1 pH = 5	C-2 pH = 9
2/13/90	cent.	0.00	0.17	0.16	0.11	0.11	0.11	0.00	0.00
2/19	cent.	2.88	0.98	0.62	*0.71	*1.82	0.25	0.00	0.00
2/23	cent.	3.32	0.25	0.25	*3.1	*1.32	*1.03	0.00	0.00
3/1	cent.	4.00	0.52	0.30	0.08	0.67	0.15	0.00	0.00
3/5	0.45 um	5.70	2.11	0.98	0.84	1.54	1.41	0.00	0.00
3/12	0.45 um	19.80	4.50	6.10	6.60	4.80	4.80	0.00	0.00
4/3								0.00	0.00

Lead Aeration Concentration

DATE	fil- tration	S-1 pH = 5	S-2 pH = 6	S-3 pH = 7	S-4 pH = 7	S-5 pH = 8	S-6 pH = 9	C-1 pH = 5	C-2 pH = 9
2/13/90	cent.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2/19	cent.	6.77	1.18	1.18	0.88	2.94	0.00	0.00	0.00
2/23	cent.	5.65	1.30	1.30	*3.91	3.04	*1.74	0.00	0.00
3/1	cent.	18.20	2.27	1.40	0.91	2.72	0.50	0.00	0.00
3/5	0.45 um	17.40	2.17	20.40	13.90	8.70	6.50	0.00	0.00
3/12	0.45 um	36.00	34.40	20.40	10.40	8.80	4.80	0.00	0.00
4/3								0.00	0.00

ALL VALUES ARE GIVEN IN PPM
* Questionable number due to particulate matter that would not settle
after centrifuging, therefore the use of a 0.45 micron filter was used
later in analysis.
cent. = centrifuge
0.45um = 0.45 micron millipore filter

COPPER - by 3/12 no oxidation had taken place

TABLE 1.1   Cadmium and lead aeration results

Cadmium - 4 replicates at pH = 5

DATE	fil-tration	*S-R pH = 5	S-S pH = 5	S-T pH = 5	S-U pH = 5	STD. DEV.
3/19	0.45 um	0.93	0.39	1.10	0.23	0.38
3/23	0.45 um	29.50	4.40	5.94	2.32	1.48
3/26	0.45 um	50.20	8.40	8.40	5.90	1.18
3/30	0.45 um	67.00	18.10	24.40	8.43	6.57

* Sample S-R had unexplainable pH fluctuation
and therefore was not used in statistical calculations

Lead - 4 replicates at pH = 5

DATE	fil-tration	S-R pH = 5	S-S pH = 5	S-T pH = 5	S-U pH = 5	STD. DEV.
3/19	0.45 um	5.60	3.20	6.40	2.40	1.65
3/23	0.45 um	25.50	19.60	23.10	18.00	2.93
3/26	0.45 um	25.80	24.20	24.60	25.00	0.59
3/30	0.45 um	33.20	29.60	30.40	32.40	1.46

TABLE 1.2   Cadmium and lead replicate results

CONCLUSION

Our bench scale model of metal oxidation has shown that lead and cadmium oxidize readily. It is shown in the data that lead oxidizes faster than cadmium, which is expected due to their solubility products. Copper did not oxidize during the course of our study due to the fact that its solubility product is much smaller than that of lead and cadmium. The reactions of each metal were quite pH dependent under our conditions which proved that the slightest change in pH can have a dramatic effect. The results showed good reproducibility, with standard deviations that were well within average acceptable limits of 0-10 percent. This study shows that metal sulfides are not stable in an interrupted environment, which proves our original hypothesis that sulfide precipitation would not be an acceptable method for metal immobilization. Future experiments by this author and other co-workers will include a more lengthy and indepth study using several collected soils in a more controlled environment.

REFERENCES

1. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 1.

2. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 57.

3. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 60.

4. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 59.

5. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines -A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 59-60.

6. Merck Index, [Rahway, N.J.: Merck and Co. (1960)].

7. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 29.

8. B. C. Kelley and O. H. Tuovinen, "Microbiological Oxidations of Minerals in Mine Tailings," in Chemistry and Biology of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer -Verlag, 1988), p. 33.

9. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 29.

10. Leadville Mine Drainage Tunnel Act of 1976, by Hon. Henry M. Jackson (Washington D. C.: U. S. Government Printing Office, 1976), p. 13-14.

11. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 1.

12. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 5-6.

13. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 70.

14. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 103.

15. M. Moxley and G. J. Catchpole, "Aquifer Restoration at the Bison Basin In Situ Uranium Mine", Proceedings, In-Situ All Mineral Symposium, Casper, Wyo., May 22-24, 1989, ch. 13.

16. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 103.

17. U.S. EPA, Ground Water Handbook, EPA Report no. EPA 625/6-27-016, 1987, p. 43.

18. D. D. Ebbing, "General Chemistry", 2nd ed., Houghton-Mifflin Co., Boston, Mass., 1987, p. 650.

19. D. D. Ebbing, "General Chemistry", 2nd ed., Houghton-Mifflin Co., Boston, Mass., 1987, p. 661.

20. D. D. Ebbing, "General Chemistry", 2nd ed., Houghton-Mifflin Co., Boston, Mass., 1987, p. 650.

21. B. C. Kelley and O. H. Tuovinen, "Microbiological Oxidations of Minerals in Mine Tailings," in Chemistry and Biology of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer-Verlag, 1988), p. 44.

22. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 33.

23. B. C. Kelley and O. H. Tuovinen, "Microbiological Oxidations of Minerals in Mine Tailings," in Chemistry and Biology of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer-Verlag, 1988), p. 44.

24. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 37.

25. C. D. Hodgmann, "Handbook of Chemistry and Physics", 42nd ed., CRC Press, Boca Raton, Fla., 1960, p. 549 and 573.

26. D. D. Ebbing, "General Chemistry", 2nd ed., Houghton-Mifflin Co., Boston, Mass., 1987, Appendix C, A-14-A- 16.

27. U.S. Geological Survey, Assessment of Water Resources in Lead-Zinc Mined Areas in Cherokee County, Kansas, and Adjacent Areas, Water-Supply Paper 2268, 1987, p. 4.28. R. W. Klusman and K. W. Edwards, "Toxic Metals in Ground Water of the Front Range, Colorado", Ground Water, 15, 160 (1977).

29. J. Hyyppa, "Use of Chemical Analysis in Studying Waters and Clays", Geologi, 33, 38 (1981).

30. R. H. Carpenter and W. B. Hayes, "Precipitation of Iron, Manganese, Zinc, and Copper on Clean Ceramic Surfaces in a Stream Draining a Polymetallic Sulfide Deposit", J. Geochem. Explor., 9, 31 (1976).

31. R. V. Nicholson, J. A. Cherry, and E. J. Reardon, "Migration of Contaminants in Groundwater at a Landfill: A Case Study", J. Hydrol. (Amsterdam) 63(1-2), 131-76. 32. I. M. Archer, N. A. Marshman, and W. Salomans, "Development of a Revegetation Programme for Copper and Sulphide-Bearing Mine Wastes in the Humid Tropics," in Environmental Management of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer - Verlag, 1988), p. 173.

33. C. Schmidt, "Groundwater Restoration and Stabilization Using Hydrogen Sulfide", Proceedings, In-situ All Mineral Symposium, Casper, Wyo., May 22-24, 1989, ch. 2.

34. U.S. EPA, Sulfide Precipitation of Heavy Metals, EPA Report no. EPA 600/2-80-139, June, 1980.

35. U.S. EPA, Water Pollution Caused by Inactive Ore and Mineral Mines - A National Assessment, EPA report no. EPA-600/2-76-298, December 1976, p. 55.

36. B. C. Kelley and O. H. Tuovinen, "Microbiological Oxidations of Minerals in Mine Tailings," in Chemistry and Biology of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer-Verlag, 1988), p. 46.

37. W. H. O. Ernst, "Response of Plants and Vegetation to Mine Tailings and Dredged Materials," in Chemistry and Biology of Solid Waste, eds. Wim Salomans and Ulrich Forstner (New York: Springer-Verlag, 1988), p. 54-66.

CHAPTER TWO
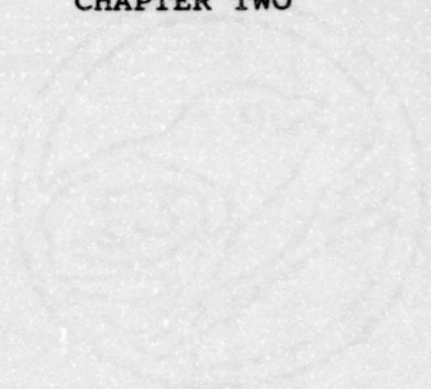
INTRODUCTION

The field of organic chemistry has experienced rapid expansion in recent years. This is due, in part, to the development of instrumentation capable of analyzing extremely small quantities of organic compounds. Gas chromatography/ mass spectrometry (GC/MS) is recognized as an invaluable tool for such analyses. Gas chromatography/mass spectrometry is a powerful technique due to the fact that it combines chromatographic separation with a two-dimensional highly selective detector which provides for an ideal analytical instrument for a wide variety of organic analyses.

A. GC/MS Applications

There are many recognized biochemically oriented applications of GC/MS, one of which is the detection of long chain fatty acid esters found in bacterium. The identity of some pathogenic bacteria is often characterized by the detection of the fatty acid esters produced by the bacterium. GC/MS has been applied for the detection of mixtures extracted from strains of mycobacterium tuberculosis.[1] Chromatographic techniques have also been used for the detection of the metabolism of unsaturated fatty acids using deuterium labeling of the stereochemical reaction catalyzed by trans-2-enoyl-coenzyme A reductase from E. Coli.[2] The use of methyl nonadecanoate as a deuteriated internal standard, for the assay of

myocardial free fatty acids has enabled the detection of concentrations at picogram (pg) levels.[3]

The detection of prostaglandins by GC/MS has shown some promising results. Thromboxane-B_2 and 6-keto-prostaglandin F_{1a} have been detected in blood plasma affording a detection limit of 50 pg.[4] For the detection of prostenoids, in human blood plasma, levels of 0.5 pg of 6-oxo- PGF_{1a}[5] and 200 fg of PGF_{2a}[6] have been recorded.

Gas chromatography/mass spectrometry has been used in the nutritional science field to quantify glucose in blood serum. Using an internal standard of C-13 glucose, two methods were compared. The derivatization of glucose to 1,2:5,6-di-o-isopro-pylidene-a-D-glucose furanose with packed column GC/MS was compared to that of 1,2:3,5-bis (butane-boronate)-6-acetate with capillary column GC/MS. Both methods showed excellent precision but the separation time for the packed column was much longer.[7] Galactose, mannose, and fructose have also been detected in blood serum. The fused silica column was used for separation with a tetra-deuterated compound as the internal standard.[8]

The behavior of o-acetyl-o-methyl galactonitriles has been evaluated by GC/MS. Several mono-o-methyl aldoses have been separated from common aldoses as isopropylidene deriva-tives.[9] Many different derivatives, including the isopropyl-idenes, have been used for the identification of hexoloses and pentosuloses.[10] Enantiomeric tetruloses and pentuloses were

characterized as their diastereoisomer derivatives using an OV-225 glass capillary column.[11]

The identification of new steroids and the quantification of known structures by GC/MS have become more prevalent in recent years. This has largely been due to the increasing interest in steroid chemistry in many areas of research including biochemical, clinical, and forensic fields. Using selective derivatization, characterization of steroids has been achieved with much success. The measurement of daily hormonal levels in humans can be monitored.[12,13] This is achieved by using deuteriated precursors and selected ion monitoring. Capillary GC/MS was favored for daily monitoring of urinary steroids.[14] Bile acids of a 3200 year old mummy analyzed by GC/MS have been shown to correspond to those of modern man.[15] Detection of steroids in athletes six weeks after administration has been achieved by GC/MS. Possible doping with 19-nor-testosterone decanoate shows up quantitatively in urine.[16] Studies of the reproductive hormones has shown detection of oestrogens in pregnant women and seminal plasma in men, bulls, stallions, and boars.[17]

In the area of clinical chemistry GC/MS has been employed for the detection of amines. Organic acids have been found and identified in human urine.[18] Approximately 40% of 500 detected acidic urine components have been identified by capillary GC/MS.[19] Studies of the volatile compounds found in normal and viral-infected tissue[20] and organic acids in

amniotic fluid at different times during gestation[21], as well as those found in human milk[22] have been analyzed by GC/MS.

The field of food and agricultural chemistry has benefitted due to the quantitative and qualitative applications of GC/MS. The quantification of vanilla beans was achieved with preference over MS/MS because of reproducibility.[23] Comparison of marijuana smoke condensates to tobacco smoke was performed using a capillary column GC/MS.[24] Toxicological aspects of nitrosamines, food packaging, pesticide residues, and mycotoxins have been determined in food chemistry.[25]

In the field of environmental science, GC/MS has proved to be a most effective technique. The detection of polychlorinated dibenzodioxins (PCDD)[26] and hazardous aromatic compounds at dump sites[27] by capillary GC/MS has been quite successful. In the case of airborne contaminants, detection levels as small as 100-200 ng/m^3 have been recorded for seven volatile nitrosamines.[28] Water analysis for the determination of organic river pollutants included the profiling of river water and industrial discharges. The retention indices of two different columns and the response factors of selective detectors were compared in order to best determine the origin of contamination.[29] Halogenated compounds in transformer oil, such as PCB's, can first be analyzed by HPLC in order to remove interferences and subsequently be analyzed by GC/MS with detection levels as small as 100 ppb.[30]

In forensic science and toxicology, GC/MS has been routinely used. Specifically, GC/MS techniques have been used in

the analysis of tissue samples, testing for drugs, pesticides, and pollutants. Chlordane has been found in fish[31] and organo-sulfur compounds have been identified in oysters, therefore indicating oil spills.[32] Techniques have also been developed for the analysis of chemical warfare agents. Four Fusarium mycotoxins, with poly ethylene glycol as a suspected carrier medium, were identified in "yellow rain."[33] The body tissues, blood, and urine of Asian chemical warfare victims as well as soil and foliage from the environment were shown to contain trichothecenes not native to that region.[34] Hair samples collected from drug users has been shown to contain chloroquine,[35] amphetamine,[36] methamphetamine,[37] and monodesethylchloroquine.[38] This has been of particular interest to the forensic scientists.

B. Rationalization for Research

Because GC/MS is such a powerful technique with obvious widespread applications in organic analysis, it is important that the scientific community train their young scientists in this area. As such, it is important to familiarize students, during their undergraduate studies, in the area of GC/MS.

Gas chromatography/mass spectrometry (GC/MS) is rapidly becoming a common instrumental technique in many undergraduate institutions. This is due primarily to the fact that the cost of GC/MS instrumentation has decreased markedly over the past several years. In addition, the external funding agencies have recognized the merit of placing such instrumentation in the undergraduate setting and have responded by making money

available to many institutions (The National Science Found-
ation ILI program has granted funding for the purchase of
GC/MS instrumentation to over 50 institutions in the past
three years). Because this is a recent phenomenon, laboratory
textbook treatment of the fundamentals of GC/MS and basic
applied GC/MS experiments has lagged. In fact, no currently
available sophomore level macroscale or microscale manuals
possess a treatment of GC/MS.

With the onset of microscale organic chemistry, GC/MS is
bound to find greater application in the sophomore-level lab-
oratory. GC/MS is ideally suited for microscale chemistry
owing to its high resolution and ability to analyze small
amounts of material(1 microliter or less). In many microscale
organic laboratory experiments the scale is too small to allow
for other types of instrumental analysis such as NMR; there-
fore, mass spectrometry will often be the ideal structural
identification and analysis tool.

The intent of this work is to present concise original
fundamental experiments in order to train sophomore level
students in the area of applied GC/MS. Three experiments are
to be presented. The first will focus on the GC aspects of
GC/MS, the second will focus on fundamental mass spectrometry,
and the third will be a combined application of gas chroma-
tography and mass spectrometry.

EXPERIMENTAL

A Shimadzu GC-8A was used to generate the experiments for the gas chromatography section of the following experiments. The detector used was a thermal conductivity detector with helium as the mobile gas. For the mass spectrometry section, a Hewlett Packard GC 5890A was interfaced to the Hewlett Packard 5970B mass selective detector (MSD); therefore the GC served as an inlet system for the detector. The MSD mainframe includes an electron impact source, quadrupole mass filter, electron multiplier detector, power supply, drive electronics, and analyzer vacuum system (see Fig. 2.1).

HP MSD 5970 B

MASS RANGE   Scans ranges between 10 and 800 amu.

RESOLUTION   Workstation controlled unit resolution over
                  the mass range SCANRATESUser controlled
                  rates of 1500, 1100, 690, 380, or 200

                  amu/sec , in 0.1 amu increments.

SENSITIVITY   Signal to noise ratio of 20:1 at 298.3 amu
                  using 1 ng methyl stearate, scanning between
                  290 and 310 amu at speed of 2 amu/sec .

DETECTOR   Electron multiplier with a max. of 3000
                  volts.

ION SOURCE   Electron impact with electron energy of 70
                  eV.

MASS FILTER   Quadrupole mass filter consisting of four
                  hyperbolic rods 203mm in length.

TEMPERATURE   Factory preset to 200 degrees C, for ion
CONTROL   source. Interface control is external with
                  range of 100-350 degrees C.

GC INTERFACE   GC can be connected to either the right side
                  or left side of MSD. Interface connections
                  accomodate fused silica columns of 0.260 -
                  0.420 mm outside diameter.

ENVIRONMENT   Operating temperatures are between 15 and 35
                  degrees C, in order to remain within
                  specifications. VACUUMHigh vacuum pump with
                  a two stage mechanical SYSTEMpump provides
                  constant high vacuum. Pump down from
                  atmosphere requires approx. 10 minutes with
                  an additional 50 minutes required for

                  temperature equilbration.

SIZE   Dimensions 14in h x 17in l x 28in w
                  Weight 46lb

Fig. 2.1   MSD 5970 specifications

DISCUSSION

The discussion section of this thesis chapter centers upon three new experiments whose focus is GC/MS. The discussion section will be reproduced in its entirety in the upcoming textbook Contemporary Microscale Organic Chemistry by R.W. Holman and R.K. Hessley. The presentation in this section is identical to that which will be given to the students. As such, the content of this section is written at the level of the undergraduate students who will perform the newly developed experiments. The format will be as follows: A) Technique Definition B) Technique Application C) Technique Theory and D) Experimental Procedure.

A. Technique Definition:

1. Gas Chromatography.

Gas chromatography is an instrumental technique utilized to separate components from mixtures. The gas chromatograph possesses a heated inlet, a glass or metal column, with a liquid or solid stationary phase, contained within a heated oven, and a detector. The components within the mixture must be in the gas phase and, as gases, they travel through the column, with each component exiting the column (and reaching the detector) at different times. The process involves separation of thermally stable and volatile organic and inorganic compounds by partitioning the

components of a chemical mixture between a moving gas (mobile phase) and a stationary liquid phase held on a solid support.

  2. Mass Spectrometry.

  Mass spectrometry is a compound identification technique which involves ionization of a sample molecule to form a parent ion and ion fragments. Once ionized the ions are sorted according to mass to charge (m/z) ratio, they are then measured relative to the abundance of ion fragments of each mass.

  3. Gas Chromatography/Mass Spectrometry

  Gas chromatography/mass spectrometry (GC/MS) is a hybrid instrumental technique in which a gas chromatograph and a mass spectrometer have been interfaced. The gas chromatograph serves as the sample inlet and as a preseparation device. The mass spectrometer serves as the detector which gives detailed molecular structure information.

B. Technique Application:

  1. Gas chromatography

  Gas chromatography finds application in the environmental analysis of complex mixtures such as PCB analysis, pesticides, water analysis, soil analysis and air pollutants. Gas chromatography is commonly employed in the petroleum industry, the medical field, the food industry, the pharmaceutical industry, and many other areas in which mixtures must be separated. However, it must be noted that GC analysis alone will separate but not directly identify the components in the mixture.

2. Mass spectrometry

Mass spectrometry is one of three fundamental identification techniques in organic chemistry. Infrared analysis (IR) provides functional group information. Nuclear magnetic resonance (NMR) provides isomeric structure information. Mass spectrometry provides for the exact molecular weight, and hence chemical formula of the compound. Furthermore, mass spectrometry provides additional structural information through detailed analysis of the fragmentation pattern. Mass spectrometry requires that a single pure compound be analyzed. A mixture of compounds analyzed by MS yields data that is too complex to obtain any useful information.

3. Gas chromatography/Mass spectrometry

Gas chromatography/Mass spectrometry is an ideal technique for analysis of a mixture of compounds. Gas chromatography alone does not provide adequate component identification, and is merely a separation technique. Mass spectrometry alone does not permit the analysis of mixtures and is merely a single component structural analysis tool. The GC/MS hybrid instrument is both an efficient tool for the separation **and** identification of components from a mixture. Gas chromatography/mass spectrometry capitalizes upon the individual strength of both GC and MS and circumvents entirely the main limitations of each instrument.

C. <u>Technique theory:</u>

1. <u>Gas Chromatography</u>

Gas chromatography (GC) is the most commonly used technique for the separation of thermally stable and volatile organic and inorganic compounds. Until the development of chromatography, volatile liquids with close boiling points had to be separated by fractionation, a cumbersome process. Gas chromatography requires less time and is a less difficult operation than is fractionation. In addition, the amount of sample required, which is less than 0.1mL, also makes GC more versatile especially when analysis is needed where only a limited amount of analyte is available. A typical GC consists of a heated injection port, carrier gas, pressure regulator to control gas flow, sample inlet, suitable column, detector, recorder, and flow meter to measure gas flow rate (Fig. 2.2).

a) Injection port. The injection port temperature is usually maintained at approximately 50 degrees higher than the boiling point of the least volatile component in the sample. This is important in order to vaporize the sample quickly and completely and to account for heat lost during vaporization. The sample is injected via a hypodermic type microsyringe. It is injected through a rubber or silicone self-sealing septum into the heated injection port where it is then vaporized. The type of column used determines the size of sample to inject.

b) Carrier Gas. The carrier gas that is used for chromatography is supplied in pressurized tanks. The gas is
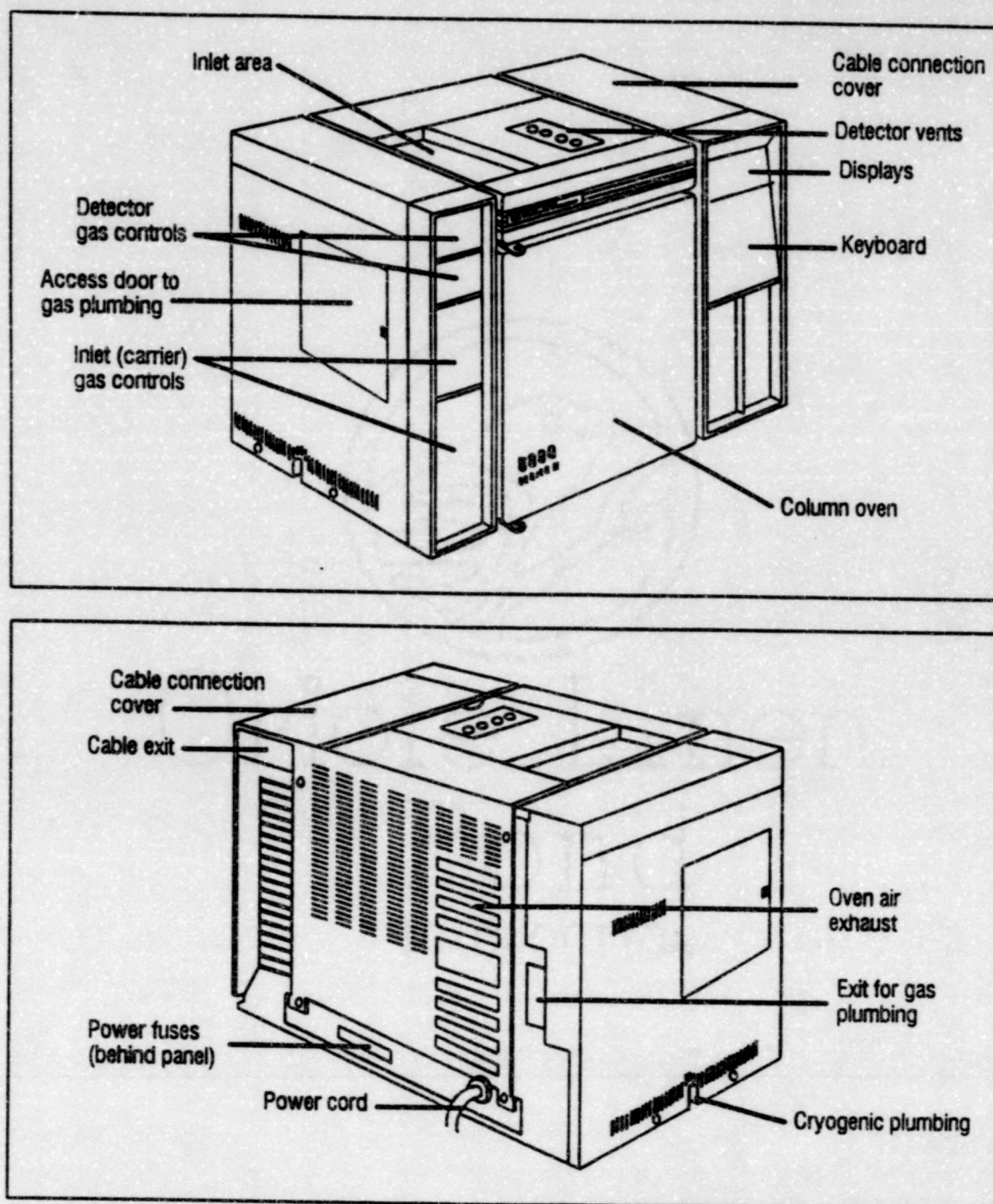
FIGURE 2.2   Diagram of a gas chromatograph
(Copyright Hewlett-Packard Company
(1987) Used with permission)

regulated and measured by flow meters and gauges. A simple soap bubble type flow meter is often used to measure flow rate. The gas must be chemically unreactive or inert toward the sample and the system components. The most common gases used are helium and nitrogen, but gases such as argon, carbon dioxide, and hydrogen can also be used. The choice is usually dictated by the types of compounds to be analyzed and the type of detector on the instrument.

c) Column. Gas chromatography involves the injection of a small amount of sample into a carrier gas, which serves as a mobile phase. The carrier gas, typically helium, sweeps the sample through a column. The column is a coiled tube packed with a stationary phase which consists of solid particles coated with a liquid. When a mixture of gases enters the packed column each component will interact with the stationary phase to a different degree. Those components that dissolve to a greater extent in the stationary phase will travel through the column slowly. Those components that dissolve to a lesser extent in the stationary phase are carried by the carrier gas through the column rapidly. Therefore, the components exit from the column and enter the detector at different times. The amount of time required for an analyte to travel through the column to the detector is called the retention time (t_R).

In gas liquid chromatography there are two types of columns available, packed and capillary (open tubular). Packed columns are used primarily to accomodate large sample

sizes and are easier to maintain and use. Packed columns are typically constructed of metal or glass formed into a coil so they can be inserted into the oven. The inner diameter of the tube ranges from 2 to 4 millimeters, while the length may range from 2 to 3 meters. A solid support is packed within the column and serves to hold the liquid stationary phase in place. This is important so that exposure of the mobile phase to the stationary phase is at a maximum. The criteria for a good support are: 1) spherical uniformity of particles 2) small size (typically 1 m^2/g specific surface) 3) chemical inertness and stability at high temperatures 4) amenability to uniform coverage by the liquid stationary phase and 5) adequate durability and strength. A perfect support is not yet available, but of the ones that are available, diatomaceous earth is the most commonly used.

Diatomaceous earth is a natural substance from which two types of packing are derived. Chromosorb P is prepared by crushing the diatomaceous earth and firing it in an oven to 900 degrees. This process forms firebricks with a surface area of about $4m^2/g$. Chromosorb G or W is prepared in the same manner as Chromosorb P but it is first mixed with sodium carbonate. The resultant compound is much more durable than the former, shows less absorbance of the solute, and has a surface area of about $1m^2/g$. The size of the support parti-cles is important since the efficiency of the column in-creases with decreasing size of the support packing. Though diatomaceous earth is the most common support used, others are

available. Teflon coated particles are available if a very corrosive substance is being analyzed. Etched glass beads are used also, especially for rapid analysis below the boiling points of the sample components.

The solid support or packing is utilized to hold the liquid stationary phase in place. This is especially important so that as much area as possible is exposed to the mobile phase. The liquid phase should be of low volatility, with a boiling point that is at least 200 degrees higher than the maximum temperature that the column can withstand. It must possess solvent characteristics that differ slightly from the solutes so that separation of the analytes can occur. There are many liquid phases available, and there is always one that is the best choice for a particular type of analysis. A good rule of thumb for choosing a suitable liquid phase is "like dissolves like," meaning the stationary phase works best when it is similar in character to that of the analyte.

Capillary columns or open tubular columns are well suited for analyses where only small amounts of sample are available. The increase in separation efficiency, based on theoretical plates (the number of partitions between the mobile phase and stationary phase the solute experiences), can be as high as several hundred thousand for a capillary column, compared to that of a packed column which is about 300 - 3000. The internal diameter of a capillary column ranges from 0.3mm to 1.0mm and they are often 100 meters in length. Capillary columns are most commonly constructed of fused silica glass

coated with a polyamide (for protection). The flexibility of this type of glass allows it to be manipulated into a variety of shapes, therefore allowing lengthy columns to be coiled for insertion into the GC oven.

Capillary columns can be packed with solid particles such as those discussed earlier, but in this case would be micro-packed. However, the most common types of capillary columns are coated internally with a stationary liquid phase (open tubular) which allows unrestricted flow through the middle of the column. Three types of open tubular columns are available, wall coated (WCOT), support coated (SCOT), and fused silica (FSOT). Each type exhibits different characteristics that have proved to be well suited for certain types of analyses.

The walls of an open tubular column are coated with the stationary phase with an approximate thickness of 0.1 to 1.0 micrometers. The liquids used are the same as those used for a packed column. One advantage to the open flow through the column is that band broadening, caused by gas flow through the packing of a packed column, is eliminated. Another important feature offered by the capillary column is the decrease in analysis time and the amount of sample required for the analysis. Therefore, nothing is currently available that surpasses the quality of separation afforded by the capillary column.

d) Detectors. After the sample leaves the column it will then enter the detector. The detector is located at the end of the separation column and is sensitive to the components that are exiting the column. As the component enters the detector, the detector will send an electrical output to an amplifier and recorder. There are four major types of detectors that can be used in the GC, thermal conductivity (TCD), flame ionization (FID), electron capture (ECD), and thermionic emission (TED).

(1) Thermal conductivity detectors: Thermal conductivity detectors (Fig. 2.3) are the most general of the detectors, being suitable for almost all applications. In a TCD analysis, the change in thermal conductivity of the mobile gas stream is measured. That is, when no sample is present the thermal conductivity remains constant, but as the sample enters the gas stream the conductivity changes according to the analyte molecules. The heating element may be a fine wire of platinum, gold, or tungsten. A semi-conducting thermistor can also be used. The resistance of the wire or thermistor gives a measurement of the thermal conductivity of the gas as it changes. Thermal conductivity detectors are non-destructive to the sample and are considered to be a general purpose type detector with sensitivities to both organic and inorganic vapors.
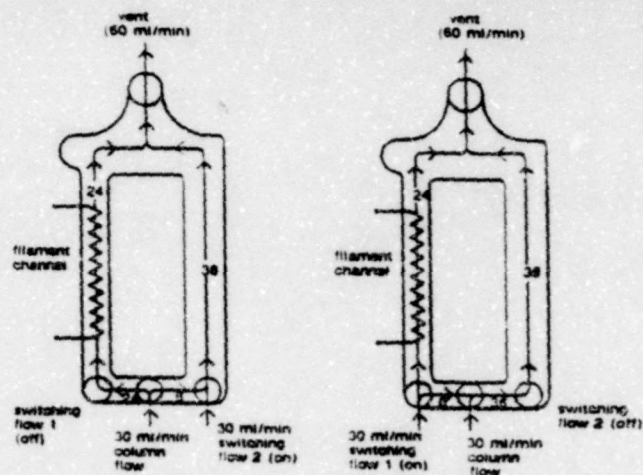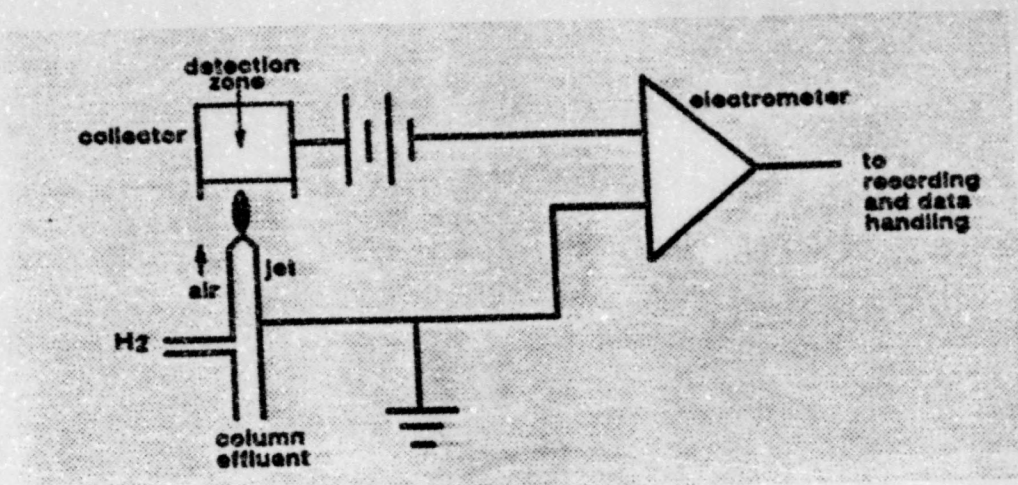
Flow diagram of a commercially available TCD cell. In the left diagram, the switching flow causes the column effluent to pass through the filament channel. When the switching flow changes (right diagram), the column effluent will pass through the empty channel. During this time the filament channel fills with the switching gas, and reference measurements are made. Switching between the column effluent and reference gas occurs every 100 milliseconds.
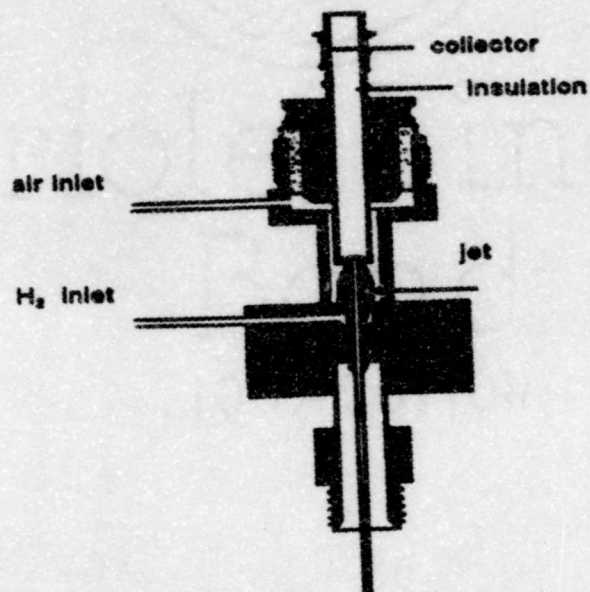
FIGURE 2.3   Thermal conductivity detector cell
(Copyright Hewlett Packard Company (1987)
Used with permission)

(2) <u>Flame ionization detector:</u> The flame ionization detector (FID) (Fig.2.4) is based upon ionization of the sample by adding hydrogen to the column effluent, passing it through a jet (where it is mixed with air), and subsequently burning the mixture. A typical FID will have a flame jet and a cylinder positioned above the tip of the flame, these will act as electrodes. A potential is applied across the electrodes subsequently lowering the resistance between the electrodes and causing a current of ions and free electrons (generated in the flame) to flow. When the analyte of interest enters the flame it will be burned and the current will increase substantially. The current will then contact a resistor, be detected as a decrease in voltage, and the signal will be sent to an output device, such as a recorder or computer. Flame ionization is a good detector for organic carbon;

51

Schematic of a flame ionization detector.

Design of a commercially available flame ionization detector.

FIGURE 2.4 Flame Ionization Detector
a) schematic of operation b) diagram of detector (Copyright
Hewlett-Packard Company (1987) Used with permission)

however, it is not good for fully oxidized compounds such as carbonyl compounds, carboxylic acids or ether groups. Moreover, the FID detector process destroys the sample. Some of the major advantages are high sensitivity, low noise, and a large linear response range.

(3) Electron capture detector: The electron capture detector (ECD) (Fig. 2.5) is composed of two electrodes, one of which has a radioisotope on the surface that emits beta particles (high energy electrons) as it decays. The electrons then bombard the carrier gas (which is typically nitrogen) thus forming a plasma of radicals, positive ions, and thermal electrons. The result of this reaction is a continuous current between the electrodes. The magnitude of this current will decrease if an organic molecule in the gas stream
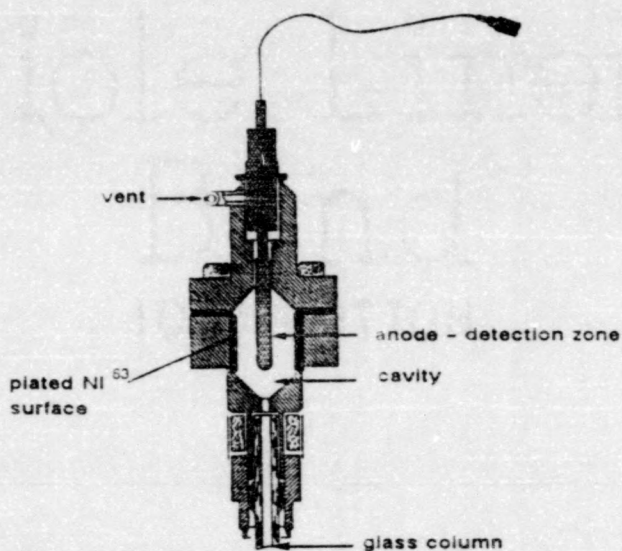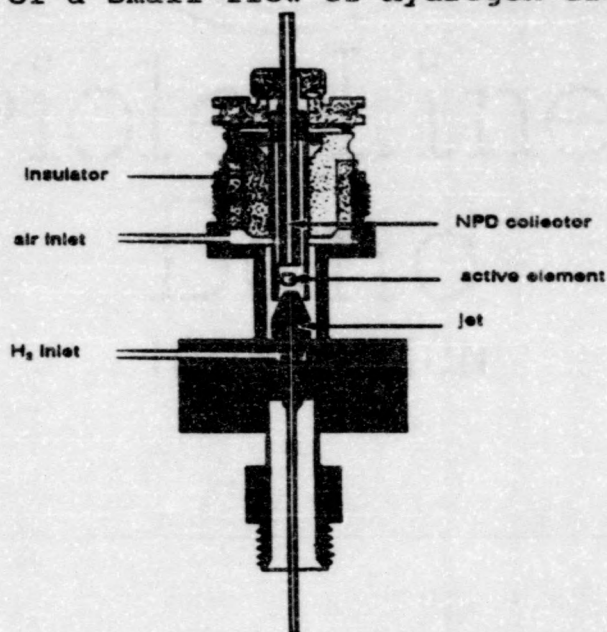

Design of a commercially available electron capture detector.

FIGURE 2.5 Electron Capture Detector
(Copyright Hewlett-Packard Company (1987)
Used with permission)

possesses the tendency to capture an electron. The decrease in the current will be measured and outputted to a recorder or a computer. The electron capture detector is very sensitive to compounds containing electronegative functional groups such as halogens, nitro groups, and peroxides. The ECD is especially useful in the environmental analyses of chlorinated pesticides and herbicides. The sample is usually unaffected by the process, in contrast to FID, but the linear response does not usually extend past 2 orders of magnitude.

(4) <u>Thermionic emission detector:</u> The structure of a thermionic emission detector (TED), also called a nitrogen phosphorus detector (NPD), (Fig. 2.6) is similar to that of an FID. It uses a fuel-poor hydrogen plasma which is formed from the introduction of a small flow of hydrogen from the column

Design of a commercially available nitrogen/phosphorus detector.

FIGURE 2.6 Thermionic Emission Detector
(Copyright Hewlett-Packard Company (1987) Used with permission)

into the flame assembly followed by subsequent mixing with excess air. After the sample enters the detector and passes through the flame it will then flow around an electrically heated rubidium silicate bead, which is centered approximately 1.25 cm above the flame tip. The temperature of the bead can be adjusted between 600 - 800°C, and the bead temperature is independent of the flame temperature. The thermionic emission detector is especially useful for the detection of phosphorus and nitrogen containing compounds. By adjusting the flow of hydrogen one can vary the sensitivity. A very small amount of hydrogen will give the ability to analyze both phosphorus and nitrogen, while a large amount of hydrogen and a change in polarity between the plasma tip and collector will allow detection of phosphorus only.

e) Recorded output: The Chromatogram

When the solute or analyte exits the column, the detector responds to the solute concentration and its signal is plotted as a function of time. In some instances the signal is plotted as a function of volume of the mobile phase (gas phase). The result of this plot is a symmetrical peak for a pure component or a series of peaks for a mixture. This printed output is called the chromatogram. A chromatogram can tell us many things about a particular mixture. The information contained in the chromatogram is representative of how many components were separated and detected. Each component can be identified by its retention time (t_R), defined as the time between injection and detection. The retention time is

considered a constant for a fixed set of conditions, such as flow rate, column, and temperature. The retention time can be used qualitatively for a compound with a unique retention time, by comparing it to a standard. Further identification may be necessary with another detector, such as a mass spectrometer. Quantitative measurements can also be achieved on the gas chromatograph. The peak area is proportional to the concentration of that component in the mixture. Peak height can also be used in a similar way.

A typical chromatogram will be a plot of separated peaks (Fig. 2.7). Along the x axis will be the retention time while along the y axis will be the recorder response (obtainable as peak height or peak area). A calibration curve can be prepared from either peak area or height using known concentrations of standards. Using this curve one can plot the concentration of an unknown sample.
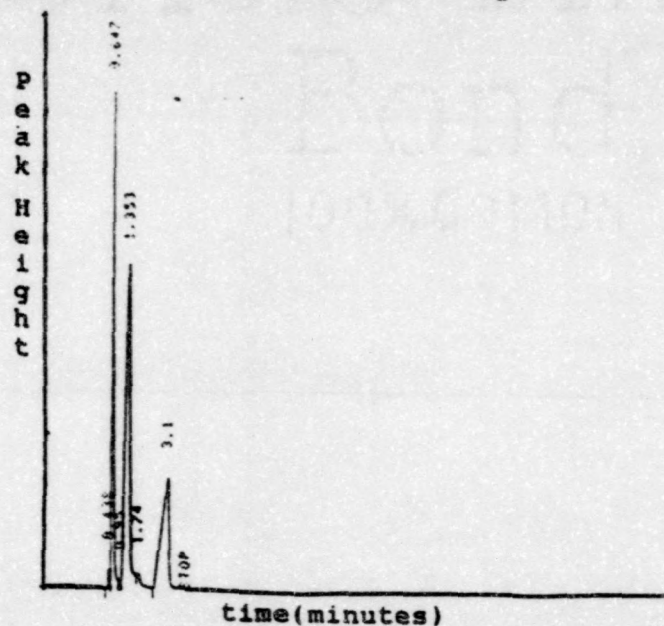
FIGURE 2.7 The gas chromatogram

f) Obtaining a Successful Chromatographic Spectrum

(1) <u>Retention time:</u> The time required from sample injection to sample detection is called the retention time (t_R). The retention time can be adjusted to allow for the time it takes for the carrier gas to pass through the column (T_m). The adjusted retention time (t_R') is represented by:

$$t_R' = t_R - t_m$$

The retention time of the carrier gas can be determined by injecting a small amount of air with the sample into a system using a thermal conductivity detector. The nature of the stationary phase controls the relative retention time between any two components in a gas chromatogram. The peak width for each component is controlled by the nature of the column itself.

(2) <u>Resolution:</u> A good separation in GC is one in which the resulting spectrum exhibits baseline resolution. Baseline resolution (R) is defined as the complete separation of two adjacent Gaussian peaks (Fig. 2.8). Peaks are said to be baseline resolved when R = 1.5; however, R values approaching R=1 are often sufficient in most analyses.

R is defined as:

$$R = \frac{t_{R,2} - t_{R,1}}{0.5(W_1 + W_2)}$$

An important factor in the efficiency of a chromatographic separation is the height equivalent to a theoretical plate (HETP), which is defined as the length of column necessary for solute equilibrium between the mobile gas phase
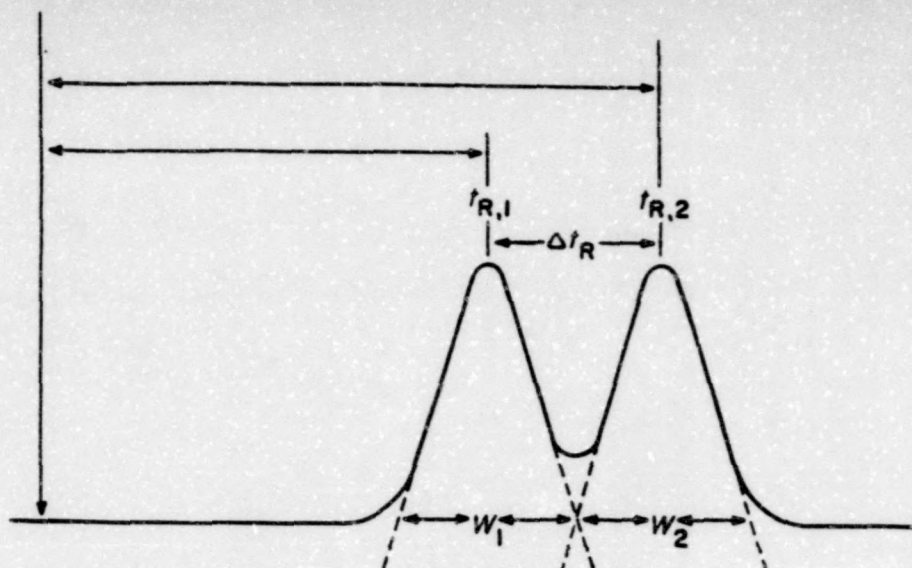
FIGURE 2.8. Complete chromatographic separation

and the stationary liquid phase, that is, the distance the solute moves while undergoing one partitioning cycle. This is represented below:

$$H=L/N$$

where L is the column length in centimeters and

N is the plate number

The smaller the value of H the greater and the better the separation. However, it should be noted that plate number is only a representation of how well the column is packed, and cannot be used as a diagnostic tool under all conditions.

The plate number (N) can be calculated directly from the chromatogram using the following equation and the chromatogram (Fig. 2.9)

$$N=\left(\frac{4t_R}{Wb}\right)^2 \quad \text{or} \quad N=5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$

where t_R is the retention time and Wb is the base peak width and $W_{1/2}$ is the width of the peak at half the peak height.
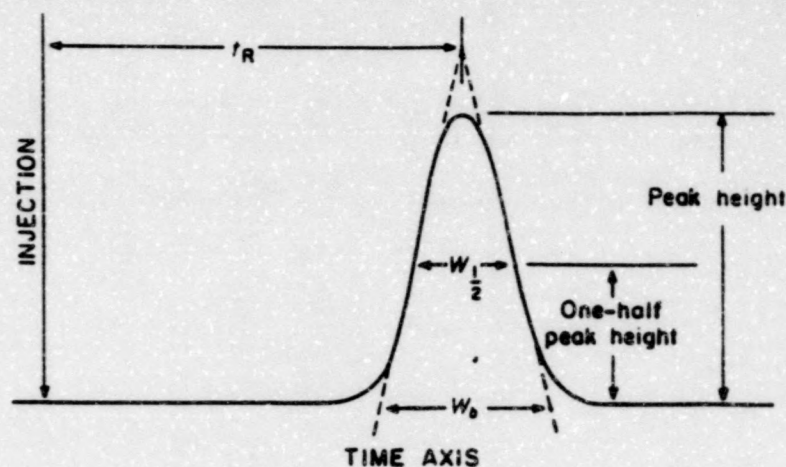
FIGURE 2.9 Calculation of theoretical plates

(3) Factors Affecting Resolution:

Injection. The sample must be injected all at once into the column to ensure immediate vaporization. It should therefore enter the column as a plug of gas. The sample should be small so that the column will not be overloaded. If the column becomes overloaded the peaks will broaden and become asymmetrical.

Flow rate. Improper gas flow can cause several problems. If the flow is too slow it will cause the sample to linger in the column too long. This will cause longitudinal diffusion and subsequent band broadening; this, however, can be corrected by increasing the gas flow. If the flow rate is increased too much, partition equilibrium between the gas phase and liquid phase cannot be maintained, which results in broadening. Therefore, there exists a flow rate for maximum column efficiency which changes with changing temperatures.

Column packing and temperature. Column packing should consist of small particles and should be placed uni-

formly in the column. In an incorrectly packed column a series of molecules may travel several different paths in order to pass through the column. Because of this, some molecules of the same species may elute more slowly or more quickly than others. This results in broadening of the elution band and it is termed eddy diffusion. The temperature must be high enough to vaporize the sample immediately in order for the sample molecules to pass through the column as a narrow band (without diffusion).

Both quantitative and qualitative information can be gained by proper interpretation of a chromatogram. The measured retention time provided can be used to characterize a sample by direct comparison with the retention time of a standard. The area under the peaks can be used to quantitate when using a series of calibration standards with concentrations similar to that of the sample. The peak area can be determined several ways, three of which are, computer integration, manual measurement, and triangulation. A computer can be interfaced to the system and can integrate the peaks as they are formed. A printout with the values is then obtainable and can then be used in conjunction with linear regression to determine concentration. To manually integrate the peaks one can cut out the peaks and weigh them on an analytical balance; this however is subject to error from non-homogeneity of the paper. Another technique used would be to triangulate the peak from the base to a point where the tangents from the base meet and from this calculate area.

Gas chromatography is the ideal separation tool for volatile organic mixtures. Because of its versatility it is currently used in many professional settings. Since GC is one of the most predominant supplemental instruments in a wide variety of environments, the need for well trained professionals is growing, and thus the training of GC experts must start in the lab of sophomore level chemistry.

2. Mass Spectrometry

Mass spectrometry is one of the most versatile and generally applicable analytical techniques available. The first mass spectrometer, developed by J.J. Thompson and F.W. Ashton, dates back to 1912. The model instrument, however was constructed in 1932 and served as an important basis for production of commercial mass spectrometers in 1940. These instruments, designed for quantitative purposes were employed mainly for the use in petroleum hydrocarbon studies. Further research and refinement permitted expansion into other aspects of organic study and thus a mass spectrometer with qualitative, as well as quantitative measurements, became available in 1950.

Elemental analyses are based on mass to charge ratio of primary ions formed in an electrical spark. This development closely paralleled the industrial research of electronic and nuclear energy. The sensitivity of this technique allows detection of trace levels of large molecules and organic contaminants.

In 1960 the emphasis of application in mass spectrometry shifted toward structural analysis and identification of complex molecules. This application is based upon the unique pattern of ion fragmentation exhibited by the ionization of large molecules. In comparison to other means of molecular analysis, such as NMR and IR, the mass spectrum is easier to interpret since the information is provided in mass units which correspond to the mass of the analyte. Thus, by the middle of the 1960's, mass spectrometry was employed as one of the most generally applicable of all analytical tools.

A typical mass spectrometer consists of several components. However, due to the complexity of the subject, only that design which applies to this study will be discussed. Mass spectrometers perform four basic functions: 1) gasification of the sample 2) ionization of sample 3) separation of ions according to mass to charge ratio and 4) measurement of the abundance of ion fragments. The essential components in order of their occurrence are (see block diagram Fig. 2.10): 1) sample inlet 2) ion source 3) ion accelerator and mass analyzer 4) ion collection system 5) detector 6) signal processor and readout. The first four components listed are considered to be the main part of the instrument and are held under a constant vacuum of approximately 10^{-5} torr.

The first step in a typical MS analysis is the introduction of the sample, via a sample inlet system, into the high vacuum of the spectrometer. The vapor then passes through a slit into the inlet chamber where it is ionized and fragmented

by collision with streams of electrons, fast atoms, ions, or photons. Once ionization is achieved, the positive ions are separated from the negative ions by a large negative potential that attracts the positive ions through a second slit into the mass analyzer, via a repeller potential. In the mass analyzer the ions are separated according to their mass to charge ratios.

```
Sample
  |
  V
┌──────────┐     ┌──────────┐     ┌──────────┐     ┌──────────────┐
│ Inlet    │──>  │ Ion      │──>  │ Mass     │──>  │ Ion          │
│ system   │     │ source   │     │ analyzer │     │ collection   │
└──────────┘     └──────────┘     └──────────┘     │ system       │
                      |                             └──────────────┘
                      V                                    |
                 ┌──────────┐                              V
                 │ Vacuum   │                     ┌──────────────┐
                 │ system   │                     │ Data         │
                 └──────────┘                     │ handling     │
                                                  │ system       │
                                                  └──────────────┘
```
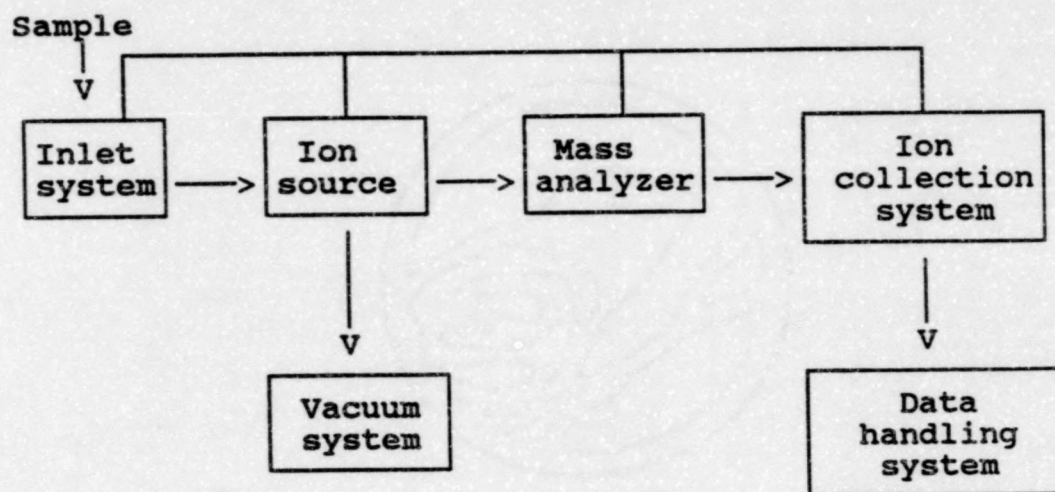
FIGURE 2.10 Block diagram of a mass spectrometer

The ions then exit the analyzer and are dispersed onto a collector plate (electrode) where the ion currents are then amplified and recorded as a function of scan time. The resultant record is the mass spectrum.

a) Sample Introduction. To obtain a mass spectrum there are several ways a sample can be introduced into the instrument. The sample can be in the form of a gas, liquid, or solid. The inlet system for gaseous samples contains a micromanometer, used to determine the amount of sample being

introduced, a molecular leak for metering the sample into the ionization chamber, and a vacuum system to pump the sample. In order to insert a gas, it is simply transferred from a heated inlet, which is a small reservoir that can be bled continuously, into the ion source through a fine leak. This type of inlet is commonly used for introducing reference compounds into the ion source for calibration of the instrument. When the sample is a non-volatile liquid, the sample is placed into a capillary tube. The capillary tube is inserted into a probe and the probe is inserted through a vacuum lock into the mass spectrometer. The sample remains in the capillary tube, via capillary action, and air can be pumped out via a differential pump. The probe is then inserted into the high vacuum of the instrument, and is heated. This gasifies the sample. Small samples with high volatility can be injected via a gas chromatograph, which can be interfaced to the mass spectrometer. The effluent from the GC serves as the sample. This system will be discussed in more detail later.

b) Sample ionization. There are several ways a sample can be ionized (Table 2.1) but one of the most commonly used and highly developed is the electron impact source. During electron impact ionization, the gaseous sample is bombarded by a beam of energetic electrons directed perpendicular to the incoming gas stream. The high energy electron beam dislodges electrons from the sample molecules (SM) and thus produces

positive ions as illustrated below:

$$SM + e- \quad ---> \quad SM^+ + 2e-$$
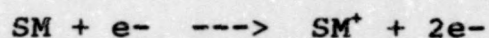
NAME	I.D.	TYPE	IONIZING METHOD
Electron ionization	EI	Gas phase	Energetic electrons
Field ionization	FI	Gas phase	High-potential electrode
Chemical ionization	CI	Gas phase	Reagent positive ions
Electrohydrodynamic ionization	EHMS	Desorption	High field
Fast atom bombardment	FAB	Desorption	Energetic atoms
Laser desorption	LD	Desorption	Laser beam
Plasma desorption	PD	Desorption	High energy fission fragments
Secondary ion MS	SIMS	Desorption	Low flux of energetic ions
Thermal desorption		Desorption	Thermal energy

TABLE 2.1   Ionization methods

The electron beam produces molecular ions in a variety of energy states. The resultant ions contain a large amount of internal energy and will be deposited into rotational, vibrational, and electronic states. This energy will be dissipated by fragmentation of the parent ion. Most fragmentation processes are endothermic. Therefore low energy molecular ions will not fragment in the source and detection of the non-fragmented ions or "molecular ions" are then possible.

In a typical electron impact source (Fig. 2.11) an electron gun is situated perpendicular to the sample flow which emits electrons from a hot filament composed of rhenium or tungsten. The electrons are drawn from the filament by a pair of positively charged slits through which the electrons

pass into the main body of the chamber. Electron acceleration is achieved by placing a 70V negative bias on the filament and
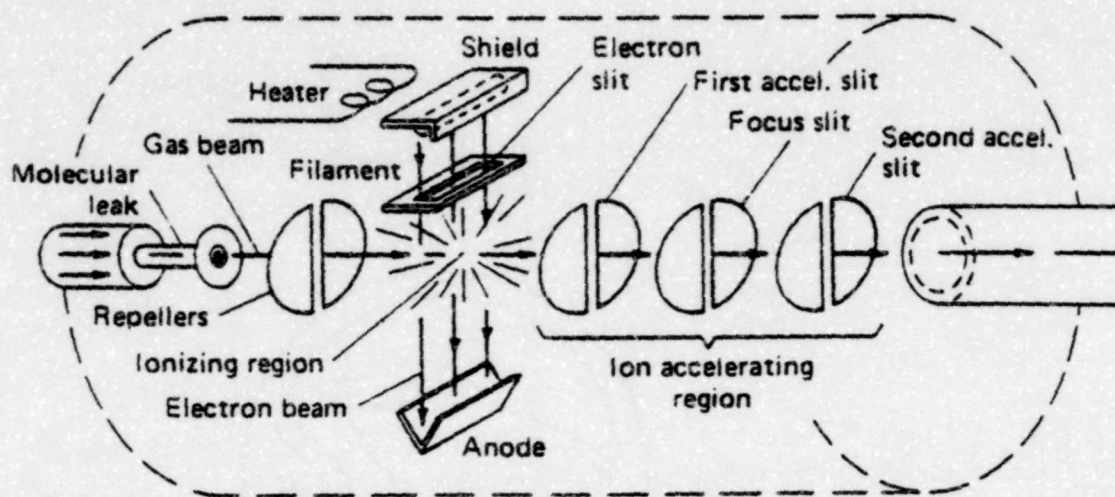

FIGURE 2.11 Electron Impact source

thus producing an electron beam which exhibits a gaussian distribution of kinetic energies with a maximum of 70eV or 1614.2 kcal/mole, an energy sufficient to dislodge an electron from the sample and produce ionic species. The ionic species will be formed with a distribution of internal energies. The electron beam is then collected on a target which possesses positive voltage target of 100V (with respect to the filament). The target and filament are combined with the proper electronic circuitry to ensure a constant flow of electrons through the ionization region. This is of particular importance in order to achieve a constant number of ionizations per unit of time. Only about 0.01% of the

molecules pass from the ionization source into the mass analyzer; the other 99.99% are pumped away from the source by diffusion pumps.

The ion gun accelerates all molecular and fragment ions out of the source and into the mass analyzer region where they are sorted according to mass to charge (m/z) ratio. The acceleration voltages range from 1,000-10,000 volts. The repellers are charged at a voltage slightly higher than the chamber in order to push the positively charged ions into the ion gun. The lens plates, which are two semi-circular discs, have an applied variable voltage to focus the ion beam. Ions formed in the source feel the effect of a repeller which is a bias positive WRT source, therefore accelerating the ions out. The amount of time that an ion remains in the ion chamber is approximately 1 micro second (usec). This, coupled with low pressure of 10^{-6} to 10^{-7} torr precludes ion-neutral reactions and thus the ions are not destroyed. Once the ion reaches this point it will then go through an exit slit then to the mass analyzer.

c) <u>Mass analyzers</u>. Several methods of mass analysis are available to a mass spectrometer. An efficient mass analyzer will distinguish between small mass differences while, at the same time, allow the passage of an adequate amount of ions to yield ion currents that are readily measured. The main difference in the types of analyzers available is their systems of separation. Two very different analyzers are the magnetic sector type and the quadrupole type.

67

(1) <u>Magnetic</u> <u>Sector</u> <u>Mass</u> <u>Analyzers:</u> In a magnetic sector instrument the ion beam leaves the source and travels down an evacuated tube toward a curved region of 180, 90, or 60 degrees between the poles of two magnets. The purpose of the magnetic sector is to disperse the ions in curved trajectories that are dependent upon the mass to charge ratios of the ion. Lower mass ions are deflected most and heavier mass ions the least. The individual ion beams are separated spatially and each beam has a unique ion trajectory (radius of curvature), r, according to its mass to charge ratio (m/z). Only ions of a single m/z value will have the proper trajectory, which will then lead it to the exit slit preceding the detector. By changing the magnetic field strength, H, the components of the total ion beams are separated according to momentum. Also, due to the variability of the magnetic field, the ions are brought into focus at the detector slit. The ion velocity, v, in the magnetic field is represented below:

$$1/2mv^2 = zV \qquad\qquad (2.1)$$

where V = potential applied to the slits

m = mass

after rearrangement:

$$v = \frac{2zV}{m} \qquad\qquad (2.2)$$

Upon entrance into the magnetic field, the ions experience a force 90 degrees to both of the magnetic lines of force and their line of flight.

In order to equate centrifugal and centripetal forces

$$\frac{mv^2}{r} = Hzv \qquad (2.3)$$

Then, solving for the radius of curvature, which is proportional to its momentum and inversely so to the strength of the magnetic field

$$r = \frac{mv}{zH} \qquad (2.4)$$

In order to eliminate the velocity term substitution of equation 2.2 into equation 2.4 is necessary

$$r = \frac{1}{H} \; 2V(m/z) \qquad (2.5)$$

Only ions that follow the path that coincides with the curvature of the analyzer tube, in the magnetic field, are focused on the exit slit. Those ions with different m/z ratios eventually hit the analyzer tube, where they are neutralized and then pumped away. The magnetic field therefore separates the ions into beams with differing m/z ratios as represented below:

$$\frac{m}{z} = \frac{H^2\,r^2}{2V} \qquad (2.6)$$

A successful mass spectrum is obtained by keeping the magnetic field constant and decreasing the accelerating voltage, so that the ever increasing ion masses are brought to focus, or by increasing the magnetic field at constant voltage. In this type of single focusing instrument, both fast scan rates and high mass range can be achieved.

2. <u>Quadrupole mass analyzers:</u> Another type of mass analyzer is a quadrupole, which performs mass separation in a

completely different manner than that of the magnetic sector. Quadrupole instruments are more widely used and produce more mass spectra than any other type of mass analyzer involved in chemical or industrial research laboratories. Quadrupole mass analyzers are not high resolution analyzers and are inferior to magnetic sectors in other respects; however quadrupole analyzers offer a number of significant advantages over the more traditional analyzers.

A quadrupole resolves ions on the basis of mass to charge ratio rather than on a momentum to charge ratio, as does a magnetic sector. Therefore, mass resolution may be preserved when ion samples of various velocities are analyzed. Because of this ability, it is therefore relatively simple to couple the mass spectrometer with another separation sources such as LC or GC.

The mechanics of a quadrupole are less complicated than other analyzers and do not rely upon the use of magnetic fields for their mass discriminatory properties. Thus, quadrupoles are substantially less costly machines and in addition they are not as bulky, heavy, or slow scanning as magnetic sector instruments. Another advantage is that resolution for a quadrupole is set electronically rather than mechanically.

The quadrupole field is formed by a set of four cylindrical shaped electrodes positioned parallel to one another in a circular form (Fig. 2.12). The opposed rods are electrically connected; one pair is attached to a negative terminal while the other pair is connected to the positive

side of a variable (time dependent) direct current (dc) source. A variable radio frequency (rf) alternating current (ac) potential, which is time dependent, is applied to each pair of rods. This combination of fields does not act to accelerate ionic species; instead it creates an oscillation of the particles around their central axis of travel. A quadrupole instrument can be considered a dual high pass/low pass filter. In effect, at any given time, two of the quadrupoles filter out ions of higher mass than the ion of interest, and the other two poles filter out ions of lower mass than the ion of interest.
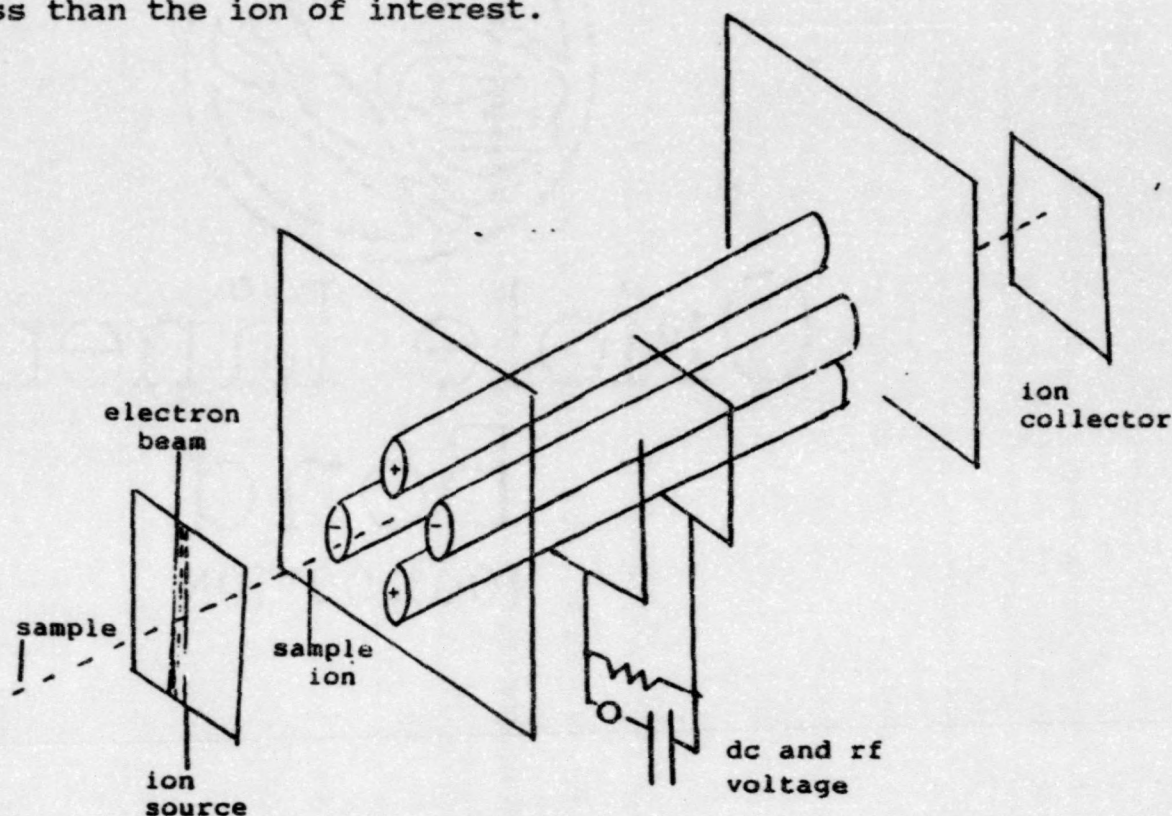

FIGURE 2.12   Configuration of the quadrupole analyzer

During a single cycle of the ac potential, in the absence of dc potential, quadrupoles **b** and **d** (Fig. 2.13) spend 1/2 cycle at a positive and 1/2 cycle at a negative potential. When the quadrupole is positive, a beam of positive ions are repelled toward the center axis (Fig. 2.14). When a negative potential is applied the inverse is observed and the ions are focused onto the center axis toward the negatively biased quadrupoles (Fig. 2.15). The distance traveled by the ion during this process determines whether or not a particular ion will strike a quadrupole and be eliminated before it reaches the detector.
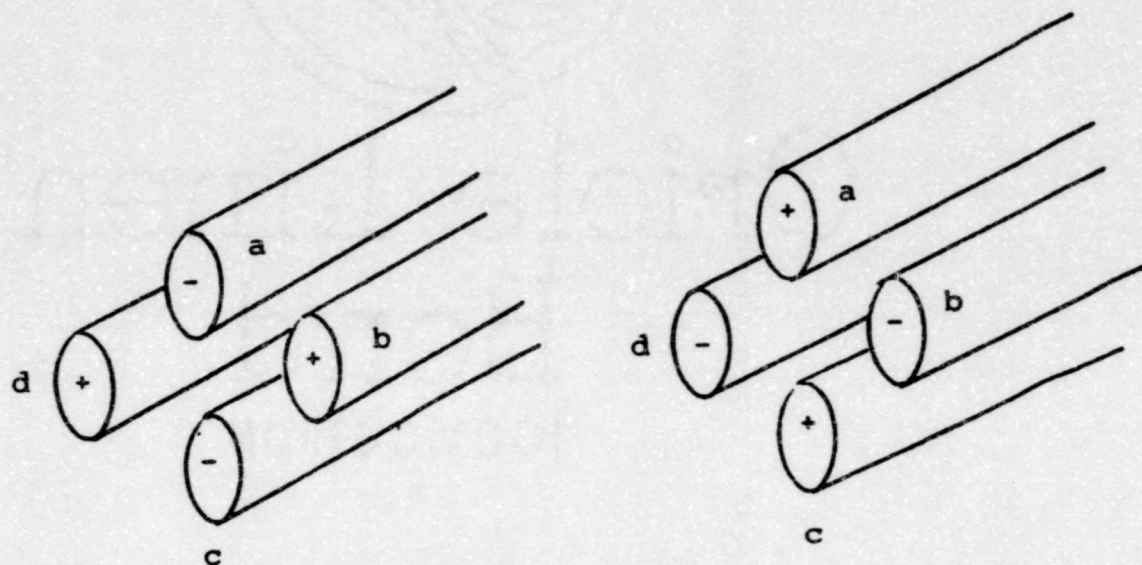

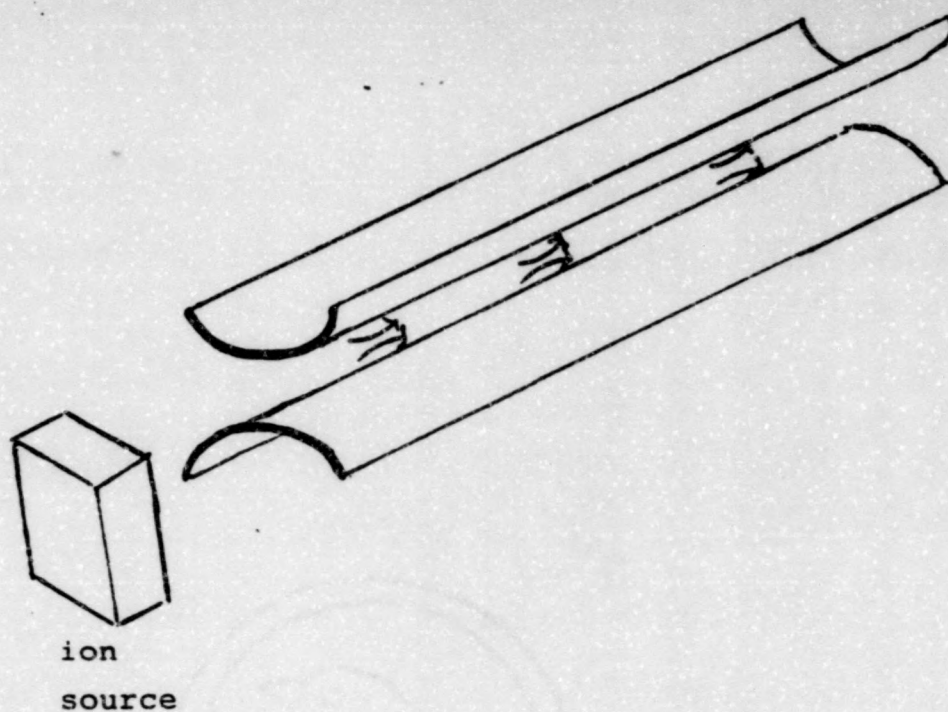FIGURE 2.13   Quadrupole field when a potential is applied

ion
source

FIGURE 2.14   Ion focus under positive potential influence
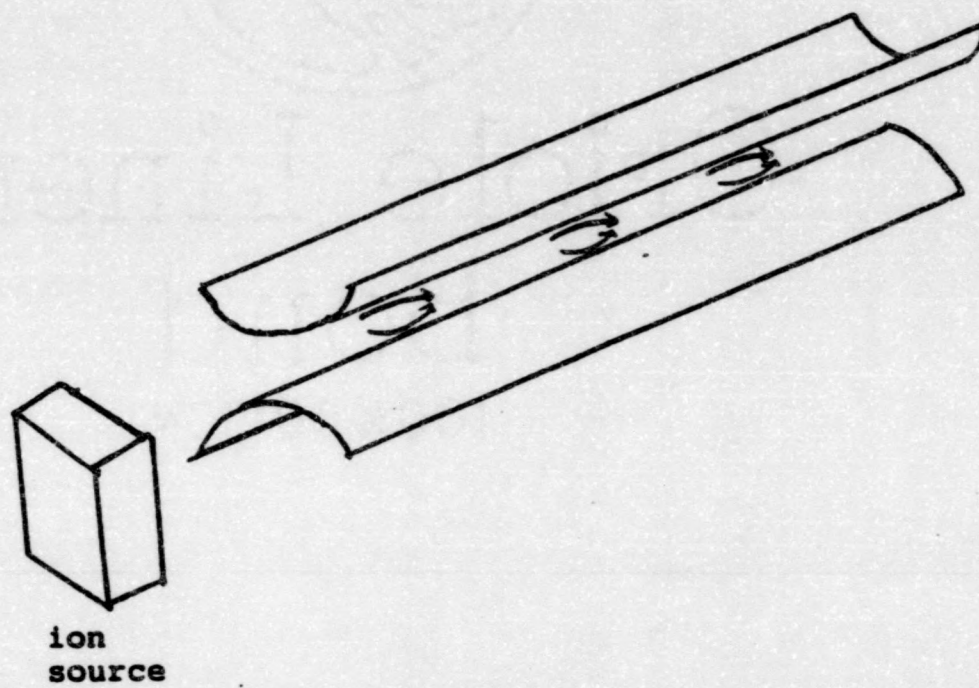

ion
source

FIGURE 2.15   Ion focus under negative potential influence

When a time independent positive dc potential is applied to electrodes **b** and **d**, the heavy ions will be most influenced. These ions will be focused onto the center of the instrument. The lighter ions may experience a sufficient amount of acceleration during a negative voltage application to cause them to collide with a quadrupole and subsequently be pumped away as a neutral species.

An ac potential is applied to quadrupoles **a** and **c** that is equal in magnitude, but opposite in sign, to that applied to quadrupoles **b** and **d**. Therefore, the rf waveform imposed upon the **b** and **d** quadrupoles are 180 degrees out of phase with that of **a** and **c**. Thus the signs of the poles alternate and are switched, generating an rf waveform. As before, ions heavier than that selected will be influenced by the applied dc potential, which is now negative. Heavy ions will tend to be eliminated due to the defocusing effect caused by the negative dc potential. In the case of ions lighter than that which is selected, response to the focusing action will result when the positive portion of the ac becomes larger than the negative portion. Therefore, if the ac frequency and magnitude are selected properly, it is essentially a correction that prevents the light ions from contacting electrodes **a** and **c**.

In order for an ion to travel from the source to the detector there must be stability in both the positive and negative poles. That is, the ion must be heavy enough not to be eliminated by the high pass filter of the **a** and **c** poles but light enough so as not to be eliminated by the low pass

filter of the **b** and **d** poles. Thus, there must be mutual stability between the quadrupoles, as well as, proper magnitude of both the ac and dc potentials, in order for an ion to pass through the analyzer and ultimately reach the detector.

Quadrupole instruments are more compact, portable, less expensive, and more rugged than their magnetic sector counterpart. They offer a much faster scan time; therefore real time chromatographic scanning is possible. Neither instrument is superior relative to resolving power and is therefore dependent upon the specific application which instrument is most useful.

Resolving power, defined as: (2.7)

$$R = \frac{m}{\Delta m}$$ where m = mass at which peaks occur
  Δm = distance between 2 resolved peaks

is one of the most important parameters of a mass spectrometer. The only representative of mass analyzer performance is peak width since ions have no natural line width. Two peaks of equal height, h, separated by a mass difference, Δm, are considered resolved when the height, h, of the valley between them is less than 10% of the peak. This is commonly referred to as the "10% valley resolution." The resolving power, given by $\frac{m}{\Delta m}$ (eqn. 2.8)

is called the half-height width definition and is the most common method of determination.

High resolution is only necessary when trying to distinguish between compounds with similar masses such as $CH_2Cl_2^+$

(83.9534 m.w.) and $CDCl_2^+$ (83.9518 m.w.). In order to resolve these masses completely, an instrument would need a resolving power of 52,470 as illustrated below:

$$R = \frac{m}{\Delta m} = \frac{83.9518}{83.9534 - 83.9518} = 52,470$$

High resolution, such as this, requires a double focusing sector and therefore cannot be achieved by a single focusing magnetic sector. Both the single focusing magnetic sector and the quadrupole can only achieve resolution of about 5000. However, this is often enough for environmental analysis of priority pollutants and some industrial applications. In many cases an instrument with higher resolution capabilities is necessary. However, in any application, once the ions are resolved they will then exit the mass analyzer and strike the detector where they will be amplified.

d) Detectors. The type of detector used in this study is the electron multiplier. If the ion currents are less than 10^{-15} amps, an electron multiplier is necessary. The resolved ion beams leave the analyzer and strikes the conversion dynode which is a metal plate used for the conversion of impinging electrons. The conversion dynode is subjected to constant high voltage which serves to accelerate and convert positive or negative ions into electrons, or positively charged ions, when they strike the plate. A dynode multiplier has 15 - 18 individual dynodes coated with a 2% copper beryllium metal alloy oxide with high secondary electron emission properties. This plate yields an average of two electrons for each positive ion. The electrons are then accelerated, by a positive

potential, to a second dynode where the electrons are again multiplied. Continous dynode multipliers, composed of leaded glass containing metal oxides, are drawn into a hollow tube and thus form a multiplier channel. Electrons are attracted by the resistance of the glass, which creates a voltage, and then travel through the tube. The gain ranges from 10^5 to 10^7, but the limiting factor is system noise and background levels.

e) Identification of molecules from mass spectra: The first and most important information that is observed from the mass spectrum is molecular weight. It is possible to obtain molecular weights as precise as eleven decimal places when a high resolution mass spectrometer is used. However, the mass of the molecular ion or parent ion (heaviest ion) gives a nominal molecular weight to the nearest whole number with low resolution mass spectrometers and exact molecular weight with high resolution mass spectrometers. For the spectrum of methanol (Fig. 16) the parent ion or molecular ion occurs at m.w. 32, also on the figure there is a tabulation of the relative abundances of each fragment. In this particular spectrum there is a p+1 peak which does not show up visually at m.w. 33, this is representative of naturally occurring carbon-13 (discussed later).

The largest peak in a mass spectrum is called the base peak and it is often assigned the height of 100. This figure is used in order to calculate the magnitude of the remaining peaks by normalizing them relative to the base peak. The normalized values are obtained as percentages of the base peak height.

PURE METHANOL

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
29.15	18	30.15	11	32.15	69	33.15	1
29.05	89	31.15	100				

TABULATE
[MS1]

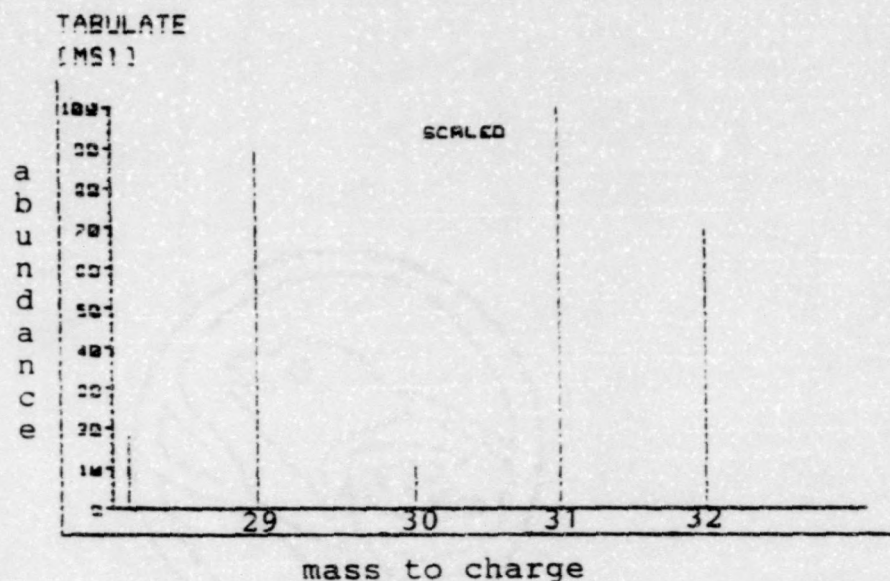
mass to charge

(enhanced actual spectrum)

FIGURE 2.16 Spectrum of Methanol

f) Interpretation of mass spectra: There are several rules to be followed in order to identify a compound in a mass spectrum. When all of these rules are combined, successful identification is usually achieved.

1. The nitrogen rule: A molecule with an odd numbered molecular weight will have an odd number of nitrogens. A molecule with an even numbered molecular weight must contain no nitrogens or an even number of nitrogens. This holds true for compounds that contain C, H, O, S, P, B, Si, As, halogens and alkali earth metal compounds.

2. <u>The hydrogen rule:</u> If the molecular weight of a compound containing C, H, O, and N is even, then the number of hydrogens it contains is also even. If the molecular weight is divisible by 4, so is the number of hydrogens.

3. <u>Heavy isotope rule:</u> A chemically pure substance will give a mixture of mass spectra because the elements are not isotopically pure. Carbon appears in two forms, carbon-12 which is the most abundant and carbon-13 which occurs at a rate of 1.11 percent. Therefore a molecular ion will be followed by an M+1 (molecular ion + 1 a.m.u.) peak that will be 1.1 percent of the height of the molecular ion peak (M). Thus, in the spectrum of an organic compound containing five carbons, the M+1 peak would be about 5.5 percent of the M.

Nitrogen also occurs in two forms N-14, the most abundant, and N-15 at a rate of 0.37 percent. A useful formula, containing both of these factors, which can be used to derive the number of carbons is shown below:

$$100 \frac{M+1}{M} = (1.11 \times \# \text{ carbons}) + (0.37 \times \# \text{ nitrogens})$$

where M = mass of molecular or parent ion
M+1 = mass of molecular ion + 1Oxygen can

also be found in a similar way, where the M+2 peak would be 0.20 percent of the M occurring as oxygen-18. This can be used once you know the number of carbons.

$$100 \frac{M+2}{M} = (0.20 \times \# \text{ oxygens}) + (1.11 \times \# \text{ carbons})$$

where M+2 = mass of molecular ion + 2

Compounds containing chlorine and bromine:

A compound containing one chlorine will exhibit a M+2 peak approximately 33 percent of the height of the M peak due to the occurrence of chlorine-37. A compound containing two chlorines will exhibit three peaks, one each at M, M+2, and M+4. See Fig. 2.17.

Bromine occurs almost equally in its two isotopic forms, Br-79 at 51% and Br-81 at 49%. When more than one bromine atom is present the ratio in the M+2 position changes and a M+4 peak will appear. See Fig. 2.18.

Once you have calculated the number of C, H, O, N, and halogens present, you can then apply the rings + double bonds formula to determine the number of unsaturated sites.

$$\text{unsaturated sites} = \#C's + 1 - \frac{\#H's}{2} - \frac{\#halogens}{2} + \frac{\#N's}{2}$$

for example, benzene = C_6H_6

$$\text{rings + double bonds} = 6 + 1 - \frac{6}{2} - \frac{0}{2} + \frac{0}{2}$$

$$= 7 - 3$$
$$= 4 \qquad (\text{1 ring and 3 double bonds})$$

g) <u>General Fragmentation Processes:</u>

(1) <u>Alkanes.</u>

Cleavage is favored at branched carbons with a tendency for the positive charge to remain with the branch.

tertiary > secondary > primary

(2) <u>Cyclic alkanes</u>.

The mass spectra of a compound containing a ring will often have a large parent peak. A large parent peak indicates a stable ring.
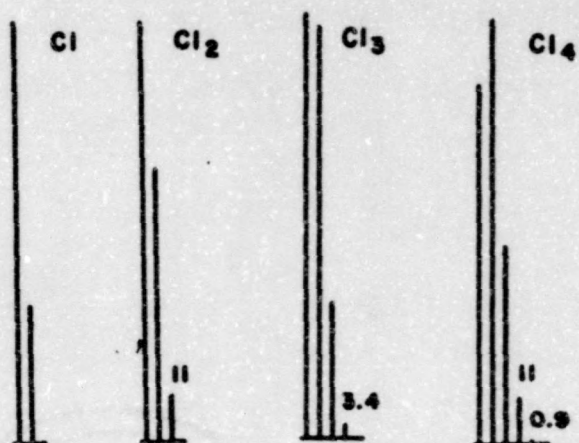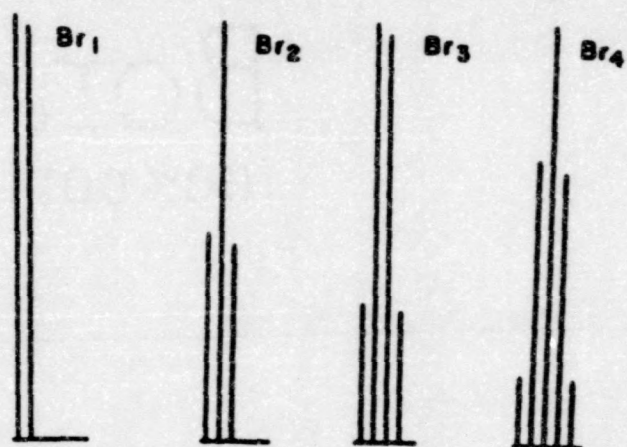
FIGURE 2.17 Spectrum of Chlorine

FIGURE 2.18. Spectrum of Bromine

A saturated ring will lose side chains at the alpha carbon.

Double bonds tend to shown cleavage beta to the bond.

Substances containing a carbonyl group will break at the carbonyl group bond and the positive charge will remain with the carbonyl group.

(3) <u>Ketones and Aldehydes</u>. Ketones and aldehydes fragment in a reproducible manner. The primary path of fragmentation is to sever the carbonyl carbon-alpha carbon bond to generate the acylium ion fragment which goes on to be detected. The fragmentation takes place on each side of the carbonyl group, thus by looking at the mass of the fragment one can determine what constitutes each "R" group in an RCOR' ketone.(See Fig. 2.19 below)

$$\underset{R-C-R'}{\overset{O}{\overset{\|}{}}} \ ---> \ \underset{R-C-R'}{\overset{O}{\overset{\|}{}}} \ ---> \ R^\cdot \ +\ \underset{C-R'}{\overset{O}{\overset{+\|}{}}}{}^\cdot \ \text{ and } R'^\cdot + \ \underset{C-R}{\overset{O}{\overset{+\|}{}}}$$
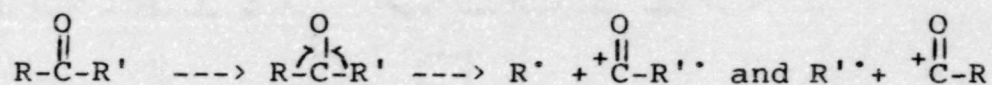
FIGURE 2.19 Ketone/aldehyde fragmentation pattern

If the R group of the ketone or aldehyde contains more than two carbons the McLafferty rearrangement (Fig. 2.20) occurs. The McLafferty cleavage is an important pathway, leading to peaks at m/z 58, 72, 86... depending on the number of carbons in R and R'.
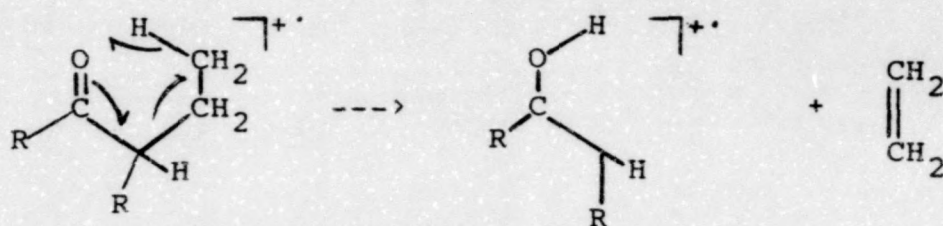
FIGURE 2.20 McLafferty Rearrangement

In the case of an aldehyde a m/z 29 peak will always be observed. Other common peaks will occur at m/z 18, 28, and 43.

(4) Alcohols. When an alcohol fragments the largest peak (base peak) is usually one at m - 18 and the molecular ion is small and may even be non-existent. This is a major process because water is a good leaving group. As shown in Figure 2.21, primary secondary and tertiary alcohols fragment differently.
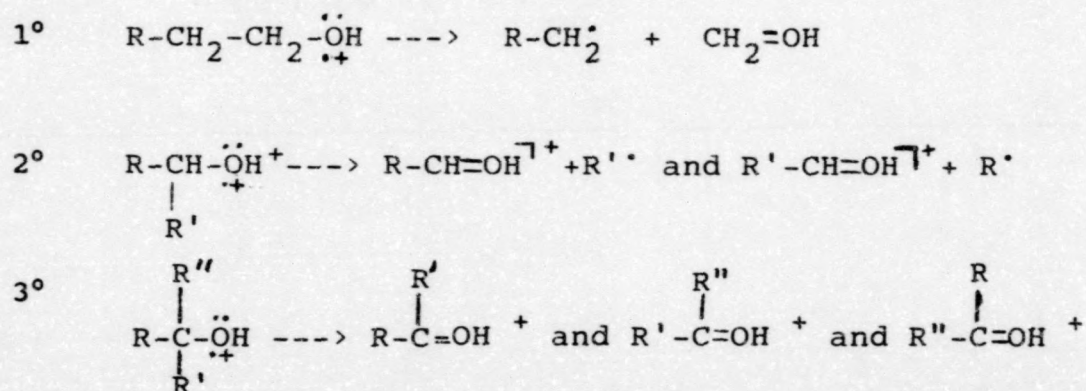
$1°$ $R-CH_2-CH_2-\overset{..}{\underset{..+}{O}}H$ ---> $R-C\overset{.}{H_2}$ + $CH_2=OH$

$2°$ $R-CH-\overset{..}{\underset{..+}{O}}H$ ---> $R-CH=OH^{\rceil +}$ + $R'^{..}$ and $R'-CH=OH^{\rceil +}$ + $R^{.}$
         $\quad\;\; |$
         $\quad\;\; R'$

$3°$ $\quad\;\; R''$
         $\quad\;\;\; |$
         $R-\overset{..}{\underset{..+}{C}}-OH$ ---> $R-\overset{R'}{\overset{|}{C}}=OH^{+}$ and $R'-\overset{R''}{\overset{|}{C}}=OH^{+}$ and $R''-\overset{R}{\overset{|}{C}}=OH^{+}$
         $\quad\;\; |$
         $\quad\;\; R'$

FIGURE 2.21. Fragmentation pattern of alcohols

Usually, if there is not a m/z 31 peak a primary alcohol is not present.

(5) <u>Carboxylic Acids</u>. Typically, carboxylic acid reactions are concerted reactions where bond breakage and cleavage occurs simultaneously without an intermediate. The molecular ion is usually very small and sometimes non-existent. The main process is the McLafferty rearrangement (Fig. 2.22).
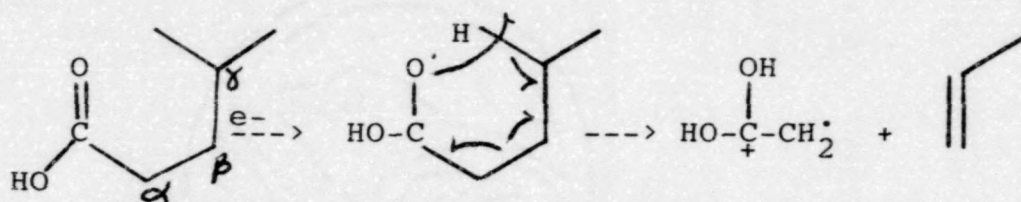
FIGURE 2.22 Fragmentation of carboxylic acids

(6) <u>Aromatics</u>. The main process in the fragmentation of alkyl aromatics is the formation of the aromatic tropyllium ion at m/z 91 (Fig. 2.23). The aromatic ring stabilizes the molecular ion; therefore the molecular ion peak is usually large.

m/z 91   m/z 91
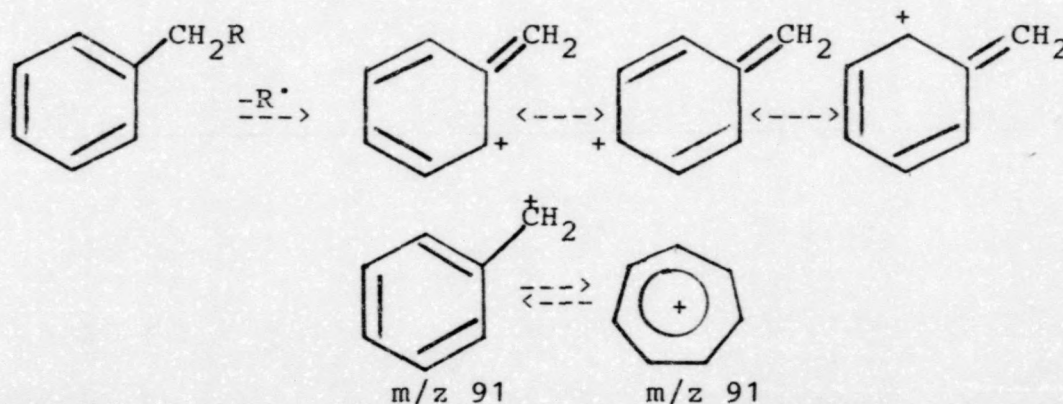
FIGURE 2.23. Fragmentation pattern of aromatics

The interpretation of mass spectra is relatively easy for simple molecules, especially if one is prepared with identification rules and has access to some spectral references. Unfortunately, as molecular complexity increases, identification becomes somewhat more tedious and therefore comparison of knowns with unknowns is used as a method of interpretation. Currently, these types of comparisons are being performed with software libraries interfaced directly with the instrument in order to isolate the most similar compound to that of the unknown.

3. Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GC/MS) is the ideal instrumental combination (fig. 2.24) for complete separation and identification. Since the mass spectrometer (MS) cannot analyze a mixture, the gas chromatograph (GC) is used to separate the mixture into its variety of components; then the MS analyzes the components and can provide information about each compound individually. The most important factor that must be considered when interfacing these instruments is to be careful not to diminish the performance of either component. A GC operates at high pressures while a MS must be ran under high vacuum. In order to have a successful interface, separators must be employed in various places between the two instruments. This is primarily due to the large ratio of carrier gas to sample eluting from the GC. An effusion separator is used to separate the carrier gas from
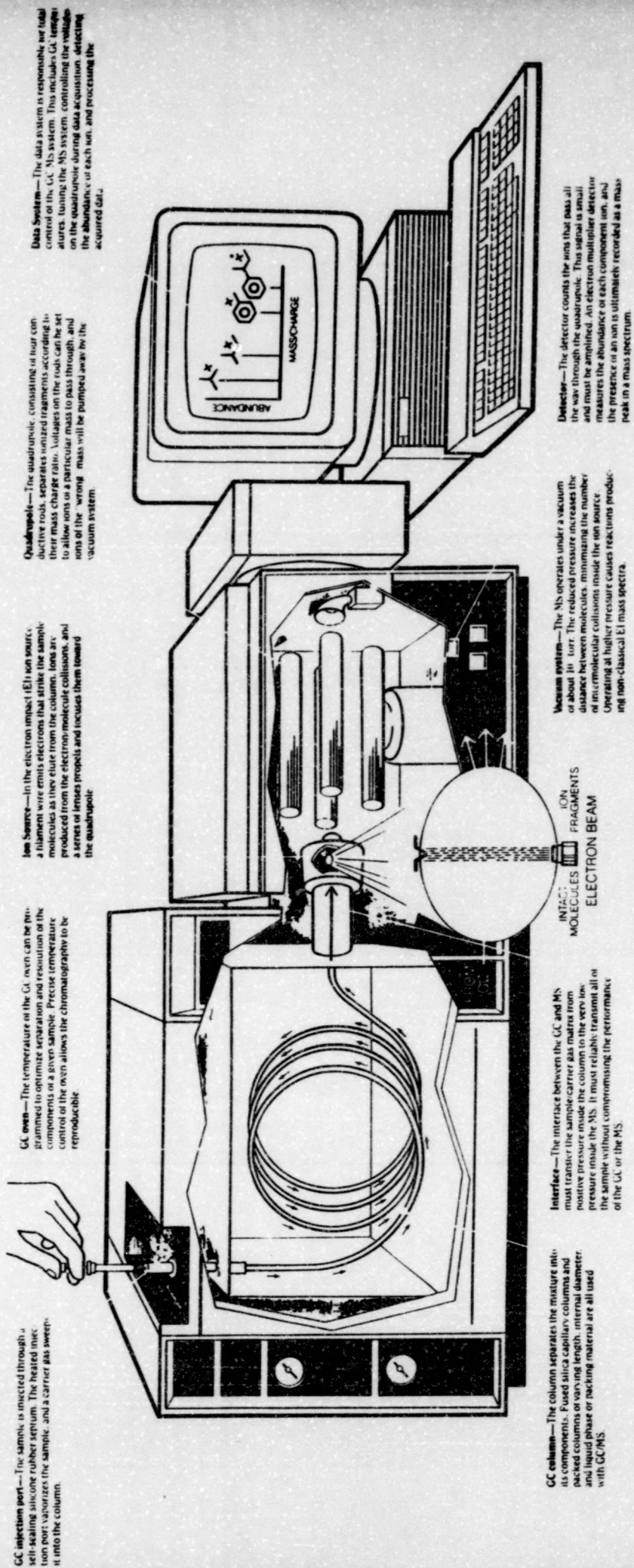
GC injection port — The sample is injected through a self-sealing silicone rubber septum. The heated injection port vaporizes the sample, and a carrier gas sweeps it into the column.

GC oven — The temperature of the GC oven can be programmed to optimize separation and resolution of the components on a given sample. Precise temperature control of the oven allows the chromatography to be reproducible.

Ion Source — In the electron impact (EI) ion source, a filament wire emits electrons that strike the sample molecules as they elute from the column. Ions are produced from the electron-molecule collisions, and a series of lenses propels and focuses them toward the quadrupole.

Quadrupole — The quadrupole, consisting of four conductive rods, separates ionized fragments according to their mass charge ratio. Voltages on the rods can be set to allow ions on a particular mass to pass through, and ions of the "wrong" mass will be pumped away by the vacuum system.

Data System — The data system is responsible for total control of the GC/MS system. This includes GC temperatures, tuning the MS system, controlling the voltages on the quadrupole during data acquisition, detecting the abundance of each ion, and processing the acquired data.

GC column — The column separates the mixture into its components. Fused silica capillary columns and packed columns or varying length, internal diameter and liquid phase or packing material are all used with GC/MS.

Interface — The interface between the GC and MS must transfer the sample/carrier gas matrix from positive pressure inside the column to the very low pressure inside the MS. It must reliably transmit all of the sample without compromising the performance of the GC or the MS.

Vacuum system — The MS operates under a vacuum of about 10 torr. The reduced pressure increases the distance between molecules, minimizing the number of intermolecular collisions inside the ion source. Operating at higher pressure causes reactions producing non-classical EI mass spectra.

Detector — The detector counts the ions that pass all the way through the quadrupole. This signal is small and must be amplified. An electron multiplier detector measures the abundance of each component ion, and the presence of an ion is ultimately recorded as a mass peak in a mass spectrum.

INTACT MOLECULES

ION FRAGMENTS

ELECTRON BEAM

ABUNDANCE

MASS/CHARGE

FIGURE 2.24 Diagram of a GC/MS
(Copyright Hewlett-Packard Company (1987)
Used with permission)

the eluent. The effusion separator is a tube of micro-fine porosity glass, surrounded by a vacuum chamber, which allows permeation of only the carrier gas molecules, while the heavier organic molecules pass on through to the MS. Another method is using an orifice or jet separator, which employs a vacuum system each for the sample and the carrier gas. The basis of sample concentration relies on the fact that helium has a faster diffusion rate than the sample and will be swept through at a higher rate. A membrane separator permeable by organic molecules is another method for separation. A silicone membrane will allow the organic molecules to pass directly into the MS, via a vacuum, while keeping the helium from passing through. This method is adversely affected by temperature and is therefore not commonly used.

There are several types of mass filters offered by the mass spectrometer, two of which are the magnetic sector and the hyperbolic quadrupole. The magnetic sector is a high resolution mass filter and can achieve mass measurement to four decimal places. The quadrupole is a relatively low resolution mass filter and is used only where nominal masses need to be measured. This type of filter is used most often.

When a mass spectrometer is used as a detector for a gas chromatograph (as in GC/MS), the data can be observed in several unique display modes. The data can be displayed in real-time as a chromatogram (intensity vs. time) and then the mass spectral information can be displayed as computer recon-structed spectra. The real-time chromatogram is called a

total ion chromatogram, or TIC. The total ion chromatogram is a plot of the sum of all ion currents as a function of time. The total ion chromatogram is always obtained first when a sample is being analyzed, the mass spectrum is then obtained from the TIC. In Fig. 2.25 there is both a TIC and a mass spectrum. The upper mass spectrum corresponds to the ion fragmentation of the molecule that gives rise to the single peak in the lower TIC.
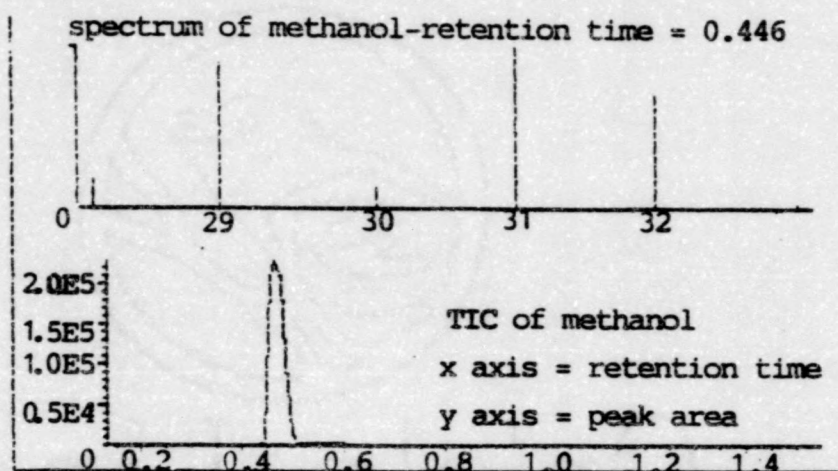
(enhanced actual data)
FIGURE 2.25 Spectrum and TIC of Methanol

There are many advantages of a mass spectrometer as a detector for a gas chromatograph. The mass spectrometer is a specific identification and confirmation tool. It can serve as a real-time detector, recording total ion current as a function of time, as well as scan for preselected ions. The ability to view fragmentation pattern provides specific information about molecular structure, as well as molecular weight.

D. Experimental Procedure:

1. Fundamental Gas Chromatography:

Instructors note: This experiment is designed to be performed on a GC utilizing a thermal conductivity detector or a flame ionization detector. Because of the bias of an electron impact ionization source in a mass spectrometer, a GC/MS should not be used in this experiment.

Purpose

To identify the components of a mixture by GC based

on retention time t_R (time required for a sample to pass

through the instrument). Assuming purity of your standards

and using peak area you should be able to derive the

percentage of each component contained in your unknown

mixture.

Procedure:

1) Your instructor will provide you with pure methanol,

   ethanol, propanol, butanol, and pentanol.

2) You will also be provided with an unknown two-

   component mixture, in which each component in the

   mixture is one of the above compounds.

3) Obtain the GC spectra of each of your five pure

   compounds as follows:

   a) Inject the proper amount of single pure sample (as

   determined by your instructor) in to the GC. The

   specific operating procedure for your gas

   chromatograph will be explained by your instructor.

b) Repeat the above analysis for the other four pure known compounds. At this point you should have five chromatograms.

c) Inject the proper amount of your unknown mixture into the GC, and obtain the chromatogram.

Data:

1) Using a chromatogram from one of the pure compounds, calculate both the resolution and the number of theoretical plates.

2) Using all five chromatograms of the known compounds, construct the following two graphs:

    a) the log of retention time vs boiling point

    b) the log of retention time vs number of carbons.

3) Compare the chromatograms of your known compounds and the chromatogram of your mixture and identify the components of your unknown mixture.

4) Using the peak area of each unknown compound and peak area of known (pure) compounds, calculate the percent of each component in your mixture.

Questions:

1) List 5 factors involved in adjusting poor resolution and how to control them.

2) If you are given a mixture of seven hydrocarbon gases and a series of halogenated hydrocarbons, which stationary phase would give a better separation, a polar or non-polar stationary phase in the column? why?

3) Explain why one would choose a capillary column over a larger bore column. What happens if the column length is too short? too long? and how does the length affect the other controlled variables concerning resolution?

RESULTS

1. Fundamental Gas Chromatography

    a) Instrumentation:

       Shimadzu model GC-8A.

    b) Detector - Thermal conductivity detector

    c) Operating conditions:

       Isothermal

    d) Temperature

       oven = 120°C

       injection port = 190°C

    e) Attenuation - 9

    f) Current - 90 mA
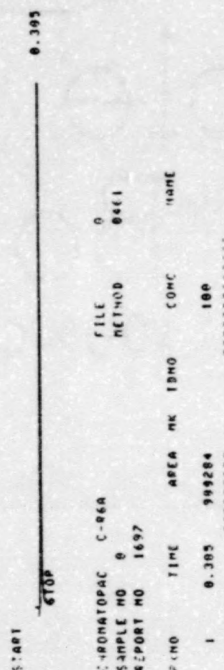
FIGURE 2.26   Chromatogram of methanol
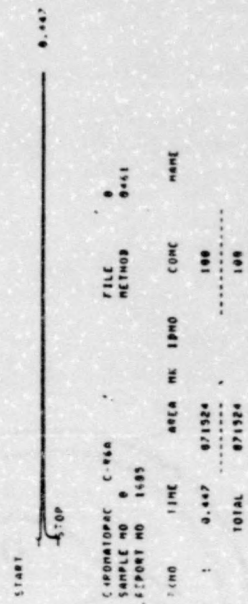
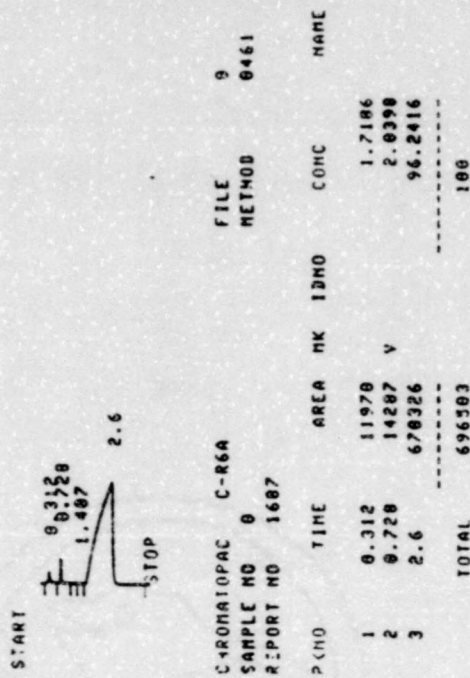FIGURE 2.27   Chromatogram of ethanol
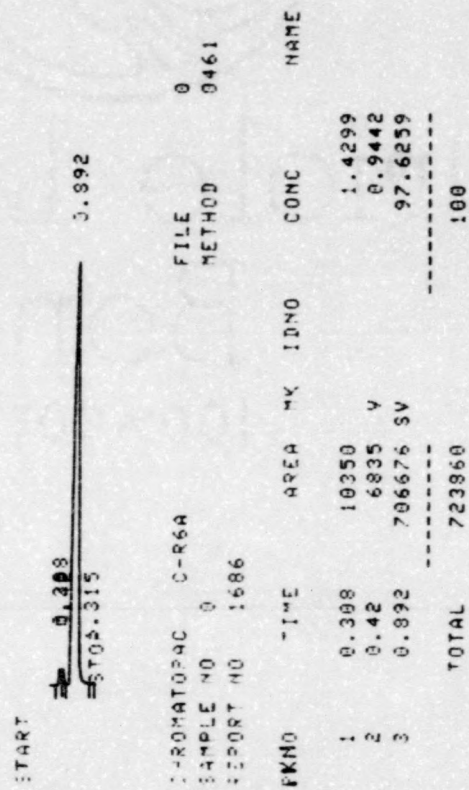
FIGURE 2.28   Chromatogram of propanol

```
CHROMATOPAC  C-R6A              FILE     9
SAMPLE NO   0                   METHOD   0461
REPORT NO  1687

PKNO   TIME    AREA   MK  IDNO     CONC    NAME
  1    0.312   11970                1.7186
  2    0.728   14207  V             2.0398
  3    2.6     670326              96.2416
               ------              --------
       TOTAL   696503               100
```

FIGURE 2.29   Chromatogram of butanol


```
CHROMATOPAC  C-R6A              FILE
SAMPLE NO   0                   METHOD
REPORT NO  1688

PKNO   TIME    AREA   MK  IDNO     CONC
  1    0.33    2766                0.6129
  2    1.787   5501                1.2189
  3    3.767   696                 0.1542
  4    7.925   442347             98.0139
               ------             --------
       TOTAL   451310              100
```
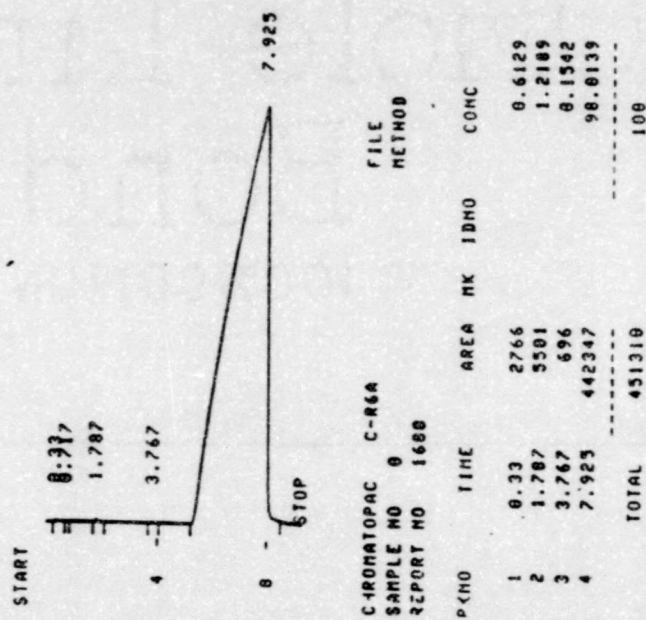
FIGURE 2.30   Chromatogram of pentanol
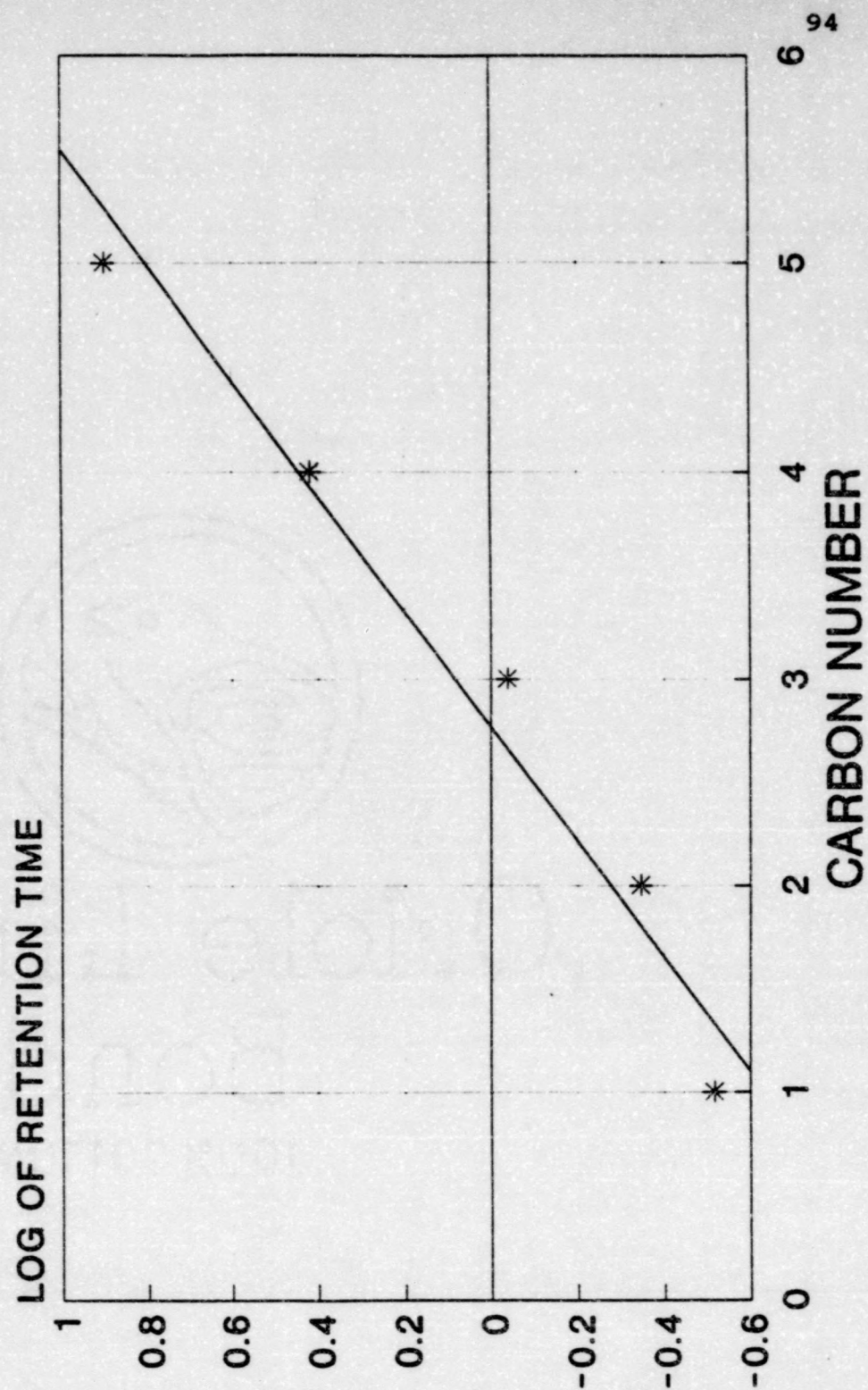
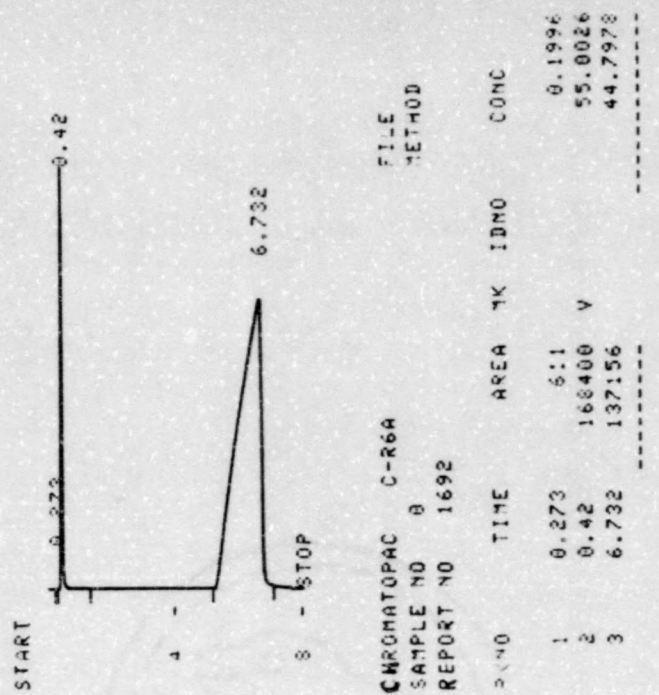Figure 2.31 Alcohols: Log of retention time vs number of
carbons

FIGURE 2.32   Chromatogram of 1:1 ethanol and pentanol

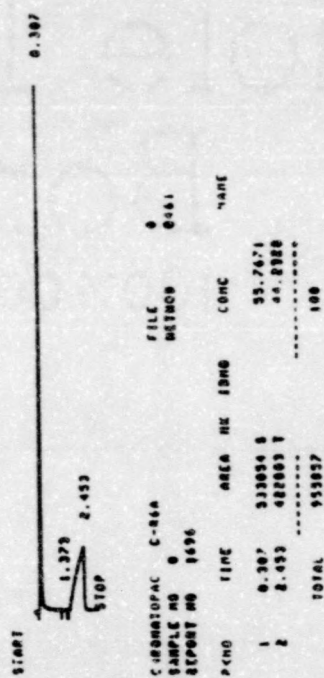
FIGURE 2.33   Chromatogram of 1:1 methanol and butanol

EXPERIMENTAL

2. Fundamental Mass Spectrometry:

Instructor's Note: This experiment does not require a GC/MS, a simple MS would suffice, because in this experiment only pure compounds will be analyzed. However, the experiment can be performed on a gc/ms. The procedure below assumes that a GC/MS is to be used.

Purpose:

To identify the components of a mixture based on the fragmentation rules of organic compounds. Using the ability of the instrument to isolate and individually view each component, the student will use various applied calculations, techniques, and fragmentation rules to derive the identity of each component.

Procedure:

1) Your instructor will provide you and a partner with three unknown compounds. The unknowns will be limited to one set of the following: three isomeric unbranched ketones or aldehydes, three isomeric substituted aromatic compounds, or three halogenated compounds. Each set will be representative of related compounds.

2) Inject the appropriate amount, as determined by your instructor and the instrument being used.

3) Obtain a mass spectrum for each of your unknowns.

a. View the total ion current and choose the best location on the peak to obtain a clear fragmentation pattern.

b. Print a copy of the fragmentation pattern and total ion current.

c. Normalize the abundances with respect to the base peak.

d. Obtain a printout of the mass to charge ratios and the relative abundances.

Data:

1) For each unknown compound:

a. Identify the molecular ion, base peak, and isotopic peaks.

b. Using the fragmentation rules and calculate the number of carbons and oxygens and rings plus double bonds.

c. Write the empirical formula.

d. Provide a mechanism for fragmentation that explains the observed fragmentation pattern in each spectra.

e. Draw the appropriate structure to represent each unknown and explain, using fragmentation rules, why each related pair fragmented differently? similarly?

RESULTS

2. <u>Fundamental Mass Spectrometry</u>

   a) Instrumentation.

   Hewlett Packard model HP 5970B mass selective detector.

   b) Isomeric Alkyl Aromatics. The following data is representative of isomeric compounds. The spectra was generated on the above-stated instrument.

   In Figure 2.34 the most predominant loss in the fragmentation of cumene is m - CH_3 which gives a large base peak at m/z 105. In Figure 2.35, mesitylene which is an isomer of cumene is somewhat different. It has a notable m - H loss at 119 which leaves a substituted tropylium ion. In Figure 2.36 n-propylbenzene has a small parent peak at m/z 120 compared to that of cumene and mesitylene. There is no peak at m/z 105 but a very large peak at m/z 91. The most distinctive loss is m - C_2H_5 which permits the formation of the tropylium ion at m/z 91.
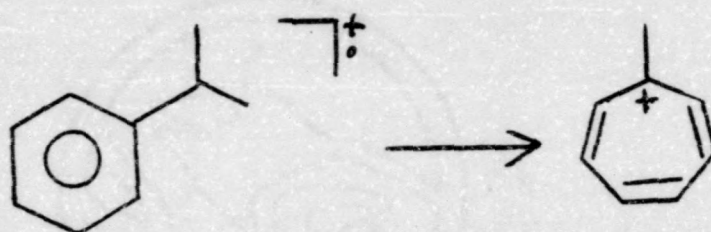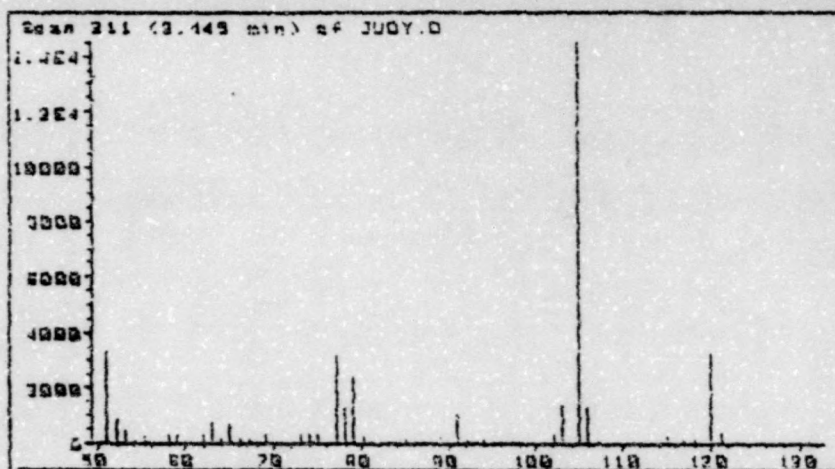
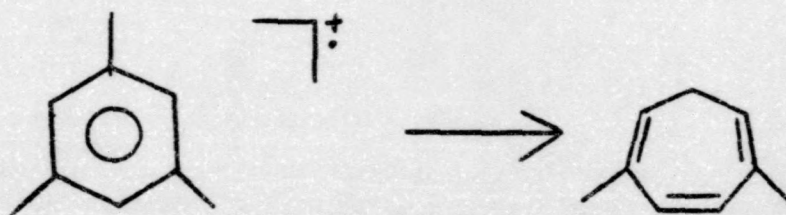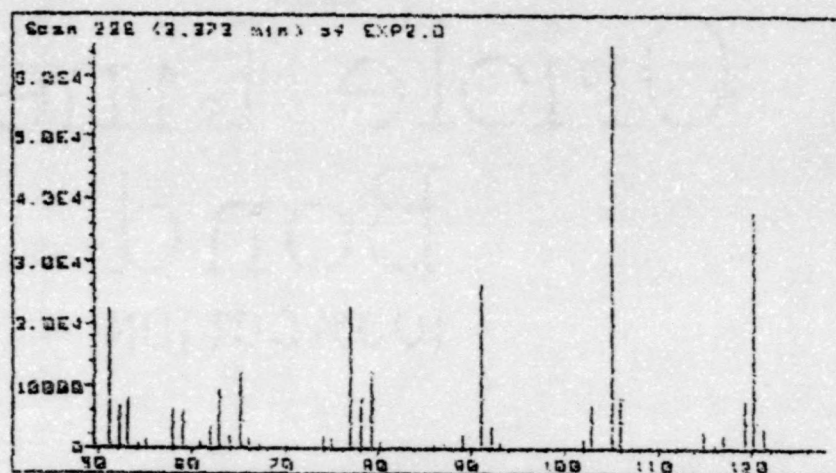FIGURE 2.34   Spectrum and main fragmentation mode of Cumene

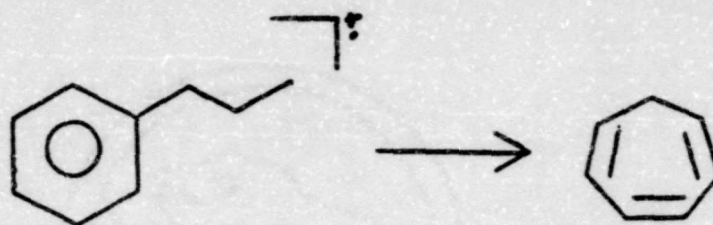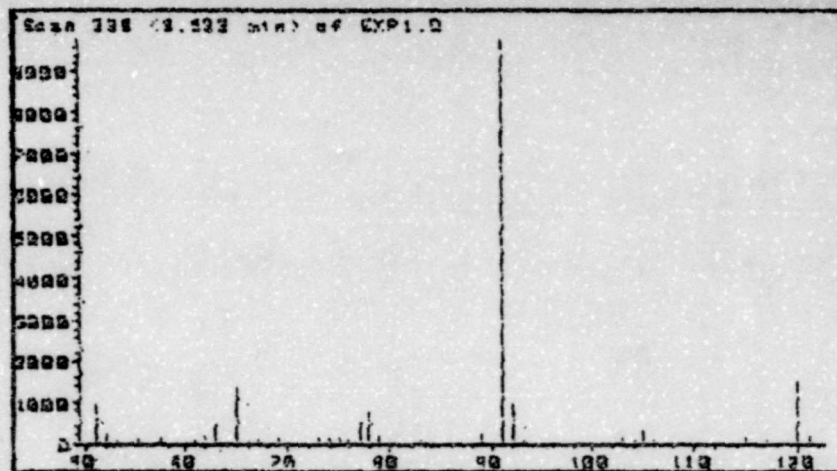FIGURE 2.35   Spectrum and distinctive fragmentation
process of mesitylene

FIGURE 2.36 Spectrum and fragmentation pattern of
n-propylbenzene

c) Halogenated Aromatics

In Figure 2.37 chlorobenzene exhibits a distinctive loss of m - 35 leaving C_6H_5 as the base peak at m/z 77. Also, there is a notable m + 2 peak at m/z 114 which is approximately 34% of the parent peak. This represents the relative abundance of naturally occurring chlorine-37. In Figure 2.38 bromobenzene has a prominent parent peak and a m+1 peak which is 49% of the parent peak. This is indicative of naturally occurring isotopic Br-79 and Br-81. The large base peak at m/z 77 is C_6H_5. In Figure 2.39 p-dibromobenzene has m-1 peak which represents Br-79 and Br-79, a parent peak of Br-79 and Br-81, and a m+1 peak of Br-81 and Br-81. These occur in a 1:2:1 ratio. At m/z 147 where m - CBr occurs the ratio of Br-79 to Br-81 represents the 1:1 ratio as in a single bromine compound. A large m/z 77 represents C_6H_5 which is common in a benzene containing compound.
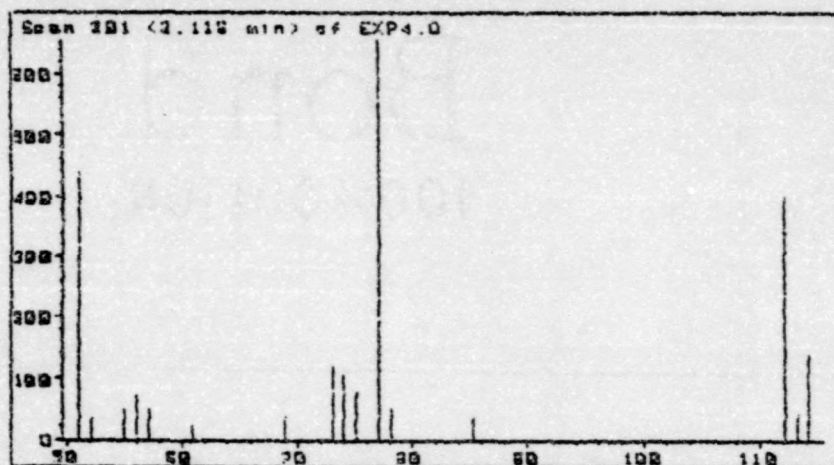

FIGURE 2.37   Spectrum and fragmentation pattern of chlorobenzene
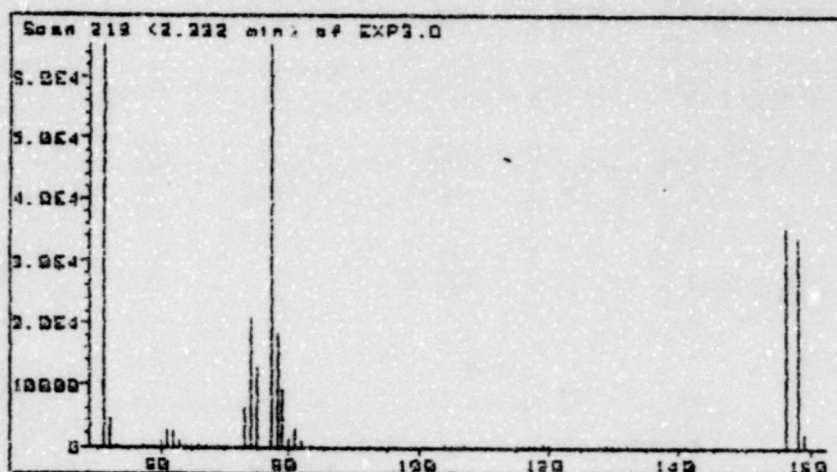
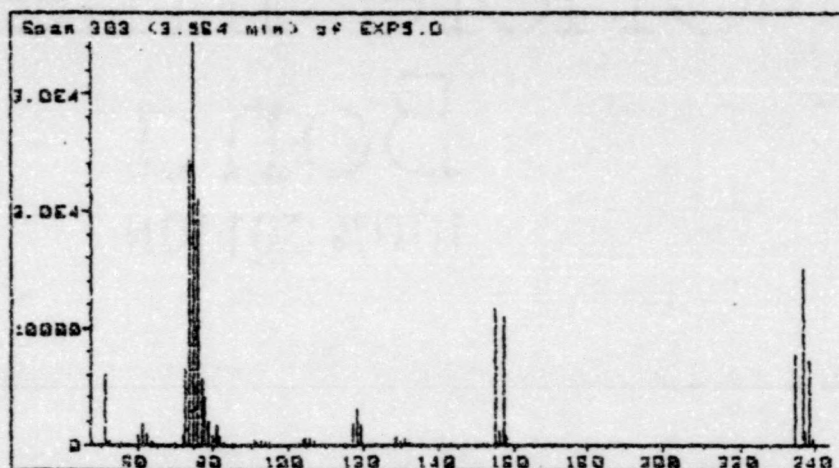FIGURE 2.38   Spectrum and fragmentation pattern of
bromobenzene

FIGURE 2.39   Spectrum and fragmentation pattern of
p-dibromobenzene

EXPERIMENTAL

3.   Fundamental   Gas   Chromatography/Mass   Spectrometry

Purpose:

To understand the enhanced performance provided by a gas chromatograph when interfaced to a mass spectrometer. Also, to understand qualification and quantification correlated to integral techniques provided by the interfacing of a computer. Familiarization with the mass spectral software which allows data manipulation and the availability of several types of spectra and tabulations will also be a key point.

The goal of this experiment is to separate the two components in a binary mixture and identify the components.

Procedure:

Part I   Functional Groups

1) Your instructor will provide you with a binary mixture of organic compounds. The functional group classes of the unknown compounds will be limited to aldehydes, ketones, alkyl aromatics, halogenated alkanes, or halogenated aromatics.

2) Obtain the total ion chromatogram of the mixture. Also obtain the mass spectra of each of the two components within the mixture.

Potential Unknowns for GC/MS experiment

Part I: Mixture analysis

    Prepare a 1:1 mixture for a total of 1.0mL

Group   Compounds

  A 2-hexanone and 2-chloropropane

  B bromo-benzene and 2-propanone

  C 1-bromopropane and cumene

  D chloroacetone and 2-bromopropane

  E 1-chloropropane and cyclopentanone

  F 2-butanone and propylbenzene

  G butanal and chlorobenzene

  H pentanal and cumene

  I acetone and cyclopantanone

The boiling point range is 34° - 159°.

The minimum boiling point difference for each set of unknowns

is 50° and the maximum is 75°.

Part II   Co-eluting compounds

In this section the student will be able to see the advantage of a MS detector for a GC. Compounds that have very close boiling points will have almost the same retention time, therefore separation by gas chromatography is nearly impossible.   This   is   called co-elution,   where   the   compounds   will   elute   at approximately the same retention time.

1) Obtain a second solution from your instructor. This solution may contain 2 compounds that will co-elute.

2) Obtain the total ion chromatogram (TIC)of the co-eluting compounds.   You should expect 1 TIC peak. Because these compounds have such close retention times the TIC will contain both compounds which can be isolated as well as the compounds as a mixture.

3) Obtain the mass spectra of each of the co-eluting compounds, and obtain the mass spectra of the components in the mixture.

Part II: Suggested Co-eluting compounds

Group   Compounds

A   cumene and n-propylbenzene

B   2-hexanone and 3-hexanone

C   1,2-chlorobenzene and 1,3-chlorobenzene

D   1-bromopropane and 2-bromopropane

Part III

Answer or calculate the following:

1) Rationalize the relative retention times for each set of unknowns in Part I and II, relating them in terms of structure.

2) Identify the molecular ion and base peak in the mass spectra of each set of unknowns.

3) Identify the major fragment components in each mass spectrum and draw out the mechanism for the formation of the fragments.

4) Identify the unknown compounds using fragmentation patterns and isotopic abundance calculations. Take note of the isotopic abundance of C, O, N, halogens, etc. relative to your unknown.

RESULTS

3. <u>Fundamental Gas chromatography/mass spectrometry</u>

    a) Instrumentation   Hewlett Packard - GC model 5890A

                          MSD model HP5970B

    b) Mixture Analysis

The following data represents mixture analysis performed on the GC/MS. This is a clear representation of the advantageous abilities of each component when interfaced in a co-operational effort to analyze a mixture.

Part a) in Figure 2.40 represents the TIC of 1:1 clorobenzene and 2-bromopropane. The large peak represents chlorobenzene while the smaller represents 2-bromopropane.

Part b) is the mass spectra of chlorobenzene. Note the 3:1 ratio of Cl-35 to Cl-37.

Part c) is the mass spectra of 2-bromopropane. Note that the parent peak is only slightly larger than that of the m+1 peak. Br-79 is 51% of naturally occurring bromine while Br-81 is 49%.

TIC of mixture

a.)

area

retention time

b.)

retention time=2.12

abundance

mass to charge
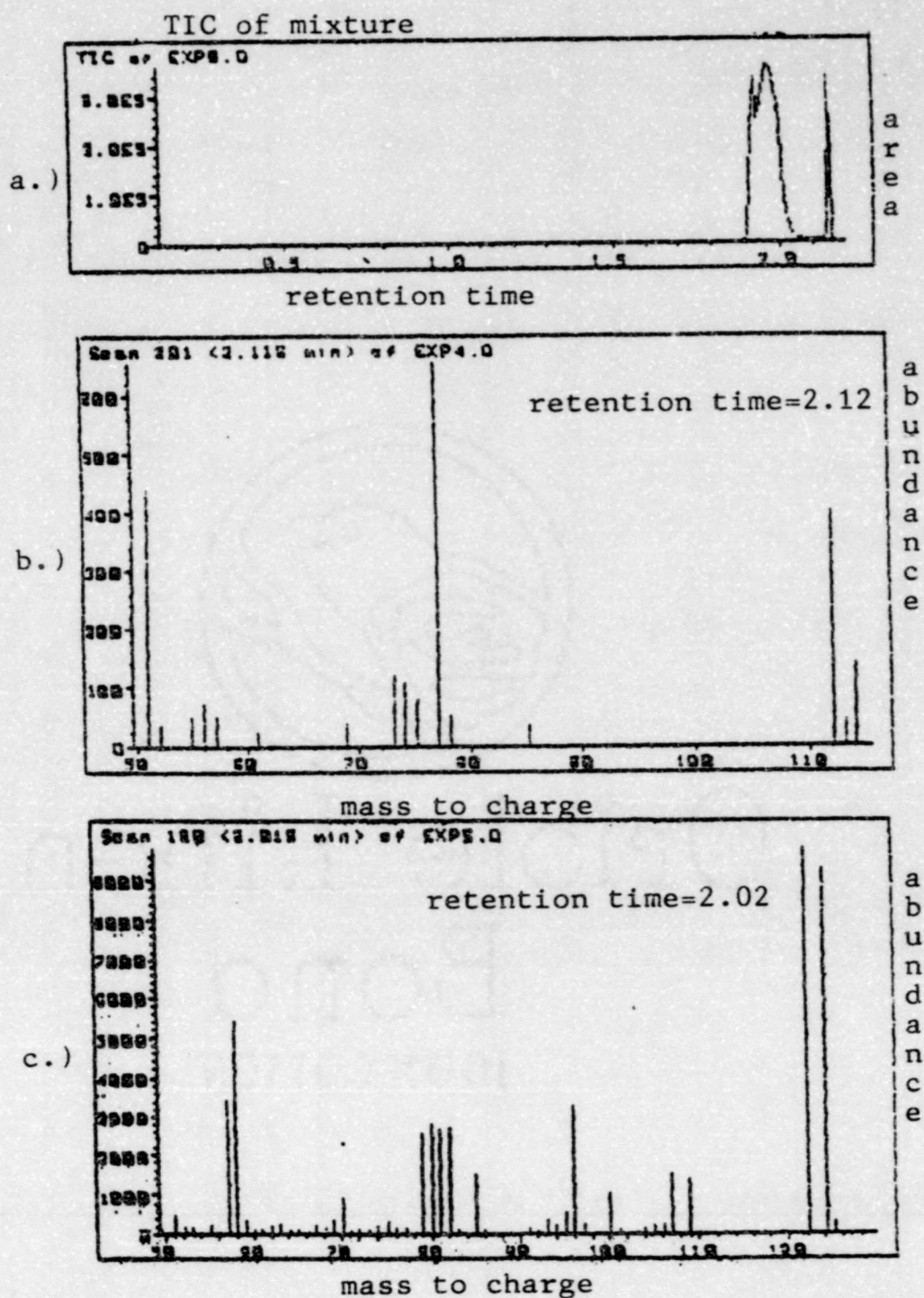
c.)

retention time=2.02

abundance

mass to charge

FIGURE 2.40   Spectra of 1:1 chlorobenzene and
2-bromopropane
a) mixture
b) chlorobenzene
c) 2-bromopropane

c) Co-elution Experiment

The following spectra in Figure 2.41 represents the separation ability of the GC/MS. In part a) the top spectrum is that of cumene and n-propylbenzene. This represents both compounds in equal amounts. The lower spectrum is the TIC of both compounds, which shows that the two compounds are not distinctly separated. In part b) at scan t=2.354 min cumene appears. The spectrum of the mixture in part c) occurs at t=2.369 while n-propyl-benzene is not found until t=2.374 in part d). Note how close the scan (retention) times are of each compound. Co-elution occurs when two compounds are closely related in boiling point and/or interaction with the stationary phase of the column.
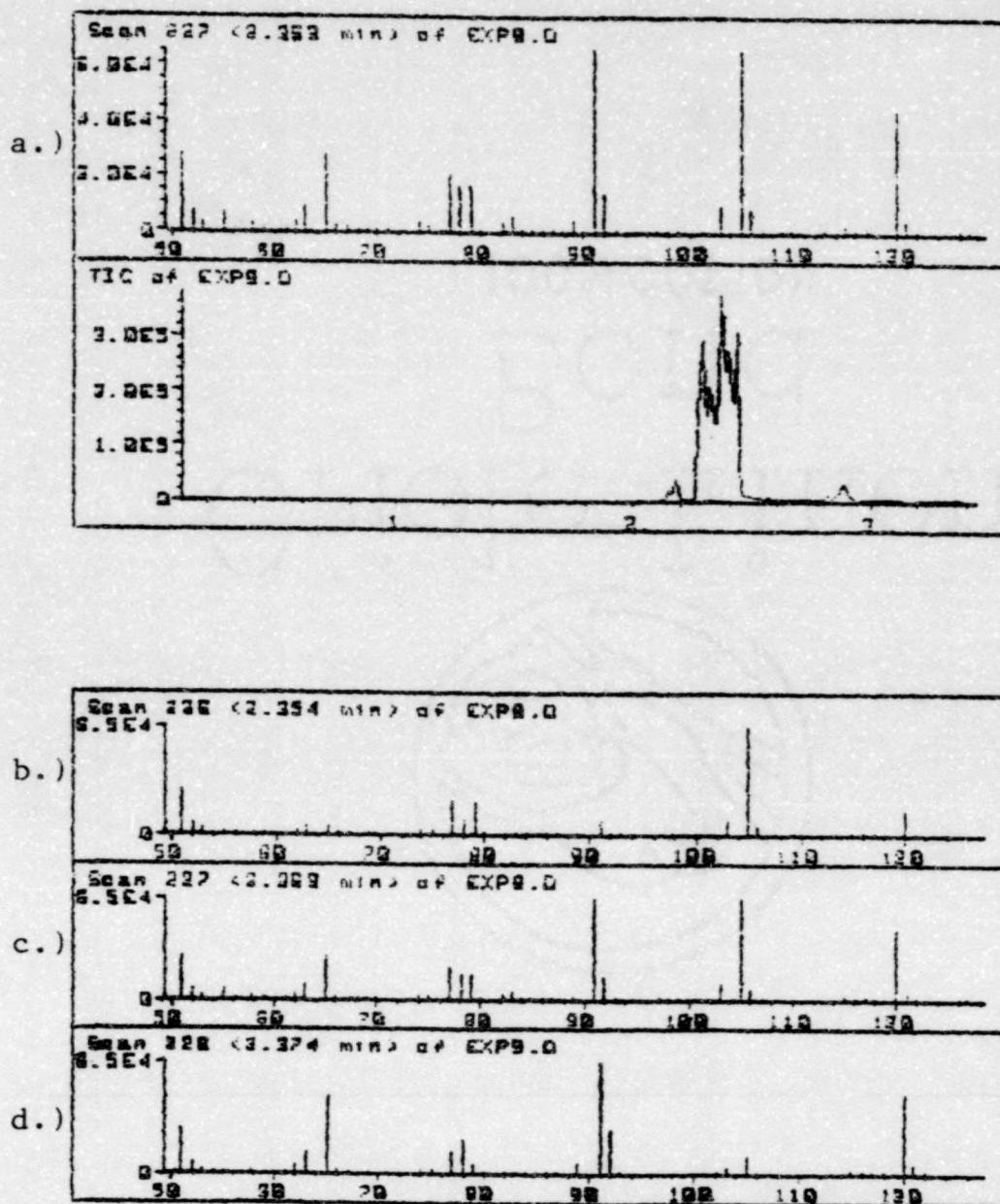
FIGURE 2.41 Spectra of 1:1 cumene and n-propylbenzene
a) mass spectra of mixture and TIC of co- elution
b) mass spectra of cumene
c) mass spectra of mixture
d) mass spectra of n-propylbenzene

REFERENCES

1. A. I. Mallet, D. E. Minnikin, and G. Dobson, <u>Biomed. Mass Spectrom</u>., 1984,11,79.

2. M. Mizugaki, T. Nishimaki, T. Shiraishi, H. Yamanaka, A. Kawaguchi, and S. Okuda, <u>J. Biochem</u>., 1982, 92, 1649.

3. D. H. Hunneman and C. Schweickhardt, <u>J. Mol. Cell. Cardiol</u>., 1982, 14, 339.

4. H. Matsuda, T. Kuzuya, M. Inui, and M. Tada, <u>Koenshu - Iyo Masu Kenkyukai</u>, 1983, 8, 155.

5. I. A. Blair, et al., <u>Adv. Prostaglandin, Thromboxane, Leukotriene Res</u>., 1983, 11, 197.

6. H. Miyazaki, et al., <u>J. Chromatogr</u>., 1984, 289, 249.

7. E. White, et al., <u>Biomed. Mass Spectrom</u>., 1982, 9, 395.

8. O. Pelletier and S. Cadieux, <u>Biomed. Mass Spectrom</u>., 1983, 10, 130.

9. K. H. Aamlid and S. Morgenlie, <u>Carbohydr. Res</u>., 1982, 111, 31.

10. P. A. Garcia Ruiz, I. Cartagena Travesedo, and A. Soler Martinez, <u>Can. J. Chem</u>., 1984, 62, 870.

11. H. Schweer, <u>Carbohydr. Res</u>., 1983, 116, 139.

12. D. W. Johnson, et al., <u>J. Steroid Biochem</u>., 1983, 19, 203.

13. D. W. Johnson, et al., <u>Int. J. Mass Spectrom. Ion Phys</u>., 1983, 48, 249.

14. J. J. Vrbanac, C. C. Sweeley, and J. D. Pinkston, <u>Biomed. Mass Spectrom</u>., 1983, 10, 155.

15. A. Kuksis, et al., <u>Can. J. Biomed</u>., 1978, 56, 1141.

16. I. Bjorkhem and H. Ek, <u>J. Steroid Biochem</u>., 1982, 17, 447.

17. A. Reiffsteck, L. Dehennin, and R. Scholler, J. Steroid Biochem., 1982, 17, 567.

18. S. Lewis, et al., Anal. Chem., 1979, 51, 1275.

19. M. Spiteller and G. Spiteller, J. Chromatogr., 1979, 164, 253.

20. A. Zlatkis, et al., J. Chromatogr., 1979, 163, 125.

21. T. M. Nicholls, R. Hahnel, and S. P. Wilkinson, Clin. Chem. Acta., 1978, 84, 11.

22. T. Masui, et al., Koenshu - Iyo Masu Kenkyukai, 1983, 8, 271.

23. D. Fraisse, et al., Analusis, 1984, 12, 63.

24. M. Novotny, et al., Chromatographia, 1982, 15, 564.

25. K. S. Webb, et al., IARC Sci. Publ., 1983, 45, 449.

26. J. R. Dahlgran and L. Abrams, J. High Resolut. Chromatogr. Chromatogr. Commun., 1982, 5, 656.

27. L. D. Betowski, H. M. Webb, and A. D. Sauter, Biomed. Mass Spectrom., 1983, 10, 369.

28. R. S. Morano, W. S. Updegrove, and R. C. Machen, Anal. Chem., 1982, 54, 1947.

29. J. C. Peterson, et al., Int. J. Environ. Anal. Chem., 1983, 14, 23.

30. V. P. Nero and R. D. Hudson, Anal. Chem., 1984, 56, 1041.

31. M. Ribick and J. Zajicek, Chemosphere, 1983, 12, 1229.

32. M. Ogata and K. Fujisawa, J. Chromatogr. Sci., 1983, 21, 420.

33. R. T. Rosen and J. D. Rosen, Biomed. Mass Spectrom., 1982, 9, 443.

34. C. J. Mirocha, et al., J. Assoc. Off. Anal. Chem., 1983, 66, 1485.

35. A. Viala, et al., J. Forensic Sci., 1983, 28, 922.

36. O. Suzuki, H. Hattori, and M. Asano, J. Forensic Sci., 1984, 29, 611.

37. S. Suzuki, et al., Eisei Kagaku, 1984, 30, 23.

38. A. Viala, et al., <u>J. Forensic Sci</u>., 1983, 28, 922.